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COMSTOCK SERIES IN VETERINARY MEDICINE

WILLIAM ARTHUR HAGAN, D V M, D SC *Consulting Editor*

THE INFECTIOUS DISEASES OF DOMESTIC ANIMALS



DR COOPER CURTICE EXAMINING TICKS ON A COW DEAD OF TEXAS FEVER

The work of Salmon, Smith, Kilborne, and Curtice on the causation and mode of transmission of Texas Fever is one of the epochal accomplishments in the field of medical history, since it was the first to show that arthropods were capable of acting as carriers of diseases of mammals Curtice championed the "tick theory" of the transmission of this disease and he was responsible in greater degree than any other in proving that the southern cattle tick (*Margaropus annulatus*) was the sole carrier of this disease. (Photograph by courtesy of *The Nation's Business*)

THE INFECTIOUS DISEASES OF DOMESTIC ANIMALS

with special reference to Etiology, Diagnosis, and Biologic Therapy

BY WILLIAM ARTHUR HAGAN D V M , D SC

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NEW YORK STATE VETERINARY COLLEGE, CORNELL UNIVERSITY



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To E. L. H.

PREFACE

This book is an outgrowth of a lecture course on pathogenic bacteriology and immunology which the author has given during the last twenty years to students of veterinary medicine. The work is less than a textbook of bacteriology in that a knowledge of the general principles of the subject is taken for granted and this part of the usual text is omitted It is somewhat more, on the other hand, in that the fungi, protozoa, and viruses that are pathogenic for the domestic animals are included in addition to the bacteria. Also, somewhat greater consideration is given to the nature of the diseases produced by the various agents and to the biological products which are available for their diagnosis, prevention, and cure than is found in most texts of this type.

Since students of animal diseases are interested in micro-organisms more because of what they do than for what they are, the work is not a systematic discussion of disease-producing organisms but rather a discussion of the infectious diseases of animals with special reference to their etiological factors

With regard to the difficult matter of nomenclature of bacteria, Bergey's Manual has been followed in general except in the case of the Gram-negative enteric organisms for which the old name *Bacterium* is retained This is done because it is felt that the numerous divisions which have been made in this group on the basis of cultural features is highly artificial The newer methods of antigenic analysis do not support these divisions but rather suggest that we have a large group in which there are minor gradations from the colon bacillus at one end to the dysentery organisms at the other without sharp divisions anywhere Until lines can be drawn more sharply it is felt that there is no justification for the creation of numerous genera within this group

In instances in which the animal pathogens are transmissible to man, this fact is pointed out and brief discussions of the nature of the human diseases are given, together with what is known of the manner in which the transmission to man occurs. It is felt that veterinarians should be informed on these matters both for their own protection and for the assistance which they often can give to physicians in such cases.

The text will be used by the author in connection with his course in Infectious Diseases of Animals. It is hoped that it will prove suitable for such

courses in other schools In addition it is hoped also that the compilation of brief accounts of the biological characteristics of the etiological agents of all of the more important infectious diseases of animals in a single volume will make it useful to veterinary practitioners, laboratory workers who are called upon to make diagnoses of these conditions, and research workers who utilize animals in their daily work. Because of the wide scope of the field covered and the necessary limitations in a book designed for student use, the discussions are not exhaustive Diseases which are known to occur in North America are treated more exhaustively than those which do not occur here Since experience shows, however, that diseases which are thought to occur only in remote parts of the world often exist here in an unrecognized form, and that it is always possible that remote diseases may be imported, an effort has been made to include brief descriptions of all of the more important of such diseases and their causative agents A few references are given at the end of each subject so those who wish to read more exhaustively may find the more important papers in the literature Since most students and practitioners do not have a working knowledge of foreign languages, the greater part of the references are to papers published in English By consulting the bibliographies given in most of these papers one can obtain leads which will open the entire literature to him.

The author is indebted to many friends for various kinds of assistance The illustrations, in particular, have been borrowed from many sources, acknowledgment being made in each case The author is especially grateful to Dr. William H. Feldman, of the Mayo Foundation for reading and making numerous criticisms of the copy, criticisms which undoubtedly have contributed to greater clarity and greater accuracy in the volume To all of those who have helped he wishes to extend his hearty thanks

In the first edition of this kind many errors undoubtedly have been included. The author will appreciate having these called to his attention in order that they may be eliminated from future editions if the reception of the work warrants future revisions In many instances it is realized that subjects are still in the stage of controversy. An attempt has been made not to be too didactic in the treating of such matters, however in the interest of good pedagogical practice some sort of a stand usually is taken in such matters after indicating that uncertainty exists.

W A Hagan
New York State Veterinary College,
Cornell University, Ithaca, N. Y.

June 1942

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PART I

THE MECHANISMS OF INFECTION AND RESISTANCE

CHAPTER I

THE CAUSES OF DISEASE

Disease may be defined as an alteration of the state of the body, or of some of its organs, which interrupts or disturbs the proper performance of its functions Functional disturbance soon is manifested by physical signs which the patient detects by his sensations and which usually can be detected by others These signs are known as *symptoms*

The cause of disease may be of external or of internal origin Little is known about the fundamental causes of the intrinsic diseases These include metabolic and endocrine disturbances, degeneration of organs from age, and neoplasms It is probable that many of these disorders are initiated by extrinsic causes as yet unrecognized The external causes of disease may be living agents such as bacteria, protozoa or filterable viruses, or they may be nonliving agents such as traumatism, heat, cold, chemical poisons, or food deficiencies

When living agents enter an animal body and set up a disturbance of function in any part, *infection* is said to have occurred The word infection is derived from the Latin *inficere*, meaning *to put into* An *infectious disease*, then, is one caused by the presence in or on an animal body of a foreign living organism, which, by its presence creates a disturbance leading to the development of symptoms

Most infections are caused by living organisms which have escaped from another individual of the same species, but sometimes they come from another species, as, for example, when man contracts rabies from a dog bite, or when a lap dog contracts tuberculosis from his consumptive master. Sometimes the infection is contracted indirectly, as when typhoid fever is contracted by a man from infected drinking water, or anthrax by a stable-fed horse in midwinter from hay which had been grown the previous summer on anthrax infected soil. Some infections originate from organisms which normally live a free existence in nature, as, for example, the bacillus of tetanus. Presumably at some remote periods in evolutionary history, all of the disease producing organisms lived a free existence, becoming parasitic and pathogenic through gradual adaptation.

THE FATES OF INFECTING ORGANISMS

Several possible fates await organisms which cause infections. This is a matter of considerable practical interest, because the transmission of the disease to other individuals depends upon the escape of the organisms from the infected one.

(A) SOME ARE DESTROYED BY THE HOST TISSUES Infections are not accomplished without resistance on the part of the host because the host-parasite relationship is not a natural one The capacity of the host to destroy invading agents is so great that the great majority of foreign living agents which manage to reach living tissues and fluids of the body are rapidly and completely destroyed. This process is going on at all times Sometimes the resistance is not great enough to prevent growth entirely and multiplication in the tissues but the infection does not become extensive and after a brief time is overcome, the invading organisms then being destroyed Sometimes the infection persists and makes slow headway against the resistance of the host, in which case the infection is called *chronic*. In a few infections such as anthrax in the herbivorous animals, the resistance of the host is so quickly overwhelmed that the organism multiplies in all parts and early death of the host ensues These cases are known as *acute* or *peracute*.

(B) SOME USUALLY ARE ELIMINATED IN THE SECRETIONS OR EXCRETIONS OF THE HOST Except in the peracute cases, when possibly no infecting organisms escape from the host, the infected animal usually eliminates, in a manner which varies with the disease, the organism which causes the disease The longer the disease lasts, i e , the more chronic it is, the more likely is the host to eliminate large numbers of the infecting agent Sometimes the infecting agent is eliminated through pus, as when an abscess bursts or is lanced, sometimes through droplets which are coughed or sneezed out when the individual is suffering from one of the respiratory infections, as canine distemper, bovine tuberculosis, avian coryza, or human diphtheria; sometimes in the intestinal discharges (feces), as in the various forms of intestinal coccidiosis of animals and in the enteric infections of man, sometimes in the urine, as in cholera of swine and in typhoid fever of man In some diseases that become extensive and even fatal, the causative organism may be eliminated in small numbers or not at all, as in some cases of tuberculosis The more chronic the disease becomes, the less likelihood there is that the host will continue to retain all of the infecting organisms. In some diseases, the mechanism by which the infection escapes from one animal to another is peculiar, as, for example, rabies in which the seat of the infection is the central nervous system and the means of escape is through the salivary glands

(c) IF THE DISEASE PROVES FATAL TO THE HOST, MANY OF THE INFECTING ORGANISMS ARE DESTROYED WITH THE CARCASS Death of the host from infection always traps in the carcass a large number of the infecting organisms. If the carcass is disposed of properly by incineration or deep burial, these organisms perish Improper disposal of dead bodies of animals may result in serious outbreaks of disease.

(D) IN SOME INSTANCES THE ORGANISM AND HOST REACH AN IMPASSE. The organism is unable to cause serious damage to the host, and yet the host is unable to eliminate the organism This situation may continue to exist throughout the lifetime of the animal, or it may be terminated either by the final elimination of the infection, or by a change in which the infection becomes more active and symptoms of disease are manifested by the host In tuberculosis of both man and animals, the tubercle bacilli may become walled off by dense tissue in some of the organs and the case is said to be *arrested.* Such cases are not entirely cured because living tubercle organisms may continue to exist in the tissues and sometimes they break forth and cause a flare-up of the disease In the human, recovery from typhoid fever usually leaves the individual with many typhoid bacilli in his urine and stools and these may persist for weeks, months, and years Individuals who discharge virulent organisms with their excretions although apparently normal otherwise, are said to be *carriers* The one who has had a recognized case of the disease in question and who has not rid himself of the infecting agent afterwards is said to be a *convalescent carrier* Sometimes individuals eliminate virulent infection although they have no history of ever having suffered from the disease themselves. These individuals are immune themselves but are a source of great danger to others who lack the same amount of resistance. They are known as *immune carriers* Sometimes, apparently, individuals harbor and eliminate a dangerous organism which they have picked up from close contact with another individual These are known as *contact carriers*

The carrier is one of the great problems in the control of many infectious diseases Animals that are obviously diseased may be recognized but there is no simple way of recognizing the carrier

SOURCES OF INFECTIONS

The courses by which infections reach new hosts often are indirect and complicated Some of the more common ways by which infections are contracted by new hosts are as follows.

1. DIRECT OR IMMEDIATE CONTACT WITH A DISEASED INDIVIDUAL This involves actual contact between a diseased and a normal surface, such as when a cow

licks the external genitals of another animal and thus picks up the organism of Bang's disease or brucellosis, or when ringworm is contracted by an animal rubbing against the affected skin of another, or when venereal infections are transferred through sexual contact.

2. CONTACT THROUGH FOMITES *Fomites* are inanimate objects which may serve to carry infections from one animal to another, as a bran sack which may convey dried discharge of an aborting cow to another cow, perhaps in a different herd, or a railroad stock car, or a motor truck which has not been properly cleaned and disinfected after carrying diseased stock and may afterwards infect other stock.

3 CONTACT WITH DISEASE CARRIERS This has already been explained. A disease carrier may infect others either directly or indirectly just as is done by a frankly diseased individual.

4. INFECTION FROM SOIL Certain spore-bearing organisms which live in soil are able to produce disease in man and animals if chance carries them into the tissues of animals, usually through wounds in the skin Blackleg of cattle, and tetanus and gas gangrene infections are of this type

5. INFECTIONS FROM FOOD AND WATER Serious infections derived from food and water are more common in man than in animals, since animals do not suffer from the typhoid and dysentery organisms which are the principal menaces to man Water often is suspected of spreading animal diseases from one pasture to another when small streams flow between them, and occasionally the suspicion has been confirmed Anthrax often is conveyed to animals through hay and straw raised on lowlands which are infected with anthrax spores. There have been a considerable number of reports of deaths of horses from eating ensilage which appears to be innocuous to cattle, and the organism of botulism has been incriminated in some of these cases.

6 AIR-BORNE INFECTIONS. Disease organisms do not spread very far through the air as was formerly believed When individuals are rather close together and especially when indoors, droplets of moisture sneezed and coughed from the upper air passages often convey the organisms of respiratory disease from diseased to well individuals. The common cold of man, influenza infections of man and animals, pneumonia, and glanders are good examples of diseases transmitted in this way. Tuberculosis of the lungs is usually transmitted in this fashion. Dust particles less often convey viable disease-producing organisms, but there are some examples such as the anthrax spore (in wool sorters disease).

7. INFECTIONS FROM BLOOD-SUCKING ARTHROPODS Some diseases of man and animals are normally transmitted through the bites of flies, fleas, mosquitoes, lice, or ticks Malaria and yellow fever are good examples of such diseases of man, and Texas fever, anaplasmosis, anthrax, and the trypanosome diseases are examples in animals. In some instances, the infecting organism must pass a part of its life cycle in the invertebrate host, as, for example, the malaria and yellow fever parasites in the mosquito, in which case the arthropod is known as a *biological vector* In other instances, such as anthrax, the black horse-fly merely carries the bacillus mechanically, not being affected by it in any way. In these cases, the arthropod carrier is known as a *mechanical vector.*

8 INFECTIONS FROM ORGANISMS NORMALLY CARRIED. Pathogenic streptococci, pneumococci, pasteurella and some other organisms can often be found on the mucous membranes of the head of apparently normal individuals. It is believed that infections sometimes occur from such organisms when the normal defensive forces of the body are weakened in any way.

INFECTION AND CONTAGION

A contagious disease is one which may be transmitted from one individual to another by direct or indirect contact All contagious diseases are also infectious, but it does not follow that all infectious diseases are contagious Tetanus, or gas gangrene infection, caused by organisms which live in the soil, are infectious but not contagious, since they are not transmitted from one animal to another The contagiousness of infectious diseases depends upon the way the parasites are eliminated from the body of the diseased animal and the opportunity they have of reaching others Some infectious diseases are highly contagious, some are slightly contagious, and a few are not contagious at all.

Properties of Pathogenic Organisms

VIRULENCE

Virulence is an attribute of all pathogenic or disease-producing organisms. The word has reference to its disease-producing power or malignancy. A highly virulent organism has great malignancy, a slightly virulent has little, and a nonvirulent has none. The property of virulence varies greatly, both between different species of parasitic forms and between different strains of the same species. In some parts of the world, smallpox is a highly malignant disease, in others it is very mild. In some years, influenzal infections are mild; in others, severe. In the laboratory, one may sometimes cause the death of a white mouse by inoculating it with as little as 0 001 cc. of a strain of strepto-

coccus, whereas another strain of the same species of streptococcus may not kill a mouse when 0.1 cc is inoculated. The one strain in this case can be said to be at least 100 times as virulent as the other.

ALTERATION OF VIRULENCE

The pathogenic power, or virulence, of many disease-producing organisms can readily be altered in the laboratory, others resist such alteration It has been suggested that possibly the ability to change virulence readily is evidence that the organism has only recently acquired the property, and thus it is not a firmly fixed characteristic

When the virulence of an organism is diminished, the process is known as *attenuation* Attenuated organisms often are used as vaccines Attenuation of virulence is readily accomplished in most instances, in fact, the mere procedure of artificial cultivation is enough to attenuate, in greater or less degree, most organisms When it is desired to reduce the disease-producing power of organisms, particularly of bacteria, many methods are available Some of the more common methods are as follows·

(1) Cultivating the organism at an unfavorable temperature Pasteur found that anthrax bacilli quickly lost virulence when they were incubated at 42–43° C. a temperature about 5° above their optimum

(2) Heating of cultures or infective material for a short time to a point a little below the thermal death point of the organism

(3) Cultivating the organism on a medium rendered unfavorable by the presence of small amounts of acids, alkalies, metallic salts, dyes, or other substances On such media many organisms can be accustomed to grow well, if the concentration of the attenuating agent is gradually increased from day to day.

(4) Injecting the organism into a species of animal which is quite resistant naturally, or whose resistance has been increased by partial immunization, and recovering it after a sojourn there

(5) Injecting the organism repeatedly into one species of animal, in which case its virulence for other animals may be decreased while it is increased for the species through which it is being passed. This phenomenon is often seen when working with viruses, seldom when dealing with bacteria.

When it is wished to increase the virulence of an organism, all methods often fail, particularly if the organism has become highly attenuated. The

procedure usually followed is to inoculate heavily an animal known to be highly susceptible with the hope of overwhelming it with the infection. If this succeeds, another animal is immediately inoculated from the first, and so on. The virulence of some disease-producing agents may be enormously increased in this way

MICROBIC DISSOCIATION AND CHANGE OF VIRULENCE

Certain growth phenomena which may be observed both macroscopically and microscopically are associated with change in virulence in many bacterial cultures. These were first described by Arkwright (1), in England, in 1920 and by DeKruif (3), in the United States, in 1921 Many of these phenomena had been seen earlier but their significance had not been fully appreciated. The changes of the type of which we speak may easily be observed on ordinary culture media, especially of the solid type When cells of a single bacterial culture are streaked on the surface of a solid medium, the colonies which develop often are not alike but may be differentiated into several types The extremes of these are the so-called S-type (Smooth type) and the R-type (Rough type). Between these two there may be several intergrading forms These types may be seen even when the culture is the progeny of a single bacterial cell, thus it is not a matter of a cultural mixture of types, except in so far as the progeny of single cells may vary from each other In most cases certain other characters are associated with each of the colony types The more important of these characters are as follows

S-TYPE COLONIES These are recognized by a smooth glistening surface and with rather regular margins The consistency of such colonies usually is soft and buttery When grown in broth, these organisms usually produce uniform clouding of the medium, and, when suspended in physiological salt solution, uniform and stable suspensions are formed When the organism is flagellated, these strains are rapidly motile When agglutinated with specific antisera, large flocculi are produced If the organism is pathogenic, this form usually is highly virulent Such organisms frequently produce capsules and are relatively resistant to phagocytosis Organisms of the smooth type usually are good antigens, i e, they are excellent immunizing agents.

R-TYPE COLONIES These colonies differ from the preceding in that the colonies have a rough or uneven contour, or at least show a granular structure under magnification and proper illumination that is not seen in the S-types. The consistency of such colonies is friable or granular. When grown in a fluid medium, the growth usually is in the form of a pellicle and sediment, and when attempts are made to suspend such cells in salt solution, the attempt

usually fails because of the cells forming into flakes and clumps which settle out. Cells from R-type colonies usually are nonmotile even though the parent culture is flagellated If the parent culture was pathogenic, the R-type variant usually is not. Capsules are not produced and such cells usually are easily phagocytosed Such strains usually are poor antigens, i.e, they immunize poorly.

INTERMEDIATE TYPES. The intermediate types usually have some of the characteristics of both R- and S-types. In some cultures, several intermediate types may be recognized, in others they are not seen

Dissociation of most bacterial cultures into S- and R-forms occurs naturally when they are growing in culture media, and in many cases also when growing in tissues. Various ways have been found by which it is possible to force dissociation to occur in culture media when it does not occur readily otherwise. Many smooth strains, for example, can be made to develop the rough form by cultivating them in culture media to which immune serum has been added.

Spontaneous dissociation from the S- to the R-form occurs readily in most cultures, sometimes to the extent that the S-form disappears entirely The R- to S-form of dissociation, however, is not often seen spontaneously and is not easily forced

The significance of dissociation with relation to virulence is clear. It affords a possible clue to the reason why pathogenic bacteria in artificial culture tend to become less virulent, why such organisms in chronic infections often are attenuated in virulence, why vaccines sometimes are efficacious and sometimes not, why some strains of organisms make satisfactory antigens for agglutination tests and others do not If virulence is to be retained, if vaccines are to be effective, and if cultures are to make good agglutination antigens, means of keeping the strains in the S-form must be found. In many cases, this is not difficult, it being necessary only to make frequent plate cultures and to select S-type colonies for propagating the strain Inoculation of susceptible animals is another way to eliminate rough and intermediate variants from a culture If the culture has not lost all virulence, the animal will act selectively, destroying the nonvirulent types and yielding finally only the smooth type

EVOLUTION OF PATHOGENICITY

Many diseases apparently are not so destructive today as they once were. The reasons for this obviously are not simple, and probably they have to do with changes in both host and parasite. Mass immunization or "herd immunity" which gradually raises the resistance of populations probably is a

factor. It will be discussed later. Better nutrition and better hygienic conditions of many kinds probably have played a part Genetic factors evidently are at work because destructive disease tends to eliminate the more susceptible and leave the resistant strains Years ago, Theobald Smith (7) suggested that it should be expected that infectious diseases would evolve into more chronic, less virulent forms in the course of time, even if the host resistance in the meantime did not change. The reasoning behind this conclusion was that in acute disease the parasite quickly destroyed its host and thus quickly terminated its own chances of escaping to new hosts, whereas in chronic disease the opportunity for escape was much better because of the prolonged course of the disease Under such conditions, Smith concluded that the chronic form had a much better chance of propagating itself and would, in time, become the predominating form.

THE MECHANISM OF DISEASE PRODUCTION BY PATHOGENIC ORGANISMS

The possession of the property of virulence distinguishes pathogenic organisms from the nonpathogenic In the final analysis virulence depends upon two properties of the organism·

1. The ability to propagate in the tissues or on the surfaces of the body.
2. The ability to form chemical substances which injure or destroy body cells, organs, or tissues.

ABILITY TO PROPAGATE IN TISSUES

The ability to grow in an animal body is something that an organism acquires in its evolution toward a parasitic existence We know very little about what actually goes on in this process Obviously the organism has to "learn" how to protect itself against forces in the body which are antagonistic to it. Virulent organisms usually, but not invariably, are of the S-type, which means that they are more or less resistant to phagocytosis and often possess capsular substance which serves to protect them from harmful influences in the tissue fluids.

The ability to invade and multiply in living tissues varies a great deal among disease-producing organisms Some organisms which are malignant disease producers have little invasive ability and do most of their damage while growing in restricted parts of the body in which they generate powerful poisons, or toxins, which are absorbed and circulated throughout the body. The tetanus organism, for example, usually remains localized in a wound which may be very insignificant in size but in this wound the powerful tetanus toxin is gen-

erated which, carried by the blood and lymph, reaches the nervous system where the damage is done The organism of human diphtheria rarely is found in the internal organs but usually is restricted to the membranes of the throat where the diphtheria toxin is generated These organisms produce systemic diseases only because of the absorption of their toxins

The organisms which lack soluble toxins must have considerable powers of invasiveness if they are to produce systemic disease, or disease of any of the vital organs Such organisms produce their principal damage at the points where they are multiplying Sometimes these organisms localize near the point where they entered the body and do not extend far from this site These are known as *local infections* Most wound infections are of this type. Others characteristically invade lymph and blood vessels whence they are carried to many other parts of the body, a process known as *metastasis,* where secondary localizations occur These are the *systemic* or *general infections*

In speaking of invasiveness we should not develop the idea that organisms actively drive or bore their way into tissues Many actively invading organisms are nonmotile In most instances, bacteria probably enter the tissues in the same way that inanimate particles do. Organisms on mucous membranes are often picked up by wandering phagocytic cells which find their way back into the tissues carrying their bacterial load with them These cells often destroy their bacterial meal, in which case nothing happens, but in other cases the bacterial load survives, destroys the cells which harbor them, and then proceeds to initiate an infection of the tissues where they find themselves. Other organisms colonize on surfaces and reach the subepithelial tissues by direct extension of growth through glands and hair follicles If the organism possesses the property of virulence, it will go on from this point to produce an infection, if it does not, it will be picked up by the fixed or wandering phagocytic cells of the tissue, and destroyed

As a general rule, bacteria do not multiply in the circulating fluids of the body. When many bacteria are found in the blood, a condition which we call *bacteremia,* it means that there are foci in the tissues from which the organisms are being poured forth in such large numbers that the blood clearing mechanism is temporarily overwhelmed.

At one time, it was seriously believed that micro-organisms might produce disease in a purely mechanical way, that is, by blocking capillaries or tissue spaces This idea is untenable because it is known that the body has mechanisms for dealing with rather large amounts of foreign solids, more than the total bulk of bacteria present even in overwhelming infections The damage caused by infecting organisms clearly is because of their chemical effects.

ABILITY TO FORM POISONS OR TOXINS

Endotoxins. As to chemical poisons, it is clear that substances mildly poisonous to animal tissues are contained in most bacteria Extracts of many purely saprophytic organisms often are distinctly poisonous when injected into animals These substances probably are a part of the bacterial cytoplasm itself, or perhaps are derived from the breaking down of the bacterial protoplasm They are known under the general name of endotoxins

Vaughan (9) observed that all proteins, bacterial or of other origin, could be split by caustic potash in absolute alcohol into fractions, one of which always is poisonous, and that this poison had practically the same pharmacological action no matter what its source He believed, therefore, that endotoxins are not metabolic products which are retained in the cell, but are protein split-products derived from the breaking down of the cells after their death

The difference between a pathogenic and a nonpathogenic organism, if pathogenicity depends upon the mildly poisonous endotoxins, must be that the one has the ability to penetrate into the body in the face of the resistance offered by the body's protective mechanism, to multiply and colonize in various tissues and organs, and there to release its poisons, while the other lacks this ability. Since most pathogenic bacteria do not form any recognizable poisons, other than endotoxins, it follows that they must have the property of invasiveness.

Exotoxins (True toxins). Certain plants and animals and a few bacteria secrete or excrete substances which are highly poisonous to animals These substances have a number of properties in common, yet each toxin is highly distinctive or specific The cardinal characteristic of all of these poisons is that they are *antigenic*, that is, that they will stimulate in animals *antibodies* which will neutralize them *in vivo* or *in vitro* The endotoxic substances of bacteria do not have this property, they are not true toxins

Toxins are divided into three groups according to the type of organism which produces them:

(a) The phytotoxins, such as *ricin* of the castor bean plant, and the *amanita toxin* of the poisonous fungi of that name (toadstools).

(b) The zootoxins, such as the venoms of certain snakes, spiders and bees.

(c) The bacterial toxins, such as those of the organisms causing diphtheria, tetanus and botulism.

Toxins usually exhibit specific affinities for certain cells or tissues; thus we have *neurotoxins*, such as those of tetanus and botulism, *hemolytic toxins*,

such as those of many streptococci and the organism of tetanus, and leucotoxins, or *leucocidins* such as those of the pyogenic staphylococci. The first type combines with and injures or destroys nerve cells, the second destroys erythrocytes or red blood cells, and the third destroys leucocytes When toxins are injected into the blood of susceptible animals, they quickly disappear from it; furthermore, in diseases in which toxins play a predominating role, seldom can more than traces be found in the circulating blood. The reason for this is that they are quickly absorbed by the cells or tissues for which they have affinities. This can easily be demonstrated *in vitro,* suspensions of nerve cells will combine with and inactivate the neurotoxins, and erythrocytes will do likewise with the hemolytic toxins. Suspensions of other types of cells will not do this. It is interesting to note also that tetanus toxin will circulate for days in the blood of some cold-blooded animals, and diphtheria toxin disappears very slowly from the blood of rats These animals are not susceptible to these toxins and their tissues have no affinity for them.

Physical Properties of Toxins

1 MOST TOXINS ARE COMPARATIVELY THERMOLABILE. Heating to 58° to 60° C. for ten minutes will inactivate most toxins. A few are more resistant.

2. ALL TOXINS DETERIORATE WITH AGE. Some lose their potency so rapidly that they are difficult to work with; others deteriorate rather slowly. The speed of deterioration depends upon the conditions under which they are kept. They usually keep best when stored in darkness and at a low temperature If carefully dried and stored in a dry atmosphere, many toxins can be kept with little change for long periods.

When toxins deteriorate, it is the poisonous portion which disappears first. Antigenicity is retained long after all traces of toxicity have been lost Toxins which have lost their poisonous properties but have retained antigenicity are known as toxoids.

3. TOXINS ARE COMPOSED OF RELATIVELY LARGE MOLECULES. They will diffuse through parchment but not through the thicker collodion membranes. Their molecular size evidently is less than that of albumens and globulins but larger than that of the amino acids.

Filtration studies always leave open the question of whether the size measured is that of the toxins, or of other substances to which a toxic radicle is adsorbed.

4. MOST TOXINS REQUIRE A "PERIOD OF INCUBATION" BEFORE SHOWING THEIR POISONOUS EFFECTS. Many of the bacterial toxins, and some of the others, do not

cause symptoms immediately after injection Even when enormous doses are given to experimental animals, there usually is a delay of several hours before symptoms appear This is quite different, of course, from the action of other poisons such as prussic acid and strychnine in which the symptoms of intoxication are immediate Most poisons have small molecules and are readily diffusible; the toxins have large molecules and do not readily diffuse through membranes. Since poisoning does not occur until the poison has diffused into the susceptible cells, the lag shown by toxins can be explained on this basis It is possible, too, that toxins have to be activated in some way before they become poisonous.

Chemical Properties of Toxins

1. ALL TOXINS ARE ANTIGENIC POISONS It already has been stated that this is the prime, or cardinal, characteristic which separates these poisons from all others The antibodies stimulated by the presence of toxin in the tissues of animals always are highly specific, i e, they will neutralize only the type of toxin which caused their production

2. TOXINS CAN BE PRECIPITATED FROM SOLUTION BY CONCENTRATED ALCOHOL, METALLIC SALTS, AND AMMONIUM SULPHATE. In these respects, their reactions are like those of proteins. After the highest degree of purification yet attained, toxins give one or more of the tests for protein. Whether this means that the toxin is protein, or is adsorbed to proteins, cannot be answered with finality.

3. TOXINS CAN BE CONCENTRATED AND PURIFIED BY ADSORPTION ON ALUMINUM HYDROXIDE GELS AND ELUTION FROM THEM. In this respect they resemble enzymes

4. MOST TOXINS ARE READILY DESTROYED BY PROTEOLYTIC ENZYMES Peptic or tryptic digestion quickly destroys the majority of toxins. The toxin of *Clostridium botulinum*, which causes botulism because of its absorption through the digestive tract, is quite resistant

5. TOXINS CAN BE CHANGED TO TOXOIDS BY CHEMICAL TREATMENT It already has been stated that toxins tend to deteriorate naturally into nonpoisonous substances called toxoids The importance of toxoids lies in the fact that they retain the immunizing properties of toxins while losing their poisonous properties. Toxins can quickly be converted into toxoids by treatment with certain chemicals, notably formaldehyde. Formaldehyde treated toxins are used for immunization against human diphtheria, and against tetanus. Ramon (5), who developed the method, calls these products *anatoxins*, and they are so

designated in the French literature. The term is used in English but the word *toxoid* is more common.

SOLUBLE PRODUCTS, OTHER THAN TOXINS PRODUCED BY PATHOGENIC BACTERIA

Many years ago (1900–05), Bail (2), and his co-workers in Germany, showed that sterile filtrates of the exudate which collects under the skin of animals after the injection of any of a number of pathogenic organisms, had remarkable properties The phenomena are highly specific for the organism used for injection Several of the more important of these observations are as follows

1 When the filtrate alone is injected into normal animals, no reactions are observed at the time, but later it can be shown that the animals have become immune to the organism

2. When the filtrate is injected with sublethal doses of the organism, the combination becomes lethal.

3 When the filtrate is injected with a dose of the bacterial culture which would have caused a chronic disease, the disease produced is acute.

4 When the filtrate is injected with a strain of the organism which has been attenuated so it no longer will produce infection when injected alone, the mixture will cause infection

The agent in the exudate which apparently increases the virulence of the specific organism was called *aggressin* Others called it *virulin*.

More recently, it has been shown that aggressive substances are present in cultures of some organisms growing on artificial media Felton and Bailey (1926), for example, showed that the specific polysaccharide, extracted from a virulent Type II pneumococcus, which is harmless in itself, would, when injected with an attenuated pneumococcus culture of the same type, render the strain highly pathogenic. Whereas the organism was quickly engulfed by phagocytes and destroyed by them when injected alone, it was not destroyed when mixed with the filtrate.

Instead of acting as a stimulant to the organism, it is evident, therefore, that the effect of the aggressive substance in such filtrates is to interfere with one of the defensive mechanisms of the body In some instances, as in the case of the polysaccharide from the capsule of the pneumococcus, the aggressive substance is relatively heat stable. In other instances, they are quite heat labile.

It is apparent, then, that the word aggressin does not refer to a single substance secreted by bacteria, as Bail supposed, but to a property which is de-

pendent upon the release from bacteria, in or out of the body, of substances such as capsular material, bacterial protein, secretions, excretions, enzymes and toxins which have a deleterious effect upon the tissues of the host, which interfere with the host's defensive mechanism, and thus permit multiplication of the organism in the tissues.

THE SHWARTZMAN PHENOMENON

By using a technic introduced by Shwartzman (6) (1928) it is possible to demonstrate the presence of toxic material of some sort in filtrates of many bacterial cultures which cannot be demonstrated by direct methods The procedure is to inject a small dose of the filtrate into the skin of a rabbit and to follow it, in from 12 to 24 hours, with a larger dose of the same material intravenously. In a large proportion of animals, the second injection is followed by the formation of a hemorrhagic lesion at the site of the local injection within an hour or two after the intravenous injection Usually necrosis of the area occurs and an ulcer is formed.

The nature of this reaction is not known, but evidently it depends upon the presence of toxic material in the filtrate The reaction cannot be elicited with filtrates of all organisms, nor can it be induced with proteins or other substances of nonbacterial origin Furthermore, the reaction is not wholly specific, since it may occur when the first injection has been made with a filtrate of one organism, and the second with that of another organism.

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CHAPTER II

THE PROTECTIVE MECHANISMS OF THE BODY

Animals are constantly in contact with many pathogenic organisms. Normal animals have rather effective mechanisms for protecting themselves against these organisms The study of these mechanisms, in particular those of the tissues which we term the internal defenses, constitute what has long been known as *immunity* or immunology. The word is rather unfortunate because immunity literally implies complete protection, whereas the subject is concerned with mechanisms which provide degrees of protection varying from the slightest to those which are complete The phrase, *resistance to disease,* is preferable to immunity from disease On the other hand, the immunologist is interested in many phenomena which, so far as is known, have nothing to do with protection from disease These will be discussed later

The protective mechanisms of the body can conveniently be divided into two types:

1. Those that serve to hinder or prevent the passage of disease-producing agents into the tissues (*Primary defenses*), and
2. Those that deal with such agents that have managed to enter the tissues in spite of the primary defense mechanism (*Secondary* or *parenteral defenses*).

THE PRIMARY DEFENSES

Before the tissues of the body can be reached, one of the epithelial coverings—the skin or a mucous membrane—must be penetrated. These integuments, the skin especially, serve as rather effective physical barriers, yet the matter evidently is more complicated than this. When cultures of many pathogenic organisms are swabbed on the skin of man, and the same facts probably will hold for the skins of animals, they die off very rapidly, much more rapidly than on the skin of cadavers and on other surfaces. The sweat contains chemical substances of unknown constitution which cause their destruction. Certain cocci, which are characteristically found on skin, are resistant to these substances.

The mucous membranes undoubtedly are protected by the mucus which

is constantly secreted This material traps small particles of all sorts, including bacteria In the digestive tract this material gathers into masses which are then carried through the canal with its contents In the respiratory tract most of it either is coughed up, or swept up into the pharynx by the action of the ciliated epithelium, whence it is swallowed Particles in the conjunctiva of the eye are carried through the tear ducts into the pharynx and swallowed. In the urinary tract foreign bodies usually are swept out with the urine. In the vagina there is no regular movement but the secretions appear to be unfavorable for the growth of most bacteria, and normally there are few bacteria there.

In the conjunctival secretion (the tears), and to a lesser extent in the secretions of other mucous membranes, and even in skin secretions Fleming (7) demonstrated a heat-stable substance which actively dissolves many saprophytic and some pathogenic organisms He gave to it the name *lysozyme*. This probably is an important protective agent.

When organisms are swallowed, they immediately come in contact with the highly acid gastric juice When much food is present, many of the ingested bacteria pass the stomach unharmed, but when it is relatively empty and thus there is little protection for them, most saprophytic and many pathogenic organisms are destroyed. The stomach thus stands guard at the beginning of the digestive canal to keep down the number of viable organisms entering it. In the beginning of the intestine, there likewise are few viable bacteria. It is only toward the end of the small intestine, and in the cecum and colon, that the intestinal flora becomes rich. It has long been known that gunshot and other penetrations of the anterior or upper portions of the bowel were not nearly so likely to lead to fatal peritonitis as when the wounds involved the lower portions.

THE SECONDARY OR PARENTERAL DEFENSES

It already has been pointed out that some pathogenic organisms possess slight powers of invasiveness yet are capable of causing serious damage because of their potent toxins, whereas others must invade the interior of the body if they are to do damage. In the first case, the principal task of the defense mechanism is to inactivate or destroy the toxin, since its entrance cannot be prevented; in the second instance the task is to hinder the multiplication and spread of the invading organisms and eventually to destroy them. The mechanism of defense is somewhat different in these two problems.

Antitoxic Immunity. Von Behring and Kitasato (12) (1890) were the first to show that the serum of animals which had received repeated sublethal doses of bacterial toxins developed the power of specifically neutralizing or inacti-

vating the toxins, *in vitro* as well as *in vivo* They worked with the toxins of the diphtheria and tetanus organisms Ehrlich (4) later showed that antitoxic serums could be produced by injecting certain phytotoxins, ricin and abrin. Calmette (2) extended our knowledge by showing that antitoxins could be produced for zootoxins (Antivenomous serum for cobra venom).

Antitoxins can be produced only by stimulating animals with nonlethal doses of toxin, and the antitoxin produced is effective only for the toxin which stimulated its production For the production of therapeutic serums, animals, usually horses, are given repeated doses of toxin As antitoxin begins to develop in them, larger and larger doses of the toxin may be given. Finally, when the animal has tolerated enormous doses, it is bled, the serum is separated from the clot, and the globulin fraction of the serum is precipitated chemically A solution of these globulins constitutes the antitoxin of commerce.

An animal which has suffered and recovered from a frank, or a mild unrecognized, infection by a toxin-producing organism has antitoxin in his blood for a long time thereafter. In practically all instances, however, the amount of antitoxin falls rapidly to a very low level after recovery. This amount usually is great enough immediately to neutralize the toxin formed if the individual should be infected with the same organism again The toxin of the new infection is thus prevented from reaching and damaging the tissues which are susceptible to it If a large dose of toxin is injected, experimentally, after the antitoxin level of the blood has become low, the animal can be fatally intoxicated because the blood is not capable of inactivating much toxin at one time. This situation cannot happen in the natural disease, however, because all infections have to have small beginnings, and it is in the beginnings that the blood antitoxin is effective

When experimental animals are injected with toxin for the first time, antitoxin production is rather slow, it may be several weeks following a single injection before the maximum level is reached. On the other hand, when a dose is given to an animal that previously had been injected with the same material, even though it may have been many weeks, months, or years previously, the antitoxin content begins to rise much more quickly. Nature evidently is not depending wholly on the small level of blood antitoxin for protection, the tissue cells are in readiness to produce more, when more is needed. Animals in this state are said to be *actively immune*. Other animals can be made *passively immune* by injecting blood or blood serum from actively immune animals into them Such animals have heightened resistance only to the extent that it is conferred by the antitoxin which is introduced, and this is fleeting since the foreign antibodies are eliminated or destroyed within a

period of several weeks The tissues of such animals are not prepared to produce more antitoxin.

Antibacterial Immunity. The mechanisms that are involved in dealing with organisms that produce disease by actively invading the tissues are considerably more complicated and less is known about them than about that which has to do only with toxins. In general it appears that bacteria are treated by the body in much the same way as inert particles of any kind

Nonvirulent organisms are destroyed by these mechanisms. Large numbers of virulent organisms also are destroyed but their ability to multiply in the body often enables virulent organisms to increase faster than they can be destroyed, in which case the defense mechanism sooner or later is broken down and the body is overwhelmed by the infection

THE BEHAVIOR OF THE FLUIDS AND TISSUES OF NORMAL ANIMALS TOWARD FOREIGN PARTICLES

When suspensions of finely divided insoluble material, such as carmine or carbon (India ink), are injected into a vein of a normal animal, the material does not remain long in the circulation After a few hours, at most, the greater part of it can be found deposited in certain organs, especially the liver, spleen, lymph glands, and bone marrow It is fixed in these locations by being taken up from the circulating fluids by certain phagocytic cells which are known as *macrophages* or *histiocytes* These cells are a part of what Aschoff (1) calls the *reticulo-endothelial* system of the body This system is a series of somewhat similar cells which are scattered through all parts of the body. Most of these cells are *fixed,* that is, they are permanently located in, and form a part of, the various organs A smaller number are not so attached and are found wandering through the tissues and circulating in the blood and lymph The sessile or fixed tissue macrophages include the endothelial cells lining the sinusoids of the liver (Kupffer cells), and those of the sinuses of the spleen, bone marrow, and lymph nodes, also the reticular cells, wherever they occur, which is largely in the spleen and lymph nodes The circulating macrophages of the blood often are called large mononuclear leucocytes, or endothelial leucocytes Besides those which are a part of the reticulo-endothelial system, another kind of circulating blood cell, the polymorphonuclear or neutrophilic leucocyte, plays an important role in many infections.

Suspensions of inert material which are relatively stable before injection usually become unstable in the blood This applies also to bacterial suspensions. The particles, or bacteria, are precipitated out of suspension and form masses which then lodge in some of the capillaries, particularly those of the lungs This process is known as *agglutination* Agglutinated masses are at-

tacked by phagocytic cells, broken down gradually, and carried away piecemeal by the phagocytes

When nonvirulent organisms are injected, they are taken up, like inert material, by the cells of the reticulo-endothelial system, and especially by the endothelial cells of the liver and spleen They are then destroyed by intracellular digestion.

Virulent organisms are taken up by the same cells but all of them are not destroyed After a few hours, the number of bacteria in the blood begins to rise because of their multiplication and from this point on, the outcome of the disease depends upon whether the phagocytic cells can remove the organisms as fast as they are produced The following table, taken from Topley and Wilson (11), illustrates a typical experiment conducted by Wright, who studied the disappearance from the peripheral blood of rabbits, of nonvirulent, slightly virulent, and highly virulent pneumococci

TABLE I

NUMBERS OF PNEUMOCOCCI IN THE PERIPHERAL BLOOD OF NORMAL RABBITS AFTER SINGLE INTRAVENOUS DOSES *

Time	Avirulent	Slightly virulent	Highly virulent
Immediately	8,900,000	1,030,000	1,070,000
Two hours	206	20,800	137,000
Five hours	2	340	25,000
24 hours	0	1,300	1,510,000
48 hours	—	134	Animal dead
96 hours	—	0	—

* From Topley and Wilson

In this experiment, nearly 98 per cent of the highly virulent culture had been removed from the blood by the end of five hours It must not be supposed that they had been killed, however, since the subsequent behavior of the infection clearly showed that this had not been the case

When inert particles, or bacteria, are injected into the body by other routes, they eventually are handled by the same types of cells that deal with them following intravenous injection Often the load of foreign materials, after subcutaneous or intramuscular injection, is so slowly absorbed that the greater part of it is handled by the local macrophages, or by those of the neighboring lymph nodes Animals frequently can be killed by smaller doses of pathogenic organisms that are injected into local areas than when they are introduced directly into the blood When injected into the tissues they usually do not encounter the full strength of the protective mechanism, as they do when they enter the blood, and thus are enabled to multiply and establish a focus from which the blood later is flooded with organisms.

The peritoneum has a characteristic defensive mechanism The peritoneal fluid usually has few cells in it. When bacteria or other particles are introduced into the cavity, there occurs an outpouring, for a few hours, of polymorphonuclear leucocytes, which phagocytose the particles Within a few hours, these cells cease to take an active part in the situation; macrophages now appear in abundance and these cells not only take up any free particles which may be left, but also the polymorphonuclear cells which contain such particles. All of these cells then tend to collect on the surface of the omentum, which contracts into a mass in the anterior part of the cavity, usually coated with fibrin In this mass, carbon particles or dead acid-fast bacilli can be recognized for months

Bacteria or other particles pass very quickly from the peritoneal cavity into the lymph spaces, then into the lymph channels and the blood. Organisms introduced into the peritoneal cavity often can be found in the blood within a matter of minutes It is believed that the pumping action of the diaphragm favors this passage

Organisms that are introduced by mouth reach the tissues, in many instances, by invasion through the tonsillar crypts, whence they are carried to the neighboring lymph nodes In other instances, they pass into the intestine, through the intestinal mucosa into the lymphatics and thence into the mesenteric lymph nodes where they often lodge After penetrating the mesenteric lymph nodes, they are washed into the thoracic duct and finally into the blood. It appears that the normal intestinal mucosa is remarkably resistant to penetration by bacteria, a fortunate circumstance, but there is no doubt that many organisms are constantly escaping through defects or injuries, if not through the normal epithelium

THE BEHAVIOR OF ANIMALS TOWARD INJECTIONS OF ORGANISMS TO WHICH THEY HAVE BEEN IMMUNIZED

Animals which previously have been treated with suspensions of living or dead organisms, or extracts of such organisms, behave toward virulent organisms in much the same way as untreated animals do toward the nonvirulent Table II (page 24), from Topley and Wilson, showing the behavior of normal and immunized rabbits to an intravenous injection of a virulent pneumococcus culture, shows this

PASSIVE TRANSFERENCE OF ANTIBACTERIAL IMMUNITY

It is possible to render an animal partially or wholly resistant to an infection by introducing into it the serum of another animal which has been actively immunized against it The passive immunity is immediately effective

TABLE II

NUMBERS OF VIRULENT PNEUMOCOCCI IN THE PERIPHERAL BLOOD OF NORMAL AND ACTIVELY IMMUNIZED RABBITS AFTER A SINGLE INTRAVENOUS DOSE *

TIME AFTER INJECTION	NORMAL		IMMUNIZED	
	Rabbit 247	Rabbit 248	Rabbit 299	Rabbit 300
Immediately	870,000	1,100,000	1,000,000	1,000,000
5 hours	1,300	3,300	12	68
24 hours	142,000	1,953,000	0	269
48 hours	2,800	Innumerable	149	79
96 hours	Dead	Dead	0	0

* From Topley and Wilson

after the injection of the serum but is short-lived The serum is cell-free, and since the passively immunized animal behaves toward the infection in essentially the same way as the actively immunized, or in the way that an unimmunized animal behaves toward a nonvirulent organism, it follows that there is something (antibodies) in the serum of the immunized animal which destroys the virulence of the organism

Protection in antibacterial immunity, therefore, is due to the co-operative effort of serum antibodies and the cellular mechanism which has been described The effect of antibodies, passively transferred in serum, on the course of the infection by virulent pneumococci in the rabbit, is shown in the following table The immunized rabbits had been given antipneumococcus serum prior to the time of the test

TABLE III

NUMBERS OF VIRULENT PNEUMOCOCCI IN THE PERIPHERAL BLOOD OF NORMAL AND PASSIVELY IMMUNIZED RABBITS AFTER A SINGLE INTRAVENOUS DOSE *

Time after injection	Normal rabbit	Immunized rabbit	Immunized rabbit
Immediately	2,300,000	2,300,000	2,000,000
5 hours	43,000	2	52
24 hours	Dead	8	14
48 hours	—	0	1
96 hours	—	0	0

* From Topley and Wilson

EFFECTIVENESS OF ANTIBACTERIAL IMMUNITY

The effects of immunization so far described have been those which tended to keep the blood stream free of bacteria and thus to prevent fatal bacteremia. Although the blood may be kept free of organisms, it does not follow, nec-

essarily, that the whole body has been freed from them In many instances organisms continue to multiply locally, producing local damage which may be so extensive as to cause serious consequences, particularly when they are located in vital organs Such animals must be regarded as only partially immunized Antibacterial immunity is much more apt to be partial, more variable, and less effective than antitoxic immunity.

Types of Immunity

Immunity or resistance to disease, may be divided into two categories, innate and acquired Innate immunity is something that is inherent in an animal, and is not due to the presence of antibodies Acquired immunity is associated with the presence of antibodies These are not inherent but are caused by contact with agents which have stimulated their formation.

INNATE IMMUNITY

One form of innate immunity is *species immunity* This may be absolute or it may be relative Man is absolutely insusceptible to hog cholera, but only relatively insusceptible to foot and mouth disease Horses are absolutely insusceptible to foot and mouth disease but only relatively resistant to tuberculosis Species immunity appears to be due to a physiological incompatibility between host and parasite

Racial immunity always is a relative matter Races or breeds of plants and animals which have greater or less resistance to particular diseases than their parent stock may be developed by selection Under natural conditions, such races develop spontaneously.

Individual immunity of innate nature always is relative and generally of slight degree When epidemics of disease occur, all individuals of populations seldom contract them This matter is complicated, of course, by the facts that acquired immunity generally is involved and that not all individuals are equally exposed.

ACQUIRED IMMUNITY

Acquired immunity may be *active* or *passive* An active immunity is acquired by having suffered from an infection, either frank or unrecognized, or by having been artificially immunized by the injection of living or dead cultures, or by culture filtrates. These substances contain *antigens*, and because of stimulation by them the animal produces *antibodies*. Active immunity is relatively long lived and sometimes life long.

Passive immunity is attained by the transference of antibodies from another animal which has been actively immunized The immunity in these cases lasts only so long as the antibodies remain in the body, which is a matter only of several weeks Passive immunity usually is a state created artificially by the injection of antibody-containing serum The only occasion when passive immunity occurs naturally is when antibodies pass to the fetus *in utero,* or to the new-born through the first milk, or colostrum, of the mother

IMMUNITY OF VERY YOUNG ANIMALS

It has long been recognized that human infants usually are resistant to many of the common diseases during the first several weeks of their lives The infant is resistant only to those diseases to which its mother is resistant, and the immunity is due to the passive transfer of antibodies from the maternal blood to that of the fetus *in utero* through the placenta. In the case of children it is known that very young infants seldom contract diphtheria Zingher (13) has shown that very young infants usually are negative to the Schick test which indicates that they carry antitoxin in their tissues Ehrlich (3) was one of the first to show that antibodies could be transmitted through the placenta Working with the phytotoxins, ricin and abrin, he showed in 1892 that young mice, born of immunized mothers, possessed protective antibodies in their blood Famulener (6) showed that hemolytic antibodies passed to the young kids from immunized mothers On the other hand, Smith and Little (9) showed that calves are not passively immunized *in utero,* and there is evidence to indicate that swine likewise are exceptions to the general rule. In these instances, nature has made up for the defective mechanism by storing a rich supply of antibodies in the colostral milk of the mother, and the new-born must depend upon this material to provide the early immunity that they need. The young animal usually suckles during the first hour after birth and thus takes in, quite promptly after birth, the antibodies which are stored in the colostrum. These antibodies would not be absorbed unchanged from the intestine of the adult animal, but the intestine of the young calf permits them to pass freely Within an hour after taking his first meal, antibodies can be detected in the blood of the calf.

The tissues of very young animals do not always react to antigenic stimuli in the same way as those of older animals, and for this reason it is believed that the antibody-forming mechanism is imperfectly developed at birth, and has to mature afterwards It is known, for example, that some of the skin reactions to tuberculin are atypical, or they may fail to appear, in very young tuberculous animals, and that very young pigs do not react to injections of virus of hog cholera in the same way that adult animals do.

LOCAL IMMUNITY

The immunities so far discussed have been those of the animal as a whole. The question has often been raised as to whether or not it was possible for one part of the body to develop resistance to an infection while other parts remain susceptible After an attack of erysipelas, it has often been observed that the skin of the affected area becomes relatively resistant to reinfection for a considerable time, while the skin of other parts of the body has little or no evidence of heightened resistance When certain toxins are injected into the skin, the injected area later becomes relatively insusceptible to a second injection, while other parts may be quite as susceptible as ever These phenomena formerly were interpreted as evidence of specific resistance, but it is now known that the greater part, if not all, of this heightened resistance is due to the inflammatory reaction which has mobilized macrophages and left other changes which together make the area less sensitive Injections of foreign serum, of egg albumin, and even of salt solution will render an area less sensitive to injections of irritating toxins or bacteria It appears probable that an increase in specific resistance in such areas is a manifestation of a general immunity

INFLUENCE OF NUTRITION AND ENVIRONMENTAL FACTORS ON RESISTANCE TO DISEASE

At times of food restriction during wars and during famines, epidemic diseases of man and animals usually appear, and these frequently are more malignant than those seen at other times One factor in some of the instances is a gross deficiency in *Vitamin A,* which occurs in man when there is an acute shortage of animal fats Experimentally, it has been shown by many workers that animals kept on diets which are deficient in this substance are much more susceptible to infectious diseases than animals which are fed adequately There also is some evidence that deprivation of *Vitamin C* from animals such as guinea pigs, which have no power to synthesize it, will render them more susceptible to certain diseases, particularly pneumonia The other vitamins, or lack of them, appear to play little part in infections

It should be emphasized that these results are obtained with animals wholly deprived of these vitamins, and it is not clear whether slight deficiencies would have any serious effect. Animals or persons who are on a varied diet probably seldom are so deficient in either of the vitamins mentioned as to appreciably decrease their resistance to disease. There is little evidence, furthermore, that the feeding of vitamin concentrates will have any effect upon infections already started, or that the intake of quantities larger than meet the requirements of the body are of any service.

Fatigue has long been recognized as a factor in increasing susceptibility to disease. This factor appears to be important in the so-called "shipping diseases" of animals

Heat and *cold* evidently have an effect upon bodily resistance In dogs held in rooms which were hot and humid, Arnold showed that the acidity of the gastric juice was diminished and that an increased passage of bacteria into the intestine from the stomach content occurred Dogs under these conditions were much more easily poisoned by *Salmonella* toxins, given by mouth, than others under normal conditions Chilling of the body surface sets up a train of physiological reactions which favor infection, particularly of the respiratory tract.

These are only a few of the more obvious conditions which have an effect upon bodily resistance to disease. It is clear that there are many others, such as the presence of finely divided solid material (calcium, silica) in the air which is breathed, or noxious gases (mustard gases used in war), and intercurrent diseases which may be quite trivial in themselves but pave the way for more serious infections by reducing the body's resistance It is quite certain that an individual whose general health is weakened in any way is an easier prey for many infectious diseases than one who is enjoying good health.

ANTIGENS AND ANTIBODIES

The immunologist supposedly is concerned only with the phenomena which have relation to an increased resistance of the host against infection Actually his work has led him to study a large number of phenomena, having to do with interactions between body fluids and tissues on the one hand, and various substances, principally foreign cells and organic bodies on the other, many of which have nothing to do with protection against infection

As defined by Topley (10), an *antigen* is "any substance that, when introduced parenterally into animal tissues stimulates the production of antibodies and, when mixed with that antibody, reacts with it in some observable way"

The same author defines an *antibody* as "any substance that makes its appearance in the body fluids of an animal in response to a stimulus provided by the parenteral introduction of an antigen into the tissues and, when mixed with that antigen, reacts with it in some observable way"

The entire study of immunity is related to the interactions between antigens and antibodies So far as resistance to disease is concerned, it is the antigens of the infecting organisms which stimulate the antibodies from which the protection is derived. These act directly in some cases, as when antitoxin neutralizes toxin, and indirectly in others, as when antibacterial antibodies so affect virulent bacteria as to render them susceptible to phagocytosis But

there are a great many substances, having no relation to micro-organisms or disease, which are antigenic. When antibodies are produced by the injection of any of these, the serum is said to be immune. Instead of calling them by this name many prefer to use the word *antiserum*

When antibodies are produced by the injection of bacteria, or of other cells, it should be realized that it is not a single antibody which is produced but many of them, for all cells contain many antigenic substances and each of them stimulates the formation of its own antibody.

Antibodies cannot be demonstrated except as they produce observable reactions when placed in contact with their specific antigens These reactions are various and often one antibody can be demonstrated in several different ways. Before this was realized, names were given to various types of antibodies according to the way they behaved *in vitro* Thus, after an animal has been immunized with a bacterial culture, the serum usually acquires the property of rendering the bacterium more susceptible to phagocytosis, of causing the clumping of the bacterial cells in masses, and of precipitating the extracted proteins of the cell from solution In some instances, it may acquire the property of actually dissolving the cell In the first of these instances, the reaction is said to be due to antibodies called *opsonins,* in the second, to others called *agglutinins,* in the third, to *precipitins,* and in the fourth, to *bacteriolysins* There is abundant evidence to indicate that these are not separate and distinct antibodies, but are different manifestations of the same one We continue to use these terms but it should be kept in mind that the name indicates only the method which was used to demonstrate it, and not that the antibody is a special one adapted only for performing the function by which it was demonstrated

THE NATURE OF ANTIGENS

The chemical nature of antigenic substances has been greatly clarified in recent years by workers who have approached the problem by synthesizing relatively simple antigens These are precipitated from their colloidal solutions by their specific antibodies, the antibody as well as the antigen being a part of the precipitate.

Until quite recently, it was believed that all antigens either were proteins or still more complicated chemical compounds having a protein component. This idea had to be abandoned when it was demonstrated that certain nonnitrogenous compounds derived from the polysaccharide of the pneumococcus could stimulate antibodies in horses and man, although not in the rabbit. Whatever their chemical nature, antigens are of relatively large molecular weight and, consequently, do not diffuse readily through organic membranes.

Antibodies may be looked upon as nature's means of attacking, digesting, and removing these agents which otherwise could not be removed from tissues when accident carried them in

The antibodies formed by the injection of highly purified, crystalline proteins are highly specific Albumins can easily be distinguished from globulins, and egg albumin of the chicken can be distinguished from that of the duck, or of other birds, by immunological means

It has been demonstrated that the immunological specificity of pure proteins can be changed by chemical means If, for example, several proteins, such as the serum albumins of several species of animals are halogenated or nitrated, the resulting compounds are still antigenic but the species specificity of the albumins are lost and instead a new specificity is shared by the several nitrated, or by the several halogenated, albumins

The synthetic antigens of Landsteiner and others are prepared in various ways, the most common being by utilizing the diazo reaction. Substances containing a benzene ring to which an amino group is attached are treated with nitrous acid to form a diazo compound When this is mixed with a protein containing either tyrosine or histidine, the compound unites with the protein. If such a conjugated protein is used as an antigen, the resulting antiserum will react either with the original protein, or with the conjugated compound If the diazo compound is attached to two different proteins, the antiserum produced by the injection of either will react with both antigens This proves, of course, that the reaction is dependent upon antibodies which have reacted with the diazo compound because the proteins have nothing in common, immunologically If the antibodies are mixed with the diazo compound alone, or with a diazo compound attached to a substance simpler than protein, such as tyrosine, no precipitate will occur If later, however, the complete conjugated protein is added to such a mixture, precipitation will be inhibited This proves that the diazo compound has united with the antibody and satisfied its combining powers even though precipitation did not occur

In the definition of an antigen, it will be recalled that it has two properties (a) the power of stimulating the formation of antibodies, and (b) the power of uniting with them, *in vitro* To substances, like the diazo compound just discussed, which have the power of reacting with antibodies, but cannot, alone, stimulate their formation, Landsteiner has given the name *haptens* or *partial antigens*. Haptens occur naturally They may be of carbohydrate nature, as the polysaccharides of the pneumococcus, they may be lipoidal, or they may be nitrogenous substances

Landsteiner (8) recognizes three kinds of antigenic complexes:

(a) *Simple haptens,* which, like the diazo compound alone, confer immunological specificity, but are not precipitated by their antiserums, and do not have the power of stimulating antibody formation

(b) *Complex haptens,* which, like the pneumococcus polysaccharides, confer immunological specificity, are precipitated by their antiserums, but do not have the power of stimulating antibody formation.

(c) *Complete antigens,* like proteins or protein conjugates, which have immunological specificity, are precipitated by their antiserums, and have the power of stimulating antibody formation

Several workers have reported success in producing antibodies with nonprotein substances adsorbed on inert material such as collodion particles and finely divided carbon Such studies, and even the work on synthetic antigens in which protein plays a part, suggests that antigenicity may not be so much a matter of structure as it is a matter of the manner in which the substance is presented to the tissues, that a carrier substance of high molecular weight is necessary.

THE NATURE OF ANTIBODIES

It has been said previously that antibodies in immune serum can be detected only by their reactions with their specific antigens The serum of an immunized animal shows no characteristic chemical differences from that of a normal animal. The antibodies can, however, be precipitated from immune serum by reagents which precipitate the proteins, and can be separated from the other proteins in the globulin fractions The antibodies usually are mostly if not entirely in the pseudo-globulin fraction It is presumed, therefore, that it is these globulins which have assumed the role of antibodies.

It has been suggested that antibodies are but altered antigens. Because of the large amount of antibody that may be produced by small amounts of antigen, this does not seem at all likely, in fact some have computed that the disparity between the amounts of antibody which can be produced by definite quantities of antigen is so great as to make the idea a mathematic impossibility

THE FUNCTION OF ANTIBODIES

Immediately after an attack of a disease, or after active immunization by artificial procedures, antibodies usually are demonstrable in the circulating blood in appreciable amounts If the animal succeeds in destroying the infecting organism and clears the system of them completely, the antibody content

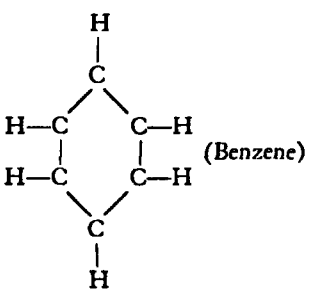
of the serum diminishes rather rapidly If this does not happen, it usually means that local foci of infection continue to exist

The disappearance of antibodies in the circulating blood of an actively immunized animal does not mean that all immunity is lost; in a passively immunized animal it does have this significance An actively immunized animal retains in his tissues a capacity of quickly producing more antibodies when the occasion demands Sometimes it is said that *sessile* antibodies exist in the tissues At any rate, the degree of resistance to a disease cannot be accurately measured by the amount of circulating antibody Circulating antibodies indicate that resistance exists, but high degrees of active resistance, or immunity, may exist when few or no antibodies can be demonstrated in the blood.

EHRLICH'S "SIDE-CHAIN" THEORY FOR THE FORMATION OF ANTIBODIES

Paul Ehrlich was, by training, a chemist, but he early became interested in bacteriological problems Some of his earliest studies were with bacterial toxins and their antitoxins These will be described later

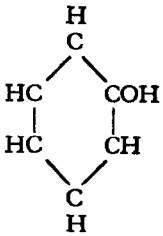
In 1885, Ehrlich (5) published a theory which he had developed to account for cellular nutrition In this, he postulated that before any cell could profit by a chemical nutrient with which it came in contact, the substance had to enter into chemical combination with it In doing this, he regarded the cells as having a central nucleus of matter, which differed with the different types of cells, but all of them had "receptors" or side chains through which the chemical affinity for the nutrient was expressed The matter generally is illustrated by comparing the situation to the chemical formula for the benzene ring.



(Benzene)

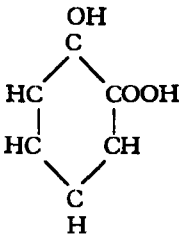
The chemical nucleus of this compound is represented by the six carbon atoms which are so firmly bound together that they cannot easily be broken down The hydrogen atoms, representing the "receptors" are quite easily displaced

by other chemical groupings such as NO_2, OH, COOH, NH_2, these completely altering the character of the compound, as, for example:



Phenol

or



Salicylic acid

The side chains thus represent affinities for other chemical groupings which combine with the chemical nucleus quite readily Thus it is with the "side chains" or receptors of cells

Ehrlich carried his nutritional theory into the field of immunity where it became best known His theory is no longer acceptable but inasmuch as Ehrlich, in connection with it, coined a large part of the terms of immunology and since this terminology continues to be used in spite of the abandonment of the theory, it is well for every student of the subject to understand the concept which he had

Knowing that toxins were complicated chemical substances which affected some types of cells and had little or no effect on others, Ehrlich reasoned that the susceptible cells had affinities, or "receptors," for the toxin molecule and the nonsusceptible cells did not But the toxins are substances which injure or even destroy the cells which have "receptors" for them If the injured cells are not wholly devitalized by the experience, repair of the damage occurs and in the course of this repair, nature tends to overcompensate for the damage done by producing more of the special "receptors" than previously existed or for which the cells have any use These supernumerary receptors are then detached from the cells and pass into the fluids of the body These free receptors retain their affinity for the toxin for which they are specific, unite with it when the opportunity is presented and so neutralize or inactivate the toxin that it becomes harmless to the tissue cells Animals with free receptors in their blood are immune to the toxin so long as there are enough receptors to unite with all the toxin present, and the blood serum of such animals, containing the receptors, can be used to protect other animals into which it is injected. The receptors of Ehrlich are what we call antibodies, in this case, antitoxin.

This is a bare outline of the concept of Ehrlich He applied the theory to types of antibodies other than antitoxins, developing eventually an exceed-

ingly complicated system made up of three orders which will not be discussed. It will be seen, however, that Ehrlich's basic idea is that antibodies, the basis of artificial immunity, are produced by certain cells of the body as a result of a reaction to an injury We might liken the process to the everyday experience of learning as, for example, the baby who burns his fingers on a stove and learns, thereby, not to repeat the process

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CHAPTER III

TOXINS AND ANTITOXINS

When toxin and its specific antitoxin are mixed *in vitro* in proper proportions, a union between the two agents occurs. This union is manifested in two ways: (a) the poisonous properties of the toxin are destroyed or inactivated, and (b) a precipitate is formed, consisting of the toxin-antitoxin combination.

It was at first thought that neutralization of toxin with antitoxin was a simple reaction, much like the neutralization of an acid with an alkali. However, it is now known that the matter is much more complicated Some of the first studies on toxin-antitoxin combinations were made by Ehrlich (2), and it was he who established some of the first units of measurement and gave names to them The first studies were with diphtheria toxin and antitoxin, substances with which students of animal disease have little concern. The reason for describing them here is that the nomenclature, if not the unit sizes in every case, has been carried over from these studies to all toxin-antitoxin studies.

TOXIN UNITS (DIPHTHERIA)

The simplest unit of toxin is the *Minimum Lethal Dose* (M.L D.). This is the amount of toxin which will kill a 250 gram guinea pig in about 96 hours after subcutaneous injection Ehrlich formulated an antitoxin unit which now is of no importance except that it was with this unit that he discovered some of the peculiarities exhibited by neutralization experiments. The Ehrlich unit was based upon the antitoxin's ability to neutralize a test dose of toxin consisting of 100 M L D Since this toxin deteriorated with age, and since deterioration was not accompanied by a change in its power to unite with antitoxin, it was found that a much larger quantity of antitoxin was required to neutralize 100 M L D of old toxin than of the freshly made. The reason for this is that the *toxoid,* formed by deterioration of the toxin, unites with antitoxin in the same way as the toxin A fresh toxin solution contains little toxoid, an old one a great deal. To obtain neutralization of a toxin solution, enough antitoxin must be added to combine with its toxoid content as well as its toxin. The amount of antitoxin required to neutralize 100 M.L.D of a toxin in which there is also much toxoid to be satisfied, naturally will be much greater than the amount required to neutralize 100 M.L.D. of another

toxin solution in which practically all of the combining power is in the form of toxin. This finding induced Ehrlich to formulate two additional units of toxin:

The L_0 dose of diphtheria toxin is the maximum amount of toxin that, when mixed with one unit of antitoxin, will be completely neutralized by it

The L_+ dose of diphtheria toxin is the smallest amount of toxin that, when mixed with one unit of antitoxin, will cause the death of a 250 gram guinea pig in about 96 hours after subcutaneous injection

The L_0 dose of toxin is determined by injecting guinea pigs, and since the size is judged to be at the point where no symptoms are produced, the determination of this point depends upon the acuity of the observer The end point of the L_+ dose, since it is determined by the death of the animal, is not subject to this objection.

It is to be noted that the L_0 dose of a toxin does not change materially as toxin deteriorates until practically all toxicity is lost, because there will be residual toxicity in a mixture of toxin and toxoid with antitoxin so long as both toxin and toxoid are not fully neutralized In deteriorating, toxin changes to toxoid and the sum total of the two remains the same, consequently the amount of antitoxin required to neutralize the sum of the two will be constant. On the other hand, the L_+ dose is quickly affected by deterioration because even a small reduction in the amount of toxin will rob it of its ability to kill the guinea pig within the proper time interval.

ANTITOXIN UNITS

The antitoxin units vary in different countries This causes much confusion, consequently the League of Nations has undertaken to establish international units for the curative serums These no doubt will be adopted by all countries in time In the United States, the present unit of diphtheria antitoxin is the smallest amount that when added to an L_+ dose of toxin will sufficiently neutralize the toxin so that a 250 gram guinea pig will die in 96 hours after subcutaneous injection of the mixture Inasmuch as this definition of a unit requires the use of a dose of toxin which can be arrived at only by having known units of antitoxin in the first place, the definition has no value unless a standard antitoxin of known unit value is kept, from which all new antitoxins can be standardized Such a standard is kept by a governmental agency, as will be described later.

The unit of tetanus antitoxin is somewhat different Tetanus toxin is relatively stable, hence it is possible to use it as a standard The *"official test dose"* of tetanus toxin in the United States is 100 M L D. (for a 350 gram guinea pig) of a standard toxin produced by a governmental agency, designated by

Congress for the purpose. The antitoxin unit is determined by titrating against this standard and it may be defined as follows The antitoxin unit (tetanus) is ten times the least quantity of antitoxin which will protect a 350 gram guinea pig for 96 hours against the official test dose of toxin.



FIG 1 Commercial Antitoxin Production Bleeding of immunized horses The blood is removed from the jugular vein into giant test tubes containing sufficient sodium citrate to prevent clotting The tubes are stored in a cool room until the cells have sedimented The plasma is siphoned off and the pseudo globulin, containing the antitoxin, is precipitated by chemical means (Courtesy of the Lederle Laboratories, Inc)

PRACTICAL PRODUCTION AND STANDARDIZATION OF ANTITOXINS

Production of Toxins. The organisms are usually cultivated in an infusion broth. The diphtheria cultures are grown in shallow layers in large flasks. The organisms form a pellicle when growing well. The tetanus cultures are grown in large flasks with a minimum of air surface The broth is seeded heavily while still warm after boiling and incubated without further attempts to create anaerobic conditions Not all cultures are suitable for toxin formation, and frequently best toxin formation occurs only when very exact requirements of H ion concentration and nitrogenous metabolism have been met. When maximum toxin accumulation has occurred, the cultures are removed from the

incubator and filtered through Berkefeld filters In some cases, the cultures are carbolized (0.5% phenol) before filtering to destroy the organisms

Immunizing the Animals. Horses are used almost entirely Healthy, strong animals receive small doses of the toxin generally mixed with antitoxin at first Later, larger doses are given without the antitoxin The doses are given regularly and are increased gradually until many thousands of fatal doses for normal horses can be withstood After several months of treatment, test bleedings are done and the antitoxic potency of the serum is determined. Many horses prove unsuitable for serum production and must be discarded. There is no way to tell which horses may be suitable, hence the manufacturer must use many animals in order to find a few which are good serum producers

Obtaining the Serum. When the serum is of sufficient potency, the skin over the jugular vein of the horse is cleaned and disinfected. From six to twelve liters of blood are drawn through sterile tubes into jars Here the blood clots and the serum may be drawn off after standing several days in a refrigerator In some cases, the blood is drawn into jars containing a concentrated sterile solution of sodium citrate This prevents clotting After standing a few days, the corpuscles settle and the clear plasma may be siphoned off

Concentrating the Antitoxin. The antitoxin is closely associated with, or is in, the pseudo-globulin fraction of the blood serum Several "salting out" methods are used for obtaining this fraction in a relatively pure state A concentration of antibodies is brought about, and a bulk of protein bodies which are of no use in the immunization procedure but which frequently bring about "serum sickness" are eliminated

Standardization of Diphtheria Antitoxin. Standard *antitoxin* is maintained and distributed by the Hygienic Laboratory of the National Institute of Health, Washington, D C An arbitrary unit was established by this laboratory when the antitoxin standardization work was begun about 35 years ago, based upon a previous arbitrary unit established by Ehrlich As new lots of antitoxin are made up for standardization purposes, each new lot is carefully compared with older lots so that the standard is kept unchanged Commercial producing laboratories in the United States must obtain this standard antitoxin by which to standardize their own product It is sent out, on request, in a dried state hermetically sealed in dark glass containers In this condition, it is relatively stable.

The manufacturer first must produce his own toxin. He tests this toxin by mixing varying quantities with one unit of the standard antitoxin. In this

way, he learns the L_+ dose of his toxin. He now mixes varying quantities of the commercial antitoxin, which he has produced, with the amount of toxin which he has found to represent the L_+ dose and injects the mixtures into guinea pigs of 250 gms weight. Those which die in from four to five days indicate the dosage which contained the equivalent of one standard unit of antitoxin

The manufacturer must state the unit value of his antitoxin on the trade packages When used, allowance should be made for deterioration since date of manufacture, also the material should be kept cold In the United States, commercial diphtheria antitoxin must contain at least 350 units per cc.

RAMON FLOCCULATION METHOD OF STANDARDIZING DIPHTHERIA ANTITOXIN. An entirely new method of standardizing diphtheria antitoxin was introduced by Ramon (3) of the Pasteur Institute in 1922 It depends upon the fact, previously observed by others, that, at the neutralization point of toxin with antitoxin, a flocculent precipitate is formed The test is carried out in test tubes Varying amounts of the unknown antitoxin solution are added to fixed amounts of a standard toxin solution The tubes are incubated for a comparatively short time and the characteristic coarse flocculation looked for. The tube in which it appears indicates the quantity of antitoxin which has just neutralized the standard toxin solution

The flocculation method gives data which correlate well with the combining powers of the toxin but there is no such correlation with toxicity except in fresh toxins In other words, deteriorated toxins flocculate the same as fresh ones

The flocculation test has proven very useful as a preliminary test during standardization. Since it does not measure the ability of sera to neutralize toxin, the test is not likely to replace the animal tests.

INTRACUTANEOUS METHOD OF STANDARDIZING DIPHTHERIA ANTITOXIN. This method is cheaper than the standard animal test and probably is more accurate It is likely to become the method of choice.

When diphtheria toxin is injected intracutaneously (intradermally) in guinea pigs, an inflammatory reaction, manifested by swelling and redness, results This reaction is the same as that obtained with the Schick test in a susceptible person

In a white-skinned guinea pig, a reaction will be obtained with toxin solutions containing as little as about $\frac{1}{500}$ M L.D The smallest amount of a toxin that will elicit a reaction is known as the M R D. (minimum reacting dose). In intracutaneous testing, the L_r dose corresponds to the L_+ dose, i e., it is the smallest amount of toxin which, when mixed with one unit of antitoxin

will still give a reaction. The tests are made with fractions of units since the total amount injected at one site must not exceed 0.2 cc Several dilutions may be tested simultaneously on the same animal.

Standardization of Tetanus Antitoxin. As for diphtheria antitoxin, the standards for tetanus antitoxin in the United States are maintained by the National Institute of Health and furnished by that organization to commercial producers The standard unit of antitoxin was determined for the United States by act of Congress in 1902 The official method of determining the unit was worked out by Rosenau and Anderson (5) The Institute maintains both a standard toxin and a standard antitoxin The two products are kept at uniform strength by measuring them against each other The units of tetanus toxin and antitoxin are not the same as those of diphtheria

Standard antitoxin is furnished the manufacturer on request The smallest amount of his own toxin that will kill a 350 gram guinea pig in from four to five days after subcutaneous injection when mixed with $\frac{1}{10}$ unit of the standard antitoxin, constitutes the L_4 dose By mixing L_4 doses of his own toxin with varying amounts of his commercial antitoxin and injecting the mixtures into standard-weight guinea pigs, the manufacturer can determine the unit value of his product The least quantity which protects the animals for four days constitutes $\frac{1}{10}$ unit

For prophylactic purposes, at least 1500 units should be employed For curative purposes, 50,000 or more units frequently are needed and even these huge quantities often will not save the patients

USE OF TOXOIDS FOR IMMUNIZATION

Animals may be actively immunized to toxins by injecting quantities of toxins too small to kill, but this procedure is too heroic to be used practically The severe local effects of toxins can be prevented, in part at least, by mixing them with antitoxin, as is done in the toxin-antitoxin method of immunizing children to diphtheria Toxins which have lost their poisonous properties through ageing (toxoids) retain their immunizing properties and may be used for practical immunization Better for the purpose are the toxoids produced by treating fresh toxins with formalin This method was first described by Ramon (4) for diphtheria and by Descombey (1) for tetanus The efficacy of these toxoids is enhanced by precipitating them with alum Alum-precipitated toxoids are now frequently used for prophylactic immunization of man against both diphtheria and tetanus. The alum-precipitated toxoid also has proved very effective in immunizing horses against tetanus Toxoids are of no value in treating cases of disease since the immunity develops slowly They

should be used on children or young animals purely as prophylactic agents. For this purpose they have the advantage over antitoxin in that the immunity produced is more enduring For further discussion of the use of tetanus toxoid on animals see page 280

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CHAPTER IV

THE LYTIC ANTIBODIES: BACTERIOLYSINS, HEMOLYSINS, COMPLEMENT-FIXATION

The Lysins

In 1894, Pfeiffer (5), in Germany, described the first of a series of studies in which it was shown that a bacterium, the vibrio of cholera, was actually broken up, or lysed, by the cell-free fluids of guinea pigs which previously had been immunized with dead cultures of this organism The observations were made by injecting the cultures into the peritoneal cavity, and following the reactions by withdrawing the peritoneal fluid, from time to time, with a hypodermic needle These observations are often known as the *Pfeiffer phenomenon* The principal facts were as follows

(a) When a suspension of vibrios was injected into an *immunized* animal, the fluid withdrawn at intervals showed that the organisms were not multiplying, but instead were swelling and assuming abnormal shapes Finally, all organisms underwent fragmentation and disappeared The organisms had been lysed, and the animal remained well

(b) When the vibrios were injected into a *non-immunized* animal, fragmentation and lysis did not occur After a few hours they began to multiply and eventually they overwhelmed the animal causing its death

(c) When the vibrios were mixed with serum from an *immunized* animal, and the mixture injected into a normal animal, the vibrios behaved as in (a), and the animal lived

(d) When the vibrios were mixed with serum from a normal animal (*non-immunized*), and the mixture injected into a normal animal, the vibrios behaved as in (b), and the animal died

Bordet (1) repeated the experiments of Pfeiffer, except that he conducted them *in vitro*, instead of in living animals. Using the same organism, the following experiments were done:

(a) When the vibrios were added to fresh, *non-immune* serum, there was only slight evidence of lysis of the organism Eventually, the organisms multiplied

(b) When the vibrios were added to fresh *immune* serum, lysis occurred

(c) When the vibrios were added to fresh *immune* serum, which had been heated at 60° C for a few minutes, lysis did *not* occur

(d) When the vibrios were added to a mixture of heated *immune* serum and fresh *normal* serum, lysis occurred.

MECHANISM OF THE PROCESS OF SERUM LYSIS

From the experiments just described, Bordet deduced that two factors were necessary for serum lysis·

1 A specific thermostable factor, which is not normally present in the body but which is produced by immunization (antibody)

2 A thermolabile factor which is present in fresh, normal serum and is not increased by immunization.

These studies were further extended by Bordet who showed that red-blood cells (erythrocytes) could be lysed with serum produced by injecting the same kind of cells into a species of animal foreign to that from which they came, thus the erythrocytes of a rabbit could be lysed by serum from a goat which had received injections of rabbit erythrocytes In these experiments, it again was demonstrated that the antibodies produced by immunization required the assistance of the thermolabile substance of normal serum When the blood cells were mixed with either of these constituents alone, lysis (hemolysis) did not occur When the cells were placed in contact with the antibody (thermostable factor) alone for a time, were then carefully washed to remove the antibody, and afterwards were placed in contact with the thermolabile factor, hemolysis occurred When the thermolabile factor was used first, and followed by the antibody, hemolysis did not occur From these experiments it was evident that the blood cells were capable of absorbing the antibody, but not the thermolabile substance, and that the antibody injured or sensitized them so that the other agent caused their lysis.

In the case of blood cells and a few bacterial cells, the antibodies, working in co-operation with the other factor, do in fact, cause lysis or dissolving of the cells for which they are specific. Such antibodies can be produced for any type of cell, but, in the vast majority of cases, the cells are not actually disrupted in the reaction In these cases, the mechanism might more properly be

called *bacteriocidins* rather than *bacteriolysins,* however, the latter term is usually used whether or not the cells are dissolved

AMBOCEPTOR

The antibodies which are demonstrated in the lytic phenomena were called *amboceptors* by Ehrlich, because he visualized them as agents which united on the one hand with the antigenic cell and on the other with the thermolabile factor Antibodies of this type were given the name *substance sensabilatrice* by Bordet Ehrlich's name is most commonly used in this country

COMPLEMENT

The constituent of fresh normal serum which is required for the functioning of the lytic antibodies (the heat labile substance of the foregoing discussion) was given the name *complement* by Ehrlich Apparently it is identical with a substance previously studied by Buchner and which was named by him, *alexin*

It is apparent that this substance is nonspecific, that is, it will function to injure, and often lyse, any kind of cell which has been acted upon or sensitized by antibodies

The nature of complement is still rather obscure It appears to act in many ways like an enzyme It quickly deteriorates in blood after it has been drawn, is easily destroyed or inactivated by chemical action and by heat, and it may be adsorbed from serum by many substances

Complement will function only when salts are present It has been shown that its inactivation in a salt-free medium is due to the precipitation of the euglobulins A solution of the precipitated globulins will not show complement activity but, if the solution is mixed with the serum from which it originally was precipitated, the complement activity is restored Evidently, therefore, complement is not a single substance but consists of two or more substances working together

THE BORDET GENGOU PHENOMENON

This name is given to an experiment conducted by Bordet (2) and his pupil, Gengou, to demonstrate that the same complement could function equally well with a bacteriolytic and an hemolytic amboceptor The experiment is of great importance because it furnished the basis for the complement-fixation test The experiment seems rather complicated but this really is not the case. It is easily understood if one understands the reagents which are used. These are five in number, as follows.

1 An antiserum (bacteriolytic amboceptor) for some species of bacterium. In the original study an antiserum for *Pasteurella pestis* was used. This serum was inactivated (Heated to 56° C. for 30 minutes to destroy any complement which may have been present)

2. A bacterial antigen homologous with this serum
In this case a suspension of *Pasteurella pestis* in saline solution.

3 Complement.
Fresh, unheated serum from a normal guinea pig.

4 Washed erythrocytes of a sheep
Defibrinated blood from a normal sheep was centrifuged and the plasma discarded The sedimented cells were then shaken up in saline solution, centrifuged out, and this process repeated several times until all trace of the plasma had been removed

5 An antiserum (hemolytic amboceptor) for sheep erythrocytes
A normal rabbit was injected several times with suspensions of washed erythrocytes of a sheep Finally the rabbit was bled and his serum obtained. This serum was inactivated, to destroy its complement, before being used.

An outline of the experiment is as follows

(a) Substances (1), (2) and (3) were mixed in a tube and placed in a water bath for one hour Substances (4) and (5) were now added, the tube was shaken and returned to the bath Hemolysis did not occur.

(b) Substances (2) and (3) were mixed in a tube and the tube placed in a water bath for one hour Substances (4) and (5) were now added and the tube returned to the bath after shaking Hemolysis did occur

Bacteriolysis had occurred in the first case, as would be expected, since the three necessary constituents, antigen (2), amboceptor (1) and complement (3) were present In the process, the complement had been used up or bound When blood cells with their hemolytic amboceptor were added, hemolysis did not occur because of lack of complement

In the second instance, because of lack of amboceptor (1) bacteriolysis did not occur and the complement (3) remained free after the first period of incubation When blood cells (4) with their specific amboceptor (5) were added, hemolysis did occur since complement was already present in a free condition

In the one instance (a) the complement was fixed by the bacteriolytic sys-

tem and hemolysis was prevented; in the other, there was no such fixation, hence the complement was free to enter into the hemolytic system On the basis of this experiment Bordet and Gengou argued that complement was a nonspecific substance since it could function in the hemolytic or in the bacteriolytic system equally well

THE COMPLEMENT-FIXATION TEST FOR THE DIAGNOSIS OF DISEASE

The principle of this test is the same as that of the Bordet-Gengou experiment Substances 3, 4 and 5 (which constitute the hemolytic system) are used irrespective of what disease is being studied The antigen (2) is a suspension

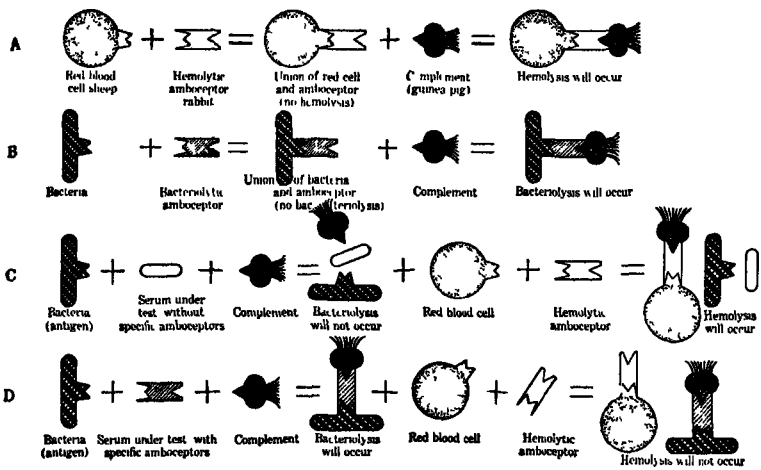


FIG 2 A Diagrammatic Representation of the Process of Complement-Fixation

or an extract of the causative agent of the disease for which the test is being made, e g, a culture of *Malleomyces mallei* when a test is being made for glanders Substance number one is the sample of serum which is being tested If this serum came from an infected individual, the amboceptors for that disease will be present, if the individual is not infected, the amboceptor will be absent The test is run only after the various reagents have been carefully standardized (titrations) The procedure in the final test is the same as that used by Bordet If hemolysis results, the suspected serum must have been devoid of amboceptor, i.e, the test is negative If hemolysis does not occur, the amboceptor for the suspected disease must have been present, i e, the test is positive.

The test has been used more or less successfully for diagnosing nearly all infectious diseases Some diseases in which the test has been successful in a practical way are syphilis, gonorrhea, tuberculosis, glanders, infectious abortion (of cattle), and trypanosome infections

The *Wassermann reaction* (7) is the complement-fixation test as applied to the diagnosis of syphilis In the beginning, since the *Treponema* of syphilis had not been successfully cultivated, the antigen used was an extract of the liver of a syphilitic fetus Later extracts of normal organs were found to function equally well At present, the acetone-insoluble fraction of an alcoholic extract of normal heart muscle, to which a small amount of cholesterol has been added, is considered the most satisfactory antigen

The Wassermann reaction depends upon the fixation of complement by precipitates which are formed by the action of syphilitic antibodies on lecithin-containing lipoids The test, then, is really a nonspecific one, and yet it has a remarkably high specificity for syphilis. For many years, it was depended upon, almost alone, for the diagnosis of occult syphilis, but in recent years various precipitation tests have been developed which depend upon observing directly the precipitates which are detected by the Wassermann test The precipitation tests are much simpler, and appear to be fully as accurate as the more complicated test The technic of Meinicke (4), Sachs and Georgi (6) and Kahn (3) have been used for the precipitation test The Kahn test, in particular, has been very popular in recent years

OTHER APPLICATIONS OF THE COMPLEMENT-FIXATION TEST

When a known antigen is at hand, this test may be used to identify the homologous antibody This is what is done when the test is used for testing serums for evidence of specific diseases On the other hand, when a known serum is on hand, the homologous antigen may be identified This is done occasionally in scientific work to identify unknown proteins, or organisms. The test may be used in medico-legal cases to identify blood stains, or other protein-bearing substances, but the precipitin tests usually are preferred for this purpose because of their greater simplicity.

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CHAPTER V

THE AGGLUTININS AND PRECIPITINS

The Agglutinins

Gruber and Durham (2) (1896) first observed the specificity of the phenomenon of agglutination of bacteria by antisera, using the typhoid bacillus and the serum of patients Later it was discovered that cells other than bacteria, such as yeasts, fungi, protozoa, blood cells, and spermatozoa will exhibit the phenomenon in the presence of specific antibodies.

The reaction may be observed *in vitro* as well as *in vivo* It consists of a coming together of the cells in suspension to form aggregates which finally may become so large as to be easily visible with the naked eye as granular masses or large flocculi, depending upon the conditions of the experiment and the substances present The phenomenon is not a vital one; suspensions of dead cells, and of inanimate particles, may be flocculated Agglutination depends upon antibodies Complement need not be present Red blood cells may be agglutinated by heated immune serum, the addition of complement then induces lysis

Agglutination is the result of the interaction of an antibody (or antibodies), known as an *agglutinin,* with an antigen (or antigens), known as an *agglutinogen,* contained in or on the cells Agglutination of cells is believed to be the same phenomenon as precipitation of antigens in solution, the difference being that in one case the antigens are attached to, or are a part of, cells, while in the other it is free in solution In agglutination the flocculation of the antigens by antibodies can occur only by clumping of the cells themselves Emulsions of inert particles, such as collodion and carbon, can be agglutinated by antiserums, after the particles have been treated with the homologous antigenic solution, followed by thorough washing to remove all antigen except that which is adsorbed by the particles Since cells always contain more than one antigen, the agglutination reactions usually are quite complicated.

Agglutination is believed to be a physical reaction caused by changes occurring on the surfaces of the cells, probably caused by a coating of adsorbed antibody How these coatings upset the normal stability of the suspensions is not known with certainty, but the cells, in the presence of specific antibodies, appear to act like hydrophobe instead of hydrophile colloids, and like the

former become unstable and are precipitated in the presence of electrolytes.

Agglutination, as well as precipitation, does not occur in the absence of electrolytes When both antigen and antiserum, in the case of precipitins, have been thoroughly dialyzed, the antigen-antibody reaction evidently occurs, but without evidence of flocculation The addition of a small amount of salt to such mixtures will cause immediate flocculation The presence of salts (electrolytes) has an effect upon the electric charge carried by the cells, or other particles in suspension The electric charge, or potential, of the particles is the factor which keeps them apart (dispersing factor) and thus maintains the suspension Anything which reduces the potential on the surface of the cells, or particles, reduces the dispersing factor and permits clumping or agglutination. The normal bacterial cell in suspension carries a negative potential If this is reduced by adding acid in small increments, the stability of the suspension will be reduced gradually to zero, at which clumping occurs, and this is at the *iso-electric point* at which the potential of the cells has been wholly discharged If additional acid is added, stability is restored because the cells resume a potential, but it now is a positive charge that is carried Agglutination of bacteria, at their iso-electric point, is called *acid agglutination* Serum agglutination is not dependent upon the iso-electric point, because the reaction occurs at a wide range of pH Acid agglutination suggests, however, that serum agglutination may have to do with an alteration of the surface potential of the cells.

CELLULAR ANTIGENS

It can easily be shown that most cells carry multiple antigens Flagellated bacteria, for example, usually have one or more antigens located in the flagella (*flagellar antigens*), and these are different from those which are found in the body of the cell (*somatic antigens*) An antiserum prepared against flagellated organisms will agglutinate suspensions of the flagella, as well as of the cells from which the flagella have been removed, by virtue of the fact that it contains both flagellar and somatic agglutinins An antiserum prepared against a suspension of the flagella will agglutinate the flagellated organism but not the cells from which the flagella are removed, and an antiserum against the deflagellated organism will agglutinate the flagellated organism but not the flagella alone

Weil and Felix (4), while studying an organism of the *Proteus* group in 1916, observed two types of colonies, one a typical spreading type and the other a dissociant of the first which was nonspreading The first was rapidly motile, the latter, nonmotile Suspensions of these two types agglutinated differently since the first contained a flagellar antigen whereas the other pos-

sessed only the somatic To the flagellar antigen, they attached the name H-antigen; to the somatic, O-antigen These names frequently are used in the literature in this way.

The somatic antigens often are numerous. Some of these antigens are thought to lie at or near the surface of the cell (surface antigens), while others are deeply embedded in the cytoplasm (deep antigens) Antiserums prepared against the superficial antigens will readily agglutinate cells of the type from which they were extracted, but antiserums for the deep antigens often will not agglutinate the cells, unless they have been treated by methods which remove the more superficial parts and expose the deeper to the action of the antibodies.

GROUP AGGLUTININS

Although antibodies are quite specific for the antigens which stimulated their formation, phenomena are sometimes observed which seem to indicate lack of specificity Thus it frequently happens that agglutinins formed by stimulating with one organism will agglutinate other organisms as well as the homologous one These are known as *group agglutinins* The best known examples of group agglutinins are those of organisms of the colon-typhoid group If one stimulates agglutinins by injecting into rabbits certain members of the paratyphoid series, these antibodies will agglutinate other members of the paratyphoid sub group, and the typhoid and colon bacillus as well Usually, although not always, the heterologous organisms are not so susceptible to the antibodies as is the specific organism and are affected only when greater concentrations of serum are used

Bacterial cells contain more than one antigenic substance, and group agglutinins may be explained by assuming that related organisms have some antigens in common Suppose, for example, that three bacilli were in a related group and that Bacillus I contained antigens A, B, and C; Bacillus II, B, C, and D, and Bacillus III, D, E, and F An antiserum for Bacillus I containing agglutinins a, b, and c would be expected to agglutinate Bacillus II but not Bacillus III An antiserum prepared with Bacillus II, on the other hand, would be expected to agglutinate all three organisms.

AGGLUTININ ABSORPTION

It has been shown that there are two steps in the phenomenon of agglutination. (a) Union of the agglutinogen with the agglutinin (antibody), and (b) Flocculation of the organisms

Some organisms cannot be flocculated with immune serum, and flocculation is impossible to determine in others because the organism does not normally form a uniform suspension in fluids In either of these cases, it is im-

possible to demonstrate a satisfactory agglutination reaction It can be shown, however, that binding of the agglutinin has occurred when such organisms are mixed with their specific antisera because, if the bacterial cells are removed by centrifugalization, the fluid which remains will be freed of its agglutinin This can be determined by testing the supernatant fluid with an organism which is known to agglutinate properly

Agglutinin absorption has proved to be a more reliable test for distinguishing between closely related forms of bacteria than the straight agglutination reaction In some instances, group agglutinins are present in such large amounts that by the ordinary agglutination reaction it is impossible to distinguish between the specific and the group agglutinins, for the specific organism may be agglutinated in no greater degree than the closely related one In this case, absorption of the serum with the two organisms under consideration will generally tell which is the specific one The specific organism should absorb all the agglutinins, while the closely related one is able to absorb only partially

Suppose, to use as an example the organisms mentioned under *Group Agglutinins,* Bacillus I, containing antigens A, B, and C, had been used for immunizing an animal and it had been found that Bacillus II was agglutinated by the antiserum to the same degree as the homologous strain The serum would contain antibodies for each of the antigens in the bacillus injected. These will be indicated as a, b, and c to correspond to the antigens If a large excess of Bacillus I is incubated for a time in a portion of this serum, and these are then removed by centrifugalization, the absorbed serum should then be free of antibodies, for each antigen would be expected to absorb its own antibody, and it would be removed with the organisms No reaction should occur when this serum is again tested for agglutinins. On the other hand, if Bacillus II, containing antigens B, C and D, is used for absorbing the serum, agglutinins b and c would be removed, but agglutinin a would remain. When this absorbed serum is tested for agglutinins, using the homologous organism, some agglutination should occur due to the presence of agglutinin a. This would indicate that Bacillus I is the homologous one, and Bacillus II, the heterologous.

NORMAL AGGLUTININS

The blood serum of normal animals frequently contains small quantities of agglutinins for a variety of bacteria Young animals do not possess these antibodies so frequently as do older animals, a fact which suggests that they may have developed as a result of infections, perhaps so mild as to be undetected. In diagnostic work, it is necessary to know the range of normal ag-

glutinins for the organism and species concerned in order that reactions within this range may not be interpreted as of diagnostic significance.

ZONE PHENOMENA

Some sera, particularly those that have been kept for some time and those that have been heated to 60°–70° C., behave peculiarly in that agglutination fails in the lower dilutions (proagglutinoid zone) but occurs in the higher. This phenomenon has been extensively studied and various explanations have been postulated. It is generally believed now that some kind of protective colloid is the interfering factor and that agglutination occurs in the higher dilutions because this colloid has been diluted out. Occasionally a serum is encountered in which agglutination will occur in low and very high dilutions, but will fail in intermediate dilutions. No adequate explanation has been given for this paradoxical behavior

PRACTICAL USES OF THE AGGLUTINATION TEST

The agglutination test is widely used for the diagnosis of disease Theoretically, it may be used for any disease caused by microscopic organisms, practically there are difficulties in the way of its use for some, as, for instance, when the organism grows characteristically in the rough form and, therefore, is spontaneously unstable in suspension It has been used with a large measure of success in typhoid and the other enteric fevers of man, in undulant fever and tularemia In animals, it has its widest uses in brucellosis and pullorum disease When the test is used for the diagnosis of disease, suitable identified cultures are suspended in a fluid This suspension is commonly known as antigen Definite amounts of the antigen are mixed with multiple dilutions of the serum of the patient, either in a test tube or in the form of drops on a glass surface The reaction may develop immediately or it may require some hours for completion, depending upon the conditions under which the test is conducted

The Precipitins

When an antigen in solution is mixed with its antiserum, a flocculent precipitate generally is formed. This precipitate is formed largely of the globulins which constitute the antibody, but the antigenic substance is also included in it. The antigen used in precipitin tests is known as *precipitinogen,* the antibody as *precipitin* Precipitins undoubtedly are the same as agglutinins, and the amboceptor of the lytic antibodies.

Most proteins will serve as precipitinogens, also the other substances men-

tioned in the discussion on the nature of antigens Gelatin, which lacks the aromatic amino acids, will not produce precipitins, and proteins which have been treated with alkali (racemized) lose their power to produce precipitins. Precipitins, like agglutinins, will not exhibit their characteristic properties in the absence of electrolytes, and in most other respects exhibit the phenomena that have been discussed under agglutinins.

PRACTICAL USES OF THE PRECIPITIN TEST

The precipitin test is a very useful one for identifying antigenic substances of all kinds It may be used for the diagnosis of disease but, when it is feasible, the agglutination test will give the same information and is preferred because of its greater simplicity The Kahn and other precipitin tests for human syphilis already have been discussed. In veterinary medicine, the Ascoli (1) test for anthrax is perhaps its most important use.

The *Ascoli test* is widely and successfully used in Europe for detecting dried hides which have come from anthrax-infected animals An antiserum for anthrax protein is kept on hand Bits of the dried hides are snipped off, minced, and soaked in water, or extracted in other ways The extract is filtered to clarify it, then it is layered in small tubes on the precipitating serum If the animal from which the hide came was infected with anthrax, there will be enough of the protein in the hide-extract to form a cloudy ring at the line of junction of extract and antiserum.

The precipitin test often is used in medico-legal cases for identifying blood stains, determining deer meat or other game taken out of season, or for identifying proteins of any kind. Identification can be made of such antigens even though they may have been dried for many years It has been claimed that muscle tissue taken from Egyptian mummies has given precipitin reactions for human protein.

For identifying the species of origin of any antigen, the laboratory must have on hand specific antiserums for the protein of as many species as may be called into question The stain, or the dried tissue, is merely soaked in water until it has been well extracted, the extract is clarified by filtration and then layered in narrow tubes with the series of antiserums A precipitate at the place where the layers come in contact will identify the homologous antigen.

ZOOLOGICAL SPECIES RELATIONSHIPS AS DETERMINED BY PRECIPITINS

An interesting use of the precipitation test was made by Nuttall (3) who tested the blood proteins of a large series of animals against an antiserum specific for human protein. He found that the blood serum of the chimpan-

zee gave practically as good reactions with it as human serum, and some of the other anthropoid apes gave good reactions but not as good as the chimpanzee. Other animals did not react He regarded the results as another proof that man is not far removed from the apes on the phylogenetic tree.

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CHAPTER VI

PHAGOCYTOSIS

Of the phagocytes of the circulating blood, the neutrophilic leucocyte, sometimes known as the polymorphonuclear leucocyte, is the most active against bacteria It is a very mobile cell and may be found wandering through almost all tissues of the body In acute inflammatory processes, this cell usually is conspicuous The pyogenic bacteria have a powerful attraction for this cell and pus always contains large numbers of them For this reason, the neutrophilic leucocyte is often called the *pus cell* It readily phagocytoses many bacteria and destroys them by intracellular digestion This cell may be easily studied in vitro, and, as a result, more is known of its phagocytic activity than of any other type of cell In many instances, the polymorphonuclear leucocytes, with their bacterial loads, are engulfed and digested by the fixed cells of the reticulo-endothelial system

The phenomenon of phagocytosis, the engulfing by ameboid activity of foreign particles of all sorts which happen to get into the tissues, was first described by Metchnikoff (1) in 1883 In a series of studies lasting throughout the greater part of his lifetime, this worker discovered most of the facts which we now have about this interesting group of cells It was recognized quite early that the activity of phagocytic cells against foreign materials depended in large degree upon the fluid in which the cells were suspended When suspended in animal serum, phagocytes were much more active than when in physiological saline solution The substance in serum which was supposed to stimulate phagocytosis was given the name *stimulin*

Denys and LeClef (1895) showed that phagocytosis of streptococci was carried out equally well by the phagocytic cells of normal as by those of immunized individuals In other words, it was shown that immunization did not alter the phagocytes and make them more active as had formerly been supposed, but altered the stimulating power of the body fluids in which they were suspended Thus, in a particular immune serum, leucocytes which were derived from normal individuals behaved precisely the same as those which came from immune individuals These experiments were carried out *in vitro* Denys and LeClef also concluded that the effect of immune serum on phagocytosis was not in stimulating the phagocytes but rather in rendering the organisms more susceptible to phagocytosis

Wright and Douglas (4) (1903) studied the mechanism of phagocytosis and it was from their studies that the greater part of our knowledge of this process was obtained Using a staphylococcus which was only slightly affected by the normal lytic power of serum, these authors found that this organism appeared to have little attraction for normal leucocytes when both were suspended in serum-free fluid or in normal serum which had been heated to destroy the complement In fresh, complement-containing normal serum, phagocytosis of the organism was active, in fresh, immune serum, the process was greatly accelerated Assuming that the stimulating substance which was present in the normal serum was the same as that which had been increased by immunization, they concluded that it was an antibody, and gave it the name *opsonin*

Experiments were conducted to determine whether opsonins affected the organism or the phagocyte When leucocytes were treated with immune serum, thoroughly washed, then placed in contact with the specific organism, little or no phagocytosis occurred When, however, the organism was treated with immune serum, thoroughly washed, then placed in contact with phagocytes, active phagocytosis occurred From this it was deduced that the immune body (opsonin) affects the bacterium and not the phagocyte

The opsonin which is present in normal serum is heat labile, quickly deteriorates with age, and is removed by substances which adsorb complement. For these reasons, it has been thought that normal opsonin was the same as complement This probably is not the case It seems probable that it is another substance which like amboceptors requires the presence of complement The antibody which is produced by immunization is not heat labile, although it also operates much better in the presence of complement Neufeld and Rimpau (2), who studied this problem practically simultaneously with Wright and Douglas, gave the name *bacteriotropin* to the heat stable antibody. In common usage today, the term opsonin is usually used for both the normal and the immune agents

The manner by which immune opsonin affects cells rendering them more susceptible to phagocytosis is presumed to be by coating them with the antibody globulin, thereby lowering the surface tension

THE OPSONIC INDEX

As a means of estimating the resistance of the body to certain infections in which immunity depends mostly upon phagocytosis, Wright (3) devised a technic for determining the "opsonic index." Using special narrow elongated pipettes, he mixed emulsions of the specific bacterium, suspensions of phagocytes from normal individuals, and serum of the individual under test, and

incubated the entire pipette after sealing. As a control, the second pipette was filled with the same bacterial suspension, the same leucocytic suspension, but with normal serum, preferably a pooled sample from several supposedly normal individuals After incubation, both pipettes were removed from the incubator, broken open, and the contents spread on slides in the form of smears. After the smears were stained, a careful examination was made of a large number (at least 100) of polymorphonuclear leucocytes, the number of organisms engulfed by each cell being noted In this way, the average number of bacteria engulfed by the leucocytes was determined The ratio which existed between the average number engulfed while under the influence of the serum of the patient to that which was engulfed by the same suspension when under the influence of normal serum, was termed the "opsonic index"

Wright used the opsonic index to determine the dosage of vaccines The size of the injections was gauged so that the index remained above one If the doses were too large or were repeated too frequently, the patient went into the "negative phase," i.e., the index became less than one, and this was considered harmful At the present time, the opsonic index is not considered to be of much value in clinical work An exception to this statement may possibly have to be made in Brucellosis or undulant fever of man, in which Huddleson claims that the opsonic index has diagnostic importance. This is discussed more fully on page 150.

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CHAPTER VII

HYPERSENSITIZATION, ANAPHYLAXIS, AND ALLERGY

The subject of hypersensitizations has been studied by a great many workers, but in spite of this much controversial material about it exists. In the immune reactions, so far discussed, previous contact with antigenic substances serves generally to lessen the sensitivity or susceptibility to them. Heightened resistance is the essence of immunity. In hypersensitiveness, we appear to have the exact antithesis of immunity. In spite of this, it is quite certain that the mechanism involved is the same as that which functions in immunity.

The nomenclature in this field is confusing, all three words which appear at the head of this chapter being used for almost every manifestation of hypersensitivity. We shall use the word *hypersensitization* in a broad sense to cover the entire subject without implications as to the mechanisms involved. Anaphylaxis, allergy, drug hypersensitivity, and serum sickness, all are forms of hypersensitization *Anaphylaxis* is a type of hypersensitization which can easily be produced experimentally in certain types of animals and which, therefore, is fairly well defined *Allergy* includes types of hypersensitization which cannot readily be induced experimentally, and, for this reason, some have argued that allergy had nothing to do with anaphylaxis. Zinsser, admitting unexplainable differences between them, nevertheless feels that the many similarities that exist between them is sufficient evidence to regard all hypersensitivities as having a similar basic mechanism.

Anaphylaxis

Anaphylaxis is a type of hypersensitiveness which may readily be produced experimentally in a number of species of animals and which is certainly concerned with an antigen-antibody reaction. The condition can best be explained by describing the essential experimental facts about it.

When any foreign antigenic substance, which may be harmless in itself, is injected parenterally into an animal and a time interval is allowed to elapse thereafter (incubation period), an altered condition is established in the

animal whereby a second injection of the same antigen may precipitate a train of symptoms which is known as anaphylactic shock The symptoms vary according to the species of animal concerned, the size of the shocking dose, and the mode by which it is administered The reaction is specific, that is, it occurs only when the substance to which the animal has been sensitized is reinjected The symptoms of shock are the same irrespective of the nature of the antigen to which the animal is hypersensitive

Reactions which we now know were anaphylactic in nature were described from time to time in the early literature It is said that Magendie described a typical anaphylactic shock in a dog which had received two injections of egg albumin as early as 1839 In 1894 Flexner (4) clearly described a similar situation.

Héricourt and Richet (5) (1898) observed that repeated injections of eel serum into dogs resulted in increased susceptibility instead of increased resistance as had been expected. These authors coined the word *anaphylaxis,* meaning decreased resistance, as opposed to *prophylaxis,* which means increased resistance

Arthus (1) observed that when repeated injections of horse serum were made into rabbits the tolerance of the rabbits for this substance decreased The first injections were innocuous, but later injections produced inflammatory swellings and, if the injections were continued, the animals were killed This is known as the *Arthus Phenomenon* This condition of local anaphylaxis has been demonstrated to occur only in the rabbit Several workers have failed to demonstrate it in guinea pigs and dogs A reaction similar to the Arthus phenomenon is occasionally observed in man

Theobald Smith (1904) observed that guinea pigs which had previously been injected with diphtheria antitoxin could be killed by injecting them several weeks later with a dose of antitoxin which was harmless for normal animals Ehrlich was told of these observations and he had Otto, one of his students, study the matter thoroughly Otto found that the reaction was due to the horse serum and had nothing to do with the diphtheria antibodies which were in it Otto referred to the matter as the *Theobald Smith phenomenon* Rosenau and Anderson (8) studied the reaction in guinea pigs which had been injected with horse serum and did much to clarify the nature of the reaction Their first paper appeared in 1906

Anaphylactic shock can be demonstrated readily in the guinea pig, rabbit, and dog, but is not so easily induced in apes, a fact which suggests that man probably is not easily rendered anaphylactic This is borne out by clinical experience Cattle may be rendered anaphylactic readily by the injection of horse serum.

SYMPTOMS OF ANAPHYLACTIC SHOCK

In all animals, anaphylactic shock is manifested by a fall in blood pressure and by a subnormal temperature The most striking symptoms are due to the effects upon the smooth muscles These symptoms vary in different animals.

In the *guinea pig,* there is evidence of extreme respiratory embarrassment due to the contraction of the abundant supply of smooth muscles in the bronchioles, and death is due to suffocation

The *rabbit* does not show marked symptoms, as a rule, except those of collapse Death is due to heart failure The muscles of the pulmonary arteries cause constriction of these vessels, with blocking of the pulmonary circulation The right side of the heart is dilated by the back pressure.

The *dog* shows epileptiform seizures, coma, and death after preliminary symptoms of restlessness, diarrhea, and vomiting The liver and intestines are congested

Cattle show uneasiness, labored breathing, edematous swellings around the eyes, udder, anus, and vulva, and diarrhea Cattle seldom, if ever, die as the result of the shock.

RECOGNITION OF THE ANAPHYLACTIC STATE

The anaphylactic condition may be recognized in two ways, viz ·

(a) By the production of shock If the shocking dose is fairly large and is administered intravenously, death of a susceptible animal may result in a few minutes Smaller doses, or doses given by routes in which absorption is slower, may result in less severe reactions

(b) By the Dale (3) method Virgin female guinea pigs are used for this work When ready for the test, the animal is destroyed and a piece of smooth muscle (a strip of the uterus) is removed and immersed in Ringer's solution held at body temperature. After the strip has been connected with a kymograph needle, some of the protein, toward which the animal is supposed to be hypersensitive, is added to the Ringer's solution If the animal is anaphylactic toward the substance, the uterine muscle will exhibit its sensitiveness by contractions which will be recorded on the kymograph drum.

PASSIVE TRANSMISSION OF ANAPHYLAXIS

True anaphylactic sensitization may be transmitted to normal animals by transfusing them with small amounts of blood or serum from the hypersensitive individual. The new individual does not become hypersensitive immedi-

ately, a few hours being required for this to take place Such individuals may be shocked and even killed by a small dose of the specific antigen.

DESENSITIZATION ANTIANAPHYLAXIS

After an anaphylactic shock, animals are desensitized and remain in this state for a considerable length of time Likewise, it is possible to forestall the development of the anaphylactic state by injecting large doses of the antigen before the expiration of the "period of incubation" in which case desensitization occurs without the appearance of shock. Also, animals may be desensitized, without the production of acute shock, by the administration of the antigen over a period of considerable time in multiple small doses

It already has been said that there is some doubt as to whether the anaphylactic condition, as we see it in some species of animals, ever occurs in man Crises similar in clinical appearance certainly sometimes occur in man, but it is possible that their mechanism may be somewhat different However this may be, physicians usually inquire about evidences of hypersensitiveness in patients who are to receive doses of serum from other species of animals and, if it appears likely that a shock may occur, it is customary to divide the dose, giving minute amounts at first, waiting for possible unfavorable reactions before proceeding with the entire amount Small quantities injected intradermally usually will indicate hypersensitiveness by causing the development of a wheal, which quickly appears and just as quickly fades away

Histamine shock will be referred to below. On the theory that this substance is the cause of anaphylactic shock, attempts have been made, apparently not wholly successfully, to destroy it by giving the patient who is receiving the foreign serum a preparation which contains *histaminase*, an enzyme capable of destroying histamine

MECHANISM OF THE ANAPHYLACTIC REACTION

The mechanism of the anaphylactic reaction is a matter which has been under investigation for many years and is still far from settled Two general theories have been advanced (a) The humoral theory, and (b) the cellular theory. There is general agreement that the reaction is a phenomenon of immunity and that antibodies are concerned in it

The humoral theory This theory was in favor a few years ago, but it has now lost most of its adherents The *anaphylotoxin* theory postulated that the initial or sensitizing dose stimulates into existence an immune or protective mechanism. After this is fully working, a large dose of the specific antigen, when injected into the animal, is rapidly attacked by the immune bodies in the fluids of the body, splitting it up and releasing from it toxic split-protein

products. This explanation found support in the work of Vaughan and Wheeler (9), who obtained toxic products from the splitting of various proteins by chemical means *in vitro.*

The cellular theory This theory is now regarded as more plausible than the anaphylotoxin theory. It would be expected, reasoning according to the humoral theory, that the greater the concentration of antibodies in the circulating fluids of the body, the greater the speed and violence of the reaction when the shocking dose of antigen is introduced This, however, is not the case; in fact, when there is a large content of circulating antibody, anaphylaxis almost never exists Furthermore, when serum from an anaphylactic animal is transferred to a normal animal nearly half of it disappears from the circulating blood within an hour If, after this time, the animal is completely exsanguinated, his blood being replaced by that of a normal animal, the first animal will be fully sensitized anaphylactically These facts and others point to the probability that anaphylaxis is due to sessile antibodies, i. e., antibodies which, in some way, are attached to tissue cells and do not circulate. In the presence of large numbers of circulating antibodies, these cells are protected from antigen introduced, but when few or no free antibodies exist, the antigen reaches and injures the tissue cells There is only speculation as to the type of antibody concerned Many believe that there is some connection between precipitins and anaphylactic sensitivity.

HISTAMINE SHOCK

The symptoms of anaphylactic shock can be reproduced very faithfully by injections of histamine This substance is derived by the decarboxylation of histidine which probably is present in every living cell One theory of anaphylactic shock is that the antigen-antibody reaction, occurring on cells, so sensitizes the cells that complement causes their chemical disruption thus releasing the histamine

HAPTENS IN ANAPHYLAXIS

Anaphylactic shock may be produced by injecting the polysaccharide of the pneumococcus The animals cannot be sensitized by this substance alone, however. Thus, haptens assume the same role in anaphylaxis as they do in other immune reactions.

SERUM SICKNESS

The term is used for certain phenomena which appear in man following the administration of therapeutic sera. It is a hypersensitiveness to the proteins of the serum, i e, generally to horse serum. The disease, except in very unusual cases is not serious, but is very distressing to the patient The symp-

toms usually appear in from 3 to 12 days after the dose of horse serum and consist of urticaria, joint pains, edematous swellings, fever, and sometimes glandular swellings, especially of the part where the injection is made The illness usually is of short duration In very rare instances the picture is of an entirely different character In these cases the patient collapses immediately after the injection, and dies

There is considerable difference of opinion as to whether serum sickness is a result of a previous sensitization In a great many cases, there is no history of any previous contact with horse serum When there has been previous contact with horse serum, in the form of previous prophylactic or therapeutic treatments containing horse serum, the incidence of serum sickness is considerably increased and the reactions usually come on earlier and may be more severe They are termed accelerated reactions

It should be pointed out that anaphylactic shock is a condition which can hardly happen without the interfering agency of man Animals may be perfectly protected by their immune mechanism against small doses of the specific antigen, against doses as large as could enter under natural conditions, but may be even more vulnerable than usual to large doses of the antigen introduced artificially and suddenly.

THE ANAPHYLACTOID REACTIONS

When normal blood serum is mixed with many inert substances, such as kaolin, talc, and barium sulphate, it becomes markedly toxic after a brief period of incubation These poisonous serums have been termed *serotoxins* The symptoms produced by the injection of serotoxins are very similar to those of anaphylactic shock, and at one time it was thought that they might be responsible for it The idea has now been given up Ordinary commercial peptone likewise is capable of producing anaphylactoid reactions The mechanism of these reactions has not been satisfactorily explained. It has been suggested that some serum component (such as antitrypsin) may be absorbed, thereby upsetting the normal balance of constituents and, as a result, releasing some toxic fractions Those who do not accept histamine as the true cause of anaphylactic shock would include histamine shock under the heading of anaphylactoid reactions

Allergy

Apparently closely related to anaphylaxis is a long series of hypersensitizations which occur among people and, to a lesser extent, among animals By common usage, this type of hypersensitiveness is called *allergy* The majority

of substances toward which this type of hypersensitization is manifested are proteins or contain protein, but some of them like drugs such as iodoform and quinine are not in this category The fact that nonantigenic substances often figure in allergy has caused many authorities to differentiate it from anaphylaxis, yet it must be remembered that haptens react in immune reactions and also that it is possible that nonantigenic substances may, in the body, attach themselves to proteins, thus forming complexes which could function as antigens, as in the artificial antigens of Landsteiner and others

A striking feature of these idiosyncrasies is that, in general, the symptoms are similar no matter what the nature of the inciting agent, and that these bear no relation to the pharmacologic action of the substance in question

In man, and possibly also in animals, allergy often is manifested by local rather than by general reactions Certain plant pollens and other substances in suspension in the air when inhaled will cause coryza (hay fever) or asthma in hypersensitive persons Certain foods will cause diarrhea and vomiting or, more commonly, skin diseases of an eczematous nature In some persons, contact with certain substances which are innocuous to most people will cause severe dermatitis The range of substances which elicit allergic reactions in people is very large, there being hardly any commonly used food substance which has not been incriminated

Very little is known about allergic reactions in animals except those which occur as a result of bacterial infections Eczematous conditions of the skin of dogs are very common, especially during the summer months and it is suspected that some, if not many, of these cases are manifestations of food allergy Pomeroy (6), in one such case, showed that the animal was hypersensitive to substances found in canned salmon Rather typical symptoms of "hay fever" have been seen in dogs, the coryza and swelling of the mucous membranes occurring annually during the pollen season In horses, a condition known as heaves is quite similar in many ways to asthma in man, and it is possible that its etiology is similar

Idiosyncrasies of the type which we are discussing have not been experimentally produced In man, they develop naturally, sometimes rather late in the life of the individual In some families, allergic individuals occur more commonly than in others, suggesting that hereditary factors are concerned Zinsser believed that allergy was not of itself inheritable, but rather a predisposition to develop such conditions In families in which such cases are frequent, one individual may be hypersensitive to butter, another to potatoes, another to rose pollen, another to lobster, and so on. It is not usual for hypersensitization to the same substance to appear in more than one individual in the same family.

No antibodies can ordinarily be demonstrated in the blood of allergic persons, although, as has been pointed out above, this does not exclude the possibility that the condition is concerned with antigens and antibodies Some lessening in the degree of hypersensitization may be brought about by injections of extracts of the provoking substance in many instances, but complete desensitization as is seen in anaphylaxis cannot ordinarily be accomplished

On the other hand, it can be demonstrated that there is a reaction between some substance in the serum of hypersensitive persons which will react with the inciting substance This is seen when extracts of the inciting substance are injected intradermally Normal persons usually give essentially no reaction at the injection site, allergic individuals react with a reddened, inflamed area. This reaction is utilized clinically to determine what substances are concerned in the hypersensitive state

That a reacting substance is present in the serum of hypersensitive persons is indicated more clearly by the passive sensitization of the skin of a nonsensitive individual by the injection of a small amount of serum from a hypersensitive one If a small quantity of serum from an allergic individual is injected into the skin of a normal one and, twenty-four hours later, an extract of the inciting substance is injected into the same site, a typical inflammatory reaction will result The reaction will not be seen if the same extract is injected into the skin of other parts This reaction has been called the *Prausnitz-Kuster* (7), or *P-K*, phenomenon, taking its name from the discoverers of the reaction

Coca (2) calls the inciting substances of these allergies *atopens*, and the substances which react with them, *atopic reagins* He does not believe that these agents are antigens and antibodies, but others look upon atopens as haptens, and reagins as antibodies.

Hypersensitization in Bacterial Infections

Hypersensitization to bacterial proteins and possibly other fractions of bacterial protoplasm, incited by the presence of the causative organism in the tissues, commonly occurs in the course of infectious diseases The *tuberculin reaction* is the classic example of bacterial allergy. Others are the mallein reaction in glanders, the brucellin reaction in undulant fever, the johnin reaction in paratuberculosis, and the typhoidin reaction in typhoid fever. Allergic reactions of this type are inflammatory in nature The allergic state may be detected by injecting whole cultures, culture extracts, or culture filtrates into the dermis of the skin, the subcutaneous tissue, the peritoneal cavity, the blood stream, or even by placing some of the inciting agent into the conjuncti-

val sac Allergic individuals react to the skin tests by the development of inflammatory swellings at the points of injection, to the ophthalmic tests by inflammation of the conjunctival mucous membrane, and to the parenteral tests by the development of fever, chills and symptoms of general illness which usually disappear after a few hours Since the allergic tests are specific, they have diagnostic value Generally speaking the allergic tests are positive only in the presence of active or latent infections Bacterial allergy cannot be passively transferred and it is not easily induced by the injection of dead cultures or of culture extracts.

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CHAPTER VIII

THE ISO-ANTIBODIES

Early in the study of immunological reactions, the question arose as to whether or not an animal might produce antibodies against antigens contained in his own body It was suggested, for example, that when blood cells escaped into tissues in the course of hemorrhage, the tissues might produce hemolytic antibodies If this should happen it would be easy to see how a condition of chronic or pernicious anemia might develop as a result of blood destruction by the new antibody

The first to investigate the matter were Ehrlich and Morgenroth (5) who injected blood cells of certain goats into other goats In some cases the serum of the recipient developed hemolysins which were active against the blood cells of the donor Such sera were actively hemolytic for the blood corpuscles of some other goats, and did not effect those of still others In no instance, however, were the blood corpuscles of the recipient affected by its own hemolysins In other words, auto-antibodies were not developed by these workers and they have not been demonstrated by any subsequent workers That antibodies could be developed for antigens derived from other individuals of the same species was, however, proved Later it was learned that such antibodies ([?]) existed normally in some species of animals

Antibodies which are effective against antigens from other individuals of the same species are known as *iso-antibodies* From a practical viewpoint, the most important of the iso-antibodies are the *iso-agglutinins* and the *iso-hemolysins* These antibodies are of interest, and of legal and clinical importance in man It is known, also that such antibodies exist normally in some species of animals and can be produced experimentally in others The blood groups of man are dependent upon the distribution of iso-hemagglutinins among the population

The Human Blood Groups

The human blood groups were discovered by Landsteiner (10) in 1901 Originally it was found that a limited group of human beings could be placed into three groups according to the behavior of their blood cells in the presence of sera of other individuals Later a fourth group was found.

The behavior of human erythrocytes when mixed with serum of other individuals can be explained by assuming that two antigens (factors), which have been designated *A* and *B*, are present in human blood corpuscles, some individuals having one, some the other, some both, and some neither Also present in man are the corresponding agglutinating factors which are designated *a* and *b* Like the antigenic factors these are distributed among people in all possible combinations, but never are the homologous antigens and antibodies found in the same blood When sera containing one, or the other, or both, agglutinins come in contact with the corpuscles containing the homologous factor or factors, the corpuscles are agglutinated and sometimes, hemolyzed

The blood groups of man are designated by the factor or antigen contained in the corpuscles Thus the following groups are recognized

Group O Contains neither the *A* or *B* antigen Agglutinins *a* and *b* are present in the serum

Group A Contains the *A* antigen in the corpuscles Agglutinin *b* is present in the serum

Group B Contains the *B* antigen in the corpuscles Agglutinin *a* is present in the serum

Group *AB* Contains both *A* and *B* antigens in the corpuscles. No agglutinins are present in the serum

Since agglutination will take place when two bloods are mixed when one contains the corpuscular antigen and the other the corresponding serum antibody, disastrous results may occur when blood transfusions are made with such incompatible bloods The following table indicates how the several human blood groups interact

TABLE IV

		Corpuscles			
		Group O	Group A	Group B	Group AB
Serum	Group O Serum factors ab	–	+	+	+
	Group A Serum factor b	–	–	+	+
	Group B Serum factor a	–	+	–	+
	Group AB No serum factors	–	–	–	–

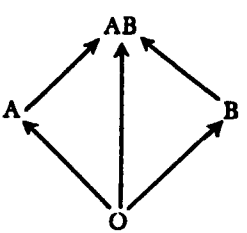
+ indicates that agglutination would be expected
– indicates that agglutination would not be expected

COMPATIBILITY TESTS

Before a blood transfusion is done on man, a preliminary test should always be made in which the corpuscles of the donor are tested with the serum of the recipient. It is well also to test the serum of the donor against the corpuscles of the recipient, although this is not of as much importance as the preceding test for reasons that will be given later. It is obvious from what has already been said that unless the donor and recipient are members of the same blood group, there will be agglutination one way or the other For this reason an effort always is made to find a donor of the same blood-group as the recipient. If such a donor is not available a transfusion generally can be made safely with a donor whose serum agglutinates the corpuscles of the recipient, for the reason that the amount of blood introduced is rather small compared with the blood volume of the recipient and the agglutinins are so diluted that ordinarily they do not cause trouble The most important thing to guard against is that the corpuscles of the donor are agglutinated by the recipient's blood, for these come in contact with concentrated agglutinin and are apt to clump, forming intravascular clots and perhaps causing sudden death, or at least severe reactions

Individuals of Group *O* are frequently called *universal donors* for the reason that their corpuscles are not agglutinated by the sera of any of the groups The fact that Group *O* individuals always have agglutinins in their sera may be ignored, in emergencies, for the reasons stated above Accidents sometimes happen when universal donors are used for transfusions into individuals of other groups because sometimes the agglutinins are present in unusual concentration in which case they are not wholly inactivated by the dilution which occurs. It is best always to use homologous donors when they are available.

The possibilities of safe transfusions among groups is indicated in the following simple diagram.



Interesting racial studies have been made on the basis of blood-groups. The Hirschfelds (7), working in the Balkan countries where there is a very great

mingling of racial types, showed that the several racial stocks differed considerably in the incidence of the *A* and *B* factors. The races can be divided roughly into three groups. The A B ratio is highest among Nordic types, is intermediate among such races as the Japanese, Arabians, Russians and Jews, and is lowest among African negroes and some of the Asiatic races The situation can be seen in the following table of the percentage population of several typical groups which fall into each of the four blood groups.

TABLE V

	Group O	Group A	Group B	Group AB
English . . .	45 4%	43 4%	7 2%	3 0%
Turkish .	36 8%	38 0%	18 6%	6 6%
African negro	43 2%	22 6%	29 2%	5 0%

INHERITANCE OF BLOOD GROUPS

The factors *A* and *B* are not limited to the blood corpuscles but may be found in all cells of the body They are somewhat variable very early in life but after the human infant is a few months old his blood type becomes established and cannot be influenced by disease or medication They are present in the newly formed cells of tumors and are transmitted through the germ plasm to the progeny

That blood groups were heritable was first shown by von Dungern and Hirschfeld (4) who demonstrated that the corpuscular factors were dominant over the serum agglutinins and that the distribution of these factors among offspring could be explained by the formula of Mendel It was their belief that the four groups were inherited as two pairs of independent factors, *Aa* and *Bb*, with the corpuscular factor dominant in each case Later it was found that this explanation did not properly account for the distribution of the several groups among men, particularly the rather rare group *AB* Bernstein (2) restudied the matter and decided that the distribution of the factors indicated that they were not inherited as had been previously thought but as triple allelomorphs, *A* and *B* being dominant to *R*, the allelomorph which determines *O*. From the mating of an *A* individual with a *B*, the offspring may be *O*, *A*, *B*, or *AB* Two *B* individuals cannot produce offspring with the *A* factor; they are limited to *B* and *O* When either parent is an *AB*, the offspring can be *A*, *B*, or *AB*, but never *O*. When either parent is an *O*, the offspring cannot be an *AB*.

Blood groups occasionally have medico-legal significance in questions of determining the legitimacy of children or in other questions of disputed parentage. Because of the great number of possibilities involved, it is not pos-

sible to identify positively the parents of a particular child through determination of their blood groups, or even to identify the second parent when one is known It is possible in such cases, however, to exclude certain individuals For example, if a child was found to belong to the *A* group and his mother to the *O*, the father could not have belonged to the *O* or *B* groups because he must have supplied the *A* factor He could only be an *A* or an *AB*

In recent years certain subgroups, designated *m* and *n* have been identified among the four primary groups described above Through utilization of these it is possible to narrow the possibilities of child-parent relationship identification and thus to increase the usefulness of the method

Blood Groups in Domestic Animals

It has already been pointed out that the first work on iso-antibodies was done by Ehrlich and Morgenroth on goats, hence it has been known from the beginning that group antigens existed in this species Later workers have demonstrated the presence of group antigens in sheep, cattle, horses, swine, dogs, cats, ducks, and chickens It seems quite likely, in fact, that they exist in most, if not all, animal species In apes the same group antigens and antibodies exist as in man In the other animals the antibodies either are not normally present or they exist in very low concentration This fact makes it possible to carry out single transfusions safely and indiscriminately within the species, without the formality of making compatibility tests

Blood groups in animals usually become evident only when repeated transfusions are made Between some individuals of the species repeated transfusions may be carried out without untoward results In other cases, however, severe and even fatal reactions may occur on the second or subsequent transfusions from the same animal, apparently as the result of the formation of immune iso-hemolysins This happens in dogs Wright (17), and Melnick and Cowgill (11) who were studying plasma regeneration in dogs found that occasional animals reacted violently to second or later transfusions from a single dog when they had not reacted at all on the first transfusion These animals showed muscular tremors, vomiting, bloody diarrhea, jaundice, hemoglobinuria, and signs of profound shock Melnick and Cowgill report that the symptoms of shock are similar to those of anaphylaxis, even to the extent of being partially alleviated by intracardial injections of adrenalin The symptoms, of course, are largely those associated with massive red cell destruction

Ottenberg and Thalhimer (12) reported the development of immune iso-agglutinins and iso-hemolysins in cats as a result of repeated transfusions The

cats showed no unfavorable reactions as a result of these antibodies except the development of hemoglobinuria Kaempffer (8) divided swine into three groups according to the antigens and antibodies present Only one antigen, designated *A* and one antibody homologous to this antigen and designated *a*, were identified One group was characterized as *Ao*, another *Oa*, and the third *Oo* Szymanowski and Frendzel (14) reported that anti-hog cholera serum often contained high concentrations of *a* agglutinins, probably because of the hyperimmunization of *Oa* animals with blood from those of the *Ao* group

Ferguson (6) claims to have identified seven different immune isolysins in cattle produced by repeated transfusions and he believes that there are many more He states that the behavior of these antigens suggests that each is controlled by a single gene and suggests that it may be possible to use immune isohemolysins as a means of proving breeding lineage in cattle In some of the cattle which were being injected a second or third time, anaphylactic-like symptoms appeared These consisted of muscular trembling, dyspnea, salivation, lachrymation, depression, and hemaglobinuria After two to four hours there often was a rise in temperature to 104° F. or higher. All of his animals recovered

Iso-antigens and immune antibodies have been studied by Schermer and Kaempffer (13) in horses, by Andersen (1) in sheep, by Keeler and Castle (9) in rabbits, by Wiener (15) in fowls and by Boyd and Alley (3) in fowls, to mention only a few of the more recent studies.

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TABLE VI

METHODS OF PRODUCING AN ARTIFICIAL IMMUNITY

- Immunity
  - Innate
  - Acquired
    - Active
      - Natural Infection and recovery
        - Not attenuated (Sub-lethal dosage or inoculation in abnormal site)
          1. Hogyes method of rabies vaccination
          2. Variolation in small pox
          3. Laryngotracheitis (Of chickens)
        - Attenuated (Vaccine)
          1. Living causative agent
             1. Drying (Pasteur rabies vaccine)
             2. Growing at unfavorable temperature (Pasteur anthrax vaccine)
             3. Less susceptible animal (Small pox), (Rabies, dog to monkey)
             4. Chemicals (Phenol in anthrax)
             5. Heat (Blackleg vaccine)
      - Artificial Means
        2. Dead causative agent (Bacterin)
           1. Heat (typhoid) (pyogenic) (cholera)
           2. Chemicals—phenol, etc
        3. Extracts of organisms
           1. Autolysates
           2. Mechanical (Cells dried in vacuo and ground)
        4. Aggressins
           1. Blackleg
           2. Anthrax
           3. Hemorrhagic septicemia
        5. Products of metabolism (Toxins, Toxoids)
        6. Combined use of virulent causative agent and immune serum
           1. Hog cholera
           2. Toxin-antitoxin mixtures
    - Passive (Artificial except for new-born and sucklings)

PART II

THE PATHOGENIC BACTERIA

CHAPTER IX

THE STREPTOCOCCI

General Characteristics

Morphology and Staining Properties. The group of cocci that characteristically develop into chains resembling strings of beads, are known as streptococci. The chains may be short (diplococci) or they may be very long. Chain length depends upon species differences and upon the medium upon which the culture is growing. Typical chain formation is best seen in fluid media; on solid mediums the chains become so entangled that their demonstration is impossible

The individual cells of streptococci are seldom perfectly spherical, and frequently there is considerable variation in the size and shape of the elements in a single culture Sometimes the cells are flattened from side to side, more often they are elongated Spores are never formed With rare exceptions they are non-motile A number of species form definite capsules when developing in tissues or in culture media containing blood serum Such strains form colonies which are mucoid and differ in appearance from those of the majority of streptococci

The great majority of streptococci are Gram-positive In old cultures many Gram-negative forms are commonly found They are easily stained with all the usual dyes. They are never acid-fast.

Cultural Features. All streptococci produce small, delicate, translucent colonies of a diameter of about 1 mm on solid media Heavy inoculations give confluent growths that are nearly transparent Their surfaces are smooth and glistening and the margins of individual colonies are perfectly circular Deep colonies in agar usually are lenticular in shape In softer media they may be globular In size they may be hardly large enough to be easily visible with the naked eye Usually there is no growth on potato When growth is obtained on gelatin, it consists of a string of delicate beads along the line of the stab, with little or no growth on the surface, and in most cases without evidence of liquefaction.

In fluid media growth usually is a little more abundant than in solids. In broth there may be a faint cloudiness or the medium may remain perfectly clear except for a fluffy sediment in the bottom of the tube The appearance

usually gives an accurate clue as to whether the coccus is growing in short or long chains; the short-chain type causes uniform clouding, whereas the long-chain type quickly sediments All streptococci grow well in milk With few exceptions the milk is soured through the formation of lactic acid from the milk sugar

Sugars are fermented by all streptococci The end product is largely lactic acid Gas is produced by only a few streptococci and by none of the types that are of importance in pathology

Physiological Characteristics. The growth range of streptococci varies from below 10° C to above 45° C The pathogenic types have a much narrower range than this

The resistance of streptococci to heat is not great The pathogenic types are usually killed by temperatures well below those used for pasteurization (about 61°–62° C for 30 minutes) It is well known, however, that the milk-souring types are not wholly destroyed by pasteurization, and some of the intestinal types have rather unusual resistance Likewise, the resistance to drying and to chemical disinfection is not very great as a rule However, when cultures are rapidly and completely dried, they often remain viable for very long periods of time, hence it is possible that streptococci withstand drying better than is generally supposed.

Habitat. The streptococci are found on the mucous membranes of men and animals, in various suppurative processes in these hosts, and in milk and milk products. It is frequently said that these organisms principally exist as animal parasites Stark and Sherman, however, have found the *Strep lactis* on growing vegetation, and this raises the question of whether these organisms may not occur more commonly on plants than it has been hitherto supposed All streptococci grow much more luxuriantly on artificial media when blood serum or tissue extracts are added This suggests that they are better adapted for a parasitic than a saprophytic existence.

DIFFERENTIATION OF THE STREPTOCOCCI

Since 1884, when Rosenbach (16) described *Strep pyogenes*, the importance of certain streptococci in pyogenic processes of man has been known. The differentiation of other streptococci, however, always has been difficult, and, indeed, the identification of *Strep pyogenes* itself when it was not associated with characteristic suppurative processes When morphological features were recognized as inadequate for differentiation, fermentative characteristics were examined Although a very large amount of work was done, fermentation tests likewise proved inadequate when applied to streptococci in general. Cer-

tain of these tests have value, however, in differentiating between types that have been grouped together by other tests applied primarily. The most useful differential test for the streptococci is that introduced by Schottmuller (17) in 1903, which depends upon their action on blood cells (erythrocytes).

1. **Action of Streptococci on Red Blood Cells.** According to their action upon erythrocytes of animals, all streptococci may be placed in one or the other of the following classes

(a) *The Hemolytic Group* includes those which form a soluble hemolysin which causes the freeing of hemoglobin from erythrocytes

(b) *The Viridans Group* includes those that cause an alteration of the hemoglobin, without freeing it from the cells, in the course of which its color is changed through various shades of green to a greenish-black.

(c) *The Anhemolytic Group* includes those that cause no noticeable change in erythrocytes.

Smith and Brown (19), pointed out that under certain conditions streptococci belonging to the Viridans Group would cause hemolysis of blood cells, and, therefore, thought it best to rename the groups They suggested the use of the first three letters of the Greek alphabet as designations Their *Alpha Group* is the Viridans Group of Schottmuller, the *Beta* is the Hemolytic Group, and *Gamma* designates the Anhemolytic Group Both systems of nomenclature are used

THE BLOOD AGAR PLATE The identification of the grouping is generally done on the blood agar plate From five to ten per cent of sterile, defibrinated horse, rabbit, or human blood is added to the melted and cooled nutrient agar just before it is poured into the plate Streak plates often are used, but it is better to use poured plates and to base the classification upon the deep rather than the surface colonies

Colonies of the *Alpha* or *Viridans* Group at the end of 18 to 24 hours' incubation at 37° C show a slight or marked discoloration of a narrow zone of blood cells immediately surrounding the colony The discoloration is easily seen with the naked eye Under the microscope it can be seen that the erythrocytes in the discolored zone are intact If the incubation is carried on for a longer period, and especially if the incubation continues at room temperature or lower, a clear zone appears outside the greenish area. Under the microscope it may be seen that the blood cells have dissolved The cells in the discolored zone remain intact, however, no matter how long the incubation is continued.

Colonies of the *Beta* or *Hemolytic* Group show no discolored zone under

any circumstances. As soon as they begin to develop, solution of the blood cells about the colonies appears After 18 to 24 hours' incubation, the minute colonies have developed clear, transparent zones about them from 1 to 3 mm. in breadth Under the microscope these zones appear perfectly clear; no intact blood cells can be found in them.

Solution of the erythrocytes occurs because of the production of a soluble hemolytic substance which is produced by the organisms in greatest abundance during the period of logarithmic increase. It may be demonstrated by adding a washed blood cell suspension to a broth culture from 4 to 15 hours old—the cells are lysed within a few minutes Older cultures show reduced or no activity The hemolysis which is produced by organisms of the Alpha or Viridans group is not of this character In this case it is probable that it is due, in part at least, to hemolyzing concentrations of acid



Fig. 3 Streptococcus of the Alpha Type on a Blood Agar Plate This plate had been incubated 48 hours at 37° C The colony appears in the center, surrounded by a wide zone in which the blood cells are discolored but are intact The color of this zone is greenish Beyond the greenish zone is a narrow zone of partially hemolyzed cells x 10

The terms *hemolytic* and *non-hemolytic* continue to be used in connection with streptococci not without considerable confusion Most authors consider all streptococci, except those of the Beta group, as non-hemolytic This usage will be followed here Certain non-hemolytic organisms of the Alpha type produce little discoloration, especially if the basic agar medium contains little fermentable sugar, or if the plates are incubated at a temperature below 37° C In these instances the secondary solution of corpuscles always exhibited by Alpha type organisms may closely simulate the Beta type of hemolysis The cleared zones in these instances are not usually so clear as those of Beta organisms, however, and young broth cultures contain no hemolytic substance

2. Limiting Hydrogen Ion Concentration (for Hemolytic Streptococci). Avery and Cullen (1) early called attention to the fact that hemolytic streptococci of human origin growing in the presence of fermentable sugar will seldom carry the pH beyond 5 0, whereas the majority of strains of bovine origin will carry it to pH 4 5 or 4 3.

3. Hydrolysis of Sodium Hippurate (for Hemolytic Streptococci). Ayers and Rupp (2) pointed out that hemolytic streptococci of bovine origin would break down sodium hippurate, whereas those of human origin would not. It appears to be true that cultures of human origin never attack this substance, but Edwards has shown that some bovine strains, as well, do not. As a matter of fact, this test is practically specific for certain streptococci associated with bovine mastitis (*Strep agalactiae* and *Strep uberis*)

4. The Fibrinolytic Test (for Hemolytic Streptococci). Tillett and Garner (20) (1933) described a reaction which appears to be valuable in differentiating hemolytic streptococci which are human pathogens Young cultures in broth (or filtrates) are mixed with oxalated human plasma A clot is then formed by the addition of calcium chloride Most organisms belonging to Lancefield's Group A will liquefy this clot in a very short time, usually within ten minutes in a water bath at 37° C With very few exceptions, streptococci of other groups will not liquefy the clot at all, or only after a long incubation period.

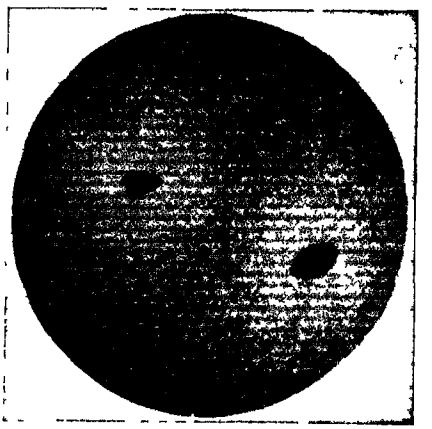


FIG 4 Streptococcus of the Beta Type on a Blood Agar Plate This plate had been incubated about 36 hours at 37° C The two colonies are surrounded by zones in which the red blood cells have apparently disappeared To the naked eye the hemolyzed zones appear much sharper than in the photograph x 10

5. Lancefield's Serological Method (for Hemolytic Streptococci). In 1933 Lancefield (9) (1933) described her method of differentiating hemolytic streptococci This method probably is the most accurate that we now possess. The method consists of the use of the precipitation test The antigens are extracts of cultures prepared with hot, dilute hydrochloric acid The antigen is carbohydrate in nature, similar to the "residue antigens" or "specific soluble substances" (S S S) which give immunological specificity to the types of pneumococci In her first study, Lancefield differentiated five types of streptococci which she designated as Groups A, B, C, D, and E. Later, three additional groups, F, G, and H have been recognized, and others probably will be found

In her first study, all cultures of human origin fell into Group A, Group B

consisted of bovine strains for the most part, Group C came from a variety of animals, Group D from cheese, and Group E from milk. Later studies have shown that some human strains fall into groups other than A, but there is little evidence that these strains are pathogenic, or numerous. It appears to be true that all of the organisms which concern public health workers belong to Group A.

6. Fermentation of Carbohydrates. Many carbohydrates have been used by workers in attempts to differentiate streptococci. Little progress was made,

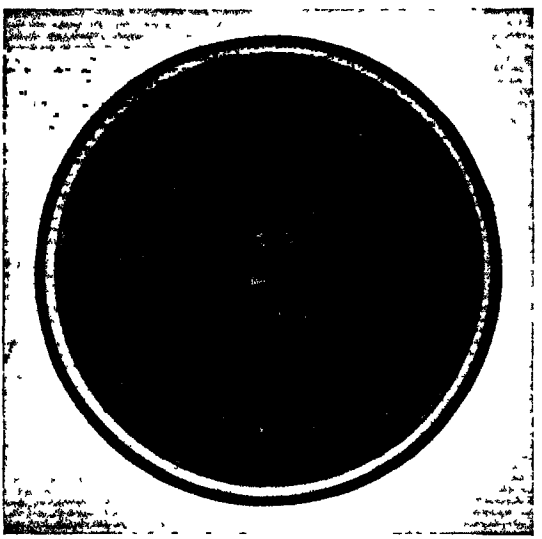


FIG. 5. *Streptococcus equi*. Colonies on a Blood Agar Plate. The colonies are seen as minute points surrounded by wide zones of Beta type hemolysis. Reduced one third.

however, until fermentation tests were relegated to a secondary role. Their value appears to be in differentiating between organisms which are grouped together on the basis of other characteristics. Thus, a certain fermentation may have great value in differentiating between closely related hemolytic organisms, but have none at all when applied to organisms of another group.

In the differentiation of mastitis streptococci from others frequently found in milk, the fermentation of esculin has been very useful. The organisms of mastitis do not attack this substance, whereas most other streptococci, likely to be found in milk, do. Edwards recommends the medium of Harrison and Vanderlek which is a 2% peptone solution in which 0.5 gm. each of esculin

and iron citrate scales are dissolved The splitting of esculin is indicated by a blackening of the medium

7. Tolerance Tests. Sherman and his students (18) (1937) have found that, in general, the streptococci of the "enterococcus" group are much more resistant to various influences than organisms, otherwise similar, which belong to the pyogenic class The enterococci, for example, will grow at temperatures as low as 10° C. and as high as 45° C., they will grow in media which contain 6 5% sodium chloride, they will grow in exceedingly alkaline media (pH 9 6), they will grow in milk containing as much as 0 1 per cent methylene blue, and they generally will survive heating to 60° C for 30 minutes, whereas the majority of other streptococci are unable to endure these conditions

8. Miscellaneous Tests. Other tests used for the differentiation of streptococci are (a) production of ammonia from peptone, (b) liquefaction of gelatin, (c) hydrolyzation of starch, (d) growth in the presence of 10 to 40% bile, (e) curdling and reducing power in milk, (f) virulence for animals, especially rabbits

In a recently published monograph, Sherman (18) (1937) has undertaken to classify streptococci upon the basis of a series of physiological tests The accompanying table is a composite from several tables given in this paper. This table includes a number of groups and species which quite certainly are not pathogenic, but they are included here, nevertheless, because one often encounters them when pathogenic forms are sought It will be noted that all hemolytic organisms fall into the first group, except two types, which are placed in the enterococcus group Since a soluble hemotoxin cannot be demonstrated in fluid cultures for the last mentioned although a definite hemolysis occurs on blood plates, the question may be raised whether they really belong to the Beta group of Schottmuller On the other hand, the hemolytic strength of *Strep agalactiae* usually is weak and often wholly absent, and the question can be raised in this instance whether the organism really belongs to the Beta group The classification, however, undoubtedly will be useful until increasing knowledge causes a realignment of the groups In this classification most, if not all, of the forms pathogenic for man and animals are included in the first (pyogenic) group

The Hemolytic Streptococci

STREPTOCOCCUS PYOGENES

This organism is generally regarded as the type species of this group. It was isolated and described by Rosenbach (16) in 1884 It long has been recog-

TABLE VII

DIFFERENTIATION OF THE STREPTOCOCCI

(Adapted from Sherman)

Division	Hemolysis	Lancefield Serological Group	Fibrinolysis	Sodium Hippurate	Growth at 10° C	Growth at 45° C	Growth in Presence of 6.5% NaCl	Growth in Presence of pH 9.6	Growth in Presence of 0.1% Methylene Blue	Survival at 60° for 30 minutes	Gelatin Liquefaction	NH_3 from Peptone	Milk curdled	Acid Produced from Lactose	Acid Produced from Trehalose	Acid Produced from Sorbitol	Group or Species
Pyogenic	++	A	−	−	−	−	−	−	−	−	−	+	−	+	+	−	S pyogenes
	++	B	−	+	−	−	−	−	−	−	−	+	+	+	+	−	S agalactiae
	++	C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	S equi
	++	C	−	−	−	−	−	−	−	−	−	+	−	+	−	+	"Animal pyogenes"
	++	C	±	−	−	−	−	−	−	−	−	+	±	±	+	−	Human C
	++	E	−	−	−	−	−	−	−	−	−	+	−	+	+	+	Group E
Viridans	−		−		−	−	−	−	−	−	−	−	±	+	±	−	S salivarius
	−		−		−	+	−	−	−	±	−	−	−	±	±	−	S equinus
	−		−		−	+	−	−	−	+	−	−	±	+	±	±	S bovis
	−		−		−	+	−	−	−	+	−	−	+	+	−	−	S thermophilus
Lactic	−				+	−	−	−	+	+	−	+	+	+		−	S lactis
	−				+	−	−	−	+	±	−	−	+	+		−	S cremoris
Enterococci	−				+	+	+	+	+	+	−	+	+	+	+	+	S fecalis
	−				+	+	+	+	+	+	+	+	+	+	+	+	S liquefaciens
	+	D		±	+	+	+	+	+	+	±	+	+	+	+	+	S zymogenes
	+	D?		±	+	+	+	+	+	+	−	+	+	+	±	−	S durans

nized as the cause of a series of malignant suppurative infections in man which frequently terminate in septicemia

Streptococcus pyogenes is the specific name for a group of organisms not wholly identical but having in common the Group A carbohydrate of Lancefield Griffiths has demonstrated at least 24 types within the group All of them are *Beta* hemolytic, and all of them produce a soluble *hemolysin* All of them are possessed also of a substance which is destructive to phagocytes (*leucocidin*) and apparently all of them produce in varying degree a toxin which causes inflammation when injected into the human skin (*erythrogenic toxin*) Strains are found in the throat and naso-pharynx of a considerable percentage of apparently normal people They are also found in tonsillitis, sore throat, otitis media, puerperal fever, scarlet fever, erysipelas, bronchopneumonia, wound infections, and other conditions of man In epidemics of sore throat caused by this organism, some individuals may exhibit the skin rash which is characteristic of scarlet fever, whereas many others do not. In other outbreaks, practically all may exhibit the rash This probably depends upon individual susceptibility in the first instance and upon especially virulent strains possessed of an unusually strong erythrogenic toxin in the second. When the skin manifestations are prominent, the cases are diagnosed as scarlet fever, when the skin manifestations are absent the condition is termed septic sore throat

So far as is known, *Streptococcus pyogenes* plays a small role in infections of the domestic animals Many of the supposed infections with this organism, which have been described in the past, are now known to be due to other types of hemolytic streptococci belonging to the Lancefield Group C. *Streptococcus pyogenes* occasionally invades the udder of cattle, as a result of contact with human beings (generally milkers) who are suffering from sore throat, or have the infections on their hands In the milk cistern of the udder the organism is capable of multiplication, as a result of which inflammation (garget or mastitis) is produced. The milk from such cattle sometimes causes large outbreaks of scarlet fever or septic sore throat, if it is marketed in the raw state It must not be supposed, however, that bovine mastitis always is dangerous to man This quite common condition is usually due to organisms belonging to Lancefield's Group B which are not known to have any disease-producing power for man

STREPTOCOCCUS AGALACTIAE

Synonym *Streptococcus mastitidis*

This organism causes the majority of all cases of bovine mastitis or garget. It may be found in the udder of one or many cows in a large proportion of

all dairy herds the world over Some workers have claimed that the organism exists in normal udders This probably is not true What constitutes a "normal" udder depends upon the clinical acuity of the observer It is true that it often may be found in the secretion of udders so little changed as to pass for normal milk, and the infected udders sometimes are so little altered that the abnormality escapes detection.

Morphology and Staining Reactions. In the secretion from infected udders, *Streptococcus agalactiae* usually appears in the form of long chains In some samples these are numerous and easily found in stained films, in other cases, even though the milk may be markedly altered in appearance, the organisms may be so scarce as to be found with great difficulty The organism is Gram-positive and is readily stained by all of the ordinary stains

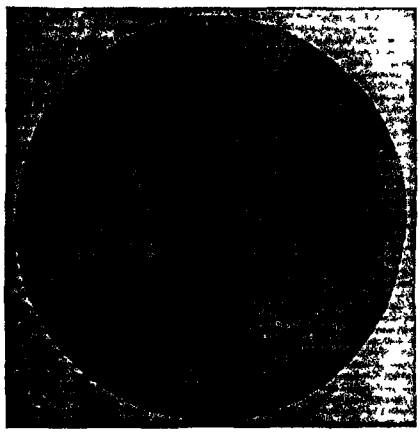


FIG 6 *Streptococcus agalactiae* From a stained film made from a sample of mastitis milk which had been incubated overnight at 37° C Similar chains often are found in the fresh udder secretion Note that the chains consist of a series of paired organisms This is characteristic but not diagnostic × 900

Cultural Features. It will be noted in the table on page 84 that this organism resembles *Streptococcus pyogenes* quite closely In morphology and in its growth characteristics on ordinary culture media the organism cannot be distinguished from it On blood agar, however, the hemolytic power of *Strep agalactiae* is much more limited, and variable The most actively hemolytic strains produce hemolytic zones not more than 1 mm broad Many strains produce only a suggestion of hemolysis on blood agar plates, and others produce none whatsoever. Some strains produce a suggestion of greenish discoloration without hemolysis on blood agar plates.

Growth in serum broth is granular or flocculent, the growth appearing in the bottoms of the tubes, the remainder of the broth remaining clear. Litmus milk, incubated at 37° C is acidified and coagulated within 48 hours. There is slight reduction of the litmus at the bottoms of the tubes. At 10° C. there is

no observable growth in five days Methylene blue milk is not reduced. In dextrose broth the final hydrogen ion concentration is from pH 4 4 to 4 7. Sodium hippurate is hydrolyzed. Dextrose, lactose, sucrose, and maltose are regularly fermented; salicin usually but not always Inulin, mannitol, and raffinose are never attacked. Aesculin is not broken down Gelatin is not hydrolyzed.

The description just given applies to *Strep agalactiae* in a narrower sense than Minett, Stableforth and Edwards (14) regard it These authors in England use the specific name to include three types, which they designate I, II, and III Most other authors now restrict the term to include their type I, but regard II and III as separate species, namely *Strep dysgalactiae* and *Strep. uberis,* respectively These will be discussed later *Strep agalactiae* belongs to Lancefield's Group B, and since there are no other known species in this group, this method of identification is the most accurate single method that we have. Many but not all strains of *Strep agalactiae* produce a brick reddish growth when growing on solid media, especially when the medium contains starch

Resistance. Resistance to heat, drying and chemicals is not great Most strains will withstand 50° C but not 60° C moist heat for 30 minutes The organism is readily destroyed by pasteurization

Pathogenicity. This organism is the cause of a large portion of all cases of chronic catarrhal mastitis in dairy cattle This disease is to be distinguished from acute mastitis in which the udder becomes greatly swollen, reddened and painful, the animal usually develops a fever, and may die. The disease caused by *Strep agalactiae* usually begins insidiously and gradually develops The milk, or udder secretion becomes altered in varying degrees, sometimes showing little or no abnormality and sometimes showing flakes, stringy masses of fibrin, blood, and thick purulent material Frequently the degree of alteration in the milk varies from time to time, being thick and purulent at one time and practically normal at another The inflammation in the udder causes the formation of new interstitial tissue and thus fibrosis changes the normal soft consistency of the gland to hardness, generally in the form of indurated masses which may not be seen but may be palpated

Normal milk has a pH slightly on the acid side of neutrality, whereas blood serum is slightly alkaline In inflamed udders the milk secretion is mixed with inflammatory exudate derived from the blood serum. The alkaline exudate causes the pH to shift to the alkaline side and this fact is the basis of the color tests for mastitis. Unfortunately it happens that in the early and late

stages of the lactation period, milk is more alkaline than normal, hence the color tests are not safely used as the sole criterion of the existence of mastitis.

As would be expected the number of leucocytes in milk coming from inflamed udders is much greater than the number found in normal milk The streptococci in most cases can be demonstrated in centrifuge sediment of the altered milk but if this is difficult, the number can be increased by incubating at 37° C overnight *The Hotis test* (8) for mastitis consists of drawing the suspected sample from the cow as nearly aseptically as possible and after adding enough brom-cresol purple to give it a distinct color, incubating it overnight at 37° C If mastitis streptococci are present the color of the dye is altered to yellow, and flakes and curdy masses appear along the sides of the tube The test is not wholly reliable but is a useful rough test which can be easily used in the field with little equipment

Transmission. *Streptococcus agalactiae* passes from infected cows to others on the hands of the milkers, or on the cups of the milking machines This is clearly shown by the fact that the disease does not ordinarily spread rapidly but gradually extends from one animal to another with relation to the milking sequence Practical dairymen long ago learned that it paid to place cows with abnormal udders at the end of the milking line in order that they might always be milked last If a milking machine is used, animals with abnormal udders are best milked last, and by hand Badly diseased animals not only are a menace to other animals in the herd but usually are unprofitable to the owner because of the reduced milk yield which the disease brings about It is best to dispose of such animals In high grade dairies it has been found profitable to have all milkers wash their hands with soap and water and to dry them on a sterile towel after milking one animal and before beginning with the next, as a means of reducing the chance of carrying the infection from one animal to another It appears to be helpful also, to dip the ends of the teats of all animals immediately after milking into a dilute solution of chlorine

Immunity. Vaccines of various kinds have been used for the treatment of bovine streptococcic mastitis The reports are somewhat conflicting but it may safely be said that such products have been of little service Recently it was hoped that sulfanilamide might prove to be a boon in treating this disease but the results have been disappointing Practically all who have tried this drug have found that a temporary reduction in the number of organisms secreted is brought about but the condition is not cured nor materially improved Fortunately it happens that the disease can rather readily be brought under control in any herd by hygienic means Also, it apparently is possible to eliminate the infection from infected udders by infusing them with dilute

solutions of some of the acridine dyes and with other substances (novoxyl) Persistent treatment with these substances will eliminate the disease from a majority of the animals treated

Considerable interest has been developed recently in the use of a new type of therapeutic compound for infections with Gram-positive organisms, particularly with hemolytic streptococci These had origin in the discovery by Dubos (6) of an aerobic spore-bearing bacillus in soil (*Bacillus brevis*) which produces substances highly lethal to Gram-positive organisms in general The name *tyrothricin* has been given to active extracts of this bacillus Tyrothricin consists of at least two active substances, *gramicidin*, which is most effective, and *tyrocidin* These substances are very effective, *in vitro*, as destroying and inhibiting agents, depending upon the concentration, of most Gram-positive organisms As effective *in vivo* agents their usefulness is diminished by their toxic effect on the animal

Little and coworkers (11) (12) (13) have used these agents for the treatment of streptococcic mastitis of cattle, apparently with good results Aqueous solutions proved too irritating to the udder tissues, but emulsions with mineral oil, in which the active agents are dissolved in the aqueous phase were tolerated well Little, Dubos and Hotchkiss (13) compared this treatment with other treatments involving udder infusion and found it to be superior to others

STREPTOCOCCUS DYSGALACTIAE

Synonym Type II of *Strep agalactiae* (Minett, Stableforth and Edwards)

This organism differs from *Strep agalactiae* only in minor cultural particulars, but the disease produced is quite different It belongs to Lancefield's Group C The chains usually are short or medium in length. The colonies are never hemolytic but show a distinct greenish discoloration Litmus milk is not always coagulated in 48 hours at 37° C Methylene blue milk, however, is regularly reduced The final pH in dextrose broth varies between 5 3 to 5 0 It never goes below 5 0 Sodium hippurate is not hydrolyzed This is perhaps the best single test for differentiating this species from *Strep agalactiae*

Pathogenesis. Unlike the organism of chronic mastitis, this species appears not to spread gradually and insidiously The infection is thus sporadic but apparently rather wide-spread The onset of the disease usually is acute, and the disease severe, but it often disappears after a time, frequently leaving a non-secreting mammary gland in its wake Unlike the *agalactiae* type of infection, the disease cannot be eradicated from herds by careful hygienic procedures The history of some of these cases indicates that the infection began in an injured teat.

STREPTOCOCCUS UBERIS

Synonym Type III of *Strep agalactiae* (Minett, Stableforth, and Edwards)

This organism differs from the previous one principally in the following details Non-hemolytic and non-green-producing on blood agar, inulin and salicin positive, hippurate positive The organism is less frequently encountered than the two previously described species, and produces, as a rule, acute cases which end in recovery after a short time

This species does not belong to either Group B or Group C of Lancefield Its classification is uncertain



Fig 7 *Streptococcus equi* Stained film from a 24 hour serum broth culture × 900

Streptococci of other types are sometimes encountered as causative agents in cases of bovine mastitis, although these are a small minority of the total number It already has been said that *Streptococcus pyogenes* sometimes infects an udder, producing there an acute mastitis which often is the source of severe and widespread outbreaks of scarlet fever and septic sore throat in people Occasionally organisms belonging to Lancefield's Group C, and classed among the "animal pyogenes" types, appear in milk These have a remarkable resemblance to the human type and before the Lancefield typing method was introduced such organism caused milk "scares" Miller and Hershman encountered a herd near Washington in which a serious outbreak of mastitis was caused by a hemolytic streptococcus belonging to Lancefield's Group G

STREPTOCOCCUS EQUI

This organism is the cause of a disease of young horses known as strangles, and it is found in a variety of suppurations in horses not clearly identified with strangles It is not found naturally in any other species of animal.

Morphology and Staining Reactions. *Strep. equi* occurs in exudates and in fluid cultures in the form of long chains, exceptionally in short chains Sometimes the chains are surrounded by definite capsular material The organism is readily stained with the usual dyes and is Gram-positive when cultures are young. Old cultures retain the Gram-stain poorly

Cultural Features. This organism has a strong hemolytic toxin which causes it to show wide zones of Beta type hemolysis around colonies on blood plates, and to hemolyze blood cells suspended in broth cultures. Acid is formed from dextrose, sucrose, maltose, and galactose. It does not ferment lactose, nor will it acidify milk, and sorbitol is not fermented In these respects this organism differs from *Strep pyogenes* on the one hand, and from the "animal pyogenes" types on the other. Sodium hippurate is not hydrolyzed. It falls into Lancefield's Group C

Resistance. *Strep equi* is said to be somewhat more resistant than most streptococci Even though this may be, it is certain that it is rather easily destroyed by chemical disinfectants, by heat, and by drying

Pathogenicity. *Strep equi* does not affect any of the domesticated animals, except members of the horse family, and it is non-pathogenic for man When injected subcutaneously, recently isolated strains will kill white mice within a day or two with septicemia, and large doses may kill rabbits and guinea pigs Older strains may produce pyemia in mice with deaths after a week or more When injected subcutaneously into horses, abscesses usually are produced. When sprayed into the nasal cavity of young colts, the symptom-complex of the natural disease, strangles, may be produced.

In the natural disease, young animals are the victims The disease begins with a respiratory infection followed by swelling and abscessation of the lymph glands of the head and neck Sometimes the infection spreads through the lymphatics to the forelegs and trunk, causing multiple abscesses Occasionally abscessation of some of the internal lymph glands occur, in which case the animal usually dies

Immunity. Unlike most streptococcus infections, one attack of strangles usually leaves the animal permanently immune This suggests that the streptococcus may not be the sole cause, and perhaps not the primary cause of this disease

Immunization against strangles with bacterins and vaccines has been attempted with rather dubious results Anti-streptococcus serum, however, often appears to lessen the severity of infections when administered early

OTHER HEMOLYTIC STREPTOCOCCI OF ANIMALS

Dimock and Edwards (4) studied a large series of hemolytic streptococci of animal origin in 1933. Exclusive of *Strep equi,* ninety-six per cent of their strains were indistinguishable from each other. These strains originated in infections of horses, cows, chickens, foxes, rabbits, and guinea pigs These strains are remarkably like *Strep pyogenes* and undoubtedly have been mis-

taken for this organism in the past It was shown, however, that these organisms fermented sorbitol and failed to ferment trehalose, whereas the *Strep pyogenes* exactly reversed this situation Later it was learned that these strains fell into Lancefield's Group C Sherman first applied the name "animal pyogenes type" to them Apparently these organisms are harmless to man How dangerous they are to animals is not fully known One organism belonging to this group is that which is known as *Strep genitalium*, an organism which causes metritis and cervicitis in breeding mares, the infection causing many mares to become barren, and others to abort Another is an organism which sometimes causes acute, fatal septicemia in chickens and which is known as *Strep gallinarum* The disease is known as apoplectiform septicemia This organism has not been studied sufficiently by modern methods to make sure that it is a definite species Hemolytic streptococci are often found in virus infections of the upper respiratory tract of chicks and adult birds These presumably belong to the "animal pyogenes" type although Edwards studied one such organism which conformed to the human rather than the animal type

The Non-Hemolytic Streptococci of Animals

A great many organisms of rather diverse characteristics are included under this heading About the only things that they have in common is a similarity in morphology, a low degree or an absence of pathogenic power, and an inability to produce a soluble hemolysin It already has been pointed out that many members of this group are capable of causing a slight hemolysis on blood plates because of acid production Some of them (Viridans group) induce chemical changes in blood cells which cause them to take on a greenish discoloration In cooked blood mediums, this phenomenon is usually more marked than in those in which fresh blood is used, and, in many instances, the phenomenon is seen only when the blood has been cooked Lancefield was unable to find, among the non-hemolytic streptococci, any antigenic fractions by which they might be grouped, and they do not precipitate with any of the sera used for grouping the hemolytic types

Organisms belonging in the non-hemolytic group are found on many normal mucous membranes, on the skin, in many animal products, and occasionally elsewhere. They frequently are found in inflammatory conditions in all species of animals, including man Most of these processes cannot be initiated by inoculation with pure cultures It is assumed, therefore, that in most instances these organisms are playing a secondary role It cannot be doubted, however, that in some instances they are primarily responsible for the disease

STREPTOCOCCUS SALIVARIUS

This organism, of which there are numerous varieties, is found as a normal inhabitant of the human mouth It may also be found in human feces, probably as a survivor from the oral cavity Apparently, a large percentage of the viridans type of streptococci found in human infections belong to this group Many strains give a strong Alpha reaction on blood media, others are weak or give no reaction These organisms form a large amount of acid from dextrose, and acidulate and coagulate milk promptly Most strains ferment raffinose and some, inulin Esculin frequently is attacked *Streptococcus mitis* of many authors is closely related to, or a variety of, this organism.

STREPTOCOCCUS BOVIS

This organism is always present in the mouths and intestinal tracts of cattle and, because of fecal contamination, is usually present in milk, where it may be mistaken for the *Streptococcus agalactiae* So far as is known, this species has no pathogenicity for cattle An organism very closely related to the *Strep bovis* has been found in the intestinal tract of man, and some have thought that it played an etiological role in ulcerative colitis This species is able to grow at relatively high temperatures, and has unusual thermal resistance. Like *Strep salivarius,* it usually ferments raffinose and inulin Most strains actively hydrolyze starch, an unusual characteristic of streptococci It does not hydrolyze sodium hippurate, and does not attack esculin, characteristics which differentiate it from the streptococci of bovine mastitis.

STREPTOCOCCUS EQUINUS

This organism is always abundantly present in the feces of horses It was first isolated from air, undoubtedly because of the presence of dried horse manure, a situation which formerly was common enough in most cities A striking characteristic of this organism is its inability to ferment lactose. It will be remembered that this also is true of *Strep equi* It does not grow well in milk, and does not cause coagulation It does not grow at temperatures lower than 20° C *Streptococcus equinus* is not known to be pathogenic for animals.

STREPTOCOCCUS LACTIS

This organism has no pathogenic properties, but because of its omnipresence in milk and milk products, pathologists should be able to recognize it and differentiate it from other organisms which may be responsible for disease *Strep lactis* is the common milk-souring organism In sour milk it usually occurs in short chains, whereas, most pathogenic streptococci form long chains. This is a fact which is of considerable differential value, however it

is not always a safe rule to follow. In culture media, particularly those which contain serum, this organism often forms long chains. A characteristic of this organism which has long been recognized is its rapid growth in milk If litmus, or other reducible dyes are present, the dye is reduced before coagulation occurs The milk souring streptococcus grows at relatively low temperatures, and also at relatively high temperatures Reduction and coagulation of litmus milk will occur at temperatures as low as 10° C. Esculin is almost always attacked.

The normal habitat of *Strep lactis* long has been a mystery Many workers have shown that it is not found in milk drawn aseptically from the udder, neither is it found in the mouth or intestines of cattle Stark and Sherman recently have succeeded in isolating typical strains from certain plants It may be that it is from such sources that initial invasion of dairies occur Once established in a dairy or milk plant, it flourishes, and, being quite heat resistant, it maintains itself on the utensils and equipment

Streptococcus cremoris is a type closely related to *Strep lactis* It usually forms longer chains, produces less acid, and is less heat resistant than the usual milk-souring organism It is sometimes used in commercial "starters" either alone or with *Strep lactis* It has not been identified elsewhere than in milk and milk products

THE ENTEROCOCCI

The term enterococcus has long been used by French workers to designate a group of streptococci which normally occurs in the intestine of man The most important of these organisms is *Streptococcus fecalis* It is known that this organism occurs in the intestines of several of the domestic animals as well as man *Streptococcus liquefaciens* is a variant of *Strep fecalis* which liquefies gelatin, and *Streptococcus zymogenes* another which is hemolytic as well as gelatinolytic.

Enterococci occasionally have been found in pathogenic processes, but generally are looked upon as harmless to man and animals. They are characterized by great hardiness Sherman separates them from other streptococci on the basis of their ability to grow in the presence of high concentrations of salt (6 5% NaCl), and in very alkaline media (pH 9 6). They grow through a wide temperature range (10°–45° C) and thrive in the presence of 0 1% methylene blue They have been found on growing plants (Sherman).

IMMUNITY TO STREPTOCOCCUS INFECTIONS

It has long been known that, as a general rule, there is little immunity remaining after recovery from streptococcus infections Various workers have

found it difficult to immunize animals effectively with heat-killed cultures, likewise there was little encouragement in the earlier work in which culture filtrates were used In 1924, however, the Dicks demonstrated that the scarlet fever streptococcus produced a potent toxin which could be neutralized with specific antitoxin, and this finding has led to new interest in the subject. Antitoxic sera for use against those streptococci which produce soluble toxins (*Strep pyogenes* group) apparently have value when given to patients in very early stages of the infection, but they have not proved wholly satisfactory. Against the streptococci which do not form soluble toxins, immune sera are of very doubtful value. What value they may have probably is measured by their opsonic content

Heat-killed and living cultures of *Strep agalactiae* have been used both for prophylaxis and cure of bovine streptococcic mastitis There are conflicting reports on the value of these products, but it is quite certain that their value is very limited

CHEMOTHERAPY OF STREPTOCOCCUS INFECTIONS

For many years workers have sought drugs which, while highly toxic for bacterial and other infective agents, were so slightly toxic for tissues as to make it possible to inject them into tissues and thus to destroy the infections there Except for quinine in malaria, and salvarsan in syphilis there were only minor successes in this field until Domagk (5) discovered the value of a drug which he called *prontosil* for the treatment of streptococcus infections. Colebrook and Kenny (3) in 1936 published the results of the treatment of a large series of cases of human infections with streptococci of Lancefield's type A which clearly indicated the remarkable value of this compound The active agent in prontosil proved to be para-aminobenzene sulphonamide, a name which has been shortened to *sulfanilamide* Hundreds of papers have appeared in recent years on the value of this substance, and of related compounds such as sulfapyridine, sulfathiazole, sulfadiazine, sulfaguanidine, and others in treating almost every kind of infectious disease of man and animals. It is firmly established that sulfanilamide is highly successful in treating many human hemolytic streptococcus infections, and that sulfapyridine and sulfathiazole are lowering materially the death rate in human pneumonia caused by types of pneumococci but so far as many other infections are concerned, the value of these drugs is not clear. They have proved disappointing in the treatment of bovine mastitis

In the concentration used for therapy, sulfanilamide is not strongly toxic for cultures *in vitro*, hence there is doubt as to its mode of action Whereas most drugs are inhibited by action of the body fluids, in this case the drugs

seem actually to be more effective *in vivo* than in the test tube. The drugs should be used with some degree of caution for, because of the benzene ring in their structure, large doses will injure the leucocytes and the blood forming elements in the bone marrow, causing anemia and leucopenia Some individuals appear to be hypersensitive to sulfanilamide this being manifested by optic neuritis, scarlatinaform eruptions, and acute hemolytic anemia The results described have been seen in man Undoubtedly the same actions occur in animals, although perhaps not to the same degree.

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CHAPTER X

THE STAPHYLOCOCCI

One can find in many places in nature, in soil, water, air, and on the surfaces of plants and animals, a group of organisms known as the micrococci. Many of these organisms apparently live a purely saprophytic existence but some undoubtedly are capable of causing disease of man and animals The most frequent pus-forming organism of man belongs to this group Several of the earliest bacteriologists recognized it in suppurative processes Rosenbach (13) in 1884 clearly showed the relationship of this organism to pus-formation in man and gave it the name *Staphylococcus pyogenes aureus* This since has been shortened to the binomial, *Staphylococcus aureus* Another organism which is quite similar except that it lacks the orange pigment and is much less virulent is *Staph albus*

These organisms are not so commonly found in animal infections as in man, yet they are by no means rare. In the majority of suppurative processes in animals in which these organisms are found, furthermore, they are associated with other species and their specific role is not so clear as in man. Studies on staphylococci from suppurative processes in various animal species have shown that they behave in experimental animals like those from man and the same toxins are demonstrable, hence there is no reason to doubt that they are identical with those of man This is confirmed by occasional accidents in which men have been infected with pus from animals

Morphology and Staining Reactions. The pathogenic staphylococci appear as perfectly spherical organisms of uniform size (about 0 8 microns diameter). In pus the organisms frequently are grouped in irregular masses which remind one of a bunch of grapes It was this appearance which caused Ogsten (14) to adopt the generic name for the group (staphylo = (Gr.) bunch of grapes) In fluid media the organisms usually appear singly or in small groups They are non-motile, do not form spores and ordinarily do not possess capsular substance The ordinary stains are readily taken and young cultures always are Gram-positive Older cultures lose part of their Gram-retaining ability The acid-fast stain is not retained.

Cultural Features. The pathogenic staphylococci either are porcelain-white or yellowish-orange when growing on solid media The orange pigment is

best seen on media that are rather dry; on coagulated blood serum or on solid media that contain starch The orange-colored cultures are the most active biochemically, and their pathogenicity usually is greater Their cultural features show quantitative rather than qualitative differences, hence they will be described together.

Broth is uniformly and rather heavily clouded A moderate amount of rather viscid sediment forms in the bottom of the tube

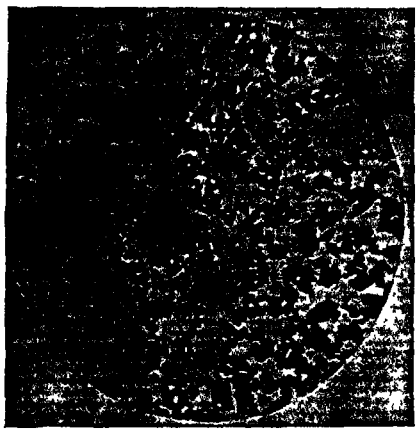


FIG 8 *Staphylococcus aureus* Stained film from a 24 hour culture on slant agar, showing typical arrangement in grape-like masses x 600

The growth on agar is quite profuse, semitransparent, moist, and glistening

Gelatin is rapidly liquefied by freshly isolated strains of *Staph aureus,* but the property is easily lost upon continued cultivation *Staph albus* usually will not liquefy gelatin even when freshly isolated

Some strains are strongly hemolytic, others have no action on blood This matter will be referred to later

Litmus milk is reddened but frequently not coagulated

Acid but no gas is formed from dextrose, lactose, sucrose, and mannitol by most cultures There is considerable variation in this group in fermenting ability, however

Unlike many of the saprophytic micrococci, the pyogenic types are unable to utilize ammonium salts as a sole source of nitrogen.

Nitrates usually are reduced

Resistance. Staphylococci are among the most resistant of non-spore-bearing organisms Most strains are capable of resisting dehydration for long periods, they are relatively heat resistant, and they tolerate the ordinary disinfectants better than the vegetative forms of most organisms

Staphylococcus Toxins. The pathogenic staphylococci, particularly those of the aureus variety, produce one or several toxins The hemolytic activity is due to a *hemotoxin,* a second, known as *leucocidin,* is destructive to leucocytes, and a third or *necrotizing* (dermonecrotoxin) toxin causes tissue destruction

around the point of injection in the skin. Some recognize a fourth poison, the so-called *lethal toxin,* which kills rabbits when injected intravenously, although this probably is the same as the necrotoxin A fifth poison, the *enterotoxin* or enteric toxin is produced by some strains. It is a toxin which is responsible for many cases of food poisoning of man, most of which appear in the form of outbreaks since usually more than one person eats of the poisonous food. In these instances the staphylococci have been given the opportunity to multiply in food materials, usually of a starchy nature, which have been prepared a considerable time before consumption and have not been properly refrigerated while being held See Dack, et al (2) (1930) and Jordan (6) (1930) This is believed to be the most common form of food poisoning in this country In the past the public usually has referred to these illnesses as "ptomaine poisoning "

Except for very young kittens which can be poisoned experimentally, the domestic animals seem not to be susceptible to the enterotoxin These organisms will form toxin when incubated in milk, hence it is possible that some food poisoning of man may be caused by the consumption of milk from cattle which are suffering from staphylococcic mastitis There are a few reports which suggest that this occasionally has happened [Minett (12)], [Crabtree and Litterer (1)]

It is quite certain that the various toxins of staphylococci are really only manifestations of a single toxin It frequently has been pointed out that the enterotoxin differs from the others in being quite heat resistant Singer (14) has found that all of the toxins of staphylococci are quite heat resistant and thus breaks down this difference We have not encountered any strains in a considerable series from both animals and man that exhibited the hemolytic toxin without at the same time also showing some evidence of the necrotoxin, and the lethal toxin Many strains from animals and man show some evidence of possessing a weak enterotoxin It is probable that all toxin-bearing staphylococci produce enterotoxin but only a comparatively few strains have sufficient potency to produce effective concentrations The available means for testing for enterotoxin are not reliable, hence this question cannot be definitely answered at the present time

In ordinary laboratory media, few strains of staphylococci produce potent toxins. The most successful media for developing the toxins consist of semisolid agar with a highly buffered base containing peptone. After such a medium has been incubated at 37° C. for several days in an atmosphere containing 80% carbon dioxide and 20% air or oxygen, the medium is squeezed through cheesecloth and the filtrate passed through a Berkefeld or other

bacteria-proof filter. The toxins deteriorate very quickly in an alkaline medium, hence the filtrates are acidified, if necessary, to keep the reaction at pH 6.5 or below.

The importance of the toxins of staphylococci in infections is not entirely clear. Strains from malignant suppurative process often show toxins of less potency than strains isolated from the skin and other sources where there is no evidence of pathogenicity.

Glenny and Stevens (5) have demonstrated that there are two antigenically different hemotoxins in staphylococci These were designated *alpha* and *beta*

In strains of human origin the A toxin was always present, in about 15% of human strains the B toxin was also present The B toxin was not present alone in any strain. Minett (11) working with staphylococci from animals, principally cattle suffering from mastitis, concluded that the B toxin was characteristic of animal strains The A toxin was present as well in most cultures but a few appeared to lack the A toxin completely Only five strains out of a series of 97 failed to produce detectable amounts of the B toxin

The A toxin, according to Glenny and Stevens, is characterized by its ability to hemolyze both sheep and rabbit erythrocytes, by its ability to produce necrosis in the skin of guinea pigs, and by its lethality to white mice when injected intraperitoneally The B toxin does not hemolyze rabbit cells, does not produce necrosis, and is not lethal to mice. The B toxin may show some hemolysis of sheep cells in the water bath but usually there is none When the tubes are cooled after incubation, however, hemolysis will occur (hot-cold hemolysis)

Duran-Reynals (3) has shown that in staphylococci (and in streptococci as well) there is a factor which increases tissue permeability This "spreading factor" probably is of great importance in the infective processes This factor may be related to the fibrinolytic action of these organisms

Pathogenicity for Experimental Animals. Minute doses of staphylococci from pathologic conditions administered intravenously to rabbits usually will cause death within 48 hours Cloudy swelling of the organs and bloody extravasations in the body cavities are found Smaller doses, or less virulent cultures will cause emaciation and death after some days or even one or two weeks In these cases multiple abscesses will be found in the kidneys, the myocardium, and sometimes in the lungs, bones, and joints In the acute cases the organism can readily be isolated from the blood, in the chronic cases from the localized lesions.

Intradermal or subcutaneous injections will lead to the formation of abscesses. Some of the earlier workers found that severe furunculosis could be produced by rubbing cultures into the human skin.

Guinea pigs and mice may be infected experimentally but these animals are more resistant than rabbits

Pathogenicity for the Domestic Animals

FOR THE HORSE Undoubtedly streptococci play a more important role in pyogenic infections of horses than the staphylococci The latter are found frequently in miscellaneous infections, however, often in association with other organisms. A pigmented staphylococcus very similar if not identical with *Staph aureus* is usually found in pure culture in the peculiar disease known as *botryomycosis* At one time this disease was thought to be caused by infection with an organism belonging to the higher fungi, but this idea has now been discarded Botryomycosis usually begins, after castration of male animals, in the stump of the spermatic cord The infected cord becomes greatly enlarged and sclerotic Small pockets of pus are found here and there in the mass of new-formed tissue, and in the pus small granules resembling those of actinomycosis are found When these granules are crushed they yield masses of staphylococci embedded in a capsular material probably furnished by the host Botryomycosis sometimes generalizes in which case there is usually a fatal ending [McFadyean (10)]

FOR CATTLE Staphylococci often are found in suppurative processes in cattle, frequently but not always in association with other organisms Pigmented, hemolytic staphylococci are occasionally found as the causative agent of mastitis Minett, Stableforth and Edwards (13) found that these cases frequently were very acute, the animals exhibiting marked toxic symptoms and a considerable proportion died within a few days from the disease Ferguson (4) studied eight such cases, six of which were critically ill and of these, three resulted in death of the cows

It is of interest to note that occasionally staphylococci induce in the bovine udder granulomatous lesions of a chronic nature which clinically are diagnosed as actinomycosis.

FOR OTHER ANIMALS Birds usually are regarded as quite resistant to staphylococcus infections, however, Madsen (8) reports serious losses in turkeys from a purulent synovitis caused by organisms of this group Earlier Jungherr and Plastridge (7) reported a similar disease in young cockerels Dogs and cats are fairly resistant, and swine are seldom affected

Magnusson (9) found that 41 per cent of the lesions in the udder of swine which were diagnosed clinically as actinomycosis were in reality caused by staphylococci.

Immunity. Repeated injections of heat-killed staphylococci will protect rab-

bits against otherwise fatal doses of *Staph. aureus* Bacterins, especially autogenous bacterins, frequently appear to be of value in combatting chronic infections in man They are sometimes used on animals although the results are not clear-cut Immune serum has been used with apparent favorable results in some cases, and with none in others Bacteriophage has been advocated for dealing with chronic infections but the evidence suggests that they are useless since the phage is rapidly inactivated when introduced parenterally Local dressings of phage, in which case inactivation by tissue fluids is retarded, may have some value.

Staphylococcus toxoid is easily made by treating filtrates with 0 3 per cent formalin for a few hours These preparations stimulate antitoxin formation very promptly and appear to have value in treating chronic infections In animals such infections are seldom treated with specific products, reliance being placed upon surgery and local treatment

Immunity in staphylococcus infections probably depends in part upon stimulation of phagocytosis (opsonic) and in part upon antitoxin In this connection it may be stated that the antigens of staphylococci appear to be identical since an antitoxin prepared with a single strain of staphylococcus will neutralize the toxins of all strains.

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CHAPTER XI

THE PYOGENIC DIPHTHEROID BACILLI

CORYNEBACTERIUM PYOGENES

This organism is the most frequent pus-forming organism in cattle, swine, and sheep It has been found in other animals occasionally but does not affect man.

Morphology and Staining Reactions. *C. pyogenes* most often occurs as small slender rods which frequently are slightly curved and often clubbed at one end The individual elements are usually short, and some strains form chains which are hard to distinguish from streptococci. There is great variation in morphology between different strains, and between individual cells of a single strain The organism is non-motile and never forms a capsule. It is Gram-positive.

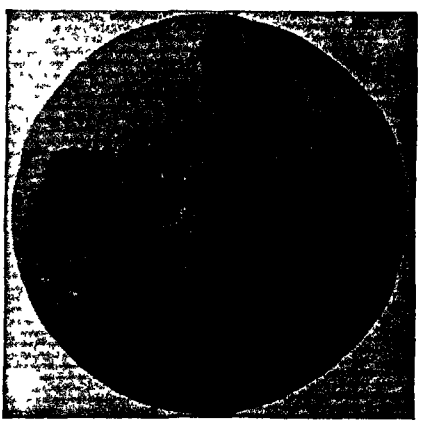


FIG 9 *Corynebacterium pyogenes* Film from vegetations on the heart valve of a calf x 900

Cultural Features. Ordinarily there is no growth on plain agar or in plain broth unless a considerable amount of blood, tissue, or tissue-debris is carried over in the inoculum It grows readily in milk which is coagulated within 48 hours The curd slowly dissolves although the medium remains acid. Growth occurs readily on coagulated serum slants. Along the line of growth a trough of liquefaction develops.

A few drops of blood or blood serum makes all of the usual laboratory media favorable for the growth of this organism In fluid media the growth is granular and sinks to the bottom of the tube In blood broth a layer of brownish-red pigmentation appears above the layer of sedimented blood cells.

This is due to the liberation of hemoglobin as a result of the hemolytic activity of the organism

On blood agar plates the organism behaves rather characteristically. The colonies always are very minute and translucent After 24 hours' incubation at 37° C. the colonies may easily be overlooked even when they are numerous for at this time they are exceedingly minute and there is little evidence of

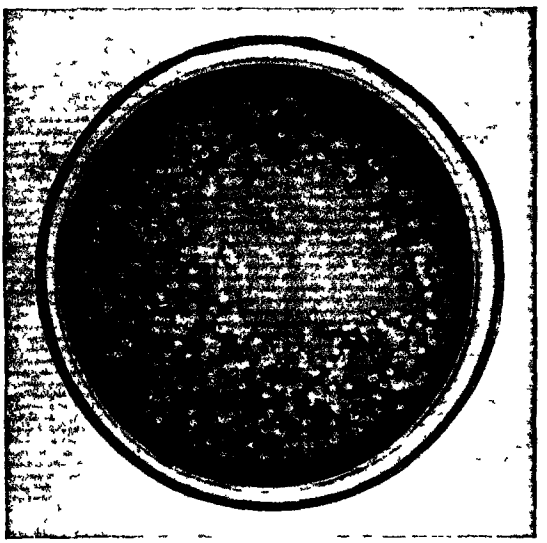


FIG 10 *Corynebacterium pyogenes* Blood agar plate incubated for 36 hours at 37° C The colonies may be discerned as minute points surrounded by narrow zones of Beta type hemolysis Blood plates incubated overnight may have numerous colonies on them but they are likely to be overlooked unless they are inspected with great care because the hemolytic zones at that time have not appeared At about 24 hours' incubation the hemolytic zones begin to appear but they are not well developed until about 36 hours Reduced about one third

hemolysis. During the second day's incubation the colonies become conspicuous because of the development of exceedingly clear zones of Beta type hemolysis These zones are very narrow, seldom if ever exceeding 2 mm in diameter

In suitable basic media dextrose, lactose, sucrose, maltose, galactose, and xylose are fermented, mannitol, raffinose, salicin, and inulin are not attacked Gas is never formed.

Growth occurs best at body temperature The minimum temperature of growth is about 24° C It is aerobic and facultative anaerobic. Indol, hydrogen

sulphide and nitrites are not produced Gelatin is liquefied. An excellent description of this organism is that of Brown and Orcutt, 1920 (1)

Resistance. *C pyogenes* is a very delicate organism It is easily destroyed by heat, drying, and by ordinary disinfectants

Pathogenicity for Experimental Animals. Guinea pigs, rats, and mice are quite resistant to infection Subcutaneous injections of cultures result in the formation of abscesses which usually develop slowly and become well encapsulated

Rabbits are most susceptible After intravenous injection no immediate effects are seen but after two or three weeks the animals begin to lose weight and may become lame or paralyzed Abscesses may develop in the kidneys, lymph nodes or in the muscular tissue but more often they are found in the bones or joints Paralysis usually is caused by the formation of an abscess in the vertebral column which brings pressure to bear on the spinal cord

Pathogenicity for Domesticated Animals

FOR CATTLE *C pyogenes* can be found in a great variety of suppurative conditions in cattle In looking for it, blood agar plates should be used and the incubation period should not be shorter than 36 hours at 37° C. When other organisms are present, as they often are, they frequently are well developed after 24 hours, whereas the minuteness of the colonies of *C. pyogenes* and the fact that they have not yet caused hemolysis will cause them to be overlooked A few hours later one often is astonished to find a plate culture filled with the characteristic colonies whose existence was not previously suspected

Ochi and Zaizen (6) claimed to have found *C pyogenes* on many of the mucous membranes of normal cattle, and of normal guinea pigs Later they concluded that these organisms were not identical with *C pyogenes* and gave them the name *C pseudo-pyogenes* From the descriptions given it would seem that the pseudo-organisms differed from the one found in purulent conditions principally in the fact that the former were less proteolytic and less hemolytic than the latter

Abscesses caused by *C pyogenes* usually develop slowly and often have heavy fibrotic capsules The pus may be thick, greenish-white, and non-odorous, or it may be thin and fetid Some lesions may develop as granulomatous tumors which resemble and often are diagnosed as actinomycotic. See Magnusson (4), Vawter (7), Davies (2)

This organism is nearly always found in necrotic and suppurating pneumonias in cattle, presumably as a secondary invader in most cases It often causes destructive arthritis in calves It causes sporadic cases of mastitis, some

of which are acute and may be fatal. On the other hand the non-functioning udders of cows and calves may be invaded, in which case chronic destructive inflammations are induced In these cases, which occur most often in calves which are kept together and which develop the habit of suckling one another, secondary arthritis may develop which leads to destruction of the animal. Umbilical infections in calves often occur.

FOR SWINE. The localizations and the character of the lesions in swine are much like those in cattle It is found very frequently in purulent pneumonia, in which case it is secondary to other agents. It is frequently found in arthritic joints.

FOR SHEEP AND GOATS Purulent pneumonia and joint infections of sheep frequently are caused by this organism The pneumonia is chronic, associated with much connective tissue formation and pleurisy with an ill-smelling pleural exudate. Abroad this condition is often called "pyobacillosis" See Jowett (3).

Immunity. There are no practicable methods of immunization against *C. pyogenes.*

Related Organisms. Whether the organism described by Ochi and Zaizen are really forms of *C pyogenes* living saprophytically cannot be decided upon the basis of present information Since this organism is practically ubiquitous around cattle, it seems almost certain that it must live upon normal mucous membranes Other types of diphtheroids frequently are found in cultures from skin and mucous membranes These usually lack the hemolytic and especially the proteolytic activity of *C pyogenes* and can easily be differentiated on these bases

Magnusson (5) in 1929 reported the existence of a pyemic disease in Swedish sheep which is very similar to that seen elsewhere, termed pyobacillosis and attributed to *C pyogenes.* According to this author, however, the causative organism is a Gram-negative organism which previously had been given the name *Bacterium purifaciens* by Christiannsen.

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CORYNEBACTERIUM RENALE

Synonyms: *Bacillus renalis bovis, Bacillus pyelonephritidis bovis*

This organism is found principally in cattle, but it also has been seen in horses and sheep, and there has been one case described in a dog (5) Female animals are affected much more commonly than males (2) It has been found only in the urinary tract where it produces a diphtheritic inflammation of the urinary bladder, of the ureters, of the kidney pelvis, and frequently of the kidney tissue itself The lesions are characteristic and are quite alike in the several species affected. Jones and Little (4) report finding the organism in the urino-genital tract of a considerable number of apparently normal calves



FIG 11 *Corynebacterium renale* Film from urine of a naturally infected cow × 900

Morphology and Staining Reactions. *C. renale* is a small diphtheroid bacillus Individual organisms do not vary greatly in morphology, all being rather short stumpy rods which usually are a little thicker at one end than at the other In exudates and in cultures the organisms are found in clumps varying from a few cells to many hundreds It is non-motile, non-spore-bearing, and non-encapsulated It is strongly Gram-positive, and stains readily with the usual stains. Bars and granules are sometimes seen when stained with methylene blue.

Cultural Features. Growth occurs in all of the ordinary laboratory media but it is greatly favored by a little blood or serum For isolation purposes blood plates are most convenient After twenty-four hours' incubation at 37° C colonies may be seen as minute opaque bodies. After forty-eight hours the colonies become larger than those of streptococci, but smaller than those of staphylococci. The older colonies are opaque, ivory-colored, and the margins are uneven. Their surfaces are quite dull in appearance There is no action upon the blood cells.

In broth and other liquid media there may be slight clouding but most of the growth appears in the form of a granular sediment. A quite character-

istic reaction is seen in litmus milk It begins with reduction of the litmus in the bottom of the tube. The casein coagulates beginning in the depths of the tube and progressing upward toward the surface The coagulum slowly digests. The litmus in the upper parts of the medium remains a deep blue.

Coagulated blood serum and gelatin are not liquefied.

Growth on potato usually is quite good It is dull and somewhat grayish at first; later, quite yellowish

Of the usual carbohydrates used for differential purposes, only dextrose is regularly fermented, occasionally levulose is fermented There is no gas In media devoid of dextrose considerable alkali is produced Much alkali is produced when the organism is grown in sterile bovine urine, and urea is broken down with alkali formation It is of interest to note that the urine of affected animals always is highly alkaline in reaction, in fact, it has been suggested that the alkali may possibly be responsible for the extreme irritation of the urinary bladder always seen in clinical cases

Resistance. No records of resistance tests have been found Laboratory strains die out quite easily however and this suggests that its resistance to physical and chemical agents probably is not great

Pathogenicity. *C renale* is non-pathogenic for the laboratory animals Inoculation experiments on cattle usually have failed, but Jones and Little (4) have reported success in reproducing the typical disease in cattle In spite of these results there is no doubt of the causative connection between this organism and the characteristic disease which causes serious losses in dairy cattle and sometimes other species

In natural infections the urinary bladder always is involved, one or both ureters in most cases, and usually also one or both kidneys Some authors have thought that the infection was of hematogenous origin but most now agree that the evidence is against this and in favor of an ascending infection Females are infected much more often than males probably because of the shortness of the female urethra The walls of affected bladders are thickened and the mucosa usually is covered with a mucoid secretion Petechiae and larger hemorrhages are present and areas of necrosis to which masses of fibrinous material adhere. The affected ureters become enormously distended and the mucosa usually is necrotic in patches or in its entirety Affected kidneys frequently are greatly enlarged and in extreme cases most of the kidney substance may be necrotic More often the kidney pelvis is found to be enlarged, the kidney papillae more or less necrotic, and abscesses are seen in the cortex The content of the pelvis consists of a grayish slimy exudate mixed with fibrin and necrotic material Calcareous material is practically always found in the

pelvic exudate and sometimes considerable sandy material is found in the bladder.

The necrotic material in the pelvis contains great masses of the characteristic diphtheroid bacilli, many of which lie in palisade fashion. Not infrequently streptococci and other organisms are present in the exudate The urine which is voided in small amounts at frequent intervals because of the bladder irritation, contains much albumin, leucocytes, fibrin and usually small, bright-red, blood clots which originate from the bladder wall. The specific organism is readily found in smears of the fragmentary material, and in centrifuge sediment

Related Organisms. Diagnosticians must be on their guard against confusing morphologically similar organisms with *C renale* It is well known that diphtheroid organisms are often present on skin and mucous membranes Jones and Little (4) in their study of the bladder and urethra of young calves, found five types of diphtheroids in apparently normal organs Most of these were found in the sheathes of bull calves Four of these species differed culturally and serologically from *C. renale,* the fifth apparently was identical with it

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CORYNEBACTERIUM PSEUDOTUBERCULOSIS

Synonyms The Preisz-Nocard Bacillus, *Corynebacterium ovis*

This organism is the cause of caseous lymphadenitis of sheep, sometimes called pseudo-tuberculosis, of ulcerative lymphangitis of horses, and of a form of suppurative lymphangitis in cattle

Morphology and Staining Reactions. This organism is a pleomorphic rod Frequently it is so short that it may be easily mistaken for a coccus In the caseous pus from lymph nodes it sometimes occurs as rod forms which resemble quite closely the organism of human diphtheria It forms no spores and is non-motile It retains the Gram stain but is not acid-fast

Cultural Features. The organism will grow on all the ordinary media, although not luxuriantly. The colonies on agar are quite characteristic. They

grow slowly and do not reach maximum size for several days when incubated at the optimum temperature (37° C.). When fully developed they have papilliform centers surrounded by concentric rings which parallel the irregular margin. The color is grayish or yellowish and the surfaces are dull and dry When touched with the needle they fragment easily

On blood agar plates the colonies are slightly hemolytic The organism grows well on Loefflers' blood serum but does not liquefy it The colonies on this medium are moist and orange-yellow in color Growth occurs in milk but there is little change in the appearance of the medium On broth a fragile pellicle is formed Dextrose and maltose are fermented without gas formation Lactose, saccharose, raffinose, dextrin, and inulin are not attacked

Toxic Products. According to Hall and Stone (3) this organism produces a soluble toxin which is similar to, but not so potent as, that of the human diphtheria bacillus The toxin is partially neutralized by diphtheria antitoxin

Pathogenicity. By inoculation *C pseudotuberculosis* is pathogenic for horses, cattle, sheep, goats, dogs, rabbits, and guinea pigs Fowls are refractory

In guinea pigs, intraperitoneal injection of large doses produces rapid intoxication and death from peritonitis Smaller doses, or less virulent strains, have a tendency to localize in the scrotal sac thus producing a condition indistinguishable from that caused by the glanders bacillus (the Strauss reaction) Since ulcerative lymphangitis of horses caused by this organism simulates glanders or farcy, this fact should be kept in mind when using guinea pig inoculation for the diagnosis of glanders

THE DISEASE IN HORSES Ulcerative lymphangitis resembles cutaneous glanders (farcy) quite closely Nodules form on the legs and these break down to form ulcers They are located around the fetlock, as a rule The ulcers heal more readily than those of glanders, the neighboring lymph nodes are not enlarged as they ordinarily are in glanders, and the mallein test is negative

THE DISEASE IN SHEEP Caseous lymphadenitis is a relatively common disease in old sheep in certain restricted areas in the Rocky Mountain area of the United States It is also seen occasionally on the Pacific Coast It occurs in many foreign countries

The disease affects the lymph nodes, especially those of the chest Eventually the infected glands become converted into large encapsulated abscesses In the younger lesions the pus is butyrous, in old ones it becomes dry and granular Calcification is not common The color of the pus is a greenish-yellow. It is non-odorous Frequently the abscess becomes several times as large as

the lymph node in which it originated Nodules frequently are found in the lungs They are not usually found in the liver, spleen, or kidneys

Old sheep, apparently quite normal, are often found at slaughter to be rather badly affected Eventually, if the animals are allowed to live long enough, they become emaciated and weak, and will die.

THE DISEASE IN CATTLE From the paucity of reports, it is probable that the disease does not often occur in cattle Kitt (4) found it in a case of bronchopneumonia in a cow It was reported by Hall and Stone (3) in a calf It is reported that a considerable number of cases of the so-called "skin-lesion tuberculosis" in Utah contain this organism (2) See "Acid-Fast Bacilli Associated with Ulcerative Lymphangitis in Cattle" on page 270

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CORYNEBACTERIUM EQUI

This organism was first described and named by Magnusson (6) in southern Sweden in 1923 as the causative agent of a purulent pneumonia in foals which frequently was associated with pyemia In 1936, Holth and Amundsen (4) described tuberculosis-like lesions in the cervical lymph nodes of swine which they attributed to a cocco-bacillus Bendixen and Jepsen (1) in 1938 first recognized the identity of the swine and the foal organisms, and because the organism occurred much more commonly in swine than in horses, Plum (7) proposed that the organism be rechristened *Corynebacterium Magnusson-Holth*. This suggestion is not likely to be accepted generally Dimock and Edwards (3) and others have found this organism in this country in foals, and Karlson, Moses and Feldman (5) have isolated it from many swine lymph nodes

Morphology and Staining Reactions. *C equi* is a rather large organism which shows considerable pleomorphism ranging from coccoid forms to bacillary forms On solid media the form usually is coccoid, in fluids it usually is bacillary. Sometimes short chains are found in fluid media Metachromatic

granules can usually be demonstrated, especially in cultures grown in milk.

The gross appearance of the growth of this organism suggests that it is a capsule former, and a number of the authors state that capsules are formed. Karlson, Moses and Feldman, however, were unable to convince themselves that capsules were present

This organism is Gram-positive and it stains readily with other dyes. A number of authors claim that acid-fast forms are demonstrable in old cultures but several other workers have been unable to confirm this Spores are not formed The fact that this organism is able to resist oxalic acid much better than most other non-acid fast and non-spore-bearing organisms is of interest

Cultural Features. Good growth occurs on all the ordinary media After two days' incubation, colonies on the surface of agar plates measure up to nearly a centimeter in diameter and are raised, moist, translucent, and regular in outline. At first they are white but a rose pink color soon appears Old cultures are distinctly pinkish especially those that are developing on potato There is a rather poor growth in milk without coagulation or other evidence of change in its chemical composition Carbohydrates are not fermented, blood cells are not attacked, and neither gelatin nor blood serum is digested Several serological types in this species can be demonstrated by agglutination tests By complement fixation, however, it can be demonstrated that there is a species specific antigen as well (2)

Pathogenicity. Magnusson found this organism in 23 of a series of 78 cases of suppurative pneumonia in foals The thoracic lymph nodes were enlarged and suppurated In some of the cases abscesses were found in the mesenteric lymph nodes, and in a few cases there were ulcers in the intestine In none of the cases were any lesions found in the lymph glands of the head, a feature by which this disease can be distinguished from strangles In only one case were there lesions in the lungs None of the cases showed lesions in the liver, spleen or kidneys

In swine the infection is one of the lymph nodes and particularly of those in the cervical region It was the belief of Holth and Amundsen (4), who first found the organism in swine that it caused a tubercle-like disease, and this view was taken also by other Scandinavian workers, notably Plum (7), who confirmed the Norwegian findings Karlson, Moses and Feldman (5), in the United States, do not accept this view since they were able to demonstrate the organism in approximately as many apparently normal as diseased lymph nodes They believe the acid-fast organisms which the European workers describe as acid-fast forms of *Corynebacterium equi* are, in reality, tubercle bacilli with which the organism is developing concurrently Karlson *et al* attempted to produce lymph nodes lesions by feeding large quantities of

cultures of *C. equi* but failed They believe, on the basis of their findings, that the organism is relatively if not wholly non-pathogenic, and that when gross lesions are evident it is because tubercle bacilli are present as well as *C. equi* The lymph nodes cultured by these workers contained other bacteria as a rule but *C equi* was readily isolated in pure culture because of the fact that it resisted treatment with oxalic acid which destroyed practically all other bacteria except tubercle bacilli Magnusson expressed the belief that this organism lives in the soil but he did not furnish any proof that this was the case.

IMMUNITY

Magnusson encountered difficulty in producing high titers in animals in which he attempted to induce immunity No practicable methods of protecting animals against this organism have been found.

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CHAPTER XII

THE BACILLUS OF GREEN PUS

PSEUDOMONAS PYOCYANEUS

Synonyms *Pseudomonas aeruginosa,* Bacillus of Green Pus.

This is an organism of comparatively low virulence found frequently in suppurative processes in cattle and swine It also is found occasionally in wound infections in man, where because of the bright green colored pus which stains the bandages, it received its common name.



FIG 12 *Pseudomonas pyocyaneus* From a culture on a slant agar incubated for 18 hours at 37° C x 900

This organism can easily be isolated from many bodies of water, particularly when they are polluted with organic material Apparently the organism is very common in nature, living generally as a saprophyte and only occasionally as a parasite

Morphology and Staining Reactions. *Ps pyocyaneus* is a straight slender rod of medium size It is so rapidly motile that young cultures can hardly be successfully watched under the microscope Motility is by means of polar flagella of which there may be only one, or several. Spores are not formed and there is no capsule The organism is Gram-negative and it stains readily with the ordinary dyes.

Cultural Characters. This organism is readily recognized as a rule because of the bright green pigment which it produces There are really two pigments One of these is yellowish green when fresh, and oxidizes to yellow. It is known as *fluorescein* This pigment is produced by a number of organisms other than *Ps pyocyaneus* which have their habitat in water The second pigment

is bluish-green which oxidizes to brown. It is known as *pyocyanin*, and this pigment is produced by the green pus organism alone One needs only to prove the existence of pyocyanin in a culture to know that *Ps pyocyaneus* is present. Both fluorescein and pyocyanin are water soluble, hence in culture the green pigment diffuses throughout the medium. This is best seen when the organism is growing on a solid medium Pyocyanin is soluble in chloroform to a greater degree than in water, whereas fluorescein is insoluble in chloroform If a few drops of chloroform are added to a culture and thoroughly shaken through it, the chloroform, when it settles out, will be colored a deep blue This is a rapid and reliable test for the presence of pyocyanin

Both of the pigments of this organism are products of oxidation and do not appear unless the organism is growing under aerobic conditions If the organism is cultivated under anaerobic conditions in a fluid medium, and then exposed to oxygen by shaking the culture or blowing air through it, the color less medium will assume the characteristic deep green color within a few seconds

Ps pyocyaneus may be cultivated upon the simplest of media The growth is smooth, shiny, moist, and spreading Colonies have thin irregular margins, and the translucent centers are cream colored although this often is disguised by the green pigment which stains the surroundings An opalescent sheen characteristically appears on the surface of growths on solid media, and in old cultures crystals usually can be seen as needle-like structures radiating into the solid medium beneath the surface colonies Of the common carbohydrate media, acid is produced only from dextrose, and even this is sometimes disguised by simultaneous production of alkali from nitrogenous bodies. Milk is rapidly digested If litmus is present, it becomes deep blue except near the surface where the blue of the litmus is mixed with the green pigment of the organism Gelatin and coagulated blood serum are rapidly liquefied Cultures have a sweetish odor reminiscent of beeswax Broth cultures are heavily clouded and have a thin iridescent, fragile pellicle

Ps. pyocyaneus produces a mild toxin against which it is possible to produce an antitoxin Rather large doses are necessary to kill experimental animals. It also produces a lipoidal substance which has a strongly antagonistic and destructive influence on many other micro-organisms This substance has been called *pyocyanase* It is heat stable.

Pathogenicity. This organism is frequently found in necrotic pneumonias of swine as a secondary invader, and it is frequent also in the intestinal lumen of cases of necrotic enteritis. It often is found in foul-smelling abscesses of the spleen and liver of both swine and cattle In traumatic pericarditis of

cattle, this organism is commonly found accompanying the foreign body. In most abscesses other bacteria usually are present and one is left uncertain as to the responsibility which each of the organisms present should bear. Poels has described a form of scours in calves in which the *Ps pyocyaneus* was thought to be the causative agent. It is found in almost pure culture in the watery stools Occasionally cases of septicemia are observed, the organism being found in pure culture in the blood and all organs shortly after death

Immunity. The organism is not regarded as sufficiently important to call for specific immune treatment, and no commercial products for such treatment are available.

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CHAPTER XIII

THE ANTHRAX BACILLUS

BACILLUS ANTHRACIS

Among pathogenic bacteria the *Bacillus anthracis* is unique in that it is the only aerobic sporulating species

The organism causes the disease known as anthrax (Ger milzbrand; Fr. charbon). The herbivorous animals, especially cattle and sheep, are affected by it Horses, deer, buffalo, and other wild herbivora, guinea pigs and mice are very susceptible to infection. Swine are not so susceptible and the disease frequently runs a chronic course Dogs, cats, rats and most birds are relatively insusceptible but can be infected artificially. Cold-blooded animals are not susceptible The disease frequently runs a fatal course in man but he is not so susceptible as the herbivorous animals



FIG 13 *Bacillus anthracis* Impression preparation from a colony on a gelatin plate The habit of the bacilli of forming long filamentous chains which lie parallel accounts for the "Medusa head" appearance of the colonies x 350

Morphology and Staining Reactions. The anthrax bacillus is a large, straight, square-ended rod usually about 1 micron in diameter and from 3 to 6 microns long In cultures it forms long chains which, unstained, appear as solid filaments since the square ends of the individual cells fit very closely together. In tissues long filaments are never seen Here the elements occur either individually or in short chains of two to five or six organisms In tissues the organism is regularly encapsulated, a single capsule enclosing as many organisms as remain in a chain. The capsules are well-marked and can be stained rather readily. Spores are formed in abundance when the organism is growing in the presence of air Because of lack of sufficient oxygen, spores are not formed in the blood and internal organs It is Gram-positive and stains easily with all the usual dyes. It is not acid-fast.

Cultural Features. The anthrax organism grows readily upon the simplest of organic infusions Best growth occurs on solid media exposed to the air. Anaerobically growth will appear but it is meager Even in a fluid medium in a tube with surface exposed to the air, in which the dissolved oxygen is short of saturation, the growth of the anthrax organism is not luxuriant.

On agar plates the anthrax organism develops into characteristic "ground-glass" surface colonies The margins of these colonies are irregular and resemble, under low magnification, locks of wavy hair It is for this reason that they are sometimes described as "Medusa-head" colonies, after the mythological maiden whose flowing locks were changed to serpents Both the ground-glass and the Medusa-head appearance are due to the fact that in such colonies the organism grows in the form of long filaments which lie in parallel wavy bundles like locks of a well-combed coiffure Deep colonies appear as small ragged, stringy colonies It happens that there are many aerobic spore-forming bacilli which form surface colonies resembling those of anthrax In differentiating the anthrax bacillus from most of these, the deep colonies will be found more useful than the more conspicuous surface ones Most of these organisms, other than the anthrax organism, either fail to develop in the depths of the agar medium, or if they do develop, the colonies are small and compact

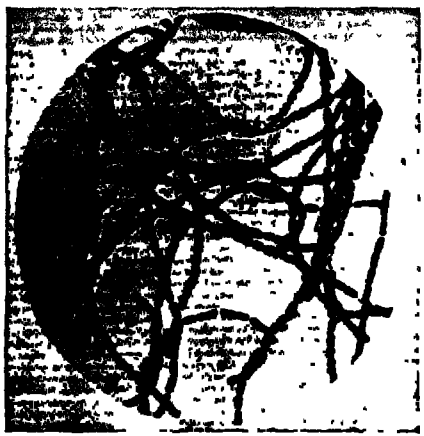


FIG 14 *Bacillus anthracis* Stained preparation from a 24 hour culture on solid media, showing arrangement of cells in long chains and the development of spores in many of the organisms x 900

On gelatin plates the characteristic Medusa-head colonies appear but the gelatin soon liquefies and this spoils further colony development Each colony sinks into a liquefied pool and further development is virtually in a liquid medium

Milk is rendered slightly acid and a soft curd usually is formed, but this quickly undergoes digestion by a rennet-like enzyme. In liquid media a fluffy growth usually begins near the surface but after a few hours of incubation this gravitates to the bottom of the tube. The fluid usually remains quite clear

except for this delicate filamentous mass at the bottom of the tube. Usually a dull whitish ring appears on the wall of the tube at the level of the fluid surface.

The anthrax bacillus grows readily upon the cut surface of many boiled vegetables, such as potatoes, beets and carrots, or on the pod of a string bean, or upon a cut surface of a banana On all of these media the growth is spreading, dull, dry, mealy, and grayish-white in color Sporulation occurs early and profusely on these substances

Acid but no gas is produced in dextrose, sucrose, maltose and salicin

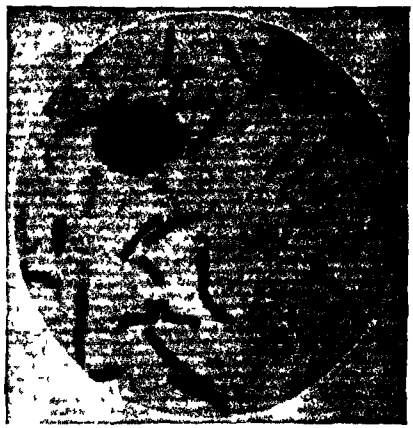


FIG 15 *Bacillus anthracis* Spleen, Bovine In tissues anthrax bacilli occur in short chains surrounded by a common capsule The capsular material shows indistinctly in the illustration It is responsible for the lack of sharpness of outline of the organisms x 900

Sources of Infection. The anthrax bacillus is not usually transmitted directly from one individual to another The organism swarms in the tissues of affected animals and is eliminated in the secretions and excretions a short time before death If animals dying of anthrax are autopsied, or if the carcasses are left to be devoured by birds or animals of prey, the organism may be widely scattered on the soil It is scattered further by floods The organism is able to maintain itself for long periods in soil Grasses growing in lowlands become infected, and the disease may be contracted in mid-winter by animals feeding upon hay grown on such lands Animals pastured on infected areas must be artificially immunized or heavy losses from the disease will occur In hot climates where flies are abundant, the disease is transmitted by the blood-sucking varieties, notably by the large horse-flies of the genus *Tabanus*

Anthrax infection often is carried long distances In the past tanneries often have been responsible for introducing anthrax into regions where it had not previously existed Dried hides from anthrax-infected animals from parts of South America, Manchuria, or the infected areas of North America, when soaked in the tannery leave anthrax spores in the soaking liquid which then is discharged into streams which, in turn, infect the low lying pastures in the

valley during flood periods Imported feeding stuffs, particularly protein concentrates, often are infected with anthrax spores The Department of Agriculture of Great Britain reported (3) that during the ten year period, 1919–1928, there were 5,512 reported outbreaks of anthrax in Great Britain, of which 4,451 were on premises where the disease had not previously occurred. In these instances 3,016 were attributed to the use of imported feeding stuffs, 191 to the use of animal fertilizers (principally ground bone) and only 70 to tannery effluents. The situation in Great Britain is quite different from that which prevails in this country inasmuch as we produce practically all of our animal feeds, and essentially all of it comes from anthrax-free districts whereas Great Britain is forced to import her food stuffs largely from South America where anthrax is much more prevalent than here

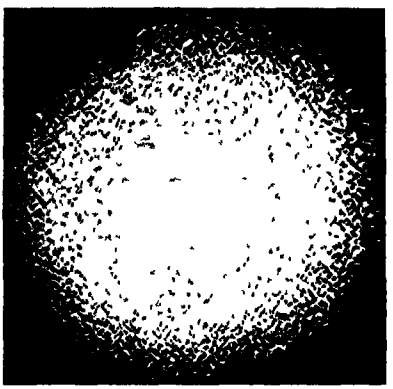


Fig 16 *Bacillus anthracis* Surface colony on agar photographed by transmitted light showing the "ground glass" appearance x 10

Infection may occur in three ways. (a) through the alimentary tract; (b) through the skin; (c) through inhalation It is much easier to infect animals through the injured skin and by way of the respiratory tract than by the alimentary tract nevertheless it is thought that the majority of natural infections occur through the ingestion of infected food materials.

Pathogenicity. The manifestations of the disease depend upon the manner of infection. When it occurs through the internal organs (no visible evidence of a localization) its principal character is the sudden onset and rapidly fatal course. The peracute form which sometimes occurs in herbivora may terminate fatally in one or two hours, the acute form usually terminates fatally in less than 24 hours The animals have a high fever and usually show bleeding from the body openings The organism can be found in the excretions or in the blood in large numbers at the time of death and this constitutes the simplest and most certain way of making an accurate diagnosis.

Localized anthrax is seen when infection has occurred through a wound in the skin This form occurs naturally more often in man than in any of the domestic animals Cutaneous anthrax takes the form of a swelling of an edematous nature which is hot and painful at first but later becomes cold and painless. In man this type of anthrax lesion is called *malignant carbuncle,*

since in its earlier stages it resembles a developing furuncle or carbuncle caused by *Staph aureus* Recoveries in both animals and man are more frequent when the disease is localized than when it is septicemic. Localized infections often become generalized, however

In swine anthrax has a tendency to assume a local form The infection presumably enters the tissues from the upper part of the digestive tract, probably through the tonsils, the disease being manifested by inflammatory edema of

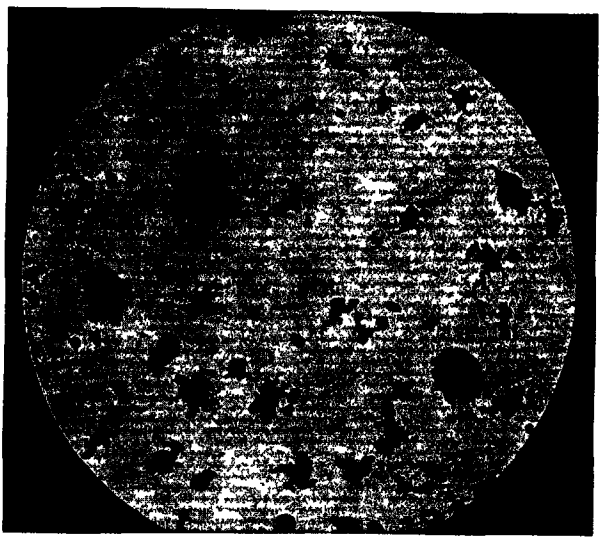


FIG 17 *Bacillus anthracis* Deep colonies in an agar plate Photograph taken by transmitted light with slight magnification The irregular colonies are characteristic Many common aerobic spore-bearing bacteria have surface colonies which resemble those of the anthrax organism but most of these produce small dense colonies in the depths of the medium

the glottis and of other tissues in the region of the throat and neck Sometimes it is localized in the intestinal wall and in the lymph glands of the mesentery, and sometimes in the spleen.

General anthrax may easily be induced in susceptible animals by the inhalation of spores. In man infections of this type commonly occur among employes of plants in which hides, wool, and hair are processed, the spores being thrown into the air from the infected materials handled. It is a rapidly fatal malady known as "wool-sorters' disease."

Resistance. In the growing or bacillary form, the anthrax bacillus is possessed of only slight resistance. It is killed by pasteurization, is easily destroyed

by ordinary disinfectants, and it quickly succumbs to the action of putrefactive bacteria.

The spores are readily formed providing the organism is under aerobic conditions In the organs of animals dead of anthrax the oxygen supply is insufficient for spore formation, consequently they are killed within a few days by the putrefactive processes. If the carcass is opened, the tissues are exposed to air, the organisms then are able to sporulate and their resistance is greatly enhanced The spores resist drying for long periods of time There are many records of spores having survived for ten or more years. Recently Umeno and Nobata (6) reported the finding of viable spores which developed into virulent cultures in the spring of 1938 from threads which had been impregnated with anthrax spores from a potato culture in 1879 Anthrax spores are not so resistant to heat as is commonly supposed, a ten minute exposure to boiling water being enough to destroy some strains

Bacteriological Diagnosis. Diagnosis of the disease by means of recognition of the organism or its products may be done in a number of ways The most important of these are

1. DIRECT MICROSCOPIC EXAMINATION OF TISSUES AND FLUIDS This method is simple and certain when the animal has just died and putrefaction has not set in The organism can be found in the blood stream or in smears from practically any organ when the disease has assumed the septicemic form In the local forms the local lesion must, of course, be examined. The organism is a relatively short, thick, Gram-positive rod, usually arranged in pairs or in chains of three, four, or five bacilli Spores are not seen With proper staining it may be observed that the chain of organisms is surrounded by a common capsule

If putrefaction has begun, it is not always an easy matter to make an accurate diagnosis by the direct smear method Many of the anaerobic bacteria of decomposition resemble the anthrax bacillus quite closely Usually these organisms are somewhat longer, and are arranged in chains longer than are formed by the anthrax bacillus in tissues When many extraneous bacteria are present it is better not to depend upon the direct microscopic method for making a diagnosis.

2. CULTURAL METHODS When the tissues and blood are fresh, there is no difficulty in cultivating the causative organism If the tissues are decomposing difficulties arise from two angles, first, the anthrax organism rapidly dies off and there may be few or no viable organisms remaining and, second, other organisms which resemble the anthrax organism very closely may be present.

The characteristic "ground-glass" type of colony is searched for on plate cul-

tures made from the organs or blood when the tissues are not absolutely fresh. Examination of the peripheries of these colonies under moderate magnification should show no motile organisms when the plates are from 18 to 24 hours old, and the other characteristics of anthrax colonies should be carefully looked for If the organism is in pure culture look for the deep colonies. If it is the anthrax organism these will be loose and ragged Most other organisms which are anthrax-like produce small compact colonies in the depths of the medium.

3. ANIMAL INOCULATION Guinea pig inoculation is usually relied upon to decide between anthrax, and anthrax-like, colonies Direct inoculation of guinea pigs with tissues, exudates, etc, is a reliable method of diagnosing the disease providing the material does not contain other organisms which destroy the experimental animal before the anthrax organism can be expected to make itself evident Organisms of the malignant edema group (anaerobes) frequently do this If viable anthrax organisms are present and if the animal doesn't die from other causes earlier, it usually will die from anthrax in from 36 to 48 hours after subcutaneous injection Occasionally death is as late as the fifth day after injection The tissues of the guinea pig will be found swarming with the organism and there will be found a gelatinous infiltration under the skin at the point of inoculation

4 SERO-DIAGNOSIS The thermo-precipitation test (Ascoli test) is used very successfully in Europe for the detection of anthrax-infected hides. It may also be used with other tissues

For the Ascoli test a precipitating serum of high titer is needed Very few animals produce a serum satisfactory for this work, hence it is rather expensive The bit of hide or other tissue is extracted with water, either by boiling or with the aid of chloroform In this way a clear fluid is obtained which should contain some of the anthrax proteins if the tissue has contained anthrax organisms This is known as precipitinogen This fluid is layered in a very narrow tube with some of the precipitating serum (precipitin) The formation of a whitish ring at the line of juncture of the two fluids constitutes a positive reaction

Immunity. The precise cause of death in anthrax is a debated question At one time the mechanical hypothesis, i. e, that the bacteria multiplied until the capillaries were choked, was seriously considered. This hypothesis has now been abandoned Toxic properties have not yet been demonstrated in cultures, either in filtrates or in the bacillary bodies, nevertheless the character of the lesions suggests that some toxic substance is produced *in vivo* if not *in vitro.*

Immunity to anthrax appears to be due largely to the production of opsonins. Resistance can easily be built up in susceptible animals by the injection

of living attenuated cultures Dead cultures and culture filtrates are generally considered useless for this purpose; however, formalin-killed cultures recently have been advocated for immunization It is difficult or impossible to produce absolute immunity to anthrax infection Vaccines will induce sufficient immunity to protect against natural infection, and thus are of practical value.

PASTEUR VACCINE The first vaccine for anthrax was made by Pasteur in 1879. Attempting to use the method which had succeeded in weakening the virulence of the fowl cholera organism, it was found that the method did not succeed with the anthrax organism because of its habit of forming spores, which then resisted attenuation Success was finally achieved when it was discovered that incubation at 42–43° C inhibited sporulation and attenuated the vegetative forms.

The vaccine strains are produced by cultivating the organism at 42° to 43° C continuously, with daily transfers to fresh medium At this temperature the virulence of the organism is gradually lost Two vaccines are used, the first consisting of a strain that has been cultivated longer at the abnormal incubation temperature, and consequently has less virulence than the second vaccine. The degree of immunity obtained appears to be directly related to the virulence of the vaccine strains used; consequently the first and weaker vaccine is to prepare the animal for the more virulent second.

The vaccine is a bouillon culture of the attenuated strain Vaccine No. 1 has been so attenuated that it will produce anthrax in mice and sometimes in young guinea pigs but not in older guinea pigs or rabbits Vaccine No 2 has been attenuated to the point where it will not ordinarily kill rabbits, but it should kill guinea pigs Vaccine No 2 is given about 10 to 12 days after No. 1, when it is used for protecting livestock

The vaccine prepared by Pasteur has been extremely successful in many parts of the world where anthrax is a menace In many areas the disease has been practically eliminated by systematic vaccination. Animals sometimes do not receive sufficient protection, and in some other cases the vaccine proves too powerful and animals die from the treatment *Owners of animals should always be warned that the anthrax vaccine is occasionally responsible for anthrax The vaccine should never be used except when a definite diagnosis of anthrax has been made on animals living on the premises.*

A definite disadvantage of this vaccine is that it does not keep well The organisms are supplied in the form of a bouillon culture to which preservatives cannot be added and in which sporulation does not occur readily; hence the organisms tend to die out rather rapidly. This disadvantage is overcome in the spore vaccine.

Neither the Pasteur nor the other vaccines for anthrax can be depended upon to give sufficient immunity to protect the animals for a period longer than one year In especially bad districts it is sometimes necessary to vaccinate more often than once a year, but usually if the animals are vaccinated early in the spring before being turned on pasture or range, sufficient protection is given

SPORE VACCINE In order to avoid the uncertainty occasioned by deterioration of the Pasteur vaccine, spore vaccines have now become popular. The anthrax cultures are attenuated as for the Pasteur vaccine The attenuated cultures are grown on a peptone-free agar for 4 to 7 days at 37° C at the end of which time the majority of the bacilli have sporulated The cultures are washed down with sterile physiological salt solution The resultant suspension is heated at 60° C for 30 minutes to destroy all vegetative forms The number of spores in the suspension is now determined by plating measured quantities The suspensions are now usually diluted until each cubic centimeter contains one million spores One cc of such a suspension constitutes a normal dose for cattle and horses, about one-fourth cc for sheep Each lot of spore vaccine should be tested for pathogenicity on guinea pigs and rabbits.

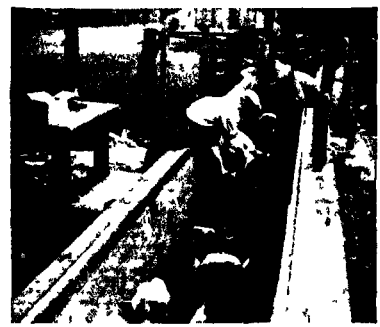


FIG 18 Vaccinating Range Cattle Against Anthrax The use of such chutes for restraining semi wild range cattle is common practice (Courtesy of the Jen-Sal Laboratories, Inc)

SPECIAL VACCINES. Besides the vaccines of standard degrees of virulence, as described under the heading of Pasteur vaccine, a number of vaccines of other grades of virulence are available and are used for special conditions. In some districts especially virulent strains of the anthrax organism abound and the ordinary vaccines will not fully protect A No. III and even a No IV vaccine, consisting of cultures less attenuated than the No II Pasteur, are used. Obviously the danger of vaccination troubles is greater when these more virulent vaccines are used; hence they should not be used unless it is known that the weaker vaccines will not protect

A special vaccine that apparently has advantages over the ordinary ones is that of Mazzucchi which has been named *Carbozoo*. This is a No 2 spore

vaccine suspended in a solution of saponin The saponin acts as a local irritant inducing a rapidly forming gelatinous infiltration at the point of injection which walls off the spores from the lymph vessels and delays their absorption It is claimed that this vaccine immunizes more solidly and more safely than the usual ones Fully virulent bacilli usually prove innocuous when introduced in this way, according to the proponents of the method Manufacturers of biologics have found that the immunizing value of many antigens is improved when they are incorporated in materials which act as local irritants, or which retard absorption in any way, and this principle undoubtedly is the one upon which the success of this special vaccine depends.

ANTHRAX BACTERIN It has long been supposed that killed anthrax bacilli had little, if any, immunizing value It has been learned in late years, however, that heavy suspensions of heat-killed vegetative forms do have some value, and such products have been marketed for practical use The immunizing value apparently is much less than that of the vaccines containing living bacilli, or living spores, and for this reason they have not become popular They have the advantage of safety, however, and thus may have a place in practical work especially with highly susceptible animals such as sheep

ANTI-ANTHRAX SERUM This may be produced from horses, cattle, or sheep Horses and sheep produce the more potent sera Horses are usually used because of the greater ease of immunization, i e, they are less likely to die of anthrax during the immunization procedure, and also because they will yield much more serum

Horses are given the simultaneous treatment for anthrax, then small quantities of virulent cultures Beginning with .005 loopful, the dose is gradually increased at intervals of 3 or 4 weeks, until finally whole agar-slant cultures and even mass cultures of young virulent cultures are used All injections are given subcutaneously since there seems to be no advantage in intravenous injections

SIMULTANEOUS TREATMENT Anti-anthrax serum is injected on one side of the neck, subcutaneously, and a spore vaccine on the other. The dose depends upon the potency of the serum Usually about 5 cc for large animals and 3 cc. for sheep is used

SERUM ALONE In treating herds in which anthrax infection already exists, the serum-alone method is used All animals not showing fever or other signs of infection are given a 50 cc dose Those showing a fever but no other symptoms are given from 100 to 300 cc Animals that are severely infected with

anthrax, if treated at all, are given larger doses. Frequently the administration of serum will markedly alleviate symptoms in a few minutes. On the second and third day after treatment, relapses may occur in which case another dose of serum should be administered Animals that are severely infected and even show bacilli in the blood may recover under serum treatment.

ANTHRAX AGGRESSIN Bail in 1904, showed that rabbits and sheep might be protected against otherwise fatal doses of anthrax bacilli by previously injecting them with filter-sterilized fluid from the edematous tissue of local anthrax lesions The immunizing substance in this material was termed "aggressin" because when mixed with virulent anthrax cultures it had the effect of increasing their virulence The nature of this substance is unknown but apparently it acts by inhibiting phagocytosis When injected alone an antagonistic substance, an "anti-aggressin," is supposed to be produced and to be responsible for the heightened resistance

Anthrax aggressin has been made on a commercial scale but it appears that the immunity produced is not always sufficiently strong to protect, and the cost of production is quite high For these reasons the product is seldom used at the present time

Comparative Efficiency of Anthrax Biologics. A study of the comparative value of the various products for producing immunity in previously unexposed animals was made a few years ago by the United States Department of Agriculture (2) The findings indicated that spore vaccines, particularly when they were injected intradermally rather than subcutaneously, are the most effective agents for producing active immunity to anthrax Well-marked immunity was also obtained with anthrax bacterin (washed culture) and anthrax spore vaccine in saponin Aggressin was found to be inferior to the products named above, and bacterin made from whole culture (broth) had practically no value. Anti-anthrax serum immunized quickly and satisfactorily but the duration of the immunity naturally was short

Treatment of Anthrax in Man. Anthrax in man may assume any of three forms, viz (a) the skin or cutaneous type (malignant carbuncle), (b) the pulmonary form (wool-sorters' disease), or (c) the intestinal form The second and third forms of the disease are usually rapidly fatal and it is unlikely that any kind of treatment would be effective, although possibly serum may be of value if the diagnosis is made early The cutaneous form is the more frequent and it is less fatal Without treatment the mortality is about 50 per cent

The modern treatment of malignant carbuncle is to abstain from surgical

procedure and to depend largely upon the efficacy of anti-anthrax serum. For human use, Eichhorn, Berg and Kelser (1) prepared a concentrated serum by precipitating out the pseudo-globulin fraction by methods similar to those used in the manufacture of diphtheria antitoxin. A highly potent product was thus obtained since it appears that all of the immunizing material exists in this fraction. The use of this serum, and similar sera, has reduced the mortality of malignant carbuncle in man to a very low figure.

Local Immunity in Anthrax. Local immunity in anthrax has already been discussed on page 27 It remains here merely to call attention to the phenomenon Besredka in 1919 made the claim that the skin was the only organ susceptible to anthrax infection, and furthermore, that this organ was the only one capable of building up a protective mechanism, that the process could be developed without the formation of demonstrable antibodies in the body fluids, and that whatever antibodies were developed were of minor importance so far as protection of the host was concerned All of these facts have been contested and it is doubtful if they can be sustained Nevertheless, whatever the explanation may be, it appears fairly certain from the results of the work of a considerable number of European workers, that immunization to anthrax can be readily obtained, perhaps more easily than by subcutaneous injection, by injecting the ordinary vaccines intradermally Very small quantities of vaccine are used, but it is claimed that the protection given is more rapidly produced, and more certain than when the materials are injected in the ordinary way

Chemotherapy in Anthrax. There has been some interest of late in the treatment of anthrax in man by chemical means and apparently there is reason for some optimism in this direction Several cases have been reported which were successfully treated with neosalvarsan (neoarsphenamine) and others with sulfanilamide alone; others were treated with these drugs supplemented by anthrax serum. Lucchesi and Gildersleeve (4) report the treatment of 67 human cases without a fatality by various combinations of these methods It must be remembered, however, that man is not highly susceptible to anthrax and undoubtedly a considerable number of these cases would have recovered if they had been untreated There are no records of such treatment of domestic animals Mitchell, Walker and McKirchner (5) attempted to protect guinea pigs from anthrax with sulfanilamide but this species is very sensitive to the drug and the necessary blood level proved toxic to them The authors conclude, however, that sulfanilamide undoubtedly controlled the infection temporarily and that this gives hope that in species which can tolerate the drug better, it may be useful.

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CHAPTER XIV

THE BRUCELLA GROUP

BRUCELLA ABORTUS

Synonyms *Bacillus abortus, Bacterium abortum, Alcaligenes abortus,* Bang's bacillus

This organism is the causative agent of the majority of cases of infectious abortion of cattle, a disease now generally known under the name of Bang's disease to distinguish it from sporadic cases of infectious abortion caused by other organisms It also produces abortion in sheep and experimentally in guinea pigs and rabbits It has been found in the uterine content of an aborting mare and in an aborted human fetus, but apparently the organism does not play an important role in abortions in either of these species It has been found in chickens and in dogs but there is no evidence that the infections in these animals are either frequent or important It has also been found in hygroma of the knees of cattle, and in inflammations of the bursae located beneath the two attachments of the ligamentum nuchae in the horse which result in fistulous withers and "poll-evil," respectively

The organism produces a generalized disease when injected into guinea pigs, rabbits, mice, and rats, and there is clear evidence now at hand to indicate that a similar disease occurs in man through the drinking of infected milk, and from contact with the organism in discharges of infected cows The organism was first described by Bang (1) (Copenhagen) in 1897 It was first recognized in this country by McNeal and Kerr (6) in 1910

Morphology and Staining Reactions. *Brucella abortus* is a small, Gram-negative, non-spore-forming rod It is frequently so short as easily to be mistaken for a coccus In exudates it is frequently found in clumps but otherwise the characteristic arrangement is singly It sometimes grows intracellularly It stains with the ordinary stains but with some difficulty

Cultural Features. The organism was first cultivated by Bang and Stribolt in a mixture of agar and gelatin containing serum This mixture is not necessary, in fact the organism grows fairly well in ordinary infusion agar without enrichment. Dextrose agar and glycerin agar are used by some in preference to ordinary agar A little serum enhances the growth. Liver agar is regarded by some as an especially favorable medium

Using the Liborius method, Bang and Stribolt discovered, after several days' incubation, a zone of colonies about 5 mm below the surface of the medium. No growth occurred on the surface nor deeper in the medium. After several transfers the organism finally acquired the property of growing on the surface and thereafter grew readily with no special attention on ordinary agar slants.

For a long time it was considered that the organism required a concentration of oxygen below that of the atmosphere Being neither aerobic in the ordinary sense, nor anaerobic, it was classed as a micro-aerobic or micro-aerophilic organism In 1921, Huddleson (5) showed that it was not the oxygen of the atmosphere which affected the growth but rather the carbon dioxide tension If a closed vessel containing the cultures is partially exhausted of air, and CO_2 introduced to give a concentration of the gas amounting to about 10 per cent, luxuriant growth of the organism occurs irrespective of the amount of oxygen present, so long as the free oxygen is not completely eliminated

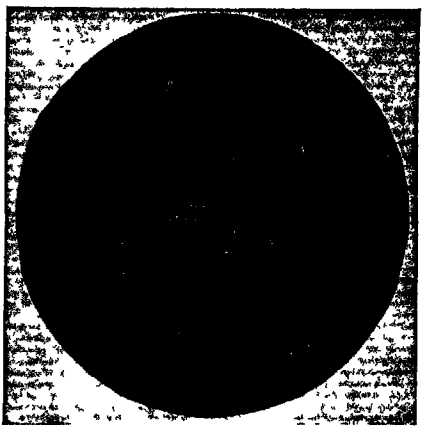


FIG 19 *Brucella abortus* From a culture on glycerin agar incubated for 18 hours at 37° C x 900

Prior to the time of Huddleson's discovery, it had been the practice in most laboratories to "lower the oxygen tension" in containers in which *Br abortus* was to be grown by connecting them with other containers in which *Bacillus subtilis* or other active aerobic bacteria were growing, a method introduced by Nowak in 1908 (7) Later it was discovered that a method introduced by Preisz in 1902 served practically as well This consisted merely in using containers in which there was relatively little air space and sealing them hermetically. In both instances the CO_2 content rises because of the respiratory activities of the bacteria and probably of viable tissue cells which usually are introduced simultaneously with the bacteria in the inoculum These methods are more haphazard than Huddleson's, and although they generally will succeed, the latter method is preferable

The organism grows rather scantily in fluid media, producing a faint clouding Carbohydrates are not fermented. Gelatin is not liquefied The organism

will multiply in milk but produces no visible changes in it. On blood agar there is no effect on the blood cells. Colonies on solid media, are smooth, shiny, and translucent.

Resistance. *Br abortus* is not very resistant to disinfectants nor to sunlight and drying Putrefaction destroys it rather quickly. When protected from complete drying it may retain its vitality for several months

Pathogenicity

FOR CATTLE The organism was first found by Bang in the utero-chorionic space of a cow in which abortion was impending A brownish, pasty, non-odorous exudate is to be found there The organisms usually are present in pure culture Many are enclosed in the protoplasm of epithelial cells Smith (9) has shown that these cells are derived from the outer of the fetal envelopes, the chorion. The chorion presents dull, later thickened, leather-like areas, due to the multiplication of this organism Generally this membrane is edematous.

It is apparent that the organism induces an inflammation of this membrane and that the circulation of the fetus is interfered with and this, no doubt, explains why the act of abortion occurs. The organism may also be found, generally in pure culture, in the alimentary tract and in the lungs of aborted fetuses. The other tissues of the fetus usually are sterile The location of the organism suggests that it had been taken into the fetus by the swallowing of the amniotic fluid rather than through the blood stream The fetus usually shows a dropsical condition, a fact which also points toward a circulatory interference. After calving or abortion the organism does not usually persist long in the uterus It may be recognized for a few days but later it seems to disappear It is thought by some that the fetal membranes represent the only medium on which the organism thrives; hence, when the membranes disappear, the organism disappears also

Besides the pregnant uterus, the organism is frequently recognized in another organ, the udder The lymph nodes adjacent to the udder and uterus are usually infected when these organs are infected Occasionally the organism may be found in some of the other organs of the body but lesions are not induced and it is probable that they harbor the bacilli only for a short time In bulls, infection of the epididymis and testicle sometimes occurs In these cases abscessation usually develops and the organs are destroyed.

A rather high percentage of infected animals develop infection of the udder, and it is in this organ that the disease maintains itself in the host from one gestation period to the next. The infected udders cannot be detected clinically but the organism may be isolated by inoculating the milk into guinea pigs. Experience has shown that non-pregnant animals which have high agglu-

tinin titers toward antigens made from *Br abortus* usually have one or more infected quarters in their udders Such animals usually are carriers for life. A few animals are able to throw off the infection Udder infection with members of the *Brucella* has considerable public health significance because of the organisms which are discharged in the milk

Br abortus may occasionally be isolated from the lymph nodes of the digestive tract and from the spleens of cattle No lesions may be recognized in these organs. It appears probable that the organism is located in these organs transiently It likewise may be isolated occasionally from the blood stream

When calves are fed upon infected milk the organism can be found in the lymph glands of the digestive canal but, within a few weeks after the infected milk is withdrawn, it generally disappears Until sexual maturity is reached, the genital organs seldom become infected Calfhood infection, therefore seldom results in permanent infection

Mode of Infection. The infection of cattle probably occurs most often through the ingestion of infected discharges of aborting animals, although it has been shown experimentally that cattle can easily be infected through the mucous membranes of the eye and this may be an important avenue of infection Another possible entry of infection which should not be overlooked is the skin, either through slight abrasions or through uninjured portions Hardy has shown that small animals may be infected through the uninjured skin

Infections of cows may occur by way of the genital tract either from the semen of infected bulls, or by contamination from sound bulls which recently have served infected cows Bulls, likewise, are probably infected by serving cows which are discharging bacilli in their genital secretions

The Disease of Guinea Pigs Caused by Inoculation with Brucella abortus. The most reliable method of detecting *Br abortus* in infected materials is by the inoculation of guinea pigs The character of the disease in this animal assumes importance because of this fact

The early workers claimed that the small laboratory animals could not be infected with *Br abortus.* This idea was corrected by Smith and Fabyan in 1912 (10) These workers found that a chronic disease, somewhat resembling tuberculosis, occurred as a result of injections of the organisms in pure culture, or when milk naturally infected with the organism was used for inoculation. This work also solved a problem which Smith had studied in 1893 in which tubercle-like lesions occurred in guinea pigs which had been injected with market milk but in which the tubercle organism could not be found A good description of the character of the lesions in guinea pigs is given by Fabyan (3) (1912)

The organisms are usually found in greatest numbers in the spleen of the infected animals This organ may remain normal in appearance and yet harbor numerous organisms, it may be nodular, or it may be enormously enlarged and engorged with blood As was shown by Smillie (8) (1918), the number of organisms in the spleen reaches its height in about three to four weeks after injection, irrespective of the number of organisms in the inoculum. The lesions at this time are not yet fully developed, the greatest development being reached only after six weeks to three months Organisms may be recovered from infected spleens months after inoculation although the number present may be rather small

The Diagnosis of Bang's Disease

1. CLINICAL MEANS The presence of an infectious abortion may be determined by simple observation Inasmuch as there are causes of abortion other than the *Br abortus*, it is not possible to be certain that this organism is the cause of the trouble

2. RECOGNITION OF THE BR ABORTUS

(a) *In the aborted fetus* Direct cultures, properly made, will usually demonstrate the *Br abortus* in the stomach content, the intestinal content, or in the lung tissue

(b) *In the placenta* Direct smears from the outer surface of the chorion, especially from the margins of the characteristic thickenings, will usually suffice to make a positive diagnosis without the necessity of recourse to cultural methods The organism occurs free, also enclosed in epithelial cells. It is these cells choked with minute organisms which can be recognized with certainty, even though many other bacteria may have invaded the placenta in the meantime For a description of these bacteria-choked cells see Smith (9) (1919) The character of the placental lesions is so clear as to be nearly pathognomonic without even applying a simple microscopic examination See Hagan (4) (1926)

(c) *In the uterine exudate* After abortion or calving, when the placenta has been infected, the *Br. abortus* is present in the lochia and may be recognized by guinea pig inoculation Within a few days, however, the organism seems to disappear, and usually cannot again be found in the uterus until the animal is again pregnant and reinfection of the organ occurs

(d) *In milk* When the udder is infected, the *Br abortus* can be readily detected by the intraperitoneal injection of milk into guinea pigs.

(c) *In abscesses* Direct cultures from abscesses of the testicle and epididymis usually give pure cultures of the organism.

3 SEROLOGICAL TESTS Both the agglutination and the complement-fixation tests are used successfully for diagnosis, however, since the accuracy of the agglutination test apparently is as great as the more complicated complement-fixation, it is favored by most laboratories



FIG 20 Localization of *Brucella abortus* in the Bovine Placenta Epithelial cells of the chorion, greatly swollen and packed with bacilli The nuclei are pyknotic After Theobald Smith (Courtesy of *The Journal of Experimental Medicine*)

(a) *Agglutination Test* The test is usually conducted with blood serum, but the plate test can be done with whole blood Milk can be used but it is better to curdle the sample with rennet and use the whey for the test.

Two methods of conducting the test are in common use The tube or "slow" method is regarded as the standard procedure, but the plate or "rapid" method is accepted by many states in disease-control work. In competent hands, the rapid test probably is as reliable as the standard method The plate test gives results sooner than the tube test, but so far as the operator's time is concerned it is doubtful if the rapid test is more economical.

The Tube Test. Blood samples are obtained by bleeding the animals from the jugular vein The blood is allowed to clot, and the serum to separate. The serum is mixed, in small test tubes, with a suspension of a specially selected strain of *Br. abortus* suspended in a carbolized salt solution. Increasing dilutions of serum are placed in successive tubes beginning with



FIG 21 Infection of the Bovine Placenta with *Brucella abortus* The surface shown is that of the chorion This membrane normally is thin and transparent Here it is transformed into a thick, opaque, yellowish white, leather like membrane covered with granular debris The fetal cotyledons are necrotic, this being evidenced by the fact that they are yellowish in color and filled with granular exudate In an earlier stage of its development, the lesions of the chorion consist of edema and dull granular thickening of the surface epithelium From these early lesions it is possible to demonstrate the bacilli choked cells shown in Fig 20 They cannot be found in such advanced lesions as the ones shown here

a dilution of 1 25, and doubling the dilution in each successive tube, viz.: 1 25, 1 50, 1 : 100, 1 . 200. Complete agglutination in dilutions of 1 100 and higher may be considered as positive, and lack of agglutination, or agglutination no higher than 1 25, as negative. Reactions in dilutions of 1 50 and no higher should be considered as suspicious, and judgment should

be based upon the history of the animal and of the herd in which it has lived, or another test should be done three weeks later in which case the status of the animal is usually cleared up Infected cows in the last several months of the gestation period often do not react. If cows in advanced stages of pregnancy are brought into uninfected herds it is advisable to segregate them until a second blood test is obtained about three weeks after calving or aborting

The Plate or Rapid Test The old slide test, used in many laboratories for identifying newly isolated cultures, has been adapted by Huddleson for

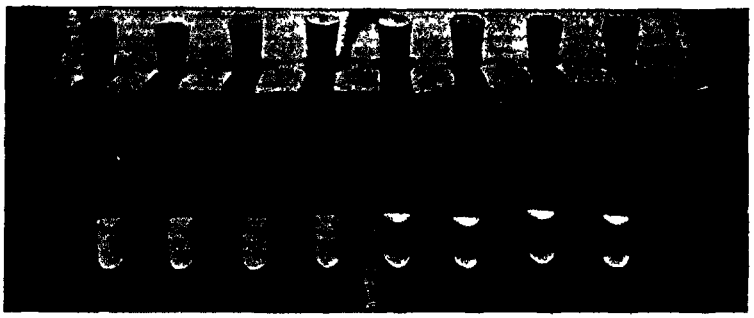


FIG. 22 The Macroscopic or Tube Agglutination Test for Bang's Disease Two tests are represented here The four tubes on the left contain serum dilutions of 1 25, 1 50, 1 100, and 1 200 respectively This serum is negative (devoid of agglutinins), indicated by the fact that the bacteria remain in suspension The tubes on the right contain the same dilutions of a strongly positive serum, indicated by the fact that the bacteria have flocculated and settled to the bottom leaving the fluid perfectly clear

dealing with Bang's disease The antigen is a very heavy suspension of specially selected strains of *Br. abortus* stained with gentian violet and brilliant green to make the tests more easily read The preparation of this antigen requires great care in order that it will have the proper degree of sensitiveness It is standardized so that it should give results comparable to those of the tube method

The test is done on a glass slide or plate Special apparatus is not necessary although a simple box fitted with a plate glass cover and containing a shielded electric lamp to supply illumination and warmth, is desirable The plate is marked off in squares for convenience. Using a 0 2 cc. pipette graduated to 01 cc. the following quantities of the undiluted serum under test is pipetted on the glass plate, each quantity into a different square 0 08 cc , 0.04 cc , 0 02 cc., 0 01 cc., 0 005 cc. Immediately afterwards one drop of the concentrated antigen is added to each lot of

serum, and mixed with it by means of a toothpick, using a new mixer for each sample and mixing the dilutions in turn beginning with the greatest dilutions to avoid carrying over enough serum to alter the concentrations appreciably. The reactions can be read immediately in many cases, but it is advisable not to make the final reading until at least 8 minutes have elapsed, since some samples agglutinate rather slowly If the antigen has been properly standardized, the dilutions used will give results comparable to those of 1 25, 1 50, 1 100, 1 200, and 1 400, respectively, in the standard tube test



FIG 23 The Rapid or Plate Agglutination Test This test may be conducted on an ordinary micro-slide or a piece of window glass If many are conducted it is convenient to use a special box, such as the one depicted here It is made of wood, painted black inside and out A portion of the top consists of a glass plate, marked into squares The serum and antigen dilutions are made on this plate A hinged cover can be closed over the tests to reduce the evaporation of fluid from the serum-antigen mixtures An electric lamp on one side of the interior of the box provides oblique illumination, which facilitates reading of the tests, and it also provides warmth which hastens the reactions

Apparently a fair degree of accuracy can be obtained by using whole blood in this test instead of serum The whole blood method has been used for testing range cattle, when it is desired to hold the animals in chutes until the results are known A drop of blood is collected on a glass slide from an incision in the end of the tail A drop of antigen is mixed with the blood and the results are obtained in a few minutes This method cannot be expected to have the accuracy of the serum test, because the presence of blood cells naturally interferes with the reading of the tests

(b) *The Complement-Fixation Test* The technique of this test differs in different laboratories When properly controlled, the test agrees in most instances with the agglutination test The discrepancies that occur usually are with sera of low titer Inasmuch as the agglutination test has proved to be wholly satisfactory, and since it is much simpler, in practical work it has replaced the complement-fixation test.

4. ALLERGIC TESTS. Many have attempted to diagnose *Br abortus* infection in man and animals by means of skin tests. Reference to these tests for man will be made later So far as animals are concerned, none have proved reliable. The test materials have consisted of suspensions of heat-killed bacilli, culture filtrates, and various extracts of the bacillary bodies The term *abortin* was applied to some of the earlier extracts made for this purpose; of late they have been known under the general name of *brucellin*. These extracts gen-

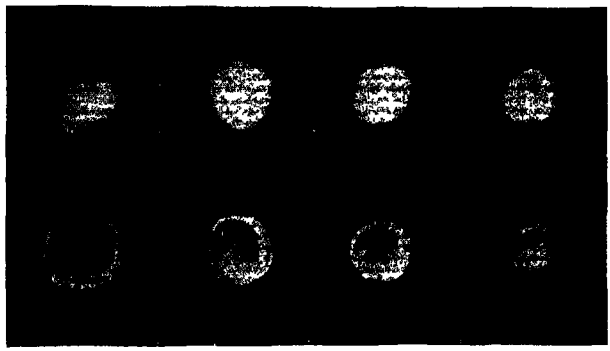


FIG 24 The Rapid or Plate Agglutination Test for Bang's Disease Two tests are depicted, four dilutions of serum being tested in each case These correspond to dilutions of 1 25, 1 50, 1 100, and 1 200, respectively, reading from left to right The sample above is negative, the one below is positive in all dilutions Slightly reduced

erally have been administered intradermally, in some cases they have been introduced into the conjunctival sac

Immunity

NATURAL IMMUNITY. Calves, infected *in utero* or from their surroundings after birth, remain infected, ordinarily, for only a short time unless they are raised on infected milk or kept in the presence of the infection Within several weeks from the time they are removed from the presence of the infection, they usually free themselves of it and develop into uninfected cattle It is not until the animal reaches puberty, becomes pregnant, and the udder begins to function, that danger of serious results appears

Adult animals which have never been in the presence of the infection are the most easily infected and the most likely to abort when infected An animal which has aborted once, or has been infected once as an adult, even though she may not have aborted, is not so readily infected a second time. A degree of immunity develops, therefore, as a result of an infection which has been overcome. This immunity frequently is not sufficient to prevent a second

abortion, or even a third or a fourth As a rule most animals, after one or two abortions, will thereafter carry their calves to full term, even though they may remain infected

There appears to be a considerable amount of variation in the resistance of individual cows to the infection. Some animals appear to be wholly resistant to natural and artificial infection even though their blood contains no antibodies, while others can be infected easily and repeatedly

ARTIFICIAL IMMUNITY

(a) The use of bacterin or killed cultures

Cultures of *Br abortus,* killed with heat or chemicals, have been extensively tried as a means of increasing the resistance of animals to Bang's disease. The method is not harmful but the immunity produced is not solid or lasting Usually the cultures are injected at intervals beginning before the animals are bred and continued throughout the gestation period The method has practically been abandoned at the present time.

(b) The use of living virulent cultures

On adult stock

Live cultures of *Br abortus* have long been used for artificially immunizing animals against Bang's disease Earlier fully virulent cultures often were used, these usually being administered sometime before the animals were bred, with the expectation that the organism would be eliminated before they became pregnant These vaccines often caused udder infections and permanent carriers, and it is claimed that breeding efficiency may be lowered by such treatment However, when the abortion rate in herds is high there is no doubt but that the rate is appreciably lowered It is doubtful whether this method of handling the disease is ever justified In any case, there certainly is no justification in using such vaccines unless it has been definitely proved that the herd is heavily infected with the disease Biologic manufacturers in the United States no longer are permitted to distribute virulent cultures for use as vaccines.

(c) The use of attenuated living cultures.

The fact that cattle could be immunized against the ravages of Bang's disease by the use of living cultures has prompted a number of workers in the past to seek non-virulent strains which might serve as immunizing agents while lacking the undesirable features of the virulent strains None of these seekers attained any considerable measure of success until Cotton and Buck (2) of the U S. Department of Agriculture introduced their Strain 19 This strain, which fails ordinarily to produce lesions in

guinea pigs, was tested on cattle and found to be non-pathogenic for this species also. Many other workers have studied it and it now seems quite clear that Strain 19 is indeed quite harmless to cattle and that it confers, for a few months at least, an appreciable degree of resistance to natural infection. This strain was furnished to commercial biologic producers and is the only strain of *Br. abortus* now permitted to be sent into interstate traffic in the United States for use as a vaccine for cattle

At present it is recommended that this vaccine be used only on calves between 4 and 8 months of age, but a great many adult cattle nevertheless are being treated with it When used on young calves the animals usually become blood reactors (i e, develop agglutinins) but the agglutinins usually disappear before the animals are of breeding age When the culture is used on older cattle, the agglutinins do not disappear so regularly and so soon. The fading of the blood reaction is accompanied by the disappearance of the vaccine culture from the animal's tissues It will be recalled that young calves are quite resistant to virulent cultures given in this way, hence it was no surprise that the vaccine strain was eliminated so readily Such animals unquestionably have much greater resistance to natural infection than unvaccinated animals, they do not often become carriers of the vaccine strain longer than a few months, and the vaccine strain apparently is not eliminated with the milk, and if it were, there is no evidence to indicate that it would be dangerous to man Vaccination does not give sufficient protection to wholly protect all animals Some vaccinated calves, living in an infected environment, develop into blood-reacting adults but most of these animals do not actually abort It is quite clear, in these cases, that the cause of the persistent blood reaction is not the vaccine strain but virulent strains from the environment against which the vaccine strain has failed to fully protect

Control Measures. Two methods of controlling the ravages of Bang's disease in herds of cattle are available One of these aims at eliminating the disease by repeated agglutination tests at short intervals and eliminating the reacting animals Usually several tests at 30 to 60 day intervals are necessary to do this, and the premises must be thoroughly disinfected on each occasion when reacting animals are removed After all reacting animals have been removed, the owner must be exceedingly vigilant to prevent re-entrance of the infection in his herd, especially with purchased animals

The second method of controlling Bang's disease in herds is by means of vaccination programs. By lessening the susceptibility of young stock by calfhood vaccination, the disease tends to diminish gradually It is doubtful whether vaccination will often succeed in eliminating the disease from herds,

but after it has reduced the incidence to a low figure, recourse can be had to the agglutination test to remove the few remaining infected animals.

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BRUCELLA SUIS

Synonyms Porcine type of *Brucella*

In the Report of the Chief of the Bureau of Animal Industry, U S Department of Agriculture, for 1914 (p 30) there is a reference to the isolation of *Brucella abortus* from a swine fetus The one who made the isolation and identification was not named Dr John R Mohler, the chief, later identified the individual as Dr. Jacob Traum

For some years it was thought that this organism was identical with the one found in cattle, although several observers noted that the organism of porcine origin appeared to be more virulent for guinea pigs than the one commonly found in cattle The lesions in this animal caused by the bovine strain are proliferative in character while those due to infection with the porcine organism are both proliferative and degenerative, i e, the swine organism commonly causes abscess formation while the bovine does not The bovine organism usually will not destroy the guinea pig, or only after a number of months have elapsed, whereas the porcine variety will frequently cause death within two or three weeks A common occurrence in guinea pigs inoculated with the porcine variety is the formation of abscesses behind the eyeball causing the eye to be protruded from the socket

It was noted by Traum, and the observation has been confirmed by all who have worked with the porcine type of *Brucella*, that the organism will grow readily in ordinary atmosphere from the first generation In other words, the peculiar CO_2 requirements of bovine strains are not shared by those from swine.

Morphology and Staining Reactions. These are identical with those already described for *Br abortus*. It is impossible to distinguish between the *Brucella* morphologically.

Pathogenicity. Brucellosis of swine occurs in various parts of the United States but it is not so widely distributed as the disease in cattle. There is very little of it in the eastern part of the United States The main centers in this country seem to be in the north central states (Iowa, Missouri and Illinois) (6), and in California (3) (4) The disease also occurs in several European countries but it does not appear to be very prevalent in any of them (7)

When porcine infections were first recognized and for a considerable time afterwards it was assumed that swine became infected through association with cattle, or by the drinking of infected cow's milk It is now known that *Br abortus*, the bovine type, has little or no virulence for swine and that *Br. suis* is transmitted almost exclusively from pig to pig (1) It is true, however, that *Br suis* sometimes infects cows and that it may be eliminated in the milk of these animals This creates the possibility that swine infection might be contracted from cow's milk, but it is not likely that this situation often exists.

In some herds of swine *Br suis* causes severe losses from abortions In others the disease is manifested principally by orchitis in the boars, the testes becoming greatly swollen and finally necrotic In other instances the disease is manifested by arthritis, in still others by necrosis of the bodies of the vertebrae (spondylitis) usually of the lumbar and sacral regions [Feldman and Olson (2)], a condition in which symptoms are usually absent and which is manifested only by positive agglutination tests Thomsen (7) claims that there are cases in which there are no symptoms or gross lesions In general the disease in swine presents a picture more like that seen in man and guinea pigs, the infection being generalized, rather than like that seen in cattle in which the infection is restricted to the genital organs (5) Laboratory methods of diagnosis are the same as those used when *Br abortus* is sought For the agglutination tests blood usually is obtained from an ear vein Vaccines have not been successfully employed.

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BRUCELLA MELITENSIS

Synonyms *Micrococcus melitensis, Bacterium melitensis,* Caprine type of *Brucella.*

This organism is a type of *Brucella* which has become adapted to living in goats. The infection was first found on the Island of Malta, later in many parts of southern Europe (south of the 46th meridian) The disease has obtained a slight foothold in the south-western part of the United States where apparently it was brought in with milking goats from Mexico An outbreak occurred in southern Texas in 1911, and another in Arizona in 1922 Attention was called to the disease in each case by the appearance of the infection in man

Morphology and Staining Reactions. *B. melitensis* characteristically grows in the form of a shorter rod than the other types The form may be described as coccoid For many years it was regarded as a micrococcus, and this explains in part why the relationship of this organism to the other *Brucella* was recognized so late Its staining characteristics are the same as those of the other *Brucella*

Pathogenicity. The disease in the goat appears to be quite like the corresponding infection in the cow Abortions sometimes occur and udder infections frequently occur, the animals then becoming milk-shedders of the organism In most instances the effect upon the goat herd is so slight that the disease is overlooked until human infections occur Diagnostic methods are the same as those used for the other *Brucella* infections.

DIFFERENTIATION OF THE BRUCELLA

In ordinary cultural characters and morphology, there are no features which will distinguish the three *Brucella* from each other Neither can one depend upon the host from which the organism has been isolated, for, like the tubercle bacilli, none of the organisms are confined to their native hosts, thus the porcine variety may be found in cattle, and all three may be found in man A number of workers have been studying methods of differentiating these varieties, and procedures are now available by which most strains can be identified with reasonable certainty Some of the more important of these are:

(A) AGGLUTININ-ABSORPTION. This procedure was suggested by Meyer and Shaw when straight agglutination tests were found to have little or no value. According to Huddleson, agglutinin-absorption will not distinguish between the porcine and bovine varieties, and the caprine cannot always be distinguished from the bovine. This method has limited value.

(B) HYDROGEN SULPHIDE FORMATION According to Huddleson (6), the porcine variety produces hydrogen sulphide continuously for at least four days of incubation, the bovine variety will produce it for only two days, and the caprine (melitensis) fails to produce a detectable amount. This test is difficult of control for variations in the amount of available sulphur compounds in the medium modifies the results obtained, and several workers have declared the method unreliable

(C) SUGAR UTILIZATION McAlpine and Slanetz (9) have shown that *Br. abortus* uses little or no dextrose when that sugar is present in the medium whereas *melitensis* and *suis* utilize from 4 to 18 per cent of the available supply (1%) Old strains are apt to lose the ability to utilize sugar

(D) DYE BACTERIOSTASIS Huddleson (5), in 1928, devised a system of differentiation by means of dyes which seems to have been rather generally accepted as being reliable The dyes are added to a liver infusion agar Basic fuchsin and thionin (certified dyes only) are used in final dilutions of 1 50,000, methyl violet in a dilution of 1 100,000 On these dye-media, the porcine variety is completely inhibited by the fuchsin and the methyl violet, but is not affected by the thionin On the thionin containing medium, the bovine organism is completely inhibited while the other two organisms are unaffected The caprine variety is not appreciably affected by any of these dyes The scheme of separation is thus as follows

TABLE VIII

GROWTH OF BRUCELLA TYPES IN DYE MEDIA

Strain	Methyl violet	Fuchsin	Thionin
Br abortus	+	+	−
Br suis	−	−	+
Br melitensis	+	+	+

+ = growth, − = no growth

(E) GASEOUS REQUIREMENTS Recently isolated strains of the bovine variety require an increased CO_2 tension for the initiation of growth Neither the porcine nor caprine varieties have this requirement

(F) VIRULENCE FOR GUINEA PIGS. The porcine variety is much more virulent for these animals than the other two types.

(6) VIRULENCE FOR MAN AND APES. For man and monkey the bovine type of *Brucella* is definitely less virulent than the other types The melitensis type has been looked upon as the most virulent for man, but it is doubtful whether its virulence is any greater than that of the porcine type Both are capable of producing severe human infections

UNDULANT FEVER OF MAN

For more than a century a disease of man has been known in the Mediterranean countries of Europe characterized by fever, chills, night sweats, and great weakness The causative agent of the disease was finally found in the blood of patients by David Bruce (1), a British military surgeon The organism which was isolated was named *Micrococcus melitensis* Since the work was done on the Island of Malta, the disease became known as Malta Fever.

The source of the infection was not discovered for nearly twenty years, although it was realized that the infection was not directly contagious from man to man A commission, headed by Bruce, in 1904 discovered that the blood of many of the milking goats on the Island of Malta contained agglutinins for the Malta Fever organism The organism was then sought and it was soon learned that infection was widespread in these goats, and that the organism was secreted in the milk The goats showed little evidence of the infection, but their milk was very dangerous for persons who had not become immunized to the infection by drinking it from early life The army and navy personnel, sent to the island from other parts of the world, suffered greatly from the disease, whereas the natives seldom were effected.

The organism of Malta Fever has been known since 1886, that of contagious abortion of cattle since 1897, and that of swine abortion since 1914 The relationship of swine abortion to that of cattle was seen from the beginning, but it was not until 1918 that it became known that these two organisms had any relationship to the one of Malta Fever The reasons for this are simple. In the first place Bruce described the Malta Fever organism as a coccus, and its relationship to caprine abortion was not appreciated In the second place, most of the active work on bovine abortion was done in parts of the world where the Malta Fever organism was not known, consequently there were few workers who had worked with both organisms

In 1918, Alice Evans (2) showed for the first time that the organisms were so alike as to be distinguishable only by serological means The work of Miss Evans was confirmed by Meyer and Shaw (1919), and the suggestion was made that the Malta Fever and the abortion-producing organisms be grouped together under the name *Brucella*, in honor of Bruce who discovered the first member This suggestion has met with general acceptance.

The discovery of the close relationship of the organisms of this new group immediately raised the question anew as to whether the abortion organisms might not at times be pathogenic for man In 1924, Keefer (8), in Maryland, reported a case of a Malta-fever-like disease occurring in a man who had not been exposed to Malta fever so far as could be determined Miss Evans determined the organism isolated from this man to be *Br abortus*, rather than *Br melitensis* Since that time several thousand cases of undulant fever in man have been found in the United States and elsewhere, due to infections derived from cattle and swine rather than from goats The disease apparently is widespread and has in the past been undiagnosed, or wrongly diagnosed as typhoid fever, para-typhoid fever, la grippe, etc

It is clear now from the work of many that the undulant fever complex may be induced in man by any of the three types of *Brucella* In the United States the caprine disease is rare in goats and in man Except for several very restricted areas in the southwest where the goat disease occurs, human infections are caused by the other two types In the eastern part of the United States, infections are almost exclusively from cattle, but in some of the midwestern states the majority of cases are of porcine origin.

The principal sources of human infections, so far as they are known, are as follows

(a) *Br melitensis* Most infections occur through the drinking of infected goat's milk, although it is likely that infections may occur by direct contact with other secretions of the infected goats

(b) *Br abortus* The fact that this organism has occurred for many years in nearly all market milk has caused many to be skeptical that human cases could be due to the drinking of such milk, the feeling being that more cases of the disease should have occurred, than have occurred Since the medical profession has become interested in this problem, however, it has become apparent that the disease is by no means as rare as was thought several years ago It is now apparent that many undiagnosed illnesses, many of slight consequence, are really mild cases of undulant fever It is probable that a large number of rural people have suffered, at some time in their lives, with undulant fever and have developed an immunity to the disease. It should be remembered also, that a large portion of the urban population of the country drink only milk which has been pasteurized and consequently are protected against this organism since it does not survive pasteurization It is probable also, that a great many people possess enough natural resistance to protect them against the relatively small dosage of organisms contained in the mixed milk of dairies

harboring the infection. Whatever the explanation may be for the apparent failure of all but a small percentage of the population to contract the infection by the consumption of infected raw milk, the fact is now clear that *many cases of undulant fever are due to the ingestion of infected raw cow's milk.*

Data collected in the United States and elsewhere agree that undulant fever may occur at all ages, but the greatest number of clinically recognized cases occur in the age groups between 20 and 45 years (3). Young infants, who consume far more milk, proportionally, than adults are seldom affected with a clinically recognized form of the disease There is also a marked difference in the distribution of the disease between the two sexes, males making up from 65% to more than 75% of the cases In spite of this fact there is no evidence to indicate that males are more susceptible than females. Considering only those cases which had had no direct contact with livestock, Hardy found that the distribution between men and women was even The explanation for the larger incidence of the infection in men lies in the fact that undulant fever may be contracted by direct contact with the secretions of animals following the act of abortion, or by contact with infected fetuses, or infected fetal membranes, and to these hazards men, naturally, are more exposed than women In Iowa, Hardy (3) found that nearly 90 per cent of the cases occurring on farms were in men In most cases it was not determined whether the infection came from cattle or from swine, and since most of the men probably had contact with infected animals of both of these species, undoubtedly many *suis* infections were in the group Veterinarians in practice in the country make up a group of persons who have especially frequent contact with the discharges of aborting animals It would be expected that this group would show a larger incidence of the disease than others Some cases have occurred in this group but in general it appears that the number of clinically recognized cases among veterinarians have been no greater than among others who have contact with farm animals Thomsen, in Denmark, and Huddleson (4) in America, have studied the blood of veterinarians in country practice for the presence of agglutinins specific for the *Brucella* group, and have found that a comparatively large percentage react positively although most of them have no record of having ever had the syndrome of undulant fever.

(c) *Br. suis* Epidemiological evidence indicates that infection of man from swine may occur either from contact with infected animals on the farm, or from contact in the slaughter house with the fresh tissues of infected animals. In Iowa, where the swine infection is common, Hardy has

shown that the slaughter house is the origin of many human infections. Infected cattle carcasses seldom cause infections Whether the difference is because of the greater virulence of the porcine type for man, or because a bacteremia frequently exists in slaughtered swine, while it does not occur so frequently in cattle, is an unanswered question. In all probability both factors have an influence.

Frequent infections of man, apparently contracted by association with animals rather than by the consumption of animal products, led Hardy to investigate the possibilities of infection occurring through the unbroken skin, or through slight wounds of the skin Using guinea pigs, he caused infection of 18 per cent by feeding, 80 per cent by applying the organisms to the skin which had had the hair clipped, 90 per cent by applying the organisms to the shaved skin, and 100 per cent by applying the organisms to the shaved and abraded skin Cotton and Buck have experimented with the possibility of causing infection of cattle by dropping suspensions of the organism in the eye and have shown that animals may easily and regularly be infected in this way They also produced infection regularly in cattle by dropping pure cultures on abraded areas of the skin, and in a number of cases by dropping cultures on normal skin, due precaution being taken in every case to make certain that the animals could not get the infection into their mouths by licking the treated areas Although the matter has not been tried on man, it is probable that infections can occur through skin wounds, or even through the intact skin It is quite certain that they may occur through intact mucous membranes

Diagnosis of Human Infections. The diagnosis of human infections often is exceedingly difficult because the symptoms frequently are not typical. The agglutination test may be used but it must be interpreted with caution, because infected individuals do not always react, especially early in the course of the disease, and agglutinins, when present, may be the result of a previously unrecognized infection

Blood cultures, when positive, are diagnostic, but the isolation of the organism from blood is usually difficult and sometimes impossible

Skin tests have value. Huddleson (7) reports successful application of such a test to man for the diagnosis of undulant fever He uses a nucleo-protein fraction of *Br abortus* He was unable to differentiate between active cases of the disease, and recovered or "sensitized" persons, hence the test alone has limited application. Those that do not react are considered as uninfected and probably susceptible to infection Reactors may be actively infected, or immune.

In differentiating between actively infected and immune persons, Huddleson (7) has found a determination of opsonic activity toward the causative organism of value The fresh blood of the patient is citrated (0.8 per cent), mixed with a heavy suspension of *Br abortus*, incubated for thirty minutes, and spreads made on slides These are stained and counts made of the numbers of bacteria ingested by the polymorphonuclear leucocytes. Normal opsonins are inhibited by the citrate so that the blood of individuals that have had no contact with the infections show practically no phagocytosis.

The test is conducted as an adjunct to the skin test. When the skin test is positive and the phagocytic test positive, the individual is considered to be immune or at any rate recovering from infection When the skin test is positive and the phagocytic test negative, the individual is considered to be suffering from an active infection.

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BRUCELLA BRONCHISEPTICA

There is considerable doubt as to the proper classification of this organism. In cultural features it has many characters in common with the *Brucella* already described (7), but the character of the disease produced is quite different. It is more than likely that when more is known about these organisms, *Br bronchiseptica* will be reclassified In placing it in the *Brucella* group, the lead of Topley and Wilson, and of Bergey, is being followed.

This organism was first described by Ferry (3) (4) in 1910. It was isolated from the upper respiratory tract of a dog which was suffering from distemper, and was believed to be the cause of that condition.

Morphology and Staining Reactions. *Br bronchiseptica* is a small, Gram-negative bacillus, not unlike the other *Brucella* in appearance It differs from the others, however, in being motile by means of peritrichic flagella.

Cultural Features. The principal features are quite like those of *Br. abortus* except that it does not have its CO_2 requirements, and it grows a little more rapidly on culture media Like the other *Brucella* it does not ferment any of the carbohydrates Characters in which it differs from other *Brucella* are the following·

(a) Litmus milk supports growth and the medium becomes progressively more alkaline.

(b) On potato the organism generally grows quite well producing a tan-colored growth which ages into a brown

(c) It is hemolytic for rabbit and guinea pig corpuscles.

(d) Freshly isolated cultures generally will produce a rapidly fatal disease in guinea pigs when injected intraperitoneally.

Pathogenicity. *Br bronchiseptica* is encountered frequently in broncho-pneumonias and other respiratory infections in rodents (rabbits, guinea pigs and rats) as well as in dogs, cats and occasionally men. In animal houses of research laboratories, epidemics of pneumonia caused by this organism frequently cause serious trouble (7) When guinea pigs are used in research work as a means of detecting *Brucella* of the abortion group, care must be taken not to confuse this organism, which may spontaneously appear, with the others. The cultural characters of *Br bronchiseptica* are sufficiently like those of *Br abortus* or *Br suis* to mislead even an experienced worker Since *Br bronchiseptica* in young cultures is actively motile whereas the others are non-motile, a simple hanging drop often will clear up doubts

Ferry (3), McGowan (6) and many others regarded this organism as the cause of the highly fatal and widespread disease of dogs which is known as canine distemper Carré (1), and a few others, contested this view claiming that distemper was caused by a virus and that the bacterial agent of Ferry was nothing more than a secondary invader The work of Laidlaw and Dunkin (5) finally settled the matter by showing that canine distemper is a virus disease, and that the disease occurs in the complete absence of the bacterial agent. These findings relegate this organism to a minor position so far as canine distemper is concerned but they do not prove that it is of no importance in canine pathology It has been clearly shown to be pathogenic, to be capable of setting up serious respiratory infection in the dog in the absence of virus (8), but its greatest role undoubtedly is in producing complications in the virus disease, in particular the broncho-pneumonia which so often is the immediate cause of death

Immunity. *Br bronchiseptica* cross agglutinates partially with the other *Brucella,* as was shown first by Evans (2) This should be remembered by the laboratory worker who runs blood tests on guinea pigs for detecting agglutinins for *Br abortus* or *Br. suis*

Prior to the time of Laidlaw and Dunkin's work, antiserum for *Br bronchiseptica* was available for use on dogs suffering from distemper Reports on these products are exceedingly conflicting It is apparent that such sera could not have had any effect upon the distemper virus, but it is probable that they may have favorably influenced the secondary infections. Suspensions of killed cultures of *Br bronchisepticus* are used by some veterinary practitioners in conjunction with other biological products for treating canine distemper and its complications. Their value has not been clearly established.

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CHAPTER XV

ORGANISMS, OTHER THAN BRUCELLA, ASSOCIATED WITH ABORTIONS IN ANIMALS

The great majority of all abortions in cattle are caused by infection with *Br abortus* but this organism has little importance in other species Sporadic abortions occur in all species In some instances the causative agents have been found and incriminated, in many instances they have not It is probable that sporadic abortions often are not caused by infections but by physiologic disturbances caused by nutritional disorders, glandular dysfunctions or other obscure causes

In addition to the *Brucella,* epizootic abortions sometimes are caused by *Vibrio fetus* in cattle and sheep, and by *Bacterium abortivo-equinus* and possibly by a filterable virus in horses Sporadic abortions have been credited to a considerable number of organisms, some of which will be described below

VIBRIO FETUS

In 1913, McFadyean and Stockman (2), in an extensive report on cattle abortion, referred to a form of abortion which had been discovered in English sheep, and which was shown to be caused by infection with a spirillum Several herds of cattle were found infected with the same organism In 1918, Theobald Smith (3) found the disease in this country in cattle and the following year Carpenter (1) found it in sheep It has since been seen by a number of workers in different parts of the country The disease does not seem to be highly prevalent, however, either in sheep or cattle

Morphology and Staining Reactions. In infected tissues, the organisms are seen as comma-shaped or S-shaped bodies, occasionally as longer spirals In young cultures the organisms are short, but in older cultures very long spirals are seen, many of them extending a considerable part of the way across the microscopic field Granules may often be demonstrated by Giemsa or other polychrome stains It is motile by means of a single polar flagellum It has no capsule and does not form spores It stains readily with ordinary dyes and is Gram-negative. A detailed description of this organism is given by Smith and Taylor (5).

Cultural Features. *Vibrio fetus* may be cultivated by any of the methods which succeed with *Br abortus*, although it is a much more delicate organism than the latter, and its growth in primary cultures is so meager that it may very easily be overlooked McFadyean first cultivated it in tall tubes of serum agar which were inoculated while liquefied. Growth appeared in a zone beneath the surface in the manner of *Br abortus* Like the *Brucella* the organism is strictly aerobic but will not grow in open tubes unless they are incubated in an atmosphere of increased CO_2 content Smith succeeded in obtaining cultures in tubes which were hermetically sealed with wax.

Primary cultures are most readily obtained on serum agar slants which are incubated in an atmosphere of 10 per cent CO_2 After several days' incubation at 37° C growth may be seen as exceedingly delicate, bluish, hazy films which push their way downward from the water of syneresis on each side between the layer of agar and the wall of the tube Slight clouding of the water of syneresis may be seen although this usually is hardly noticeable On primary cultures no growth is evident on the surface of the agar slant After a few transfers the strains usually grow a little better, causing opalescence of the water of syneresis and sometimes even producing a bluish film on the agar surface

In fluid media growth usually is very meager, even when the culture has become adapted to artificial cultivation In serum-broth a little opalescence may be produced Growth in milk occurs meagerly without changing its appearance Gelatin is not liquefied None of the sugars is fermented

Resistance. Cultures are exceedingly hard to maintain unless they are given constant care. Evidently the resistance of this organism is not great

Pathogenicity. *Vibrio fetus* is non-pathogenic for laboratory animals After intraperitoneal inoculation of guinea pigs, the organism often will survive for three or four days and may then be re-isolated from the spleen There are no lesions evident and later the organism disappears. This method has been used to isolate this organism when mixed with other bacteria, as, for example, when it occurs in a soiled placenta

The lesions in the fetal membranes of cattle are very similar to those produced by *Br abortus* infection It is assumed that the mechanics of their production are identical, i e., interference with the circulation of the chorion Definite lesions in the fetus are not seen but vibrios usually can be cultivated and sometimes detected microscopically in the stomach content and in the lungs because of the habit of the fetus of swallowing the amniotic fluid which contains organisms.

Immunity. Little is known about immunity in this disease. It has been noted in both cattle and sheep that the disease tends to die out Sometimes this happens abruptly, a single disastrous season being followed by no further evidence of the disease Whether or not this is a result of immunization is not known.

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BACTERIUM ABORTIVO EQUINUS

Infectious abortion in mares is a serious problem in some horse-breeding centers. As in cattle there appears to be more than one agent responsible for these happenings but the most common agent is an organism belonging to the *Salmonella* group and known as *Bacterium* (*Salmonella*) *abortivo-equinus* This organism is described on page 192.

Abortions occur in some horse-breeding establishments in Kentucky that are not associated with the organism just mentioned nor with any other organism, so far as has been determined Dimock and Edwards regard these abortions as caused by a filterable virus Anderson and Goodpasture have cultivated this virus by a special method. The disease and its causative agent are discussed on page 624

SPORADIC ABORTIONS IN CATTLE

In 1920 Theobald Smith (4) detected and isolated a mold (*Mucor spp.*) from a diseased chorion in a bovine uterus obtained from a slaughter-house. From the conditions present Smith concluded that abortion had been impending. The spores of the organism produced focal lesions in rabbits following intravenous inoculation Cattle were not inoculated In 1925, Gilman and Birch (1) reported the finding of a *Mucor* in several cases of abortion in cattle on a single farm. These animals were negative to the agglutination test for *Br. abortus* and other tests indicated that the Bang's bacillus had nothing to

do with these cases. The mold was isolated from the intestinal tract of the fetuses Pregnant cows, free from Bang's disease, which were inoculated intravenously with the mold culture, developed infection of the fetal membranes and one animal aborted as result of the disease.

It is assumed that the organisms found by Smith, and by Gilman and Birch were identical but the descriptions given do not permit one to judge, since neither studied their organisms in any detail Smith stated that the organism with which he worked resembled *Mucor rhizopodiformis* (Lichtheim) very closely. Gilman and Birch evidently believed that their organism was of the same species as Smith's.

Molds other than mucors have been isolated from the placentae of aborting cattle It is probable that some of these were the cause of sporadic abortions.

Reference is made on page 240 to abortions in cattle caused by avian tubercle bacilli (*Mycobacterium tuberculosis,* avian type). According to Plum (3) such abortions are not rare in Denmark but oddly enough no cases of this type of infection have ever been reported from the United States, in parts of which avian tuberculosis is quite common In these cases there is a chronic tuberculous metritis When such animals become pregnant, the infection extends into the fetal membranes producing lesions which grossly are not unlike those caused by the Bang bacillus Such cows tend to abort repeatedly, the infection carrying over in the uterine wall between gestation periods

Graham, Hester and Levine (2) recently isolated an organism belonging to the *Listeria* group from a premature bovine fetus This organism apparently is *Listeria monocytogenes,* which is most commonly found in cattle in infections of the nervous system (See page 206) With the culture these workers produced abortion in 10 days in a pregnant cow by intravenous inoculation, and the organism was re-isolated from the fetal membranes

Sporadic abortions, not caused by any of the agents which have been discussed above, occur in many herds of cattle The causes of these abortions remain obscure

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CHAPTER XVI

THE PASTEURELLA GROUP

In 1880 Pasteur (12) described the organism which causes cholera in fowls Later it was learned that the fowl cholera bacillus could not be differentiated culturally from the organisms of rabbit septicemia, of swine plague, and of hemorrhagic septicemia of cattle. The apparent identity of these organisms, and the similarity of the diseases produced by them in the various animal species led Hueppe (5) in 1886 to group them under one name, *Bact septicemiae hemorrhagicae* Trevisan (14), the following year, proposed that the several disease-producing agents be recognized as separate species but that they be grouped in a single genus *Pasteurella,* named in honor of Pasteur Lignieres (9), in 1901, applied the name *Pasteurelloses* to the group of diseases caused by these organisms, a name which has come into rather common use

In addition to the hemorrhagic septicemia organisms, several others have been added in more recent years These are similar culturally to the hemorrhagic septicemia organisms but the diseases produced are of a quite different type Two of these are the causative agents of diseases of rodents which are transmissible to man *Pasteurella pestis* is the cause of bubonic plague of man which has its natural reservoir in rats, and *Past tularense,* is the cause of tularemia which occurs principally in wild rabbits *Past pseudotuberculosis* causes a disease of guinea pigs and other rodents but is not pathogenic for man

The Hemorrhagic Septicemia Organisms

For many years it has been recognized that the cultural and biochemic features of the pasteurella organisms isolated from birds, cattle, swine, sheep, rabbits, reindeer, American bison, and other wild animals were essentially identical In spite of this, most authors have preferred to follow the lead of Flugge (1), who in 1886, proposed to set up a different species for each host affected by them Under this plan the following species became known:

Pasteurella aviseptica The cause of fowl cholera

Synonyms *Bacterium cholerae gallinarum, Bacillus avicida*

Pasteurella suiseptica The cause of swine plague.

Synonyms. *Bacillus suisepticus, Bacterium suicida*

Pasteurella lepiseptica. The cause of snuffles, pneumonia and septicemia in rabbits.

Synonyms *Bacterium cuniculicida,* Bacillus of rabbit septicemia.

Pasteurella boviseptica. The cause of hemorrhagic septicemia of cattle.

Synonyms *Bacillus bovisepticus, Bacterium bipolare multocidum,* Bacterium of *Wild- und Rinderseuche*

Pasteurella oviseptica. The cause of pneumonia and hemorrhagic septicemia in sheep

Synonym: *Bacillus ovisepticus*

In view of the fact that these organisms cannot be differentiated from each other on any basis now known, including serological tests, Rosenbusch and Merchant (13) recently have proposed again, as Hueppe and Kitt had done many years ago, that the hemorrhagic septicemia organisms of the various animals be recognized as a single species Since Kitt (8) was the first to make the suggestion, they propose that the name of the species be *Past multocida,* derived by eliminating the middle name of the trinomial *Bacterium bipolare multocidum* which was proposed by him. This seems logical and is being adopted here.

Many years ago, Moore (10) found members of the hemorrhagic septicemia group present on the mucous membranes of the respiratory tract of apparently normal cattle, sheep, swine, dogs, and cats These observations were confirmed by Jorgensen (7) in cattle One of the strains isolated by Jorgensen was tested on a cow after finding it of unusual virulence for rabbits It proved to be highly virulent for the animal, destroying it with typical pasteurella pneumonia in three days following spraying of the culture into the nostrils The majority of the strains isolated from mucous membranes of cattle were non-pathogenic, but this is not surprising since the majority of strains derived from acute infections of cattle show little virulence for rabbits and other experimental animals.

PASTEURELLA MULTOCIDA

As explained above this name is adopted for all of the hemorrhagic septicemia organisms, the strains found in chickens, cattle, swine, sheep and other animals being regarded as a single species

Morphology and Staining Reactions. These are very small ovoid rods measuring about 0.3 microns wide by 0.4 to 0 5 microns long. When seen in carefully stained films from tissue, the ends of the rods are more deeply stained than the central portion, giving to them a distinct bipolar appearance. This

characteristic is not so marked in bacilli from cultures, and in any case, it may be easily obscured by overstaining Wright's or Giemsa's stains are recommended for demonstrating it, although careful staining with methylene blue usually is satisfactory In fresh material, unstained, the bipolar appearance usually can be seen Pasteur referred to the fowl cholera bacillus as his "figure-of-eight bacillus."

The *Pasteurella* are Gram-negative and non-spore-forming. Many strains form capsular substance when freshly isolated but this property usually is quickly lost



FIG 25 *Pasteurella multocida* A stained film made from the spleen of a rabbit which had died as a result of inoculation with blood of a hen dead of fowl cholera The minute bacilli are characterized by their tendency to stain deeply at their ends and faintly in the middle portion, the so-called bipolar staining x 900

Cultural Features. Organisms of this group are easily cultivated on ordinary infusion agar although growth is never very luxuriant Media made from meat extract is not suitable unless enriched with a little blood or serum.

In infusion broth growth is manifested by slight clouding and a viscid sediment Growth in broth is greatly increased by the addition of a few drops of sterile serum

On agar the surface colonies usually appear as small, translucent "dew drops" although there are some differences in colonial appearance among these organisms depending upon whether the organism is in the "smooth" or the "rough" form The rough type colonies are more opaque and less glistening than the smooth Deep colonies appear as minute pin points. On blood agar plates the colonies develop a little larger than on plain agar The blood is not altered except that some strains may exhibit a slight greenish haze around the deep colonies. Gelatin is not liquefied. Milk supports growth but is only slightly changed; the litmus usually changes from a blue to a violet color Indol is usually produced. Dextrose and sucrose are fermented with acid but no gas production Lactose, maltose, and salicin are not attacked by most strains Mannitol is not attacked by some. Most strains are not soluble in ox-bile; a few are easily dissolved. Toxins are not formed.

Resistance. Cultures of the *Pasteurella* die out quickly and transplants must be made at least twice each month Cultures dried on cover-glasses in the air but protected from light usually die in less than 24 hours. Cultures also are easily and quickly killed with all ordinary disinfectants Some authors have thought that these organisms lived widely as saprophytes It is clear that many of them live on normal mucous membranes but it is doubtful if they thrive elsewhere.

Pathogenicity. Of the experimental animals, rabbits and mice are highly susceptible to inoculation Guinea pigs and rats are resistant The pathogenicity of different strains for these animals varies enormously Certain strains injected subcutaneously will kill rabbits and mice overnight In these animals the blood and tissues are teeming with bipolar bacilli Other strains, even when freshly isolated, will cause only an edematous swelling at the point of inoculation but after a few days the rabbit begins to emaciate and it dies after a week or more In these instances the pleural cavity is filled with fluid and fibrinous material and the lungs are pneumonic, the affected portions being very solid In still other cases the edematous area may disappear after a few days and the animal shows no further effect of the inoculation It has been our experience that strains from fowl cholera and swine plague usually are highly virulent, those from cattle and sheep are variable, a great many of them being practically avirulent for experimental animals (See discussion of *Past hemolytica* below).

The naturally occurring hemorrhagic septicemias seem to be diseases of devitalization Presumably under conditions of lowered vitality, organisms which already are being carried by the animals on their respiratory mucous membranes are unleashed and assume a pathogenicity which they formerly did not possess Once the disease begins in a herd or flock, it is likely to spread rapidly

Some of the well recognized predisposing factors in the several pasteurelloses are·

Fowl cholera Poor sanitation and ventilation of buildings, too much crowding of birds

Rabbit septicemia Poor sanitation and crowding

Swine Plague Usually accompanies hog cholera

Hemorrhagic septicemia of cattle and sheep Exposure to cold, wet weather, shipping and driving long distances

The naturally occurring diseases seldom spread to species of susceptible animals other than the one in which the infection started That is to say, an outbreak in chickens seldom spreads to cattle or to sheep, even though they may be in intimate contact with them, and the cattle disease will not spread

to sheep or birds If the strains of organisms concerned in outbreaks are isolated and injected into other species, however, often they prove capable of producing acute fatal septicemic diseases Thus certain cultures isolated from birds are capable of producing rapidly fatal septicemia when inoculated intravenously into horses, cattle, sheep, rabbits, and white mice. Gouchenour (2) isolated an organism from an outbreak of hemorrhagic septicemia in American bison in Yellowstone National Park which proved highly pathogenic for nearly all species of animals in which its virulence was tried

FOWL CHOLERA, the pasteurellosis of birds, affects chickens principally, although ducks, geese, and swans have also been affected. In many cases the disease is peracute In these instances the poultryman first becomes aware of trouble in his flock by the finding of many dead birds under the roost in the morning In most cases the bird lives for several days It is depressed, refuses food and develops a watery diarrhea In some cases the disease lasts much longer Hendrickson and Hilbert (3) were able to cultivate the *Pasteurella* organism from the blood stream of affected chickens in many cases several days before death, in several cases for as long as a week or more, and in one case for as long as 49 days On the duck "ranches" of Long Island, cholera often takes heavy toll of the ducklings which are raised in large numbers on small areas of ground under poor hygienic conditions

RABBIT SEPTICEMIA may be very acute with hardly any premonitory symptoms. The organism may be found in large numbers in the blood after death. The most common form of the disease is less acute, the disease taking the form of a fibrinous pneumonia. The lungs are very solid, in these cases, and the pleura is covered with heavy deposits of fibrin *Snuffles* is the common name applied to a milder infection of the upper air passages The external nares and the eyes are plastered with a muco-purulent exudate The animals have difficulty in breathing The noises made by a colony of affected animals are characteristic and the source of the name These cases frequently end in fibrinous pneumonia and death

SWINE PLAGUE usually consists of a fibrinous pneumonia accompanied in some cases by septicemia. The lungs present a characteristic appearance quite similar to those seen in the other hemorrhagic septicemias of mammals. The anterior and sometimes the diaphragmatic lobes are involved The lung surface is covered with a sero-fibrinous exudate and a turbid fluid usually is encountered in the pleural cavity The involved lung tissue is solid and mottled in color, some lobules being dark red in color and neighboring ones grayish. The interlobular connective tissue usually is filled with sero-fibrinous material and thus is quite conspicuous.

HEMORRHAGIC SEPTICEMIA OF CATTLE AND SHEEP usually takes the pectoral or pneumonic form and is similar to that described in swine A septicemic form has been described but apparently seldom occurs except when animals are inoculated with very virulent cultures such as that of Gouchenour which was derived from Yellowstone buffalo This strain when inoculated into animals produced a tremendous local reaction followed by septicemia manifested by multiple hemorrhages in all tissues, high fever, and early death.

It is not uncommon to find pure, or nearly pure, cultures of *Pasteurellae* in various pneumonic processes in calves Some of these are almost surely secondary to other organisms such as the *Actinomyces actinoides* of Theobald Smith, and many suspect that in other cases one or more viruses may be primarily concerned with the *Pasteurella* playing a secondary role Strength is given to this idea by the facts that the organisms present have little pathogenicity, as a rule, when injected into other calves, and that similar organisms are found on the mucous membranes of apparently normal calves.

PASTEURELLA HEMOLYTICA

Jones (6) in 1921 studied the organisms found in an outbreak of hemorrhagic septicemia in a large herd of cattle, together with a few cultures from cattle in other herds The strains in the large herd were collected over a period of several years, during which there had been a sharp outbreak of disease in adult animals and an outbreak among the calves The strains were found to fall into three groups which were designated I, II, and III.

Group III of Jones is only slightly different from Group II and one of the groups was regarded as a variant of the other These groups possess the characters which have been described under the name of *Past multocida* The organisms of Group I, however, possessed characters quite different from the others, and Jones expressed the belief that this group deserved a special species designation but did not offer a name for it Later, Newsom and Cross (11), who found this group to be important in sheep, suggested the name with which this section is headed.

Pasteurella hemolytica occurs in pneumonia in cattle and sheep producing a process which is not distinguishable from that caused by *Past multocida* In most cultural features it is not distinguishable from the latter The distinguishing feature which is responsible for its name is the fact that horse and cow blood cells are hemolyzed Around deep colonies on blood plates there are narrow zones of Beta type hemolysis This organism does not produce indol In addition to the sugars fermented by *Past multocida,* lactose and maltose are fermented by *Past hemolytica* This organism appears to possess low pathogenicity for laboratory animals.

IMMUNITY TO PASTEURELLA INFECTIONS

For immunization against the pasteurelloses a number of products have been used with success. The first of these was the vaccine of Pasteur for fowl cholera, of historical importance since it was the first bacterial vaccine used. His method of using a living attenuated culture now has been abandoned.

1. Bacterins. Cultures of the specific organism are killed with heat or chemicals. They are used on cattle and sheep some time before it is intended to ship them, since it is at the time of shipment that the great danger from the disease appears To be effective they should be administered from two to three weeks before the shipping date to allow time for antibodies to form Some have advocated the use of this product on herds in which the disease is raging, but this is theoretically wrong, and the reports of success are not convincing. There is a considerable difference of opinion, as a matter of fact, as to whether bacterins are useful in immunizing cattle which later are to be shipped There are many reports of failure of such methods

Outbreaks of cholera in chickens often can be stopped by a single injection of bacterin into all stock Bacterins have been used very successfully in controlling cholera in ducklings (4)

2. Aggressin. Weil produced aggressin by injecting rabbits intrapleurally with fowl cholera bacilli, collecting the exudate which formed in the cavity and sterilizing it with chemicals When injected into rabbits and hens it conferred a solid immunity against otherwise lethal doses of virulent culture given several weeks later In this country, Gouchenour (2) in 1924 produced an aggressin with a *Pasteurella* which he had isolated from an American bison and which proved to be unusually virulent for most of the domestic animals Calves were inoculated with pure cultures and the fluid in the large edematous area which formed subcutaneously at the injection point was used to make the aggressin For a time such aggressin was marketed by a number of commercial companies in the United States but its popularity was short lived and it is doubtful if it is now available

3. Immune Serum. Two kinds of immune serum are available for treating cattle The one which is known as *homologous* is made by immunizing cattle, the other is made from horses The homologous serum has the advantage that there is no danger of serious serum reactions which sometimes are seen when horse serum is given to cattle Otherwise, so far as is known, the one is as good as the other

Large doses of serum are useful in treating the disease in its early stages, and in smaller doses it will protect animals that are exposed When cases

appear in a herd it is advisable to take temperatures on all animals and administer serum to all which show abnormal elevations. Since the serum is relatively expensive this procedure is more economical than using serum on all animals not showing symptoms.

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Members of the Pasteurella Group not Associated with Hemorrhagic Septicemia

PASTEURELLA PESTIS

This organism is the cause of *plague* or *pest* of man. This disease in past ages spread time after time over Europe from its endemic centers in Asia, destroying millions of people and spreading terror to the population. The disease became known as the black death. In the warmer climates the disease usually assumes the *bubonic* form, so called from the swollen lymph nodes, known as *buboes,* which characterize it. In colder climates the disease is apt to take the *pneumonic* form, a much more fatal and contagious type

Bubonic plague is not a disease of any of the domestic animals. It occurs naturally in rodents, especially in rats, in which the disease spreads rapidly at times In rats the disease is quite similar to that in man and the mortality

is about as great The infection spreads from rat to rat, and from rat to man, through the agency of the rat flea In recent times it often has been observed in plague centers that great human epidemics have been preceded by great rat epidemics.

In addition to rats, the disease also occurs naturally in marmots, ground squirrels and other rodents Unlike the rat, these creatures often live in areas sparsely inhabited by man When plague spreads in such areas, it is known as the *sylvatic* form In some of the wild animals which do not live in close association with man, plague has a much greater tendency to assume the more highly contagious pneumonic form, than it does in the rat, and it appears that the disease contracted from them by man is also more likely to assume the pneumonic form

Plague exists in a half dozen areas of the world, known as plague centers With the exception of one African and one American center, the others are in Asia, especially in the region of the Himalaya Mountains The American center took origin in San Francisco where infection was brought in with oriental rats in 1900 In spite of a relentless warfare by public health officials against rats, the disease continues to smolder in the Bay Region of California in rats, and has spread among ground squirrels to at least five of the western states, where it constitutes an ever present threat to man Since the relationship of man with ground squirrels is not nearly so intimate as with rats, the disease of man in this country has usually been sporadic

Morphology and Staining Reactions. The plague bacillus is a little larger than the organisms of hemorrhagic septicemia but otherwise resembles them very closely Organisms in tissues usually measure about 0 5 microns by 1 5 microns. They may be readily stained with ordinary stains and the bipolar appearance is easily demonstrated if diluted stains are used. The organism is Gram-negative and capsules are not ordinarily developed In media containing 3 per cent salt solution great pleomorphism is exhibited and this characteristic is used for diagnostic purposes

Cultural Features. The growth on most media resembles that of the hemorrhagic septicemia organisms but is a little more luxuriant. Colonies on agar become a little larger, and they develop a little more rapidly Gelatin is not liquefied, milk is slightly acidified but not coagulated Indol is not formed. Acid but no gas is formed from dextrose, levulose, maltose, galactose, and mannitol Lactose, saccharose, dulcitol, raffinose, and inulin are not attacked

The organism grows best at temperatures somewhat below that of the body. It grows poorly when the temperature is below 20° C. and above 38° C. It is non-hemolytic and does not form a true toxin.

Control Measures. In dealing with plague, dependence is largely placed upon warfare on rats Rat proofing of buildings, wharves, and ships has done much to keep the disease down, and to prevent its spreading to parts of the world where it has not previously existed Shipping from plague centers is subjected to cyanide fumigation before discharging cargoes in plague-free areas in order to destroy the rats which may have found their way aboard. Modern steel ships also are built so as to be rat proof, or so that rats will find few places to hide.

Immunity. Many vaccines have been used in preventing plague in man The most widely used of these is Haffkine's vaccine in which virulent cultures are grown for several weeks and then killed with heat and phenol This vaccine contains the products of autolysis of the organism as well as intact bacilli It has been used very successfully in India as a prophylactic procedure. There are no products of demonstrated value for use in treating the disease

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PASTEURELLA TULARENSE

This organism is the cause of tularemia, otherwise known as deer-fly fever, rabbit-fever, and Ohara's disease The disease affects various rodents, especially the wild cottontail rabbit, and occasionally certain birds and sheep Man becomes infected from some of these animals either through direct contact with them or their carcasses, or through the agency of ticks or blood-sucking flies.

The disease was first recognized in ground squirrels in California by McCoy (6) in 1911 These animals become fatally infected, the lesions resembling those of bubonic plague with which it was at first confused McCoy and Chapin (7), in 1912, isolated and described the causative organism

Some years later Francis identified the organism as the cause of a serious disease of man in Utah It was Francis (2) who gave the name "Tularemia" to the disease, the name being derived from Tulare County, California from whence came the ground squirrels with which McCoy was working when the disease was discovered For some years it was thought that the disease was

confined to the United States It is now known that it exists in the Scandinavian countries, in Soviet Russia, and in Japan In these countries, as well as in the United States, the disease has become of considerable importance as a human infection.

Morphology and Staining Reactions. This organism is much more pleomorphic than any of the others included in this group In cultures bacillary forms up to 2 and even 3 microns in length may be seen Coccoid forms usually are mixed with the bacillary types Polar granules sometimes can be seen but these do not regularly occur The organism is Gram-negative, stains with the usual stains. It has neither capsules nor spores

Cultural Features. *Past tularense* will not grow on ordinary agar or in plain bouillon Media containing cystine is necessary (3) This may be supplied by adding egg yolk, or by adding salts containing cystine to ordinary types of culture media Francis recommends a blood-glucose-cystine medium for this organism

On suitable media, *Past tularense* develops readily, forming a smooth, viscous, grayish-white growth It does not liquefy gelatin, grows poorly in milk It forms acid but no gas from dextrose, levulose, and glycerin

This organism differs considerably from other members of the *Pasteurella* group It is doubtful whether it should be included It is included for convenience rather than from conviction

Pathogenicity. The guinea pig is easily infected with this organism, and therefore is frequently used in diagnostic work A generalized, fatal disease develops in which the most striking lesions are multiple necrotic areas in the liver and spleen Great care must be taken when working with this disease for many laboratory workers have contracted the infection (5) Apparently the organism is capable of entering the unbroken skin.

The only domestic animal which is affected with this disease is the sheep. Parker and Dade (9), 1929, have reported severe losses in lambs pastured on land in Montana which is heavily infected with wood-ticks, and have shown that it is this tick which carries the infection The ticks become infected, of course, by feeding upon wild rodents in which the disease is enzootic

The greatest reservoir of tularemia in the United States is in the wild rabbit population, especially in many of the midwestern states It is from the handling of the carcasses of such rabbits that most of the human cases in this country originate A few cases are contracted from the bites of blood-sucking insects which have fed upon infected rabbits (4) The organism sometimes becomes prevalent in water holes and in small streams, it being spread from

the bodies of infected water animals such as water rats and beaver (1). Human cases have been reported from contact with such water (8).

Immunity. One attack of tularemia gives a very solid and lasting immunity Individuals who have suffered from the disease develop agglutinins which persist for long periods—sometimes for many years after all symptoms have disappeared These agglutinins will react with *Brucella abortus,* and *Brucella* agglutinins will react with the organism of tularemia, and this is something which should be kept in mind when using serological tests for diagnosis Usually the titer for the homologous organism is very much higher than for the heterologous, hence if both organisms are tested it is simple to judge which is specific

There are no biological products of value for treating this disease

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PASTEURELLA PSEUDOTUBERCULOSIS

Synonyms *Bacterium pseudotuberculosis rodentium, Corynebacterium pseudotuberculosis, Corynebacterium rodentium*

This organism is the cause of a disease of guinea pigs, sometimes of rats, and occasionally of other rodents which has been called *pseudotuberculosis* This disease and its causative organism should be differentiated from *Corynebacterium pseudotuberculosis,* the Preisz-Nocard bacillus, already described The organism now being considered has little importance in animal pathology except in stocks of guinea pigs, although rare infections with it have been reported in cattle, horses, pigs, goats, rabbits, birds, monkeys, and man. This organism resembles that of plague so closely, and the lesions in guinea pigs are so similar, that one needs to be on his guard not to confuse one with the other.

Morphology and Staining Reactions. In form this organism varies from coccoid to bacillary forms 5 microns or more in length. They generally appear

single; occasionally in chains Bipolar staining can sometimes be noted This organism is somewhat larger than the hemorrhagic septicemia bacilli It is Gram-negative, non-acid fast, and not encapsulated.

Cultural Features. This organism differs from others in the *Pasteurella* group by being motile. Motility, however, is seldom seen in strains grown at body temperature, young cultures grown at 18° to 26° C are most favorable for detecting it.

On plain agar good growth occurs without the addition of serum or other enrichment Blood agar shows no evidence of hemolysis The colonies are small, translucent, and granular. In old colonies the centers are raised and more opaque than the periphery which frequently shows radial striations The consistency is soft and butyrous

Growth in broth is fairly good There is moderate turbidity in 24 hours at 37° C but later the growth sediments into a viscid mass A surface ring usually appears.

On potato a thin growth appears which is cream-colored at first, later becoming yellow and then brown Litmus milk slowly becomes alkaline Indol is not formed Acid but no gas is formed from dextrose, maltose, mannitol, salicin, arabinose, xylose, rhamnose, and glycerol Sucrose is sometimes fermented Lactose, raffinose, dulcitol, and sorbitol are not attacked

Pathogenicity. The disease is most often seen spontaneously in stocks of guinea pigs The affected animals sicken, lose weight, develop diarrhea, and die in three to four weeks In such animals the mesenteric lymph nodes are greatly swollen and caseous, and there may be nodular abscesses in the intestinal wall originating in follicles Similar nodules usually thickly stud the liver and spleen Ramon claims that there are acute cases in which the animals die in one or two days, and another, more chronic type, in which the infection is localized in the lymph nodes of the cervical region

The disease is easily produced in guinea pigs and many other animals by inoculation A caseous local lesion develops at the site of inoculation, the regional lymph nodes become enlarged and caseous, and nodules form in the liver and spleen Inoculated animals usually die in from 1 to 3 weeks.

Natural infection is supposed to occur through ingestion of the causative organism since primary localizations appear in the intestinal wall and the mesenteric lymph nodes.

Immunity. Not much is known about immunity to this organism Some workers have claimed excellent results in protecting susceptible rodents by the use of heat killed cultures, but others have not been able to achieve such pro-

tection It is interesting to note that injections of bacterins made from this organism seem to confer considerable immunity to the plague bacillus

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CHAPTER XVII

THE ENTERIC ORGANISMS

The bacillus causing human typhoid fever was discovered by Eberth in Germany in 1880 Later it was learned that there were many organisms similar to this in the normal intestinal tract of man and animals, and that there were many more closely related types which caused illnesses by their presence. The identification of many of these organisms has proved to be exceedingly difficult, and some can be identified with certainty only by using elaborate serological technics All members are Gram-negative, non-sporulating rods. The majority are motile by means of peritrichic flagella With but few exceptions, gelatin is not liquefied All are easily cultivated on ordinary media, and all form colonies which are similar although some species grow more luxuriantly than others They are facultative anaerobic but thrive best in the presence of air All ferment dextrose and usually a number of other carbohydrates with the formation of acid and frequently of gas as well. Most members of the group are found in the intestinal canal of man and animals, and in intestinal discharges Some species occur in other locations A few plant pathogens are included in the group

Although Eberth had seen the typhoid bacillus in 1880 in the spleen of a man dead of typhoid fever, it was not until 1884 that Gaffky isolated it in pure culture and described some of its characteristics. In the following year the colon bacillus was described The earlier work on this group of organisms was concerned with methods of differentiating between the comparatively harmless colon bacillus and the deadly typhoid bacillus In the course of time many other organisms having characteristics of both of these organisms became known (*Bact cholerae-suis,* 1886; *Bact enteritidis,* 1888, *Bact. gallinarum,* 1889, the paratyphoid organisms, 1898, and others) Soon authors began recognizing three sub-groups, one centering around the colon bacillus (Sub-group I), another centering around the typhoid bacillus (Sub-group III) and a third including forms intermediate between these (Sub-group II). The line of demarkation between these three sub-groups never has been sharply drawn and as more information about them has accumulated the division lines have become hazier rather than clearer. For descriptive purposes the three sub-groups usually are retained by most authors but it should be realized that from the colon bacillus at one extreme to the dysentery bacilli

and the typhoid bacillus at the other, we have a long series of organisms which vary from each other only slightly as we proceed down the scale, step by step.

Many writers recognize a number of genera in this group, such as *Escherichia* for the colon bacilli, *Aerobacter,* for the aerogenes types, *Salmonella* for the greater part of the intermediate group, *Eberthella* for the typhoid organism, and *Shigella* for the dysentery organisms and related types Undoubtedly this group should and will eventually be broken up into several clearly defined genera but until these lines become clearer it seems best to retain the old genus *Bacterium* for the entire group.

The fermentation of lactose is a criterion which appears to be quite reliable for separating the comparatively harmless colon-aerogenes organisms from the remainder of the group, most of which have pathogenic properties The colon-aerogenes types ferment lactose with the production of both acid and gas, the other members of the group do not attack lactose One often encounters members of the colon-aerogenes group, however, which ferment lactose very slowly, and these types may be easily mistaken for pathogenic species

The Lactose Fermenting Group

The organisms which ferment lactose represent a group in which there are two well recognized species, in both of which there are many varieties The species are *Bact coli* and *Bact aerogenes* Both of these species may be found in the alimentary tract of man and animals *Bact coli,* however, is quite strictly parasitic in its habits and therefore is not found abundantly anywhere in nature except in intestinal tracts, in feces, and in materials which have been subjected to fecal pollution *Bact aerogenes,* on the other hand, grows freely in nature and its presence in any substance does not necessarily mean that fecal pollution has occurred The differentiation of one group from the other is of some importance in water analysis, in which the presence of *Bact coli* types is interpreted as evidence of fecal pollution, whereas the presence of a

TABLE IX

	Bact coli	*Bact aerogenes*
Methyl Red Reaction	+	−
Voges-Proskauer Reaction	−	+
Citrate Utilization	−	+
Sodium Malonate Utilization	−	+
Reduction of Methylene Blue	−	+
Indol Formation	+	−

few organisms of the aerogenes group is not so interpreted. Some of the criteria for differentiating these species is given in tabular form on page 172

Inasmuch as *Bact aerogenes* is not a pathogenic organism, it will not be considered further.

BACTERIUM COLI (ESCHERICHIA COLI)

This organism is a normal inhabitant of the lower bowel of all warm-blooded animals It usually is absent from the intestines of fish, and other cold-blooded animals. Few or none are found in the stomach and anterior portions of the bowel Carnivora and omnivora usually harbor the organism in greater abundance than the herbivora The feces of cows and horses frequently show very few

Morphology and Staining Reactions. *Bact coli* is a small rod-shaped organism which varies widely in morphology under varying conditions Usually it is a short, plump rod, sometimes rather long filaments are seen. Spores are never formed and capsular material is absent from most but not all strains. It stains readily and evenly with ordinary stains and is Gram-negative.

Cultural Features. *Bact coli* grows readily on all ordinary media Its optimum temperature is about that of the body but it will grow through a wide range It is aerobic and facultative anaerobic Most strains show motility of a sluggish type

In broth there is uniform clouding within 12 to 18 hours On old cultures friable pellicles form and very old cultures show considerable viscid sediment. Agar surface colonies are slightly raised, smooth, glistening, unpigmented, and circular in outline Deep colonies usually are lenticular in shape and brownish On slants the growth becomes confluent, and the water of syneresis, turbid Gelatin colonies are thin, bluish-white, translucent, and glistening The surface usually shows radial ridges and the margins are somewhat irregular, giving the colonies the shape of a grape leaf The gelatin is not liquefied. Growth on potato is less abundant than on agar, is brownish and rather dry. Some strains are strongly hemolytic, producing wide zones of Beta-type hemolysis around colonies on blood agar plates. Others have no hemolytic action. Litmus milk is acidified and coagulated within 24 hours at 37° C. The litmus is reduced except near the surface Dextrose and lactose are attacked by all strains, both acid and gas being formed On other carbohydrate media, acid and gas are formed from some and not from others depending upon the strain.

Indol is formed, usually in abundance, nitrates are vigorously reduced and

the Voges-Proskauer reaction is negative. This last reaction is considered valuable in distinguishing *Bact coli* types from *Bact. aerogenes* which give a positive reaction.

Resistance. *Bact coli* is fairly resistant to drying and to the action of many chemical disinfectants. It is destroyed by pasteurizing temperatures

Pathogenicity. *Bact coli* is usually regarded as a harmless parasite That the organism may be pathogenic at times, or that certain races may be pathogenic, has been known for a long time Moderate doses injected into the peritoneal cavity of guinea pigs will result in death overnight It is found frequently as the apparent cause of peritonitis following abdominal operations, rupturing of the intestines, and perforating wounds of the digestive canal

Bact coli is one of the most common organisms encountered in cultures made from animal tissues, especially when the tissues have been removed from the body some hours after death It appears that the organism escapes from the digestive tract into the blood stream and is disseminated to all parts of the body about the time of death If the animal is killed quickly when in a state of health, these organisms are not found, or at least not so frequently

Organisms of the colon bacillus type are generally regarded as the causative agents of the disease of young calves commonly called *white scours* or *calf scours* There is no doubt about the fact that tissues of animals suffering from this disease are teeming with *Bact coli* shortly before and after death The organisms found differ in no way from those commonly present in the intestinal canal of normal calves, and cultures of these organisms will not ordinarily cause disease in other calves when fed to them experimentally For these reasons, it is very doubtful that they are the primary cause of the disease It is likely that other, as yet unrecognized, agents are primary and that these pave the way for invasion of the tissues with colon bacilli from the digestive tract In the most acute form of this disease the calf shows weakness, appears sleepy, and soon dies without other symptoms In the usual form the calf shows a severe diarrhea, the fecal material often being whitish because of lack of bile, and full of gas bubbles The animal may die after a few days, or it may recover In the more protracted cases, lameness may appear because of acute inflammation in one or more joints In such joints the capsule is distended with a cloudy fluid in which there are myriads of colon bacilli These animals nearly always die eventually. Calves which have passed through these infections successfully frequently exhibit whitish fibrotic areas in the cortex of the kidneys.

Smith and Little (3) pointed out the interesting fact that calves which are deprived of the first milk (colostrum) of their dams nearly always develop

colon bacillus septicemia In a series of papers by Smith and co-workers, (2, 4, 5, 6) the mechanism of this septicemia is made clear. It appears that the function of colostrum is to carry protection to the digestive tract of the new-born calf against miscellaneous bacteria, principally colon bacilli, which are harmless to older animals but which rapidly invade the tissues of very young calves from intestinal canals which have not had the protection offered by colostrum

The colostrum, it has been shown, is very rich in globulins, and also rich in antibodies When colostrum is fed to the new-born calf, the globulins containing the antibodies appear in the blood stream almost immediately The structure of the bovine placenta is such that antibodies are not transmitted from the mother of the calf before birth, as happens in many other species, and the colostrum appears to exert a compensatory function When colostrum is withheld, the new-born calf appears to be entirely devoid of a resisting mechanism and the tissues may be overrun with ordinary organisms, principally colon bacilli

It was shown by Smith and his co-workers that normal cow serum would function almost as well as colostrum in protecting the new-born calf Normal cow serum contains antibodies for the colon bacillus, hence these antibodies rapidly appear in the blood of the calf when the serum is fed mixed with milk The calf is protected best, however, when cow serum is injected subcutaneously and fed as well.

Howarth (1) has reported a large outbreak of abortion in sheep in California in which an organism belonging to the colon group seemed to be the causative agent Of a band of 820 ewes, abortions occurred in 219, and of the aborting ewes, 101 died shortly after the act The organism was readily isolated from the body fluids and tissues of the fetuses, from the placentae, uterus and uterine discharges of the ewes With the cultures abortions were readily produced by intravenous injection, but not by subcutaneous injection nor by feeding The flock had been forced to drink from stagnant pools containing a great deal of organic material, and it was thought that these pools had been the source of the infection The organism isolated from these ewes differed in cultural features in no way from organisms found commonly in the intestinal canal

Colon Bacillus Toxin. Organisms of this group are regarded ordinarily as non-toxin formers Smith and Little (4), however, showed that filtrates of comparatively young broth cultures of certain strains of colon bacilli isolated from cases of calf septicemia were toxic to calves and older cattle when injected intravenously Grave symptoms were produced often followed by death The symptoms consisted of rapidly developing and severe dyspnea.

and after death the lungs were found to be filled with a foamy, edematous fluid. Large doses of such filtrates did not produce symptoms when injected subcutaneously. The authors found that similar results could be obtained with filtrates of some paratyphoid bacilli They did not determine the nature of the toxic material.

Immunity. Calf scours serum made by immunizing horses or cattle with strains of colon bacilli isolated from calves suffering from "white scours" has been widely used as a means of treating animals suffering from this disease. The results reported have varied widely It seems very doubtful that such sera have materially altered the course of the disease Shortly after injection the calves frequently appear better for a time, but a similar effect can be obtained by injecting blood or blood serum from normal cattle.

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3 SMITH AND LITTLE Jour Exp Med, 1922, 36, 181.
4 SMITH AND LITLE Jour Exp Med, 1922, 36, 184
5 SMITH AND LITTLE Jour Exp Med., 1922, 36, 453
6 SMITH AND LITLE Jour Exp Med, 1927, 46, 125.

The Non-Lactose-Fermenting Enteric Organisms

In morphology and in general cultural features, these organisms are not unlike Bact coli, however they do not ferment lactose Milk usually is lightly acidified but this is followed within a few days, in most instances, by a progressive alkalinity which may go so far as to cause solution of the casein, in which case the milk loses its normal appearance and becomes translucent and opalescent

Many organisms of the group are associated with specific diseases which are readily recognized clinically The organisms associated with such diseases ordinarily are easily identified A group of these organisms, however, are associated with enteritis and diarrheas of both man and animals, and in these cases it often is a difficult matter to definitely identify the causative species even when it has been obtained in pure culture.

Formerly it was customary to divide the organisms of this group into (a) those which form acid and gas from dextrose (The Intermediate or Salmonella Group), and (b) those which form acid only (The Typhoid-Dysentery Group). This arrangement placed the typhoid organism in one group and the

paratyphoid organisms in another, a classification which cannot be justified by any other criteria, and placed together the typhoid and dysentery organisms which are not closely related otherwise The recently developed methods of serologic classification (antigenic analysis) indicate that antigenically the typhoid organism of man is closely related to the paratyphoid organisms.

It long has been known that many antigens existed in this group and that some of these were specific and others existed in common Antigenic analysis depends upon the identification of the various types of antigens present in the bacterial cell, such identification being sufficient to positively identify the species, since no two species have identical antigenic structures The method was first used by Andrewes, in England in 1922, and has been extensively used in recent years In order that the world should be saved from great confusion a committee of the International Microbiological Congress has set up a series of typing laboratories in many countries under the general direction of a central laboratory, the State Serum Institute of Copenhagen, Denmark, the function of which is to keep on hand type cultures and sera by which unknown strains may be identified

Group agglutination already has been described hence we will discuss here only a few pertinent facts as they relate to the Salmonella organisms which constitute the greater part of the non-lactose fermenting members of the enteric group Since most of these organisms are motile, they possess both the H (flagellar) and the O (somatic) antigens Organisms like *Bact gallinarum* and *Bact pullorum* which are non-motile naturally possess only the O antigens. The cultures are classified primarily on their O antigens of which at least thirteen different types have been recognized These are designated by Roman numerals A single species may have more than one O antigen. Some of the O antigens are specific, that is, they are found in one species only, and others are not specific, since they may be found in several different species (group antigens) The H antigens, of which more than thirty are known, may be absent (non-flagellated types) or there may be from one to six in a single species These are of two types, the *specific phase* and the *non-specific phase* When both of these types are present in a single species, the organism is said to be *diphasic,* when only one, *monophasic* The specific phase antigens are those which occur in single types or species and these are designated by small letters The non-specific phase antigens are those which occur in several species (group antigens) and these are designated by Arabic numbers. Thus the antigenic formula of *Bact typhimurium* is IV, V . 1 : 1, 2, that of *Bact choleraesuis* is VI, VIII c 1, 5, and that of *Bact pullorum* is IX · –, –.

Since the method of antigenic analysis in this group requires a rather elab-

TABLE X

SOME DIFFERENTIAL CHARACTERISTICS OF ORGANISMS OF THE NON-LACTOSE-FERMENTING ENTERIC GROUP WHICH ARE OF IMPORTANCE IN ANIMAL PATHOLOGY

ORGANISM	CULTURAL FEATURES										ANTIGENIC STRUCTURE		
												H antigens	
	Dextrose serum water	H_2S Formation	Dextrose	Lactose	Arabinose	Xylose	Dulcitol	Inositol	Trehalose	Maltose	O antigens	Spec phase	Non-spec phase
Bact typhimurium	−	+	ag	−	ag	ag	ag	ag	ag	ag	IV, V	1	1, 2
Bact enteritidis	−	+	ag	−	ag	ag	ag	−	ag	ag	IX	g, m	−
Bact anatum	−	+	ag	−	ag	ag	ag	ag	ag	ag	III, X	e, h	1, 6
Bact choleraesuis	+	−	ag	−	−	ag	−	−	−	ag	VI, VII	c	1, 5
Bact typhisuis	+	−	ag	−	ag	ag	±	−	ag	ag	VI, VII	c	1, 3, 4, 5
Bact pullorum	±	±	ag	−	ag	ag	−	−		−	IX	−	−
Bact abortivoequinus	+	−	ag	−	ag	ag	ag	−		ag	IV	e, n, x	−
Bact gallinarum	±	±	a	−	a	a	a	−		a	IX	−	−
Bact equirulis			a	a	−	a	−			a			

ag = acid and gas

a = acid without gas

orate set of type sera, this method probably will be used only in a few laboratories especially equipped for this work Differentiation of species by fermentation tests and other biological methods which are less complicated undoubtedly will continue to be used by most workers These, taken in conjunction with a knowledge of the host from which they were isolated and the nature of the pathological processes with which they were associated, make identification by the older methods reasonably satisfactory In special instances, especially in the group which are associated with intestinal intoxications, cultures had best be submitted to a typing laboratory * for analysis

BACTERIUM TYPHIMURIUM

Synonyms *Bact aertrycke, Bacterium pestis caviae, Salmonella aertrycke* and many others

This organism, frequently called the mouse typhoid bacillus, was first isolated by Loeffler (2) in 1892 It is pathogenic for rodents, especially mice and guinea pigs, but also for sheep, pigs, a number of species of birds, and man For many years it was not recognized that the mouse typhoid organism was identical with those isolated from other species Nocard, in 1893, isolated his *Bact psittacosis,* from parrots which were suffering from the disease known as psittacosis, erroneously believing the organism to be the causative agent The error with respect to the etiology of psittacosis was recognized long before it became evident that the organism was identical with Loeffler's bacillus Wherry (6), in 1908, studied a plague of guinea pigs from which his *Bacillus pestis caviae* was isolated It is now known that this organism is the same as Loeffler's The specific name, *aertrycke,* proposed by deNobele in 1898 has been used for this organism for many years and is well established in the literature, however, *typhimurium* has six years priority over it and since it has been adopted by Bergey's Manual, it will be used here

Morphology and Staining Reactions. These are typical of the group. It is motile by means of peritrichic flagella

Cultural Features. These are typical of the group as a whole. Differential characters may be noted in Table VI The fermentation of inositol distinguishes this organism from *Bact enteritidis* and related organisms In antigenic structure there are several varieties. The typical organism has the following formula IV, V, XII : 1 : 1, 2, 3. Strains from pigeons usually lack the somatic antigen V.

* For the United States, the Department of Animal Pathology, University of Kentucky, Lexington, Ky

Pathogenicity

FOR GUINEA PIGS. Stocks of guinea pigs occasionally are completely destroyed by this infection If the disease becomes prevalent in a colony it is exceedingly hard to eliminate except by disposing of all stock, rigorously disinfecting and beginning anew with fresh, uninfected stock Animals dead of the disease show enlarged, dark livers, frequently with small flecks of necrotic tissue The spleen usually is enlarged up to five or six times its normal size It is dark, friable, nodular, and may show minute necrotic foci like those of the liver. There may be fluid in the chest cavity, and a fibrino-purulent pericarditis is common This disease apparently was first described by Smith and Stewart in 1896 The name *Bacillus pestis caviae* was given it by Wherry in 1908

FOR MICE Epizootics of mouse typhoid occur frequently in mouse colonies in research laboratories, the mortality varying from 20 to 80 per cent depending on environmental factors, state of nutrition, genetic constitution, and no doubt other factors not recognized The disease may be acute or chronic Many of the chronic cases and some that show no symptoms at any time become chronic carriers, shedding the organism in their feces for long periods of time Mice dead of the disease show enlarged livers and spleens, in which pin-point necrotic areas may or may not be seen Other lesions are inconstant. Usually microscopic examination will demonstrate damage in the bone marrow, apparently due to toxic material, and this interferes with leucocyte formation, so there is a characteristic leucopenia in this disease

Amoss (1), Topley (3), Webster (5) and others have used the "mouse typhoid" organism for studies in experimental epidemiology with results that have thrown much light on human disease dissemination. Webster has shown that highly resistant and highly susceptible strains of mice can be produced by breeding from siblings of individuals that have shown unusual resistance or susceptibility, respectively

Apparently *Bact enteritidis* is capable of initiating a disease in mice in every respect like that produced by *Bact typhimurium*

FOR RATS Young rats are susceptible to infection with *Bact. typhimurium* but older ones are somewhat resistant Certain strains, supposedly well suited to the purpose, have been used as "rat viruses" for rat extermination but there have been many accidental infections of other animals, and of man, with such strains and this has discouraged the practice

FOR HORSES, CATTLE, SWINE, AND SHEEP *Bact typhimurium* is said to occur in these species. *Bact enteritidis* infections have been reported frequently in the past, especially in Europe, in these animals Whether the organisms were really of the species indicated is impossible to tell in many cases because of

insufficient evidence It may be that *Bact typhimurium* is more frequent in domestic animals than has been thought.

FOR VARIOUS SPECIES OF BIRDS Infections have been reported in chickens, ducks, turkeys, parrots, pigeons, canaries, and others In most species the infection is manifested by enteritis and diarrhea with septicemia in fatal cases. In pigeon lofts, losses from this organism often are very great The losses are in the squabs The pigeon fancier often calls this disease *megrims*. The squabs either die soon after hatching or develop swollen wing-joints which render them unable to fly The joint swelling is due to the collection of a gelatinous exudate in the joint capsule In this exudate the organism is readily found by making cultures. The adult stock shows no evidence of the disease ordinarily but when destroyed for examination the ovaries of some of the females are found to be diseased The organism can be found in many of the developing yolks, presumably passes in this way into the egg and then into the developing embryo

FOR MAN Some strains of this organism probably are capable of infecting man The organisms of outbreaks in mice and guinea pigs in animal houses seldom, if ever, cause infection in human attendants The same may be said for the infections in birds On the other hand there have been a number of outbreaks in man which have been attributed to the contamination of human food with the droppings of rats and mice

Immunity. Infections with this organism occur so sporadically in the larger animals and in man that there is no need of immunizing products Cultures of the organism, killed with heat or chemicals, have been used in attempting to stop epizootics among mice and guinea pigs but without great success Resistance can be enhanced in this way, but the protection usually is not great enough to stop outbreaks

Attempts have been made to control the squab disease by using the agglutination test to detect infected adult female pigeons The antibody content of the birds is so low, however, that the method has failed

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BACTERIUM ENTERITIDIS

Synonyms: *Salmonella enteritidis,* the Gartner bacillus; the meat poisoning bacillus.

This organism was incriminated by Gartner, in 1888, as the cause of an outbreak of acute illness in a group of 57 persons who had eaten the flesh of a recently slaughtered cow The animal had suffered from diarrhea at the time it was slaughtered Similar stories have been repeated many times in some of the European countries but are rare in the United States Apparently this organism is not so widely scattered or so prevalent here as it is abroad The system of emergency slaughter, permitted abroad but not here, may have a good deal to do with this difference By emergency slaughter is meant the slaughtering for food of animals which are frankly sick.

Intestinal infections of cattle with this organism are quite common in some parts of the world Apparently they are very rare in the United States, since there have been few reports. It is from the eating of the uncooked meat from such animals that human "meat poisoning" occurs.

Morphology and Staining Reactions. These are typical of the group The organism is actively motile.

Cultural Features. These are typical of the group as a whole In most cultural features this organism cannot be distinguished from *Bact typhimurium.* The principal cultural difference is with respect to inositol which is fermented with acid and gas formation by *typhimurium* and is not attacked by *enteritidis* The antigenic composition of *Bact enteritidis* indicates that there are a considerable number of varieties All contain the same O antigens, IX, and XII, but differ in H antigens.

Pathogenicity. Like *Bact. typhimurium,* this organism occurs naturally in rodents, principally rats and mice, in which a disease essentially like mouse typhoid is produced. Infections of larger animals, especially of calves, is frequently reported abroad. The only case which has been found in the United States which we have been able to discover is one reported by Meyer (1) in 1916 In this instance the culture which had been isolated from a calf suffering from diarrhea caused infection of an attendant who was handling infected milk.

The infection in man takes the form of an acute intestinal infection or intoxication. The organism possesses a potent endotoxin which is regarded as having an important part in the disease process.

Immunity. Little is known about immunity to this organism since outbreaks of disease are sporadic, and immunizing products therefore are seldom called for.

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BACTERIUM ANATUM

Synonym *Salmonella anatis*

In 1920, Rettger and Scoville (2) described *Bact anatum* as the cause of a destructive disease of ducklings on Long Island and in Connecticut and Massachusetts The disease was called "keel" by the duck raisers because many of the affected birds collapsed suddenly with few premonitory symptoms

Morphology and Staining Features. These are typical of this group. It is actively motile.

Cultural Features. In 1927, Edwards and Rettger (1) reported that serological tests of strains of *Bact anatum* had shown two serological types, one of which was identical with *Bact aertrycke* (*typhimurium*). On all ordinary differential tests this organism cannot be distinguished from *Bact. typhimurium* but the antigenic composition is quite different (III, X e, h 1, 6).

Pathogenicity. The greatest mortality in ducklings occurs during the first ten days of life It sometimes amounts to practically 100 per cent Affected birds are listless, sleepy, crowd close to the heaters, and do not eat well. Thirstiness is a prominent symptom There is no diarrhea Death usually occurs suddenly

The infection may occur in the egg from organisms originating in the ovary of breeder animals as in pullorum disease.

Attempts to control the disease by testing the adult stock for carriers by the agglutination test has not been successful since the antibody content of such birds is low.

The organism evidently is not highly pathogenic for man since there have been no recognized infections among the duck raisers

Immunity. Specific treatment of infected ducklings is impracticable and probably useless.

REFERENCES

1. EDWARDS AND RETTGER. Jour. Bact, 1927, *33*, 73.
2. RETTGER AND SCOVILLE. Jour. Bact, 1920, *26*, 217.

BACTERIUM CHOLERAESUIS

Synonyms *Bacillus cholerae suis*, the "hog cholera bacillus"; *Bacillus suipestifer, Salmonella suipestifer*

This organism was isolated and described by Salmon (2) and Smith in 1885, and was the first of the paratyphoid organisms to be recognized It is because of this fact that these organisms are frequently known as the Salmonella The authors believed the organism to be the cause of the destructive and prevalent disease known as hog cholera, but later work demonstrated that this is not the case, that hog cholera is caused by a filterable virus Nevertheless this organism plays an important role in porcine pathology, principally as a secondary invader in the virus disease

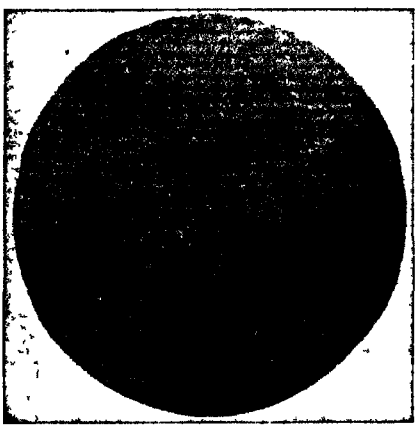


FIG 26 *Bacterium choleraesuis* From a culture on slant agar incubated for 18 hours at 37° C x 900

Morphology and Staining Reactions. These are typical of the group Most strains are actively motile but non-motile variants are known.

Cultural Features. These are similar to those of other members of the group. For differential features see Table VI It is to be noted that hydrogen sulphide is not formed (i e, lead acetate negative) and that arabinose, dulcitol, inositol, and trehalose are not fermented In these respects this organism differs from the rodent and meat poisoning types The reaction in litmus milk is very characteristic After a slight initial acidity, the reaction becomes progressively more alkaline and finally after several days' incubation the casein goes into solution and the fluid becomes translucent and opalescent This reaction is exhibited by other members of the paratyphoid group but usually not to such a degree

The organism described is the one which is commonly found in this country. Sometimes it is called the American type of *Bact. choleraesuis* to distinguish it from another type which occurs in Europe and which is known as the Kunzendorf type The latter differs from the American type only in that

it forms hydrogen sulphide, and has been found much more commonly in human infections of a food poisoning type

The antigenic formulae of the two types are the same VI, VII · c : 1, 5

Occurrence. In some of the older literature it was reported that *Bact. choleraesuis* occurred in a considerable number of apparently healthy pigs as an inhabitant of the intestinal tract, but apparently this was an error caused by failure to differentiate this organism from other paratyphoid organisms It now seems clear that this organism is found only in sick swine and in material contaminated by them.



FIG 27 The So-called "Button Ulcers" of Hog Cholera These crater-like ulcers, their centers filled with caseous material, are due primarily to the activities of the *Bact choleraesuis Actinomyces necrophorus* usually has an active part in their formation but is not primary

Pathogenicity

FOR SWINE This organism is very commonly associated with the virus of hog cholera *Salmon* and *Smith* (4) succeeded in isolating it from about four-fifths of a large number of animals sick and dead with naturally occurring cases of hog cholera With their cultures they produced fatal septicemia in swine by artificial inoculation This disease resembled cholera and was mistaken by them for cholera They noticed, however, that the acute inoculation disease did not transmit to pen mates, as the disease in the field did, and this should have warned them that they did not have the primary cause of the disease Filterable viruses at that time, it should be remembered, were not known.

There is no doubt that *Bact choleraesuis* plays an important role in the clinical disease known as hog cholera, and that in many cases it plays a determining role so far as death or recovery are concerned The enteritis manifested by the so-called "button ulcers" in the region of the ileo-cecal valve, a characteristic of the more chronic cases of hog cholera, is caused by the activities of the hog cholera bacillus rather than by the virus.

Bact. choleraesuis is capable of causing disease in young pigs in the absence of the virus of cholera. *Infectious necrotic enteritis* is a destructive disease of

young pigs kept under unhygienic conditions Diseased animals are unthrifty and exhibit diarrhea Many die and those which survive usually are so stunted that they make unsatisfactory meat animals The lower end of the small intestine and the large intestine are the seats of the lesions On the mucous membrane of these parts there may be numerous ulcers filled with dry, caseous material, or the entire mucosa may be changed into a dry caseous mass which adheres tightly and can be separated from the intestinal wall only by tearing out the entire mucous membrane

In necrotic enteritis, the virus of hog cholera may be present and should be suspected, but the disease can occur in cholera immune pigs Murray (1) and his associates in Iowa have produced necrotic enteritis regularly by feeding cultures of *Bact choleraesuis,* a fact which removed all doubt about its causal relationship to the disease

FOR EXPERIMENTAL ANIMALS *Bact choleraesuis* is highly pathogenic for white mice, rabbits, and guinea pigs Rabbits inoculated intravenously die in from 5 to 8 days. The lesions consist of a very engorged and enlarged spleen, and necrotic foci throughout the liver Guinea pigs and mice show similar lesions, but guinea pigs are more resistant and usually live longer than the others after inoculation

FOR MAN This organism has been isolated from a few outbreaks of food poisoning in man, but the type usually found (Kunzendorf) is different from that which commonly occurs in swine in this country.

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3 SALMON U S Dept Agr, Bur Animal Industry, Ann Rpt for 1886, p 20
4. SMITH. U S. Dept. Agr, Bur. Animal Industry, Bull No. 6 (1894).

BACTERIUM TYPHISUIS

Synonyms *Bacillus typhisuis, Salmonella typhisuis,* the "ferkeltyphus" (German) bacillus.

This organism is very similar to *Bact. choleraesuis* and probably should be regarded as a variety of that organism It differs from it only in that it ferments arabinose and trehalose, whereas the former does not. Its antigenic structure is the same. This organism, so far as is known, does not occur in the western hemisphere. In Europe it causes an intestinal disease of young pigs known as "ferkeltyphus."

BACTERIUM PULLORUM

Synonym· *Salmonella pullorum.*

Bacterium pullorum is the cause of pullorum disease, sometimes called bacillary white diarrhea, of young chicks which frequently causes heavy losses to the industry. This organism was first isolated and described by Rettger (1) in 1909.

Morphology and Staining Reactions. These are typical of the group. The organism lacks flagella, consequently is non-motile

Cultural Features. The organism grows readily upon ordinary culture media but the growth is never luxuriant On agar small, translucent, convex, glistening colonies appear in 24 hours or less The organism grows on gelatin delicately but produces no liquefaction In broth a faint clouding is produced Milk is slightly acidified but is not curdled Of the ordinary carbohydrates, dextrose and mannitol are fermented with gas formation Lactose, sucrose and maltose are not fermented Some strains of *Bact pullorum,* otherwise characteristic, fail to produce gas The property of gas formation may be lost when under artificial cultivation The non-gas-forming strains are generally known as the B type while the gas formers are called the A type The B type is usually found in adult stock and not in chicks. The two types interagglutinate freely.

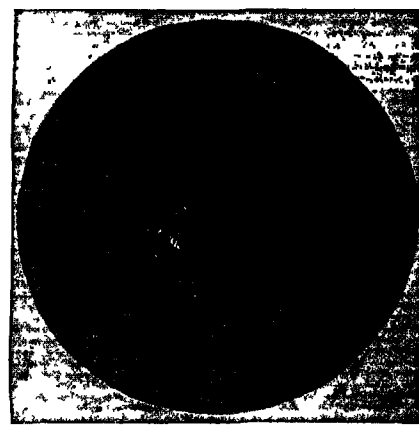


FIG 28 *Bacterium pullorum* From a culture on plain agar incubated for 18 hours at 37° C x 900.

The antigenic structure is IX, XII — — It possesses only somatic antigens. In serologic make-up this organism is identical with *Bact gallinarum*

Pathogenicity. *Bact pullorum* is highly fatal if cultures are fed to young chicks during the first few days of life, and particularly if the chicks are allowed to become chilled by lowering the temperature of the brooder house *Older chicks become progressively harder to infect in this way, but occasion*ally even adult birds may be killed. Old birds can be infected by subcutaneous or intravenous injection. In these cases the infection may remain localized, or

septicemia may result Large doses, given intraperitoneally, will kill guinea pigs.

In its natural host, pullorum disease proceeds in cycles. The infection is carried in the ovaries of some hens but there are no symptoms to indicate this fact. Some of the eggs laid by such hens will contain the organism in their yolks If these eggs are incubated, many will fail to hatch but the ones that do will give rise to chicks which harbor the infection in their yolk sacs Some of these chicks appear not to be seriously harmed by the presence of the organism and become in turn ovarian carriers of the disease when adult age is



FIG 29 Pullorum Disease, Ovary, Chicken The diseased ovary is depicted on the right On the left is a normal ovary for comparison The ova of the diseased bird are small, misshapen, discolored, and sometimes hemorrhagic

reached Others, especially those which are shipped very young and which are chilled or otherwise devitalized, may become ill from an acute diarrhea accompanied by septicemia The diarrheal discharges are highly contagious for the other chicks and soon a large part of the birds associating with a few sick ones are infected with the disease In incubator-raised chicks the disease is especially malignant, for the infected down from a few diseased chicks will be blown around the entire machine by the fan which circulates the air, and all or many of the chicks will contact the disease by inhaling the organism.

The affected chicks huddle near a source of heat, they do not eat, they appear sleepy, they may show diarrhea, and they usually die within a few hours. The lesions vary according to the method of infection The chicks that are hatched containing the infection, usually show unabsorbed yolk sacs with dry cheesy material in them, the liver frequently appears pale and half-cooked,

and the intestines are inflamed Chicks infected by inhaling the organism usually show caseous areas in the lungs Similar caseous areas often are seen in the wall of the gizzard and in the heart muscle The losses vary greatly depending upon how the chicks are handled It not infrequently occurs that a hatcheryman will have no trouble in chicks which he raises himself but there will be great losses in other chicks of the same lot that are shipped to distant points.

The traffic in day-old chicks is very great, and it is in this traffic that the greatest losses from pullorum disease occurs The disease is propagated from year to year in the ovaries of infected hens

Control Measures. Treatment of the diseased chicks is impracticable since birds of a few days of age have very little resistance and quickly succumb

Efforts at control must be aimed at preventing the infection. These efforts are of two kinds 1 The detection and elimination of the hens which are carrying the infection and are laying infected eggs, and 2 The better control, including fumigation of the incubators, of commercial hatcheries

The elimination of the infected hens cannot be done on the basis of any kind of physical examination The carrier hens can be detected only by means of a serological test, or possibly by means of an allergic reaction

Serologic Tests. The complement-fixation test may be used but it has not been shown to be any more accurate than the agglutination test. The simpler test, therefore, is the method of choice The birds are bled from the wing vein into small vials The antigen is, of course, a suspension of *Bact pullorum* Three methods of performing the test are in use, viz

(a) *The tube method* This is the older and standard test The serum is separated from the clot The test may be set up in a number of tubes, using decreasing concentrations of serum as is done in the test for Bang's disease of cattle, but usually, to save expense, only a single tube is used Into the clean, dry tube $\frac{1}{20}$ cc of undiluted serum (some merely add one drop without bothering to measure the quantity more accurately) is placed. The suspension of bacilli (antigen) is then pipetted into the tube and caused to mix with the serum If 1 cc of antigen is used, a dilution of 1 20 is obtained; if $1\frac{1}{2}$ cc , 1 30; if 2 cc , 1 40 Birds that react in dilutions of 1 25 or higher are regarded as infected

Some chicken sera yield a flocculent, fatty material which floats on the surface of the fluid. This interferes with the reading of the test. These cloudy reactions can be avoided by making the antigen alkaline (pH 8.0) by the addition of NaOH just before the tests are set up.

(b) *The serum-plate method* This is done in the same way as the plate method for Bang's disease (see p 137). When done by experienced operators it probably is as accurate as the tube method

(c) *The whole blood plate method* This method can be done while the birds are being held to await the outcome of the test A drop of blood is collected on a clean glass slide and immediately mixed with a drop of the concentrated antigen which has been especially prepared for this test The accuracy of the test is not as great as that of the other methods but it is frequently used, nevertheless, because of the cheapness of the method

Allergic Reaction. Several workers have prepared extracts of *Bact pullorum* and have used these after the manner of using tuberculin The injections are usually made into one of the wattles, intradermally Infection is indicated by swelling of the wattle The reactions are judged on the following day or after 48 hours The test is in an experimental state at the present time From the evidence at hand it does not appear that the test can be depended upon as a diagnostic procedure

Immunity. No attempts have been made to immunize birds against *Bact pullorum* infection.

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1 RETTGER Jour Med Res, 1909, *21*, 117.

BACTERIUM GALLINARUM

Synonyms *Bacillus sanguinarium, Salmonella gallinarum, Eberthella gallinarum, Shigella gallinarum,* the fowl typhoid bacillus

This organism was isolated and described by Klein (2) in 1889 as the cause of a disease known as *fowl typhoid* In 1895 Moore (4) isolated an organism which later proved to be the same as Klein's from an outbreak of an acute disease in chickens which he named *infectious anemia* Moore applied the name *Bacillus sanguinarium* to his organism since he was not aware that it was the same as the one with which Klein had worked The name *gallinarum* has priority over Moore's name, and therefore is the one now used

Morphology and Staining Reactions. These are typical of the group The organism lacks flagella, therefore is non-motile

Cultural Features. This organism is quite typical of the paratyphoid group except that it is non-gas forming In this respect it resembles the typhoid and dysentery bacilli of man, and it is for this reason that the organism has been

placed in the genus *Eberthella,* with the typhoid organism of man by some authors, and in the genus *Shigella* with the human dysentery organisms by others Still others, in consideration of the fact that it contains antigens in common with typical paratyphoid organisms have placed it in the *Salmonella* This confusion illustrates the fact that lines of division in this group are not sharply drawn at present, and is the reason why we are retaining the generic name *Bacterium* for all members of the group

Bact gallinarum is very closely related to *Bact pullorum* It differs in that gas is not formed from carbohydrates The same carbohydrates are fermented, however, and in addition to those attacked by *pullorum,* maltose is fermented *Bact gallinarum* grows somewhat more luxuriantly than *pullorum* It has long been known that these two organisms cross agglutinate to full titer, and cultures of *gallinarum* have even been used as antigen in pullorum disease-control work because of the greater ease with which it can be manufactured. It was not surprising to learn, when antigenic analysis was applied to these organisms, to find that they were identically constituted This raises the question of whether this organism is not a non-aerogenic variety of *Bact. pullorum* The antigenic is IX — —

Pathogenicity. Fowl typhoid affects adult birds The symptoms are those of an acute septicemic disease, that is, wasting, weakness, drowsiness, and diarrhea. There is rapidly developing anemia, and a leucocytosis In many cases the birds are found dead under the roosts in the morning without any symptoms having been noticed The lesions consist of thin anemic blood, multiple small necrotic areas in the liver and heart, and an enlarged spleen. The best means of making a diagnosis is the isolation of the causative organism which is easily accomplished

Some workers claim to have isolated this organism from diseased ovaries of hens, to have found it in eggs, and to have observed outbreaks of typical bacillary white diarrhea in chicks in which this organism was the causative agent On the other hand outbreaks of disease in adult birds which resembles fowl typhoid in every way have been attributed to *Bact. pullorum.*

Like other members of this group, pure cultures usually will cause fatal septicemia when injected into mice, guinea pigs, and rabbits

Immunity. Killed cultures have been used in attempts to control this disease, but the evidence indicates slight success.

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BACTERIUM ABORTIVOEQUINUS

Synonyms *Bacillus abortivus equinus, Bacillus abortus equi, Bacterium abortum equi, Salmonella abortivo-equinus.*

F. L. Kilborne (4), in 1893, made cultures from the vagina of an aborting mare in Pennsylvania. These were turned over to Theobald Smith (7) who studied the pure culture which developed on the media The organism was so like the hog cholera bacillus, with which he was then working, that he could not differentiate it with certainty The same organism was isolated by Good (1), in Kentucky in 1911, and by Meyer and Boerner (6) in Pennsylvania in 1913 It has been isolated from aborting mares in several European countries, from Pennsylvania, Kentucky, Iowa, and Minnesota in the United States, and from the Province of Ontario in Canada Without doubt it exists in many other parts of the world.

Morphology and Staining Reactions. These are typical of the group It is rapidly motile in young cultures

Cultural Features. These are typical of the group as a whole, although on solid media the older colonies tend to become dry and parchment-like Often such colonies can be pushed along the surface of the medium with the inoculating loop. For differential cultural reactions see Table X In fermentation reactions this organism is quite like *Bact enteritidis* except that it does not produce hydrogen sulphide In antigenic content it is related to *Bact typhimurium* The antigenic formula is IV, XII e, n, x : —.

Pathogenicity

FOR HORSES The organism apparently is spread mainly in the pasture Infective discharges from aborting animals contaminate the grass which then is eaten by susceptible animals Abortion can readily be produced in mares by mixing pure cultures with their feed, and by injecting cultures intravenously. Just before the act of abortion occurs the affected mare usually shows fever and other signs of a general reaction Some believe that the mare suffers a brief period of septicemia. If so, it disappears and the only lesions found after the abortion are in the fetal membranes which are edematous and frequently show hemorrhages and areas of necrosis.

FOR OTHER ANIMALS. Rabbits and guinea pigs may be killed by parenteral injection. For small experimental animals this organism has about the same degree of virulence as the other members of the paratyphoid group.

FOR MAN So far as is known, this organism is non-pathogenic for man.

Diagnosis. The specific organism may easily be cultivated by ordinary cultural methods from the placenta, fetus, or uterine exudate Agglutinins are produced in the course of the infection and the diagnosis may be made by making use of them According to Good and Corbett (2), normal animals may agglutinate the specific organism in dilutions of $\frac{1}{200}$ and occasionally as high as $\frac{1}{300}$ Infected animals usually agglutinate in dilutions from $\frac{1}{500}$ to $\frac{1}{5000}$

Immunity. Various workers have induced abortion in pregnant mares by injecting pure cultures of the organism intravenously Against such a method of infection it has not been possible to protect animals by methods of artificial immunization Against natural modes of infection (ingestion) bacterin treatment has proven highly satisfactory as a prophylactic procedure, according to Good and Dimock (3) In about 7,000 cases treated during a period of more than 10 years, only one case of abortion caused by *Bact abortivoequinus* has been found following vaccination The method of controlling abortion has also been practiced successfully by the U S Army, according to Koon and Kelser (5) On the other hand, some European authorities are skeptical of the immunizing value of dead cultures of this organism.

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BACTERIUM EQUIRULIS

Synonyms: *Shigella equirulis, Bacillus nephritidis equi, Bacterium viscosum equi, Bacterium pyosepticus equi, Shigella equi, Shigella viscosa.*

This organism is found frequently as the causative agent in purulent infections of the joints and in kidney abscesses in very young foals. It has occa-

sionally been found in adult horses *Bacterium equirulis* was first described by Meyer (4) who found it in kidney abscesses in horses in South Africa and who gave it the name *Bacillus nephritidis equi* Magnusson (3), finding the organism in foals in Sweden and not recognizing it as the same as the organism described by Meyer, named it *Bacterium viscosum equi* McFadyean and Edwards (2) recognized that the two organisms were identical

Morphology and Staining Reactions. This is a rod-shaped organism typical of the group but rather smaller than most of the others Short chains and filaments are often seen Capsules have been described but generally it is believed to be non-encapsulated It is non-motile, stains easily with the ordinary stains, and is Gram-negative

Cultural Features. *Bact equirulis* grows readily in ordinary media, producing rather abundant growth Colonies on agar plates are smooth, rather dry in appearance and tough Dissociation readily occurs, especially when the medium is acid and the incubation temperature high, the product being smaller, smooth, glistening colonies, some of which are dwarf types On agar slants the growth is diffuse, grayish-white and very mucoid A ropy sediment forms in broth and old cultures become very cloudy and mucoid A grayish pellicle sometimes appears Gelatin stabs show a filiform growth. There is no liquefaction Litmus milk is slowly acidified and sometimes coagulated The uncoagulated cultures generally are very slimy Indol is not formed and the Voges-Proskauer test is negative No toxins are generated Blood is not hemolyzed. Acid but no gas is formed from dextrose, lactose, sucrose, galactose, maltose, raffinose, xylose, and mannitol

Pathogenicity. In some horse-raising districts many foals are lost each season in from several days to several weeks after birth through the development of joint inflammations frequently accompanied by septicemia and pyemia Many of these cases are caused by streptococci, and some are attributed to colon bacilli, but a large number, one-half or more, are caused by *Bact equirulis* According to Dimock (1), who has had a large experience with this condition, the greater part of the natural infections occur *in utero*, the foal often being born dead, or it dies during the first, second or third day of life The joint lesions are not seen in those which die so early Many show no lesions whatsoever, others suffer from enteritis These cases usually show acute nephritis and the organism often is present in the joint capsules even though no sign of the disease can be seen in them Foals which live longer show more marked lesions; joint swelling and lameness, multiple abscesses in the cortex of the kidneys. The hock, knee, and hip joints are most often involved.

Mode of Infection. *Bact equirulis* is an inhabitant of the intestinal canal of many horses where it apparently exists harmlessly Dimock demonstrated it in cultures taken from the tonsillar region in 10 out of 12 horses which had died of causes unrelated to this disease, and others have had similar success He also noted that the common verminous aneurysms of the mesenteric arteries often are infected with this organism, even when it cannot be demonstrated elsewhere He believes, therefore, that the larvae of *Strongylus vulgaris,* migrating from the intestinal lumen into the arteries, carry *Bact equirulis* with them and in this way set up infections in young susceptible animals This would account only for the colts which develop trouble when several weeks or months old The others are thought to be prenatally infected, in a manner not known It is possible that strongyle larvae of the dam may invade the fetal circulation carrying the infection with it

Bact equirulis is not pathogenic for man Unless very large doses are given, it is not pathogenic even for the small laboratory animals.

Immunity. Specific treatment of foals with an antiserum prepared from *Bact equirulis* has been attempted but without encouraging results. After symptoms become evident, the prognosis is not good

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CHAPTER XVIII

THE HEMOPHILIC BACTERIA

The organisms of this group require iron-containing compounds for growth These compounds can be supplied most easily by adding small quantities of hemin, hematin, or methemaglobin, all constituents of red blood cells, to the basic medium. In addition most species require a substance (or substances) contained in fresh plant and animal tissues, others apparently are able to synthesize the second substance for themselves and therefore will grow in media devoid of it. Both of the necessary substances, or factors, are present in fresh blood. The factor which contains the iron compound is known as factor X. It is heat stable The second substance has been called factor V This can be destroyed by boiling, and it can be extracted chemically It is present in the fresh tissue juices of plants and animals, in yeast cells, and in many bacterial cells. The hemophilic bacteria cannot be cultivated in media enriched by serum alone, but the addition of a very small amount of the hemoglobin extract makes such media suitable for them

On plate cultures these organisms often exhibit the phenomenon of satellitism When colonies of other bacteria develop on plates containing hemophilic bacteria it can be observed that in the immediate vicinity of these extraneous colonies, the colonies of hemophilic bacteria develop to a much greater size. This has been shown to occur because of the diffusion of the V factor from such colonies

The first organism of this group to be described is that which is now known as *Hemophilus influenzae* This was described by Pfeiffer (3) in 1892 It was isolated from the upper respiratory tract of persons suffering from influenza and was believed to be the cause of that condition In the great pandemic of human influenza in 1918–1919, however, it was discovered that Pfeiffer's bacillus was not invariably present in the disease, and it was shown by English and American workers that influenza was caused by a filterable virus. The situation that exists between the virus of influenza and the bacillus of Pfeiffer is quite analogous to that existing between the virus of hog cholera and *Bact. choleraesuis*. The influenza bacillus undoubtedly plays a secondary role in human influenza, and has a part ofttimes in the complications which so often develop, but true influenza can occur in its absence.

During the pandemic of human influenza in 1918, there appeared in the

mid-western part of the United States a respiratory disease of swine which had not previously been seen. Koen who worked with the disease in Iowa was so impressed with its resemblance to the human disease that he dubbed it "swine flu" (1), and the disease soon became well known under that name. Only a few at that time believed that the swine disease had any connection with the human disease Its probable relationship became known a dozen years later through the work of Shope

HEMOPHILUS SUIS

Synonym *Hemophilus influenzae,* var *suis*

This organism was first described by Lewis and Shope (2) in 1931 In subsequent studies Shope (4) clearly demonstrated that whereas *Hemophilus suis* was relatively harmless to pigs when inoculated alone, it became highly pathogenic for susceptible swine when inoculated with a filterable virus The virus alone is also relatively harmless Swine influenza is a disease produced only by the concerted action of the bacillus and the virus The relationship between these two agents will be discussed more fully later under virus diseases (see p 603).

Morphology and Staining Reactions. *Hemophilus suis* is indistinguishable morphologically and tinctorially from the Pfeiffer bacillus of man. It is a very small rod-shaped organism, non-spore-bearing, non-motile, and Gram-negative Usually it is quite pleomorphic In young cultures it usually appears as thin rods measuring about 0 2 microns in breadth and from 0 5 to 2 0 microns in length Long thread-forms frequently are seen On some media these thread-forms may predominate Cultures older than 48 hours usually consist largely of coccoid elements, often arranged in large masses Giant coccoids, club-shaped forms, and comma-shaped cells often are seen in old cultures The organism stains rather poorly with ordinary stains Old cultures in particular often can hardly be stained satisfactorily, no matter what stain is used

Cultural Features. No growth is obtained on plain or glycerol-containing agar, in broth, gelatin, milk, potato, egg media or on coagulated blood serum, unless the growth factors X and V are added in the form of fresh blood, or extracts of blood Cultures must be incubated at about 37° C

For isolation Shope prefers to use agar slants to which 0 5 to 1 0 cc. of defibrinated blood has been added There is no growth on the slant The growth in the bloody fluid at the base of the slant is not evident until the fluid is examined microscopically. In such cultures the organism usually remains viable for about two weeks On blood agar plates, well-established cultures grow feebly in the form of minute colonies which have no observable effect upon

the medium. When other bacteria grow on the plates, the influenza bacillus colonies near the contaminating colonies grow very much more vigorously. Good growth usually occurs in blood broth

Another medium upon which this organism thrives is chocolate agar. This medium is made by adding defibrinated blood to agar at a temperature of 70° to 80° C. The surface colonies are circular in outline, grayish, flattened, semi-transparent, and have sharp edges Under the most favorable of conditions the colonies do not become larger than about 1 mm. in diameter Best growth usually occurs around the margins of colonies of other bacteria

Growth does not occur in litmus milk unless a little blood is added to it, and even then the growth is meager The milk is not changed in appearance. Carbohydrates are not fermented All strains reduce nitrates Neither hydrogen sulphide nor indol are formed

Pathogenicity. *Hemophilus suis* is only slightly pathogenic for normal swine After intranasal instillation a mild transitory illness sometimes occurs, but frequently there are no detectable symptoms When illness is evident, the disease does not transmit to pen-mates. When cultures are added to the virus of influenza, which alone will not cause serious illness in swine, typical influenza results and this disease will transmit naturally to pen-mates

The organism ordinarily is non-pathogenic for rabbits, guinea pigs and white rats. It occasionally proves pathogenic for white mice

Immunity. *H suis* will not immunize swine against influenza While it is essential to the production of the disease, it is clear that the role played is wholly secondary to that of the virus.

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HEMOPHILUS HEMOGLOBINOPHILUS

Synonym *Hemophilus canis.*

This organism is quite similar in morphology and cultural characteristics to the influenza bacillus It was first isolated and described by Friedberger (1) in 1903 under the name *B hemoglobinophilus canis.* Rivers (2) who studied this organism in 1922 reported that it formed acid from dextrose, levulose, galactose, saccharose, xylose, and mannitol Indol was produced and nitrates were reduced. The organism requires the X factor but can synthesize the V

factor. It has been isolated by several workers from the prepucial secretion of male dogs, where it seems to live a parasitic existence without doing much, if any, harm. It is non-pathogenic for laboratory animals.

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HEMOPHILUS GALLINARUM

Synonym *Bacillus hemoglobinophilus coryzae gallinarum.*

The name, *H gallinarum,* was proposed in 1934 by Eliot and Lewis (3) for an organism which had been described first by DeBlieck (1) in Holland under the name given as a synonym and which is invalid because it is not a binomial It had also been previously studied by Nelson (4) in New Jersey, by Delaplane, Irwin and Stuart (2) in Rhode Island, and by Pistor, Hoffman, Beach and Schalm in California (5) It is the cause of a serious and widespread disease of chickens known as *fowl coryza* The organism is very much like the influenza bacilli of man and swine, and the disease has some similarities, but unlike the other diseases this one seems not to be associated with a virus.

H gallinarum is isolated with some difficulty. Nelson succeeded first by using rather coarse Berkefeld filters which removed all ordinary bacteria but regularly passed this minute organism Later he found that colonies could be obtained on blood agar plates seeded with nasal exudate, providing the plates were sealed with wax DeBlieck, and Eliot and Lewis appear to have recovered it without sealing It is not wholly certain that the organisms isolated by these workers were identical.

Morphology and Staining Reactions. The organism is of about the same size and shows the same pleomorphism as the swine influenza organism. If examination of cultures is postponed for a few days, the organism stains poorly, or perhaps has disintegrated. It is Gram-negative, non-motile, and facultative anaerobic.

Cultural Features. Both X and V factors are necessary for growth. For original cultures slant agar with a small amount of sterile defibrinated blood added to the water of condensation is a favorable medium. The organism grows in the bloody fluid at the foot of the slant without altering its appearance. After the first two days of growth it is difficult to demonstrate the organism microscopically.

On blood plates the organism grows best in the vicinity of colonies of other bacteria. Nelson found that best growth was obtained by sealing the plates. The colonies are very small, bluish-white, and transparent.

Growth on chocolate agar resembles that of the influenza bacillus There is no growth in gelatin, plain broth, milk, potato, and carbohydrate media. So far as has been determined no carbohydrates are fermented, and indol is not formed Nitrates are reduced to nitrites, however

Pathogenicity. The exudate of natural cases of fowl coryza is highly infectious when introduced into the palatine cleft of susceptible birds Filtrates of this material are not infectious except, as Nelson showed, when coarse Berkefeld candles are used for the filtration, and the filtrates are incubated for a time in the presence of fresh chicken blood Pure cultures will reproduce the disease and such cultures will retain their virulence for chickens after many generations in artificial media Rabbits and guinea pigs are resistant to injections of pure cultures Turkeys, pigeons, and many other species of birds are also refractory

Immunity. Successful methods of immunizing against fowl coryza have not been developed Even natural immunity appears to disappear after a few months and birds that have recovered may again suffer a new infection Many recovered birds remain carriers of virulent bacilli and it is in this manner that the disease appears to be propagated from year to year.

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HEMOPHILUS OVIS

This organism was found and described by Chas. A Mitchell (1) in 1925 There have been no other reports on it. It occurred in a single herd of sheep in Canada, where it apparently was the active agent in a disease which caused acute illness and death in a considerable number of animals The symptoms and lesions of the natural disease were produced artificially by inoculating sheep with a pure culture

Morphology and Staining Reactions. *H. ovis* is a small, Gram-negative rod, rather pleomorphic and non-motile. It stains poorly with all stains Coccoid forms which stain better than the other cells frequently are found in cultures. Dilute carbol fuchsin is the best stain for routine use

Cultural Features. Cultures grow best at 37° C. but some growth occurs at 28° C. It does not form indol but reduces nitrates. Blood is not hemolyzed. No growth occurs on potato or in litmus milk In stab cultures growth occurs only at the surface In blood-milk, no gross changes occur On plain agar and in plain broth, no growth occurred in the beginning but after a few generations on chocolate agar, feeble growths were obtained in the unenriched media. After persistent effort strains were adapted to grow fairly well on the plain media

Best growth was obtained on heated blood (chocolate) agar slants Colonies came up in from 24 to 36 hours as pinhead size, glistening, moist, grayish colonies When touched with the needle they were found to be viscid.

H ovis requires only the heat stable factor (X factor) although growths were more luxuriant when the blood was heated below the point at which the Y factor is destroyed Acid but no gas is formed from dextrose, lactose, saccharose, maltose, mannose, mannitol, galactose, levulose, raffinose, and sorbitol. Rhamnose, arabinose, salicin, and inositol are not attacked

Pathogenicity. The onset of the disease was sudden and featured by great prostration, marked cyanosis, labored and distressed breathing, and fever in the early stages Sixteen out of seventeen naturally infected animals died within a few days Some animals discharged a bloody fluid from the nostrils, and most of them discharged bloody feces. The lesions consisted of bronchopneumonia which was always bilateral and involved the anterior lobes, intense congestion of the abdominal organs, especially the kidneys, petechiae on various organs, and a yellow, friable liver having a cooked appearance. The lesions were reproduced faithfully in a sheep which received culture intratracheally

Pure cultures killed guinea pigs and rabbits but proved innocuous for chickens.

Immunity. There is no information on this point. Mitchell failed to demonstrate toxin in culture filtrates.

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CHAPTER XIX

THE BARTONELLA GROUP

From very ancient times, a disease of man, characterized by fever, anemia, and a fairly high mortality, has existed in Peru under the name of *Oroya Fever.* Milder cases often show warty eruptions and this form is known as *Verruga Peruviana* In 1905 Barton (1) found a small cocco-bacillus within and attached to the red blood cells in Oroya Fever The name *Bartonella bacilliformis* was given to this organism by Strong (9) in 1913 The nature of the organism was unknown until Noguchi (8), in 1926, cultivated it in artificial media, and reproduced the essential manifestations of both Oroya Fever and Verruga Peruviana in monkeys. It is now regarded as a bacterium, although very little is known about it The organism can be transmitted by the tick, *Dermacentor andersoni,* but it is transmitted principally by a gnat belonging to the *Phlebotomus* group. The group is briefly described here because similar organisms cause disease in rats and dogs, and both diseases appear to be rather widespread although masked It may be that these infections will ultimately be found to be much more important than is now believed

BARTONELLA MURIS

This organism was first described by Mayer (7) in Germany in 1921 It was found in laboratory rats which had been infected with trypanosomes The organism closely resembles the one previously described, and like the previous one is found in and on the surface of the red blood cells The organism can be cultivated on blood agar, on which small translucent colonies are produced. The organism is motile It grows best at about 25° C It usually will not cause infection in rats, unless the animals are infected with trypanosomiasis, are given certain blood-destroying poisons, or are splenectomized. Of great interest is the fact that rats in many parts of the world, including the United States, carry this organism latently, as can be demonstrated by the fact that removal of the spleen often will lead to prompt development of the disease It appears that the spleen, possibly through a protective activity of its reticulo-endothelial system, is able to hold the disease in check. The disease is manifested by a rapidly developing anemia and by the appearance of the organism in the blood The animals may die of the disease, or they may recover after a few days

BARTONELLA CANIS

In 1928, Kikuth (2), in Germany, described an organism similar to the other *Bartonella* which he believed to be the cause of an infectious anemia of dogs. Workers in other countries have, more recently, seen the same organism. None have succeeded in cultivating this organism, however, hence its relationship to the other *Bartonella* has not been proved The disease appears to be rather mild Knutti and Hawkins (3), in the United States (1935) encountered the condition in splenectomized, bile-fistula dogs Spontaneous periods of anemia, associated with excess bile production, were regularly associated, in some dogs, with the appearance of *Bartonella*-like bodies in the blood Simple splenectomy would not regularly produce the disease, but inoculation of blood of dogs containing the *Bartonella* into such animals was regularly followed by anemia and the appearance of the parasite.

BARTONELLA BOVIS

Donatien and Lestoquard (4) reported *Bartonella* in the blood of cattle in 1934 Lotze and Yiengst (6) found similar forms in American cattle in 1942. Since they were found in animals infected with anaplasmosis the American workers were not certain that they did not represent a stage of the life cycle of *Anaplasma marginale* Later Lotze and Bowman (5) found them in an anaplasma-free calf shortly after it had been splenectomized It is clear, therefore, that *Bartonella bovis* is not necessarily associated with anaplasmosis. Usually only a very few parasites are found in the blood, but they may become much more numerous during the incubation period following inoculation with anaplasms There is no evidence, at present, that *Bartonella bovis* is of any economic importance

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CHAPTER XX

THE LISTERIA GROUP

In 1927, Pirie (12) in South Africa isolated an organism from a plague-like disease of the gerbille (a rodent) for which he created a new genus called *Listerella* Inasmuch as it was pointed out to him later that this name had been pre-empted for a genus of slime molds, he proposed in 1940 that the name be changed to *Listeria* (13), a suggestion which will be followed here

Closely related, and possibly identical with Pirie's organism is one which Murray (of England) (9) first found in stock rabbits, producing in them a mononucleosis, one which Gill first found in sheep in New Zealand which produces "circling disease" of sheep and cattle, one which Ten Broeck found in a chicken in New Jersey which produces necrotic myocarditis and pericarditis, and one which Schultz in California and Burn in Connecticut found in cases of fatal meningo-encephalitis of man The name *Listeria monocytogenes* has been applied to the rabbit organism It is not quite clear that the species found in the other animals are identical with that of the rabbit, but at least they are very closely related They will all be described here under the name of the rabbit organism

LISTERIA MONOCYTOGENES

Synonym *Listerella monocytogenes*

Morphology and Staining Reactions. This organism occurs in the form of small rods, one to two microns in length, which frequently show slight clubbing and therefore appear like diphtheroids Coccoid elements are commonly found Young cultures are actively motile. Spores are not produced It is Gram-positive and non-acid-fast Usually Gram-negative cells can be found in young cultures and old cultures often are nearly wholly Gram-negative

Cultural Features. Growth occurs on most of the ordinary laboratory media although it is never abundant In general the gross features of cultures resemble those of streptococci

For isolation we prefer blood plates The colonies may be seen after 24 hours' incubation at 37° C as minute points (deep colonies) and as small, flat, bluish-white, transparent surface colonies The deep colonies are surrounded by narrow zones of hemolysis of the Beta type, and this characteristic makes

them conspicuous The surface colonies seldom if ever exceed 1 mm. in diameter.

Broth is faintly and uniformly clouded Growth is favored by the presence of a little sterile serum or defibrinated blood When blood cells are present, hemoglobin, released from them, diffuses upward from the layer of sedimented cells Dextrose greatly favors growth

Stab cultures in gelatin appear as a line of discrete colonies along the stab Seastone says that an especially characteristic growth may be seen in semi-solid agar containing dextrose Stab cultures in this medium usually show a cloud of minute colonies surrounding the line of the stab



FIG 30 *Listeria monocytogenes* Culture from a serum agar slant incubated for 18 hours at 37° C x 900

Acid without gas is formed from dextrose, rhamnose, and salicin within 48 hours Sucrose and dextrin are fermented, but more slowly Results are inconstant with maltose, lactose and glycerol These substances usually are fermented slightly and slowly

Litmus milk supports growth but there is little change in the appearance of the medium as a rule Sometimes it is slightly acidified There is no growth on potato Hydrogen sulphide and indol are not formed, and nitrates are not reduced

Pathogenicity

FOR RABBITS Large doses given intravenously cause a marked mononuclear leucocytosis of the myeloid type, focal necrosis of the liver, necrotic areas in the myocardium, and extensive involvement of the meninges. These lesions are also found in the guinea pig and other rodents The natural disease in the gerbille, known as the Tiger River Disease, described by Pirie (12), presented lesions of these types

FOR CATTLE AND SHEEP Infection in sheep was first described by Gill (4) of New Zealand in 1931 It was he who gave the illness the name of "circling disease" because of the characteristic actions of affected animals. This infection obviously is quite widely scattered in the eastern part of the United

States. It was first diagnosed in cattle by Jones and Little (6) in New Jersey in 1934, in 1935 by Fincher (3) in New York cattle, and by Olafson (10) in 1936 in New York sheep Later the disease has been reported in cattle in Illinois, and in sheep in Connecticut, Illinois, and Iowa

In sheep and cattle the disease caused by this organism is an encephalitis The cerebrospinal fluid may be cloudy and there may be some congestion of the meninges Usually the visceral organs show little or no evidence of disease. Sections of the brain of such animals show polymorphonuclear and

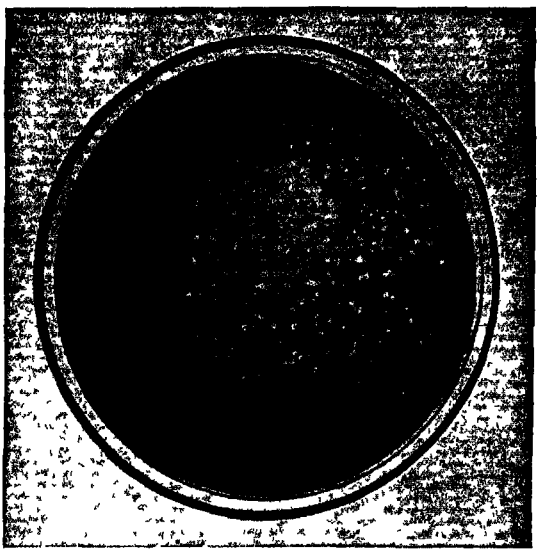


FIG 31 *Listeria monocytogenes* Blood agar plate incubated at 37° C for 24 hours The colonies are not discernible in the photograph They are very minute, each being surrounded by a sharply defined but narrow zone of Beta-type hemolysis Reduced one third

mononuclear foci in the white matter of the cerebrum and cerebellum and perivascular cuffing with mononuclear cells The causative organism can be readily isolated from these brains, but the organisms often appear to be present in small numbers, hence it is well to inoculate the cultures liberally. Affected sheep show signs of depression, weakness, inco-ordination of movement, fever, walking in circles, pushing against objects, progressive paralysis, and death within two or three days The symptoms in cattle are similar

Graham, Hester and Levine (5) isolated this organism from the stomach content of an aborted bovine fetus, and from the liver and brain of a rabbit

which had been inoculated with the same material. There was no history of listeriosis in the herd from which the animal came The aborting cow had been slaughtered immediately after aborting and was not available for study.

FOR CHICKENS Ten Broeck, whose observations were recorded by Seastone (16) in 1935, isolated this organism from a case of necrotic myocarditis in a chicken in 1932 Patterson (11) recorded the disease in four flocks in England in 1937. The latter found only one case of necrotic myocarditis in seventeen fowls studied, hence it appears that this is not a common finding The affected birds suffered from emaciation and general weakness The lesions consisted of edematous tissues, fluid in the body cavity and in the pericardial sac, and focal necrosis of the liver The organism was readily isolated from all birds by culturing the liver

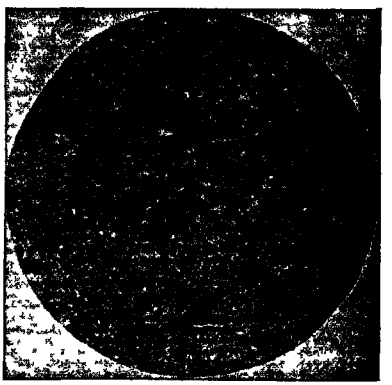


FIG 32 Listeriosis, Brain, Cow The cells infiltrating the brain substance are both mononuclears and polymorphonuclears There is marked perivascular cuffing x 100 (Courtesy of S H McNutt)

FOR SWINE Biester and Schwarte (1) have seen several outbreaks of listeriosis in swine in Iowa Young animals were affected more commonly than old The symptoms were vague but suggestive of disturbances of consciousness. The lesions in the central nervous system were not marked, but were similar to those seen in sheep It was their belief that diagnosis of this condition in swine could be made with certainty only by recovering the causative organism

FOR MAN. Burn (2) found an organism which he concluded was identical with strains isolated from cattle by Jones and Little (6) in four human infections. Three of these were new-born infants who died within a short time. All showed focal necrosis of the liver and meningitis with a thick green exudate in the subarachnoid space covering the medulla, pons, and parietal lobes. In one case a pneumococcus was isolated in addition to the *Listeria*, in the other two the *Listeria* was in pure culture A fourth case was of a 53-year-old man suffering from bilateral otitis media followed by meningitis A type II pneumococcus and *Listeria* were isolated from the brain at autopsy. The lesions resembled those seen in the infants Schultz, Terry, Brice, and Gebhart

(15) also saw a case of meningoencephalitis similar to those described by Burn.

Pons and Julianelle (14) isolated a culture of *Listeria* from the blood of a girl suffering from a disease which was diagnosed as infectious mononucleosis or glandular fever The patient showed fever, angina, enlarged axillary and cervical glands, a palpable spleen, and a leucocytosis varying from 11,000 to 17,000 with 40 per cent monocytes These authors (7) observed that young cultures, when applied to the conjunctiva of rabbits, guinea pigs or rats, caused a distinct, purulent conjunctivitis which developed in from 1 to 5 days When the swollen lids were forced apart a thick, heavy exudate composed largely of mononuclear cells was expelled The conjunctiva was acutely inflamed, the cornea became opaque and pitted, and the blood vessels pushed out to the pupillary margin The reaction subsided in from 5 to 10 days and the lesion healed in from 1 to 3 weeks They suggested the value of this test for diagnosis Graham and co-workers found that strains isolated from sheep and cattle gave this reaction in the rabbit's eye

Immunity. Julianelle and Pons (8) found that eight strains which they studied fell into two agglutinative groups Their Type I was composed of two rabbit and two human strains, and Type II contained one cow, one goat, one sheep, and one human strain Seastone had previously noted that a rabbit strain agglutinated differently than strains from other animals It was suggested that possibly it would be found that Type I was essentially a rodent type (rabbit, gerbille) and Type II a ruminant type (sheep, bovine) The organism found by Graham et al (5) in a bovine fetus was tested with type serum from Julianelle and found to fall into the rodent group

Olafson (10) succeeded in immunizing several sheep so they would partially withstand intravenous inoculation of culture The disease is so sporadic that immunizing procedures, even if successful ones were available, would not be of great service

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CHAPTER XXI

THE SWINE ERYSIPELAS GROUP

Two species are generally recognized in this group although it appears that these organisms probably are but variants of a single species The "mouse septicemia bacillus" was first described by Koch (8) It was obtained by inoculating a mouse with putrid blood It is known as *Erysipelothrix muriseptieae* In 1885 Loeffler (9) recognized and isolated the organism of swine erysipelas, *Erysipelothrix rhusiopathiae* The microscopic and cultural features of these two organisms are identical but the pathogenicity differs Organisms apparently identical have been isolated from lambs suffering from arthritis, and from sick fowls They also have been isolated from apparently normal tonsils and mucous membranes of swine, from decaying plant and animal tissue, and from the slime on the bodies of both fresh and salt-water fish They are found in a local skin affection of man known as "erysipeloid" This condition is seen in persons who handle pork and pork products, and fish.

ERYSIPELOTHRIX RHUSIOPATHIAE

Synonyms *Bacillus rhusiopathiae suis, Bacterium erysipelatos suum, Bacterium rhusiopathiae*, the swine rotlauf bacillus

This organism is the causative agent of swine erysipelas, a disease of tremendous economic importance in continental Europe Until recently the disease was thought not to exist in this country although Moore (12) in 1892, isolated an organism from the tissues of diseased swine which he believed to be that of swine erysipelas In 1920 Ten Broeck (18) isolated the organism from the tonsils of 5 of 16 pigs affected with hog cholera in New Jersey In 1921 Creech (4), of the United States Bureau of Animal Industry, succeeded in isolating it from the skin lesions of the "diamond skin disease," a condition which had been recognized for years as similar to the mildest form of the disease as it exists in Europe This finding removed all doubt as to the presence of the disease in this country In 1922 Ward (21) called attention to the fact that a form of polyarthritis in swine in this country is due to this organism In 1931 acute swine erysipelas with serious losses occurred in some isolated areas in South Dakota Since that time the disease has been found in many parts of the United States In some parts of the swine belt (South

Dakota, Nebraska, Iowa) the disease has developed into one of the major problems of the industry as it has been for many years in continental Europe.

Morphology and Staining Reactions. The organism usually occurs as a small, slim rod which is straight or slightly curved In cultures made from rough colonies long filaments are commonly found These often are beaded and exhibit swollen areas It stains readily with ordinary stains and is Gram-positive It is non-motile and does not form spores



FIG 33 *Erysipelothrix rhusiopathiae* Culture on serum agar, incubated for 24 hours at 37° C x 900

Cultural Features. On agar and coagulated blood serum small, delicate colonies, so fine that they may readily be overlooked, are formed On gelatin the colonies are very small and have a fuzzy appearance When magnified this is seen to be due to filaments which radiate from the central core Gelatin stabs are particularly characteristic. Beadlike colonies form along the line of stab, these finally coalesce to form a spike from which fine filaments push outward into the medium at right angles, giving the whole growth the appearance of an inverted delicate test tube brush The gelatin is not liquefied Broth is slightly clouded and a flaky sediment collects in the bottom of the tube There is no growth on potato Milk is not usually altered, but occasionally it is slightly acidified Narrow discolored zones appear around deep colonies on blood agar plates. Hydrogen sulphide is produced and nitrates reduced Indol is not formed. Fermentation reactions differ according to strain Some strains apparently do not ferment any carbohydrates but most ferment dextrose, lactose, and levulose

Resistance. The organism is rather resistant to drying, and to such procedures as smoking, pickling, and salting Farms which are once infected with the disease will usually experience recurrences of it from year to year It is thought that the organism may live over from year to year on the pastures and in filth It will survive for relatively long periods in putrefying flesh and

in water It is not resistant to heat. Cultures are destroyed by exposure to moist heat at 55° C for 10 minutes

Pathogenicity

FOR LABORATORY ANIMALS White mice and pigeons are very susceptible to infection by inoculation and are commonly used in diagnostic work After subcutaneous inoculation they usually die in from 18 hours to four days Rabbits are not highly susceptible Usually a local reaction occurs and the animal may die after six or seven days Guinea pigs are quite resistant Inoculated mice usually show evidence of conjunctivitis, first serous and later purulent, which glues their eyelids together They sit with arched backs, roughened hair, and do not eat The lesions consist of enlargement of the spleen, discrete grayish foci in the liver, and occasionally congestion of the lungs The blood and spleen contain large numbers of organisms, many of which have been taken up by phagocytes Pigeons which have been inoculated into the breast muscles, show swelling and hemorrhagic inflammation around the point of inoculation The spleen is swollen and the liver may show focal necrosis The organism is abundant in the blood and tissues Wayson (22) has given a good account of a natural outbreak of this disease in wild mice and Balfour-Jones (1) has described an outbreak in a stock of laboratory mice

FOR SWINE Inoculation or feeding of cultures does not regularly produce the disease, yet it is quite certain that natural infection occurs principally through ingestion In the acute septicemic form of the disease in swine, the erysipelas organism may be found in the intestinal content in large numbers, and sometimes it occurs in the urine The infection is spread through the discharges of such animals and the contamination of food materials thereby The organism has been found in the tonsils of apparently healthy pigs and in the flask-shaped glands that occur on the ileo-cecal valve It is apparent that such animals act as carriers of virulent infection

Several forms of swine erysipelas are recognized The acute form of the disease is a septicemia The spleen and lymph nodes are enlarged and reddened, and the mucosa of the stomach and small intestine is acutely inflamed, hemorrhagic, and sometimes ulcerated The kidneys generally show cloudy swelling and are often petechiated Red patches commonly occur on the skin, particularly of the ears, abdomen, and insides of the legs The mortality from this form of the disease is very high

The chronic form of the disease nearly always takes the form of a vegetative endocarditis The heart valves, particularly the mitral, are eroded and become so covered with fibrin deposits that their functioning is seriously impaired.

Affected animals invariably die from this condition, sooner or later, and often suddenly.

The arthritic form of the disease generally occurs in older animals, although arthritis may be part of the picture in the more acute forms of the disease. The joints become enlarged and painful, the animals are reluctant to move, the gait is stilted, and the animals become stunted in growth

The urticarial, or skin form of the disease, often occurs in association with internal lesions, or it may occur without evident involvement of the viscera. The lesions consist, in the beginning, of reddish or purplish rhomboidal blotches on the skin, several centimeters in diameter and found principally



FIG 34 Diamond Skin Disease, Pig Characteristic lesions of one type of swine erysipelas (Courtesy of R A McIntosh)

on the abdomen The shape of these blotches is like that of a diamond of a playing card and has given the disease its common name of "diamond skin disease" The urticarial areas later become necrotic, the affected skin dries into dense scabs which finally peel off leaving a bleeding area if removed too soon

FOR SHEEP Pools (15) in 1913 described a polyarthritis in sheep caused by the swine erysipelas organism The disease was first recognized in this country by Ray (16) in 1930, and by Marsh (11), working independently It has been described in several countries of Europe and in New Zealand.

The disease is seen in lambs, beginning when they are from two to three months of age It is thought that the disease is contracted through umbilical infection but apparently this has not been proved The affected animals develop a stiff gait. They eat well but do not thrive. Advanced cases often get down and find difficulty in arising Affected animals seldom die from the disease. Lesions are not present in the visceral organs, in fact they are found

nowhere except in some of the joints of the legs One joint or several of them may be affected The involved joint usually is swollen and the joint capsule is thickened Granulation tissue occurs on the inner surface of the capsule The fluid usually is thin but pus cells can be found on smears The specific organism usually cannot be found in smears, but cultures are easily obtained. The organism is in every way typical

FOR BIRDS According to Van Es and McGrath (19), the swine erysipelas organism is pathogenic for turkeys, chickens, geese, ducks, mud hens, pigeons, parrots, quail, and many small wild birds and larger species often found in zoological parks In this country the species most often and most seriously affected is the turkey The first outbreak of this kind was recognized by Beaudette and Hudson (2) in 1936 In 1938 Van Roekel, Bullis and Clark (20) described three outbreaks occurring in Massachusetts, Vermont and New York Madsen (10), shortly before, described an outbreak in Utah It is apparent that the disease is an important one from an economic viewpoint in this country as well as abroad

Affected turkeys usually are adult, or nearing adult age They exhibit a cyanotic skin which is most obvious as a "blue comb" The birds become droopy, develop diarrhea, and die The lesions consist of massive hemorrhages and petechiae in the muscles of the breast and legs, also large hemorrhages on the various serous membranes, particularly those of the heart Hemorrhages occur in the mucosa of the gizzard and of the small intestine, and the content of the intestine often is bloody. The liver and spleen are ordinarily congested and enlarged The causative organism can easily be isolated from any of the tissues

Graham, Levine and Hester (5) described an outbreak of erysipelas infection in a large flock of ducks in which between ten and twelve thousand ducklings about ten weeks of age were lost

FOR MAN Many cases of wound infection of the hands have been reported In Europe most of the human cases have been attributed to the handling of infected swine and pork, but some have occurred in fishermen and fish dealers who have had no contact with swine According to Klauder, Righter and Harkins (7), the disease is fairly common among fish handlers along the entire Atlantic seaboard of the United States Human infections with this organism were well discussed by Klauder (6) in 1938 More than half of the cases recognized in the region of Philadelphia were in slaughter-house employes who, presumably, were infected from pork and pork products.

Klauder studied a number of cases among veterinary students who apparently had contracted infection from horse carcasses in the dissecting room.

Morrill (13) has also reported on student infections contracted in this way. In this instance the specific organism was isolated from one of the horse carcasses on which one of the infected students had been working.

The infection in man is known as "erysipeloid" to distinguish it from human erysipelas which is caused by a hemolytic streptococcus Rarely the disease is manifested by septicemia which is likely to be fatal. The usual type is manifested by a local lesion developing from an abrasion of the skin where the infection enters The lesion usually is on one of the fingers The intensity of the inflammation varies The infected finger swells, and usually the swelling extends throughout the entire hand There is no suppuration, and no pitting on pressure A throbbing and burning pain which usually prevents sleep is a conspicuous symptom There is marked erythema of the infected region, sometimes local arthritis In the majority of cases the infection runs a course of about three weeks, a few heal in a shorter period and some require much longer Local treatment of the wound is of little value Wet antiseptic dressings are applied and the hand often can be immobilized with advantage by splinting Erythema doses of ultra-violet light sometimes exert a favorable influence Specific antiserum is used when there is evidence of arthritis and when the infection tends to spread unduly Klauder doubts its value in the usual, uncomplicated cases Fortunately the disease usually is self-limiting and will heal ordinarily within a month



FIG. 35 Erysipeloid Hand of a butcher affected with erysipeloid A small puncture wound in the palm had been made by a sharp bone splinter from a pig carcass Note the small wound and the large erythematous area surrounding it (Courtesy of J V Klauder)

Source of Infection. The excreta of infected swine contain large numbers of organisms and it is through the ingestion of food contaminated with such material that infections usually occur. Carrier animals exist and such animals probably serve to spread the disease. Community sales-yards and stock-yards frequently are sources of infection Pork trimmings in garbage probably account for many sporadic cases. It should be remembered that this organism is unusually resistant to smoking and salting, hence pickled and smoked products often are capable of causing infections. It has been suspected also that fish products, often used as protein supplements in animal foods,

may be responsible for introducing infections. We have repeatedly isolated the erysipelas bacillus from samples of commercial ground fish meal, indicating that the processing of such material is not always associated with enough heating to destroy this organism

Diagnosis. In swine the differential diagnosis between erysipelas and hog cholera often occasions considerable difficulty In recently infected lots of swine, erysipelas often sweeps through them with symptoms and lesions somewhat resembling those of cholera Schoening, Creech and Grey (17) introduced a rapid, whole-blood, agglutination test which can be used in the field and which is highly accurate in detecting the sub-acute and chronic cases A heavy suspension of the causative organism, taking care that only smooth-types of the organism are used, is mixed on a slide with a drop of freshly drawn blood, in the proportion of one drop of blood to two of the bacterial suspension Clumping of the bacilli within two minutes is indicative of infection Breed (3) has described a precipitation test which he considers to be reliable This test can be done only in a laboratory

Immunity. Three procedures for immunization of swine have been used successfully These are known as (1) The serum alone method, (2) The vaccine method, and (3) The simultaneous method, in which living culture and immune serum are used together

IMMUNE SERUM Immune serum is of value both for prophylaxis and for treatment For treatment, 10 to 30 cc of serum is injected as early in the course of the disease as possible Losses may occur in spite of such treatment For prophylaxis the serum is very successful, the principal disadvantage being that the immunity is short-lived It can be depended upon to protect for not more than 15 days For animals weighing less than 100 pounds, five cc of the serum is sufficient For larger animals, one cc additional is allowed for each 20 pounds in excess of 100 pounds, body weight

Unless special permission is obtained from the U S. Department of Agriculture, this method of immunization is the only type permitted in the United States This restriction is believed advisable because swine erysipelas is not prevalent, except in some of the mid-western states, and danger of spreading the disease through the unwise use of living cultures is thus avoided.

VACCINE METHOD This is the oldest method of immunization to this disease. A vaccine was developed and successfully used by Pasteur and Thuillier (14) in 1883, and their method continues to be used until the present time

Attenuation of the culture for swine was accomplished by passing it through rabbits In the course of time such cultures acquire great virulence for rabbits but simultaneously they lose virulence for swine Two strains are used,

the most attenuated being injected first, and the second about a week later. The procedure usually immunizes safely for periods of eight months to one year which ordinarily is as long as is necessary since the life span of swine is not much longer than that Breeding stock may require reimmunization after one year

This method is not wholly safe since vaccination erysipelas often occurs For this reason, it has been largely superseded by the following method

SIMULTANEOUS METHOD The animals are given a dose of virulent culture and at the same time a dose of immune serum The serum protects the animal from the full effects of the culture while the latter stimulates an active immunity This method is commonly used in countries where losses from the disease are great enough to warrant active immunization of herds The live culture is capable of maintaining the infection in herds and in spreading the infection, of course, and for this reason active immunization of swine to erysipelas has been prohibited in the United States until quite recently In some of the middle western states where the disease has become prevalent rather extensive experiments have been conducted on the effect of the use of the serum-culture method of immunization during the last several years More than 500,000 pigs have been treated on farms in this area On the basis of this experience, the U S Bureau of Animal Industry announced on May 18, 1942 that limited commercial licenses had been issued for the manufacture of live culture for simultaneous immunization of swine and its sale in areas where state and national authorities agree that use of this method of immunization is justified.

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CHAPTER XXII

THE ORGANISM OF GLANDERS

The causative agent of glanders has stood for many years in a group by itself Quite similar to it, and classified with it by some authors is the bacillus causing *melioidosis*, a disease of man and rodents in Malaya and other parts of southeastern Asia This organism is known as the bacillus of Whitmore, or *Malleomyces whitmori*

MALLEOMYCES MALLEI

Synonyms *Bacterium mallei, Bacillus mallei, Pfeifferella mallei, Loefflerella mallei, Corynebacterium mallei, Mycobacterium mallei*

M mallei* is the cause of glanders, a disease primarily of solipeds (horses and the horse family) It also affects man and occasionally other kinds of animals, especially the members of the cat family which have fed on infected horse meat The disease is one of the oldest known It was described by the ancient Greeks and Romans As early as the 17th century it was recognized as contagious, but there were dissenters from this belief as late as the middle of the 19th century The infectious nature was proven by inoculation tests long before the causative agent was found The organism was isolated and shown to be the etiological agent by Loeffler and Schutz (8) in 1882

Morphology and Staining Reactions. In young cultures the cells are long slender rods Older cultures often are quite pleomorphic, the bacilli varying in size and shape from coccoid elements to long slender filaments The longer rods usually are distinctly beaded, the shorter may be bipolar because of granules lying in each end of the cell In size the width is from 0 3 to 0 5 microns and the length from 0 7 to 5 0 microns The cells are always Gram-

* The proper name for the genus in which the glanders bacillus belongs is a matter of considerable uncertainty Buchanan, in 1916, proposed that it represent the type species of a new genus for which he proposed the name *Pfeifferella* Inasmuch as Pfeiffer had had nothing to do with the organism, the appropriateness of the name was questioned and this brought from Buchanan the admission that the name was the result of a clerical error and that he had intended to propose the name *Loefflerella* in honor of Loeffler who had first described it Since the new generic name, inappropriate as it might be, had been published and therefore was valid, Buchanan, in order to avoid further confusion, did not attempt to correct the error Gay and Associates, in their recent text, have adopted the name *Loefflerella* but this lead has not been followed by others The fifth edition of Bergey's manual has dropped both *Pfeifferella* and *Loefflerella* and has adopted the name *Malleomyces* which had been proposed earlier than either of the others This seems logical and this name, therefore, is used in this text

negative With the weaker dyes, it stains rather poorly Spores are not formed, there are no capsules, and there are no flagella

Cultural Features. *M mallei* grows well but rather slowly upon ordinary laboratory media, particularly if they contain glycerol It is rather insensitive to acidity and will grow well on media which are too acid for most pathogenic bacteria

After a few days' incubation at 37° C the surface of glycerin agar slants becomes covered with a confluent growth slightly cream colored, smooth, moist, and viscid Continued incubation causes the blanket of growth to increase in thickness and the color to darken until it is a dark brown It is now so viscid as to make it difficult to remove bits of growth with the inoculating loop Upon plain agar the growth is much less luxuriant



FIG 36 *Malleomyces mallei* Film from exudate in the scrotal sac of a guinea pig infected by intraperitoneal inoculation of a pure culture x 900

Very characteristic is the growth upon glycerol-potato Old potato cultures generally become exceedingly luxuriant, the blanket of growth being slimy and then viscid, light tan in the beginning and a mahogany brown finally

In glycerol broth a viscid sediment forms and if the cultures are not disturbed a heavy, slimy pellicle forms from which stalactites stretch in the medium toward the bottom of the tube or flask The broth gradually darkens Cultures several weeks old become coffee-colored

The growth on gelatin usually is poor and ordinarily there is no liquefaction, although some authors have described strains which caused slow liquefaction Litmus milk is slightly acidified and coagulation may occur after long incubation Carbohydrates usually are not fermented but dextrose media may be slightly acidified

Indol is not produced, nitrates are not reduced, and blood is not hemolyzed

Resistance. The organism possesses only slight powers of resistance to drying, heat and chemicals Outside of the body it probably cannot, under the most favorable of conditions, exist longer than two or three months.

Pathogenicity. The organism is highly pathogenic for horses, mules, and asses It is less so for cats (wild and tame), dogs, goats, and man Sheep, swine, and cattle are highly resistant. Guinea pigs are easily infected artificially, rabbits less easily The disease occurs almost entirely in the horse species and in carnivora which have consumed infected meat Man is infected only occasionally when handling animals.

The infection in horses may be either acute or chronic The latter is by far



FIG 37 Lesions of Glanders, Lung, Horse This lung is extensively involved Not only are there nodules but the hemorrhages indicate that there is a diffuse involvement with glanderous pneumonia

the most common form In mules and asses the acute form is more frequent than in horses

The mode of infection is a disputed question, however, it appears probable that ingestion is more important than inhalation Infection of wounds of the skin occurs, though probably rather rarely According to the work of Nocard (16), and of McFadyean (11), infection can easily be produced by feeding infected materials. In some cases lesions occur in the mesenteric lymph glands, but many times the lesions appear in the lungs and the mucosa of the upper air passages without evidence of disease processes in the intestine where the infection took place The intestinal tract therefore probably has a considerable degree of organ immunity The lungs appear very susceptible since they are nearly always involved irrespective of the port of entry.

The lung lesions may take the form of nodules, or of a diffuse, pneumonic process The nodules have a characteristic histologic structure by which they may be recognized (3) (11) This structure is not unlike that of a tubercle Through the rupture of lung nodules into bronchi and the carrying of infective material upward, the upper air passages frequently become the seat of characteristic lesions Apparently, also, these lesions can occur in animals by direct metastasis from the portal of entry for sometimes well-marked



FIG 38 Lesions of Glanders, Nasal Septum, Horse Shown are ecchymotic hemorrhages and the superficial ulcers from which a sticky discharge exudes

lesions occur in the upper air passages when few or none exist in the lungs. The lesions in the nasal passages begin as submucosal nodules which quickly break down forming shallow, crater-like ulcers which exude a thick, sticky purulent material This is discharged from the nostrils constituting a highly dangerous exudate

Glanders nodules may be found in other organs, in the liver and spleen, especially Frequently nodules form under the skin, particularly of the legs These occur in the lymph channels, the infection localizing here and there forming chains of nodules connected by indurated cords The nodules usually break down forming crater-like ulcers which discharge a sticky, honey-like exudate containing the glanders bacillus This form of glanders is known as *farcy* Farcy can be a manifestation of local wound infection, but usually it is accompanied by lesions in the internal organs McFadyean (11) who had a large experience with glanders in London, stated that "No case of glanders

with lesions elsewhere than in the lungs and with those organs healthy, has ever been recorded"

Distribution and Mode of Infection. Infection is contracted in most instances through ingestion, according to McFadyean (11), although it probably can occur through inhalation and through wound infection Glanders has always, until recently, been the scourge of army horses From ancient times until the World War of 1914–1918, wars had always caused the disease to flourish and the distribution of army animals into civilian service afterwards had served to spread the disease far and wide The American Civil War served to spread the disease over the eastern parts of the United States It flourished mostly in the cities where there were great concentrations of horses in the days of the horse-cars and the great livery and delivery stables, but it was by no means unknown in the rural districts During the early part of the present century, after excellent diagnostic tests had been developed, the disease was rapidly brought under control, and the advent of the motor car and the motor truck, which diminished the horse populations of all cities helped greatly in stamping out the disease. At the present time the disease has been practically eliminated from the United States and the countries of western Europe, but in eastern Europe, particularly in Russia, where less vigorous measures were taken, the disease continued to flourish until very recently.

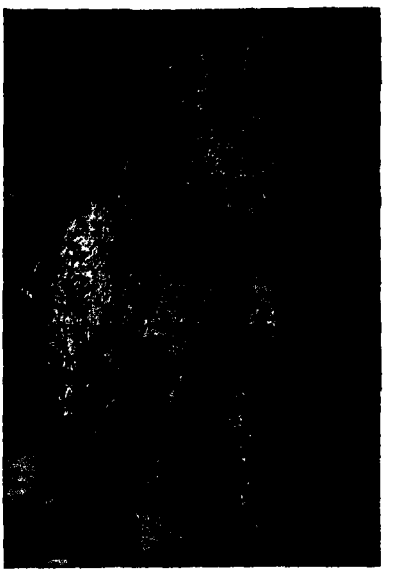


FIG 39 Skin Glanders or Farcy of the Horse

Infections are contracted mostly from the highly infectious nasal discharges which contaminate the surroundings, especially harnesses, feeding troughs and the old-fashioned watering troughs Carnivorous animals usually are infected by the eating of meat of glanderous horses A number of serious outbreaks have occurred in zoological parks from the practice of feeding horse meat to members of the cat family (7). Human infections occur principally among persons whose work brings them into close association with diseased

horses Infections may also occur through wounds while conducting autopsies or while handling meat from glanderous animals, but this danger apparently is not so great as had once been supposed, since McFadyean (12) reports that cases among the personnel of horse slaughtering establishments in London which handled thousands of diseased carcasses were very rare A large number of laboratory infections have been reported, a rather surprising fact in view of the comparatively slight infectivity of glanderous carcasses for man

Diagnosis of Glanders. There are a number of ways by which this disease can be definitely diagnosed The most important of these are

1. PHYSICAL EXAMINATION AND POSTMORTEM LESIONS Well developed clinical cases of glanders are generally easily diagnosed by the symptoms, and the lesions are easily recognized at autopsy in the majority of cases Unfortunately such diagnoses can be made only after the animals are well advanced in the disease and have become dangerous spreaders of the infection

2 DETECTION OF THE ORGANISM *M mallei* may be readily cultivated from closed lesions on plain or glycerin potato, or upon glycerin agar (preferably acid in reaction) When the lesion is open and other organisms are present, it is surer to inoculate several guinea pigs rather than to depend on cultures If male guinea pigs are inoculated intraperitoneally with not too great a number of the *M mallei*, a localized peritonitis involving the scrotal sac usually (not always) develops As a result of this process the scrotal sac becomes enlarged and painful Usually the process requires several days to reach its height The testicle itself becomes involved in a short time and the whole organ is reduced to a mass of caseous pus which will break through the skin and discharge to the surface This is known as the *Strauss Reaction* (5) A similar reaction sometimes occurs due to other organisms, e g , *Ps pyocyaneus* and the Preisz-Nocard bacillus, thus it is not diagnostic of glanders If too many glanders organisms are injected the guinea pig will develop a generalized peritonitis and die in a day or two without showing the scrotal changes If the injection is made subcutaneously instead of intraperitoneally, an ulcer usually forms at the site of injection and the animal will die after four or five weeks with nodules in many of the internal organs

3. SEROLOGICAL TESTS

(a) Complement-fixation This test has proved to be the most accurate of the serological methods of diagnosing glanders It was first applied to the disease by the Germans about 1909 and soon afterwards was introduced into this country as a diagnostic procedure by Mohler and Eichhorn (13)

(b) Agglutination This was first used by McFadyean (9) in 1896 It is generally admitted to be not so accurate as the previous test The New York City Health Department (1) reported it as about 84 per cent accurate. The failures were in the more chronic cases Normal agglutinins exist in a concentration as high as 1–500 in many horses Infected animals usually react in dilutions of 1 1000 and higher (15)

(c) Precipitation This test is done with the serum of the animal suspected of being glandered and with an extract of the *M mallei* In the hands of some the test has been quite successful, although it probably is no more reliable than the agglutination test A positive precipitation test is definitely indicative of glanders, a negative test does not exclude glanders

4 MALLEIN TESTS. Mallein is a product in every way analogous to tuberculin. In the early days it was made by extracting, with glycerinated water, the glanders bacillus which had been grown on potato Later the crude mallein was, and is at the present time, produced by growing the organism on glycerin broth A slimy, slowly developing film appears on the surface, the broth is clouded, and a gummy sediment forms The medium gradually darkens After several months' incubation the culture is killed by steaming and the organisms are removed by filtration The filtrate constitutes the crude mallein For special purposes this mallein is concentrated by evaporation, or is precipitated by alcohol in the form of a white powder

Mallein, for normal animals, is an inert or, at the most, only a slightly toxic substance Animals affected with glanders, however, are hypersensitive to it and symptoms of intoxication occur when it is injected into them It is, accordingly, a valuable diagnostic agent Mallein has been used by some for treating glanders The value as a curative agent is doubtful

Mallein is used in three ways (a) Subcutaneous test, (b) Ophthalmic test, (c) Intrapalpebral test When injected subcutaneously it gives rise to fever which appears and subsides again within twenty-four hours after the injection There is usually a marked swelling at the point of injection Normal horses may show some swelling at the point of injection but the temperature curve is absent The ophthalmic test consists of instilling some concentrated mallein into the eye (conjunctival sac) A pus-forming inflammation of the eye occurs within a few hours when the animal is glanderous. The intrapalpebral test consists of injecting a small amount of concentrated mallein into the skin of the lower eyelid A local swelling and a pus-forming inflammation of the eye occurs The intrapalpebral test is more accurate than the ophthalmic and accordingly is more often used.

Immunization. It has been generally believed that animals never recover from glanders Recovery certainly is not usual but there have been many reports of horses which, after showing symptoms of the disease and after reacting to diagnostic tests, have made clinical recoveries and have become negative to the laboratory tests It appears, then, that recoveries do occur (10)

Many attempts have been made to protect animals against glanders by the use of a variety of biological products but none has proved successful (14). Control of the disease has been accomplished wholly by methods which involve early diagnosis and elimination of the reacting animals Clinical examinations at frequent intervals, the use of mallein and the serological tests, and the destruction of animals which give evidence of infection by any of these methods has proved adequate These methods have practically eliminated the disease from the United States and from many European countries.

Glanders in Man. Man is not highly susceptible to glanders, yet numerous infections have occurred in persons caring for glanderous animals, especially stable men and veterinarians (2) The disease is characterized by swelling and pain at the point of infection (usually the hand, the lip, or the eye) which comes on in from three to five days, swelling of the neighboring lymph glands, development of nasal and mouth ulcers (in about half the cases), development of abscesses and pustules in the skin, joint inflammations, and general symptoms accompanied by fever The cases usually end fatally in from two to four weeks A few cases of glanders in man occurred in Russia during the World War Chronic glanders in man has been vividly described by Gaiger (6), a British veterinarian who contracted the disease in India

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CHAPTER XXIII

THE ACID-FAST ORGANISMS

The property of acid-fastness is possessed by a large group of rod-shaped organisms A great many species of these organisms are found in the soil, where they live a saprophytic existence A few species are wholly parasitic and pathogenic in the sense that they cause transmissible diseases In another sense all acid-fast organisms are at least mildly pathogenic for all of them contain irritating lipoids and proteins and when inoculated into tissues of living animals they cause the formation of granulomatous lesions which are called *tubercles* Tubercles may be produced by the inoculation of heat-killed cultures, or by certain extracts of cultures as well as by the living organisms Sabin (26), working with chemical fractions prepared by Anderson, showed that tubercles are produced by tissue stimulation with a constituent of the phospholipid fraction of the bacillary bodies, a substance that proved to be a saturated fatty acid to which the name *phthioic acid* was given It appears, therefore, that the principal difference between the pathogenic and the non-pathogenic species of this group is that the former possess the power of multiplication in the tissues whereas the latter do not The similarity of the chemical composition of some well-known acid-fast organisms is indicated in the following table which is taken from Chargoff, Pangborn and Anderson (7)

TABLE XI

THE CHEMICAL COMPOSITION OF SEVERAL TYPICAL ACID FAST ORGANISMS

	Myco tuberculosis Human type	Myco tuberculosis Bovine type	Myco tuberculosis Avian type	Myco phlei (Timothy bacillus)
Phosphatid	6 54	1 53	2 26	0 59
Acetone soluble wax	6 20	3 34	2 19	2 75
Chloroform soluble wax	11 3	8 52	1 79	4 98
Total lipoids	23 78	13 40	15 26	8 37
Polysaccharide	0 78	1 09	1 02	3 90
Dried bacterial residue	75 01	85 50	83 71	87 70

The simple rod-shaped acid-fast organisms referred to above belong to the genus *Mycobacterium* Besides the mycobacteria, the property of acid-fastness is possessed in variable degree by some of the higher bacteria, actinomycetes, and molds Some have claimed to have induced acid-fastness in bacteria which normally do not have this character by cultivating them on media of high fat content Bruner (5) was unable to accomplish this and decided that such results are artifacts On the other hand organisms which are normally acid-fast often are found in a non-acid-fast form, and many of them can be induced to develop in a non-acid-fast form by cultivating them in media in which there is little available carbon Often it is impossible to demonstrate acid-fast organisms in smears and sections of diseased tissue but cultures are readily obtained Some have thought that the acid-fast form is but one stage in a cycle in which other stages are non-acid-fast but there is little to support such a hypothesis When acid-fast organisms cannot be demonstrated microscopically it means, in most cases at least, that the numbers present are very few, but it may be that in some cases the organisms present are starved as in carbon-free media and exist temporarily in a non-acid-fast form.

MYCOBACTERIUM TUBERCULOSIS

Synonyms *Bacillus tuberculosis, Bacterium tuberculosis,* the tubercle bacillus

The disease caused by this organism was described over two thousand years ago, and bone lesions found in Egyptian mummies prove that it existed among people long before that Quite naturally there was much confusion between tuberculosis and other diseases of man in ancient and medieval times and this confusion lasted until after the middle of the 19th century. Many early writers claimed the disease to be infectious, but others regarded it as a form of malignant tumor and non-infectious until after the causative agent had been found and experimentation had removed all doubt as to its nature Although he was not the first to claim infectiousness, Villemin (38) demonstrated, in 1865, that tuberculous tissue from man and cattle produced the disease in rabbits by inoculation

The tubercle bacillus probably was first seen in tissues by Baumgarten (2) in 1882 In the same year Robert Koch (17) succeeded in demonstrating the organism in diseased tissues by staining them with alkaline methylene blue and counterstaining with Bismarck brown (vesuvin) With this method the tubercle bacilli remained blue while all other organisms and tissues lost the blue and took on the brown color of the counterstain. Koch also found that the organism could be cultivated in pure culture on a medium consisting of coagulated bovine serum With such cultures he readily reproduced the disease in experimental animals and thus removed all doubt as to its etiologi-

cal relationship. His final report on this work was published in 1884 (18).

Koch was unable to induce the organism to grow on any of the ordinary media Nocard and Roux (26) in 1887 reported that cultures could be successfully grown on ordinary nutrient agar and broth fortified by the addition of from 5 to 8 per cent of glycerol, and this is the simplest medium for tubercle bacilli that we have today These media are not suitable for making primary isolations but serve very well for strains that have been accustomed to artificial growth on media containing blood serum or egg yolk The egg medium of Dorset (8), described in 1902, is still one of our most useful for primary cultures

The organism grows fairly readily though slowly on suitable artificial media It requires much free oxygen for vigorous growth, hence it does not multiply in the depths of most fluid media and it will not grow under anaerobic conditions Even the sealing of the culture tubes with wax partially inhibits growth, presumably through the reduction in the amount of free available oxygen

Biologic Characters. The *Mycobacterium tuberculosis* produces a characteristic, dry, waxy growth on artificial media The appearance differs according to the culture medium upon which it is grown, and the type and age of the culture All of the true tubercle bacilli grow slowly, especially when recently isolated On fluid media heavy pellicles are formed, whereas the underlying fluid remains practically free of organisms

The organism does not form spores and possesses only moderate resistance to heat It is destroyed by pasteurization It is fairly resistant to desiccation Direct sunlight is rapidly fatal to it In moist soil the organism may remain alive for more than a year It resists putrefaction fairly well To most of the disinfectants the organism has no unusual resisting powers, but to acids and alkalies it has unusual resistance Antiformin destroys it very slowly, hence it may be used to isolate tubercle bacilli when they are mixed with other organisms

Pathogenicity. The name of the disease caused by this organism is derived from the Latin word *tuberculum* which means a small nodule or lump As has already been explained, wherever acid-fast organisms lodge and multiply in living tissue a reaction is induced resulting in the formation of a granulomatous nodule, hence the disease is well named The disease is essentially one of the lymphatic system Beginning at the port of entry into the body the bacilli are carried in the lymphatics, usually lodging in the first lymph node where multiplication occurs and a tubercle is formed Escaping from the primary focus, secondary foci often are formed, and this process of

extension may go on until eventually the bacilli may reach the blood stream by which they are carried to all parts of the body Most cases do not go as far as this, and the disease remains restricted to certain parts of the body related to the portal of entry. If bacilli manage to reach the blood stream in large numbers, they are phagocytosed and most of them are carried to the lungs, liver, and spleen where many small tubercles are formed The individual in this case is said to be suffering from *miliary* or *generalized tuberculosis* and usually death soon occurs

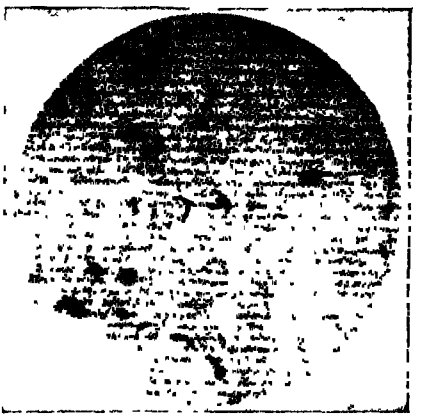


FIG. 40 *Mycobacterium tuberculosis*, Human type In sputum of a consumptive patient x 900

In mammals the most frequent localization of tubercle bacilli is in the lymph nodes of the pharyngeal region and in the nodes of the chest Of the principal visceral organs, the lung is most often involved. The form of the disease varies from one, or a few tubercles which often heal and never cause symptoms, to extensive tuberculosis abscesses The latter are often found in the lungs The large masses of tuberculous granulation tissue containing caseous material which has a tendency to calcify may occupy the greater part of the tissue of one or both lungs The breaking down of this tissue leads, in man to a greater extent than in animals, to the formation of great cavities because of the discharge of the caseous content through the air passages Severe and often fatal hemorrhages occur in man through rupture of corroded blood vessels into these cavities Such individuals suffer from continued, low-grade fever, weakness, emaciation, hemorrhages from the lungs (*hemoptysis*), and a cough which raises sputum which often is rich in tubercle bacilli

Of the domestic animals, cattle, horses, swine, and chickens are highly susceptible and often suffer from tuberculosis Sheep, goats, carnivorous animals, and water-fowl are relatively resistant, although the disease is occasionally found in all of these

The Types of Tubercle Bacilli. Villemin (38) showed that tuberculosis could be produced in rabbits by inoculating them either with sputum from human cases or with tissue from the nodules which occur on the chest wall

of tuberculous cattle He believed, therefore, that the diseases of man and of cattle were identical and caused by a single virus This belief was also held by Koch at first since, after demonstrating the acid-fast bacilli in human material, he found what appeared to be the same organism in bovine tissues Rivolta (31), however, in 1889 showed that the bacillus of birds was not identical with those of mammals, and Theobald Smith (34), in 1898, pointed out certain differences in cultural characteristics and pathogenicity between the types ordinarily found in man and those found in cattle



Fig 41 *Mycobacterium tuberculosis*, Human type From a culture on glycerin egg medium incubated six weeks at 37° C × 900

Three types of tubercle bacilli are now recognized as responsible for tuberculosis in warm-blooded animals These are the human type, the bovine type, and the avian type Of these, the mammalian types (human and bovine) are very closely related The differences between them are quantitative rather than qualitative The avian type, on the other hand, differs from the mammalian in many respects This has been recognized in the Fifth Edition of Bergey's Manual by segregating the avian type under the name of *Mycobacterium avium* Here we shall continue to regard the organism as *Mycobacterium tuberculosis*, variety, *avium*

Instead of dividing the mammalian tubercle bacilli into the two types, human and bovine, English authors, especially Griffith, refer to them as the *eugonic* and the *dysgonic* types These terms have reference to the comparative ease with which primary cultures of the human type usually are obtained and the greater difficulty with primary cultures of the bovine type The term eugonic is equivalent to the human type, and the dysgonic, the bovine

Differentiation of the Types of Tubercle Bacilli. Identification of the types of tubercle bacilli frequently cannot be based upon the host from which they are isolated for, as will be shown below, the host relationship is far from a fixed one. Some idea of the type may be gained by microscopic examination of the organisms but this is uncertain because there is considerable variation in the size and shape of each type. Cultural features generally will serve to

differentiate the avian type from the mammalian, but distinguishing between the two mammalian types on cultural grounds is quite uncertain The most satisfactory method is based upon pathogenicity for experimental animals, although this sometimes fails Strains which have grown in unusual hosts sometimes show unusual properties which makes it difficult to assign them certainly to any of the recognized fixed types

1 COMPARATIVE MORPHOLOGY The avian type is the most pleomorphic of the three types In infected tissue it usually appears as very short, solid-staining rods In cultures it often appears as very long, beaded forms The bovine type appears in cultures and tissues as straight, solid-staining rods with comparatively little variation The human type is usually somewhat curved, is longer than the bovine type as a rule, and often has clubbed ends Beading is frequent in the human type, both in tissues and in cultures, but long filaments are not usually found All three types are strongly acid-fast

2 COMPARATIVE CULTURAL FEATURES The avian type bacillus is much more readily cultivated than the mammalian types Primary cultures on glycerin agar usually succeed in the case of the avian type but rarely or never in the case of the mammalian The well-developed growth of the avian type on solid media is soft, smooth and rather butyrous On fluid media a slimy pellicle is formed and slimy stalactites extend downward into the medium In very old cultures a viscid sediment collects in the bottom of the culture vessel Except for the slimy stalactites, the fluid beneath the pellicle remains clear



FIG 42 *Mycobacterium tuberculosis* Bovine type From a culture on Dorset's egg medium incubated six weeks at 37° C These long beaded forms are seen only in cultures In tissues this type ordinarily is solid staining and shorter than the other types x 900

Primary isolations of the mammalian types of tubercle bacilli usually require a solid medium containing egg yolk, or coagulated blood-serum The bovine type develops with greater difficulty and more slowly than the human type and does better if glycerin is omitted from the medium. The human type develops on the same media but thrives

much better if 5 per cent glycerin is included in the medium After the strains have become accustomed to growing on artificial media, both types will grow much faster and the final volume of growth is much greater, but at best growth is relatively slow The effect of the presence or absence of glycerin in the medium then is not so marked although the preference of the human type for the glycerin is never lost Old, well developed cultures of the two mammalian varieties cannot be distinguished with certainty even by the experienced worker The appearance of the growth differs according to the type of medium used but generally both produce rather dry, granular growths which heap up on the slants reminding one of the castings of earthworms except in color The color depends upon whether or not the cultures have been exposed to light Those grown in complete darkness are grayish-white, those which have had considerable exposure to light take on a tan or even a distinct brick-red color On fluid media both types grow poorly until they have become accustomed to it If the delicate pellicles are carefully lifted to fresh media and caused to float on the surface, they spread over the surface in the form of a dull, grayish, translucent pellicle which later becomes thickened and thrown into folds After several weeks, these pellicles become quite thick, opaque, and dry in appearance, and they push up at the margins on the sides of the culture vessels At all stages of growth the pellicles are fragile and brittle, thus contrasting sharply with those of avian strains which are slimy The broth beneath the pellicles remains quite clear and there is very little sediment even in very old cultures



FIG. 43 *Mycobacterium tuberculosis*, Avian type Film from liver lesion of a naturally infected chicken × 450

Theobald Smith (35) early pointed out a growth character by which mammalian strains generally may be differentiated from each other. This depends upon the fact that the human type utilizes glycerin very actively whereas its utilization by the bovine type is very limited When cultivated upon a broth containing 3 per cent glycerin, human types ferment the glycerin producing enough acid to maintain a terminal acidity of the medium, whereas

the bovine types leave the medium alkaline Fully developed cultures are tested with phenolphthalein. If the reaction is acid, the presumption is that the strain is of the human type, if alkaline, that it is of the bovine type If the glycerin content of the medium is below one per cent, both types produce alkali. This reaction generally holds true but it is of little practical differential value because of the necessity of training the strains to develop well on a fluid medium before the test may be made

3 COMPARATIVE PATHOGENICITY. The avian type of the tubercle bacillus usually is highly pathogenic for chickens and rabbits It is nearly non-pathogenic for guinea pigs as a rule Frequently local lesions are produced in this species of animal but only rarely does the disease become generalized The mammalian types are almost wholly non-pathogenic for chickens, but most strains will produce progressive infections in guinea pigs leading to their death from generalized tuberculosis These features serve to distinguish the avian type bacillus from the mammalian types

The differentiation of the mammalian types from each other is more difficult. On the whole the bovine type is perhaps a little more virulent for guinea pigs than the human, as measured by the rapidity of the development of the lesions and the time of death of the animals, but this difference is too slight to make it useful in differentiating the types In the rabbit, however, there is generally an appreciable difference in the virulence of the two types as was first pointed out by Smith (34) When this animal is given a small dose of bovine type tubercle bacilli of normal virulence, a progressive disease is set up which leads to its death in from three weeks to three months The same size dose of human type bacilli of normal virulence usually produces only local lesions from which the animal recovers At the end of three months rabbits which have been inoculated with human material usually not only are living but they have taken on weight and appear to be thriving. A clear presentation of the value of the rabbit test



FIG. 44 *Mycobacterium tuberculosis*, Avian type From a culture on Dorset's egg medium which had been incubated six weeks at 37° C. x 900

for differentiating human type tubercle bacilli from the bovine type may be found in the papers of Park and Krumwiede (27) who used the method extensively

When it is desired to determine the type of tubercle bacilli present in tissues, the best available procedure involves the experimental inoculation of

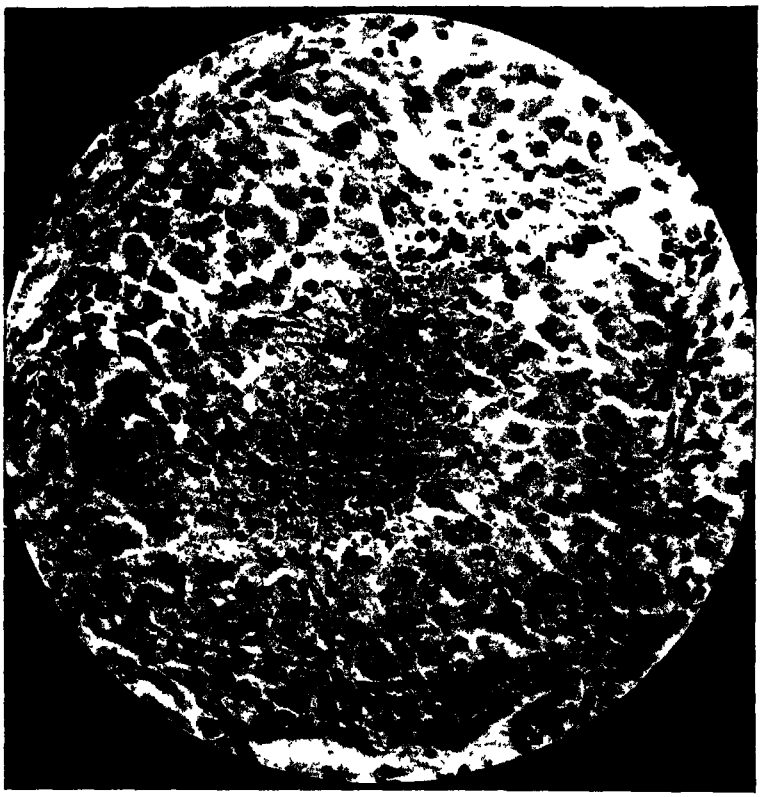


FIG. 45 A Primary Tubercle A very early tubercle in the lung of a rabbit caused by *Mycobacterium tuberculosis*, avian type The structure is typical of all primary tubercles irrespective of the type of bacillus concerned The central area of necrosis is surrounded by a layer of epithelioid cells which make up the greater part of the field The periphery of the lesion consists of fibroblasts and lymphocytes Giant cells appear a little later near the margin of the necrotic area × 300

guinea pigs, rabbits, and chickens Care must be taken to be sure that the experimental animals are not naturally infected There is comparatively little chance of this happening in rabbits and guinea pigs but there is real danger

of mistakes in case of chickens if one is not sure that they came from a tuberculosis-free flock The safest procedure in all cases is to inject mammalian tuberculin intradermally into the mammals and avian tuberculin into the chickens before they are used If no reactions are obtained one may proceed with reasonable assurance

It is best always to isolate the organism in pure culture, since in this way the dosage may be better regulated A small bit of the culture is scraped from the surface of the solid medium and weighed It is then placed in a small mortar and ground until the larger clumps are broken up Broth or saline solution is added to make a smooth emulsion of the bacilli and this is diluted until there are about 0 1 mg of tubercle bacilli per cubic centimeter of fluid About 0 1 cc of this fluid is injected into each of the experimental animals, making a dose of about 0 01 mg After a little experience one can judge sufficiently accurately the amount of material that he has scraped from the culture so it is unnecessary to do the laborious weighing process on each sample

The guinea pigs should be inoculated subcutaneously in one of the thighs, or it may be injected into the muscular tissue The rabbits are inoculated in the ear vein, and the chickens in the brachial vein where it crosses the second joint on the under surface of the wing

If the strain is a typical avian type, the chickens and rabbits will gradually lose weight and usually will die after several weeks but the guinea pigs will remain well If it is a bovine type, the rabbits and guinea pigs will sicken and die but the chickens will remain well If it is a human type, the guinea pigs will die but the rabbits and chickens will remain well All animals which remain alive after 8 to 10 weeks should be destroyed and autopsied

Pathogenicity of the Types of Tubercle Bacilli for the Domestic Animals. Whereas the well-known tubercle types are in their native habitat when they are in man, cattle, and fowls, respectively, all of them are frequently found in other species of animals Some are susceptible to only one type, others may be infected by two types and still others suffer from all types The disease picture usually differs somewhat according to the type involved

Table XII, p 238, is taken from Griffith (14) who has done a great deal of work in identifying the types in man and animals in England

Pathogenicity for Animals of the Human Type of Tubercle Bacillus. It will be noted in Table XII that the human type of tubercle bacillus plays a relatively small role in animals.

FOR SWINE Infection of swine with the human type tubercle bacillus generally occurs through the feeding of uncooked garbage. Garbage originating in tuberculosis sanatoria is particularly likely to cause these infections, however,

TABLE XII

TOTAL NUMBER OF CASES OF NATURAL TUBERCULOSIS IN DIFFERENT ANIMALS IN WHICH THE TYPE OF TUBERCLE BACILLUS HAS BEEN DETERMINED (FROM GRIFFITH)

Species of Animal	Number of Cases	Types of Tubercle Bacilli Found		
		Bovine	Human	Avian
Horse	25	24	–	1
Pig	163	118	5	43
Cat	20	20	–	–
Dog	4	1	3	–
Goat	1	1	–	–
Sheep	4	2	–	2
Cattle	52	50	1	1
Fowl	13	–	–	13
Guinea pig	6	5	1	–
Rabbit	8	4	–	4

Feldman (11) found human tubercle bacilli in the lesions of 12 swine which had been fed on general garbage collected in a city, indicating that human sputum probably enters garbage more often than would have been supposed The lesions usually are minor in character, consisting ordinarily of a few small lesions in the lymph nodes of the pharyngeal region, and in the mesenteric nodes. The disease seldom or never generalizes and the animals show no clinical evidence of the infection except for their newly acquired capacity to react to tuberculin

FOR DOGS Tuberculosis of dogs is not common Occasionally clinical cases are encountered with extensive involvement of the lungs, liver, and spleen. More often the animals display no symptoms, and lesions in the lymph nodes are found unexpectedly at autopsy The majority of such cases are caused by bacilli of the human type, but an appreciable percentage prove to be bovine.

FOR CATTLE Cattle are quite resistant to tubercle bacilli of the human type but minimal infections of the lymph nodes of the pharyngeal region and of the mesenteric nodes occasionally are found A careless tuberculous owner or caretaker who expectorates freely around a cow barn has been known to cause multiple infections in the herd The infection is of little importance except for the fact that the animals become tuberculin reactors

FOR BIRDS All birds, except members of the parrot family, are highly resistant to mammalian tubercle bacilli A considerable number of tubercle infections in parrots which have been companions of tuberculous people have been reported.

FOR HORSES, CATS, SHEEP, AND GOATS. These species appear to be quite resistant to tubercle bacilli of the human type

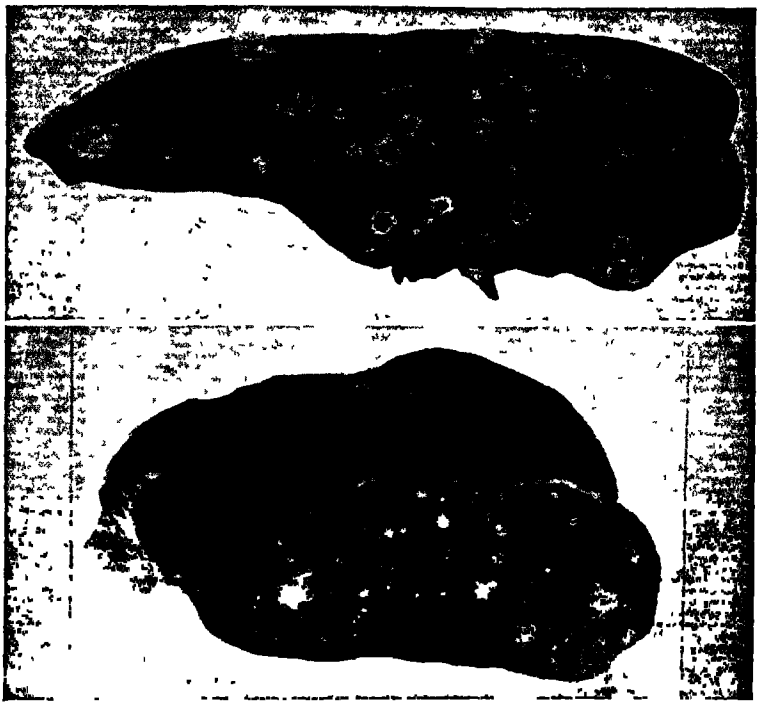


FIG 46 Tuberculosis, Liver, Chicken *Upper,* Gross lesions in cut section *Lower,* Lesions as seen from the surface

Pathogenicity for Mammals of Tubercle Bacilli of the Avian Type

FOR SWINE The avian tubercle bacillus readily infects swine In the majority of cases the disease is limited to the lymph nodes of the head, neck, and alimentary canal In these nodes well-marked caseous lesions are often found. It is generally believed that generalized tuberculosis of swine usually is caused by tubercle bacilli of the bovine type rather than by the avian, but Feldman (9) found that avian type bacilli were responsible for 24 out of 26 cases in which the carcasses had been condemned by the meat inspectors in a midwestern state because of generalized tubercle infection, hence it seems that this idea needs revision.

Since bovine tuberculosis has been reduced to a low incidence in the United States, by far the greater number of tuberculous swine carcasses now detected in meat inspection examinations are the result of activities of the avian bacillus In a study conducted in 1925 when the bovine disease was still quite prevalent, Van Es and Martin (36) showed that in a series of more than 200 porcine lymph nodes affected with tuberculosis, approximately 88 5 per cent contained the avian type alone, an additional 6 per cent contained avian as



FIG 47 Tuberculosis, Intestine, Chicken Intestinal ulcers are common in avian tuberculosis These ulcers gradually become very deep, the tissues of the intestinal wall forming sac like structures which appear on the serous surface like tumors The centers are filled with dry, caseous material which gradually discharges into the intestinal lumen

well as mammalian, and only about 5 per cent contained mammalian tubercle bacilli alone

FOR CATTLE Avian tubercle bacilli have quite frequently been found in lesions in cattle in the north-central part of the United States where tuberculosis in poultry is prevalent These organisms are picked up from the soil which is polluted by infected birds. The lesions are insignificant, as a rule, and are located in the lymph nodes which drain the digestive canal They have no effect on the health of the animals Such animals will regularly react to avian tuberculin and johnin and thus may be mistaken for cases of paratuberculosis or Johne's disease It is probable that such animals also occasionally react to mammalian tuberculin

In Denmark, Plum (28), Bang (1), and others have found cases of infectious abortion of cattle in which avian tubercle bacilli are the apparent causes.

In these instances tuberculous lesions were found in the uterine wall and the infection had extended to the placental structures. The diagnosis of such cases can readily be made by examining the afterbirth or the uterine discharge after abortion The infection may remain in the uterine wall from one gestation period to another, and such animals become chronic aborters In these animals the bacillus is not ordinarily found in other parts of the body except occasionally in the mesenteric lymph nodes, a fact which suggests that the disease may have been contracted from the digestive tract Plum succeeded in producing abortion in one animal by injecting avian tubercle bacilli into the jugular vein

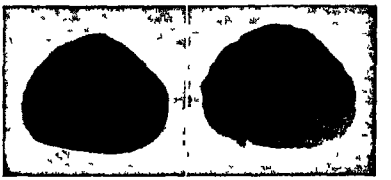


FIG 48 Tuberculosis, Spleen, Chicken *Right*, Lesions as seen on the spleen surface *Left*, Lesions seen in cut-section (Courtesy of F L Brunett)

Mitchell and Duthie (24) caused infection of the udder of a cow by injecting avian tubercle bacilli into the jugular vein Naturally occurring cases of this kind have not been reported



FIG 49 Advanced Tuberculosis, Lung, Cow The entire diaphragmatic lobe is involved in the tuberculous process There is extensive necrosis and fibrosis The necrotic tissue contains large amounts of calcareous material

FOR SHEEP AND GOATS Tubercle infection in these animals is comparatively rare. Most of the cases which have occurred abroad in which the organism was typed have proved to be bovine In the United States the greater part

have been avian, having arisen in the mid-western states where poultry tuberculosis is prevalent. Harshfield, Roderick and Hawn (16) described 19 cases of avian type infection of sheep in North Dakota and referred to 7 additional cases found earlier Lesions of the lungs and thoracic lymph nodes were found in all of these cases, a majority showed liver tubercles, and the spleen was affected in about half of the cases In the annual report of the Chief of the Bureau of Animal Industry, U S Department of Agriculture for 1940, it is stated that 8 cases of ovine tuberculosis had been investigated and that 7 had proved to be infections with the avian type bacillus and one, bovine

FOR RABBITS Naturally occurring tuberculosis of the rabbit is practically unknown, however, it is rather remarkable that rabbits can be very easily destroyed by inoculating them with tubercle bacilli of the avian type If very small doses are administered the animals usually develop a chronic disease and die after several months The autopsy then shows large, well-developed tuberculous masses in the lungs, liver, and spleen If large doses are given the animals usually become emaciated rapidly, and die in from two to three weeks The lungs, liver, and spleen, in these cases, are filled with myriads of minute, semi-translucent tubercles, or there may be no tubercles that can be differentiated with the naked eye, but the organs are swollen The situation is that the tubercles are so closely set and so small that they may not be distinguished This condition was described by Yersin (39) and is often called the *Yersin type of tuberculosis*, a tuberculosis in which there are no visible tubercles

FOR MAN. Human beings are very rarely infected with tubercle bacilli of the avian type Feldman (10) who reviewed the circumstances in about 40 cases which have been reported in the literature, concluded that sufficient proof had been supplied in 13 cases to warrant incriminating avian type tubercle bacilli in human infections Van Es and Martin (37) examined 227 specimens of tuberculous tissue from human cases, finding human type bacilli present in 219, the bovine type in 9 and the avian type not at all Since much of their material originated in a region where poultry tuberculosis is prevalent, their work clearly shows that human beings are quite resistant to infection with the avian tubercle bacillus

Pathogenicity of the Bovine Type Tubercle Bacillus for Animals other than Cattle. The bovine type of tubercle bacillus affects a much wider range of animal species than either of the other types Nearly all mammals are at least partially susceptible to this type.

FOR FOWLS. Except for the members of the parrot family, which exhibit the same susceptibility to the bovine as to the human type (but are less often in-

fected with it because of lesser exposure), birds are highly resistant to tubercle bacilli of the bovine type.

FOR HORSES. Horses are relatively susceptible to bovine type tubercle bacilli. When horses associate with tuberculous cattle, the incidence of the disease may be high Actually equine tuberculosis is not frequent under the condi-



FIG 50 Tuberculosis, Spleen, Horse Lesions in horses often resemble tumors, being white or gray in color, uniform in consistency, and lacking obvious gross evidence of necrosis

tions in which horses ordinarily are kept, because the opportunities for infection are not great

The lesions of equine tuberculosis are most frequent in the lymph nodes of the pharyngeal region and in those of the mesentery but lung, spleen, and liver involvement is not rare In many instances, especially in the lungs, the lesions do not have the appearance of ordinary tubercles but consist of whitish masses of rather soft tissue, without evidence of necrosis These masses have often been mistaken for lung tumors In other instances the lesions are typical of tubercles with caseation and calcification

Many strains of tubercle bacilli isolated from horses are sub-virulent for experimental animals and some of them are practically avirulent

FOR SWINE Pigs are very susceptible to tubercle bacilli of the bovine type. Infections lead to extensive and progressive lesions which often produce death

within a few months The infections do not usually occur from pig to pig but more often from cattle to pigs, especially in regions where pigs are commonly kept in the same barn-lots with cattle In the early part of the present century when bovine tuberculosis was common in our cattle, the losses from tuberculosis in swine were much greater than at present Earlier many of the cases of tuberculosis were generalized but at present the majority are localized and thus do not lead to condemnation of the carcasses This change has occurred because infection with the bovine type tubercle bacillus has become rare, whereas that caused by the avian type continues to be common

FOR SHEEP AND GOATS These animals are relatively resistant to tubercle infection, and the majority of cases found in this country are caused by bacilli of the avian type Bovine type infections seem to predominate in other parts of the world These frequently are progressive and commonly lead to emaciation and death

FOR DOGS AND CATS Tuberculosis is relatively rare in both of these species Dogs may be infected with either of the mammalian types of tubercle bacilli but the disease in cats is almost always caused by bacilli of the bovine type Infections occur most frequently because of their having consumed infected cow's milk The lesions in cats are located most commonly in the lymph nodes of the digestive tract, in the liver and spleen and, less commonly, in the lungs In dogs the lesions are most often in the thoracic lymph nodes and in the lungs

Tuberculosis in Wild Animals. Tuberculosis in wild animals is not common except when they are in captivity The disease has been found on several occasions in wild birds (3) (23) living a natural life, and there have been a considerable number of reports of the disease in wild deer Cases in deer are due in almost all cases to the bovine type bacillus and infection is caused by the association of these animals with cattle on pasture, or by browsing on land which is used for pasturage for cattle It is not unlikely that some of the "breaks" in tuberculosis-free herds are caused by the introduction of the disease by infected wild deer

Tuberculosis has always been one of the major problems of zoological parks The most severe losses have been among birds, but extreme care is necessary to keep the losses among members of the monkey family within reasonable bounds It is the human type which causes the losses among the apes The bovine type causes losses among reindeer and other ruminants Tubercle bacilli of the cold-blooded type, to which reference will be made later, cause serious losses among reptiles and amphibia.

Human Tuberculosis. The most common form of tuberculosis of man is that which is known as *phthisis,* or *pulmonary consumption* This disease affects primarily the lungs and pleura and the associated lymph nodes In many individuals the disease becomes arrested early and no symptoms are induced Such individuals will, however, react to tuberculin, and X-rays may show one or several small encapsulated and calcareous lesions In others, unfortunately, the disease spreads from the original focus and causes large exudative and destructive lesions The victim suffers from a low-grade, intermittent fever, weakness, shortness of breath, a hacking cough, loss of weight and finally great emaciation Large amounts of purulent sputum are raised and this often is very rich in tubercle bacilli Frequently there are severe and often fatal hemorrhages from large blood vessels which have been damaged by the necrotizing process This type of tuberculosis is caused by tubercle bacilli of the human type in most cases The disease is transmitted from man to man, animal infections playing little part in it except in a few cases in which the bovine type bacillus is involved Until a few years ago it was thought that bovine tubercle bacillus infections of the human lung were extremely rare, but in recent years it has become evident that such infections are much more numerous than had been supposed Griffith (15) in England, and workers in the Scandinavian countries where bovine tuberculosis is prevalent have shown that from 1 to 6 per cent of human pulmonary infections are caused by the bovine type This situation does not exist in the United States where bovine tubercle infection is now comparatively rare

Extra pulmonary tuberculosis of man is often caused by tubercle bacilli of the bovine type These infections occur more often in children than in adults and are caused by the drinking of infected cow's milk These infections involve the lymph nodes of the pharyngeal region, and the abdominal organs instead of the organs of the thorax Bovine tubercle bacilli are often found in infections of the bones and joints, of the skin (*lupus*), and in tuberculous meningitis All of these forms of tuberculosis are caused more often by bacilli of the human type rather than by those of the bovine type except, possibly, the infections of the neck glands (*scrofula*) in which the bovine type may be more frequent than the human type Since bovine tuberculosis has been reduced in incidence in the United States, and since a large part of all milk consumed is pasteurized, it has been often noted by surgeons that those forms of tuberculosis in which the bovine type bacillus frequently occurs are becoming rare. Whereas in the early part of the century scrofula among children was not uncommon in many parts of this country, it now has become a rare disease.

That tubercle infection of children with bacilli of the bovine type was an important matter earlier in this century in the United States and elsewhere is clearly shown by statistics collected by Park and Krumwiede (27) which were published in 1912 Some of the more significant of their findings are shown in the following table Nearly half of the cases included were studied by the authors in and around New York City It can be noted in this table that the bovine type infections were largely in young children and that they were largely extra-pulmonary forms of the disease

TABLE XIII

TYPES OF TUBERCLE BACILLI ISOLATED FROM CASES OF HUMAN TUBERCULOSIS

Type of Disease	Age Groups of Patients					
	16 years and older		5 to 16 years		Under 5 years	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary tuberculosis	497	3	6	0	28	1
Cervical adenitis (Scrofula)	27	1	17	14	9	11
Abdominal	15	4	7	8	9	11
Generalized	33	1	7	5	72	16
Total of all kinds	625	14	85	37	201	51

Routes of Infection in Tuberculosis. The localization of the lesions in tuberculosis and the character of the disease depends in considerable degree upon the manner in which the infection enters the body It is clear that there are several routes that may be followed.

1. INHALATION The fact that tubercle lesions in adult human beings and cattle occur more frequently in the chest cavity than elsewhere suggests that infection commonly occurs through inhalation Experimentally it has been shown by McFadyean (22) and others that infections can easily be produced in guinea pigs by spraying them with tubercle bacilli This fact, coupled with the knowledge that in pulmonary tuberculosis both man and cow cough into the air droplets of secretion containing tubercle bacilli which can readily be inhaled by others near them, is rather convincing evidence On the other hand it is more difficult to produce infections, even in highly susceptible animals, with moderate doses of tubercle bacilli fed in gelatin capsules, and when infections are induced in this way lesions are almost invariably found in the mesenteric lymph nodes Since these nodes often are free of lesions when the chest organs are severely infected, it does not seem likely that the infection

generally enters by the intestinal route. On the other hand, Ravenel (29), many years ago, showed that tubercle bacilli suspended in butter could be found in the lacteals shortly after feeding, hence it appears to be possible for these organisms to reach the lungs, in some cases at least, without leaving lesions in the digestive tract Lung infection can also be produced experimentally by causing susceptible animals to inhale tubercle bacilli suspended in dust.

2. INGESTION Ingestion of tubercle bacilli in considerable numbers in infected milk readily produces tuberculosis in young animals In these cases lesions usually occur in the lymph nodes of the alimentary canal, tuberculous ulcers frequently are found in the intestine, and lesions occur in the liver and spleen more frequently than in the organs of the chest. Before pasteurization of skim milk and whey became rather uniformly practiced, many calves and pigs were infected with tuberculosis from these products brought back to the farm from the creamery or milk station Human beings and animals affected primarily with pulmonary tuberculosis, often develop intestinal lesions from the swallowing of quantities of infective sputum Infection in birds is nearly always the result of ingestion of organisms picked up with the feed from the ground Intestinal ulcers and liver and spleen lesions are commonly found in avian tuberculosis

3 WOUND INFECTION Tuberculosis of the skin, or *lupus,* occasionally occurs in man but is very rare in animals In man it is often known as "pathologists' warts" since the infection usually is contracted while conducting autopsies. The bacilli in such lesions frequently become greatly attenuated in virulence. In cattle tubercle-like lesions of the skin are rather common. These contain acid-fast bacilli and for a long time they were regarded as "skin tuberculosis" but many workers have failed to cultivate or to infect experimental animals with this material and it is now believed that the causative organisms are not true tubercle bacilli

4 CONGENITAL TUBERCULOSIS A few instances have been described in which new-born calves were infected with generalized tuberculosis In these instances a tuberculous lesion has developed in the placenta and this has eroded into the fetal blood vessels, thus showering the fetal tissues with organisms. Such animals die shortly after birth

Congenital tuberculosis should be carefully distinguished from hereditary tuberculosis of which much may be heard but which is, in reality, fictitious. Neither this nor any other infectious disease is inherited Any disease-producing agent of which we know, if carried in the germ plasm, would destroy that heredity-carrying bit of protoplasm Young animals, born into an en-

vironment where tuberculosis is rampant naturally are very apt to acquire the disease from their surroundings The "tuberculous taint" which people and animals were supposed to carry in their family trees is, in reality, merely familial infections as a result of intimate association in the same environment.

Immunity in Tuberculosis. The waxy substance present in tubercle and other acid-fast bacilli serves to protect them against the antagonistic influences of the body Even in naturally non-susceptible species of animals, tubercle bacilli usually are destroyed only after a long time Frequently the immune reaction in such species consists of a walling-off reaction in which the nest of bacilli is surrounded by a dense wall of tissue which keeps the organisms more or less dormant

That a type of immunity occurs in the course of tubercle infection is well established This was first shown in the so-called *Phenomenon of Koch* It was observed by Koch (20) that guinea pigs which already were infected with a low grade tubercle infection reacted differently to a second inoculation of a culture of high virulence than animals which had not suffered the primary infection The animals which were already infected proved to be refractory to the second dose Whereas the previously normal animal developed an acute, progressively fatal disease, the previously infected developed only a swelling at the point of inoculation This became a local abscess which opened to the surface and sloughed away the necrotic tissue and the virulent bacilli without involvement of the neighboring lymph nodes Calmette and Guerin (6), much later, using a dose of virulent tubercle bacilli intravenously which produced acute, fatal, miliary tuberculosis in normal cattle, found that tuberculin-reacting cattle (infected) could not be so killed These animals showed an immediate reaction from which they rapidly recovered and then they continued on their course of life as if nothing had happened These experiments show that a new, more acute infection cannot be superimposed upon one already established, that a chronic disease is a protection from a more acute form

These altered reactions are manifestations of a state of allergy The relation of allergy to immunity is a question over which there has been a great deal of controversy Some believe that allergy and immunity are identical; others think that they are different properties which have no relationship to each other The allergic condition evidently has an important role to play in the course of the disease When small infective doses of tubercle bacilli reach individuals of a susceptible species which have not previously encountered infection (initial infection), there is a marked tendency of the tissues to wall off the infection and to reduce it to a latent state In the allergic individuals, on the other hand, there is a tendency for the tissues to react with an acute,

inflammatory reaction when tubercle bacilli, escaping from a primary lesion, find themselves deposited in a new location These lesions do not tend to heal but rather to spread and to destroy large amounts of tissue Some believe that one or two latent tubercles in the lungs of adult persons derived from old childhood infections render them somewhat resistant to serious reinfection later in life Others think that the latent infections, because of the state of allergy produced, favor a spreading destructive disease when reinfections occur.

Artificial Immunization. Because of the great importance of this disease, it is likely that more attempts have been made to find immunization methods for tuberculosis than for any other disease of man or animals Unfortunately these methods have not met with a large measure of success The earlier attempts at immunization have been reviewed by Mohler and Schroeder (25) The matter will not be discussed in any detail here but a few of the products that have been tried will be discussed briefly

1 TUBERCULIN This name has been given to a variety of aqueous extracts of tubercle bacilli It was first made by Koch (19) in the hope that it would have immunizing value, but these hopes have not been realized In the course of the work of testing it on patients its diagnostic value was discovered and it is for this purpose that it is used today

2. KILLED TUBERCLE BACILLI When large numbers of tubercle bacilli, killed by heat or chemicals, are deposited in tissues, tuberculous tissue is produced around the deposit and abscessation is likely to occur When used as vaccines, therefore, the dosage must be kept small There is evidence that such vaccines enhance the resisting power of experimental animals somewhat, but the products have never been of service in practical work

3 LIVING CULTURES Numerous experiments with attenuated tubercle bacilli, and with acid-fast organisms other than tubercle bacilli, as vaccines for enhancing the resistance of animals to tuberculosis have been tried but with little success Perhaps the best known of these vaccines which have now been discarded was the "bovovaccine" of von Behring, a vaccine which was widely heralded for protecting cattle. The nature of the vaccine was not disclosed for a long time but finally it became known that it consisted of a virulent strain of human tubercle bacilli The vaccine will, indeed, confer rather a strong resistance to cattle, and with little damage to them, but the method was given up when it was learned that some of the vaccinated cattle were eliminating human tubercle bacilli in their milk

Another highly publicized vaccine was that of Friedman, who came to the United States in the early part of the century with a widely heralded

"turtle serum" treatment for tuberculosis Later it developed that the product was a suspension of living acid-fast bacilli which had been isolated from a turtle suffering from "cold-blooded" tuberculosis This vaccine proved worthless except as a means of enriching the pocket of the vendor.

B. C G stands for the *Bacillus of Calmette and Guerin* (6) These workers cultured a bovine type tubercle organism continuously on a bile-saturated medium for thirteen years, passing the organism through more than seventy generations in this time It gradually changed in physical characteristics and lost its virulence, until at the end of this period it had lost its tuberculogenic properties completely, according to its sponsors The culture was used as a vaccine on guinea pigs, calves, monkeys, and finally on babies It is used in two ways, by injection and by mouth, the latter being used especially for infant vaccination Recently the method of injection seems to be gaining favor over the oral method as being more certain For calves or babies, Calmette urged that the vaccine be administered as early in life as possible, even as early as the first or second day A strenuous effort is then made to keep the young child or animal in a tuberculous-free environment for three months or as long as is practicable It is then returned to its normal life, i e, into a tuberculous environment Calmette terms his method of immunization, *premunizing*, and the result a state of *premunition* These terms have been generally adopted for this situation in which immunization against virulent infection is accomplished by establishing a more or less persistent benign infection It is supposed that the heightened resistance in these cases lasts only so long as the more benign infection persists

In general the results from the use of this vaccine appear to be good The vaccine will not entirely prevent tubercle infection but it appears to increase the resistance of the infant or animal so that it can hold the disease in check and prevent it from progressing to a serious state When infants or animals are developing in environments heavily laden with tubercle bacilli, many of them die early in life from actively progressive tuberculosis *B C G* seems to be able to prevent this from happening Some have claimed that the vaccine is not safe, that under certain conditions it may resume virulence This question is not yet settled but many thousands of infants have received the treatment, and there is little evidence that damage has been done Accidents may happen, of course, and this seems to be the explanation for the disaster at Lubeck, Germany, where about seventy-five infants died of tuberculosis following vaccination The German investigating committee was not able to determine the precise cause of the disaster but considered it probable that the vaccine culture and virulent cultures had been mixed in the laboratory where the vaccine was made

Park, and associates, in New York City have vaccinated infants with the vaccine, and report favorable results therefrom Some experimental work has been done on animals in this country, but it is unlikely that it will ever be put to practical use for the reason that tuberculosis is being eliminated by the eradication method In countries where the disease is widespread, there seems to be a real field for its use

Recently there has been considerable interest in the findings of Brooke and Wells (4) that an acid-fast organism which they had isolated from the vole (field mouse) was capable of conferring a rather high resistance on guinea pigs to both human and bovine type tubercle infections This organism is almost non-pathogenic for guinea pigs The authors claim that the degree of immunity conferred is considerably greater than that due to *B. C G* The outbreak of war has prevented extensive work on the value of the vole bacillus as a vaccine for mammalian tuberculosis

Chemotherapy in Tuberculosis. A large number of substances have been tried in the hope of finding a chemical agent which would destroy tubercle bacilli *in vivo* and would, at the same time, be tolerated by tissues of the body Until very recently there were no hopeful results In 1938 Rich and Follis (30) reported that sulfanilamide inhibited the development of experimental tuberculosis in the guinea pig In 1940, Feldman, Hinshaw and Moses published a preliminary report on the efficacy of a new drug, "promin" in inhibiting the development of tuberculosis in guinea pigs Their final report (13) appeared in 1942 Chemically the new drug is Sodium p, p′ Diaminodiphenylsulfone -N, N′-Didextrose Sulfonate It belongs to the group of "sulfa" drugs as will be noted by its formula When this material was incorporated in the feed in the proportion of 1 per cent it was readily consumed by the animals with no serious ill effects, although it is apparent that it is slightly toxic in this concentration Guinea pigs fed daily on this drug showed a greatly heightened resistance to the development of experimental tuberculosis Whereas control animals, which did not receive the drug, developed a progressive disease which led to death in every instance, the treated animals in about one half of the cases showed no evidence of tuberculosis, grossly or microscopically, and in the remainder the lesions were definitely fewer and less progressive than in the controls These results are very encouraging to the belief that progress is being made toward the finding of a cure for tuberculosis

Tuberculin. Tuberculin is a protein or a protein-derivative produced by tubercle bacilli It is contained in any aqueous extract of the bacilli and in media upon which tubercle bacilli have grown Until recently the tuberculin of commerce was made from cultures grown on glycerin broth. Most of it is

now made from cultures grown on synthetic media. After these cultures have reached their maximum growth, they are boiled to destroy their vitality and to extract the bacilli, after which the organisms are removed by filtration. The clear filtrate is evaporated to the desired concentration

The nature of tuberculin remained obscure from the time it was discovered by Koch in 1890 (19) till Long and Seibert (21) finally obtained it in a purified form in 1926 It was long believed to be a protein but chemical analysis was impossible so long as the organism was cultivated on a medium containing protein By cultivating the organism on a synthetic, protein-free medium, Long and Seibert were able to precipitate from the filtrate a crystalline protein which apparently is the active principle



Fig 51 *Mycobacterium tuberculosis*, Bovine type Growth on glycerin broth In the right hand flask the culture is about two weeks old in the left it is about two months old Growth appears as a dull, grayish white pellicle which finally covers the surface of the fluid medium, becomes thick, opaque, folded into creases and pushes up on the sides of the flask at the margins The fluid remains perfectly clear

So long as impure tuberculins were used, their concentrations were expressed in terms of Koch's OT (Old Tuberculin) OT is a glycerin broth filtrate which has been evaporated to $\frac{1}{10}$ of its original volume Different lots vary in potency although when standardized conditions of production are adhered to, the variation is not great Since the active principle can now be obtained in relatively pure form, the concentration can be accurately measured in terms of this principle

It was first discovered by Koch that tuberculin was highly toxic for the tuberculous while being nearly innocuous for normal animals When tuberculin is injected into animals harboring one or more tubercles, a ***general reaction*** manifested by fever and constitutional symptoms occurs if the dose is sufficiently large If the animal is destroyed while at the height of the reaction it will be found that an inflammatory reaction has occurred around the tubercles present in the organs This is termed the ***focal reaction*** When sufficient tuberculin is used, tuberculous animals sometimes react so violently as to cause death If the tuberculin is injected into a tissue from whence it is not quickly disseminated, such as the dense layers of the skin, a ***local reaction*** at the point of injection becomes manifest.

Tuberculin Tests. Prior to the last twenty years the tuberculin test was not extensively employed in human practice, inasmuch as nearly all adults harbored latent tubercles and reacted because of them The test had little diagnostic value, *therefore, because the clinician could not determine whether the* reaction was due to an active infection or to an old, healed, childhood lesion. In this country the active campaign against tuberculosis has changed conditions so that not nearly all persons now react to tuberculin, and consequently the test has greater significance

In using tuberculin diagnostically in human beings, great care is taken to avoid constitutional reactions, since it is probable that the inflammatory reactions in the foci of the disease may spread the infection This is not an important consideration in animals since reactors usually are destroyed anyway. The test has been of great service in dealing with the disease in cattle, swine, and chickens When testing for infections due to the mammalian types, tuberculin made from either the human or bovine bacillus may be used When testing for infections due to the avian type, the tuberculin must have been prepared from that type

1 THE THERMAL TEST This was formerly the standard method of testing cattle for tuberculosis In later years it has been largely replaced by tests which require less time The tuberculin (10% Koch's O T , 2 cc or more) is injected subcutaneously after several temperatures have been taken at two-hour intervals to make certain that the animal is not suffering a fever from some other cause After eight hours, temperatures are again taken at two-hour intervals through the 16th or 18th hour after the injection. A typical reaction consists in a rise in temperature of at least 2 degrees F which appears some time between the 8th and 18th hour and subsides within twenty-four hours

As has been explained above, this form of test is not used on human beings because of the danger from the constitutional reaction

2 THE INTRADERMAL TEST This test is now extensively used on cattle, swine, chickens, and man The tuberculin is more concentrated than that used for the thermal test (usually 25% O T or some precipitated tuberculin) A small quantity, usually about 0 1 cc is injected into the dense tissue of the skin In cattle the tail-fold is usually used, swine are injected into the ear, chickens into the wattle A positive reaction is indicated by a local swelling which is firm and sometimes persists for days The reactions in cattle are judged on the third day (72 hours) or later The same period is used for the swine tests The *reactions in chickens are judged on the second day*

The tuberculin now supplied by the Bureau of Animal Industry of the U S Department of Agriculture for testing cattle is made from a human type

culture grown on a synthetic medium. The filtrate is concentrated by evaporation. The product is much purer than the old O T, but the active principle is not isolated This product has been designated Tuberculin, Special F, to distinguish it from the older product made from cultures grown on glycerinated beef broth.

The Mantoux test for tuberculosis in man is an intradermal test done with tuberculin which has been greatly diluted so as to avoid the possibility of systematic reactions. Formerly O T. 1/5000 was used, from 0 05 to 0 1 cc being injected into the skin of the forearm A few years ago Seibert (33) produced



FIG 52 The Intradermal Tuberculin Test in Cattle *Left,* Making the intradermal injection into the right tail fold *Right,* Typical swelling at the point of injection 72 hours later (Courtesy of I T Faulkner)

a very pure and active tuberculin by precipitating a proteose-like substance with trichloracetic acid from filtrates of cultures grown on synthetic media This material seems to have great advantages over the older products in that it is freed of impurities which may complicate the tests and also it can be accurately standardized It is manufactured and sold under the name P P D (*Purified Protein Derivative*) When using this material it is customary to give a dose of very highly diluted material, and, if this proves negative, to follow in a few days with a much more concentrated solution

3. THE OPHTHALMIC TEST This test is sometimes used on cattle A concentrated tuberculin (Koch's O T) is instilled into the conjunctival sac with a camel's hair brush or with a medicine dropper A positive reaction is indicated by an inflammation of the conjunctiva during the course of which pus is formed and appears at the inner canthus. One instillation of tuberculin serves to render the eye more sensitive to another; hence it is common practice to sensitize the eye by one treatment and to repeat the treatment two or

three days later, the reaction being observed and judged after the second instillation The inflammation and appearance of the exudate is rather prompt. The test is usually read from 4 to 6 hours after the application of the second dose of tuberculin.

Opinions differ as to the reliability (comparative) of the three types of tests when used on cattle The intradermal is probably the most sensitive. The ophthalmic alone is not to be relied upon since many animals fail to react to it when tuberculous On the other hand, the ophthalmic test will sometimes be positive when others are negative Many workers employ all three tests and this procedure undoubtedly gives the highest percentage of accuracy

The tuberculin test is highly reliable for detecting tuberculosis in man and animals, yet it is subject to some errors When the lesions are very extensive, the tissues often are so saturated with the by-products of the tubercle bacillus that they will not react to tuberculin As a rule these cases present clinical symptoms and a correct diagnosis can be reached by a physical examination On the other hand there are a certain number of animals that exhibit typical reactions to tuberculin which fail at autopsy to show lesions of tuberculosis. In the cattle tuberculosis-eradication program in the United States these animals have amounted to less than one per cent of the animals tested, but where the number of reacting animals has been low the percentage of no-visible-lesion reactors amounts to ten per cent or more of the total number of reactors The cause of these reactions has not been determined but several plausible explanations have been offered

1 That many such animals are in the early stages of the disease at which time reactions to tuberculin will occur though there are no visible lesions

2 That many animals have small lesions which may be located in parts of the carcass not ordinarily examined in routine meat inspection work

3 That animals which have been in contact with avian tubercle bacilli will ordinarily exhibit slight or no visible lesions but some of these will react to tuberculin Also, cattle may be sensitized to tuberculin by contact with human tubercle bacilli.

4 That some, perhaps many animals come in contact with acid-fast organisms other than tubercle bacilli which are able to sensitize them to tuberculin.

Mechanism of the Tuberculin Reaction. Despite the fact that the tuberculin reaction is the best known and most studied of the allergic reactions, its mechanism is still largely unknown The reaction can be elicited by the injection of living or dead tubercle bacilli, or by tuberculo-proteins High grade allergic

sensitization can be produced most easily by the use of living cultures, less easily with dead cultures, and with considerable difficulty with the extracts of cultures In all cases it seems to be clear that tubercles must have formed, or at any rate that the granulomatous tissue reactions characteristic of infections with acid fast organisms must have formed The reactions are quite specific, in that the tuberculins must have been made from the type of organism which is responsible for the infection if reactions are to be obtained consistently * It is true that a certain amount of cross-reacting occurs, that is, animals affected with avian tuberculosis occasionally will give reactions with mammalian tuberculin, and vice-versa

After an intradermal or intracutaneous injection of tuberculin an early polymorphonuclear and eosinophilic cell infiltration occurs at the point of injection but these are soon replaced by histiocytic and mononuclear cells Oedema appears early and lasts for five to seven days Changes occur in the arteries of the region resulting in thrombosis of the vessels These changes are those which occur in cattle according to the studies of Feldman and Fitch (12)

The Eradication of Bovine Tuberculosis in the United States. After much discussion of the matter over a period of years, a plan was developed in 1917 which looked forward to the eradication of tuberculosis from the cattle of the United States This plan involved co-operation between the federal and state governments in a uniform method of approach to the problem The plan became known as the *Accredited Herd Plan of Bovine Tuberculosis Eradication* In outline the project was simple, in practice the agencies concerned had to meet many serious obstacles not the least of which was serious opposition from a small portion of livestock owners The plan was that every bovine animal in the country was to be subjected to tuberculin tests and those that reacted were to be removed and slaughtered All slaughtered animals were subjected to veterinary meat inspection examinations to determine the extent of the development of the disease in each case and to determine whether or not any portions of the carcasses might be salvaged for human food Before condemned animals were slaughtered, their market value was determined by experienced appraisers and the owners were reimbursed for their losses from the public treasuries of the federal and state governments

The Accredited Herd Plan has met with excellent success Whereas it was estimated in 1922 that about 4 0 per cent of all cattle in the country were infected with tuberculosis, by 1940 the estimated number had been reduced to about 0.46 per cent In other words the incidence of this disease had been re-

* For testing for mammalian tuberculosis tuberculin may be made from either of the mammalian types of tubercle bacilli Since the human type grows somewhat more profusely on artificial media, it frequently is used for making tuberculin for use on cattle The tuberculin made by the U S government for official testing is a human type tuberculin

duced in less than 20 years by more than 85 per cent. Continued testing and retesting is still further reducing the incidence of the disease and we may look forward to a time, not too far distant, when the disease will have been wholly eliminated

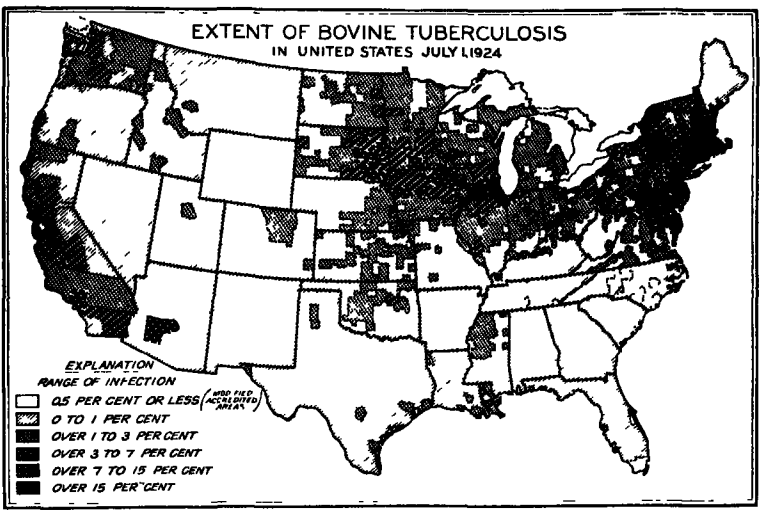


FIG 53 The Federal-State co-operative plan for the eradication of bovine tuberculosis in the United States The Accredited Herd Plan was launched in 1917 but at the time the above map was made little progress had been made The map conveys a good idea of the distribution of this disease before eradication was begun The work progressed rapidly after it was well organized In November 1940 all parts of the country were declared to be modified accredited, which means that less than 0 5 per cent reacted on the last tuberculin test of all the cattle (Courtesy, Bureau of Animal Industry, U S Dept of Agriculture)

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MYCOBACTERIUM PARATUBERCULOSIS

Synonyms The bacillus of Johne's disease, Johne's bacillus

This organism is a rod-shaped bacillus somewhat smaller than any of the tubercle bacilli. It causes a disease of cattle and sheep which in English-speaking countries generally is known as Johne's disease. It also is known as

chronic bacterial enteritis, chronic hypertrophic enteritis, and paratuberculosis The organism was recognized in diseased tissues for the first time by Johne and Frothingham (15) These authors mistook the organisms for avian tubercle bacilli and thought they were dealing with an isolated case of a peculiar nature Later it was recognized that many cases of this disease occurred but they were generally regarded as a peculiar form of tuberculosis until Bang (2) clarified the matter by careful study The causative organism was isolated in 1911 by Twort (17), in London Howarth (14), and Dunkin (6) who worked with the disease in sheep failed to obtain cultures of the organism from tissues by the use of technics which succeeded in cattle This leaves some question about the identity of the sheep organism, but since the lesions are identical it is assumed that it is the same as that of cattle. Several years ago we successfully infected several sheep by feeding them with infective material from cattle, the manifestations and lesions being typical of the naturally occurring disease (8)

Morphology and Staining Reactions. *Mycobacterium paratuberculosis* appears both in tissues and in cultures as a short, thick rod measuring about 0 5 micron thick by about 1 0 micron long It is strongly acid-fast and Gram-positive It has neither spores nor capsules In tissues it commonly develops intracellularly in the epithelioid cells which proliferate at the sites of localization of the organism

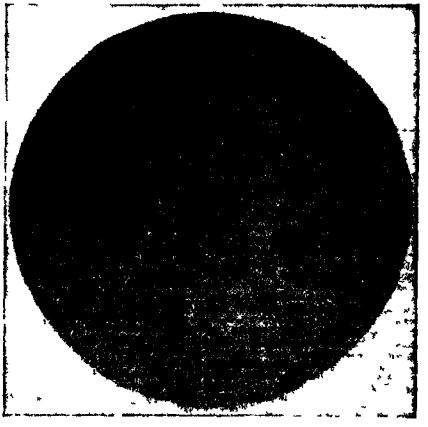


FIG 54 *Mycobacterium paratuberculosis* From a culture about two months old on a synthetic medium containing heat-killed acid-fast organisms For comparative size see illustrations of tubercule bacilli taken at same magnification x 900

Cultural Features. The causative agent of Johne's disease was first obtained in artificial culture by Twort (7) in 1911 This worker succeeded in obtaining growth after earlier workers had failed by the expedient of incorporating in his media a suspension of heat-killed acid-fast organisms of other species This was done after he had experienced repeated failures to obtain growth of this organism on a variety of media which are suitable for tubercle bacilli. It occurred to

Twort that perhaps some essential substance for growth of the Johne bacillus was absent from his media and that possibly whatever was needed might exist in the substance of other acid-fast organisms Having proved the correctness of this hypothesis, he sought to isolate this "essential substance" by extracting the cultures, and by using extracts of many other substances. It was found that both water and fat solvents were capable of removing the necessary substance from several species of acid-fast organisms, but extracts of other bacteria and of plant and animal tissues did not contain it All of this work was described in detail by Twort and Ingram (18) in 1913 Little more was learned of the nature of this "essential substance" until the work of Wooley and McCarter (21) appeared in 1940 These authors showed that "phthiocol," which Newman, Crowder and Anderson (16) had isolated from the human tubercle bacillus in 1934, had the qualities of the necessary factor Almquist and Klose (1), in 1939, had shown that phthiocol possessed the anti-hemorrhagic qualities of Vitamin K, therefore Wooley and McCarter tried the synthetic Vitamin K—2 methyl naphthoquinone—and found it also to be effective The "essential substance" of Twort therefore appears to be the fat and water soluble Vitamin K, the antihemorrhagic factor. The authors admit, however, that their cultures did not thrive quite as well with the extracts as with the suspensions of acid-fast bacteria, therefore it is possible that one or more additional factors which favor the growth of the Johne bacillus have not yet been recognized Incidentally it is of interest to note here that while the water-soluble vitamins, or food accessory factors, are known to be needed for the growth of a number of species of bacteria, this is the only instance in which it is known that one of the fat soluble vitamins is required



FIG. 55 Johne's Disease (Paratuberculosis) A clump of *Mycobacterium paratuberculosis* in intestinal scrapings × 900

Primary cultures of the Johne organism grow very slowly on all media, much more slowly than tubercle bacilli On Dorset's egg medium, fortified with about five per cent, by weight, of the moist cells of one of the rapidly

growing saprophytic acid-fast organisms added before sterilization and coagulation, the organism can be grown without great difficulty from infected lymph nodes where it often exists in the absence of other bacteria The cultures must be incubated at 37° C for about six weeks before evidences of growth appear During the period of incubation the tubes must be partially closed so that evaporation will not cause dehydration of the media Growth appears in the form of very small, dry, irregular colonies, not unlike primary cultures of mammalian tubercle bacilli When these are subcultured on fresh media of the same type, a confluent growth is obtained This usually is visible after two or three weeks incubation but reaches its maximum only after five or six weeks Cultures accustomed to growth on culture media are dry and flaky and of a cream color Even after many years of artificial culture, the organism will give little or no growth on most of the media which serve for tubercle bacilli if the essential supplement is not added Meager growths may occur, but if so, they will fail after two or three transplants on the same unfortified medium

It is difficult to "train" this organism to grow on fluid media In glycerin broth to which the "essential substance" has been added in the form of heat-killed cells of *Mycobacterium phlei*, growth is always poor After several months' incubation small floating islands may develop on the surface, and there may be a fairly heavy granular sediment on the bottom of the flasks It finally was discovered that certain synthetic media, when supplemented by the necessary growth factor, were far more favorable than the usual types The medium which we have found most successful is that which was described by Dorset, Henley and Moskey (5) It has the following composition

Dipotassium phosphate	1 0	gm
Magnesium sulphate	1 0	"
Sodium citrate	0 5	"
Asparagin	5 0	"
Ferric ammonium citrate	0 06	"
Glycerol	70	cc
Water	1000	"

To make this medium suitable for the Johne bacillus, 0 3 to 0 5 gm of dried cells of *Myc phlei* must be added prior to sterilization The medium is adjusted to about pH 7 2 if the final reaction is far from this point On this medium very heavy growths of *Myc paratuberculosis* can be obtained after the strain has become accustomed to it Very good growths can be obtained from some strains when the supplement is omitted, but such strains usually die out after a few transfers The growth on fluid media resembles that of the mammalian tubercle bacilli in that the fluid remains clear and the growth occurs in the form of a dry, dull pellicle which spreads across the entire sur

face of the medium and then thickens into a heavy, wrinkled blanket of growth.

Pathogenicity

FOR EXPERIMENTAL ANIMALS Rabbits, guinea pigs, rats, mice, chickens, and animals other than ruminants are not susceptible to infection If large doses



FIG 56 Johne's Disease An advanced case of the disease The animal is emaciated, has a rough dry coat and a harsh skin, is constantly scouring, and is so weak that she must brace her legs to keep from falling This animal had been artificially infected slightly more than one year previously by feeding of infective material

of culture are introduced into any animal, however, local lesions at the point of injection should be expected, since such reactions are induced by all acid-fast organisms If the bacilli are introduced into the peritoneal cavity, tubercle-like masses will appear on the great omentum

FOR CATTLE. Johne's disease is a disease of young animals and it is rather seldom that old animals develop clinical symptoms The great majority of cases are seen in two- and three-year-old females, and the disease most frequently

becomes apparent after the first or second calf is delivered. Greatest losses occur among high producing animals and it is presumed that the strain of lactation is responsible for breaking down the resistance of animals which are already carrying the infection The infection involves the intestinal canal and the lymph nodes of the mesentery The lower end of the small intestine, the cecum, and the beginning of the colon are the parts most often involved, but advanced cases may have lesions extending from the duodenum to the rectum. The lesions in the intestinal wall take the form of diffuse masses of

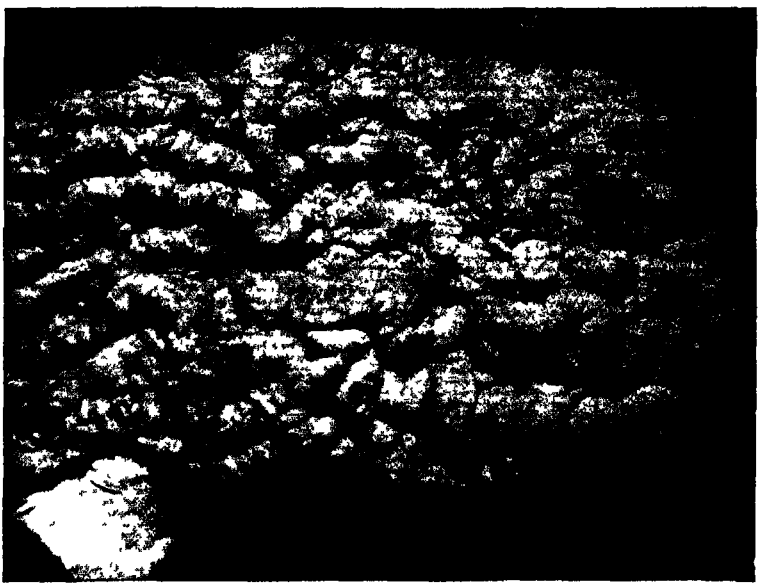


FIG 57 Johne's Disease Mucous membrane of a portion of the ileum The wall is greatly thickened by large deposits of epithelioid cells in the sub-epithelial tissue of the mucosa The irregular folds and plaques are characteristic There is a complete absence of ulcers and necrosis

epithelioid cells which often cause great thickening of the mucous membrane and the submucosa Unlike the tubercle infections, this organism does not cause the formation of tubercles and ulceration The intestinal wall may be grossly thickened, and when stretched the plaque-like thickenings may easily be seen, but the epithelium remains intact. The mesenteric lymph nodes may show no gross lesions, or at most a slight enlargement Sometimes the lymphatics on the serous surface of involved bowel portions are thickened into the form of tortuous cord-like structures conspicuous because of their en-

largement Great numbers of small acid-fast organisms usually can be easily demonstrated in films made from scrapings of the mucosa of the bowel or of scrapings from the cut surface of the lymph nodes The organisms usually are found in clumps, and frequently intracellularly. In sections most of the organisms are seen in the epithelioid cells of the lesion

As it occurs naturally, Johne's disease does not cause the loss of many animals at one time but the usual experience is that the losses occur gradually, one or two animals at a time In the aggregate the losses often are great since they occur persistently over long periods of time This accords with our experiences in an experimental herd which we have maintained for some years (12) It has been our experience in this herd that it is easy to produce infections by allowing uninfected calves to associate with infected adult animals but that adult animals seldom contract the disease in this way By administering infected material by mouth, we have found it possible to infect young calves and older animals as well, but whereas the greater part of the calves ultimately developed into clinical cases after an incubation period of from one to two years, the older animals became reactors to allergic tests for a time, these finally faded out and clinical evidence of disease failed to appear It is clear that the majority of cases of this disease are contracted very early in life, that many but not all of these animals develop symptoms in early adult life, and that adult infections are not common except as they have been carried up from early calfhood (9).



FIG 58 Johne's Disease (Paratuberculosis) Section through the subepithelial tissue of the mucosa of the small intestine of an acute case, showing the epithelioid cells which infiltrate this tissue and cause the characteristic thickening of the mucosa A typical giant cell is shown x 450

The symptoms are quite characteristic Usually the first evidence is general unthriftiness, with roughness of coat and a dry skin A diarrhea begins and frequently becomes very profuse The hindquarters and tail of the affected animal soon becomes plastered with the liquid discharge The animal becomes

dehydrated, emaciation rapidly advances, the eyes sink into their sockets, and the animal becomes weak The appetite fails and for many days the animal can be induced to eat nothing. There is no fever, the bodily temperature often being subnormal Death may occur within a week or so from the time the diarrhea began On the other hand, some animals cease scouring after a few days, improve in condition for a time, only to have a recurrence of the symptoms after periods which vary from a few days to a year or more Frequently the recurrence of symptoms appears after the termination of the following pregnancy (10).



FIG 59 Johne's Disease Epithelioid cells in the mucous membrane of a rapidly progressive case of the disease The cells are packed with masses of *Mycobacterium paratuberculosis* This preparation had been stained with the Ziehl Neelson technic for acid fast bacteria In preparations stained with hematoxylin and eosin these cells appear quite normal x 900

FOR SHEEP AND GOATS In these animals the symptoms, and the lesions are similar to those in cattle It has already been pointed out that several workers have suggested that the sheep disease may be caused by a different organism than that which causes the disease in cattle, since methods which succeed in isolating and cultivating the cattle organism have not succeeded with the organism of sheep and goats

Mode of Infection. The natural route of infection undoubtedly is by the digestive tract Experimental evidence indicates that infections are relatively easily produced by this route, but that other factors determine whether the disease will progress to the point where clinical evidence of the disease develops, or whether the disease remains quiescent, or dies out

Methods of Diagnosis. Several methods are available for detecting the presence of this disease in animals The principal ones are as follows.

1. CLINICAL METHODS. Animals which persistently scour and emaciate should be looked upon with suspicion. If such animals die or are slaughtered a por-

tion of the lower end of the small intestine should be sent to a laboratory to be examined

2. SCRAPINGS FROM THE RECTAL MUCOUS MEMBRANE In a certain number of advanced cases, probably not more than one-fourth of all cases, the disease spreads to the lower bowel and even the rectum In such cases the thickened rectal mucosa may be recognized by palpation If no thickening is recognized, a bit of the mucosa may be pinched out with the thumb nail for microscopic examination The fragment should be well-rinsed with clean water, placed on a slide, and crushed Smears made from the crushed fragment then are stained for acid-fast organisms It should be kept in mind that acid-fast organisms occur in the feces of all cows and that these must be differentiated from the bacillus of Johne's disease The Johne bacillus is smaller than most of the saprophytic forms, and it occurs in characteristic clumps An experienced observer usually can be sure of the identification of the organisms seen

3 THE ALLERGIC TESTS In 1909, before the bacillus of Johne's disease had been cultivated artificially, Oluf Bang (3) called attention to the fact that many infected animals would react to avian tuberculin administered subcutaneously This test was used by many workers with fairly satisfactory results One of Twort's first efforts after obtaining pure cultures of the organism causing Johne's disease was to make a product analogous to tuberculin for use in diagnosis Twort's cultures grew rather poorly, however, and the products which he was able to make were not successful, although it was demonstrated that reactions could be obtained in some animals Beach and Hastings (4), in this country, were the first to use johnin, or paratuberculin, intravenously Their work, and that of others, has demonstrated that this method of administration is more effective than by subcutaneous injection It has not been demonstrated, however, that johnin is any more effective, or has any advantages over avian tuberculin, when used intravenously

The physiological reaction following the intravenous injection of johnin and avian tuberculin is the same Normal animals, if not overdosed, show little or no reaction Diseased animals, however, usually begin to show signs of discomfort within an hour or so There is depression, the animal ceases to eat, the head is held low, the hair coat stands on end, and the animal may shiver Some animals begin to scour profusely and this may last for several days. The fever curve begins from the third to the fifth hour after injection of the test material and reaches its height from the fifth to the eighth hour depending upon the size of the dose and the potency of the test fluid After the peak has been reached, the temperature usually falls quickly to normal by the 10th or 12th hour (11).

Both avian tuberculin and johnin may be used intradermally The crude filtrates may be concentrated, or it is possible to precipitate an active agent from such filtrates by the method used by Seibert for preparing her P P D from tuberculin We have tried all of these products and believe them to be inferior to avian tuberculin, administered intravenously Others, however, report successful results with the intradermal test Such tests are much simpler and less time consuming than intravenous tests, and undoubtedly will replace the latter if it can be shown that they give results that are as accurate

4 SEROLOGICAL TESTS Animals affected with Johne's disease regularly fix complement with antigens made from any of a series of acid-fast organisms In other words the test is group- and not species-specific It will not distinguish between tuberculosis and Johne's disease, and furthermore many animals that have neither disease will fix complement, presumably because of sensitization with other kinds of acid-fast bacilli Our experience has shown that a negative test on an animal showing suspicious symptoms indicates that the disease is something other than Johne's disease, but that positive tests have to be interpreted with great care (13)

Immunity. Animals which develop clinical evidence of Johne's disease seldom recover permanently Occasionally animals which have been far advanced in the disease will apparently recover, but there are no records of animals which have completely freed themselves of the disease after having shown clinical evidence of infection One animal which we observed remained well for five years after having suffered with the disease, but minimal lesions were found at autopsy It seems probable, judging by the results of repeated allergic tests on a herd of experimental cattle, that many more animals become infected than ever exhibit symptoms, and that in these symptomless animals the disease develops only to a limited extent and ultimately is thrown off completely

Vallee and Rinjard (19) in 1926 began a series of experiments in France to determine whether the bacillus of Johne's disease might be used as a vaccine to protect cattle. They found that when cultures were injected subcutaneously they produced only harmless tumors, and that these tumors apparently gave a considerable measure of protection against the naturally acquired disease In a report published in 1934 (20) it is stated that over 12,000 animals had been treated and that the method had proven to be safe In a small experiment conducted in this country it was shown that vaccination in this way did not prevent infections of calves but that vaccinated calves withstood the disease better than the control animals (7) The method has not been tried

on commercial herds in this country, and no further information is available on the results of the French workers

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MYCOBACTERIUM LEPRAE

Synonyms *Bacillus leprae*, the leprosy bacillus

Human leprosy is one of the oldest of known diseases In 1874 Hansen (2) observed the organism, which has come to be known as Hansen's bacillus, in the granulation tissue of lesions of this disease In certain types of the disease this organism may easily be demonstrated in large numbers, in others its demonstration is more difficult Its constant presence leaves little doubt that it actually is the causative agent of the disease It is strongly acid-fast and appears in the form of slender solid-staining rods which usually are clumped Many workers have isolated acid-fast organisms from leprous tissue and have believed them to be the bacillus of Hansen It is quite certain that most of these cultures are not the true causative agent of leprosy, and it is not unlikely that the true organism has not as yet been cultivated. The term *Myco leprae*

has been applied to many diverse cultures Until proof can be presented that a culture is, in fact, the bacillus which may so easily be demonstrated in naturally infected tissues, this name ought not to be applied to it

The lepra bacilli, i e, the group of types which various workers have isolated from cases of leprosy, differ among themselves but the group stands apart from the ordinary acid-fast organisms which are so numerous in soil, and from those which affect cold-blooded animals (1) Such organisms do not appear to exist in or on normal human skins What their relationship is to the disease, is not clear at the present time

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THE ACID-FAST ORGANISM OF RAT LEPROSY

In a rat destruction campaign waged in Odessa in 1901 Stefansky (2) found about 5% of the animals to be suffering from a disease which resembles human leprosy somewhat, particularly in the fact that the lesions contain large numbers of acid-fast organisms The disease has been seen in other parts of the world (1), but usually only a fraction of one per cent of the population is involved In some cases there is enlargement of the lymph nodes, particularly those of the axillary and inguinal regions The glands become enlarged and hardened but do not suppurate Myriads of acid-fast bacilli can be found in such glands, usually located intracellularly, in large cells which probably are epithelioid in nature. In other cases the disease affects the skin and subcutaneous tissue and sometimes the underlying muscle The hair is lost from such areas, and

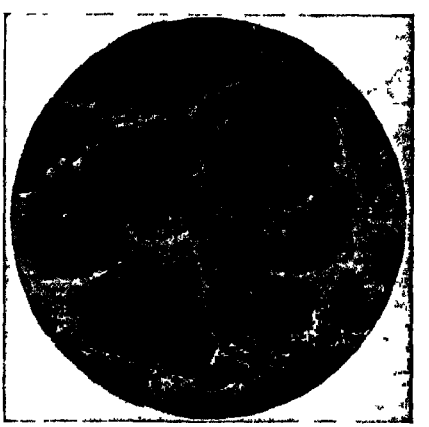


Fig 60 The Acid Fast Organism of Rat Leprosy Large epithelioid type cells located in the granulomatous lesion of the subcutaneous tissue and packed with the acid-fast lepra bacilli x 900

sometimes ulcers are formed from which a thick discharge, rich in bacilli, exudes In the granulation tissue which forms beneath the skin, bacilli are plentiful Lesions in the internal organs are rare, except that nephritis usually exists, in which cases bacilli cannot be demonstrated in the kidneys

It is not at all certain that the bacillus of rat leprosy has ever been successfully cultivated Several have isolated cultures which they have thought to be the causative agent, but these cultures usually fail to reproduce the disease, whereas it can be induced by injecting infected tissue The rat leprosy organism probably has no relation to that of human leprosy, inasmuch as rats are resistant to inoculation with leprous tissue from man The disease is not transmissible to any of the domesticated animals

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ACID FAST BACILLI ASSOCIATED WITH ULCERATIVE LYMPHANGITIS IN CATTLE

In the course of the work of eradicating bovine tuberculosis in the United States, much attention has been given to a condition which occurs in many parts of the country to which the name "skin lesion tuberculosis" was early attached Traum (8) appears to have been the first to call attention to the fact that these lesions sometimes caused cattle to react to the tuberculin test. Animals in some areas are more often affected with these lesions than in others, and the distribution of cases does not correspond to the distribution of orthodox tuberculosis It is now fairly certain that these lesions are not caused by tubercle bacilli

The lesions usually occur on the skin of the lower parts of the legs They first appear as nodules which seem to be attached to the skin but are actually located in the subcutaneous tissue In the course of time these nodules usually soften, and ulcerate through the skin In the meantime other nodules usually appear along the course of the lymphatics It is not uncommon to see animals having from four or five to as many as twenty-five nodules, many of which have broken through the skin After discharging their contents the lesions usually heal In some cases instead of discharging, the lesions coalesce forming large dense masses formed mostly of connective tissue in which areas of suppuration occur The pus may be fluid, pasty, or dry, inspissated, and calcareous The neighboring lymph nodes usually do not become involved, a situation which invariably occurs in the presence of true tubercle infection

The histological structure of these nodules resembles that of tuberculous tissue Acid-fast bacilli which cannot be distinguished morphologically from

bovine tubercle bacilli can be found in most cases, although usually they are not numerous Although many workers (1) (2) (3) (4) (5) (7) (8) have studied these lesions, none has succeeded either in obtaining cultures of the acid-fast organism, or in causing infections in laboratory animals Neither has anyone succeeded in transferring the infection to other cattle, even by inoculating them with material directly from the nodules Several workers have occasionally isolated cultures of acid-fast bacilli but the strains isolated usually have the characteristics of saprophytes, i e , they have been incapable of producing more than an abscess at the point of inoculation (8)

Animals affected with these lesions do not always react to tuberculin and when reactions occur they are somewhat atypical in many instances Such animals may react at one time and fail to react at another When the lesions are removed surgically, gradual loss of sensitivity occurs The disease is not a serious one, *per se*, although the blemishes produced are distasteful to owners of fine cattle The most serious feature about them is the fact that they confuse the diagnosis so far as tuberculosis is concerned Reactions to tuberculin cannot be safely ascribed to the presence of such lesions, unless the history of the animal makes the occurrence of genuine tuberculosis in the same animal highly improbable, for they are found in tuberculous as well as in non-tuberculous cattle Until quite recently one could have assumed because of the absence of references to these lesions in the literature that the condition did not occur in Europe This was not true, however Identical lesions were described in English cattle by Robertson and Hole (7) in 1937, in Danish cattle by Gotzsche and Plum (1) in 1938, and in Swedish cattle by Krantz (4) in 1938 As early as 1913, Perard and Ramon (6) described a similar if not identical condition in France.

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THE TUBERCLE BACILLI OF COLD-BLOODED ANIMALS

Bataillon, Dubard and Terre (3) in 1897 isolated an acid-fast organism from fish (carp) which were living in polluted water. Since that time, dis-

cases having some resemblance to tuberculosis of warm-blooded animals and associated with acid-fast organisms have been seen in various cold-blooded animals such as frogs, tadpoles, snakes, turtles, and other species of fish These organisms are readily cultivated upon media which support growth of true tubercle bacilli They differ in cultural features but are alike in that they grow rapidly at low temperatures Several workers have attempted to show a relationship to tubercle bacilli of warm-blooded animals but it is clear that they are quite different The diseases are seen most often in animals kept in captivity and are the cause of deaths of many animals of this type kept in zoological parks and aquaria In many instances the diseases can be reproduced readily by feeding pure cultures (2) (4) Although these organisms resemble closely some of the saprophytic acid-fast organisms commonly found in soil and water, it is clear that in most instances the tubercle bacilli of cold-blooded animals are true parasites that have a specific affinity for their hosts and are not found commonly in nature except in their hosts and materials contaminated by them (2) Friedman attempted to use a turtle strain for immunizing people to tuberculosis but the experiments failed Apparently the tubercle bacilli of cold blooded animals have little in common with tubercle bacilli of warm blooded animals other than the fact that both are acid-fast A good review of the subject of tuberculosis in cold-blooded animals is that of Aronson (1)

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THE SAPROPHYTIC ACID FAST BACILLI

Acid-fast organisms belonging to the mycobacteria are widespread in nature Nearly all soils harbor them (1), and they are common on vegetation and in the alimentary tracts of herbivorous animals They have also been found on the mucous membranes and skins of animals

These organisms apparently are harmless, for the most part, although abscesses and tubercles may be produced by injecting them into animals They frequently show a very close resemblance to tubercle bacilli but may be distinguished from them by lack of pathogenicity for animals, rapid manner of growth on culture media, and the fact that they will grow well at room temperature Most of these organisms will develop luxuriantly on plain glyc-

erol agar, on plain agar, or on solutions of simple mineral salts. They grow on fluid media in the form of pellicles, in most instances, and produce filtrates which resemble tuberculin Usually these filtrates will not give reactions in animals affected with tuberculosis but will in animals which have been inoculated with the homologous organisms

The studies of Thomson (4), Gordon (2), and Gordon and Hagan (3) have made it clear that many of the acid-fast organisms that have been isolated from a variety of sources by different persons in the past and have been endowed with different names depending usually upon the source from which they were obtained, are in reality alike Thus of a collection of 331 strains, most of which had been isolated by the authors from soil and water, but which included about fifty named strains of other authors, the greater part fell into three principal groups Thus the smegma bacillus of Alvarez and Tavel, *Myc gramins* and *Myc stercusis* of Moeller, *Myc berolinense* of Rabinowitsch, the *nasenschleim* bacillus of Karlinski, several of the so-called leprosy bacilli and others, together with 104 strains isolated from soils, proved to be indistinguishable from each other Based upon this work, the Fifth edition of Bergey has classified all of these organisms as a single species under the named *Myc lacticola* In the present state of our knowledge of these organisms it is inadvisable to attempt naming such cultures We prefer to regard them merely as "saprophytic acid-fast bacilli"

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CHAPTER XXIV

THE PATHOGENIC SPORE-BEARING ANAEROBIC BACTERIA

The anaerobic spore-bearing organisms which are classed together under the generic name *Clostridium* are quite similar in morphology and staining qualities. All are rather large, rod-shaped, and Gram-positive when young. The rods usually are straight. Some species commonly appear in tissue fluids singly or in pairs whereas others usually are found in long chains. The spores in most species are oval, are located somewhat centrally in the rod, and usually are greater in diameter than the rod itself. Because of their great similarity in form, it usually is not possible to be certain of the identity of these organisms without studying the cultural characteristics. In many instances the symptoms and lesions of the diseases produced by them are sufficiently characteristic to enable a reliable diagnosis to be made.

These organisms may be divided into two groups on the basis of their disease-producing mechanisms. The first consists of those species which have little or no power to invade and multiply in living tissues. Such organisms owe their pathogenicity to their power of forming powerful toxins which are produced in localized areas, or outside of the body. The damage in these instances is almost or wholly due to the absorption of the poison. The organisms of this group which will be described are *Cl. tetani, Cl. botulinum* and *Cl. parabotulinum*. The second and larger group consists of species which have the power to invade and multiply in tissues. These organisms in most cases also produce toxins but they are much less potent than those of the first group and the damages to the tissues are not due wholly to the toxins. These organisms are sometimes referred to as the "gas gangrene" group since many of them are concerned in wound infections of the gas gangrene type in man. Wounds of animals sometimes become infected with pathogenic anaerobes in which case the condition is similar to that of man. A number of these organisms, however, find their way into animal tissues through the digestive tract and thus we have rapidly developing, highly fatal infections of animals without the existence of wounds of the skin.

The number of species of organisms which have been found in these gan-

grenous infections of animals is large. The ones most frequently encountered are the only ones which will be considered here These are

Clostridium chauvoei	The cause of blackleg in cattle and sheep
Clostridium septicum	The cause of braxy in sheep and of malignant edema infections in other species
Clostridium perfringens	The cause of lamb dysentery, "struck," and "pulpy kidney disease" of sheep, and occasionally of malignant edema infections of other species
Clostridium novyi	The cause of "black disease" of sheep, and occasionally of malignant edema infections of other species
Clostridium hemolyticum	The cause of bacterial icterohemoglobinuria or "red water" of cattle

The spore-bearing anaerobic bacteria cause animal diseases which are infectious without being contagious, that is to say, the diseases produced seldom if ever are transmitted from one animal to another All of these organisms have their habitat in the soil, and it is from soil, or vegetation, that the infections are derived Epizootics seldom occur, although it is possible for them to happen when conditions become favorable for a large number of animals to become infected from the same source simultaneously

CLOSTRIDIUM TETANI

Synonym *Bacillus tetani*

The causative agent of tetanus is the best known of all anaerobic, spore-bearing bacilli, principally because the symptoms of the disease are so well-known and characteristic that it has not been confused with any other of the anaerobic infections The organism was isolated in impure culture by Nicolaier (8) in 1884 from white mice which had been inoculated with garden soil In 1889 Kitasato (4) obtained pure cultures by heating impure cultures from infected wounds, thus destroying the ordinary bacteria of suppuration but leaving the heat-resistant tetanus spores In the following year (1890) von Behring and Kitasato (2) published their classical work announcing the discovery of bacterial toxins of which the first was that of *Cl tetani*.

Morphology and Staining Reactions. *Cl tetani* is a slender straight rod from 0.4 to 0.6 microns in width and from 2 to 5 microns in length In both tissues and cultures it most often occurs singly but sometimes chains

of organisms forming long filaments are seen In old cultures the rods and threads disappear leaving the spherical spores These spores are formed after 24 to 48 hours' incubation, and appear in the ends of the rods, swelling them so they have the appearance of badminton rackets or drumsticks In some media spores are formed in abundance, in others even after prolonged incubation they are few in number Cultures are Gram-positive when young but after a few days most of the cells become Gram-negative Young cultures are actively motile by means of peritrichic flagella

Cultural Features. The tetanus organism grows on all of the ordinary media of the laboratory provided only that fairly good anaerobic conditions are maintained It will grow also in aerobic cultures in association with aerobic organisms Deep agar colonies are fluffy, cottony spheres When blood is present, hemolysis occurs Broth becomes slightly clouded but clears from sedimentation Gelatin stabs first develop a spike of growth along the stab, next cottony filaments extend from the stab at a right angle into the medium, giving a brush-like effect, then liquefaction and blackening of the medium occurs, and gas bubbles are formed Litmus milk is not usually changed A soft clot may be formed Coagulated blood serum is softened and in old cultures may be blackened but is not liquefied Cooked-meat medium and brain medium are not digested but become turbid and give off a very foul odor Carbohydrates are not fermented but dextrose greatly favors growth in the simpler types of media Growth occurs best at 37° C but slow growth occurs at 20° C



FIG 61 *Clostridium tetani* Stained preparation of a 48 hour culture in a meat piece medium The terminal spherical spores are characteristic x 900

Natural Habitat. The organism of tetanus was found originally by Nicolaier (8) in garden soils, 12 out of 18 samples being positive Fildes (3), in England, examined 70 soil samples including both cultivated and uncultivated and found 33 positive A number of workers have found the organism to be commonly present in horse manure Some have found it in the feces of cows,

sheep, dogs, chickens, rats, and guinea pigs, whereas others have failed to find it in some of these species, a fact which suggests that these animals may only be transitory hosts for the organism Noble (9), for example, found it in 11 out of 61 samples of horse feces, but failed to find it in 21 samples from cows Human feces also are sometimes infected with tetanus bacilli The experiences of different workers on this subject have differed widely, some being unable to find the organism in large series of cases, others finding it in a relatively large proportion TenBroeck and Bauer (11) demonstrated tetanus



FIG 62 Tetanus in a Pig Tetanic spasms of the muscles manifested by rigidity of the parts is characteristic of tetanus in all animals (Courtesy of the Jen-Sal Laboratories, Inc)

bacilli in the stools of 27 in a series of 78 individuals living in Peking, China, and concluded that the organism must be living in the intestinal canal since certain individuals who had lived on an almost sterile diet for more than a month continued to yield several million tetanus spores per stool

Pathogenicity. Infections in all animals and man occur as a result of wound contamination Clean wounds rarely result in tetanus It is the dirty wound which contains foreign material, particularly soil, that is most dangerous. Deep penetrating wounds are much more dangerous than superficial ones. During the World War of 1914–1918, the study of tetanus was greatly stimulated by the large number of human cases which developed in troops fighting on the cultivated fields of Flanders It had been known previous to that time that tetanus spores, washed free from toxin, did not ordinarily produce

tetanus when injected into animals; that the spores in such cases did not germinate, were taken up by phagocytes and ultimately destroyed It was learned that tetanus spores would not germinate in living tissues probably because of there being too much oxygen present, and that germination occurred only when the spores lodged in destroyed tissues Cultures of the tetanus organism will produce tetanus upon inoculation because the toxin present is a local tissue destroyer and thus a suitable environment is provided. When soil, or foreign materials of other kinds, accompany tetanus spores into a wound, they pave the way for the germination of the spores and the setting up of an infection Tulloch (12) showed that when washed spores were introduced with the toxins of some of the organisms causing gas gangrene, tetanus resulted with regularity It was also observed by other English workers that the injection of washed tetanus spores suspended in a dilute solution of calcium chloride produced tetanus, and that infections regularly occurred in animals receiving washed spore suspensions by inoculation into areas of the skin into which calcium salts had previously been injected When spores were injected intravenously and a calcium solution subcutaneously, tetanus resulted through local multiplication of the tetanus organism at the site of the injection of the salt Calcium salts seem to alter tissues in a manner to make them more favorable for germination of the tetanus spore.

Of the domesticated animals, the horse is by far the most frequently affected with tetanus Sheep are rather frequently affected, cattle and swine occasionally, carnivorous animals very rarely, and birds never The occurrence of the naturally acquired disease seems to correspond fairly closely to the susceptibility of the animals to tetanus toxin. The amount of toxin needed per gram of body weight to kill a chicken is about 350,000 times as great as for the horse For the dog it is about 600 times as great

In tetanus infections the bacilli do not multiply in any part of the body other than the local area where the infection occurred As soon as toxin formation is begun, it and pyogenic bacteria which usually are also present in the wound prepare a nidus where conditions are favorable for the tetanus organism and it is here that the organisms multiply and the toxin is generated The local effects of tetanus are negligible, the principal agent in the production of the disease is the toxin which is one of the most poisonous substances known.

There is considerable controversy about the manner by which tetanus toxin reaches the central nervous system where its principal effects are produced. If a fatal dose of tetanus toxin is injected into the foot of a susceptible animal, the onset of the disease may be greatly delayed by severing the principal motor nerve trunks of that leg (5), or by infiltrating the nerve trunk with antitoxin (7) Fatal tetanus may be produced by injecting a dose of toxin into one

of the peripheral nerve trunks which is not great enough to cause death when injected intravenously These and many other similar experiments seem to indicate that the tetanus toxin is absorbed by the peripheral nerves and that most of it passes through the nerves centripetally until it reaches the motor cells of the anterior horn of the cord at which time general symptoms of the disease make their appearance. The mechanism by which the toxin passes up the nerve trunks has not been adequately explained, and there are those who doubt that this explanation is the true one (1)

The symptoms of tetanus are similar in all animals They consist of chronic or tetanic spasms of the muscles Sometimes these begin in one part of the body, where the infected wound is located, but generally the disease extends to all parts The frequency with which the facial muscles are affected, thus making it difficult for the victim to open his mouth, is responsible for the name "lockjaw" by which the disease is commonly known

Infections in horses occur most often as a result of nail wounds in the foot. In sheep the infection is seen most often after lambs are castrated or docked In cattle it may be a puerperal infection following calving, or it may follow dehorning, castration, and nose ringing of bulls In all animals it may occur as a result of infection of otherwise trivial wounds, and cases are even seen when no wounds can be found Umbilical infections of new-born animals often occur.

Immunity

NATURAL IMMUNITY Birds and other animals which are naturally resistant to tetanus have no antibodies in their tissues The brain tissue of such animals, however, seems to have no affinity for the toxin, as was first demonstrated by Metchnikoff (6) The blood of most cattle contains neutralizing antibodies, and small amounts are found in the blood of sheep and goats. It has been suggested that perhaps this comes about from the activity of tetanus bacilli in the fore-stomachs of these ruminants The blood of horses, dogs, pigs, and men does not normally contain antitoxin The brain tissue of all susceptible animals possesses the power of uniting with tetanus toxin *in vitro* and thereby neutralizing it

ACQUIRED IMMUNITY Effective means of rendering susceptible animals immune prophylactically are available. This may be accomplished either actively or passively. When an animal has suffered a wound from which it is feared tetanus may develop, it is necessary to use a method by which resistance can be conferred quickly In this situation *tetanus antitoxin* is indicated. Usually 1,500 units is adequate to give complete protection for a period of several weeks which ordinarily is long enough. If another wound is incurred a few months

later, another dose of antitoxin is needed, and repeated doses of antitoxin may result in anaphylactic shocks in all animals except the horse, for which the serum is homologous

When symptoms of tetanus have appeared, the efficacy of the antitoxin is not so great as when it is used prophylactically In these instances the antitoxin is administered as soon as possible and is given preferably in a single dose of from 100,000 to 200,000 units Animals frequently die in spite of such treatment On the other hand when the infected wound is thoroughly cleaned out surgically and treated with antiseptics, and the animals are kept quiet in a darkened room, one-third or more of them will recover without treatment with antitoxin.

Active immunization of horses against tetanus was introduced by Ramon (10) of the Pasteur Institute in Paris, and has proven to be a thoroughly practical procedure The immunizing substance is *tetanus toxoid* or *anatoxin* This is made by incubating highly potent toxin with 0.4 per cent formalin until the toxicity has been completely destroyed This requires several weeks The solution may be used as it is, but an improved product results from precipitating the toxoid from solution with alum (potassium aluminum sulphate) The precipitate contains the active principle freed from extraneous material After it has been washed it is suspended in saline solution A single injection of this material will produce an appreciable degree of immunity, but it is best to give a second dose in about one month * Such animals will have sufficient antitoxin in their blood to protect them from natural infection for at least a year. If dangerous wounds are contracted later it is best to administer another dose immediately This will cause an immediate increase in antibodies

If the animal has not previously been injected with toxoid, it is useless to use toxoid in the face of an emergency because initial production of antitoxin is too slow

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CLOSTRIDIUM BOTULINUM AND CL PARABOTULINUM

Synonym· *Bacillus botulinus*

As a disease of man botulism has been known for many years The disease was given its name by Muller (13) in 1870 The causative agent was found in 1897 by van Ermengem (5) who studied an outbreak occurring in Ellezelles, Belgium, in a group of persons who partook of an imperfectly preserved, smoked ham at a dinner The organism was found in the ham and the tissues of one of the persons who died of the disease The toxicogenic properties of the organism were recognized by van Ermengem, also the fact that the toxin wrought its damage by attacking portions of the nervous system

In Europe a considerable number of outbreaks of the disease have occurred during the last thirty years, the greater number being traced to hams, sausages, and other meat products Beginning about 1919, a series of outbreaks have been recognized in the United States, but these have been traced, with but few exceptions, to canned vegetables It has been shown by Meyer and co-workers (11) that the organism is commonly present in the soil in all parts of the world. Botulism is due, therefore, to food materials which have been contaminated at some stage of their preparation with soil, which have been imperfectly sterilized thereafter, and then allowed to stand, with the air excluded, sufficiently long to allow the organism to generate its powerful toxin The disease is not a bacterial infection, it is an intoxication with toxin generated in food materials before they are eaten The organism has little or no ability to generate toxin in the alimentary tract

Botulism as a disease of domesticated animals has been recognized only in recent years In 1917 it was studied by Buckley and Shippen (3) as a disease of the horse in the United States. This horse disease was studied by Graham and associates (7) in 1919 and later. In 1920, an outbreak in a large flock of chickens was described by Hart (9) Dogs, sheep and pigs are very resistant to botulism and it is doubtful that natural outbreaks ever occur in these species Sporadic cases of botulism frequently are diagnosed on a purely clinical basis in this country but it is likely that most of these diagnoses are incorrect. *Lamziekte,* a disease of cattle in South Africa, and *Loin disease* of cattle in parts of Texas apparently are botulism. Seddon has described botulism in cattle in Tasmania.

Clostridium botulinum and Cl parabotulinum are described together since both produce the disease known as botulism The principal difference between the two species is that the first is non-proteolytic and the second proteolytic There are differences, also, in the antigenic properties of the toxins, but such differences occur between types within each of these species.

Morphology and Staining Reactions. The species and types within the species cannot be distinguished on a morphological basis All are relatively large rods which usually occur singly, but may form short chains They measure from 0 5 to 0 8 microns in width and are from 3 to 6 microns long Motility occurs in young cultures, the cells being provided with peritrichic flagella Spores form readily and abundantly They are oval, are located centrally and excentrically, and cause slight bulging of the cells The organism is Gram-positive but in old cultures the cells usually decolorize



Fig 63 *Clostridium botulinum* Culture in meat piece medium incubated 48 hours at 37° C x 900

Cultural Features

CLOSTRIDIUM BOTULINUM The original culture of van Ermengem has been lost and since it was inadequately described in the light of present-day knowledge, present descriptions are based upon a strain from the Lister Institute (No 94) which corresponds in its principal features to van Ermengem s organism

Colonies in deep agar are fluffy Better growths occur in media made from liver than in those made from muscle tissue Gelatin is rapidly liquefied Milk is slowly acidified but is not coagulated. Coagulated blood serum and coagulated egg albumin are not liquefied Brain medium and meat medium are not digested Acid and gas are formed from dextrose, levulose, maltose, dextrin, glycerol, adonitol, and inositol Lactose, sucrose, galactose, raffinose, inulin, dulcitol, mannitol, xylose, arabinose, rhamnose, and salicin are not fermented (Type C, described below, differs from the above principally in that it ferments galactose and adonitol)

CLOSTRIDIUM PARABOTULINUM. This organism corresponds to the foregoing description except in the following particulars. Milk is slowly curdled, the

curd partially digested and darkened Coagulated blood serum and egg albumin are digested and blackened and a foul putrefactive odor is emitted. Brain medium and meat medium are digested, blackened, and emit a putrefactive odor. Acid and gas are formed from dextrose, levulose, maltose, dextrin, glycerol, and salicin Galactose, lactose, sucrose, rhamnose, raffinose, inulin, adonitol, dulcitol, mannitol, xylose, arabinose, and inositol are not fermented

All organisms included in these species are strict anaerobes but are not fastidious, otherwise, in their growth requirements They grow best at temperatures around 30° C but are capable of growing in a wide range of temperatures up to that of the body The strains which are not proteolytic nevertheless give off a strong odor which is suggestive of putrefaction but is not so powerful as that produced by the protein liquefying types

The Cultural and Toxicogenic Types. In 1919 Burke (4) found that the strains in her possession, which had been isolated from California products, were not alike toxicologically, although agreeing in cultural features The toxins of all strains produced practically the same effects on animals, but antitoxins which would neutralize the toxins of one group of organisms would not neutralize those of another, and vice versa On the basis of her experiments she differentiated two types and designated them as Types A and B The Type A organism appears to be the more common in California and is the one which was found in most of the outbreaks of botulism originating in canned goods packed by commercial firms in California between 1919 and 1925 The Type B organism, on the other hand, seems to be more widely distributed and is the one which predominates in the middle west and eastern parts of the country In 1922, Bengston (1) described another type which she had isolated from fly larvae This organism differs from those previously described in certain cultural features, and in the toxin produced She designated the organism as Type C. In 1929, Meyer and Gunnison (12) studied the organism causing the South African *lamziekte*, found it to be different from those previously described, and so designated it as Type D In 1928, Theiler (14) found that two mules which had died after eating out of the same manger, had been poisoned by botulism toxin which came from a decomposing rat carcass in the hay. This type proved to be different from those previously described and was designated *Cl botulinum*, Type E This organism is similar to Type C and may prove to belong to that type Some authors recognize two kinds of Type C, designating them as C_a and C_b. The C_a type is associated with fly larvae and affects only birds, whereas C_b affects birds, monkeys and laboratory animals

In 1924, Bengston (2) studied all available strains of the organism of botulism in a comparative way She found that the Type A and Type B strains,

which apparently are most common and most important in this country, were strongly proteolytic, whereas, the original description of van Ermengem was clear in that the Ellezelles organism was not proteolytic (the original strain could not be studied because of its having been lost) On the other hand, she found her fly-larva strains (Type C) to be non-proteolytic, and since the two South African animal strains are non-proteolytic, she proposed that Types C, D, and E be called *Cl botulinum,* and A and B, *parabotulinum* Some authors prefer to regard all of these types as members of a single genus.

Certain characteristic differences aside from properties of the toxins and proteolytic activity may be noted among the several known types of the organism of botulism The Type A organism produces spores which are extraordinarily resistant to heat, thus Esty (6) and Meyer have reported maximum resistances for organisms of this type up to five and one-half hours at 100° C. Organisms of the other types show much less resistance, most of them being destroyed by boiling for a very short time Undoubtedly this difference in heat resistance explains why there has been so much difficulty in controlling botulism in canned goods packed on the Pacific Coast where the A type is common

Only Types A and B (the parabotulinum types) have been incriminated in human botulism, although it has already been said that the original strain of van Ermengem probably did not belong to either of these types

The Type A organism is usually possessed of the more virulent toxin, in fact, no more poisonous substance has ever been found than the toxin of certain strains of this type By using properly graded doses of unknown strains, Graham (8) has been able to make a rapid differentiation between the A and B types by the inoculation of chickens and of dogs These species are relatively resistant to the B type of toxin while very susceptible to the A type, thus, unless excessive doses are used, they will remain free of symptoms in the presence of the B type but show typical botulism in the presence of the A type

The toxins of both A and B types affect a great variety of animals, both vertebrate and invertebrate Earthworms, snails, tadpoles, frogs, fish, birds, dogs, cats, horses, monkeys, and man have been poisoned. Cattle may be poisoned, but apparently are somewhat less susceptible than most other mammals, and swine are quite resistant.

Although it is not poisonous for man, the Type C organism has proven to be important economically Bengston found it originally in the larvae of a blowfly, *Lucilia caesar,* which obtained it from putrefying carcasses upon which they had fed When these larvae were fed to chickens, a characteristic disease which had long been recognized as a disease entity, "limber neck," was produced. In this disease the birds exhibit sleepiness, and they are unable to

hold up their heads finally because of a flaccid paralysis of their neck muscles Such birds usually die Kalmbach (10) proved that a devastating disease in wild duck and other water birds in an area centering around Great Salt Lake in Utah was due to botulism, the organism belonging to Type C In shallow stagnant water in many pools which dry up during the hot months, decaying vegetation provides a favorable medium for the growth of this organism and the generation of its toxin, and many thousands of ducks, feeding upon such vegetation on the bottom of the water holes, die each year The disease has long been known as the western duck sickness or as the "alkali disease" since it had been believed that it was caused by salt poisoning

The Type D organism has been found only in the disease of cattle of South Africa which is known as *"lamziekte,"* or the lame sickness The etiology of this disease was finally cleared up by Theiler and his co-workers (14) in 1926 The disease occurs only in certain restricted areas in cattle on the range In these areas the cattle have the habit of bone chewing which, it was shown, is due to a phosphorus deficiency which creates the abnormal appetite The bones of animals dying on the "veld" (range) were eagerly sought and chewed Some of these bones were in advanced stages of decomposition, and in them the botulinum toxin often was present A disease which evidently is the same as lamziekte occurs in certain parts of the Texas plains where phosphorus deficiency exists and bone chewing is common The symptoms are the same as those of the South African disease

The origin of the Type E organism from a rat carcass in hay has already been mentioned Gunnison, Cummings and Meyer proposed that a strain isolated from spoiled fish in Russia be designated as Type E It is evident that the last word on the classification of these types has not yet been said

Pathogenicity. The toxin of the organism of botulism enters the body through the intestinal tract but the damages caused by it are almost wholly in the nervous system Histological changes have not been found in the nervous system, however, hence there is much doubt as to the manner by which damage is caused It has been suggested that the damage is to the peripheral nerves *rather than to the central nervous system.*

The symptoms are essentially those of paralysis Disturbances in vision occur, there is difficulty in locomotion, the tongue often becomes paralyzed, swallowing becomes impossible because of pharyngeal paralysis, and respiratory paralysis finally terminates the disease.

Sources of Infection for Animals. It has been said that most cases of botulism of man occur from the eating of preserved food, especially canned vegetables. These are not ordinarily eaten by animals but several large outbreaks have

been caused by feeding spoiled canned goods to chickens. It is difficult to see how conditions favorable for the generation of botulinum toxin can develop in the food of the herbivorous animals, as they are ordinarily maintained In moldy hay and grain which have been damp or wet for a time, it is possible to have the toxin develop We believe that most reported cases of botulism in horses and cattle are the result of erroneous diagnoses.

Diagnosis. The bacteriologic diagnosis of botulism in animals is difficult, and there are many clinical diagnoses that cannot be sustained in this way. The finding of the organism in the intestinal tract of the affected animal, or the proving of its presence in food materials is not sufficient to prove its connection with the disease It must be remembered that organisms of this group are wide spread in nature and are likely to be encountered in any food stuffs that may be contaminated by soil (dust) To prove that a food is guilty it must be shown that it actually contains toxin in an amount sufficient to cause poisoning.

Immunity. Homologous antitoxins protect animals very well against botulism. In practice they are of little use since the disease does not appear frequently enough in any one herd to warrant prophylactic immunization, and for curative purposes antitoxins are of little value Polyvalent antitoxins (Types A and B) are made commercially and are available If given very early in the disease it is thought that they have some curative effect

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CLOSTRIDIUM CHAUVOEI

Synonyms. *Bacillus chauvoei; Bacillus carbonis, Bacillus anthracis symptomatici.*

This organism affects cattle principally, sometimes sheep and goats. Guinea pigs may readily be infected by inoculation The other domesticated animals, and man, are immune In cattle the disease is known as blackleg, black quarter, quarter ill or symptomatic anthrax In England it is reported that the disease is common in certain areas where it usually occurs as a post-parturient infection in sheep The infection is widespread in large areas of the "range country" of the United States where the losses are principally in cattle In this species the disease occurs without any evident portal of entry.

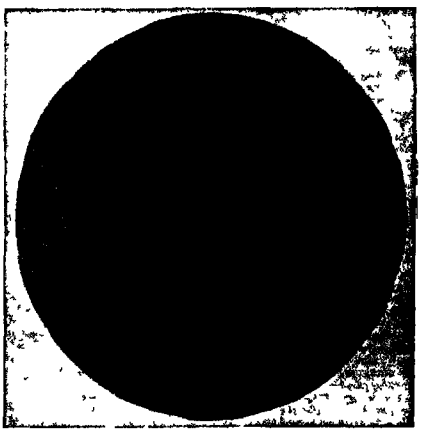


FIG 64 *Clostridium chauvoei* Film from the surface of the liver of a guinea pig dead as a result of artificial inoculation with a pure culture The bacilli are always arranged singly or in pairs, never in long chains as in *Cl septicum* infections x 900

Morphology and Staining Reactions. This organism appears in tissues and cultures as a straight, round-ended rod about 0 6 microns in width and from 3 to 8 microns long It usually appears singly or in chains of three to five organisms in the peritoneal exudate of inoculated guinea pigs, and this is useful in distinguishing it from *Cl septicum* and other anaerobic bacilli which frequently occur in materials suspected of blackleg The latter organisms usually occur in long chains Spores are oval and appear excentrically, swelling the rods into lemon-shaped structures Very young cultures are motile by means of peritrichic flagella The cells stain somewhat unevenly The Gram stain is positive when the cultures are young but erratic after they are a few days old.

Cultural Features. *Clostridium chauvoei* is a little more exacting in its cultural requirements than most of the organisms in this group It is strictly anaerobic, and will not grow on ordinary dextrose agar except when tissues are carried over in the inoculum. The addition of blood or tissue make ordinary

broth and agar favorable for it It will grow luxuriantly on all media made with a liver infusion base, without enrichment

Deep colonies on agar are delicate and compact, being irregularly spherical. When blood is present there is evidence of slight hemolysis but definite zones are not formed around surface colonies In plain broth there usually is no growth unless blood or tissue has been carried over with the inoculum In liver broth, the fluid becomes moderately clouded Gelatin containing a little serum is slowly liquefied and a few gas bubbles are formed. Growth on coagulated blood serum and coagulated egg is poor, and there is no liquefaction Cooked meat medium becomes pinkish and the fluid is slightly clouded Liver-brain medium gives excellent growth and is a good medium on which to maintain cultures It is not digested Acid and gas are formed from dextrose, levulose, galactose, maltose, lactose, and sucrose Inulin, salicin, mannitol, glycerol, and dextrin are not fermented Cultures of this organism give off a characteristic odor by which experienced workers frequently can identify the species It is not putrefactive but rather butyric. Some of the toxins reported by earlier workers evidently were the result of working with impure cultures.

Normal Habitat and Mode of Infection. The organism of blackleg exists in the soil. Whether it multiplies there, or whether it merely lives there in the spore form and multiplies in the intestinal canal of animals, is not known. In any case it is known that when pastures or grazing grounds once become infected the disease will reappear regularly in susceptible animals year after year In sheep, the disease seems to be quite often a wound infection, occurring after lambing, docking, and shearing (4) In cattle, wounds are seldom found and it is believed that infections occur mostly through the digestive tract.

Pathogenicity. Guinea pigs are easily infected by inoculation and are the best animals for diagnostic work Mice can sometimes be infected and also rabbits Experimental animals usually die in about 48 hours The muscles in the region of the point of inoculation are hemorrhagic, darkened, and there may be a little edema but no gas present The abdominal cavity usually is moist and the liver may have a semi-cooked appearance Smears from the liver surface show numerous typical bacilli arranged singly, in pairs, or in very short chains. Pure cultures are easily obtained from the peritoneal fluid, and usually from the heart blood.

Bovine infections occur mostly in young animals, from four months to two years old Lameness is the first manifestation A diffuse swelling then usually appears in the region of the shoulder or of the rump, and the animal shows

fever and great depression If the swollen region is palpated it is found to be soft and when pressed a crackling sound is heard because of the gas in the muscular tissue The affected animals usually die within a day or two The lesions consist of blackened muscular tissue where the swelling existed. The tissues are quite dry and gas bubbles are found throughout. They give off a characteristic rancid odor Cases of blackleg occur in which muscular lesions are not found, or the lesions may be small and located in such obscure muscles as the psoas group, or those of the diaphragm In addition to the characteristic muscle lesions, the liver may be swollen and show collections of small gas bubbles, especially if some hours have elapsed since death occurred In a great many calves vegetative lesions may be found on the heart valves, the heart muscle is often pale and friable, and there may be fluid and fibrin in both chest and abdominal cavities

The disease is prevalent in the states of the Mississippi Valley and in the range regions beyond It also exists in many small areas in nearly all parts of the world where cattle are kept

Immunity. The few animals that recover from an attack of this disease appear to be permanently immune In regions where the disease is common, young cattle are regularly immunized in order to prevent losses The immunization is ordinarily done in late winter or early spring because the infections are contracted on the range or pasture during the grazing season Several methods for successfully immunizing animals are available For many years vaccines were used Later these were largely replaced in the United States by tissue or culture filtrates (aggressins) These were better but were much more expensive to manufacture In recent years the blackleg bacterin has been used to the exclusion of all former methods Bacterins are very satisfactory and cheaper than filtrates Immune serums have been prepared These will protect when administered prophylactically but they are expensive and the duration of the immunity is too short for practical use. When given in large amounts and very early in the course of the disease infected animals may sometimes be saved

VACCINES A number of different vaccines have been used more or less successfully for the prevention of blackleg The two best known of these are those of Arloing, Cornevin, and Thomas (1) (known generally as the Lyon vaccine, since the workers were at the Veterinary School at Lyon, France) and the Kitt (2) vaccine, which is, essentially, a modification of the Lyon vaccine In both of these vaccines, the diseased muscular tissue is used as a basis. By a process of drying and heating the vegetative forms of the blackleg bacilli in the muscular tissue are killed and the spores are attenuated until they no

longer have sufficient virulence to be dangerous The original Lyon vaccine consisted of two vaccines, one weaker and intended for use first, and another stronger, intended for use about ten days after the first. The Kitt vaccine consisted of only a single injection of material which had been attenuated For many years a modification of the Kitt vaccine was made and distributed free-of-cost to the cattlemen of the midwest by the Federal Bureau of Animal Industry (6). This vaccine gave reasonably good results and certainly prevented heavy losses from the disease The vaccine consisted of blackleg muscle which was ground up and spread upon plates or shallow pans in this form and heated for six hours at 94° to 95° C The dry scale was afterwards scraped off, ground to a brownish powder, and stored until needed When ready to use, weighed amounts were soaked up in water, the material was filtered and the filtrate used Insignificant reactions to the vaccine usually resulted. Occasionally a few losses from blackleg occurred from this vaccine and sometimes animals appeared to lack proper protection after its use This vaccine is no longer made since better products are now available

AGGRESSIN It has been shown by several workers that filtrates of the blackleg tumor fluids possess immunizing properties Schoenleber, Haslam and Franklin (7) (1917) applied the method in field practice and found it very successful. The aggressin, when properly prepared, gives a strong immunity and is perfectly safe The immunity is active It persists longer than is the case with the older vaccines.

GERM-FREE FILTRATE (ARTIFICIAL AGGRESSIN) Nitta (5), of Japan, observed that filtrates of blackleg cultures grown in the presence of meat fragments had immunizing properties which were nearly as great as those of true aggressin These substances do not appear in ordinary cultures The method of production is to cultivate the organism in flasks which contain a meat-piece medium, to squeeze the juice out after growth is completed and to free the juice of the organisms by filtration This product can be made more cheaply than the true aggressin which necessitates the sacrifice of calves in its production

BLACKLEG BACTERIN BLACKLEG ANACULTURE Following the discovery that formalin had the power of destroying the poisonous properties of toxins while preserving their antigenic value, Leclainche and Vallee (3) tried the procedure on cultures of the blackleg organism and discovered that formolized whole cultures constituted a safe and effective immunizing product About the same time, or perhaps previously, the same procedure had been discovered by the research staff of an American commercial company The product has been extensively used in this country and is unquestionably a safe, reliable, immunizing agent for cattle. The immunizing substance resides in the bacterial

cells, hence the cultures are concentrated by sedimentation before the formolization is carried out The material can be produced more cheaply than either naturally or artificially produced aggressin, for the reason that Berkefeld filtration is eliminated, and this is the most expensive procedure in the aggressin manufacture Since the product does not depend upon a detoxified toxin, or antitoxin, for its immunizing properties, but rather upon the antigenic proteins of the killed bacterial cells, the term *anaculture* has been proposed for it.

BLACKLEG SERUM By immunizing horses with washed cultures of the blackleg organism a highly potent serum can be obtained which is useful, when used in large amount, in protecting valuable calves when blackleg is present in a herd, and in treating cases which have already developed. For treatment the serum is not highly successful though some calves have been saved with it.

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CLOSTRIDIUM SEPTICUM

Synonyms *Vibrion septique, Bacillus septicus* Probably Ghon-Sachs bacillus. Also erroneously, *Bacillus edematis-maligni, Bacillus edematis.*

This organism was first identified by Pasteur (6) in 1877 from carcasses of animals thought to have died from anthrax About this time Koch (5) isolated his *malignant edema bacillus* from animals which had been inoculated with soil Koch regarded the two organisms as identical but since his organism was strongly proteolytic and Pasteur's was not, it is evident that they were not identical. Koch's organism has been lost, hence it is not possible to know precisely what organism he had. It is possible that his cultures contained *Cl. septicum* contaminated by a proteolytic anaerobe such as *Cl sporogenes* This organism is generally called the malignant edema bacillus and the condition produced in animals is termed malignant edema, but it should be kept in mind that it is not the malignant edema bacillus first described by Koch.

Morphology and Staining Reactions. *Cl septicum* is a rather large rod having the shape and size of the blackleg organism. It is from 0 6 to 0 8 microns in width and 3 to 8 microns long. It usually is straight and the ends are rounded. In cultures it usually occurs singly or in short chains, but in animal exudates it appears in long chains It has already been pointed out that the tendency of this organism to form long chains on the surface of the liver of inoculated guinea pigs is a feature by which it may be distinguished from *Cl chauvoei* which occurs singly or in very short chains. Young cultures show active motility because of peritrichic flagella The spores are oval, occur excentrically, and swell the cells in which they are formed It is Gram-positive, but like most of the other organisms of this group, old cultures usually decolorize It stains readily with all of the ordinary stains.

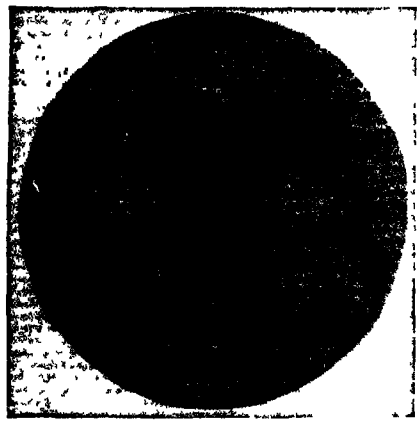


FIG 65 *Clostridium septicum* Culture in meat-piece medium incubated for 48 hours at 37° C One sporulated bacillus is seen in the upper part of the photograph x 900

Cultural Features This organism grows readily in all ordinary media so long as good anaerobic conditions prevail In its growth vigor it differs from the blackleg organism with which it often is confused, the latter being much more fastidious in its requirements than *Cl septicum*

Colonies in deep agar usually are cottony and filamentous In blood agar plates the colonies are surrounded by hemolytic zones Gelatin is liquefied and a few gas bubbles are formed in it Plain infusion broth is lightly clouded Litmus milk is coagulated and some gas may be formed in the curd The curd is not digested Coagulated blood serum and coagulated egg albumin are not digested There is good growth in meat medium and in brain-liver-medium but there is no digestion or darkening. Acid and gas are formed from dextrose, levulose, galactose, maltose, lactose, and salicin. Sucrose and mannitol are not fermented A mild toxin is generated by this organism.

Natural Habitat and Mode of Infection. *Cl septicum* exists in all fertile soils and in the intestinal tracts of herbivorous animals As in the case of *Cl. chauvoei*, it is not known whether it multiplies in the soil or whether it merely

exists in the form of spores formed by organisms present in animal excrement.

Infections occur through wounds in many cases but not all cases can be accounted for in this way. The disease of sheep occurring in Norway, Iceland, and Scotland which is known as *bradsot* or *braxy* is caused by *Cl septicum*, the lesions being internal. It is supposed that the infection occurs through the digestive tract

Pathogenicity. Pure cultures will kill guinea pigs, which are highly susceptible also to *Cl chauvoei*, and rabbits, mice and pigeons which are resistant to the blackleg organism The lesions in guinea pigs cannot be distinguished from those of blackleg. A blood-tinged gelatinous exudate is found beneath the skin at the point of inoculation and the muscular tissue is dark red in color. Gas is not usually present in the tissues The peritoneal cavity usually is moist and may have a little more fluid in it than normal The liver is lighter than normal, having a semi-cooked appearance Stained films from the liver surface show long, jointed chains of cells

Wound infections in animals are known under the general name of *malignant edema* Such infections are characterized by rapidly extending swellings which are soft and pit on pressure The affected animals show fever and other signs of intoxication and most of them die within a few hours to one or two days The affected tissues are infiltrated with large quantities of gelatinous exudate most of which is in the subcutaneous and intermuscular connective tissue The muscular tissue is dark red, but unlike blackleg, contains little or no gas Infections in cattle sometimes resemble blackleg very closely Recognizing this, the organism in the German literature is referred to as the para-blackleg bacillus It is believed that in many cases cattle and sheep suffer from a mixed infection with the blackleg and other anaerobic organisms, and that the organism of blackleg in such cases often is overlooked



FIG 66 *Clostridium septicum* Stained film from the surface of the liver of a guinea pig inoculated with a pure culture The long filaments are characteristic They are helpful in differentiating this organism from *Cl chauvoei* which never forms such filaments x 900

because it grows more delicately than the others and is crowded out of cultures.

Cl. septicum causes infections not only in cattle and sheep which are also susceptible to blackleg, but also in horses, swine, and man, who are not susceptible to blackleg.

Braxy or bradsot destroys large numbers of sheep each year in northwestern Europe, principally in Norway, Scotland, the Faroe Islands, and Iceland (4) The disease is apparently caused by *Cl septicum*, by ingestion, although experimenters have had little success in producing the disease in that way It is thought that some accessory factors, as yet unrecognized, are responsible for the infections. Affected animals die very suddenly without showing symptoms previously, or after only a few hours of symptoms at most The walls of the fourth stomach and the first part of the small intestine are edematous, hemorrhagic and sometimes necrotic The internal organs show only degenerative changes.

Immunity. *Cl. septicum* produces a toxin of moderate potency Usually 0 5 to 1.0 cc. of a filtrate of a culture will kill guinea pigs within a few minutes. According to Dalling (2) an antitoxin can be prepared by inoculating animals with the toxin, but the toxin always is highly irritating and produces necrosis at the point of injection Animals may be immunized by injecting them with toxin partially neutralized with antitoxin For use in England where malignant edema seems to be a more important infection in cattle than it is in the United States, Dalling recommends simultaneous immunization against this disease and blackleg by injecting with a mixture of blackleg aggressin and toxin-antitoxin for malignant edema For protection against braxy in sheep, formolized whole cultures of *Cl septicum* seem to be quite satisfactory (3) The potency of antitoxic serums is too low to make them of service in treating wound infections.

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CLOSTRIDIUM NOVYI

Synonyms Novy's *B edematis maligni II, Clostridium edematiens.*

This organism was first described by Novy (2), in 1894, who isolated it from a guinea pig which had been inoculated with unsterilized milk protein. It was lost sight of for many years until Weinberg and Séguin (6) rediscovered it in gas gangrene infections of man in 1915 It was they who gave it the name *Cl. edematiens,* and this name still is used by most English and French workers. It is quite similar to *Cl septicum* in its cultural features and in pathogenicity

Morphology and Staining Reactions. This is one of the largest of anaerobic bacilli It measures 0 8 to 1 0 microns in breadth and is from 3 to 10 microns long The rods usually are quite straight and the ends rounded The spores generally are present in abundance They are located subterminally and are oval Young cultures are motile by peritrichic flagella Young cultures are Gram-positive, older cultures usually lose this property.

Cultural Features. This organism is more strictly anaerobic than most of the other disease producers To obtain surface growths, the anaerobic apparatus must be in good working order In deep agar cultures the colonies grow well, especially when dextrose is present. The colonies vary in form, some being compact and of a yellowish tinge, others are loose and woolly The medium is disrupted by gas formation. On blood agar the colonies are surrounded by hemolytic zones Gelatin is liquefied Broth supports a poor growth, most of which sediments to the bottom Litmus milk is reduced but not coagulated Coagulated blood serum and egg albumin are not liquefied There is good growth in cooked-meat medium The meat fragments become reddish in color and a rancid smell is emitted Acid and gas are formed from dextrose, levulose, maltose, xylose, starch, and glycerol Lactose, sucrose, mannitol, dulcitol, inulin, and salicin are not fermented A toxin which is more potent than those of most of the tissue-invading anaerobes is formed.

Pathogenicity. *Cl novyi* is pathogenic, by inoculation, for a wide variety of species of animals, and naturally occurring cases have been found in many species The horse, cow, sheep, goat, pig, dog, cat, guinea pig, rabbit, white rat, white mouse, and fowls are susceptible. Guinea pigs inoculated subcutaneously die in from 24 to 48 hours At the injection spot there is edema extending into the intermuscular connective tissue. The muscles are dark red in color There may be a little gas present. In the abdominal cavity there usually is considerable clear fluid which coagulates upon exposure to air.

This organism is occasionally found as the cause of malignant edema in cattle and sheep The condition begins in a wound and cannot be distinguished from that caused by *Cl septicum* without a bacteriological examination In Australia two peculiar diseases of sheep are attributed to this organism. One is known as "swelled head" or "big head," a condition seen in young Merino rams, rarely in older males or females of any age (1) It is believed to be due to infection of head wounds obtained in fighting An infiltration of the tissues of the head and often the neck and brisket with a gelatinous, nongaseous fluid occurs Death occurs in practically 100 per cent of the cases The other disease has been given the name of "black disease," and this has been reported from Europe as well as Australia The most characteristic lesion in this disease is necrosis of the liver The extensive hemorrhages seen on the inner surfaces of the hides gave origin to the name "black disease" Affected animals almost always die, usually within a few hours after showing the first symptoms Turner (4) showed that this disease developed only in regions where the liver fluke abounded, and that the organism multiplies in parts of the liver damaged by the fluke Wardle (5) reports that excellent progress in bringing the disease under control has been achieved by campaigns aimed at eradicating the fluke through destruction of the snail which is the intermediate host

Black disease has been found in a number of countries other than Australia, including New Zealand, Germany, Roumania, France, Chile, and the United States It probably occurs wherever the sheep liver fluke occurs

Immunity. It is possible to prepare highly potent antitoxin by immunizing animals against culture filtrates, and such antitoxins have been used prophylactically in dealing with lacerated wounds of man in which there is great danger of gas gangrene infection. Such serums are of little value in animals because the disease progresses too rapidly to make any kind of specific treatment possible

Turner (4) had very good results in prophylactically immunizing sheep in "black disease" districts with formolized whole broth cultures Several doses are needed to give the necessary degree of immunity Tunnicliff and Marsh (3) used an alum precipitated toxoid given in a single dose of 5 cc and found that it protected sheep against toxin, and against the natural disease which occurs in the Bitter Root Valley in Montana

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CLOSTRIDIUM PERFRINGENS

Synonyms *Clostridium welchii, Bacillus aerogenes capsulatus, Bacillus phlegmonis emphysematosae,* the Welch bacillus, the gas bacillus.

This organism was first isolated and described by Welch and Nuttall (11) from a decomposing human cadaver in which the tissues were gaseous It was named by them *Bacillus aerogenes capsulatus,* a term which does not conform to accepted rules of nomenclature and therefore is invalid The name *Bacillus perfringens* was given the organism by Veillon and Zuber (10) in 1898 In 1900 Migula termed it *Bacillus welchii,* the name by which it is best known to American workers, however, Veillon and Zuber's name seems clearly to have precedence, hence it will be used here This name is used in the last edition of Bergey's manual but is not used by many American authors. The name "Welch bacillus" probably will continue to be used even if the formal name is divorced from Welch's name since it is pretty well intrenched

Cl perfringens is wide-spread in the soil and is found in the alimentary tract of nearly all species of warm-blooded animals It is frequently found as a postmortem invader from the alimentary tract in the tissues of bloating cadavers of man and animals. For this reason some caution is necessary in drawing conclusions based upon the presence of the organism in the tissues collected after death It is found more often in the so-called "gas gangrene" infections of man than any other organism, although it generally is associated with other species of anaerobes in these processes It is found also in maligna it edema-like infections of animals, particularly sheep Certain varieties of the organism, the L D bacillus (lamb dysentery bacillus), *Bacillus paludis* and *B ovitoxicus* produce fatal toxemias in sheep.

Morphology and Staining Reactions. *Cl perfringens* occurs as thick, straight-sided rods, generally singly and in pairs, seldom in chains The individual cells are about 1.0 micron in width and from 4 to 8 microns long The spores are oval and small enough that they cause little swelling of the rods Spores do not form in highly acid media, hence they are not apt to be found in media which contain fermentable carbohydrate Strains vary in their ability to sporulate, in some cases it is difficult to find spores no matter what the nature of the culture medium In old cultures many queer forms may be found clubbed types, ballooned cells, and filaments Capsules are formed in tissues and in

some types of culture media There are no flagella; the organism is therefore non-motile. Young cultures retain the Gram stain, older ones frequently decolorize.

Cultural Features. In deep agar, colonies are small and biconvex. If fermentable sugar is present the medium will be fragmented and even blown out of the tube by the abundance of gas formed If blood is present it will be hemolyzed Sharp hemolytic zones are formed around colonies on plates. In broth there is excellent growth, the fluid becoming greatly clouded Gelatin is rapidly liquefied Coagulated egg medium and Loeffler's blood serum are not liquefied There is good growth in cooked-meat medium with considerable gas formation The meat fragments are pinkish and not digested A sour odor is emitted A very characteristic reaction occurs in litmus milk—the "stormy" fermentation The milk quickly coagulates and the curd is fragmented by active gas formation Acid and gas are formed from dextrose, levulose, galactose, mannose, maltose, lactose, sucrose, xylose, trehalose, raffinose, starch, glycogen, and inositol. Some strains attack glycerol and inulin

Cl perfringens is divisible into at least four toxicogenic types which Wilsdon (12) has named A, B, C, and D Each type produces a toxin which is qualitatively different from the others, but the specific antitoxins often neutralize the toxins of some of the other types The A type which is the one found in human infections produces a toxin which is neutralized by antitoxins specific for all other types, but its own antitoxin will neutralize only the homologous toxin Type B antitoxin usually will neutralize the toxins of all four types, Type C that of A, B and C, and Type D that of A and D

With relationship to the diseases produced the types are distributed as follows

Type A This is usually found in human infections and not in animals

Type B This type is usually found in the disease known as lamb dysentery. It is frequently called the L D bacillus

Type C A type found in a disease of sheep in England and known as "struck " The organism is also known under the name *B paludis*

Type D. A type found in a common and destructive disease of sheep, described in Australia, New Zealand, Wales, and the United States. It is known as "entero-toxemia," "pulpy kidney disease" and "overeating." The organism is also known under the name *B ovitoxicus*

In addition to differences in toxins there are minor cultural differences among these types.

Pathogenicity. There is great variation in pathogenicity between different strains Most will kill white mice, guinea pigs, and pigeons by inoculation. Rabbits are more resistant The lesions in inoculated animals are similar to those produced by *Cl septicum*.

LAMB DYSENTERY is a disease which destroys many lambs during the first two weeks of life In many cases symptoms appear within a few hours after birth It is prevalent in the border country between England and Scotland, where it was first described by Gaiger and Dalling (4) A disease which apparently is the same was described in Montana by Tunnicliff (9) in 1933. The disease consists of an enteritis. In some cases there may be extensive ulcerations In the diarrheal discharges and in the intestinal content *Cl perfringens*, differing from the normal type in that it produces a powerful toxin, can be found. The organism does not ordinarily invade the tissues of the body but a potent toxin is formed in the intestine and the absorption of this toxin accounts for the disease Affected lambs usually die within a few hours The L. D bacillus of Dalling and associates differs from the classical Welch bacillus in that it liquefies coagulated serum and clots an alkaline egg medium. Tunnicliff's strain did not show these characteristics.

"STRUCK" is the local name for a disease of sheep which has been reported only from the Romney marsh in England and in North Wales Adult sheep are affected The stricken animals die so suddenly that the only symptoms are the death convulsions The mortality is very high If carcasses are examined immediately after death the only lesions are severe enteritis and peritonitis. If the examination is postponed for a few hours, the muscles present the appearance of gas gangrene The Type C organism found in this disease is much more toxic than the other types.

ENTEROTOXEMIA of sheep is another disease which is associated with a highly toxic intestinal content The situation is about like that of lamb dysentery except that the latter occurs only in very young lambs whereas this disease is seen in older animals It was described first by Bennetts (1) in western Australia, but has been seen since in other countries including the United States Newsom and Thorp (8) say that this disease causes greater losses among feedlot lambs in Colorado than all other diseases combined. These authors call the disease "Over-eating." They claim that the primary inciting cause is overeating of too much concentrated food such as corn, barley, peas, and cane and say that losses can be stopped abruptly by withholding the grain ration for a day or two The intestinal content of affected lambs contains toxin of Type D. Filtrates of this material will kill laboratory animals and lambs

when injected parenterally, and such animals can be protected from the filtrates by administering antitoxin of Type B as well as Type D.

PULPY KIDNEY DISEASE is the name which Gill (5) applied to a disease of lambs which he studied in New Zealand The disease is characterized by severe degeneration of the kidneys and is ascribed to Type D of this organism. The disease has been described in Wales. The relationship of this disease to enterotoxemia is not clear Newsom and Thorp found sugar in the urine of their cases but do not mention the severe kidney damage which Gill described.

Immunity. Dalling (3) has reported good results with two methods for controlling lamb dysentery One method is to immunize the ewes before lambing time with a toxin-antitoxin mixture, or with formolized cultures Antibodies are then secreted in the first milk (colostrum) and these give adequate protection The second method is to inject antitoxin into the lambs as soon as they are born

For preventing "struck," McEwan (6) advises immunization with toxoid

Bennetts (2) reports success in immunizing sheep against enterotoxemia by vaccination with formolized whole cultures According to Newsom and Thorp, immunization is not necessary since the disease can be satisfactorily controlled by careful management to prevent the lambs from overeating.

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CLOSTRIDIUM HEMOLYTICUM

Synonyms *Clostridium hemolyticus bovis, Bacillus hemolyticus*

This organism is the cause of a disease of cattle, occasionally of sheep, commonly known as "red water disease." It is also known as "hemorrhagic dis-

ease" and infectious icterohemoglobinuria One case in a hog has been described by Records and Huber (3).

So far as is known the disease occurs only in rather restricted districts, especially in the poorly drained mountain and valley pastures of the Sierra Nevada, Cascade, and Rocky Mountains in the western part of the United States It has been reported also in the delta parishes of Louisiana, in central Mexico, and in Chile The disease occurs principally during the summer and early fall months The organism was described by Vawter and Records (6) in 1926. The disease was first described by Meyer (2) in 1916, later by Mack and Records (1) and by Records and Vawter (4) As a result of the earlier studies it was believed that the disease was caused by *Cl. perfringens,* but it is now known that this organism is merely a secondary or agonal invader



FIG. 67 *Clostridium hemolyticum* Bacilli in the characteristic liver infarct A few of the organisms are beginning to form spores × 900 (Courtesy of Edward Records)

Morphology and Staining Reactions. This organism is somewhat larger than most of the other tissue-invading anaerobic bacilli It measures from 1 0 to 1 3 microns in breadth and from 3 0 to 5 6 microns in length It has straight sides and rounded ends It occurs singly, as a rule, but may form short chains in tissues and cultures The spores are oval and are located subterminally They cause bulging of the cells in which they lie. The cells are actively motile when young. Young cells are Gram-positive but when they are more than 24 hours old they rapidly lose their ability to retain this stain.

Cultural Features. Deep agar colonies are lenticular at first, later becoming woolly Little or no gas is formed unless fermentable sugar is added to the medium When blood is present it is rapidly hemolyzed Gelatin is liquefied in from two to four days Coagulated serum and egg media are not softened or liquefied Cooked-meat media and brain-media support good growth but there is no digestion of the solids and no blackening unless iron salts are added Even in the presence of an abundance of iron salts the blackening is but slight. Milk is not changed Dextrose and levulose are the only carbohydrates fermented. These are actively destroyed with the evolution of both acid and

gas Hydrogen sulphide is formed in large amounts in liver media, and in media containing proteose-peptone. The methyl-red and Voges-Proskauer tests are negative, and nitrates are not reduced.

A striking feature of this organism is the powerful hemolytic toxin which it forms This toxin is the principal reason for the great pathogenicity which it exhibits. The hemolytic toxin is rather unstable and reaches its greatest concentration in cultures within 16 hours, after which it rapidly disappears. A second toxin having necrotizing properties is present in cultures. This substance is present in young cultures but it is most marked in older cultures

This organism is rather exacting in its cultural requirements Good anaerobic conditions are necessary, and the media must contain tryptophane for optimum growth and toxin-formation. Vawter and Records depend principally upon a peptic, liver-digest medium in their work

Pathogenicity

THE NATURAL DISEASE The disease presents a quite uniform picture which is readily recognized by those who have had experience with it Appetite, rumination, lactation, and bowel movement suddenly cease and the afflicted animal stands apart from the rest of the herd, presenting the picture of an acutely ill animal The back is arched, the abdomen tucked up, and it is difficult to make the animal move Breathing is shallow, and there is grunting with each step The temperature varies from 104° to 106° C in the early stages but becomes subnormal before death The feces become deeply bile-stained or bloody The urine is a dark red or port wine color, clear but foamy The color is due to large amounts of hemoglobin There are no intact erythrocytes in the urine. Sugar is absent but albumin tests are strongly positive

At the time when hemoglobinuria appears, as much as 40 to 50 per cent of all of the erythrocytes of the body have been destroyed The red cell count at this time may not be greater than two millions per cubic millimeter and the hemoglobin readings may be as low as 3 5 gms per 100 cc of blood The leucocyte count increases, sometimes to as high as 30,000 per cu mm Death is due to anoxemia because of the wholesale destruction of erythrocytes. The mortality is high, varying from 90 to 95 per cent in untreated animals.

The most characteristic lesion is the large infarct which always is found in the liver This is a mass of necrotic tissue, varying from 5 to 20 centimeters in diameter, often mottled, and usually lighter in color than the normal liver tissue. This lesion may be located in any part of the organ The lesion is formed as a result of an occluding thrombosis of one of the branches of the portal vein. The tissue has undergone coagulation necrosis. In the sinusoids of these areas

large numbers of large rod-shaped bacteria containing subterminal or terminal spores may be seen.

Extensive hemorrhages are found on the serous membranes, in the subcutaneous connective tissue, and in the substance of the visceral organs Acute

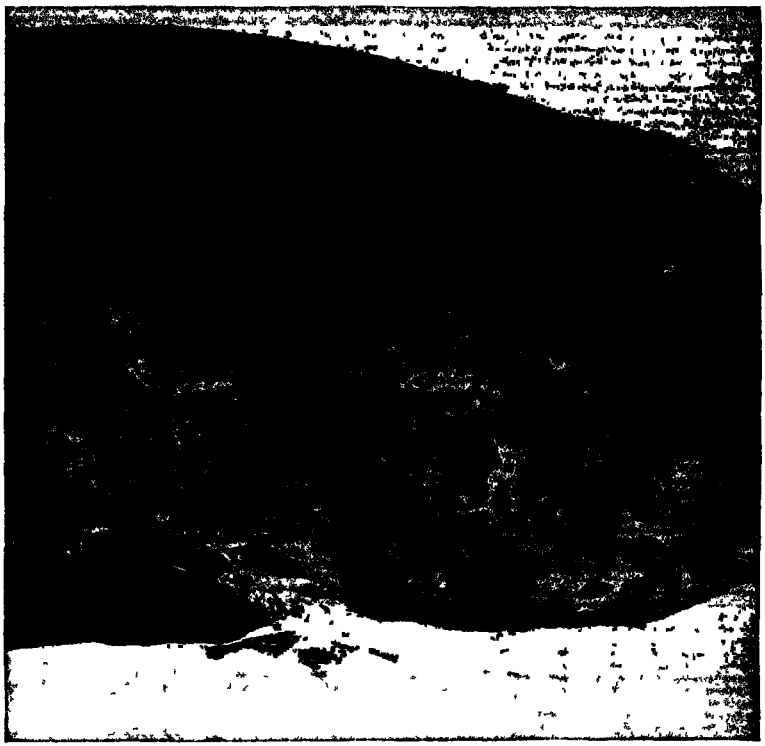


FIG 68 Massive Infarct in a Bovine Liver Caused by Infection with *Clostridium hemolyticum* This lesion is characteristic of the "red-water" disease of cattle (Courtesy of Edward Records)

degenerative changes occur in the organs, and the peritoneal and pleural cavities usually contain large quantities of hemoglobin-stained transudates. Besides the subserous hemorrhages which regularly occur in the intestinal wall, there is a severe hemorrhagic enteritis, the mucous membrane often being practically wholly undermined with extensive hemorrhage.

THE EXPERIMENTAL DISEASE Vawter and Records (7) readily killed cattle by inoculating them with pure cultures of *Cl. hemolyticum,* the animals dying

with typical symptoms within 36 hours The lesions in such animals are typical except that the liver infarcts are absent Repeated attempts to produce the disease by feeding large amounts of the pure cultures have failed. Records and Vawter (5) fed encysted cercariae of the liver fluke to young cattle and followed the feedings with large amounts of pure culture of *Cl hemolyticum*, thinking that fluke invasion might pave the way for the entrance of the organism, but the experiments failed even though the flukes invaded the liver tissue of all the experimental animals The mode of natural transmission of this disease therefore is unknown.

Rabbits and guinea pigs may be readily killed by toxin-containing cultures. Subcutaneous injection leads to the formation of a hemorrhagic, edematous area at the point of inoculation with little or no gas formation Intravenous inoculation of rabbits usually leads to death in from two to four hours with great blood destruction and hemoglobinuria

Immunity. Records and Vawter (7) developed an immune serum which proved highly effective in protecting animals against artificial inoculation of otherwise fatal doses of culture This serum also exhibited considerable curative power when given in large doses to animals which were just beginning to show hemoglobinuria and before the temperature receded to subnormal. The same workers developed a phenolized whole-culture vaccine, and a glycerinated vaccine which served to protect quite well Recently they have developed an improved, formolized bacterin, adsorbed on aluminum hydroxide, which they claim to be especially successful This vaccine protects for a full pasture season and often for a full year

Serologic studies by Vawter and Records (8) indicate that all strains of *Cl hemolyticum*, with one exception, are uniform in composition. Their studies dealt with agglutinins and toxin neutralization. No antigenic relationship with any of the other pathogenic anaerobes was found

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DIFFERENTIATION OF THE TISSUE-INVADING SPORE-BEARING ANAEROBES

The spore-bearing anaerobic bacteria which produce phlegmonous conditions in animals are not always easily identified, since they are similar in their morphology, cultural features, and often in pathogenicity. In addition to those which have been described and which are the most important ones, a great many other species have been described and are often encountered in animal infections The following table gives the more important cultural characteristics of the anaerobic bacteria which commonly infect animals.

TABLE XIV

	Cl chauvoei	Cl septicum	Cl perfringens	Cl novyi	Cl hemolyticum
Spores	S T	S T	Cent	S T	S T
Motility	+	+	–	+	+
Deep agar colonies	Pin point	Fluffy lenticular	Lenticular	Lenticular	Lenticular
Brain medium	o	o	o	o	o
Coagulated albumin	o	o	o	o	o
Milk	Coagulation	Coagulation	Stormy ferm	o	Coagulation
Gelatin	Gas Liq	Gas Liq	Gas Liq Black	Gas Liq Black	Gas Liq
Dextrose agar	No growth	Growth	Growth	Growth	Growth
Dextrose	+	+	+	+	+
Lactose	+	+	+	–	–
Saccharose	+	–	+	–	–
Maltose	+	+	+	+	–
Galactose	+	–	+	+	+
Salicin	–	+	–	–	–
Pathogenic	+	+	+	+	+
Toxin formed	–	+	+	+	+
Liver surface smear	Single	Single and filaments	Single and short chains	Single and chains	?

S T = Subterminal
Cent = Central
o = No change

CHAPTER XXV

THE PATHOGENIC NON-SPORE-BEARING ANAEROBIC BACTERIA

Our knowledge of the non-spore-bearing obligate anaerobes is very fragmentary and their classification is very confused and certain to be changed as more information about them is acquired A number of anaerobic streptococci have been described, usually from disease processes The organism of true actinomycosis of cattle and man is at least partially anaerobic Gram-negative, non-spore-bearing bacilli are present in the intestinal canal of man and animals, often in large numbers (Classified in Genus *Bacteroides*). Whether these have any role in pathology is unknown. Certain long organisms with tapering ends occur in the human mouth and often are associated with troublesome inflammations These are sporeless and are strictly anaerobic (Genus *Fusiformis*) Many spirochetes are anaerobic and non-spore-bearing

ACTINOMYCES NECROPHORUS

Synonyms *Bacillus diphtheriae vitulorum, Streptothrix cuniculi, Corynebacterium necrophorum, Cladothrix cuniculi, Bacterium necrophorum,* the calf diphtheria bacillus, the necrosis bacillus

The classification of this organism is questionable It certainly has few of the characteristics of the other members of the *Actinomyces,* and therefore should not be placed there Topley and Wilson place it in the genus *Fusiformis* This is more logical, but according to definition, it could belong also to the genus *Bacteroides* Not being certain of its proper classification, it seems best to leave it where it is for the present, inappropriate though the present classification is, rather than to complicate matters still further by assigning it elsewhere from whence it is likely to be moved as more information about it is accumulated

Morphology and Staining Reactions. In infected tissues this organism is ordinarily seen in the form of long filaments, but shorter elements and even coccoid forms occur The rods are about 1 micron in width and may be in excess of 100 microns in length In some cultures swollen rods are seen which

may be nearly twice as thick as the usual forms Freshly isolated strains growing in cooked-meat medium usually show a predominance of long filaments. The sides of these filaments are parallel and regular, and are either straight or form sweeping curves. After prolonged artificial culture the predominating forms usually are short. Very young cultures stain uniformly as a rule but the filaments in cultures older than 24 hours usually are vacuolated, that is, the stained portions are separated by portions which are almost or quite free of stain The irregular distribution of cytoplasm along the filaments can easily be seen in unstained preparations Some early authors have described branching but most of those who have studied this organism agree that it does not branch. Flagella have not been demonstrated and motility is absent Ordinary dyes stain young cultures readily The organism is always Gram-negative



FIG 69 *Actinomyces necrophorus* Lung Abscess, Calf The long filaments with irregular distribution of chromatic material are characteristic x 900

Cultural Features. The necrosis bacillus is very sensitive to oxygen and under usual conditions growth does not occur unless good anaerobic conditions are obtained (7) Beveridge (2) claims, however, that growth occurs readily when the organism is grown in association with staphylococci, and that surface colonies on solid media which are well started anaerobically will continue to increase in size when incubated aerobically It is difficult to obtain primary growths on solid media incubated in anaerobic jars because exposure to air damages the cells so they frequently will not grow, the damage coming about before anaerobic conditions are established

Growth in ordinary media is poor and frequently fails entirely. Ordinary agar, gelatin, and broth, for instance, are not suitable media for this organism. When enriched with serum or blood, they become suitable, but cultures quickly die out Growth in litmus milk usually fails unless peptone or serum is added. Cooked-meat medium or liver-brain medium are very favorable and are recommended for isolations. Even in these media cultures die out in most cases within one or two weeks, although occasionally a strain will remain

viable for several months Tunnicliff (13) reports that a liver-brain medium to which calcium carbonate is added will retain viability for a year or more. Under favorable conditions acid and gas are formed from dextrose, lactose, sucrose, maltose, and salicin The amount of acid formed is not great and gas formation is limited. In cooked-meat medium, covered with a vaspar seal (petrolatum and paraffin equal parts) a large bubble of gas is regularly formed. Hemolysis of horse blood occurs Serum gelatin is not liquefied and coagulated serum is not digested In clear solid media, colonies are fuzzy when the medium is fairly soft and dense when it is more solid.

Natural Habitat. This organism has been found in the cecum of apparently normal swine (1) and it is likely that it exists in the alimentary canals of other species of animals. Infections in animals generally occur when they are kept in filthy surroundings, especially when there are accumulations of manure under foot It does not seem likely that the organism could multiply outside of the body but it undoubtedly remains viable in soil for short periods of time Marsh and Tunnicliff (9) were able to demonstrate the organism in a wet pasture ten months after sheep affected with foot rot had run on it, but they could not demonstrate it after a second ten-month period. Under these conditions, which apparently were unusually favorable, the organism was able to survive through one winter in the rigorous climate of Montana.

Pathogenicity

FOR EXPERIMENTAL ANIMALS Progressive disease is produced in rabbits and white mice by subcutaneous injection of pure cultures Guinea pigs are more resistant, but local lesions may develop The rabbit is the most satisfactory animal for diagnostic use If the material injected is contaminated with many other bacteria, as it is when taken from an intestinal ulcer, for example, it is best to introduce a small bit of dry material from the depths of the lesions into a small subcutaneous pocket, rather than to inject it ground up and suspended in a fluid Injection with considerable fluid seems to favor the contaminating organisms, whereas the necrosis bacillus thrives better when it is not unduly exposed to air, as in grinding, and the dryness seems to retard the other pathogens present At the point of inoculation a spreading subcutaneous necrosis occurs and the rabbit rapidly loses weight The rabbit usually dies in from 4 to 7 days in a greatly emaciated condition The autopsy examination in these cases usually reveals no lesions in the internal organs. Extending for a considerable distance from the inoculation point, a pasty, whitish, necrotic material is seen At its lower points there usually is considerable edema Bits of this material smeared on slides and stained with dilute fuchsin generally shows many filamentous forms, mixed with any other organisms

that may have been present in the inoculum Pure cultures can seldom be obtained from the local lesions when badly contaminated material has been used for inoculation, but if cultures are made from the heart blood, liver, spleen, and kidneys in cooked-meat medium, quite often one or two of the cultures will prove to be pure Some strains are less virulent for rabbits and the animals may live for two weeks or longer In these cases, necrotic areas usually are found in some of the internal organs, in the heart muscle, the lungs, liver, or kidneys, and pure cultures are readily obtained from them. Intravenous inoculation of cultures usually kills in from a few days to two or three weeks, depending upon the dosage and the virulence of the strain Multiple necrotic areas are then found in the internal organs Sometimes a fibrinous pleuritis or pericarditis is found Inflammation of one or more joints is observed occasionally

Natural Infections. *Act necrophorus* is found in association with a wide variety of lesions in horses, cattle, sheep, swine, and some birds It has been found also in many wild animals such as reindeer, antelope, buffalo, monkeys, and even in several species of cold-blooded animals It has been reported a number of times in man but there is some question about the identity of the human cultures and the necrosis bacillus of animals Carnivorous animals appear to be highly resistant to this organism

The role of this organism in many of the pathological processes with which it is associated, and of which it has been regarded in the past as the etiological agent, is not clear This organism at one time was regarded as the cause of necrotic enteritis of swine but Murray, Bicster, Purwin, and McNutt (10) have demonstrated that *Act necrophorus*, although practically always present in the lesions, is not capable alone of producing the disease, but that it could be reproduced regularly by feeding cultures of *Bact choleraesuis* In the ex-experimentally-produced cases the typical ulcers were produced and *Act. necrophorus* was present in them, proving that the latter existed in the intestine as a saprophyte and took part in the ulcerating process only when another organism had initiated the process *Act necrophorus* has long been regarded as the causative agent of foot-rot in sheep, a contagious disease which causes heavy losses in some sheep-raising countries. Recently Beveridge (3) working in Australia has challenged this idea and has brought forth convincing evidence that the necrosis bacillus is not the primary agent It has long been known that *Act necrophorus* often appeared in the ulcers following the rupture of the vesicles of foot and mouth disease in cattle, and of the vesicles of contagious ecthyma of sheep The evidence appears to indicate that this organism has little or no ability to invade normal mucous membranes or the skin but frequently thrives in wounds of the surfaces produced by mechanical

injury or bacterial action On the other hand, such lesions as the characteristic liver abscesses of cattle usually present pure cultures of *Actinomyces necrophorus* and the organism may be seen in numbers at the margins of the necrotic areas, hence it can hardly be looked upon as a purely saprophytic type

The diseases in which this organism appears prominently are known under the collective name of *necrobacilloses* Necrobacillosis of horses usually takes the form of a gangrenous dermatitis of the feet and lower parts of the legs, and occasionally as a necrotic pneumonia In cattle the lesions may be found in various parts The common "foot-rot" or "fouls" is thought to be caused by it Infections of the mouth and pharynx of calves (calf diphtheria) is an especially malignant form of the disease Lesions are often found in the liver as firm, dry, sharply circumscribed areas of a light yellow color, and sometimes as well-encapsulated abscesses In the latter instances other bacteria usually are present as well as the necrosis bacillus, in the former type, it usually is present in pure culture Lesions not infrequently appear on the skin, especially the skin of the udder and teats Uterine infections are not infrequent and ulceration of the mucosa of the abomasum is often ascribed to this bacillus In sheep the diseases known as lip and leg ulceration, and foot-rot have been attributed to *Act necrophorus* but Beveridge has apparently shown that this accusation is false In swine it has been regarded as the causative agent of a common disease known as ulcerative stomatitis (sore-mouth) and the condition known as "bull-nose' in which there is an infection of the subcutaneous tissues of the face frequently originating in the wound made by the placing of a ring in the nose The organism also has been reported as a secondary invader in the virus disease of chickens known as avian diphtheria (fowl pox)

Internal lesions caused by pure infections with this organism usually are firm yellowish-white, tumor-like nodules consisting of tissues which have undergone caseation necrosis The cut surface is dry and very firm Around the younger lesions there may be inflammatory zones In sections made of these lesions the specific organism frequently can be seen as long filaments lying in parallel bundles and radiating outward from the center of the lesion The organism cannot always be demonstrated microscopically in the older lesions but cultures usually succeed These lesions are practically never encapsulated. When the infection occurs on the surface of the skin or mucous membranes, the lesions are characterized by dry whitish patches, consisting of necrotic material, which extend deep into the underlying tissues. Usually a foul odor is present Often abscesses are found in the internal organs in which the necrosis bacillus is associated with other bac-

teria In these instances the pus may be fluid or thick, and always malodorous Such lesions usually have a thick fibrous capsule around them.

Toxin Formation. The fact that rabbits usually die after great emaciation when the only lesion is a comparatively small subcutaneous area of necrosis has led most workers to conclude that this organism produces an exotoxin Filtrates of cultures, however, exhibit very little toxicity. A mild inflammatory lesion may be produced in rabbits by subcutaneous injection of filtrates, hence it is probable that the poisonous property is endotoxic in nature That endotoxins exist in the bacilli cannot be doubted, since heat-killed cells will cause inflammation and necrosis when injected intradermally into rabbits The endotoxic substance is strongly heat-stable

Immunity. All attempts to produce immunity to this organism have failed Cultures killed by heat, phenol, or formalin may prolong the life of treated rabbits for a day or two, but doses of cultures which kill the controls will also kill the treated animals Antibodies in low titer may be produced by injecting repeated doses of killed culture According to Feldman, Hester, and Wherry (6) there is no evidence to indicate that different animal species suffer from distinct strains of this organism These workers found that agglutinins were present for *Act necrophorus* in the blood serum of a large percentage of normally-appearing horses, cattle, sheep, and swine, but that they were absent from the sera of calves, lambs, rabbits, and human beings They conclude that the agglutination test for the detection of obscure lesions of necrobacillosis in mature horses, cattle, sheep, and swine is useless

Human Infections. A number of cases of human infections ascribed to the necrosis bacillus have appeared in the literature The earlier reports dealt with local infections some of which were in persons working with the animal disease [Schmorl (11)] Later ones were cases of purulent pneumonia (12), of deep-spreading abscesses (4), of ulcerative colitis (5), and infections of the uterus (8) The cultural features of the organisms indicate that they are closely related to *Act necrophorus* in all cases and in some instances they appeared to be identical Dack and his associates (5) have called attention to the close relationship of the organism known as *Bacteroides funduliformis* to *Act necrophorus* It is evident that these organisms are sufficiently similar that they ought to be classified together

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ACTINOMYCES NODOSUS

Synonym *Fusiformis nodosus*

This organism was described by Beveridge (2) in 1941 He regards it as the cause of "foot-rot" of sheep The work was done in Australia but he also examined material in the United States and found that the conditions in the two countries were the same The organisms isolated in Australia were serologically related to a strain isolated in the United States

According to Beveridge, two organisms usually predominate over all others in cases of foot-rot One is a spiral organism which had previously been found and described by the author in 1936 (1) and the other a motile fusiform bacillus Both of these organisms were obtained in pure culture and with them attempts were made to reproduce the disease by introducing them into scarified areas on the feet of sheep Foot-rot was not produced in this way Less abundant in the foot-rot lesions but usually present was *Actinomyces necrophorus* Many attempts to produce foot-rot with this organism also failed Rather scarce in stained films of the lesions was a fourth organism, a large, Gram-negative, non-motile bacillus with clubbed ends With pure cultures of this organism rather mild cases of foot-rot were produced by inoculation Typical foot-rot was readily produced when animals were inoculated with the large non-motile bacillus and the spirochete The non-motile bacillus (*Act nodosus*) therefore is regarded as the primary cause of the disease with the spirochete (*Sp penortha*) as an accessory factor The role of the motile fusiform bacillus in the lesions is not clear.

Morphology and Staining Reactions. The organism is a large, rod-shaped bacterium characterized by the presence of terminal enlargements, usually at

both ends. These enlargements are more pronounced in organisms seen in tissue smears than in those developing in culture The rods usually are straight but may be slightly curved They are from 0 6 to 0 8 microns in diameter and from 3 to 10 microns in length, although few are more than 6 microns long In cultures the organisms tend to be shorter and in old cultures they may even be coccoid in form It is non-motile and does not form spores or capsules It stains readily with all ordinary dyes It is Gram-negative and non-acid-fast Organisms stained with methylene blue often show one or several metachromatic granules, usually located at the ends of the rod.

Cultural Features. The organism is an obligate anaerobe Growth is enhanced when 5 to 10 per cent or more of carbon dioxide is introduced into the anaerobic culture jar Growth occurs best at 37° C At room temperature very slow growth occurs Cultures grow best in neutral or alkaline media

Practically no growth is obtained on any of the ordinary media unless horse serum is added to them in a proportion of 10 per cent Not all lots of horse serum prove satisfactory, and sheep serum not only failed to promote growth but actually inhibited it in the presence of horse serum Rabbit and cow serum were not satisfactory

Best growth was obtained on "V-F" agar, which is a peptic digest of beef muscle and liver Veal infusion media were not very favorable even when horse serum had been added Growth did not occur on inspissated horse serum or egg medium Growth was never luxuriant in any fluid media, and ordinary types even with serum added often failed to promote growth

On "V-F" agar plates containing horse serum and 0 1 cystein-hydrochloride as a reducing agent, surface colonies are obtained These are generally of a smooth surface, develop up to a diameter of 1 mm , and usually lie in small "etched" depressions in the agar surface If blood is added to the medium instead of serum, no hemolysis is observed Heavy inocula often will cause curdling of milk after several days' incubation without change in reaction, and later the curd is digested In cooked-meat media the fragments are partially digested In old cultures tyrosine crystals are formed None of the ordinary carbohydrates are fermented Nitrates are not reduced but hydrogen sulphide is formed

Natural Habitat. *Act. nodosus* has been found only in the lesions of foot-rot of sheep Since it has been shown that foot-rot virus will remain virulent in pastures, even when continually moist, for only a few days it is unlikely that the organism can survive long in nature away from animal tissue. Beveridge claims that apparently recovered sheep often harbor small, incon-

spicuous lesions in which the organism will remain viable for months and believes that the disease is kept alive in flocks in such animals.

Pathogenicity. Subcutaneous inoculation of sheep, rabbits, guinea pigs, and mice with large doses of the pure cultures of this organism produced nothing more than transitory local lesions When *Sp penortha* was added to the inoculum, the effect was not materially changed. Only by inoculating the scarified skin around the margins of the claws of sheep were any significant lesions produced

Toxin Formation. No evidence of any type of toxin was observed

Immunity. Agglutinins were readily produced by rabbit immunization Antisera prepared with an American and an Australian strain cross-agglutinated but not completely Sheep affected with foot-rot failed to agglutinate these antigens even in low dilution This is ascribed to the rather superficial character of the lesions of the disease

The naturally occurring disease confers little immunity An animal with one or two infected feet of long standing can be artificially infected on another foot Attempts by Beveridge to immunize sheep by vaccines of several kinds containing heat-killed cultures were not successful The disease is successfully controlled in Australia by carefully examining the feet of all sheep at the beginning of the dry season, when the disease does not spread, and eliminating all of those that show evidences of the disease.

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CHAPTER XXVI

THE ACTINOBACILLUS GROUP

This group contains three species which are quite dissimilar and which ought not to be classified together So far as is known, only one of them is of importance, the *Actinobacillus lignieresi* The *Actinobacillus actinomycetamcomitans* has been found in association with the organism of actinomycosis in the lesions of that disease Its significance in the disease is unknown. The *Actinobacillus actinoides* occurs in certain types of broncho-pneumonia in calves and white rats, and probably plays a role in the causation of some cases. These organisms are placed in the same genus by Bergey and that classification is used to avoid further confusion Thompson (5) has called attention to certain resemblances between the organism of glanders (*Malleomyces mallei*) and *Actinobacillus lignieresi* and on this basis has proposed that the glanders organism be placed in this group This suggestion has not been followed by other authors

ACTINOBACILLUS LIGNIERESI

This organism was first described by Lignieres and Spitz (2) (3) in 1902 It had been isolated from Argentine cattle suffering from a disease which clinically resembles actinomycosis Later this disease was recognized in Europe (4) and in the United States (7) and is quite common Many continue to confuse it, however, with actinomycosis.

Morphology and Staining Reactions. In the pus of the lesions of the disease the small rod-shaped organisms are encased in small cheese-like granules These are quite similar to the "sulphur granules" of actinomycosis but generally they are much smaller, measuring less than 1 mm in diameter as a rule. If these granules are picked out of the pus and crushed between slides, moderate magnification will show club-like bodies radiating out from the centers of the masses Stains made from the crushed granules show small Gram-negative bacilli

In cultures the organism exhibits considerable variation in morphology depending upon the medium used and whether surface or deep growth on solid media is examined. Diplococci and slender rods are seen in fluid cultures. Long curved forms often are seen in colonies growing in the depths of solid

media. The bacilli are about 0.4 micron in width and from 1 to 15 microns in length. They are non-motile. They stain with the usual dyes and are Gram-negative.



FIG. 70. *Actinobacillus lignieresi*. Club bearing rosettes in pus from a lymph node lesion. Unstained. x 360. (Courtesy of L. R. Vawter.)

Cultural Features. This organism is quite serophilic and little growth occurs in most media unless a little serum or blood is present. Also, it is quite strongly aerobic, to the extent that growth practically always fails under anaerobic conditions. On the other hand, primary cultures in fluid media or in stabs in solid media are more apt to succeed than surface cultures. Primary cultures succeed best when the cultures are incubated in an atmosphere consisting of 10 per cent carbon dioxide.

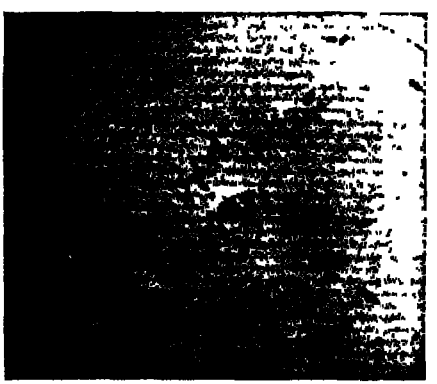


FIG. 71. *Actinobacillus lignieresi*. From a culture on serum agar incubated 24 hours at 37° C. x 900. (Courtesy of L. R. Vawter.)

In serum-agar, delicate, nail-like growths appear along the length of the stab. Surface colonies are bluish-white and very delicate. They are smooth, glistening, convex, and vary from 0.5 to 2 mm. in diameter. There is good growth in serum-gelatin but the medium is not liquefied. Dextrose serum

broth usually shows a characteristic growth consisting of small grayish gran ules which adhere to the sides of the tube but are easily broken loose by shak ing The remainder of the broth is clear Litmus milk usually remains un changed after 30 days Sometimes it develops slight acidity Excellent growth occurs on coagulated blood serum The medium is not softened or liquefied When dissolved in serum broth, dextrose, lactose, sucrose, maltose, raffinose, and mannitol are regularly fermented Xylose is fermented irregularly Arab inose, dulcitol, salicin, and inulin are not attacked Indol is formed in small amounts Cultures must be transferred at frequent intervals, otherwise they lose viability

Pathogenicity. This organism is only slightly pathogenic for guinea pigs and rabbits and not at all for rats and mice In guinea pigs which have been inoculated intraperitoneally, a lo calized peritonitis in the scrotal sac may occur not unlike the Strauss re action caused by the glanders bacil lus

The natural disease in cattle is manifested most commonly by slow ly developing tumors which may occur in any part of the body but are seen most frequently in the re gion of the lower jaws and neck. These are hard and often lobular. Sooner or later softened areas be come evident and these fluctuate upon pressure indicating the pres ence of fluid, which is, in reality, a mucoid, non-odorous pus This breaks through the skin creating a deep ulcer which will not heal In the meantime the tumorous mass usually continues to enlarge and ad ditional ulcers may form A char acteristic form of this disease is the so-called "wooden tongue" of cattle In this disease the hard tumorous mass forms in the substance of the tongue causing serious disability Lesions in the internal organs, particularly of the lymphoid structures, the lungs, and the walls of the stomachs, are not un common



FIG 72 Actinobacillosis Purulent focus in a case of wooden tongue ' The lesion consists principally of granulation tissue con taining such foci as is depicted here The rosette often is surrounded by a number of giant cells, epithelioid cells, and polymorpho nuclear leucocytes Stained with haematoxy lin and eosin x 400

This disease often is confused with actinomycosis The true actinomycosis,

which is caused by the "ray fungus," *Act bovis*, seldom occurs in the soft structures but is found mostly in the bone of the lower jaw

Actinobacillosis is a relatively common disease of cattle in the western hemisphere. Usually it occurs as sporadic cases but occasionally small epizootics are seen. The mortality is not high, since the subcutaneous lesions yield readily to surgery if taken before they involve too much tissue Cases of "wooden tongue" are likely to prove fatal and such animals should be slaughtered as soon as the condition is recognized in order to realize the beef value of the carcass before emaciation begins

Several human infections with this organism have been reported The pathogenicity for man apparently is not high

Immunity. Magnusson found that all strains of this species isolated from cattle were serologically identical He believed that the agglutination test might prove useful in diagnosis but others have not been able to confirm this idea.

There is no evidence that animals may be successfully immunized to this disease, and no immunizing products are available It is interesting to note that this infection, like actinomycosis, is iodine sensitive and that local lesions may be successfully treated by injecting them with an aqueous solution of iodine (Lugol's solution) For inaccessible lesions, potassium iodide or sodium iodide may be administered intravenously

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ACTINOBACILLUS ACTINOMYCETAM-COMITANS

Synonym *Bacterium actinomycetum-comitans*

This organism was first described by Klinger (3) in 1912, who found it in a case of human actinomycosis in association with the ray fungus Later Colebrook (2) in England and Bayne-Jones (1) in the United States found the same organism associated with human actinomycosis Although human actinomycosis is caused by the same organism that so commonly affects cattle,

this organism has never been reported from cases of bovine actinomycosis. The significance of the organism in the disease is not clear. It has been claimed that when metastatic lesions are present in a patient, this organism can be found only in the primary lesions It is present in the interior of the "sulphur granules" and may be demonstrated there by a simple microscopic examination of the crushed granules, as well as by cultural examinations

Morphology and Staining Reactions. The organism occurs as cocco-bacilli or as rods 1 0 to 1 5 microns long by 0 6 to 0 8 microns broad It is non-motile and Gram-negative

Cultural Features. On dextrose agar small, smooth, slightly yellowish, adherent colonies are formed Growth occurs in gelatin along the stab but the medium is not liquefied When the gelatin is incubated at 37° C a characteristic growth appears Grayish-white granules form along the sides of the tube and by fusion these eventually form a complete ring around the tube and a pellicle over the surface A similar effect appears in broth cultures, but in this case the fluid finally becomes turbid Growth does not occur in milk or on potato Acid is formed from dextrose and lactose but there is no gas formation. Growth in all media is enhanced by the addition of serum

Pathogenicity. Except when very large doses are used, this organism is non-pathogenic for laboratory animals

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ACTINOBACILLUS ACTINOIDES

Synonym *Bacillus actinoides, Actinomyces actinoides.*

This very peculiar organism was found by Theobald Smith (2) in cases of calf pneumonia in 1917 It was described more fully in 1921 (3) Apparently it has not been observed by any other, except perhaps by Jones (1) who found a similar, if not identical, organism in a type of pneumonia commonly found in old white laboratory rats

Morphology and Staining Features. In tissues this organism usually appears as a slender rod In cultures it may be bacillary or coccoid Growing on media containing serum, the organisms usually are embedded in a non-stainable material resembling capsular substance Long filaments and coccoid elements

may be found in such masses When growing on blood agar, the capsular material is not formed and the organisms appear in the earlier stages as long granular rods Later the rods disappear and only coccoid elements are seen. It is non-motile and Gram-negative.

Cultural Features. This organism is cultivated with difficulty and strains quickly die out Smith and Jones found coagulated horse serum slants, to which calf serum-water had been added, the most suitable medium When bits of the affected lung tissue were rubbed over the slant and then deposited in the serum-water at its base, growth was obtained The slants had to be sealed air-tight with sealing wax and incubation had to be at 37° C The first evidence of growth was seen as scintillating flakes in the serum water after 3 or 4 days These gradually enlarged, becoming mulberry-like masses in which crystals were embedded When these granules were crushed on a slide, magnification showed delicate filaments with club-like bodies not unlike those of *Actinobacillus lignieresi*

Growth on the surface of the slants does not occur at first but after several generations of growth some strains develop tiny translucent colonies Smith did not succeed in obtaining growth in broth, gelatin, milk, or potato

Pathogenicity. Smith believed that this organism was the primary etiological agent in a certain type of pneumonia of calves, and Jones believed his organism to be the cause of the rat pneumonia Neither of these beliefs were substantiated by experimental evidence Cultures injected into the trachea of calves produced circumscribed areas of lung necrosis, and when injected subcutaneously, resulted in an indurated lesion which later became necrotic

In calves the pneumonic process becomes clinically evident when the animals are from two to three months of age, although it is obvious that it is a slow-moving process which begins much earlier By the time pneumonic symptoms are evident, the lungs usually are filled with abscesses from which pus forming organisms, especially *Corynebacterium pyogenes,* may be isolated From such lesions it is impossible to isolate the organism which Smith believed to be the primary pathogenic agent

Immunity. Nothing is known about immunity to this organism.

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CHAPTER XXVII

THE PATHOGENIC ACTINOMYCETES

The actinomycetes are organisms that evidently are somewhat higher in the evolutionary scale than the ordinary bacteria Usually they grow in the form of a much-branched mycelium In many forms this mycelium frequently breaks up into fragments which cannot be distinguished from ordinary bacteria Some have referred to them as the "Higher Bacteria" Henrici, in discussing the relationship of these forms to bacteria and molds mentions three possibilities (1) That they are bacteria which have evolved into a higher form, (2) That they are molds which have degenerated, (3) That they are forms from which the bacteria have developed by degeneration and the molds by evolution Some of the actinomycetes are acid-fast and evidently are closely related to the acid-fast bacteria

Large numbers of actinomycetes may be found in garden soil A few of these are, at times, pathogenic for man and animals Other types have been found only in disease processes and evidently are obligatory parasites The pathogenic forms produce low grade inflammatory reactions with a tendency to form nodules which undergo degeneration and suppuration in a manner quite like the lesions of tuberculosis Some of the actinomycetes have a tendency to grow in tissues in the form of colonies at the periphery of which club-like protuberances are seen.

ACTINOMYCES BOVIS

Synonyms *Streptothrix actinomyces, Discomyces bovis, Nocardia bovis; Streptothrix israeli,* and others

This organism is the cause of the common disease of cattle known as actinomycosis or "lump jaw" It also affects swine where the seat of localization is the mammary gland Human infections occasionally occur, the manifestation being similar to those in cattle

It should be pointed out that conditions that resemble actinomycosis clinically, and are often called actinomycosis, are due to other organisms particularly *Actinobacillus lignieresi* and *Staphylococcus aureus* The true actinomycosis of cattle usually is an affection of bony structures, particularly the

mandible (lower jaw) The condition known as "wooden tongue" which is commonly called actinomycosis really is actinobacillosis in nearly every instance. This is true also of the subcutaneous nodules of the region of the jaw and neck, and the nodules which occasionally are found in the liver, lungs, and other internal organs Magnusson found that "actinomycosis" of the bovine udder was, in every instance, actinobacillosis, and in the sow, in about a third of his cases, staphylococcus infections

Morphology and Staining Reactions. In the "sulphur granules" in the tissues, *Actinomyces bovis* is seen as a tangled mass of filaments around the

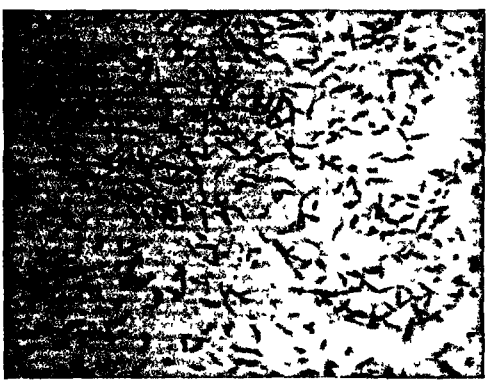


FIG 73 *Actinomyces bovis* Diphtheroid forms in a culture on Loeffler's blood serum incubated for seven days at 37° C under increased CO_2 tension × 900 (Courtesy of L R Vawter)

periphery of which is a considerable mass of acidophilic capsular material The filaments stain Gram-positively, and also retain the usual basophilic stains When stains are made of crushed granules a great diversity of forms are seen, which leads one to think that he is dealing with a mixed infection. They are coccoid, rods of varying size, filaments, branching forms, club-shaped forms, and spiral elements Actually all of these are forms of the one organism. In cultures, the organism usually appears in the form of diphtheroid bacilli when young; older cultures may show filaments of all kinds. When grown in an atmosphere of carbon dioxide branching filaments and clubs are frequent.

Cultural Features. *Act bovis* frequently is regarded as an obligate anaerobe. This conception is false. Growth cannot be obtained on the surface of solid media incubated in the air, but it may be obtained when the media are

enclosed in a tight vessel in which from 10 to 15 per cent carbon dioxide is introduced When shake cultures are made in solid media, growth does not occur on the surface The optimum zone, in this case, is about 1 mm. below the surface, but scattered colonies usually are found throughout the depths of the medium Cultures sometimes will develop on the surface if the tubes are hermetically sealed, a procedure which results in an increase in the carbon dioxide content of the imprisoned air



FIG 74 *Actinomyces bovis* From a serum broth culture incubated six days at 37° C Clubs, filaments and diphtheroid forms present x 900 (Courtesy of L R Vawter)

Act bovis is a scrophilic organism, i e, little or no growth can be obtained in ordinary media unless animal fluids are present It does not grow at temperatures very much below those of the animal body

In stab cultures in serum agar a nodular growth occurs along the lower parts of the stab There is no growth on the surface, nor in the upper centimeter of the stab In shake cultures small, biconvex colonies appear throughout the medium except in the upper layer Growth on serum agar slants will occur only if the tubes are incubated in a carbon dioxide-containing atmosphere, or anaerobically

The growth in serum broth is not abundant If incubated in the air the medium should be in tall columns and it should be heated shortly before the serum is added and inoculation is done The growth is in the form of granules which collect along the sides of the tube and in the bottom The fluid is clear except for the granules

Loeffler's blood serum slants are good for isolation providing they are incubated in a carbon dioxide jar Growth is evident after two or three days in the form of fine colonies which may easily be scraped off of the medium After five or six days' incubation at 37° C the colonies will have reached maximum size, which is about 0 5 mm in diameter The condensation water at the bottom of the slant usually contains excellent growth in the form of a slimy deposit.

On blood agar plates the colonies are small and non-hemolytic.

Little growth occurs in milk unless serum is added to it In serum-milk

medium there is little change in the appearance of the medium Sometimes there is bleaching of the litmus in the bottom of the tube

No growth occurs in gelatin unless serum is added. Serum-gelatin is not liquefied.

In serum-containing broth under a vaseline seal, dextrose, levulose, maltose, galactose, sucrose, and salicin are slowly fermented without gas formation

Pathogenicity. *Act bovis* is non-pathogenic for laboratory animals As a matter of fact, cattle cannot regularly be infected by inoculation with material from lesions, or with cultures Magnusson (3) succeeded in eight in-

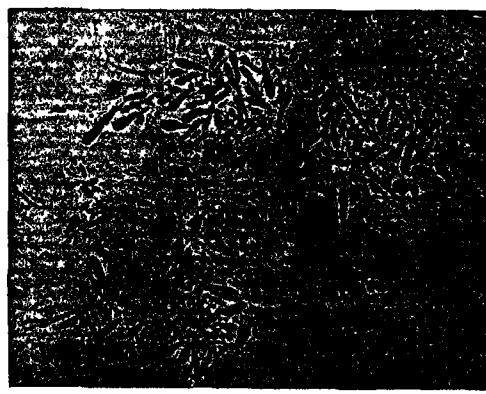


FIG 75 *Actinomyces bovis* Clubs and rosettes in pus of a bone lesion Unstained x 360 (Courtesy of L R Vawter)

stances in a total of thirty-two attempts He succeeded twice in infecting swine; in one instance by injecting culture into the mammary gland, in the other by injecting it into the testicle

Actinomycotic lesions are characterized by the formation of a soft, granulomatous tissue After a time this tissue exhibits necrotic areas which break down into abscesses These abscesses then coalesce to form sinuses or fistulous tracts and at the same time the connective tissue hardens into dense masses or tumors. A thick, mucoid, tenacious, greenish-yellow, non-odorous pus is characteristic of the disease The pus contains cheese-like granules varying in size up to 3 or 4 mm in diameter These are the colonies of the organism, and are commonly called "sulphur" granules

If these granules are examined in the fresh condition, simply by pressing a clean cover glass on them, the "ray-fungus" appearance can be easily dis-

cerned. This is the most rapid way to make a definite diagnosis. The borders of the crushed granules show radiating, swollen, club-like filaments. The club-like forms are not seen in stained preparations of the pus as a general rule, but can be seen in histologic sections Apparently, the swollen filaments are

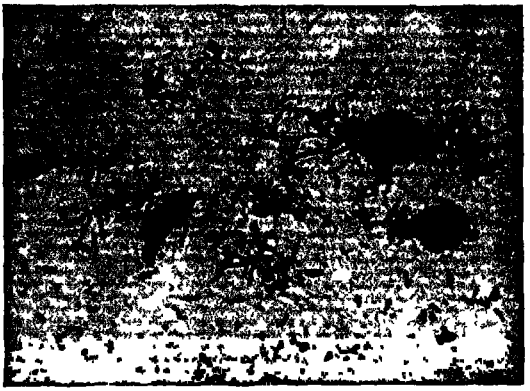


FIG 76 *Actinomyces bovis* Branched filaments and coccoid bodies in actinomycotic pus x 900 (Courtesy of L R Vawter)

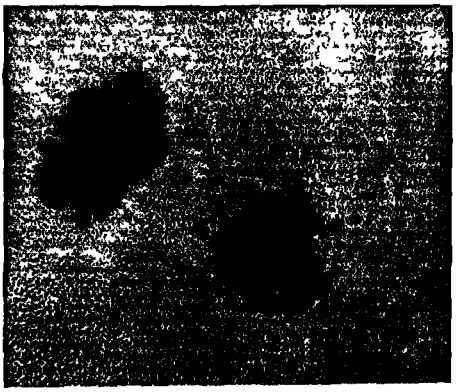


FIG 77 *Actinomyces bovis* Rosettes in pus of a bone lesion Picro-fuchsin stain x 360 (Courtesy of L R Vawter)

the result of a mantle of capsular material, and this material probably is produced as a result of contact with the tissue fluids

"Sulphur granules" are found in the pus of actinobacillosis, and also in those actinomycosis-like lesions which are caused by staphylococci. Fresh im-

pression preparations show radiating, club-like forms, not unlike those of true actinomycosis The sulphur granules in the non-actinomycotic lesions usually are much smaller than those of true actinomycosis, and frequently are so small that they are difficult to find on gross examination The granules may be differentiated, of course, by making stained preparations, when the morphology of the causative organisms can be determined true actinomycosis showing Gram-positive elements, short rods, filaments and branching forms; actinobacillosis showing small Gram-negative rods; and staphylococci showing their typical morphology When making such examinations it is well to select the granules from the pus, wash them, and crush them on clean slides. If the slide is made at random from the pus, it usually happens that no organisms at all will be found The granules usually can be obtained rather easily by placing some of the pus in a tube of broth or salt solution, shaking the tube to dissolve the mucin which holds the pus together, pouring the solution into a flat dish and searching for the granules which do not break up

Sources of Infection. It frequently is stated that the organism of actinomycosis is widespread in nature, occurring in the soil and on vegetation, and that animal infections are caused by injury of the mucosa of the mouth through which the organism enters Support for this theory is afforded by the observation that the incidence of this disease often is high when cattle are fed upon very rough forage, and especially on barley straw in which sharp awns are found It is not uncommon to find fragments of such awns buried deeply in actinomycotic tumors of the jaw.



FIG 78 Actinomycotic Lesion Showing rosettes embedded in pus in center of the lesion The greater part of the actinomycotic nodule consists of granulation tissue x 90

On the other hand, this organism never has been isolated from soil or animal foodstuffs, and since it is rather delicate many have doubted that it could maintain itself outside of the animal body Recently, Emmons (2), and Bibby and Knighton (1) have studied actinomycetes from the human mouth, some of which are very closely related, if not identical with, *Act. bovis* Such organisms have not been identified from the mouths of

bovine animals, yet it is quite probable that this is the natural habitat of this species It formerly was said that the infection of the udder of sows came about from coarse vegetation which injured the low-hanging gland Magnusson doubts that this is the true explanation and suggests that the infection probably originates in the mouths of the suckling pigs and reaches the sow's udder through teat injuries made by the sharp teeth of the pigs



FIG 79 Actinomycosis, Bovine This is a case of true actinomycosis, involving the bone of the jaw and caused by *Actinomyces bovis*

Immunity. No attempts have been made to immunize animals against this organism, and there are no records of attempts at diagnosis by serological means Magnusson found that agglutinins could be produced experimentally By this means he found that there were three serological types, A, B, and C Type A was characteristic of cattle, B and C of swine These types also showed some cultural differences

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ACTINOMYCES FARCINICUS

Synonyms *Nocardia farcinica, Streptothrix farcinica, Streptothrix nocardii; Actinomyces nocardii,* and others.

This organism is the causative agent of a disease of cattle which was first described in France under the name *farcin-de-boeuf* (bovine farcy) (3). The disease is said to be enzootic on the island of Guadeloupe in the French West Indies It is not known to exist in North America

Morphology and Staining Reactions. Stained films show filaments varying in length and averaging perhaps 0 3 microns in width. Branching is frequently seen. The filaments easily break up into fragments, many of which resemble

bacilli. These elements are Gram-positive and most of them retain the acid-fast stain.

Cultural Features. The growth on solid media resembles that of many of the saprophytic actinomycetes which are so common in garden soil. Growth occurs readily on plain agar slants Small ragged colonies quickly coalesce to form a tough yellowish-white, dry pellicle which becomes wrinkled and powdery. The powdery appearance indicates that aerial hyphae are formed In broth, the growth occurs principally as whitish granules, although small islands of growth may appear on the surface Gelatin is not liquefied and milk is not changed An abundant, dull pellicle forms on the surface of potato slants. No pigment is formed Growth is best at 37° C.

Pathogenicity. By inoculation this organism is pathogenic for cattle, sheep, and guinea pigs Musgrave and Clegg (2) produced miliary nodules in monkeys. Other authors say that the monkey is not susceptible to inoculation.

When inoculated intraperitoneally into guinea pigs, the animal usually dies within 10 to 20 days Autopsy shows general emaciation and numerous tubercle-like nodules scattered over the surface of the peritoneum When the inoculum is administered intravenously, miliary nodules are formed in the lungs and the animal dies within one to two weeks, depending upon the size of the dose and the virulence of the strain Subcutaneous inoculation produces only an abscess

Cattle and sheep are somewhat more resistant to inoculation than guinea pigs but death usually occurs after several weeks following intravenous inoculation. The animal becomes very emaciated before death, and the lungs are found to be riddled with myriads of small nodules

The naturally occurring disease in cattle appears first as a chronic, indurative lymphangitis and lymphadenitis of the subcutaneous tissues, usually of one of the extremities, the lesions eventually breaking through the skin forming sinuses communicating with cold abscesses The disease is of long duration Eventually lung involvement generally occurs, the animal becomes emaciated, and death ensues Cultures are easily obtained from the freshly opened nodules

Immunity. There are no immunizing products Some affected animals react to tuberculin.

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ACTINOMYCES ASTEROIDES

Synonyms: *Cladothrix asteroides, Streptothrix eppingeri.*

Eppinger (3), in 1890, isolated an acid-fast actinomycete from a brain abcess of a man who died of a generalized disease which resembled tuberculosis. Besides a purulent meningitis, there were caseated bronchial lymph nodes and miliary lesions of the lungs from which the organism was isolated. Perhaps 30 or 35 other human cases have been described in more recent years, but the cultural characters of the organisms isolated have not, in many cases, exactly agreed with those of Eppinger's organism Henrici and Gardner (5) described such a case in 1921 and reviewed the literature up to that time For further details the reader is referred to their paper In 1933, Bishop and Fenstermacher (1) described an organism belonging to this general group which had been isolated from a tuberculous-like process in a cow The organism was highly pathogenic for guinea pigs Earlier Burnett (2) had described two cases in bovine lungs which clearly were caused by members of this group but since the organism was not isolated, its specific characters were not learned Gordon and Hagan (4), in 1936, described several acid-fast actinomycetes which had been isolated directly from soil and which proved to be pathogenic for guinea pigs, producing in them fatal infections in which the lungs were the principal organs involved.

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THE SOIL ACTINOMYCETES

Members of the actinomycete group are very common in soil, and a large number of species have been described Colonies may be found on almost any agar plate which has been exposed to the air and thereafter incubated for some days at room temperature, or which has been allowed to stand around in the laboratory The colonies are several millimeters in diameter, are very convex, and appear to be resting in depressions in the agar surface They usually are chalky white, yellowish, or orange colored The chalky surface is due to the fact that these forms possess aerial hyphae which are too fine to be seen by the unaided eye Many of these species excrete a tyrosinase which results in

browning of the medium in the vicinity of the colonies Many of these colonies produce a strong penetrating odor which is evident as soon as the plate is opened, and which is reminiscent of freshly ploughed ground

A small proportion of these actinomycetes are acid-fast, and since they readily fragment, the elements cannot be distinguished with certainty from acid-fast bacilli This fact should be remembered by those who are dealing with materials which may have been contaminated with soil Some of these forms have proven pathogenic for experimental animals when cultures are inoculated, and it is possible, therefore, that occasional spontaneous animal infections may be due to such organisms.

PART III

BACTERIA-LIKE PATHOGENIC ORGANISMS OF UNCERTAIN CLASSIFICATION

CHAPTER XXVIII

THE SPIROCHETES

The organisms known as the spirochetes sometimes are grouped with the bacteria and sometimes with the protozoa Probably because of their close resemblance to organisms that undoubtedly are bacteria, the spirilla, they now are generally regarded as bacteria If this is done it should be recognized that they possess a number of features which are seen in no other bacteria Some of the general characteristics of the group are as follows

Morphology. The form is spiral In some species the spirals are tight, in others quite open and variable Terminal filaments are seen in some forms but these do not behave like flagella When examining tissue fluids in the dark field, especially blood, for the presence of spirochetes, one must be very cautious in the interpretation of findings, inasmuch as mistakes can be very easily made even by experienced workers Filaments of elastic tissue, fibrin shreds and other artifacts often resemble spirochetes so closely as to deceive all but the most wary Such materials even create the illusion of motility

Staining Properties. Most of the spirochetes are difficult to stain Few of the pathogenic species may be stained with methylene blue. All are Gram-negative The Giemsa stain is useful, some staining red, others blue Those that stain blue generally are saprophytes that can be stained with methylene blue For demonstration in tissues, Levaditi's stain is most useful This stain contains silver nitrate After the tissue block has been saturated with the silver compound, it is treated with a reducing agent which removes the silver from the tissues and other bacteria but not from the spirochetes These then are seen as intensely black organisms. The dark-field method of examination often is used when searching for these organisms, since it is practically impossible to see them unstained in ordinary light because of their extreme tenuousness India ink, nigrosin, and similar background-filling agents also are useful.

Motility. The motility of spirochetes is derived from their rotatory motion In some species it is so rapid as to make it impossible to follow them with the eye Many spirochetes exhibit various bending movements which probably have little to do with their movement of translation.

Resistance. In general the resistance of spirochetes is very low Drying is rapidly fatal Temperatures of 50 to 60° C generally kill within a short time. Resistance to chemical disinfectants is not great

Cultivation. The spirochetes generally are less easily cultivated than bacteria. Some, especially the *Leptospira,* can be cultivated without great difficulty but others require elaborate media and the results are uncertain at best The majority are strict anaerobes Even the aerobic forms thrive best when the oxygen tension is lowered None can be cultivated upon the surface of solid media Either fluid media must be used, or the inoculum must be incorporated in the depths of solid media Growth of the pathogenic species practically always requires the presence of serum or other animal fluids

Classification. Six families of spirochetes are recognized The genera *Spirochaeta* and *Saprospira* include a number of species all of which are free living and are relatively large Species of the genus *Cristospira* occur as parasites of various molluscs, principally oysters, mussels, and scallops The species which are pathogenic for birds and mammals belong to one of three genera, *Borrelia, Treponema,* or *Leptospira* Members of the genus *Borrelia* are relatively large forms with open, irregular coils which move by active lashing movements and slow rotation These forms are readily stained by many of the aniline dyes which stain bacteria Members of the *Treponema* are smaller, with close, rigid coils, and these do not stain readily with dyes other than the Giemsa stain The *Leptospira* resemble the *Treponema* in that they have close coils, but these are not rigid, the organism frequently stretching out into a straight filamentous form which relaxes into the coil once more A further characteristic feature of the *Leptospira* is the almost constant presence of a bend or hook at one or both ends of the organism Like the *Treponemata,* the species of this group are difficult to stain except with Giemsa's stain

The three genera which contain the species pathogenic for the higher animals (*Borrelia, Treponema,* and *Leptospira*) may be distinguished from the saprophytic groups by the fact that they may be readily dissolved by a 10 per cent bile solution The *Treponemas* may be distinguished from the *Leptospiras* by the fact that they may be dissolved by a 10 per cent saponin solution

With one exception, all of the spirochetes known to be pathogenic for man and the higher animals, occur in the genera *Borrelia* and *Leptospira.* The exception is *Treponema pallida,* the cause of syphilis of man, a disease which does not naturally affect any of the domestic animals but which may be transmitted by inoculation to the rabbit

In Europe, India, Africa, and America a disease of man, known as relapsing

fever, occurs In the febrile paroxysms of this disease, members of the *Borrelia* can be demonstrated in the blood, and these organisms undoubtedly are the cause of the disease The organisms differ in the various localities, however, and the means of transmission differ In Europe, the common bed bug, *Cimex lectularius,* is the transmitting agent In India it is another species of bed bug. In Africa and America, ticks are usually incriminated Rats and mice usually can be infected by means of the agent which transmits the disease to man, and it is evident that in some of these instances, rodents and possibly other animal species constitute the reservoir from which human infections occur

BORRELIA THEILERI

Synonyms *Treponema theileri, Spirocheta theileri*

This organism was found by Sir Arnold Theiler (1) in the blood of South African cattle in 1902 It is a large, loosely twisted spiral, measuring from 20 to 30 microns in length The organism can be easily demonstrated in the blood during the febrile stage of the infection but it disappears later It is actively motile Artificial cultivation of this organism has not been reported

The disease produced apparently is quite benign The symptoms resemble those of anaplasmosis but are less severe One or more febrile attacks are followed by recovery Transmission is by the ticks, *Margaropus decoloratus* and *Rhipicephalus evertsi*

The same, or at least a similar, organism has been found associated with febrile attacks in sheep and horses These diseases are not serious

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BORRELIA ANSERINA

Synonyms *Spirocheta anserina, Spirocheta gallinarum, Borrelia gallinarum, Spironema gallinarum*

This organism was first described by Sakharoff (4), in Russia, as the cause of "goose septicemia" in 1891 It is probable that it is the same as the cause of "fowl spirochetosis" or "fowl spirillosis" which was recognized in Brazil by Marchoux and Salimbeni in 1903 The disease was found a little later in the Sudan in Africa. The spirochete of the chicken disease is regarded as a separate species by some under the name *Borrelia gallinarum* A similar disease has been reported in ducks It seems likely that geese, chickens, and ducks are affected by the same species If this is the case the correct name of the organ-

ism, inappropriate as it may seem, is that which was first applied to the goose infection.

The disease is manifested by symptoms of acute septicemia The affected birds develop fever, they are depressed, a profuse diarrhea occurs, and they soon die. The mortality rate is very high Autopsy examination reveals a swollen spleen, a pale and swollen liver, and a sero-fibrinous exudate in the pericardial sac During the early stages of the febrile reaction the spiral organisms can readily be found in the blood At the time of death they usually are absent, or abnormal or clumped forms may be found The disease is transmitted by ticks, generally *Argus persicus* or *Argus miniatus,* occasionally by others. The fowl mite, *Dermanyssus gallinae* has also been suspected of being a transmitting agent

Morphology and Staining Reactions. This is a loosely spiralled organism varying in length from 8 to 20 microns It is actively motile Older cells contain definite granules The Gram stain is negative but ordinary aniline dyes stain the organism very well It can be seen best by dark field illumination, but the India ink method and the Levaditi stain are good methods of demonstrating it

Cultural Features. Noguchi (3) succeeded in cultivating *B anserina* by using the methods which he had found to be successful with the relapsing fever spirochetes of man The medium consisted of a tall column of ascitic fluid containing a bit of sterile rabbit kidney, overlaid with paraffin oil A few drops of infected blood were used as the inoculum, and the tubes were incubated at body temperature In such tubes the spirochete grew rather rapidly for four or five days then appeared to disintegrate into granules Such cultures remained virulent for birds for several weeks

Pathogenicity. The disease is easily transmitted to a variety of birds, including, besides the ones which suffer from the natural infection, guinea fowl, sparrows, and canaries Pigeons are quite resistant, rats and guinea pigs wholly so. The inoculation disease is quite like the naturally occurring type

Immunity. Recovery from the disease leaves the bird refractory to further infection for a considerable period of time Gabritschewsky (1) found that the fresh serum of recovered geese caused disintegration and destruction of the spirochetes in a short time when incubated at 37° C This is similar to the action that goes on in the blood of the recovering bird The same worker found that immune horse serum was effective as a prophylactic agent but was ineffective when administered to birds in which the organism had begun to multiply.

Marchoux and Salembini (2) prepared an effective vaccine by heating the fresh blood of affected birds to 55° C. for five minutes They also found that the organism loses its virulence when stored in blood for 48 hours, and that such blood may be used as a vaccine Arsphenamine and its derivatives are used successfully in treating some of the relapsing fever infections of man. No record has been found of its use against this organism

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LEPTOSPIRA ICTEROHEMORRHAGIAE

Synonyms *Spirocheta icterohemorrhagiae*

This organism is the causative agent of a disease of man known variously as leptospirosis, leptospiral jaundice, and Weil's disease The disease is contracted by contact with rats or rat urine Surveys conducted in various parts of the world indicate that the infection of rats is very prevalent The disease ordinarily is sporadic except in situations where men come in intimate contact with rats as those who work in sewers, in rat infested mines, and in rat infested trenches in time of warfare The disease in rats is chronic, the principal seat of infection being the kidneys It is probable that most human infections occur through contact of the skin with fresh rat urine, with water, and less often with food contaminated in the same way.

This organism can easily be transmitted by inoculation to dogs and foxes, and natural infections undoubtedly occur The human disease can be contracted from such animals. It appears that the majority of infections of dogs are caused, however, by a closely related species, *Leptospira canicola,* which will be described later As a matter of fact it is not entirely certain that any of the canine cases which have been studied in the United States are due to the *L. icterohemorrhagiae,* since all strains that have been isolated and studied appear to be of the canicola type Walch-Sorgdrager and Schuffner (6) in Holland, and Meyer, Eddie and Stewart-Anderson (1) in the United States, upon the basis of agglutination tests, believe that *L. icterohemorrhagiae* is responsible for some canine cases Okell, Dalling and Pugh (4) who first described canine leptospirosis believed that the causative organism was the same as that which causes Weil's disease in man but their data are not conclusive on this point They did show, however, that rat strains produced a disease in

dogs, experimentally, which they regarded as identical with the one which they observed in naturally infected hunting dogs. Also it cannot be said that Rubarth (5), who described enzootics of the disease in foxes and dogs in Sweden, proved that the leptospira which he found were identical with those of Weil's disease rather than *L. canicola*

LEPTOSPIRA CANICOLA

A number of European workers had recognized spirochetes in the kidneys of dogs suffering from jaundice and from a hemorrhagic-uremic disease of dogs commonly known as Stuttgart disease prior to 1930, and it had been demonstrated that pathologic pictures similar to some of the naturally occurring forms of the disease could be produced by inoculation with the Weil's disease organism. It had been assumed, therefore, that the causative organism was *L. icterohemorrhagiae*. Klarenbeck and Schuffner, working in Holland, showed in 1931 that a spirochete which differed serologically from the classical Weil organism, was the causative agent of many of the canine cases. This organism later became known as *L. canicola*. It is now known that it differs from the classical type of man and the rat in the character of the disease produced in the dog and in the fact that it has slight pathogenicity for rats. Present evidence indicates that *L. canicola* is much more prevalent in dogs than *L. icterohemorrhagiae* and that instead of being a more or less accidental infection, it spreads readily from dog to dog and frequently produces enzootics, especially in large kennels. In many European countries *canicola* leptospirosis appears to be quite common, and it has become evident recently that there are many areas in the United States in which the disease is prevalent.

Morphology and Staining Reactions. Since *L. icterohemorrhagiae* and *L. canicola* cannot be distinguished from each other except by serological means, they will be described as one organism.

The organism is usually most easily found in the urine, in which it should be concentrated by centrifugation. It may also be found in the kidneys and with greater difficulty in other organs. Sometimes it is found only after prolonged search. Sometimes it may be recovered by guinea pig inoculation when it cannot be found at all in the dog.

In form these organisms are typical leptospira. The coils are so fine that in silver nitrate preparations and in the dark field they can be distinguished only by very careful observation. In fresh preparations they possess very active motility. The ends of the organism are characteristically hooked.

Cultural Features. These organisms are very easily cultivated The medium should be slightly alkaline, and should contain 5 to 10 per cent serum Growth can never be obtained on the surface of solid media Semisolid media may be used, such as Noguchi's leptospira medium, or fluids will suffice In fluid media it is well to have a fragment of fresh sterile tissue This may be a fragment of the kidney of the infected animal in which case the tissue serves the double purpose of supplying inoculum, and also reducing the oxygen tension to a favorable degree for the leptospira It is well to cover the surface of fluid media with oil or vaseline, although this is not necessary if rather tall columns of fluid media are contained in the culture tubes. Incubation may be conducted at a wide range of temperatures, although several degrees below body temperature appears to be the most favorable Growth usually is evident after about a week Cultures retain their viability, and pathogenicity for several weeks

Differentiation between L. canicola and L. icterohemorrhagiae Infections. The differentiation between these two infections in dogs and man is practically impossible except by the use of serological reactions Some contrasting characters of the two kinds of infection are given by Walch-Sorgdrager and Schuffner (6) from their large experience with both types in Holland Their principal points of differentiation are summarized below

1 THE DISEASE IN DOGS *Canicola* infection seldom produces icterus whereas this is a prominent feature of Weil's disease in dogs as in man (Experience in this country indicates that this feature is not a reliable means of differentiation, since many of the *canicola* infections exhibit icterus)

2 THE DISEASE IN MAN *Canicola* infection is more benign than Weil's disease, since it seldom results in death In man, icterus is seldom seen in *canicola* infection whereas it is common in Weil's disease In man *canicola* infection is likely to produce severe symptoms of meningitis, whereas these symptoms are not common in Weil's disease.

3 THE DISEASE IN EXPERIMENTAL ANIMALS *Canicola* infection is not so pathogenic for guinea pigs as is Weil's disease (more than half of injected animals fail to develop clinical disease) In guinea pigs icterus occurs only in a few animals injected with material from dogs when the causative organism is *canicola*, whereas 90 per cent or more develop icterus when injected with the Weil's organism Guinea pigs injected with *canicola*-containing materials, even though they may show no symptoms, usually shed the *Leptospira* in the urine for considerable periods of time. Those injected with the Weil's disease

organism usually die from an acute infection before they become urinary shedders

The water rat, which is the reservoir in all parts of the world of the *Leptospira icterohemorrhagiae,* and also other varieties of rats, are resistant to *L. canicola* The latter organism usually multiplies for a few days in the tissues and disappears without appearing in the urine, whereas infection with the former practically always results in urine shedders Furthermore, serological tests of rat populations indicates that the infection carried is always *icterohemorrhagiae* and never *canicola* It is clear, therefore, that *canicola* is not a rat-borne infection.

4 EPIDEMIOLOGY Epidemiological studies indicate that *canicola* infection is spread directly from dog to dog Infected animals usually are urinary shedders It is probable that the infection is taken in through the mouth and nose when dogs are smelling and licking the urino-genital organs of other animals It is significant that male dogs are infected more than twice as often as females It is not impossible that the infection is spread through copulation

5 SEROLOGICAL REACTIONS These two leptospira cross agglutinate only to a small degree The greater number of infected dogs agglutinate *canicola* to a high titre and *icterohemorrhagiae* to a low titre or not at all, whereas naturally infected rats agglutinate the Weil's organism and not the *canicola*

Pathogenicity. Three types of the disease in dogs are recognized, the acute hemorrhagic type, the icteric, less acute type, and the uremic type, commonly known as Stuttgart disease or canine typhus *L. icterohemorrhagiae* causes the first and second type but rarely, if ever, the third type *L. canicola* causes most, if not all, of the third type, some of the second, and a few of the first.

The first type is characterized by high fever, prostration and early death Hemorrhages occur throughout the organs, especially in the lungs and alimentary tract The second type is less acute and is characterized by intense icterus, hemorrhages with blood-stained feces, and pigmented urine The third type is characterized by uremia, because of extensive kidney damage, by a foul odor from the mouth because of ulcerative stomatitis, hemorrhagic enteritis, coma, and death in a high percentage of cases

The clinical picture of the true Weil's disease in man is similar to that exhibited by dogs. That caused by *L. canicola,* which Meyer has dubbed "canicola fever" often is severe and prolonged but recovery usually occurs The symptoms are quite varied and cases often are mistaken for undulant fever, influenza, epidemic meningitis, and other diseases. Undoubtedly many cases pass unrecognized Meyer, Stewart-Anderson, and Eddie (2) have warned veterinarians and dog owners to be careful in the handling of dogs which

show symptoms of icterus or of Stuttgart disease, because of the hazard to their health.

Diagnosis. The symptoms of the disease in dogs are sufficiently characteristic to enable a tentative diagnosis to be made A definite diagnosis can be made by conducting an agglutination test with a suspension of the specific organism Agglutinins appear rather late in the disease, hence agglutination is most useful in determining the type of infection after the animal has recovered, or in making surveys to indicate the prevalence of these infections By this test Meyer, Stewart-Anderson, and Eddie (2) determined that 34 per cent of the dogs in one area in San Francisco had had the *canicola* infection, and that, of a large number of serum samples collected in New York City, 9 per cent had antibodies for *canicola* fever and 2 8 per cent for Weil's disease

Immunity. After recovering from an attack of leptospirosis both man and animals are thereafter immune Immune sera have been used in treating Weil's disease in man with fairly good results Such sera are difficult to produce and can be expected only to ameliorate the symptoms rather than effect a complete cure There are no reports of the use of immune serums on dogs Arsphenamine and related compounds which are effective in some types of spirochetal infections are not effective against leptospiral infections

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CHAPTER XXIX

THE RICKETTSIAE

The name *Rickettsia* designates a group of small, bacteria-like organisms which are commonly found in the tissues of arthropods They were named in honor of Howard Ricketts who saw and described the one which causes Rocky Mountain spotted fever of man in 1909, and who contracted and died from the disease he was studying shortly afterwards This disease is transmitted to man by ticks, principally *Dermacentor andersoni* Ricketts demonstrated this fact in 1907 and he also showed that the disease in the tick is hereditary, the human infections being merely incidental

Non-pathogenic forms of rickettsiae occur in many arthropods such as ticks, mites, spiders, blood-sucking and non-blood-sucking insects, and lice Most of the rickettsiae occur intracellularly, in fact there are some workers who refuse to recognize as true members of this group any organisms which multiply extracellularly

Typical rickettsiae resemble small bacteria, morphologically In some instances there is a great deal of pleomorphism, in other instances they are quite uniform in size and shape Most of them occur in groups in the cytoplasm of the parasitized cells, in some instances they occur intranuclearly Nearly all measure less than 0 5 micron in diameter They stain poorly with ordinary dyes but can be well and characteristically stained by Giemsa and similar stains They are Gram-negative Most species of rickettsiae have been cultivated successfully in tissue culture None of the true members of the group have been cultivated in the absence of living cells

All of the diseases caused by rickettsiae are contracted by contact with infected arthropods These diseases are similar in that the principal lesions which result in hemorrhages and skin rashes are located in the walls of blood vessels The most important human rickettsial diseases are spotted fever, typhus fever (not typhoid fever), Wolhynian or trench fever and tsutsugamushi or Japanese flood fever There is only one animal disease of importance, heartwater.

Successful vaccines for Rocky Mountain spotted fever, and typhus have been developed by phenolizing or carbolizing tissues rich in the causative organism The first vaccines were made by using the viscera of infected arthropods—of ticks in the case of spotted fever and of lice in the case of typhus.

Later methods have been developed in which the rickettsiae have been cultivated in media containing minced tissue containing susceptible types of cells. The effectiveness of such vaccines depends upon obtaining a heavy concentration of rickettsiae in the cultures. The method of Zinsser, FitzPatrick, and Wei (4), successful in typhus, is probably applicable to other rickettsial diseases This method consists essentially of serum agar slants the surface of which has been spread with minced tunica vaginalis of the guinea pig, minced mouse embryo, or minced chick embryo The rickettsiae develop in the living cells whose vitality is preserved for a considerable time because the agar medium acts as a nutritive substance and as an absorber of waste products of cellular metabolism The infected cellular material is washed from the slanted medium when the rickettsiae have reached maximum development and made into vaccine by treatment with formalin Cox (3) discovered that large concentrations of spotted fever rickettsiae could be obtained by inoculating the yolk-sacs of developing chick embryos Growth of these organisms can be obtained on the chorio-allantoic membrane of developing chick embryos but the yield is too meager to make good vaccine

Passive immunity can be obtained by the transfer of serum from recently recovered animals Several weeks after recovery the serum is of little value.

RICKETTSIA RUMINANTIUM

This organism was first described by Cowdry (2) in 1925, who was working at the time in South Africa It is the cause of a disease of cattle, sheep, and goats commonly called "heartwater," because one of the characteristics of the disease is hydropericardium This disease occurs only in South Africa, French West Africa, the Belgian Congo, Kenya, and Tanganyika and possibly also in Madagascar The disease has long been known in South Africa and associated with the "bont" tick, *Amblyomma hebraeum,* which is the transmitting agent (1) Many other kinds of ticks occur in the heartwater districts but apparently the bont tick is the only vector Larval ticks retain the infection through the molts to the adult form, but the parasite is not transmitted through the egg to the next generation.

The disease can be transmitted by inoculation with blood taken from sick animals during the early febrile period, but transmission is not certainly accomplished in this way. Subcutaneous inoculation of blood succeeds in not more than 25 per cent of the trials, intraperitoneal and intratracheal inoculations are even less certain, and ingestion practically always fails It is clear that the disease is transmitted naturally solely through the activities of the bont tick.

The affected ruminants develop a high fever, show signs of gastroenteritis,

edematous swellings, and nervous symptoms. The temperature falls to subnormal before death.

The rickettsiae are found in the cytoplasm of the endothelial cells of the renal glomeruli and in parts of the brain They are not so readily demonstrated but they also occur in the capillaries of many of the internal organs. The affected cells become greatly swollen. Many capillaries become completely blocked in this way

If the animal survives an attack of the disease it becomes solidly immune. This is usual in all the rickettsial diseases

Rickettsia ruminantium apparently has never been cultivated artificially. In all probability, methods which have succeeded with other pathogenic rickettsiae would also succeed with this organism

There are no reports of the development of successful vaccines for this disease.

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CHAPTER XXX

THE PLEUROPNEUMONIA GROUP

In 1898, a group of French workers headed by Nocard (11) succeeded in cultivating a peculiar, very minute, organism from the exudates of a destructive cattle disease known as contagious pleuropneumonia The organism is so small that it passes filters as readily as many of the filterable viruses and for this reason it was long classified as one of the viruses It is now regarded as belonging to the bacteria and not to the virus group, since its only resemblance to the latter is in its minute size All true viruses are obligate intracellular parasites and cannot be cultivated on artificial media except when living host cells are present The pleuropneumonia organism, like other bacteria, can be cultivated on lifeless media.

In 1923, Bridré and Donatien (2) cultivated an organism closely related to the one of pleuropneumonia from the joints of goats which suffered from a disease known as contagious agalactia In 1934, Shoetensack (17) found an organism belonging to this group in dogs suffering from distemper In 1935, Klieneberger (7) made the surprising discovery that all strains of a bacterium known as *Streptobacillus moniliformis*, an organism which is normally present in the naso-pharynx of many rats, contained minute forms which have the characteristics of the pleuropneumonia organisms She succeeded in separating and cultivating the minute organism and believes that it is a symbiont of the *Streptobacillus* She gave it the designation L_1 Dienes (4), however, believes that L_1 is a variant of the *Streptobacillus* In 1938, Sabin (13), and Findlay et al (6) independently described a disease of mice (rolling disease) which was shown to be a neuro-intoxication caused by the growth of organisms of this group which multiply in the joints and in the mesenchymal cells of the peritoneum and pleura In 1939 Sabin (14) isolated another such organism, immunologically distinct from the one of rolling disease, which developed in the joints of mice producing an ankylosing arthritis Still later Sabin and Johnson (16) showed that such organisms were carried by normal mice on their nasal and conjunctival mucous membranes, and that at least five immunologically distinct types existed. These authors, thinking that organisms of this type might be responsible for the baffling lesions in the joints of man suffering from rheumatoid arthritis made a number of attempts to

demonstrate them but unsuccessfully Quite recently Dienes (5) has reported the finding of pleuropneumonia-like organisms in the cervical exudate of women suffering from gonorrheal vaginitis The significance of this finding is not known In 1936, Laidlaw and Elford (9) reported the finding of organisms of this group in filtrates of raw sewage These organisms are nonpathogenic for experimental animals Other organisms which probably belong to the group have been found in rats and chickens

The work of the last few years has made it clear that a considerable group of minute bacteria exists of which the bovine pleuropneumonia organism is the type Borrel and co-workers (1) gave the name *Asterococcus mycoides* to the bovine organism but the name has been little used Sabin (15) in his recent monograph on this group proposes to create a new class for these organisms since he feels that they are sufficiently different from ordinary bacteria to warrant taking them out of the class of *Schizomycetes* The name proposed for the new class is *Paramycetes* Two families are created, the *Parasitaceae* including the forms found in animals and *Saprophytaceae,* the forms found in sewage. The *Parasitaceae* are divided into a number of genera named according to the hosts in which they are found, thus, *Bovimyces, Capromyces, Canomyces, Murimyces* and *Musculomyces* Only the two species which affect domestic animals will be discussed here

BOVIMYCES PLEUROPNEUMONIAE

Synonyms *Asterococcus mycoides,* The organism of bovine pleuropneumonia.

This organism is the cause of a destructive disease of cattle which occurs in all parts of the world except Western Europe, North America, and a few other smaller areas. The disease has been known for more than 200 years From time to time in the past the disease has spread over the greater part of Europe In the early part of the 19th century the disease became widespread in Europe, and from there disseminated to South Africa, Australia, and the United States in exported cattle According to Moore (10), the disease was imported into the United States in 1843, 1847 and 1859 The disease was restricted to some of the eastern states until 1883 when it appeared in Ohio By 1886 it had reached a few herds in Illinois, Kentucky, and Missouri It was the spread of this disease that led to the establishment of the Bureau of Animal Industry, of the U S Department of Agriculture In 1887 Congress made available to the Bureau of Animal Industry adequate funds to deal with the disease During the next five years the disease was hunted down, all affected animals were destroyed, and in September 1892, the Secretary of Agriculture

issued a proclamation declaring the country to be free of the disease It has not occurred in this country since March 1892.

Morphology and Staining Reactions. The causative organism of bovine pleuropneumonia is exceedingly pleomorphic and varies widely according to whether it is grown on fluid or solid media, whether the cultures are young or old, and according to the method used for its demonstration Minute granules, and larger bacilliform elements, spirals, ring forms, globules, and bud-like forms are seen Even amorphous masses containing chromatic bodies are seen in preparations from solid media In tissues usually nothing recognizable as organisms can be found. The size varies considerably but the units capable of reproduction evidently are very small since ultrafiltration studies indicate that they are of the order of 125 to 175 millimicrons in diameter, a size which places them with many of the filterable viruses

Cultural Features. Nocard, Roux, Borrel, Salimbeni, and Dujardin-Beaumetz (11) first succeeded in cultivating this organism by inoculating serum broth with pleural exudate, enclosing the mixture in collodian sacs and placing the latter in the peritoneal cavity of rabbits The animals became emaciated and finally died, whereas others treated with similar sacs containing uninoculated media remained well The broth in the sacs had become slightly clouded, and when examined with the highest powers of the microscope, minute refractile dots were observed The organism was cultivated serially in this manner through many generations, remote cultures retaining virulence for cattle Later it was learned that the organism would grow in broth to which 10 per cent serum had been added, hence the collodion sacs were not necessary

Serum broth cultures in which this organism is growing become very faintly clouded This is so faint that it is advisable to incubate uninoculated tubes of the same media for purposes of comparison

In the presence of 2 per cent peptone and 10 per cent serum in broth, the organism produces acid from a number of carbohydrates, dextrose, fructose, mannose, maltose, and dextrin Sucrose and trehalose are only slightly attacked

Surface colonies may be obtained on agar containing 30 per cent serum or ascitic fluid Plates or tubes should be sealed to prevent drying In from 2 to 7 days' incubation at 37° C the characteristic colonies may be seen. These are from 10 to 600 microns in diameter and so transparent that they are very easily overlooked They may be seen with a hand lens under reflected light, or better under the 16mm objective of the compound microscope under oblique illumination.

No growth occurs in plain broth, litmus milk, blood agar, blood broth, and Loeffler's blood serum.

Pathogenicity. This organism is pathogenic only for cattle, normally, although several workers have reported that strains grown in the presence of horse or sheep serum acquire pathogenicity for sheep and goats

Artificial inoculation of naturally infected materials or cultures seldom reproduces the picture of the natural disease The pneumonia which is the principal lesion in natural cases does not often occur even when virus-containing material is introduced into the trachea Sabin (15) suggests that possibly the cattle lung worm may be concerned with the naturally transmitted disease, a suggestion which has not been investigated Daubney (3) succeeded in producing typical lung lesions by the device of incorporating infective material into small agar plugs which were injected into the jugular vein These lodged in the blood vessels of the lung, constituting infective emboli Exudates and cultures inoculated subcutaneously produce large inflammatory swellings and a toxemia from which the animals may die, but this does not resemble the natural disease, and the inoculation disease is not naturally transmissible

The Natural Disease. The natural disease spreads slowly and is difficult to eradicate Walker (18) who has had a large experience with this disease in Nairobi found that 58 per cent of the cattle in a large infected herd had not contracted the infection after a period of seven months.

The disease may be quite acute, resulting fatally within a week, or it may be very chronic. The disease has a way of becoming arrested by the walling off of diseased lung foci in which case the animal may appear to have recovered, but the sequestration is likely to break down at any time, perhaps weeks or months later, with an extension of the disease, the reappearance of symptoms, and the discharge of virulent material It is by the movement of such animals into new herds and the reopening of the lesions there that the disease is spread

The pleural cavity of acutely diseased animals contains a great deal of fluid—as much as 15 or 20 liters The surface of the lung is injected and covered with a thin deposit of fibrin The subpleural tissue is thickened and filled with fluid, and the same kind of fluid distends the interlobular septa. When the affected lobes are incised these fluids run out, coagulating after a few moments exposure to the air.

The pneumonia begins as nodules or foci which spread until entire lobes are involved These areas are hepatized and are bright red, brownish-red or grayish in color depending upon the stage of the process. The surface of the

cut section presents a marbled effect, the various colored lobules being separated from each other by wide bands of infiltrated interlobular tissue Necrosis occurs in the chronic cases, large portions of lung tissue often being necrotic and sequestered by connective tissue

Bacteriological Diagnosis. Agglutinins, precipitins, and complement-fixing bodies are formed by infected animals and may be used as means of diagnosis. Antibody formation develops rather slowly, however, and these tests have not proved very useful in detecting early cases They have been useful, however, in detecting chronically infected animals which may show few or no clinical symptoms

Immunity. Animals which have recovered from the disease cannot again be infected for a long period of time Methods of artificial immunization have been used for many years in the badly infected areas The earlier method consisted in the subcutaneous injection, usually in the tail, of pleural fluid This method gives protection from the lung disease but reactions often are severe and some animals even die from the inoculation Experience with the use of living culture as a vaccine have varied Purchase (12) found that cultures which had been maintained for many generations were harmless to cattle and resulted in effective immunity, but Kurotchkin (8) found such cultures to be ineffective in controlling the disease Large doses of immune serum will usually protect animals before infection has actually occurred, but the immunity is short-lived, and after infection has occurred it appears to be useless Various methods of chemotherapeutic treatment have been tried but none of these appear to have been successful In the past the disease has been eradicated from many countries by the method of slaughtering all infected herds, and this appears to be the only practicable method now available for dealing with it in areas where most herds are not already infected

Resistance. The organism of pleuropneumonia possesses very little resistance to drying, heat, and chemical action The virus is kept alive in herds, not because of its persistence on the premises, but because it is harbored and excreted for long periods by apparently recovered animals

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CAPROMYCES AGALACTIAE

Synonym The Organism of Contagious Agalactia of Sheep and Goats

The disease caused by the organism here considered is known to occur only in parts of southern Europe and in northern Africa Agalactia or mastitis in sheep and goats occasionally occurs in the United States, but this disease is related to other causative agents

The organism undoubtedly belongs to the pleuropneumonia group, but it is distinguishable from the bovine organism on serological grounds, and also on the basis of species pathogenicity (3)

The causative organism was first isolated and studied by Bridré and Donatien (1) in 1923 Its cultural features are not materially different from those of the pleuropneumonia organism of cattle. The organism can be found in the blood in the early stages of the disease, later in the joints, eye, and mammary secretions.

The name suggests that the disease is localized in the udder and is misleading in this respect. Actually it is a generalized disease which affects males and females alike. The principal lesions are located in the joints, the eyes, and in the mammary glands of females. The disease may be acute but usually is chronic. The involved joints may become ankylosed but usually do not The mastitis is manifested by the usual symptoms, and milk secretion diminishes

and even ceases. Pregnant females often abort Chronically affected animals become weak and emaciated.

Attempts to immunize goats in various ways have proved rather disappointing Hyperimmune serum gives transient protection only. Much more encouraging in this disease has been the use of a chemotherapeutic agent, the sodium salt of stovarsol (acetylamino-hydroxy-phenylarsonic acid) Bridré, Donatien, and Hilbert (2) report that this substance has a specific curative effect upon animals affected with this disease

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PART IV

THE PATHOGENIC FUNGI

CHAPTER XXXI

THE DERMATOPHYTIC FUNGI

The simplest, least differentiated of known plants belong to the phylum *Thallophyta* These plants have no leaves, stems, seeds, and roots but consist wholly of single cells or chains of such cells which form filaments The mass of such filaments which constitute a single individual plant or colony is known as a *thallus* It is from this word that the group derives its name

Some of the thallophytes contain chlorophyll and thus are capable of photosynthesis These are known as *algae* Others do not have chlorophyll, hence they are not green and are not capable of photosynthesis. These are called *fungi* The fungi, being unable to utilize the energy of sunlight, live a saprophytic or parasitic existence, and generally thrive best in darkness

The fungi are divided into three groups, the *Schizomycetes,* or bacteria, the *Eumycetes,* or true molds, and the *Myxomycetes,* or slime molds The last group contains no species of medical importance This chapter will be devoted to a consideration of the organisms belonging to the *Eumycetes.*

THE EUMYCETES

Some of the molds are unicellular, others are multicellular. Some are dimorphic, that is, they may grow as unicellular individuals under some circumstances and as multicellular under others This occurs among some parasitic fungi, the unicellular stage occurring in tissues and the multicellular in cultures Most pathogenic species are multicellular The yeasts, however, are always unicellular

The multicellular fungi or molds are made up of cells which, placed end to end, form filaments known as *hyphae* The tangled mass of hyphae forming a single colony is known as the *mycelium*

In some molds the mycelial filaments are not divided by septa or crosswalls but consist of single, multinucleated cells in which the protoplasm streams back and forth as it does in some of the algae These are said to have a *coenocytic mycelium,* and such species are classified in a group known as the *Phycomycetes.* The greater number of species, however, have *septate mycelums,* and such species belong to the *Basidiomycetes,* the *Ascomycetes,* or the *Fungi Imperfecti.* The *Basidiomycetes* consist largely of the mushrooms, bracket fungi, and other fleshy forms which have no importance in animal

infections, hence they will be considered no further here. The *Ascomycetes* are characterized by the possession of membranous sacs called *asci* (sing. ascus) which contain spores, generally eight in number, called *ascospores.* Similar to the *Ascomycetes* are the large number of forms which are now relegated to the *Fungi Imperfecti* Probably the greater part of these forms actually belong to the *Ascomycetes* but since the sexual or ascospores have not been observed, they are not placed there. The imperfection indicated by the name of the group is probably, in most instances, imperfection in our knowledge rather than imperfection in the fungi themselves The group is diminished, from time to time, by the discovery of the sexual phases which permits the transfer of such species to one of the groups previously mentioned

In all molds except the *Fungi Imperfecti* both sexual and asexual spores are recognized In most instances the asexual spores are much more readily formed, are more numerous, and are more conspicuous than the sexual types *Oospores* are sexual spores produced by the fusion of two unlike cells one of which is regarded as a male element and the other a female *Zygospores* are sexual spores, occurring among the *Phycomycetes* only, produced by the fusion of two similar sexual cells *Ascospores* are regarded as sexual spores since the parent cell from which the ascus is formed possesses two nuclei which fuse

The simplest asexual spore is the *arthrospore,* which is merely a portion of hypha which breaks off and which is capable of reproducing the species In many species of fungi, spore-bearing hyphae develop and these form spores by a process known as abjointing, i e, by pinching and breaking off special elements In some forms special cells appear, generally at the end of the hyphae, which enlarge and become ballooned into a sac-like structure in which the spores are formed This is known as a *sporangium* In other forms the spores are formed exogenously at the tip of special hyphae These are known as *conidia* and the specialized structure which bears them is called a *conidiophore Chlamydospore* is a general name used for thick-walled structures of many kinds which are formed in any part of the thallus and which apparently are resting forms

The diseases caused by fungi fall into two categories:

a The *dermatomycoses,* the skin mycoses of the "ringworm" group These diseases are caused by many different species of fungi, most of which are classified among the *Fungi Imperfecti* These organisms cause superficial infections of the skin which are annoying rather than fatal, as a rule.

b. The mycoses of internal organs, consisting of destructive lesions of the mucous membranes, of tumor-like, mycotic granulomas and abscesses of the

internal organs Some of these begin as skin infections. These infections generally are chronic in their course and frequently have a high fatality rate

The Dermatomycoses of Animals

These infections are common in both man and animals They are generally known as "ringworm" from the fact that typically they begin in small areas and spread centrifugally, the region of greatest inflammation at the periphery forming an ever widening circle If the cases are not treated, new areas usually become infected and finally large areas of the skin may become involved through the coalescence of the primary lesions Ringworm is also known under the name *tinea* A certain kind of ringworm is known as *favus*

The fungi of the ringworm group grow almost wholly on the keratinized layers of the skin, including the hair and other horny structures They show a preference, in most cases, for the hairy portions of the body, however some species occur on the glabrous, or hairless, areas The infections usually involve the hair follicles and the hairs themselves The latter become brittle and often break off near their bases, or sometimes they split Commonly they appear dry and lustreless. The skin of the affected areas becomes scaly and harsh and crusts are formed Bacterial infection often complicates the picture, pustules being formed in the hair follicles This condition is known as *sycosis* The appearance of the infections differ considerably depending in part upon the nature of the infecting species.

Generally speaking, ringworm thrives best in young animals and in older ones which have been devitalized by disease or malnutrition It is seen more often in stabled animals than in those on pasture, and more often in winter than in summer. Ringworm infection which has become widespread in groups of calves often will clear up spontaneously in the spring several weeks after they have been turned out into the sunshine This may be a result of better nutrition or it may be due to the direct influence of the light Ultraviolet light has proved to be useful in treating many forms of ringworm The infections occur most frequently on the face, neck, and around the tailhead, but they may be found on any part of the body Many types of ringworm are highly contagious, not only to other members of the same species but often also for members of other species Usually when the infection occurs in an abnormal species the disease does not spread in the new one but dies out in the initial host. Ringworm of cats, horses, and cattle readily infects people, and most of the other animal types have been known to infect man These infections are very annoying and sometimes very resistant to treatment.

CLASSIFICATION AND IDENTIFICATION OF THE FUNGI OF THE RINGWORM GROUP

To the ordinary bacteriologist the classification and identification of genera and species in this group is a baffling problem. Even the mycologists find it a difficult group and disagree on the classification. The botanic classifications of the mycologist do not meet the needs of the dermatologist who seeks a relationship between the classification and the nature of the lesions produced. Sabouraud, a French mycologist, proposed a scheme which has been widely adopted by dermatologists and it is proposed to use his nomenclature and his classification in this work, in so far as it applies to the lower animals. In this plan three genera are recognized, *Microsporum, Trichophyton* and *Achorion*

The group to which the causative agent of any particular case of ringworm belongs often can be determined by a careful inspection of the lesions When members of the genus *Microsporum* are involved the diseased skin is very scaly and these scales tend to pile up to form crusts The hairs may break off at some distance from the surface of the skin, but often they remain intact until the disease is well advanced, so much so that, in cats especially, the lesions can be felt rather than seen beneath the fur coat This disease is very highly contagious to animals of the same species and to others Microscopic examination of hairs plucked from follicles in the involved area often show masses of polyhedral spores clustered around their bases These are not arranged in chains, but are clustered in irregular formation

Members of the genus *Trichophyton* are divided into an *endothrix* variety, in which the hyphae and spores are found within the hair shaft, an *ectothrix* variety in which these elements are on the outside of the hair, and a third group, called the *neo-endothrix* variety in which most of the elements are inside the hair but a few are found outside So far as is known, only the ectothrix varieties are of importance in animal pathology The spores of the trichophyta are arranged in chains like giant streptococci In the animal infections they can generally be demonstrated easily by placing a few plucked hairs on a slide, wetting them with 10 per cent sodium hydroxide in water, covering with a cover glass, and heating gently before examination under a low power of the microscope.

Members of the genus *Achorion* are the causative agents of *favus,* a type of ringworm characterized by the production of structures called *scutula* which have the general appearance of shields (Latin *scutum* = shield) These are formed as a consequence of the habit of these parasites of radiating out from the hair follicles between the Malpighian and the outer cornified layers of the skin, a process which separates the latter from their attachments. The separated layers are thoroughly invaded by the fungus hyphae which form a

felted mass within them The hairs remain more or less intact, projecting out through the shields. The mycelium can be demonstrated inside the shafts of the plucked hairs, as well as in the substance of the scutula. It tends to break up into fragments of varying length, many of which are nearly spherical and thus resemble spores

Specific identification of these dermatophytes can be accomplished only by cultural means Most of them are rather easily cultivated on artificial media, the principal problem being to separate them from miscellaneous saprophytic fungi which usually are abundant on animal skins Even when isolated in pure culture, their specific identification is a task for the expert It is based upon the gross characteristics of the colonies developing on solid media, to a limited extent upon physiologic characters, and upon microscopic characteristics of the developing hyphae and spores, which often are quite different from those observed in preparations made directly from the skin The morphology of cultures often varies widely when they are grown for a time on the same kind of medium, and different types of media usually profoundly influence their morphology The study of such cultures is a very specialized one which ordinarily will not be fruitful except to one who has had a good basic training in mycology The scope of this work will not permit further discussion of the subject Those who desire more information are referred to special works such as those to which references are given at the end of this chapter

Before special types of ringworm fungi are discussed, it is of interest to note the fact that many types of dermatophytes have the property of fluorescing in ultra-violet light Not all of them fluoresce and there are other fungi which do, hence the property must be used cautiously as a diagnostic procedure, however, it often proves useful in this connection The patient is examined in a darkroom with a lamp which emits ultra-violet rays from which most of the visible rays have been removed by a Wood's filter A yellowish green fluorescence is characteristic of fungi Plucked hairs and epidermal scales from patients may be examined in the same way, in which case fluorescence of the epidermal material around the root hairs is significant. In making such examinations care must be taken to exclude the possibility of confusing with fungi other fluorescing substances, especially medicaments which may have been used in treatment Mineral oils and petrolatum, for example, emit a strong bluish-green fluorescence in ultra-violet light.

Among domestic animals in the United States, ringworm is seen most commonly in cattle, especially in calves The fungus in this case belongs to the genus *Trichophyton*, or *Ectotrichophyton*. Occasionally serious outbreaks occur in horses, these cases generally being caused by members of the *Tricho-*

phyton group but sometimes by members of the *Microspora* and occasionally by members of the favus group (*Achorion*). Ringworm of dogs and cats is common in many localities Most of these are caused by members of the genus *Microsporum*, but some cases are favus which probably are contracted from rodents, especially rats and mice Chickens and other birds sometimes suffer from favus which affects their combs, principally. Ringworm of sheep and of swine has been reported but these infections do not appear to have much economic consequence Many cases of ringworm have been described in which the nature of the causative agent is in doubt Obviously the ringworm infections of animals are in need of much more study.

MICROSPORUM CANIS

Synonyms This species is apparently identical with *Microsporum lanosum* (of man) and *Microsporum felineum* (of cats).

Ringworm infection in which this species is the etiological agent is quite common. The disease spreads readily in kennels and catteries and among household pets which associate with stray animals and pets of other owners in the neighborhood.



Fig 80 Ringworm Infection in a Dog (Courtesy of the Lederle Laboratories, Inc)

The disease appears as small scabby areas on any part of the body but is seen most frequently on the ears, face, neck and tail These areas do not appear to cause much irritation, nor do they have any appreciable effect upon the general health of the animal as a rule The hair is not shed and, in cats especially, if the disease affects the long haired breeds and occurs on parts which are especially well covered with fur, the lesions may be overlooked until the disease has spread over a considerable part of the body. Such cases are stubborn to treat, and such animals readily infect other cats, dogs, and often humans with which they come in contact. Guinea pigs may be readily infected experimentally. In man the disease may appear on the scalp, or circinate lesions may appear on the relatively hairless parts of the body There are records of the disease being transmitted from one cat to another through the agency of an intermediate human being.

MICROSPORUM EQUINUM

This species has been described from the horse, but apparently this type of ringworm is not so common as the *Trichophyton* type which will be described later. The lesions are relatively benign, the disease does not easily transmit to man, and the guinea pig is said to be resistant to experimental infection. These facts indicate that the species is not identical with those which ordinarily infect small animals Two other species, *M rubrum* and *M marginatum* have been described from horses in France and Algeria, respectively It is not clear whether these are really different species, but in any case they do not appear to be important

TRICHOPHYTON MENTAGROPHYTES

Synonyms *Ectotrichophyton mentagrophytes, Trichophyton gypseum; Trichophyton asteroides.*

Ringworm of cattle is a very common disease, especially in young animals kept indoors during winter months The causative agent is easily demonstrated, but the lesions are so characteristic that demonstration of the fungus is unnecessary for diagnosis Hairs plucked from the margins of the lesions, examined in a strongly alkaline solution which has a clearing action on the keratinized epithelium, show masses of spherical spores around their bases These spores are arranged in chains Filamentous hyphae may also be seen around the hairs but these are not so easily recognized as the refractile spores

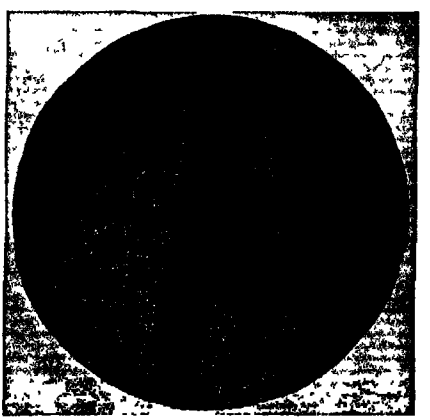


FIG 81 *Trichophyton mentagrophytes* Scrapings from a ringworm lesion on the skin of a calf, unstained but cleared with caustic potash The dark band running vertically is a pigment containing hair in its follicle Around the shaft of the hair large numbers of fungus spores can be seen Mycelium can be seen in some instances but not in this photograph x 150

It has been observed that normal calves, placed in quarters where ringworm-infected animals have previously been kept, often will promptly develop the disease. This happens at times when infected animals have been absent from the premises for months. This suggests that the fungus survives on the premises for long periods, perhaps

even from one winter to the next. Muende and Webb, in England, succeeded in finding colonies of the calf ringworm fungus growing on semi-dried fecal material in such a stable. The fungus produced colonies large enough to be macroscopically visible.

The lesions are usually found on the face, particularly around the eyes. They also occur on the neck, and occasionally on other parts of the body. Well developed lesions consist of raised, dry, crusty, grayish-white masses from which a few broken hairs protrude If the crusty material is pulled loose, bleeding occurs The disease spreads rapidly among calves kept in a common room, especially if the quarters are dark and damp Human infections occasionally occur.

TRICHOPHYTON GRANULOSUM

Synonyms *Ectotrichophyton granulosum, Favotrichophyton caballinum; Megatrichophyton equinum, Trichophyton equinum* *

Ringworm of horses occasionally causes a great deal of trouble in large stables and in military units The disease spreads readily, principally through the use of common grooming tools, harnesses, and blankets Outbreaks can be controlled only by treatment of affected animals and by thorough disinfection of all stable equipment.

The disease is seen on parts of the body where harness or blanket straps rub the skin The face, breast, croup, flanks, and the back where the saddle and saddle girth rub, are the areas most often involved The hair on these areas breaks off and much of it comes out leaving semi-bald patches The skin becomes progressively thickened and overlaid with flaky crusts The underlying skin is dry and has a dull lustre Infections often complicate the picture, making the areas moist and reddened The disease is transmissible to man, although apparently its contagiousness is not so great as that of the type caused by *Microsporum* These infections apparently cause little inconvenience to the animal The principal damage is the disfigurement, temporarily, of the animal's coat If untreated the disease may spread over large areas

ACHORION GYPSEUM

This is the causative agent of one type of favus which apparently occurs most commonly on the horse, but has been found also on cats, rats, mice, and man Typical scutula are produced Little information is available about the nature of the disease. It is said to occur occasionally in many European coun-

* All of these have been described as etiological agents of ringworm infections in horses It is probable that they represent more than one species and possibly more than one genus They are listed here as synonyms for convenience and because they have not been adequately differentiated

tries. Cases in the United States and in South America have been attributed to it.

ACHORION CANINUM

Showing cultural features differing from the preceding species, this one has been found in dogs and is inoculable to mice and man. Little is known about it.

ACHORION QUINCKEANUM

This species occurs most commonly in mice in which typical favus results The species readily transmits from mice to cats in which lesions most commonly occur around the paws and on the ears

ACHORION GALLINAE

Favus of fowls, particularly of chickens and turkeys is a disease of considerable importance It appears as small white patches on the comb (usually of male birds) These enlarge and coalesce so that finally the comb may be covered with a dull, white, moldy layer several millimeters thick The disease usually is self-limiting, healing after several months if untreated. Scutula are not found on the comb lesions but occasionally the disease extends into the feathered parts in which case typical shields are formed So long as the disease is limited to the comb, there is little effect upon the health of the birds When the feathered portions are involved, however, the bird becomes emaciated and may die.



FIG 82 Lesions of Favus in a Chicken This condition is caused by *Achorion gallinae*

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CHAPTER XXXII

THE FUNGI CAUSING MYCOSES OF THE INTERNAL ORGANS OF ANIMALS

The dermatophytic fungi are definitely parasitic in habit, existing nowhere in nature, so far as is known, except on the skin of infected animals, on hairs and epidermal scales from such lesions, and to a limited extent upon materials in intimate contact with such epithelial debris On the other hand, the fungi which cause the deep-seated infections generally, if not always, live saprophytically except when chance places them in positions where a parasitic habit is forced upon them The superficial mycoses are definitely contagious, the deep-seated are not The agents of the deep-seated infections apparently live in the soil and on vegetation Some of these diseases occur only rarely and in widely separated localities They have no definite areas of localization The causative agents evidently are widespread, the cases of disease depending upon chance infection rather than the distribution of the causative agent Other fungi of this group are numerous in certain localities and absent in others, hence the diseases are limited to definite areas

The fungi which cause the superficial mycoses are usually associated with disease in young animals Those which cause the deep-seated infections are found most often in mature and aged animals Excepting aspergillosis, all of these infections are chronic ones Henrici (1) believes that animals are highly resistant to infection with such organisms as those concerned in the internal mycoses, and that active, progressive disease does not occur until the tissues have become hypersensitized (allergic) through repeated contact He thinks that repeated minor infections must occur before the conditions become right for the progressive disease to begin This opinion is based upon considerable experimental evidence but it cannot be said to have been definitely proven. If this theory is substantiated, an adequate explanation for the relative absence of the diseases in young animals is provided.

The lesions of deep-seated fungus infections usually take the form of tumor-like masses commonly called infectious granulomas These granulomas have necrotic foci in their centers surrounded by heavy walls of fibrotic tissue. Around the margins of the necrotic foci, epithelioid and giant cells may usually be found The structure, therefore, is similar to that of a tubercle.

In the necrotic tissue, instead of tubercle bacilli, one generally can easily demonstrate the hyphae of the fungus. In some cases spores can be found, in others yeast-like cells, depending upon the nature of the fungus present Frequently stellate bodies made up of fine hyphae radiating from a central mass, may be seen. The tips of the radiating filaments often are surrounded by hyaline, acidophilic material, the whole having a distinct resemblance to the colonies of the "ray-fungus" of actinomycosis Henrici believes that these actinomycetoid bodies are produced by the fungus as a result of specific stimulation by the allergic host tissues That allergic sensitization does occur in fungus infections can easily be demonstrated by making skin tests with extracts of the causative agent

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Mucor Infections in Animals

Members of the family *Mucoraceae* belong to the *Phycomycetes* Some members are very common in nature, particularly the common bread mold, *Rhizopus nigricans* A bit of bread, moistened and kept in a Petri dish usually will furnish a luxuriant growth of this organism within a few days It is recognized as a maze of loosely felted white mycelium which completely fills the dish If the lid of the dish is lifted it will be noted that the hyphae are attached and the disturbance of these attachments results in collapse of the entire structure Close examination of well developed cultures reveals black spherical bodies which are masses of spores encased in sporangiophores

R. nigricans is not pathogenic but species quite similar in morphology are encountered, rather rarely, in animal infections.

RHIZOPUS EQUINUS

Dodge (4) includes in this species the fungus which Christiansen and Nielson (3) found in swine and which was regarded by them as a new species, *R suinus*, also the mucor found by Theobald Smith (6) in bovine fetal membranes Bendixen and Plum (1) who studied similar, if not identical, infections in Denmark identified the mucors which they encountered as *Absidia ramosa*. Whether Smith's organism, and the one with which Gilman and Birch (5) worked, belong to *Rhizopus equinus* or to *Absidia ramosa*, it is not possible to say They will be described under the latter name

R. equinus, as its name implies, was found in a horse where it was regarded as the causative agent of a tumor in the region of the withers An-

other case, possibly due to the same species, consisted of a soft, fat-like tissue in the maxillary sinus of a horse.

RHIZOPUS SUINUS

In 1922 Christiansen (2) described two cases of generalized mucormycosis in swine One of the cases was attributed to *R equinus*, the other to *A ramosa* In 1929, Christiansen and Nielson (3) described a larger series consisting of nine cases, including the two which the senior author had described earlier The seven additional cases were attributed to *A ramosa*, therefore the larger series included only the one case caused by *Rhizopus* which had been described originally In this paper they conclude, however, that the species is not *R equinus* and they propose a new name, *R suinus*.

The affected pig was emaciated A large vascular tumor was found in the abdominal cavity This was made up of a conglomerate of small nodules, each surrounded by a connective tissue capsule The interior of the nodules consisted of dry firm tissue in the center of which there was necrosis Around the periphery there were hemorrhages In each lung there were about 20 nodules similar in structure to those making up the abdominal tumor. A few were also found in the liver Mycelium was easily demonstrated in the caseous tissue and the mold was readily cultivated on maltose medium.

ABSIDIA RAMOSA

The genus *Absidia* differs from *Rhizopus* only in minor cultural details. This species was originally found in an infection of a human ear, later in head infections of horses It proved to be the causative agent in eight of nine cases of generalized mucormycosis of swine described by Christiansen and Nielsen (3) It was found by Bendixen and Plum (1) in nine out of eighteen cases of bovine mycotic abortions, being in pure culture in two cases and mixed with *Aspergillus fumigatus* in seven additional cases The mucor described by Smith, and by Gilman and Birch may have belonged to this species.

The swine infections, as described by Christiansen and Nielsen (3), were manifested by the formation of nodules in the wall of the small intestine and in the mesenteric lymph nodes, with occasional nodes elsewhere The intestinal lesions frequently ulcerated into the lumen of the bowel forming large ulcers The histological make-up is similar to that described under the preceding heading Hyphae were easily demonstrated microscopically, and the mold was readily cultivated

In the fungus-infected placentas of cattle, the hyphae are readily found in fresh films. The cotyledons are usually necrotic and dry. Sometimes the entire chorion is necrotic, dry, thickened, and leather-like.

Gilman and Birch (5), and Bendixen and Plum (1) were able to cause placental infections by inoculating pregnant cattle intravenously. Christiansen was able to kill rabbits, guinea pigs, rats, and mice by intravenous and intraperitoneal inoculations The principal lesions are found in the kidneys, spleen, and liver

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ASPERGILLUS FUMIGATUS

The *Aspergilli* belong to the *Ascomycetes* Most species are saprophytic and colonies are frequent on plate cultures as a result of aerial contamination The most characteristic feature of the *Aspergilli* by which they may be readily recognized is the structure of the expansions of the tips of certain of the aerial hyphae which bear the spores or conidia These expansions bear small papillae on which the spores are borne externally. Colonies are woolly and dense and quite unlike the loose mycelium of the *Mucors* Most of the *Aspergilli* have pigmented spores and these give color to the entire colony. *Aspergillus fumigatus* has dark green spores, hence colonies have a dusty, dark green color.



FIG. 83 *Aspergillus fumigatus* Photograph of the aerial hyphae and the fruiting bodies in an Henrici slide preparation x 60

A fumigatus is fatal to rabbits and other experimental animals when spores are inoculated intravenously. If the dosage is rather

large the rabbit will die within a few hours, the principal lesions being multiple hemorrhages. If the dose is smaller the animal will live longer, and multiple granulomatous lesions will develop, principally in the lungs but also in other organs.

This organism was isolated by Bendixen and Plum (1) from the placentae of fifteen cows which had aborted The lesions are identical with those described under *Absidia ramosa,* in fact in seven of the cases both types of molds were isolated. According to these authors, it was generally possible to distinguish the type present in the fetal tissues by determining the cross walls of the *Aspergillus* Inoculation of pregnant cows intravenously proved that the organism could localize in the fetal membranes and set up a train of changes which would result in abortion

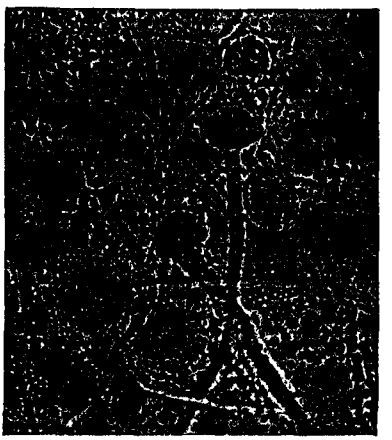


FIG. 84 *Aspergillus fumigatus* Unstained preparation showing mycelium, fruiting bodies, and free spores The straight, stiff stalks are aerial hyphae The bulbous expansions at their free tips contain finger-like processes (sterigmata) which bear long chains of highly refractile spores of a yellowish-green color The photograph was made from a bit of culture removed from a solid medium and immersed in a clearing solution The majority of the spores have broken loose from their attachments x 400

For birds this organism is very dangerous, producing a disease which is known as aspergillosis This disease sometimes occurs in epizootic form in which case large losses may be sustained Unlike most of the other fungi which produce deep-seated diseases, this one produces disease in young birds more often than in the older.

The disease is limited to the upper air passages and sometimes the mouth, the lungs, and the air sacs In these locations the mold has access to air and it vegetates readily Tubercle-like bodies containing giant cells and lymphocytes usually form and these quickly go on to caseation The lungs therefore may show caseous areas, and the air sac walls may be thickened Sometimes the air sacs are lined with greenish areas because of the presence of large numbers of spores of the organism In other cases the air sacs are not uniformly thickened, but many small, whitish bodies of dense composition are present. The mold hyphae in many of these lesions may be recognized by crushing them with a little caustic potash. In the green areas

the spores are readily found In some of the denser lesions it is difficult or impossible to find evidence of the mold except by cultural means

Culture of the causative organism is very easily accomplished on plain agar, or better upon maltose or wort agar The organism grows best at about 30° C although it will grow at body temperature Fine woolly colonies appear. After a day or so these enlarge and greenish yellow specks appear in the aerial hyphae These are the spores Later the entire surface is covered with a thick matted mycelial growth, yellowish-green in color, and dusty

A. fumigatus apparently is introduced into flocks principally in moldy grain feeds and in moldy litter The species seems to be widely scattered in nature and can readily multiply in feeds that become wet, or are stored in a damp room. The inhalation of spores from such sources seems to be the manner in which outbreaks are produced The disease has been reported in chickens, pigeons, turkeys, ducks, geese, canaries, and many kinds of wild birds. So far as is known all birds are susceptible.

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COCCIDIOIDES IMMITIS

This *Ascomycete* was originally thought to be a protozoon The form which occurs in the lesions resembles an oocyst of a coccidium, and it is from this resemblance that the generic name was derived.

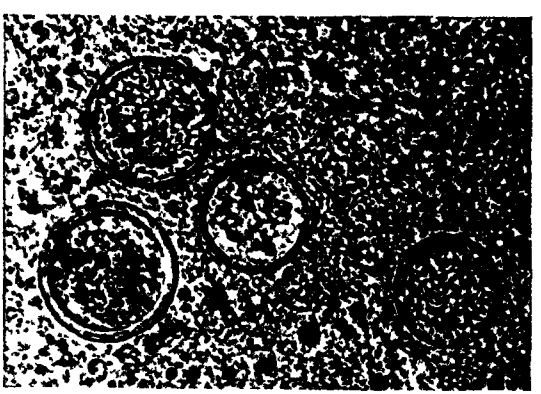


FIG. 85. *Coccidioides immitis* Several of the spherules contained in pus expressed from a lesion in a lymph node x 600 (Courtesy Stiles and Davis, *Jour Amer Med Assoc*)

It is the causative agent of a human disease of considerable importance, especially in the valleys of central and southern California where the disease is endemic. The disease is not contagious, since neither human nor animal cases have ever been traced to infection from previous cases It is generally thought that the disease is contracted from the inhalation of chlamydospores for it has been observed that the disease is prevalent during the dry dusty season and is rare during the wet season It has been supposed that the organism lives saprophytically in the soil, but Emmons (4) recently has questioned this belief and has shown that rodents in the infected districts carry the infection Inasmuch as the organism has been isolated from soil by several workers, there is no question about its existence there, but perhaps it reaches the soil through animal excreta Most of the early cases of human infection originated in the valley of the San Joaquin river and the disease became well-known as the San Joaquin Valley disease Until quite recently only the chronic cases, characterized by the formation of granulomas in the internal organs and especially in the lungs, and by a mortality rate in excess of 50 per cent, were recognized In 1938, Dickson (3) showed that the disease occurred in another much more prevalent form This is the "valley fever" or "desert fever," an influenza-like disease which had long been known in the valleys of central California without its real nature being suspected It is now known that large numbers of residents and transient workers in these valleys become infected with this disease Most cases recover in from three to six weeks Only a few lapse into the chronic form characterized by granulomatous lesions.



FIG 86 *Coccidioides immitis* Hanging drop preparation from a culture showing mycelium of the organism x 200 (Courtesy Stiles and Davis, *Jour Amer Med Assoc*)

In 1918 Giltner (6) identified this organism in an infection of a cow which had lived in the San Joaquin Valley More recently the disease has been recognized in many cattle (1) (2) (9), in one sheep (1), and in several dogs (5) (8). Most of the cases have occurred in the original area, however isolated

cases have been recognized in Arizona, Texas, and Colorado, indicating that the fungus is more wide-spread than was originally believed.

Morphology and Staining Reactions. As it occurs in the purulent material and the granulation tissue of lesions, the fungus appears as spherical bodies which vary greatly in size from about 5 to more than 50 microns in diameter The wall is double contoured and highly refractile The protoplasm is finely granular In many of the larger spheres a number of ascospores may be seen as spherical bodies varying from 3 to 5 microns in diameter Mycelium is never seen in tissues.



Fig 87 *Coccidioides immitis* A single colony of the organism growing on a solid medium Note the cotton like appearance x 2 (Courtesy Stiles and Davis, *Jour Amer Med Assoc*)

When tissues are planted on suitable culture media, protoplasmic shoots appear from the spheres These develop into hyphae and soon a well-developed mycelium is formed The hyphae branch extensively and exhibit well-marked septa In time aerial hyphae appear and a white woolly colony is formed Microscopically, numerous chlamydospores may be seen and some arthrospores The spherical structures found in tissues are never present in cultures unless they are incubated under special conditions semi-anaerobically (7).

All forms of this parasite can be stained but for most purposes fresh material unstained is preferable for study

Cultural Features. *Coccidioides immitis* will grow on all of the common media of the bacteriological laboratory On most of the solid media, the growth is similar in appearance When cultures are incubated at 20° C growth does not appear for three or four days, but at 37° C it is usually evident within 24 hours The colonies are circular in outline, of a silvery gray color, and slightly raised The mycelium penetrates deeply into the medium so the colonies cannot be removed except by digging out the medium. After a few days the cultures develop a whitish, moldy appearance because of the development of short aerial hyphae In some tubes these are abundant and from 2 to 3 mm long, in others they may be scarce and short. In old cultures the medium develops a brownish discoloration but the growth remains white. Gelatin and coagulated bovine serum are slowly liquefied. Milk is gradually digested Broth cultures develop as fluffy masses in the bottoms of the tubes, and some tubes show rather tough pellicles. Sugars are not fermented.

Pathogenicity

FOR CATTLE. Coccidioidomycosis in cattle (1) (2) (6) (9) is a benign disease which ordinarily involves only the lymph nodes of the chest—the posterior mediastinal and the bronchial. In a few cases small granulomatous lesions have been found in the lungs Symptoms are not elicited The affected glands are enlarged and contain a yellowish, glutinous pus, similar to that of actinomycosis The sulphur granules are not present, of course, and microscopic

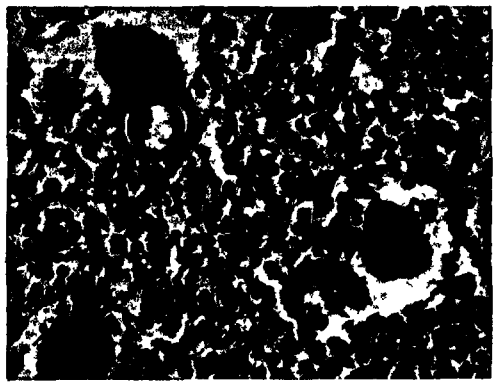


FIG 88 *Coccidioidal granuloma* Section of a bovine lymph node showing granulation tissue and several giant cells, one of which contains a spherule of the causative agent x 300 (Courtesy Stiles and Davis, *Jour Amer Med Assoc*)

examination readily reveals the spherical coccidioides The abscess wall consists of granulation tissue Calcification is not present

With but few exceptions all cases have originated in the inland valleys of California where the human disease occurs The diseases in the human and bovine species have no direct relationship to each other since the infection is not transmitted from man to animals, and vice-versa. Both species contract the disease from the same source, that is, from dust infected with chlamydospores. Several cases have been found in cattle which were raised in Colorado and in Arizona It is evident that the infection is not restricted to California.

FOR SHEEP One case of coccidioidomycosis in sheep (1) has been reported. The lesions were like those in cattle

FOR DOGS. Two cases have been described (5) (8) Multiple lesions were found in the lungs and there were lesions also in the liver, spleen and kidneys. The picture grossly resembled tuberculosis. The affected animals were in poor physical condition when destroyed.

Artificial Inoculation. Intravenous inoculation of cultures into guinea pigs, rabbits, sheep, calves, and dogs results, ordinarily, in the rapid development of multiple lung lesions and sometimes multiple lesions in other organs The animals emaciate rapidly and soon die Swine resist the infection much better than other animals Lung lesions develop in this species but the general health of the animals does not seem to be seriously impaired by them.

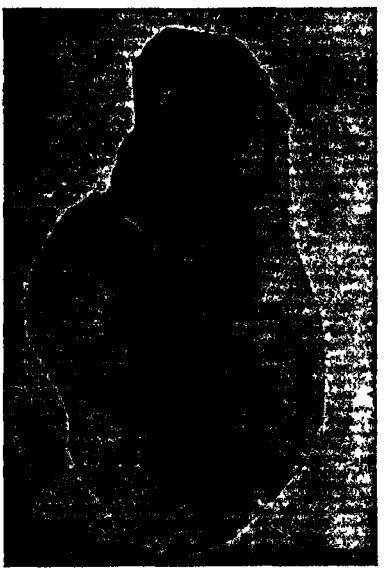


FIG 89 *Coccidioidal granuloma* Lesions in a bovine lymph node which strikingly resemble those of tuberculosis The lesions vary in size They are caseous in their centers Note the hemorrhages and the encapsulation About x 2 (Courtesy Stiles and Davis, *Jour Amer Med Assoc*)

Subcutaneous inoculations result in the formation of local abscesses which finally rupture and form ulcers. The larger animals usually show no extension of the disease, however in guinea pigs the disease usually generalizes giving a picture not unlike that of tuberculosis

Immunity. No biological products for the prevention or cure of this disease are known A product, *coccidioidin*, made from filtrates of old broth cultures, has been successfully used for diagnostic purposes This material is quite analogous, in composition and use, to tuberculin.

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CRYPTOCOCCUS FARCIMINOSUS

Synonyms: *Zymonema farciminosum, Saccharomyces farciminosus, Endomyce farciminosa, Histoplasma farciminosa, Saccharomyces equi; Blastomyces farciminosa*

The name *Cryptococcus* is used for those members of the *Fungi Imperfecti* which appear only as round or oval cells that reproduce by budding. They are closely related to the yeasts except that ascospores are not formed. Mycelium is not found either in tissues or cultures. Colonies on solid media are soft and pasty, like those of yeasts. There are several species which are pathogenic for man, but only one which affects animals.

Cryptococcus farciminosus is the causative agent of epizootic lymphangitis or pseudofarcy of horses and mules. A few cases have been reported in cattle but cattle evidently are not highly susceptible. The disease has long been prevalent in parts of Italy and France, in Russia, Japan, the Philippine Islands, and South Africa. The disease caused a great deal of trouble during the Boer War and cases were brought back to England after its conclusion. It also was of much concern during World War I. Some doubtful cases have been reported in the United States. Most of these, possibly all, were cases of sporotrichosis.

The organism was first demonstrated in the pus, by Rivolta, in 1873. It was not successfully cultivated until 1896. The first pure cultures were obtained by Tokishiga (2), in Japan.

Morphology and Staining Reactions. In pus, the organism appears as a double contoured oval or ovoid body, measuring 2.5 to 3.5 by 3 to 4 microns. The cells resemble those of yeasts very closely. The cytoplasm is granular, and here and there bits of cytoplasm may be seen extruding from a break in the cell wall, forming buds from which daughter cells are formed. In cultures the cells resemble those seen in pus except that they have a tendency to form short chains.

The fungus cells can be stained though not very satisfactorily. Structural details are best seen in fresh, unstained preparations. The Gram stain usually is retained.

Cultural Features. *C farciminosus* is strongly aerobic. It has been successfully cultivated on a variety of media but growth is slow and uncertain. When incubated under the most favorable of cultural conditions now known, growth usually is not evident for one to three weeks or longer, and of many tubes inoculated similarly a considerable part may fail to exhibit growth. Growth may be obtained on plain agar and broth, on potato, coagulated egg medium, coagulated serum medium, and various other special media. On the

solid media growth appears in the form of small grayish-white granules which have a dry appearance and may become leather-like in appearance. In liquid media, growth generally occurs in the form of a scanty, granular sediment. Sugar media are not fermented

Pathogenicity. *Epizootic lymphangitis of horses is characterized by inflammation of the superficial lymphatic vessels and nodes, principally of the legs, the chest, and the neck Infection is believed to occur through wounds* In severe cases lesions may be found on any part of the body and even on the mucous membranes

The lymph channels become enlarged and appear as tortuous cords beneath the skin, connecting the swollen lymph nodes The nodes soften and rupture forming crater-like ulcers from which a thick pus exudes The yeast-like organism can easily be demonstrated in such pus When mucosal lesions occur they are most likely to be found in the nasal passages, but there are records of their occurrence on the genitalia and of the transmission of the disease from stallions to mares by copulation

Bennett (1) a few years ago described a type of equine pneumonia which was believed to be caused by *C farciminosus* It was of an interstitial type beginning with infiltrations of lymphocytes and then monocytes. Syncytia and giant cells next appeared and in these the cryptococci could be seen The organisms then multiplied profusely leading to extensive destructive changes, and fatality The condition was not associated with skin manifestations The organism was not cultivated, and therefore was not certainly identified

The disease is very chronic as a rule, though some cases heal spontaneously after a few weeks The chronic cases usually are incurable There are no biological agents of value for the diagnosis or control of this disease.

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MONILIASIS

The genus *Monilia* is used by medical mycologists to designate simple, yeast-like fungi which reproduce by budding, form septate mycelia, and are not known to produce ascospores The spores are formed by successive budding of specialized cells at the end of hyphae, or at nodes in the filaments. Both yeast-like cells and mycelial elements occur in tissues. Colonies on solid media do not form aerial hyphae and therefore they do not become fuzzy but are fleshy, like colonies of true yeasts

Members of this group cause a variety of lesions of the mucous membranes,

of the skin, and sometimes of internal organs of man. The best known form is the condition known as *thrush* which is an infection of the oral mucosa of human infants, particularly of malnourished ones In several species of animals thrush-like infections are said to occur Apparently these are not frequent or serious except in birds Many kinds of *Monilia* occur on normal mucous membranes as saprophytes, thus care must be used in attributing pathogenicity to forms found in surface lesions

MONILIA ALBICANS

Synonyms· *Oidium albicans, Saccharomyces albicans*

This is the species which causes thrush in human infants The types found in birds cannot be distinguished from it

Morphology and Cultural Features. Young cultures consist of oval, budding yeast-like cells which measure 3 5 by 5 5 microns Older cultures show septate hyphae and occasional spherical, thick-walled chlamydospores In lesions the yeast-like cells in process of budding, and fragments of mycelium as well, can be seen



FIG 90 *Monilia spp* Unstained preparation from a deep colony in agar showing mycelium and the yeast-like spores which arise at the end of filaments and at mycelial nodes Surface colonies of monilia consist largely of yeast like cells. Mycelium is produced only under conditions of reduced oxygen tension Both mycelium and yeast like forms are found in lesions x 350

On Sabouraud's agar, soft creamy colonies which are very convex appear in 24 to 48 hours when incubated at 37° C In gelatin stabs short villus growths extend out from the main spike of growth. The medium is not liquefied Acid and gas are formed from dextrose, levulose, maltose, and mannose A little acid but no gas is formed from sucrose and galactose Lactose, raffinose, and inulin are not attacked

Pathogenicity. Some authors refer to monilia infections of the oral mucosa in calves and colts No additional information about them is available Presumably they are of little consequence.

Thrush-like lesions of birds—chickens, pigeons, turkeys, pheasants, and grouse, are quite common and often very serious. They involve the mouth, the crop, the proventriculus, and the gizzard. The lesions consist of whitish circular areas or of elongated patches along the crests of folds of the mucosa The areas may become confluent and finally involve large parts of the linings of these organs. The involved tissues finally slough off leaving superficial ulcers Epizootics in very young birds may cause a heavy mortality. In older birds the infections occur but recovery is the rule.

OIDIOMYCOSIS

Closely related to the *Monilia* are the *Oidia* The latter are simple yeast-like forms consisting of oblong elements which form chains or hyphae These break up forming spherical or oblong, spore-like elements Most of them are saprophytic, the best known being *Oidium lactis,* commonly associated with milk and therefore often in the mouths of young animals which are on diets containing milk Some species have been regarded as pathogenic for man but recent authors appear to doubt their disease-producing power Jungherr (1) has found a member of this group associated with the crop and gizzard lesions of birds described above and believes it plays a part in the infection Pure cultures were shown to be pathogenic for birds He proposed the name *Oidium pullorum* for it.

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SPOROTRICHOSIS

Sporotrichosis is a disease of man and horses, rarely of other species, caused by fungi belonging to the genus *Sporotrichum* which belongs to the group of *Fungi Imperfecti.* The disease is characterized by chronic granulomatous lesions of the skin, skin lymphatics, and occasionally of the internal organs The superficial lesions have a tendency to ulcerate. Elongated yeast-like bodies occur in the pus but these frequently can be demonstrated only with difficulty and sometimes not at all Mycelium is not found in tissues The condition in horses resembles epizootic lymphangitis. Early in the century epizootic lymphangitis was reported in the United States (2), but Page, Frothingham, and Paige (1) showed that the condition was sporotrichosis rather than cryptococcosis. Sporotrichosis in both man and horses has been reported rather frequently from the states of the upper Mississippi valley and infrequently from various other parts of this country. It is supposed that the organism is a saprophyte which lives in the soil.

SPOROTRICHUM SCHENCKI

Synonyms: *Sporotrichum beurmanni*

Some authors regard *S schencki* and *S beurmanni* as distinct species. The former was described from the United States; the latter from Europe It is evident that they are very similar if not identical The description given below is taken largely from that given by Page, Frothingham, and Paige who worked with equine material originating in Pennsylvania and North Dakota

Morphology and Staining Reactions. In pus the organism usually is difficult to demonstrate microscopically The only forms that can be recognized are cells resembling elongated yeasts, sometimes described as "cigar shaped." They vary in length from 2 to 10 microns and in breadth from 1 to 3 microns

In cultures the morphology is best studied in fluids, unstained. The filaments are rather delicate measuring about 2 microns in diameter They are septate and branching Short irregular lateral branches are numerous On the ends of the branches clusters of the oval or pear-shaped spores are found Such spores are also found along the sides of the filaments The hyphae and spores stain readily with ordinary dyes and with the Gram stain, but the process of staining shrinks the hyphae and dislodges the spores

Cultural Features. Growth occurs on all the ordinary laboratory media, but solid media are more productive than fluids Acid media are more favorable than those used for the cultivation of bacteria Maltose favors growth Good growths are obtained on potato and carrots and on these media the characteristic brownish-black pigment is best seen

Small whitish filamentous colonies appear on potato slants on the second day of incubation These gradually enlarge and gradually darken until finally the color is almost black The surface is woolly because of the short aerial hyphae Old cultures develop convoluted surfaces suggestive of the convolutions of the brain.

The growth on sliced carrots is similar to that on potato

On agar the growth is similar to that on potato but the colonies remain a whitish color They are adherent because of the penetration of the mycelium into the medium

Gelatin is slowly liquefied. Most of the growth is near the surface but spike-like growth occurs along stabs. There may be some blackening of the surface growth.

Loeffler's blood serum shows a slight depression under the colonies but general liquefaction does not occur

In litmus milk there is little growth except on the surface where white fila-

ments grow around the tube in the form of a ring which becomes black in about one week The milk may coagulate after three weeks but the reaction remains alkaline.

In fluid media growth occurs principally as a pellicle or a ring, but if undisturbed a few fluffy colonies, which remain permanently white, may form along the sides of the tube or on the bottom If the pellicle is caused to sink by shaking, a fresh one replaces it.

Poor growth occurs in sugar-free broth and in peptone water. It is much better in dextrose and especially in maltose broth Growth occurs in broth containing chloroform and in broth which contains more than 5 per cent salt

Acid is formed from dextrose but not from lactose, sucrose, maltose, mannitol, dulcitol, adonitol, raffinose, and inulin Gas is not formed in any medium.

Pathogenicity. The disease in horses is characterized by the formation of nodules, spherical in form, and of sharp contour There is no tendency for the nodules to coalesce Although they may become multiple and spread over areas of the body, in neither the nodular nor ulcerative forms is there any evidence of "cording" of the lymphatics The skin over the nodules becomes moist, the hair falls out, and crusts form The pus discharged from the crateriform ulcers is yellowish and rather scanty in amount The ulcers heal slowly and usually leave hairless cicatrices Internal lesions have not been reported in horses

Sporotrichosis has been reported in dogs but few details are available Rats also are said to contract infections naturally Experimentally, guinea pigs, rats, and rabbits can be infected but usually only a local abscess is formed

Immunity. Little is known of this aspect of sporotrichosis The disease is not common enough to warrant special biological treatment, furthermore, the disease yields readily to systemic treatment with iodides The serum of chronically infected animals will agglutinate spore suspensions, and filtrates of old fluid cultures (*sporotrichin*) will give specific skin reactions.

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PART V

THE PATHOGENIC PROTOZOA

CHAPTER XXXIII

THE PATHOGENIC PROTOZOA

General Considerations

Generally protozoa are regarded as the first, and the lowest, phylum of the animal kingdom All animals which consist of single cells, capable of carrying on all life processes independently, are included. The great English protozoologist Dobell insists, however, that the protozoa are non-cellular animals and thus are different from all other members of the animal kingdom which are cellular He raises the protozoa in rank equal to all others, and calls the others *metazoa* While the protozoa are generally regarded as the most primitive of animals, it should be noted that, physiologically, the protozoan cells must be more complex than the cells of the metazoa because they must assume all of the necessary functions of life, whereas the cells of multicellular animals have these functions divided between them

The soil, surface waters, vegetation, and the intestinal tracts of man and animals are teeming with protozoa which live saprophytic existences. Only a small proportion are parasitic in habit and a still smaller proportion of all are disease-producing. Nevertheless a number of very important diseases of man and animals are caused by members of the group.

STRUCTURE

All protozoan cells consist essentially of two parts, cytoplasm and nucleus The nucleus generally is relatively large and easily recognized. It contains a substance (chromatin) which has an affinity for the basic dyes The cytoplasm stains with acid dyes and consists of a foamy protoplasm in which vacuoles often are seen Embedded in the cytoplasm of some species are special organs which have to do with locomotion, and others which act as skeletal supports.

LOCOMOTION

The simplest mode of locomotion among protozoa is by ***pseudopodia.*** These are processes of protoplasm extruded from the body surface into which the cytoplasm flows like water in a rubber sac which is pushed across a table. This type of motility characterizes the group of amoeba, thus this type is often called ***amoeboid movement.***

Permanent special organs of motility are possessed by many groups of protozoa These are in the form of whip-like processes which lash the medium in which the creatures live and thus cause movements of translation Sometimes these lashes are small and numerous; sometimes they are fewer and large. The small lashes are known as *cilia* and such forms belong to the *Ciliophora* The cilia arise from minute granules, lodged in the ectoplasm of the cells, which are homologous with the blepharoplasts of the flagellates. When the lashes are few and large they are known as *flagella,* and the flagellated forms constitute a special group known as the *Mastigophora* Flagella consist of a central filament, known as the *axoneme* which takes origin in a body embedded in the cytoplasm, usually near the nucleus, called the *blepharoplast.* As the axoneme passes out through the cell wall to form the lash it becomes covered with a layer of material derived from the cell wall.

MULTIPLICATION

Most protozoa multiply by a process of cell division in which two, more or less equal, individuals are formed This process is known as *binary fission* Some forms divide characteristically into two unequal individuals This form is called *gemmation* or budding A large group of parasitic protozoa, the *Sporozoa,* reproduce by a process known as *schizogony* In this method the nucleus divides and the daughter nuclei divide and subdivide until there are a large number of nuclei in one cytoplasmic mass These nuclei arrange themselves around the periphery of the cell and are pinched off, each with a small amount of cytoplasm, to form a shower of new individuals called *merozoites.* These grow into adults which may again reproduce by a repetition of the schizogonic process

SYNGAMY

Sexual differentiation occurs among many protozoa, the differentiated cells being known as *gametes* The *macrogametes* are the equivalent of the ovum of higher creatures, and the *microgametes,* of the sperm cells The fertilization of a macrogamete by a microgamete gives rise to cells known as *zygotes,* or *oocysts,* which frequently develop resistant capsules and thus are prepared to await more favorable conditions, or a new host, before developing

PROTOZOAN CYSTS

A characteristic of many of the parasitic protozoa in particular, but of many of the saprophytes also, is their ability to form structures which resist drying. These are called cysts and are formed by the secretion of substances which

harden into rather dense capsules. Cyst walls often are quite clear and refractile. Unlike the spores of bacteria, to which these structures are comparable, at least in function, the protozoan spore is not materially more resistant to high temperatures than the vegetative organism.

IMMUNITY IN PROTOZOAN DISEASES

Generally in protozoan diseases the parasite multiplies rapidly for a time, then, if the host continues to survive, gradually diminishes in number until it can no longer be found In some instances it does not wholly disappear and may give rise to a recrudescence of the disease. This loss of vigor on the part of the parasite is similar to that seen in most bacterial infections and may be due to the same cause, namely, the development of antibodies which are antagonistic to the parasite Antibodies do develop in protozoan infections, and they may be transmitted passively, often protecting completely an otherwise susceptible host Agglutinins, precipitins, complement-fixing antibodies, lysins, and opsonins for protozoa have been demonstrated It is probable that developing immunity is not the only factor in causing disappearance of protozoan parasites, for in many instances, new infections can be superimposed on the old one Whereas the original infection is fading out, the new one may be vigorous and progressive This indicates a failing vigor of the parasite probably due to inherent properties which permit only a limited number of generations by one mode of multiplication, after which another mode must intervene Schizogony, or asexual multiplication, of coccidia of animals and of the malaria parasites of man, can go on in the original hosts only for a limited number of generations, after which the parasite assumes the sexual form and further multiplication in the same host does not occur After sojourn in another host, as in malaria, or in the soil, in the case of coccidia, the parasite is then ready to institute a new series of generations in another host or in the very host from which it was derived

In some protozoan diseases, such as human leishmaniasis and East Coast Fever of cattle, recovery results in complete and absolute immunity In most cases, however, the immunity is only a relative matter and the disease may run for years in a single host because of repeated reinfections Immunity in these instances does not suffice to protect from new infections.

Many attempts have been made to induce active immunity to protozoan infections by vaccinating the animals with attenuated strains, or suspensions of killed cultures These have met with very little success The only immunity which is successful is that which is produced as a result of suffering from an actual infection. In some instances it has been noted that young animals withstand certain protozoan infections much better than adult animals and

this fact has been put to practical use as, for example, in Texas fever immunization.

ARTIFICIAL CULTIVATION

The pathogenic protozoa fall into two classes a Those that live in the fluids of the body extracellularly, and b Those that live all or part of their lives intracellularly Most of the species that live extracellularly have been cultivated in artificial media. The culture media usually are rather simple but most of them contain blood, or blood serum. The species that live intracellularly have been refractory to artificial cultivation, as might be expected Success has been attained only in tissue cultures in which the host cells are growing, or at least in which the viability of the host cell is preserved for a time.

Detailed classification of the protozoa will not be considered We are concerned only with the disease-producing forms All of these fall into four classes *Rhizopoda, Mastigophora, Sporozoa,* and *Ciliata*

THE RHIZOPODA include the organisms which move and ingest food by means of pseudopodia. The only pathogenic species of importance in this group is *Entamoeba histolytica,* the cause of amoebic dysentery in man This species will develop in young kittens and these animals are frequently used as experimental hosts but natural infections are unknown

THE MASTIGOPHORA are sometimes called the *Flagellata* This class includes all of the species which possess flagella during the greater part of their life span Flagella are seen temporarily at certain stages of development in some species which are not members of this class Some members of this group live in the intestinal and genital tracts and others live in the blood The group of trypanosomes, the leishmanias, the trichomonads, and the hexamitae are members of the *Mastigophora.* The histomonas of blackhead in turkeys is assigned to this group, although it is not a typical member

THE SPOROZOA include forms which are exclusively parasitic in habit and which at some stage in their development produce resistant spores enclosing one or more sporozoites which carry the infection to new hosts This group includes the coccidia and many species of blood parasites, the hemosporidia

THE CILIATA are protozoa which possess cilia in all stages of their development. Most of them are free-living, non-parasitic forms Only one species is of enough importance to warrant consideration here This is *Balantidium coli.*

CHAPTER XXXIV

THE MASTIGOPHORA

The Trypanosome Group

The most important disease-producing members of the *Mastigophora* belong to the family *Trypanosomidae* This family is divided into five genera: *Leishmania*, which occurs in both vertebrates and invertebrates, *Leptomonas*, *Crithidia* and *Herpetomonas* which occur only in invertebrates, usually in the digestive canal, and *Trypanosoma*, which occurs in vertebrates as well as invertebrates, usually in the blood plasma

All of these forms resemble each other in that they possess a nucleus and a single flagellum which arises from a small body, embedded in the cytoplasm and known as the *blepharoplast* Just posterior to the blepharoplast and very near to it is a deeply staining structure known as the *parabasal body* The composite structure consisting of the blepharoplast and the parabasal body is known as the *kinetoplast*

The *Leishmania* usually are spherical or oval cells in which the nucleus and kinetoplast are visible but the flagellum is rudimentary, or absent and in no case does it extend outside the cell wall These cells, therefore, are non-motile. *Leptomonas* consists of an elon-

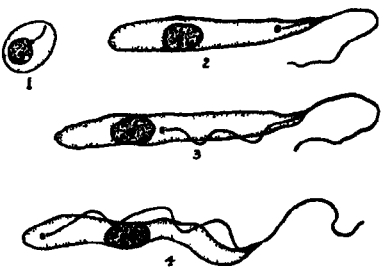


FIG 91 Diagrammatic Representation of the *Trypanosomidae* (1) *Leishmania* The kinetoplast is located near the nucleus and the flagellum is absent or rudimentary In no case does the flagellum extend beyond the cell body, thus these forms always are non-motile. (2) *Leptomonas* The cells are elongate, the nucleus is located near the center of the cell, the kinetoplast is near the anterior end of the cell and the flagellum extends well beyond the cell wall, constituting an effective means of locomotion There is no undulating membrane (3) *Crithidia* The cells are elongate, the kinetoplast is located near but slightly anterior to the nucleus, the flagellum passes out of the cell at one margin and runs in the edge of an undulating membrane to the anterior end of the cell and thence forward as a free flash These forms are actively motile (4) *Trypanosoma* These are similar to the preceding except that the kinetoplast is located posterior to the nucleus and usually well toward the posterior end of the cell. The flagellum and undulating membrane are similar to those of the crithidia

gated cell with a nucleus located more or less centrally The kinetoplast is located near the anterior end of the cell and the flagellum extends well beyond the cell wall, constituting an effective means of locomotion An undulating membrane is not present in this group. The cell body of the *Crithidia* is elongated and similar to that of the previous group but the kinetoplast is located near but slightly anterior to the nucleus, i e, near the center of the cell body. The flagellum passes out of the side of the cell into a protoplasmic ridge which is known as an *undulating membrane* of which it constitutes the outer or free margin At the anterior end of the cell the flagellum leaves the undulating membrane and extends well forward as a free lash The *Trypanosoma* resemble the *Crithidia* except that the kinetoplast is located well behind the nucleus toward the posterior end of the cell and the flagellum leaves the side of the cell near its posterior end into an undulating membrane which runs nearly the entire length of the cell At the anterior end of the cell the flagellum leaves the membrane and passes farther forward as a free lash The free margin of the undulating membrane in both crithidia and trypanosomes is longer than the attached margin, hence it is thrown into folds or ruffles which have an undulating movement when in action.

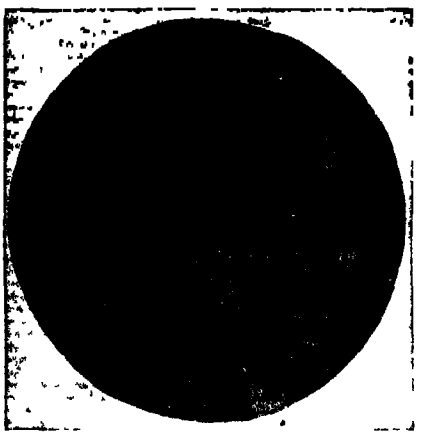


FIG 92 Crithidia form of *Trypanosoma melophagium* Stained film of a culture on artificial media Note that the flagellum originates in a kinetoplast located near but slightly anterior to the nucleus and that an undulating membrane is present

The Leishmania

Flagellates of the genus *Leishmania* occur in the form of leptomonads as well as leishmania and they may be found in both vertebrate and invertebrate hosts Members of the genus *Leptomonas* occur in both forms but they are confined to invertebrate hosts Those of the genus *Crithidia* may appear in their invertebrate hosts as leishmania, leptomonads or crithidia Those be-

longing to the genus *Herpetomonas* are confined to invertebrates and may appear as leishmania, leptomonads, crithidia, or trypanosomes Members of the genus *Trypanosoma* are like the herpetomonads in that they may occur in any of the four forms but differ in that they are found in vertebrate as well as invertebrate hosts

All of this sounds, and is, very confusing, The fact is that the groups represent evolutionary stages in which the more complicated forms tend to retrogress at times Thus a culture of a trypanosome on artificial media may show leishmania, leptomonas, and crithidia forms only, but upon inoculation of such a culture into a susceptible host the parasite may again assume the form of a trypanosome In the intestine of a blood-sucking insect which has fed upon blood containing trypanosomes, the same phenomenon can be seen. On the other hand there are flagellates in arthropods which never assume the more complex forms and these are classified as belonging to the most complicated group to which its development entitles it The terms are used as nouns, in which they refer to the genera, or as adjectives when they refer to a developmental stage of another form We may speak of crithidia forms of trypanosomes, or the leptomonad form of leishmania On the other hand we say that the causative agent of human kala-azar is *Leishmania donovani* It will have been noted that only the leishmania and the trypanosomes are parasitic and pathogenic for vertebrates These will be the only genera which will be considered further

LEISHMANIA DONOVANI

Synonym The Leishman-Donovan bodies

This parasite is the causative agent of a malaria-like disease of man known as kala-azar or dumdum fever which is endemic in India, China, southern Russia, and the countries of Europe and Africa which border on the Mediterranean Sea The parasite usually is found within large endothelial cells of the capillaries and is concentrated in the spleen, bone marrow, lymph nodes, and liver. Usually very few are found in the blood stream and diagnosis by means of blood smears, therefore, is very uncertain For diagnosis, spleen or liver puncture is frequently done Usually the endothelial cells of these organs contain large numbers of the parasite.

In man another disease, known as "oriental sore" or "Delhi boil" is caused by a member of the group, *Leishmania tropica* This is regarded as a separate species although it cannot be distinguished with certainty from *L. donovani.*

That the leishmania are flagellates was first demonstrated by the development of flagellated forms of the leptomonas type in citrate solution to which

infected splenic material had been added It is believed that the transmitting agent of the disease is an arthropod although the precise vector has never been identified. Gnats of the genus *Phlebotomus* have been suspected. The belief in an invertebrate vector is based partly on the fact that the cultural leptomonad form is characteristic of the type usually found in invertebrates

Morphology and Cultural Features. *L donovani* usually is ovoid in outline. It measures from 1 5 to 2.5 by 2 to 5 microns Multiplication is by binary fission and dividing forms are frequently seen in spleen pulp films. Artificial culture was first obtained by immersing bits of infected spleen pulp in a sodium citrate solution but continued cultivation by this method is not successful. The best medium for initial as well as continuous cultivation is the well known N N N medium, now commonly used for the cultivation of most of the pathogenic trypanosomes This medium consists merely of an agar jelly made up of agar and saline solution to which 10 per cent fresh defibrinated rabbit blood is added just before use The medium is slanted and growth of the flagellates occurs in the water of syneresis This medium will support vigorous growth of *L donovani* indefinitely The medium takes its name from the fact that it was described by Nicolle and is a modification of a medium first used by Novy and McNeal (Nicolle-Novy-McNeal).

Pathogenicity for Dogs. Dogs in the kala-azar districts, and especially in the Mediterranean region, have frequently been found infected with this parasite (2) (3) Some have suggested, in fact, that the dog is the natural reservoir from which human infections are derived but there is little support for this idea Apparently dogs are exposed to the same sources of infection as man, and obtain the infection from the same sources

The disease in dogs is much like that of man It may be acute or chronic and the mortality may be great, frequently because of intercurrent infections, sometimes because of the uncomplicated disease There is great enlargement and hardening of the spleen and liver, there is fever, leucopenia, anemia, loss of weight and of general vitality.

In dogs leishmania are rarely found in the peripheral blood and often they are scarce in the liver. Since the spleen is rather hard to puncture in dogs, bone marrow is often collected by trephining one of the long bones of the legs (1).

Immunity. Animals that recover from leishmaniasis are said to be immune thereafter. The mortality of the disease has been markedly reduced by the use of tartar emetic for treatment. This is given intravenously and has a markedly specific effect upon the parasite.

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The Non-Pathogenic Trypanosomes

TRYPANOSOMA LEWISI

This species of trypanosome occurs in wild rats the world over and can very easily be transmitted by artificial inoculation to white rats The rats show very little evidence of the infection, as a rule, but some strains appear to possess some pathogenicity

Morphology. Shortly after inoculation the forms which appear in the blood vary greatly in appearance from leishmania-like cells to very broad trypanosomes with long flagella After the multiplicative stage has passed, the individuals become much more uniform in size and shape As seen in unstained blood, they are exceedingly active, dashing here and there, knocking the cells about The parasites are about 25 microns long, have pointed ends and a curved body The kinetoplast is situated at some distance from the posterior end The nucleus is a little forward of the center of the body. The undulating membrane is not markedly folded and thus the flagellum is fairly straight The sharp posterior end and the curved body gives this species a characteristic appearance

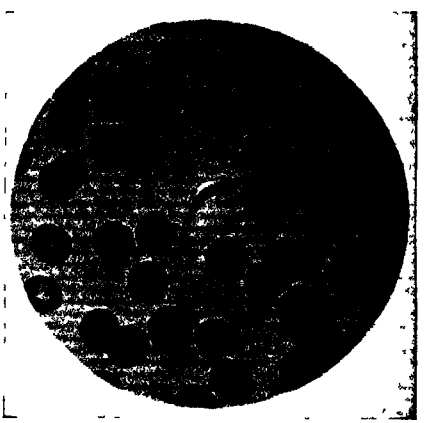


FIG 93 *Trypanosoma lewisi* Stained blood film from a naturally infected wild rat This trypanosome is relatively long and slender and it has a long flagellum which may be seen extending from the anterior end which is uppermost in the photograph The nucleus is seen in the anterior part of the cell and the kinetoplast near the posterior end The undulating membrane with the flagellum in its free margin may be seen along the left hand side of the cell x 600.

During the multiplicative period parasites in various stages of development may be seen in the blood. Division of the kinetoplast first occurs, followed by that of the nucleus Two flagella are now formed and the

cell splits between them forming two individuals. Before one division is complete others may begin, hence compound structures sometimes are seen.

Cultural Features. *Trypanosoma lewisi* is readily cultivated in N N N. medium All of the forms of the trypanosomidae are produced in cultures, the trypanosome-form gradually giving way to the simpler ones. The virulence for rats is maintained for a long time

Transmission. Various rat fleas are the transmitting agents Infection is not conveyed by the bite, however, since the mouthparts of the fleas are not infected. Infection apparently occurs through the ingestion, by the rats, of fecal material from the fleas, or from ingestion of whole fleas The newly-infected flea does not become immediately infective for rats. The parasite undergoes definite cyclical changes in the flea which require about six days for completion, and before the infective stage is again reached

Immunity. The serum of rats which have recovered from infection with *Tr. lewisi* agglutinates and frequently lyses suspensions of the organism In agglutination the parasites form clumps in which the individuals are attached by their posterior ends. The serum of recovered rats inhibits the development of the parasites in culture.

TRYPANOSOMA MELOPHAGIUM

This trypanosome occurs in a large percentage of all sheep, in some flocks occurring in 80 per cent or more of all animals It is similar to *Tr theileri* in form and size and in the fact that it is wholly non-pathogenic It undergoes its life cycle in the sheep "tick" or ked Infection of the sheep is not through the bite of the insect since the head parts are not infective but rather the lower parts of the insect's intestine Infection apparently occurs through the rubbing of the excrement of the ked into bite wounds or other abrasions of the skin.

The parasite is found rather sparingly in the blood of the affected sheep but can be demonstrated readily with the cultural method described under *Tr theileri* In the ked the parasite develops in the intestinal canal largely in the crithidia form If all keds are removed from an infected sheep, the parasite rather quickly disappears from the sheep's blood, showing that the reservoir of the infection is really the ked

TRYPANOSOMA THEILERI

Synonyms· *Trypanosoma americanum, Trypanosoma franki,* and others.

Theiler, in 1903, found a large trypanosome in the blood of a cow which

apparently was normal. Laveran later named it *Tr. theileri* in honor of it's discoverer. Many observers in later years have seen such trypanosomes, and many new names have been given to them. All of these may not be identical with Theiler's but they are similar in morphology and similar in that they are non-pathogenic to their hosts.

Morphology. This is a very large trypanosome, some individuals measuring as much as 60 to 70 microns in length Others are only half as large.

Transmission. The parasite evidently is transmitted by several types of blood-sucking flies. Tabanids have definitely been incriminated in a few cases.

Pathogenicity. Cattle seem not to be affected in any way by the presence of the trypanosome and no other animals are known to harbor it The parasite usually is very scarce in the blood, so scarce that it can be found only occasionally on direct microscopic examination In case the parasite cannot be found, it may often be demonstrated culturally by using a simple technique. One part of the animal's blood is secured aseptically and added to two parts of plain infusion broth The tube is incubated at 25° C or at room temperature In about one week growth of the trypanosome becomes evident as small whitish colonies which lie on the surface of the mass of sedimented blood cells, contrasting sharply with the dark color of the blood In these colonies every flagellate type from leishmania to large trypanosomes are seen In many areas 50 to 75 per cent of the cattle may be shown in this way to be harboring *Tr. theileri*

AVIAN TRYPANOSOMES

Trypanosomes occur quite commonly in many species of wild birds. They are very rare in the domestic species. Many of these trypanosomes have been given specific names, especially related to the host in which they are found but these are not of importance in this work

The morphology varies considerably The transmitting agents generally are not known In some cases mosquitoes have been incriminated. The degree of pathogenicity in most instances is not high, and many of them seem to be practically harmless.

The bird trypanosomes are readily cultivated in N.N.N. medium and even on the surface of blood agar plates. Infections can often be diagnosed by cultural means when the organisms are not readily found microscopically.

The Pathogenic Trypanosomes

TRYPANOSOMA BRUCEI

This trypanosome was discovered by Bruce in 1895 and is the cause of a destructive disease of domestic animals which are taken into many parts of the African continent, notably Rhodesia, the Sudan, Uganda, Tanganyika, and, in general, nearly all parts of tropical Africa The disease is called *nagana.* Some differences are noted in the manifestation of the disease in different areas, and there are morphological differences in the parasite, however it is thought that these are only varieties of the same species.

Morphology. *Tr. brucei* varies considerably in shape and size, even in the same host. There is a short broad form without a flagellum, a long slender form with a long flagellum and an intermediate form In some of the broad forms the nucleus is displaced posteriorly, especially when the parasite is growing in rats, mice, and guinea pigs and this characteristic is of some diagnostic importance The parasitic cells average 22 microns long but they vary from 12 to 35 microns

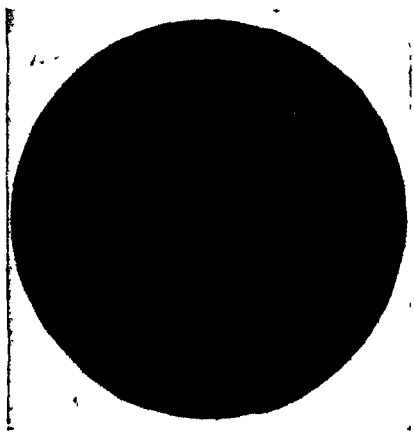


FIG 94 *Trypanosoma brucei* Two parasites in a stained blood film of an artificially infected rat This trypanosome is rather broad and the flagellum is short ×600

Transmission. *Tr brucei* is transmitted by the tsetse fly, *Glossina morsitans,* and other species of glossina, as was shown first by Bruce in 1897 These flies may transmit the disease by contamination, i e, by carrying the blood parasite directly from one animal to another A short time after feeding on an infected animal, however, the fly becomes non-infective and remains so for 18 to 20 days It then again becomes infective and remains so for the remainder of its life During this time the trypanosome undergoes cyclical changes in the fly, first in the intestine, then in other organs, and finally it reaches the salivary glands Here the crithidia forms await the opportunity to infect a new host at the time the next blood meal is taken by the host

Reservoir Hosts. In his early investigations in Zululand, Bruce showed that the tsetse flies were infective for dogs which he had taken into the country with him Since there were no domestic animals in the area it was obvious that there were reservoirs of the infection in some of the wild animals which were abundant there Later studies showed that about 30 per cent of the wild animals harbored the parasite in small numbers apparently without being materially harmed by them

Pathogenicity. Horses, mules, cattle, sheep, goats, swine, dogs, and cats are naturally susceptible to nagana The disease is most severe in animals of the horse family, in swine, and in dogs These animals usually die from the disease in from two weeks to three months Cattle are more resistant, although most infected individuals eventually die, if untreated Sheep and goats are still less susceptible By inoculation, *Tr brucei* is the most virulent of all trypanosomes It will infect practically all mammals, except certain races of monkeys and man Even man is occasionally infected

Nagana gets its name from a native word which signifies weakness The disease is manifested by swellings around the neck and legs, fluctuating fever, loss of appetite, and muscular weakness The affected animals become very anemic and the mortality rate is high The parasite is found in large numbers in the bone marrow after death but only a few can be found in the blood stream The spleen is greatly enlarged and the pericardial sac usually is filled with fluid.

TRYPANOSOMA RHODESIENSE

Human trypanosomiasis occurs only in restricted areas of Africa, especially in lowlands close to the water courses in the more tropical parts of the continent In some of these areas the causative agent is a trypanosome which can be distinguished morphologically from those which infect animals. Furthermore while most of the domesticated animals can be infected with this trypanosome, experimentally, the infections ordinarily are mild and the parasites so few in them that they can be demonstrated in many cases only by inoculating blood or tissues into the smaller, more susceptible, animals This parasite is regarded as a definite species and is known under the name *Trypanosoma gambiense* The human infections are chronic, leading to final emaciation, weakness, and encephalitis which gives to the disease its common name, "sleeping sickness." This parasite, like those of animals, is transmitted by members of the tsetse fly group

In some areas where nagana is prevalent and severe, human cases are practi-

cally unknown. In others, however, human cases are frequent and much more acute and fatal than the typical sleeping sickness disease. These cases are caused by trypanosomes which morphologically are like *Tr brucei*. Strains derived from such cases when inoculated experimentally, produce severe animal infections, which resemble nagana in every way Some workers prefer to look upon this parasite as a distinct species and have called it *Tr. rhodesiense*. Others regard it as a strain of *Tr brucei* which has become specifically adapted to man It is suspected that this form is harbored by wild animals and causes disease of man and his domesticated animals alike

TRYPANOSOMA CONGOLENSE

This is a small trypanosome which affects cattle, horses, pigs, and dogs in many parts of tropical Africa The parasite averages 14 microns in length, varying from 9 to 18 There is no flagellum, that is, the axoneme or intracellular part of the flagellum is present, arising in the kinetoplast and running along the margin of the undulating membrane, as usual, but it does not extend forward as a free whip or lash as in most forms The nucleus is located in the middle of the cell

Tr congolense produces a chronic wasting disease associated with fever and anemia Transmission is by means of several species of tsetse flies in which a definite life cycle occurs Wild game animals are the natural reservoir of infection.

TRYPANOSOMA VIVAX

This trypanosome is widely distributed throughout the tsetse fly areas of Africa Wenyon found 76 per cent of the humped cattle of the Gold Coast of West Africa to be infected It occurs most commonly in cattle, sheep, and goats, but also affects horses The disease is similar to, but less virulent than, that caused by *Tr. congolense*, nevertheless the average mortality is high Dogs, guinea pigs, rats, and mice are refractory to inoculation The disease is transmitted by several species of tsetse flies in which a definite life cycle occurs Antelopes and other members of the deer family constitute the natural reservoir of infection

Tr vivax obtained its specific name from the fact that it is an exceedingly active parasite It measures from 18 to 26 microns in length The posterior end of the body is broader than the anterior, consequently the greater part of the cytoplasm lies posterior to the nucleus. The undulating membrane is less well developed than in most trypanosomes The flagellum, as a consequence, is straighter than usual.

TRYPANOSOMA EVANSI

This organism is the cause of a disease which occurs principally in southern Asiatic countries and is known under the name of *surra* The disease is most virulent for horses but also affects camels, elephants, and dogs Cattle and the water buffalo (carabao) are susceptible but the disease in them is very mild and recovery generally occurs Man is not susceptible

The disease occurs in India, Burma, Ceylon, South China, Siam, Sumatra, Java, the Philippines, Madagascar, Persia, and Arabia Infected horses have been imported into Australia and the United States but vigilance has prevented the disease from getting a foothold A disease of camels, which apparently is surra of a mild type, occurs in the French Sudan, Algeria, and Egypt. It affects horses as well as dromedaries, the disease being milder in this species than in Asiatic camels

Morphology. The trypanosome of surra is of more uniform morphology than those of the African animal diseases. It averages 25 microns in length The body is slender, both ends are pointed, and the body usually is bent into the form of a crescent. The nucleus is centrally located and the flagellum is long It is actively motile in blood films

Reservoir Hosts. The water buffalo and other ruminants are the reservoirs of infection in Asia Camels in other areas are chronic carriers The greatest losses occur in horses, especially military animals, which are brought into infected areas and quartered near herds of water buffalo

Transmission. Surra is transmitted by a number of blood-sucking flies, principally members of the group of *Tabanidae*, or "horse-flies." There is no evidence of any kind of life cycle, such as occurs in the tsetse flies infected with the African trypanosomes, in the case of *Trypanosoma evansi*. Apparently transmission is a purely mechanical process Flies held in captivity for a few hours after feeding on a surra-infected animal lose their ability to infect other animals. Blood-sucking arthropods other than horse-flies have been incriminated experimentally as potential spreaders of surra but there is little evidence to indicate that any of them have been important natural spreaders.

Pathogenicity. The natural disease is almost always fatal for horses, death occurring in from a week or two to as long as six months after the date of infection. The affected animals exhibit fever, weakness, wasting, anemia, and edematous swellings. The disease in camels and elephants is similar, but considerably more chronic. Cattle and buffalo are readily infected but the disease does little harm to them Dogs are highly susceptible and heavy losses

have been reported in kennels of hunting dogs. Ticks and fleas have been believed to be the principal transmitting agents among dogs.

Rats and mice are highly susceptible to inoculation and usually die in about one week with massive invasion of the blood stream Guinea pigs and rabbits are more resistant but usually succumb to the disease, without showing great numbers of parasites in their blood, in from one to four months.

TRYPANOSOMA EQUINUM

This parasite is the causative agent of a disease known as mal-de-caderas, which occurs in Brazil, Bolivia, Paraguay, and Argentina in South America. The disease affects horses principally Mules are less susceptible than horses. Cattle, sheep, and goats have the disease in a very mild form

Morphology. *Trypanosoma equinum* resembles *Tr evansi* very closely It is regarded as a variant of the Asiatic species It has one remarkable feature and that is the fact that it does not have a parabasal body This is the only species which lacks this structure The parasite measures about 22 microns in length and shows the general features already described for *Tr evansi*

Transmission. This trypanosome probably is transmitted by blood-sucking flies of the Tabanid and Stomoxys groups

Reservoir Host. In the mal-de-caderas districts lives a large rodent, the capybara. This animal appears very much like a giant guinea pig It is found along the water courses It has been noted that this animal often suffers severe epizootics in which a trypanosome indistinguishable from the one of mal-de-caderas occurs It is thought that the capybara may serve as a reservoir of infection for domestic animals

Pathogenicity. The name, mal-de-caderas, refers to a weakness of the hind quarters and calls attention to one of the prominent symptoms of the disease in the horse. Emaciation, weakness, conjunctivitis, remissions of fever, and edematous swellings are seen The disease is fatal to horses in from one to four or five months Rats and mice and other small laboratory animals may be readily infected experimentally.

TRYPANOSOMA HIPPICUM

This trypanosome is the causative agent of *murrina* or *derrengadera*, a disease affecting horses and mules in the Panama Canal Zone, in the Republic of Panama, and possibly in the Republic of Colombia. The disease is spreading.

Morphology. *Trypanosoma hippicum* is indistinguishable from *Tr. evansi* and from *Tr venezuelense* which will be described next. Both of these species are considered to be variants of the trypanosome of surra.

Transmission. The disease can be transmitted by blood-sucking flies particularly those of the Tabanid family. It is interesting to note, however, that an important means of transmission, perhaps the most important means, is the vampire bat This creature (*Desmodus rotundus*) is a vicious blood-sucker which preys upon livestock at night by alighting on their backs and puncturing the skin in the region of the withers with their sharp incisors These bats also prey upon each other The trypanosome infects these bats, producing a disease which is fatal to them in from nine to twenty-seven days While infected they continue to prey upon animals, transmitting the disease to them

Reservoir Hosts. Cattle and the native burro appear to be important reservoirs of infection

Pathogenicity. The symptoms in horses are characteristic of trypanosome infections in general There is irregular fever, progressive emaciation, anemia, slight icterus, weakness, and edema of the lower portions of the body. Death usually occurs after several weeks or months The mortality is very high. Cattle and the native burro contract the disease but in them it is very benign and recovery occurs

A large variety of animals can be infected by inoculation, most of them fatally Cattle, sheep, goats, and swine can be infected but recover Cats, burros, deer, and the wild hog (peccary) develop very chronic infections. Chickens are resistant

TRYPANOSOMA VENEZUELENSE

This trypanosome occurs in Venezuela where it causes a disease of horses and dogs which resembles surra. The parasite is indistinguishable from those of surra and murrina, and it is doubtful whether it should be regarded as a separate species Rangel, the discoverer, claims that the parasite occurs naturally in Venezuela in the wild dog, the capybara, and a type of howler monkey.

TRYPANOSOMA EQUIPERDUM

This parasite affects horses and members of the horse family in which a serious disease known as *dourine*, or *maladie du coit* is produced. The disease is transmitted almost exclusively by sexual contact, in which respect it is

unique among the trypanosome diseases Dourine is the only trypanosome disease of importance in the United States The infection had long been known in Europe. About 1850 it became prevalent in France It was first recognized in the United States in 1885 by Williams, in DeWitt County, Illinois. It is believed that it was imported from France in 1882 in an infected Percheron stallion. Before it was recognized the disease had been spread from the region in Illinois where it was first recognized to other areas It later appeared in Nebraska, Iowa, North Dakota, South Dakota, and the western provinces of Canada. Still later it was recognized in New Mexico and Arizona and, very recently, in southern California It is presumed that the infection in all of these localities had its origin in the early focus in Illinois.

By the use of the complement-fixation test and the destruction of all reacting animals, the disease was brought under control and by 1920 it was believed to have been eradicated. In 1941, however, some infected animals were found in Arizona and southern California It is believed that the disease had existed in the intervening period in horses owned by Indians living in isolated areas of the southwestern deserts The infected area is small, hence the vigorous efforts which are now being made to stamp out the disease should succeed within a short time.

Morphology. The trypanosome of dourine resembles that of surra It varies in length from 25 to 35 microns

Transmission. Dourine is transmitted from stallion to mare and from mare to stallion by contact of the mucous membranes of the genital tract during coitus It has been proved, too, that the disease can be transmitted by blood-sucking flies (Tabanidae and Stomoxys), but fly transmission appears to be the exception rather than the rule

Reservoir Hosts. Members of the horse family are the only animals that are known to contract infection naturally. There is no reservoir, therefore, other than naturally infected horses

Pathogenicity. The disease is chronic in nature. In tropical climates it has a tendency to be more acute than in cooler regions. The disease may continue for months or years, the affected animals alternately improving and relapsing. Finally emaciation and nervous symptoms make the animals worthless, and usually they are destroyed when this stage has been reached.

The symptoms begin with swellings of the genitalia The prepuce, penis and testicles of the stallion become swollen and reddened. The vulva and vagina of the mare swells and emits a mucoid discharge. In these discharges the trypanosome generally can be found, and it is through them that the

disease is transmitted during coitus After the acute signs in the genital tracts have subsided, peculiar raised plaques of the skin appear. These are frequently called "dollar" plaques because they have the feeling of a disc, like a silver dollar, under the skin. They vary in size from ones much smaller than a silver dollar to ones which are several times as large These appear quickly and disappear within a few hours or after several days to be replaced by others At this time depigmentation of the mucosa of the genital tract may occur Symptoms of paralysis gradually develop, an inconstant fever occurs, emaciation progresses, and death occurs

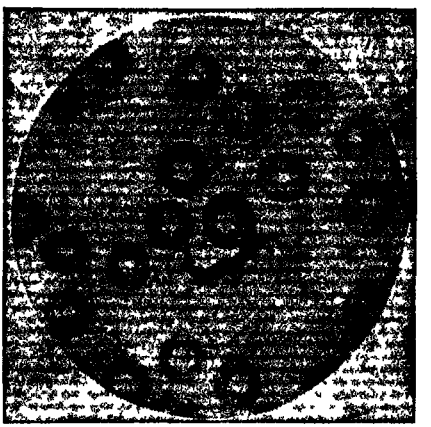


FIG 95 *Trypanosoma equiperdum* Stained film of the blood of an artificially infected guinea pig x 600

Tr equiperdum often is difficult to demonstrate in cases of dourine The trypanosome is present in the blood in very small numbers, this probably being the reason why the disease is not more often insect-borne It may be demonstrated during the acute stages of genital swelling in the mucoid discharge, and it may often be demonstrated by scarifying and squeezing the dollar plaques. The edema fluid often will show parasites

Tr equiperdum is readily inoculable into dogs as a rule, and in diagnostic tests, a dog may be given from 100 to 200 cc of blood or other fluids from the suspected horse Not all strains will infect dogs, however, so a negative test is not conclusive Rabbits are fairly susceptible—much more so than guinea pigs, rats, and mice The latter can sometimes be infected, in which case the strain may rapidly become adapted to growing in them.

THE DIAGNOSIS OF TRYPANOSOMIASIS OF ANIMALS

Diagnosis of trypanosome infections of animals depends upon the recognition of the clinical symptoms and the finding of the parasite in the blood films. Frequently it is impossible, even though thick films have been examined, to find the parasites This is especially true in dourine. In these cases the complement-fixation test often is useful.

Dourine has been eradicated from all of Canada and from all of the United

States except for a small area in the southwest, by the use of the complement-fixation test and the elimination of all reacting animals. The test is done in the usual manner except for the preparation of the antigen For antigen, a suspension of *Tr equiperdum* is made from the blood of heavily infected white rats. The rats are inoculated and killed when their blood is teeming with the parasites The blood is drawn into a citrated-saline solution and centrifuged at high speed The greater number of trypanosomes will be found as a whitish layer on top of the packed red cells but many will be mixed with the cells The supernatent serum, which is practically free of parasites is discarded, and the tube is filled with distilled water which will hemolyze or lake the erythrocytes A second centrifuging will give a small amount of sediment which consists largely of trypanosomes, leucocytes and cellular debris. This material is washed with saline solution and then suspended in saline solution containing 50 per cent glycerin This constitutes the stock antigen

Complement-fixation with trypanosome antigens is not strictly specific since one may obtain cross reactions between certain species Positive fixation therefore is indicative of trypanosome infection but one usually needs to determine the infecting species by other means In the United States the test has been very useful in detecting occult cases of dourine that may not be detected in any other way

TREATMENT OF TRYPANOSOMIASIS IN ANIMALS

Serological treatment of trypanosome infections has been very disappointing. In not a single instance has it been possible to effect a cure by serum treatment Much more encouragement has been met with in the treatment of these diseases with chemotherapeutic agents The chemotherapeutic attacks on the trypanosomiases were among the first attempts to attack infectious diseases in this way.

The most successful compounds have been those of arsenic and antimony Arsenious acid was used quite early but it was too poisonous to the host to make it a successful remedy *Atoxyl*, an organic arsenic compound introduced in 1905, proved to be a great improvement and it was extensively used Antimony, in the form of sodium-antimony-tartrate (tartar emetic) also proved useful In the case of both of these drugs, the blood-stream may be readily cleared of trypanosomes but after a few days they usually reappear Many small doses of arsenic and antimony, given alternately, may bring about a cure but often the compounds gradually lose their effectiveness because the parasite tends to develop a tolerance to them. This is a phenomenon known as "drug-fastness."

In 1920 the Bayer dye corporation in Germany announced a new synthetic trypanocidal substance under the name of Bayer 205. Later it was named Germanin. The formula was kept secret but French workers apparently have been able to duplicate the material. It is a non-metallic compound having a large molecule and with urea as its base This material has proven very effective in sterilizing the blood in many, but not all, types of trypanosome infections Unlike previous compounds, the action of this compound on the body is prolonged. In the United States, Louise Pearce developed another compound which has been very useful in treating some of the human trypanosome infections This substance is known as *tryparsamide*, which is the sodium salt of N-phenylglycine-p-arsonic acid.

The compounds mentioned above are effective, some on one type of trypanosome, some on others In general it seems to be true that drug treatment of human trypanosomiasis has been more effective than in those of animals. *Trypanosoma brucei* infections of animals have proven especially refractory to treatment All of the drugs mentioned have been used on animals with varying success. Tartar emetic, administered intravenously, probably is the most common type of treatment for animals Although this drug often does not eliminate the disease, it serves to reduce the number of parasites and improve the condition of the animals When relapses occur later, as they usually do, a new course of intravenous injections is given.

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CHAPTER XXXV

THE MASTIGOPHORA (*continued*)

The Trichomonads

The trichomonads are flagellates characterized by the possession of a variable number of flagella, a definite cytostome (an opening into the cytoplasm through which food particles are ingested), and a hyaline rod-shaped supporting structure known as an *axostyle* which arises from the blepharoplasts anteriorly and passes backward through the body to the posterior end from which it usually protrudes In some species one flagellum is directed backward and there is a well developed undulating membrane These organisms frequently are quite pleomorphic and may easily be confused with other types of flagellates The body usually is more or less pear-shaped Reproduction is by means of binary fission Under unfavorable circumstances the organism may assume a spherical form surrounded by mucoid material. This is known as encystment

Members of this group are rather widely scattered through the animal kingdom as parasites, involving many invertebrates as well as vertebrates Many forms have been found in the intestinal canal of animals There is disagreement regarding the pathogenicity of some of these If they are pathogenic the disease-producing power is low. *Trichomonas hominis* occurs in the intestines of man Apparently it is relatively harmless *Trichomonas vaginalis* occurs in the vagina of women where it causes a vaginitis accompanied by a whitish secretion The disease is commonly called leucorrhoea. The species of importance in animal pathology are *Trichomonas fetus* in cattle, *Trichomonas gallinae* and *Trichomonas gallinarum* in birds.

TRICHOMONAS FETUS

Synonym: *Tritrichomonas fetus*

This organism causes early abortions, pyometra, and sterility in cattle. The parasite was recognized early in the 20th century in Europe but its significance was not appreciated until about 1925 In 1932, Emmerson (3) described the parasite and the condition caused by it in the United States for the first time. It quickly became apparent thereafter that the disease existed in nearly

all parts of this country, although the total of all infected herds apparently is not great

Morphology. This parasite normally is spindle- or pear-shaped but there is considerable pleomorphism, some forms appearing almost spherical. It averages about 16 microns in length, the variation being from 10 to 25 microns. The width usually is from one-third to two-fifths of the length.

There are three anterior flagella instead of the four which occurs in most members of the trichomonad group and because of this fact Riedmuller (8) proposed the generic name *Tritrichomonas*. These flagella originate in a blepharoplast located in the extreme anterior part of the body. They extend forward as free lashes which are approximately as long as the cell body. The posterior flagellum originates in the blepharoplast, runs along the free margin of the undulating membrane, and extends posteriorly as a free flagellum of about the same length as the anterior flagella. The nucleus is large and is located somewhat anterior to the center of the cell. The axostyle extends axially from the blepharoplast to the posterior end of the cell. It is a tubular structure with an expanded portion anteriorly. It projects from the posterior end of the cell as a pointed structure which ends in a slender spine. The mouth or cytostome is not easily seen. It is triangular when open, located at the anterior end of the cell, ventral to the blepharoplast. The cytoplasm of this species is rather dense and only slightly vacuolated. Rounded individuals are seen which superficially resemble cysts but it is doubtful if true cysts are formed. A detailed description of this species is found in the paper of Wenrich and Emmerson (9).

Good stained preparations are difficult to obtain. Wet fixation must be employed otherwise the cells are so shrunken and distorted as to be unrecognizable. In fresh preparations the motility is so great as to make accurate observations difficult. The type of motility exhibited is, however, so characteristic that an experienced observer can recognize them without difficulty. The organism rotates counter-clockwise as it progresses forward, jerkily, along an irregular path.

Cultural Features. *Trichomonas fetus* is readily cultivated artificially but there may be difficulty in obtaining pure cultures from exudates in which bacteria are also present. Glaser and Coria (4) made use of a semi-solid medium in U-tubes, for purposes of isolation. The motile flagellates outdistanced the more slowly moving bacteria and appeared in the opposite arm of the U-tube before the bacteria. Andrews and Lyford (1) used this method successfully. They also succeeded in obtaining pure cultures by inoculating

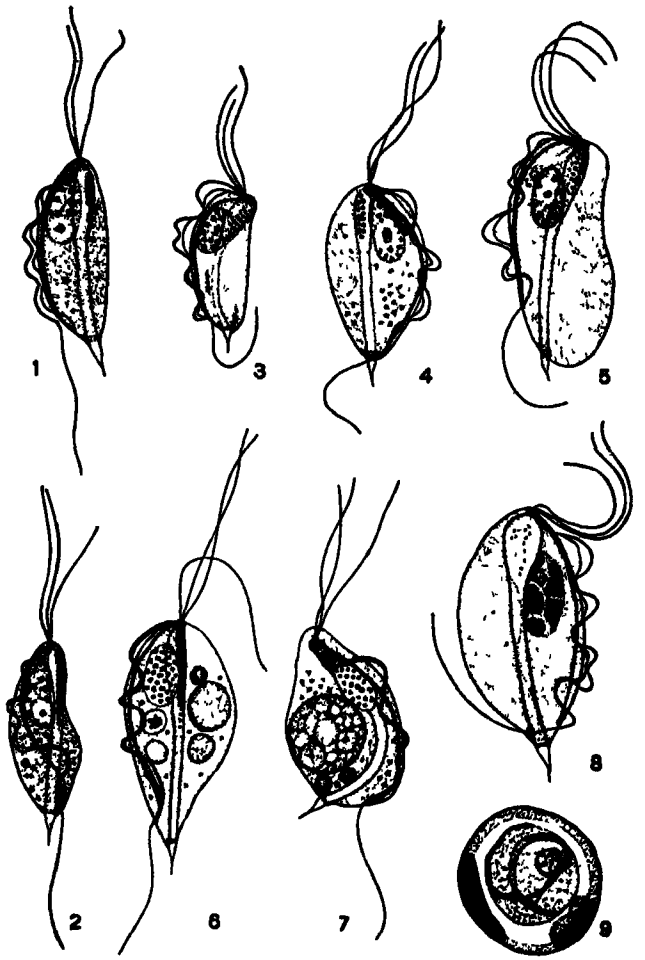


FIG 96 Morphology of *Trichomonas foetus* (1 to 3) Typical individuals 1 and 3, side views, 2, dorsal view, showing spiral course of undulating membrane

(4 and 5) Left side and right side views, respectively, showing parabasal body 5 shows cytostome

(6 and 7) Individuals showing included food bodies

(8) Individual stained with Giemsa Note heavy staining of nucleus and axostylar granules

(9) Leucocyte containing an ingested flagellate

Drawings were outlined with the aid of a camera lucida Magnification about x 2500. From plate by Wenrich and Emmerson (Courtesy of *Journal of Morphology*)

soft media in Petri dishes and depending upon the flagellates to migrate from the point of inoculation more rapidly than contaminating forms

For obtaining and maintaining pure cultures, coagulated whole egg slants upon which a few cubic centimeters of defibrinated blood mixed with Ringer's solution has been added, is satisfactory Loeffler's blood serum slants or liver infusion agar slants may be substituted for the egg medium Growth occurs in the fluid part of the medium The appearance of the parasite is essentially the same as that of parasites growing in exudates Whole blood is not necessary for growth Serum may be substituted, or both serum and blood may be eliminated The organism will grow actively for a short time in serum dextrose broth Morisita (6) found whole egg slants covered with neutral broth containing 5 per cent dextrose to be a favorable medium

The best incubation temperature is from 30 to 37° C Acid and gas are produced in dextrose, lactose, maltose, galactose, mannose, saccharose, raffinose, dextrin, and starch media Glycerol, adonitol, dulcitol, mannitol, and sorbitol are not attacked Cultures on egg-Ringer-blood medium will remain viable for about one month Those on simpler media often die out in a few days

Nelson (7) succeeded in propagating *Tr fetus* in the allantoic cavity of the developing chick embryo Profuse growth was obtained About 25 per cent of the embryos died within the first four days

Pathogenicity. This flagellate is transmitted as a true venereal infection in cattle The cow is infected at the time of coitus from an infected bull, or vice-versa (2) The disease may rarely be spread in other ways

The affected cow may show some evidence of vaginitis shortly after infection occurs but usually this is overlooked The infection begins in the vagina and soon invades the uterus As a result of this the animal may fail to conceive If conception occurs the animal may abort as a result of the infection within two to four months In other cases the fetus dies but is not discharged, in which case it becomes macerated and lies in a thin, nearly odorless fluid in which many trichomonads often may be found, and from which pure cultures may be obtained

Non-pregnant cattle may readily be infected by introduction of the parasite into the vagina but in these cases the disease is transitory and the flagellate can rarely be found after one or two months The disease tends to die out rather quickly in the infected cows but is maintained in herds by infected bulls in which the disease becomes chronic In bulls the organism is found in the prepuce and on the penis In some cases the epididymis and vas deferens becomes involved.

Immunity. Nothing is known about immunity to this disease. The disease ordinarily is controlled by disposing of infected bulls and withholding all breeding operations on infected females for three months.

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TRICHOMONAS GALLINAE

Synonyms *Tr columbae, Tr diversa*

A disease of young pigeons caused by a trichomonad was first described by Rivolta, in Italy, in 1878 This parasite has long been known under the name of *Trichomonas columbae* but recently Stabler (6) has called attention to the fact that the name *Cercomonas gallinae* applied by Rivolta had priority, so far as the specific name was concerned, therefore it should be known as *Trichomonas gallinae*

A disease affecting the upper digestive tract of young turkeys and causing serious losses at times has been known for some years to be caused by a trichomonad Volkmir (8) who suggested the causative connection of the flagellate to the disease process suggested the name *Trichomonas diversa*, by which it has been generally known Comparing the organisms from pigeons and turkeys, Stabler (7) could find no significant differences Furthermore he found that he could readily infect turkeys with strains derived from pigeons, hence he concluded that the two organisms are identical.

Levine and Brandly (4) encountered a disease of young chickens which resembles that of turkeys quite closely. They concluded that the causative agent, a trichomonad, was probably the same as that which occurs in pigeons.

Morphology. This organism is nearly round as a rule. Occasionally it is pear-shaped It measures from 7 to 10 microns in diameter Active motility is maintained by means of three or four anterior flagella, and one posterior

which runs along the free margin of the undulating membrane as far as the posterior end of the cell. In this species the flagellum does not extend beyond the posterior end of the cell as a free lash as it does in most species.

Cultural Features. Waller (9) cultivated this organism in Locke's solution to which 5 per cent dehydrated Loeffler's blood serum had been added. Evidently most of Waller's cultures contained bacteria. He states that the presence of bacteria is not detrimental to the trichomonads providing the cultures are transferred every 24 hours Pure cultures were obtained by Cauthen (1), Stabler (7) and others In Locke-egg-serum medium, and modifications, this flagellate grows readily.

Pathogenicity

FOR PIGEONS The disease occurs principally in squabs from three weeks to several months of age The most conspicuous lesions are in the liver This organ is enlarged and congested The specific lesions consist of necrotic areas of a yellowish color, varying in size from microscopic to four or five centimeters in diameter These are is occur throughout the liver tissue but are readily seen on the surface of the organ Here they are slightly depressed and smooth The peritoneum of the body cavity is often eroded and contains a sero-sanguineous fluid Lung lesions are seen in some cases These are somewhat like those in the liver but are softer and darker in color Both solid lesions and the fluids contain many trichomonads

This organism occurs commonly in the crop and mouth of older birds where it appears to do little or no harm Occasionally ulceration of the crop occurs Waller believes that the squabs become infected from the adults through the "pigeon milk," a fluid secreted by the glands of the crop, with which the old birds feed their young

FOR TURKEYS This flagellate is believed to be the cause of an unusual type of erosion of the mucosa of the crop, the upper and lower esophagus and sometimes the back part of the mouth in turkeys The disease is seen most often in mature birds but Hawn (2) noted that the young birds (poults) were much more susceptible to inoculation than older birds and suggested that the low incidence of the natural disease in young stock is due to the unusual protection which is ordinarily given, in not allowing them to associate with older birds and in keeping them off of ground on which birds have previously been raised This disease was first described by Jungherr (3) in 1927 but he ascribed the disease to the activities of a fungus The trichomonas associated with the disease was first described by Volkmar (8) who, however, presented little evidence linking the parasite with the disease Hawn (2) furnished the

evidence upon which this protozoan is believed to be the causative agent of the disease.

This disease has been seen in many parts of the United States and probably occurs wherever turkeys are raised The losses sometimes are very great The flagellate causing the disease has been known by the name applied to it by Volkmar, *Tr diversa*. It is not entirely certain that the species found in turkeys is the same as that of pigeons but it seems best, for the time at least, to regard them as identical

The early lesions in turkeys are found in the crop and upper esophagus. They appear as small, whitish nodules located in the mucous glands and varying in size from 0 5 to 2 mm in diameter. The content is semi-caseous and can be expressed by pressure The lesions may be so numerous that they may be scraped off from the epithelium as a single layer quite easily. The characteristic older lesions appear as necrotic areas of grayish color These become conical, horny growths which project well above the surface of the mucosa and are tipped with thorn-shaped processes from 1 to 3 mm in length. Large areas of the mucous membrane may become necrotic The lower or thoracic esophagus sometimes becomes totally occluded by masses of such material The crop usually contains a viscid, colorless mucus with a foul odor This fluid usually is rich in flagellates

Hawn succeeded in setting up the characteristic disease in 30 out of 56 turkeys by feeding them with cultures of *Tr gallinae* which had been kept in cultures for periods varying from two days to seven months The cultures were contaminated with bacteria, but such cultures in which the flagellates had died never produced the disease, hence the author felt sure that the pathogenic agent was the trichomonad

FOR CHICKENS Levine and Brandly (4) encountered a disease which destroyed a large number of pullets on one farm Small, multiple lesions, necrotic foci, were present in the mucosa of the crops of some of the birds, larger lesions with thickening of the crop wall were seen in others, and in still others the crop appeared to be normal In all, trichomonads indistinguishable from *Tr gallinae* (*columbae*) were found Attempts to transmit the infection to chickens, chicks, turkeys, and pigeons failed except in one case in which a large caseous lesion appeared in the crop of a pigeon four weeks after feeding

FOR OTHER BIRDS Cauthen found this trichomonad present in a large percentage of ring doves and mourning doves held in captivity on a farm with pigeons, and Stabler (5) found large numbers in the crops of five hawks. Since these prey upon pigeons, he believed the infection to have been con-

tracted from pigeons. Several workers have suggested that the pigeon may be the normal host of *Tr gallinae*, and that all infections of other species are derived directly from pigeons.

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TRICHOMONAS GALLINARUM

This species, according to Allen (2), is associated with a disease resembling blackhead in turkeys and chickens (See *Histomonas meleagridis*) She claims that trichomoniasis occurs as a separate disease, and that it is also often associated with histomoniasis, the two parasites developing in the same lesions According to her the liver lesions of trichomoniasis are yellowish and less well defined than those of true blackhead and furthermore they are raised above the liver surface rather than depressed The general resemblance of the lesions of trichomoniasis to those of histomoniasis is close enough, however, to cause confusion in diagnosis In mixed infections the lesions are similar to those of true blackhead in that they have depressed centers and a mottled appearance, but their margins in these cases are raised and have a nethke appearance Allen contends that it is possible to distinguish by gross inspection alone between the liver lesions caused by the pure blackhead infection, the pure trichomonad infection, and mixed infections In addition it is possible to cultivate the trichomonad rather easily, whereas the histomonad is refractory to cultivation in pure culture The organisms may also be distinguished morphologically in films from the lesions

By feeding pure cultures of the trichomonad isolated from liver lesions, Allen produced 10 cases of disease in young poults out of a group of 75 Both cecal and liver lesions were present The blackhead parasite could not be found in these lesions but the trichomonad was recognized and recovered in culture. On the other hand she produced 24 cases of true blackhead by feeding cultures of *Histomonas meleagridis* to 26 poults In the lesions in these birds the histomonad was present but not the trichomonad.

Tr gallinarum, according to Allen, is usually found in the ceca of turkeys and only occasionally enters the liver Chickens carry it in their ceca but only very rarely develop liver lesions In very young poults and chicks, a severe cecal diarrhea sometimes occurs which she believes to be caused by this trichomonad Allen suggests that the severe losses of chicks in flocks in the northwestern states, described by Weinzirl (3) and attributed by him to a parasite which he called *Tr pullorum* probably was due to *Tr. gallinarum* The following morphological facts about this parasite are taken from a detailed description by Allen (1).

Morphology. The cells usually are nearly spherical, some are pear-shaped. They average 5 by 6 6 microns There are five anterior flagella and another that runs along the border of the undulating membrane and ends in a free lash The parabasal body is elongated and located along the base of the undulating membrane The blepharoplast consists of a small group of granules located at the anterior end of the cell Below the blepharoplast and nucleus and located on the side of the cell opposite from the nucleus is the cytostome, a small curving opening The nucleus is round or oval The parasite has a characteristic type of movement which serves to distinguish it from other trichomonads It shows rapid jerky motions which causes the cell to turn from side to side and often to spin around but little or no progress from place to place is made This is in sharp contrast to *Tr gallinae* which moves so rapidly as to make it difficult to follow under the microscope

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Hexamita Infection in Birds

The *Hexamitae* constitute a group of flagellates which differ from all others in that the nucleus and other structures are duplicated This makes them bilaterally symmetrical

The *Hexamitae* have pear-shaped bodies Six flagella arise from the anterior end and are directed forward, two arise posteriorly Dujardin who first described a free-living protozoon of this type thought that there were only four anterior and two posterior flagella, six in all, and this was the reason for the name which he coined for the group The name has been retained although it is known that there are, in reality, a total of eight flagella.

Members of the group are found free-living in stagnant water and others occur in the intestinal tract of frogs and mice. The only species of any importance in animal pathology, so far as is known, is the one described below.

HEXAMITA MELEAGRIDIS

This species is thought to be the cause of an enteritis of young turkeys A similar disease was described by McNeil, Platt, and Hinshaw (5) in quail and Chukkar partridges and a similar organism found in them The turkey disease had been recognized as an entity by many workers in various parts of the United States and had been generally regarded as a trichomoniasis The causal relationship of the *Hexamita* to it was recognized by Hinshaw, McNeil, and Kofoid (1) in California in 1938

Morphology. This organism measures about 2 5 by 6 microns but varies considerably in size and shape The flagella are typical in form and number There is no undulating membrane and this serves to distinguish it from the trichomonads The lack of an undulating membrane also is responsible for a different type of motility, active, but lacking the rotating movement of the trichomonads This species also lacks the axostyle with its posterior projecting "tail-piece," so characteristic of trichomonads

Cultural Features. There have been no reports of the artificial cultivation of this species.

Pathogenicity. Hinshaw, McNeil, and Kofoid (1) (2) claim that this organism is the cause of a disease they and other authors previously had regarded as a trichomoniasis The disease affects young turkeys and may be the cause of serious losses The affected poults are listless, they require more heat than usual, and their droppings are foamy or watery, or both Greatest losses occur in birds from three to five weeks of age The course of the disease in individuals is from one to six days The mortality varies from 20 to 90 per cent Birds which recover lose a great deal of weight In the flock the disease usually runs its course in about three weeks

The crop usually is empty and the intestinal content is thin and watery and usually contains gas bubbles The ceca frequently appear enlarged and the wall of the entire intestine is flabby from lack of tone The specific lesions are in the upper part of the intestine (duodenum and jejunem) Areas of inflammation are located here and frequently regions of bulbous expansion of the intestinal wall are seen The other organs show no evidence of disease

In the upper part of the intestine only *Hexamita* is found but in the ceca

trichomonads and other species of flagellates occur as well and tend to obscure them. The *Hexamitae* have been found constantly in the bursa of Fabricius. In convalescent cases the parasite disappears from the upper bowel but tends to persist in the lower in association with other flagellates Adult birds two years old have been proved to be carriers

Inoculation experiments using material from naturally infected birds were carried out by Hinshaw, McNeil, and Koford (2) The disease was reproduced regularly when *Hexamita* was present and never when various other flagellates, including the trichomonad which formerly was believed to be the causative agent, were present without the *Hexamita* The authors feel that the failure to recognize the causative agent earlier was due to the fact that most workers had been studying the protozoan fauna of the lower bowel where the trichomonads are the most numerous and most conspicuous parasites They evidently feel that the trichomonad is purely parasitic and has nothing to do with the causation of the disease McNeil and Hinshaw (5) showed that young chicks can easily be infected with *Hexamita meleagridis* The disease in chicks is relatively harmless but they remain carriers of the infection for a long time and may be the source of infection for turkeys

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HISTOMONAS MELEAGRIDIS

This protozoon is regarded as the causative agent of infectious enterohepatitis or "blackhead," a destructive disease of young turkeys The same disease occurs in chickens pheasants, quail, and pea-fowl but these species are rather resistant and large mortalities are seldom experienced in them They often are the means of introducing infection into lots of turkeys, however.

The systematic relationship of this organism to other protozoa has never been definitely settled Originally it was thought to be an amoeba but later it was found to be a flagellate Wenyon classifies the organism in the family *Monadidae*, the simplest of the flagellates, which are practically amoeba-like organisms except that they have rudimentary flagella. Amoeboid motility occurs as well as flagellar motility The flagella arise in a blepharoplast which is located on the nuclear membrane, or at least very close to the nucleus.

Morphology. The organism is seen embedded in solid tissues as small spherical bodies from 8 to 10 microns in diameter A few cells are larger. These cells possess a rather small nucleus, usually located excentrically, and a small "extranuclear" body which was noted quite early (19) but whose function was not known until recently

When small bits of tissue containing the parasites are placed on a warm slide and crushed in warm fluid, the organisms can be found in abundance. They are quite transparent Amoeboid activity can readily be seen if the slide is kept at a temperature of 42° C, which is the body temperature of the bird, but there is little if the temperature is kept at 37° C This usually is the only type of motility exhibited by parasites removed from the tissue lesions If the material is taken from the lumen of the intestine (cecum) the parasites resemble those seen in tissue preparation except that a different type of motility is present The organisms rotate in a jerky manner, always going anti-clockwise, each sudden jerk turning the cell approximately 45 degrees Tyzzer (14), who first observed this motion, correctly decided that it could be caused only by a kinetic apparatus within the cell It is a flagellar motion, quite different from the amoeboid motion exhibited by the tissue parasites Tyzzer noted that when warm-stage preparations were left standing for several hours, the original amoeboid activity gradually gave way to the pulsating, jerky variety. Properly stained preparations indicate that the cells living free in the intestinal lumen have one, sometimes three or four, flagella These are not seen on the tissue inhabiting parasites

The small "extranuclear" body seen by early observers is the blepharoplast This is located on the nuclear membrane or near the nucleus The flagellum or flagella arise from it These are rudimentary structures which often do not protrude beyond the cell wall There is no undulating membrane or anything comparable to an axostyle or a cytostome

The parasite of blackhead was first described by Theobald Smith (11), who gave it the name *Amoeba meleagridis*, since it resembled amoeba more than any other type of protozoa Hadley believed the organism to be a stage in the life cycle of a coccidium and then changed his mind and decided it was a trichomonad Tyzzer (14) showed that it was not any of the types which it had previously been considered to be but differed from any other protozoon known, hence he created a new genus for it, *Histomonas*

Cultural Features. The first to cultivate the protozoon of blackhead was Drbohlav (7) The medium used was blood agar slants covered with Locke's solution, and coagulated egg medium covered with the same solution Later he found that slants made of coagulated egg albumin covered with a blood broth containing 1 per cent peptone was better than either of the original

media Cultures were obtained from the cecal content and always contained bacteria upon which the *Histomonas* feeds. At times the protozoon also ingested blood cells He did not succeed in obtaining pure cultures. Tyzzer (16), DeVolt and Davis (6), and Bishop (2) have reported success in cultivating the blackhead organism using methods similar to those used by Drbohlav. DeVolt and Davis found that the addition of a small amount of serum and a little rice starch to the coagulated egg medium gave better growths but care had to be observed that the bacteria did not overgrow the protozoa Transfers were made every 48 hours and incubation was carried on at 42° C. If the bacteria appeared to be overgrowing the parasites, one or more transfers were made on egg medium covered with plain Locke's solution, in which the bacteria do not thrive Cultures have been maintained by several workers for more than one year, and infections have been produced with them by rectal injections No one has so far succeeded in obtaining cultures free of bacteria Cultures from the liver lesions usually do not succeed

In dealing with cultures one has to be on his guard against interpreting other flagellates as *Histomonas* In practically every normal ceca there are flagellates belonging to the genus *Chilomastix*, and amoeba and trichomonads are frequent All of these will develop in the mediums described above

Histomonas in cultures has the same appearance as the motile forms found in feces If examined on a warm stage, the large spherical forms show the pulsating motion and the jerky anti-clockwise movement already described. Sluggish ameboid activity may also be observed

Pathogenicity. Turkeys are native American birds, at one time roaming the North American continent from southern Mexico to the northern border of the United States The domesticated types of today are certain improved varieties originating in Mexico Turkeys have been exported to all parts of the world, and blackhead has followed these birds This disease has been known in America since domestication of the bird began In more recent years it has been found in various European countries, in South Africa, Brazil, Japan, the Philippine Islands, and Australia The disease probably exists wherever turkeys are raised

This disease has been the principal reason for the difficulties encountered in successfully raising this species in flocks Under flock conditions the disease tends to build up in intensity from year to year, and after a few seasons it becomes economically impracticable to raise these birds without using special methods of husbandry

The disease is seen most often in young birds, that is, in birds varying in age from three weeks to four or five months Losses occur in old turkeys but these usually are sporadic The losses among young turkeys frequently exceed 50

per cent and sometimes all of the young birds die. The losses among chickens are almost wholly in those of three to five weeks of age Serious losses sometimes occur but generally the disease disappears spontaneously after a few birds have been lost. An occasional case is seen among adult chickens

Affected turkey poults are inactive and refuse their food They lose weight and walk with a stilted gait There is diarrhea with light sulphur-colored droppings They usually show symptoms for a number of days before they

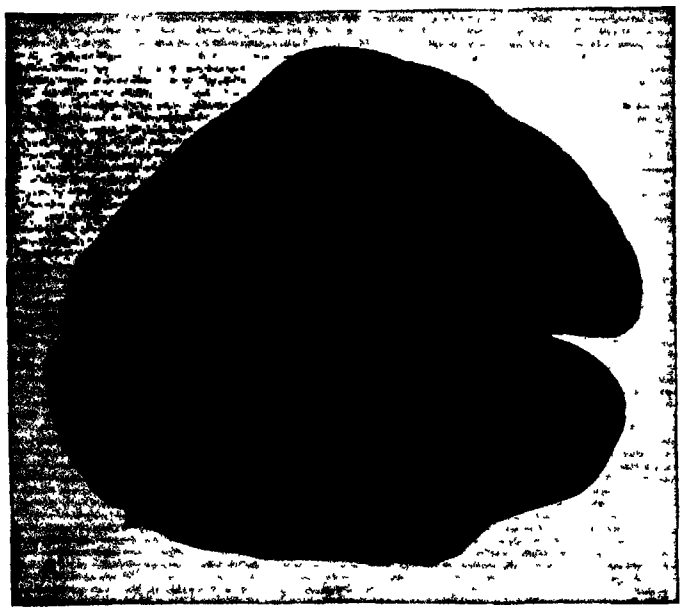


FIG. 97 Lesions of Blackhead (Histomoniasis), Liver, Turkey

die In chicks the disease runs a short course The birds are depressed, they gather around the brooder stove, pass bloody droppings, and soon die.

The lesions of blackhead occur in the ceca and the liver Other organs as a rule are normal Sometimes there is fluid in the body cavity. The lesions have the same appearance whether in chickens or turkeys

The affected ceca are enlarged and their walls are thickened. Usually a core of grayish-yellow necrotic material mixed with excrement fills their lumen. When this core is removed the wall of the organ is seen to be necrotic

The liver lesions are quite characteristic in appearance. They consist of round necrotic areas, varying in diameter from microscopic size to several centimeters. They appear on the liver surface as smooth areas, somewhat

firmer than normal liver consistency, and somewhat depressed or concave. The color is mottled with green, yellow, and brown intermixed. Often concentric rings occur. Outside of these areas, the liver substance is very dark and the entire organ is enlarged

Allen (1) claims that *Trichomonas gallinarum* sometimes produces cecal and liver lesions which may easily be confused with those of blackhead and also that this trichomonad often is associated with the blackhead organism in the lesions of the liver She describes three types of liver lesions. (a) Those caused by pure infections with *Histomonas meleagridis*, already described above, (b) Those caused by pure infections with *Tr gallinarum*, which produce yellowish lesions up to 2 cm in diameter but which have less well defined borders than those of true blackhead and are raised rather than depressed with respect to the liver surface, and (c) Those caused by mixed infections with these two organisms which are large, circular, well defined lesions, mottled in color and with markedly depressed centers but raised borders Allen claims that it is possible to distinguish these three types of lesions from one another by gross inspection When in doubt both organisms are easily identified in preparations made from the lesions For further consideration of this subject, see *Trichomonas gallinarum*

Microscopic examination of the cecal wall shows myriads of the characteristic parasites located free in the tissue spaces They are never found intracellularly except an occasional one which has been phagocytosed and probably is dead In ordinary sections the parasites usually have shrunken and appear to lie in spaces a little larger than their own diameter They stain with the acid dyes and their nuclei are poorly stained and often not visible The numbers are so great as to account for a considerable part of the thickening of the cecal wall Necrosis probably is due as much to pressure as to toxic materials which they may secrete Parasites may be seen in the necrotic material in the lumen of the organ These have the same appearance in sections as those embedded in the tissues. When examined in fresh preparations these forms show flagellate motility whereas those from the tissues usually show the amoeboid type

The liver lesions are caused by parasites which escape by the blood stream from the cecal lesions The parasites multiply in the sinusoids of the liver, causing pressure and possibly toxic necrosis of the neighboring liver cells. The lesions attract large numbers of lymphocytes in the early stages Necrosis occurs and gradually the lesions enlarge The older lesions consist of myriads of parasites embedded in necrotic tissue Epithelioid and giant cells usually appear in limited numbers in the older lesions The necrotic tissue is surrounded by a mild inflammatory zone but there is no capsule formation.

Mode of Infection. *Experimentally, blackhead is not easily produced by the* feeding of tissues or discharges of acutely infected birds. If large amounts of material are given, a few birds will be successfully infected The disease can be produced much more regularly by injecting infective material per rectum. Very young poults and chicks can be infected fairly regularly in this way If infected birds are placed among normal birds on clean ground, i e, ground upon which chickens and turkeys have not previously lived, the disease will not spread (12) If old turkeys or chickens are allowed to run with young turkeys, or if the young turkeys are allowed to run on ground which had previously been used for raising either chickens or turkeys, severe outbreaks can be expected

These peculiarities were explained by the finding of Graybill and Smith (9) that a common nematode, belonging to the ascarid family and having its habitat in the ceca of turkeys, chickens, and other birds, *Heterakis papillosa*, played an important part in the transmission of this disease These parasites, living in the lumen of the ceca, ingest the protozoon of blackhead in considerable numbers The protozoon penetrates the intestinal wall of the worm and reaches its body cavity These stages may readily be followed with the microscope The succeeding stages have not been followed microscopically but apparently the organism reaches the ovary and in some form exists in the ova of the worm The evidence for this was furnished by Tyzzer and Fabyan (19) who showed that ova which had been embryonated in 1 5 per cent nitric acid, which renders them bacteriologically sterile, were capable of producing blackhead when fed to young turkeys Tyzzer (15) and others have studiously examined many infected worm eggs without being able to find any morphological evidence of the protozoon The Japanese investigator, Nimi (10), claims to have found minute forms varying from 1 to 1 4 microns in diameter in infected worm eggs which he believes to be the protozoon

The cecal worm is distributed widely and is present in practically every adult chicken, turkey, pheasant, grouse, and other species which are susceptible to blackhead Apparently also the protozoon of blackhead is widely distributed among adult birds of these species, and therefore, most of the ova of the cecal worm carry the parasite of blackhead To produce blackhead experimentally one has only to feed fairly large doses of the embryonated eggs of the cecal worm, or to place the birds at their most susceptible age on soil which is infected with such ova as a result of having been used previously for poultry range The feeding of embryonated *Heterakis* ova collected from single adult birds will not always produce blackhead, thus indicating that not all *Heterakis* ova are infective, yet infection seldom fails when ova from several birds are used whether or not there has been clinically recognized

blackhead in the flock from which the turkeys originated. Apparently the damage caused by the localization of the young nematodes in the cecal pouches provides a portal of entry into the cecal wall for the blackhead parasite. The feeding of blackhead material without the worm eggs usually fails to infect, presumably because of the lack of the preliminary damage from the larval worms

Outside the host, the protozoon of blackhead will maintain viability only for a few hours It has been well demonstrated, however, that the infective agent of the disease will remain alive in the soil of poultry yards for many months and may survive one or more severe winters It is obvious that these persistent forms are those which are harbored by the *Heterakis* eggs

Experimental Blackhead. Tyzzer and Fabyan (18) demonstrated that an experimental disease of turkeys, different from the natural form, could be produced by the subcutaneous injection of material containing the *H meleagridis* This type of the disease can be propagated indefinitely This disease is manifested by the development of a local lesion at the point where the inoculum has been deposited This can be seen after a few days and then rapidly develops into a granulomatous lesion of considerable size After several weeks metastasis occurs to the lungs where several lesions usually develop. From the lung lesions tertiary lesions in many organs develop if the bird lives long enough to permit it The disease is always fatal. It is characterized by weakness, loss of appetite, weight loss, the appearance of sulphur-colored feces, all of which are characteristic of the natural disease, and in addition, coughing and dyspnea The lesions contain myriads of the characteristic parasites

In chickens, subcutaneous inoculation results only in the production of a local lesion which rapidly heals Similar lesions develop in about one third of inoculated pigeons Rabbits, guinea pigs, and mice are not susceptible to such inoculations

DeVolt and Davis (6) produced what they called an "histomonal sinusitis" by placing small bits of infected liver tissue into the facial sinus Lesions developed causing distortion of the facial bones, metastasis to the lungs, and death The whole picture is similar to that produced by subcutaneous inoculation

Immunity. Tyzzer (16) (17) has succeeded in conveying a considerable degree of immunity to young turkeys by inoculating them with a culture strain of *H meleagridis* which had become avirulent About two weeks after inoculation with the vaccine strain, both turkey poults and chicks showed a high degree of immunity to natural infection with fully virulent strains. The im-

munity is lost rather early unless the birds are exposed continuously to natural infection. In birds which have lost their immunity a high percentage of recoveries indicate that all resistance has not been lost

Chemotherapy. Various attempts at chemotherapeutic treatment have failed. Recently Blount (3), in England, and Bolin and Vardiman (4), in this country, have reported considerable success with an arsenic-containing drug called "marphasan" The drug must be administered individually, hence it is not likely to become popular.

Surgical Prevention. Durant (8) has shown that blackhead can be prevented by surgical abligation of the ceca This involves opening the body cavity and ligating the ceca so there is no connection thereafter with the remainder of the intestinal lumen Delaplane and Stuart (5) vary Durant's procedure by the use of aluminum clamps which accomplish the same purpose. The operations are attended with considerable surgical mortality and are time consuming, hence the methods are not likely to be used as practical procedures However, they are of interest as indicating that the walls of the ceca are the only natural points of entry of this infection.

Practical Control. Practical control of this disease has been accomplished by large growers through the institution of sanitary measures The young poults are incubator-hatched and kept in clean surroundings away from adult birds (turkeys and chickens) until they are well-grown Infections are thus prevented.

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CHAPTER XXXVI

THE SPOROZOA

General Considerations

The sporozoa constitute a group of protozoa which live a parasitic existence and many of which are highly pathogenic to their hosts Most of them live, at some stage in their life cycle, intracellularly The cycle of development is complicated by an *alternation of generation*. A period of repeated asexual multiplication (*schizogony*) finally terminates in the formation of sexually differentiated cells known as *gametes* The female gametes are fertilized (*syngamy*) by the male elements, the product of the union being known as *zygotes* It is the function of the zygotes to carry the infection into new hosts

In some instances as in the coccidia, for example, the zygote develops a protective membrane around itself and is then known as an *oocyst* The oocyst escapes from the host into the soil There, in the presence of moisture, and an abundance of oxygen the zygote multiplies by cell division (*sporogeny*) to form a number of *sporozoites* within the oocyst If the "ripened" oocyst is taken into a suitable host, the walls dissolve liberating the motile sporozoites each of which then penetrates an epithelial cell and another period of asexual reproduction follows

In other instances, as in the hemosporidia, the alternation of generation involves an *alternation of hosts* In these cases instead of escaping from the host into the soil the zygotes are taken up by blood-sucking arthropods in which sporogony occurs Eventually the sporozoites, the final products of sporogony, find their way into the salivary apparatus of the arthropod from whence they escape into a new host at the time blood sucking occurs

The damage to the vertebrate hosts results largely during the period of schizogony since this is a period of rapid multiplication, each generation giving rise to greater numbers of new individuals (*merozoites*) each of which enters and destroys another host cell

The sporozoa of interest to animal pathologists belong to the Sub-order *Eimeridea*, which includes the coccidia, the Sub-order *Hemosporidiidea*, which includes the malaria parasites and the Sub-order *Piroplasmidea*, which includes the organisms of Texas fever and East Coast Fever. In addition

there are a number of organisms of doubtful classification such as the anaplasmas, the toxoplasmas, and the sarcosporidia.

The Coccidia

Most species of coccidia are parasites of the intestinal epithelium In a few cases they occur in other epithelial organs. In many instances they cause extensive destruction of the intestinal epithelium which results in acute enteritis accompanied by diarrhea Such cases may result fatally, especially in young animals The severity of the disease depends in large measure upon the size of the infecting dose and the opportunities for multiple reinfections, since coccidia, unlike bacteria and many other pathogenic organisms, are not able to multiply without limit in the new host but are limited to a definite number of asexual generations after which they assume the relatively harmless sexual form If the host survives the acute stages while asexual multiplication of the parasite is occurring, it recovers but retains the infection for long periods and serves as a source of infection for others If the sanitary conditions are not good, animals usually become reinfected from their own feces and this serves to keep the infection alive in herds and flocks

In the process of schizogony the entire growth period is passed within the cytoplasm of host cells It begins when the host cells are penetrated by sporozoites released from ingested oocysts The elongated sporozoites are transformed in the host cells to spherical bodies which rapidly grow at the expense of the cytoplasm These are known as *trophozoites* or *schizonts* As they grow the cell nucleus is crowded aside and when the schizonts are fully developed there is little left of the host cell The cytoplasm of the schizonts undergoes multiple division forming numbers of smaller elements The schizont then ruptures releasing the smaller bodies which are known as *merozoites* These are motile and immediately seek out other cells which they penetrate, one merozoite into each host cell, forming a new crop of schizonts

After a number of generations of merozoites have been formed, the number varying according to peculiarities of the species, a crop of schizonts are formed which become transformed into *macro-* and *micro-gametocytes* which when mature are usually of about equal size Each microgametocyte gives rise to a large number of small serpentine elements which are known as *microgametes* These correspond to the male sperm cells Released by rupture of the parent cell, these seek out the macrogametes, the female elements, which they fertilize The fertilized macrogametes then secrete a thick capsule around

themselves forming oocysts. These are discharged into the lumen of the organ and thus escape from the host The oocysts are more readily identified than any other stage in the life cycle of coccidia and are commonly sought as a means of diagnosis

Two genera of coccidia occur among domestic animals The genus *Isospora* can be readily distinguished from the other, the *Eimeria,* by examining the

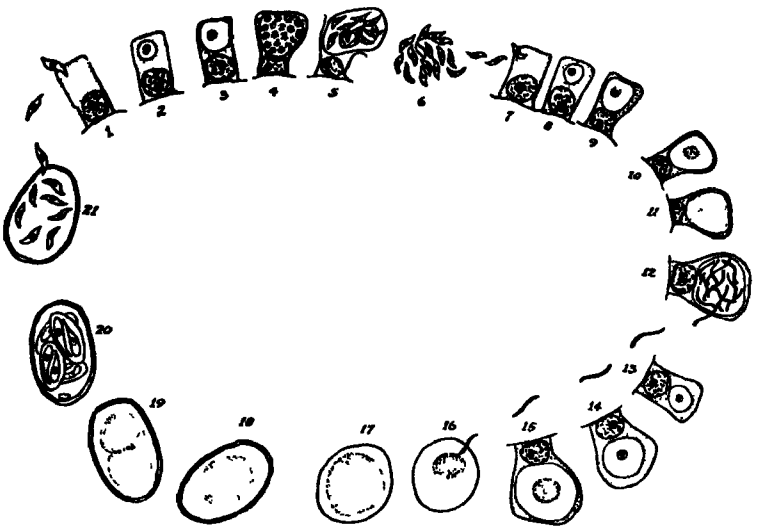


FIG 98 Diagrammatic Representation of the Life Cycle of a Coccidium (1) Sporozoite entering an epithelial cell (2, 3, 4) Schizonts developing in epithelial cells (5, 6 7) Merozoites released from the schizonts and entering other cells (8, 9) Second generation of schizonts developing in other epithelial cells (10, 11, 12) Formation of microgametocytes and release of microgametes (13, 14, 15) Formation of a macrogametocyte (16) Fertilization of a macrogamete by a microgamete (17, 18) Formation of a zygote or oocyst (19) Beginning of the process of sporulation of the oocyst Two cell stage (20) The fully sporulated oocyst Four sporocysts are present, each containing two sporozoites, in this instance, thus indicating that the species belongs to the genus *Eimeria* Members of the genus *Isospora* develop only two sporocysts, each containing four sporozoites (21) The release of sporozoites from the ripe or sporulated oocyst Stages 1–18 inclusive occur in a single host Stage 18 usually is eliminated from the body in the excretions Stages 19 and 20 usually develop in moist soil Infection of new hosts, or reinfection of the same host occurs through the contamination of food and water with stage 20 Stage 21 occurs in the new host through excystation of the sporozoites through action of the digestive enzymes

ripened oocysts. The cytoplasm of the oocyst of *Isospora* divides into two bodies known as *sporoblasts* each surrounded by a membrane known as a *sporocyst.* In each of these sporocysts four sporozoites are formed. In the

Eimeria four sporocysts are formed, each containing two sporozoites. In both cases, it will be noted, eight sporozoites are formed

HOST SPECIFICITY OF THE COCCIDIA

It was once believed that the coccidia were relatively non-specific so far as hosts were concerned, that is, that infections in one species might readily be transferred to another. It was thought, for example, that rabbits often infected domestic livestock It is now known that there is a high order of host specificity in this group and that infections seldom are contracted from other species This is especially true of members of the *Eimeria* which are responsible for the most serious infections of domesticated animals. Occasionally certain species may be found in closely related species of animals, but this seems to be the exception rather than the rule The members of the *Isospora* seem to be somewhat less specific than the *Eimeria*, thus we find certain kinds which infect both dogs and cats.

IMMUNITY IN COCCIDIAL INFECTIONS

There is definite evidence that animals develop immunity to coccidial infection Tyzzer showed that young chicks can be readily immunized by infecting them with small doses under conditions in which the birds are unable to reinfect themselves The mild infections run a definite course and clear up In some cases the bird may be infected a second, and sometimes several additional times but eventually it becomes so resistant that additional infections cannot be obtained with the same species Similar results have been obtained with dogs It is probable that this is a rule applicable to all coccidia. This idea is at variance with older ideas It has long been known, for example, that older animals of the species frequently harbored coccidia without showing any symptoms, and that these animals served as sources of serious epizootics among the younger animals which lived on the premises It was thought that these animals were chronic carriers, that the disease was not thrown off by such animals but that the parasites were carried in a low stage of activity. This may be true in some cases, but it is quite certain that the carrier state often is a result of constant reinfections, and that when such animals are maintained in such a way as to prevent reinfections they usually eliminate their infections completely.

Studying *Eimeria caviae* of the guinea pig, Henry was shown that these animals not only are immunized by a single contact with the infection but that they develop a skin sensitization to proteins derived from the oocysts. She was also able to show evidence of anaphylactic sensitization to these proteins

The Coccidia of Dogs and Cats

Coccidiosis of dogs appears to be a very common infection In practically all parts of the world where surveys have been made the incidence has been at least 5 per cent and in many places it has been much higher. Lee (3) reports that the incidence in the clinics of the Veterinary Division of Iowa State College over a ten year period was 13 8 per cent Of 320 dogs examined by Gassner (1), of Colorado State College over a short period of time, 79 per cent proved to carry coccidia All of the infections in these studies proved to be *Isospora*, the *I. bigemina* being the most frequently encountered. In Gassner's series, for example, *I bigemina* was found in 74 per cent of the dogs, *I rivolta* in 20 per cent and *I felis* in 6 per cent

In dogs, the infections with the *Isospora* are undoubtedly the most common A member of the *Eimeria, E canis* is encountered occasionally Yakimoff and Matschoulsky (6) found that the incidence of this species in Leningrad was about 2 5 per cent whereas *I rivolta* amounted to 14 per cent *E canis* has been reported in single cases by Honess (2), in Wyoming, and by Skidmore and McGrath (4) in Nebraska These authors think that the species may be more common than reports would indicate

There have been few reports of coccidial infections in cats Cats are susceptible to experimental infection with all three of the dog species, and natural infections with all three species have been reported Wenyon (5) states that *I felis* infections of young kittens is common in London Lee reports a single natural infection in an Iowa cat

It appears that the majority of coccidial infections of dogs and cats are light and that there is little evidence of serious damage to the hosts In some cases, however, there is diarrhea with excessive amounts of mucus in the feces, and occasionally there may be a bloody and even fatal dysentery. Evidently the severity of the disease is dependent upon the numbers of the coccidia present, since Lee has clearly shown that all of the *Isospora* are capable of causing serious damage when large doses are given experimentally

The differentiation of the parasites is relatively easy To distinguish between the *Isospora* and the *Eimeria* one needs only keep some fecal material containing oocysts a few days diluted with one per cent potassium bichromate solution (to prevent putrefaction) in a shallow dish The presence of four sporocysts in the oocysts, each containing two sporozoites, indicates that the organism belongs to the *Eimeria*, if there are two sporocysts each containing four sporozoites, it belongs to the *Isospora* The examination can be readily made with the 4 mm. or 16 mm lens of a reasonably good microscope. The differentiation of the species within the genus is more difficult. It is

most readily done by noting the characteristics and the measurements of the sporulated oocysts. The measurements of the *Isospora* as given by Gassner are as follows

TABLE XV

	Oocysts	Sporocysts
Isospora felis	40 – 42 × 31 – 32 microns	19 - 22 × 13 - 15 microns
I. rivolta	23 - 24 × 15 16 "	15 - 16 × 9 7 × 11 5 "
I bigemina (small variety)	10 – 12 × 8 – 9 6 "	7 8 6 × 8 5 - 9 "
I bigemina (large variety)	14 8 × 12 5 "	9 5 - 10 8 × 8 – 9 4 "

ISOSPORA BIGEMINA

This coccidium occurs in both dogs and cats, apparently being more common in the former Lee (3) was able to produce acute infections with a bloody diarrhea in cats with cultures derived from a dog He also succeeded in infecting a fox with a strain from a dog

Two types of *I bigemina* are recognized, one producing a small oocyst and the other a much larger The small variety is by far the more common Both ordinarily develop in the subepithelial tissues of the small intestine but Wenyon and Sheather found one acute case in which the epithelial cells were filled with schizonts and oocysts It is believed that during acute phases of the infection the epithelial cells are attacked and during the chronic stages the infection remains in the subepithelial tissues During the chronic stages of the disease the oocysts undergo sporulation in the tissues, frequently rupture there, and release the sporocysts which are found in the feces instead of the oocysts Immature oocysts may sometimes be found in the feces along with the free sporocysts but usually they are very scarce By purging his pa

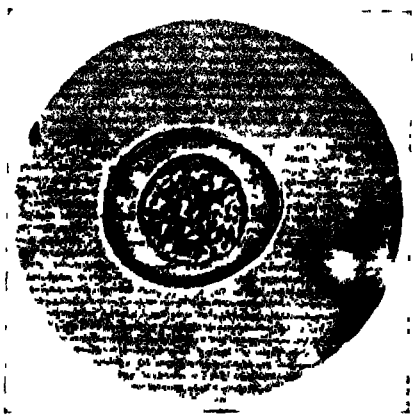


FIG. 99 *Isospora bigemina* Non sporulated oocyst of the common coccidium of the dog Unlike most coccidia, the oocyst of this species often sporulates while in the intestinal canal × 1100

tients with arecoline hydrochloride, Gassner found that he could force the appearance of oocysts in the feces

Lee found that when small numbers of sporulated oocysts were fed to dogs, a few oocysts were eliminated on the 6th or 7th day and elimination continued for about one week. No symptoms were observed. When larger doses were given, a profuse, hemorrhagic diarrhea began on the 3rd or 4th day, and oocysts were discharged in abundance on the 6th and 7th days One dog died of the disease, but the others recovered after a few days although oocyst discharge continued for several weeks Doses of intermediate size produced a catarrhal diarrhea, the feces often being streaked with blood Mixed infections of *I bigemina* with one or both of the other species of *Isospora* occur naturally, and experimentally such infections often are rather severe

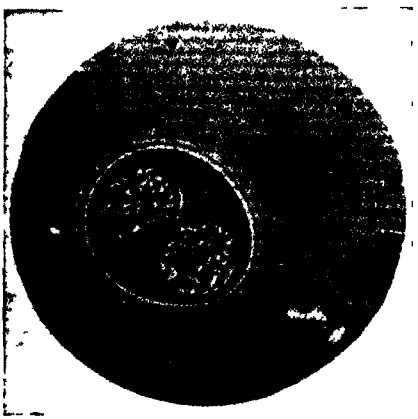


FIG. 100 *Isospora bigemina* Oocyst in early sporulating stage In this instance a dense membrane will form about each of the two cells and each will become a sporoblast containing four sporozoites This will identify it as a member of the genus *Isospora* × 1100

ISOSPORA RIVOLTA

The multiplicative stages of this species occur in both epithelial and subepithelial tissues of the small intestines of dogs and cats Lee, in his series, found them only in the epithelium near the tips of the villi The species is regarded as relatively harmless Coccidia indistinguishable from *I rivolta* have been found in a number of wild animals and in some cases infections of dogs have been produced with them This form is frequently found in clinical cases in association with *I bigemina.*

ISOSPORA FELIS

This species is easily distinguished from the others by its very large oocysts. As its name indicates it is found principally in cats but dog infections occur. Wenyon states that this species is common in London cats It has been found less frequently in the United States than the other two species and generally in mixed infections Pure infections in cats generally are relatively harmless although diarrhea may be produced The multiplicative stages are

found in the small intestine, being located in the epithelial cells near the tips of the villi Some are found also in the mucosa of the cecum. Lee reports a pure experimental infection in a dog. Following a rather large dose of sporulated oocysts by mouth, a bloody diarrhea began on the 6th day and continued until the 11th. Oocysts appeared on the 8th day and continued until the 29th day

EIMERIA CANIS

This species has been identified only on a few occasions It was first seen by Wenyon in London Two isolated cases have been described in the United States, by Skidmore and McGrath (4) in Nebraska, and by Honess (2) in Wyoming The Nebraska dog was said to be unthrifty and suffered from vomiting and diarrhea Blood was not detected in the stools Honess sporulated the oocysts from his case and fed them to two experimental dogs. Some of the oocysts were said to have been sporulated when passed After three days all had sporulated The number fed was not large, and the dogs showed no symptoms at any time Oocysts appeared in the feces on the 8th day in one case and on the 17th day in the other The cases were followed for two weeks during which time oocyst shedding continued

The oocysts were colorless, whereas Wenyon described those of the case with which he worked as being pinkish They were ellipsoidal and assymetrical, being more curved on one side than on the other The sporocysts were elongated The oocysts averaged 12 by 18 microns in size, the sporocysts 5 8 by 10 4 These measurements are smaller than those given by the European workers, but since otherwise there was agreement, Honess regarded his organisms as *E canis*

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The Coccidia of Rabbits

At least four types of coccidia affect the rabbit Of these, two types are common and destructive. One of these, *Eimeria stiedae*, was the first coccidium known.

EIMERIA STIEDAE

Young rabbits are especially susceptible to this species and nearly all deaths from it occur in animals less than four months of age. The mortality rate is especially severe in commercially raised rabbits, particularly when they are raised in hutches or confined spaces where opportunities for massive infections are great. Under such conditions heavy losses from this and other coccidial infections can be prevented only by scrupulous cleanliness, frequent changing of bedding, and feeding and watering from containers which cannot readily be soiled with the animal feces. Disinfection in this disease, as in all coccidial infections, can best be accomplished by heat, since chemical disinfectants have little effect upon the oocysts. Moist heat is very effective, but it should be borne in mind that if it is employed, means of thoroughly drying the hutches and cages should be sought since moisture favors the maintenance of viability of the sporulated oocysts.

Eimeria stiedae differs from the other coccidia of the rabbit in that it localizes in the bile ducts of the liver. The liver often becomes greatly enlarged, and white or yellowish lesions which resemble abscesses may be seen on its surface. In acute cases these may not be present. The gross lesions referred to are spherical or elongated and vary in size from minute to 2 cm. in diameter. When these lesions are relatively young they are filled with a thin whitish fluid, when older the content may be thick and caseous, when very old the lesions may be dense and even calcified. The fluid of the fresh lesions consists largely of oocysts, which may be as numerous as leucocytes in pus. In the caseous material they usually may be found but are not numerous, and in the inspissated, calcified material they are usually absent, having degenerated.

Sections of affected livers show that the bile duct epithelium, under the stimulus supplied by the multiplicative stage of the parasite, has proliferated and formed cystic adenomas, the linings of which are filled with every developmental stage of the coccidium. The epithelial growths push out into the liver tissue and become filled with fluid and the products of growth of the parasite, forming the whitish gross lesions already mentioned.

The oocysts escape from the cystic dilatations of the bile ducts to the intestine through the gall bladder and the common bile duct, and thus out of the body with the feces. In the presence of an abundance of oxygen and a little moisture they undergo sporulation in about 60 hours under the most favorable conditions. The oocysts are large and have a yellowish color. They average about 35 microns in length by a little more than half this dimension in width. One end is slightly flattened and here there is a thin place in the

capsule, the *micropyle*, which the sporozoites penetrate in escaping from the cyst. The cytoplasm of the cell is granular and ordinarily does not occupy more than one half of the space within the capsule, the remainder being empty. The sporocysts are quite elongated, measuring about 10 by 18 microns

Infection of the bile ducts of the liver occurs by the passage of sporozoites



FIG 101 Hypertrophied bile duct epithelium of a rabbit caused by infection with *Eimeria stiedae* A number of gametocytes and one oocyst are shown x 350

through the intestinal wall into the tributaries of the portal vein, which carries them to the liver When very heavy infections occur, the rabbits die within 3 to 4 weeks In more chronic infections death may be delayed for 6 weeks or more, or recovery may occur The affected animals generally become pot-bellied because of the liver enlargement, and diarrhea usually develops especially if they are obtaining much succulent feed.

EIMERIA PERFORANS

This species probably is just as common in commercially raised rabbits as the preceding but the evidence of its existence is not so conspicuous, thus it is not so well known As a matter of fact, this organism was confused with *E. stiedae* for many years Infections with this species can be distinguished from those of the liver-infecting variety by a simple fecal examination of the discharged oocysts. Those of *E perforans* are colorless, i e., they lack the yellow color of those of *E stiedae,* furthermore they are much smaller and more nearly spherical. They measure between 24 and 30 by 15 to 20 microns, averaging 15.5 by 25 5 microns The oocyst walls of this species are slightly thicker than those of *E. stiedae* Sporulation under favorable conditions occurs in 48 hours or less After experimental infections, oocysts appear in the feces on the fourth or fifth days, whereas those of *E stiedae* do not appear in the feces for six or seven days *E perforans* can be separated from *E stiedae* by feeding the mixed culture to a young rabbit and saving the fecal material discharged on the fourth and fifth days at which time only the oocysts of the first species will be present

Development occurs in the epithelial cells of the small intestine and, to some extent, of the cecum The small intestine, especially the duodenum, becomes dilated to several times its normal diameter The walls of the affected gut are pale and sometimes edematous Reddened streaks are found on the mucosa Sometimes almost every epithelial cell in parts of the intestines are infected

Diarrhea is characteristic of this disease, but if the animal is fed only on dry feed the feces may be soft rather than fluid The victim becomes pot-bellied, anemic, dehydrated, and in general presents a miserable aspect Death usually occurs within two weeks of the time when symptoms appear.

EIMERIA MAGNA

This organism originally was regarded as a variety of *E perforans* but now is regarded as a separate species. Like *E perforans* it occurs in the epithelial cells of the small intestine where it produces similar changes The oocyst is much larger than that of *E perforans* but about the same as that of *E stiedae.* Like the latter it is of a yellowish or even a brownish color It measures 28 to 40 by 20 to 26 microns A broad micropyle is located at the more tapering of the ends This species is not so widespread or common as *E perforans.*

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The Coccidia of Chickens

Among domesticated livestock coccidial infections do greatest damage to poultry Acute infections of young chicks frequently result in mortality rates approaching 100 per cent. The more chronic infections of older birds are not often fatal but result in malnutrition, unthriftiness, and decreased production.

Until Tyzzer (8) published his first work on the differentiation of the coccidia of chickens in 1929, it was thought that there was only a single species, and this was known as *Eimeria avium* Tyzzer called attention to the fact that Railliet and Lucet had differentiated one species adequately in 1891 and had given it the name *E tenella* This species was confirmed by Tyzzer, and three additional, *E acervulina, E maxima* and *E mitis* were named and described. In the following year, 1930, Johnson (3) differentiated two additional species, *E necatrix* and *E praecox* Levine (5), in 1938, added *E hagani* Thus what formerly was regarded as a single organism now has been differentiated into seven distinct species Of these *E tenella* and *E necatrix* are highly pathogenic and destructive parasites, *E maxima,* and *E hagani* possess virulence of a medium grade and generally are associated with chronic infections, whereas *E acervulina, E mitis* and *E praecox* are nearly harmless. All of these species, except possibly *E hagani,* for which data are not available, are widespread Several species often occur simultaneously in a single fowl, in fact this situation generally exists in the infections of older birds *

All of the coccidial infections of birds are self-limiting, that is, birds that survive the original acute attack will recover completely and eliminate all traces of the infection, providing means of reinfection are eliminated Chronic coccidiosis of birds, therefore, is a disease kept alive by repeated reinfections of the same species, or by repeated infections with different species Immunity of a rather high order is produced by one or two light infections with some species; with others a number of reinfections is necessary. In all cases apparently enough resistance is established eventually to wholly prevent reinfection or to make them so slight as to be symptomless. Both Johnson and Tyzzer have shown that age is not the essential factor in immunity to *E tenella,* an organism which causes severe infections in young chicks but does not often affect adults When birds were reared in the laboratory free of all coccidial infection, they were found to be susceptible to *E tenella* infection when they were more than two years old Infections do not ordinarily occur in old birds reared naturally because almost invariably they

* Since this was written, P P Levine has discovered and differentiated an eighth species of *Eimeria* in the chicken The description will be published soon A photograph of the sporulated oocyst is included in Fig 102 This species has been named *E brunetti*

come in contact with the organism in repeated small doses earlier in life and are immunized by such contacts. As in other coccidial infections, whether or not symptoms of the disease develop depends upon the size of the infecting doses. The practical control of these diseases depends upon keeping the environment of the birds sufficiently clean so they will not pick up massive

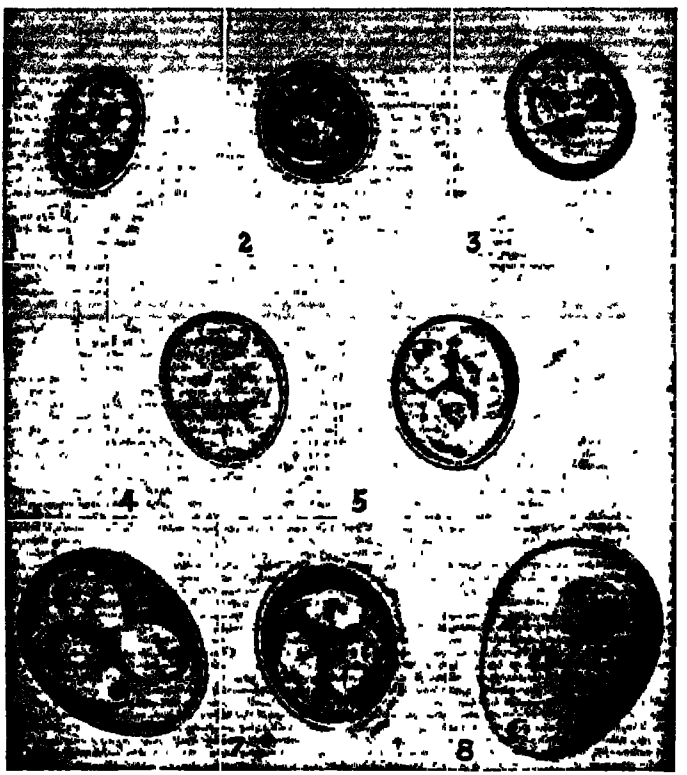


FIG 102 Sporulated Oocysts of the Coccidia of Chickens All photographs taken at a magnification of x 1100 to show relative size (1) *Eimeria acervulina* (2) *E mitis* (3) *E hagani* (4) *E necatrix* (5) *E tenella* (6) *E brunetti* (7) *E praecox* (8) *E maxima* Species number 6 has recently been differentiated by P P Levine It has not been named or described at the time this book goes to press

doses of infection at their first exposure This is accomplished by cleaning, thoroughly and frequently, the floors of buildings and runs, using feed and water containers that cannot easily be contaminated with droppings of the birds, cleaning and scalding these containers at frequent intervals, keeping

the birds on clean range of adequate size so that the infected droppings are scattered widely, and ploughing and planting contaminated ranges. Good poultrymen have found it possible to control coccidiosis in their flocks by the use of these methods.

EIMERIA TENELLA

This species affects young chickens, most commonly birds from six to twelve weeks of age, but they may be as young as three weeks and as much as several years old The affected birds suffer from what is commonly called acute coccidiosis, a disease in which bloody diarrhea is conspicuous and death losses often are large.

E tenella develops only in the ceca or blind pouches and it is here that the only lesions are found These consist of gross hemorrhage, the pouches usually being distended with wholly or partly clotted blood. The tissues of the remainder of the body are anemic The birds actually bleed to death internally in these cases

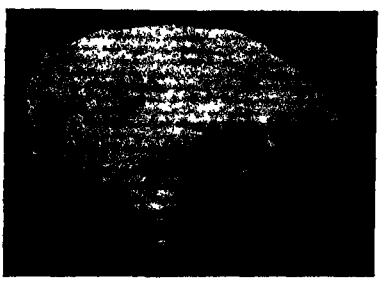


Fig 103 Chicken Affected with Cecal Coccidiosis (*Eimeria tenella*)

The sporozoites liberated from the infecting oocysts enter and develop in the epithelial cells of the ceca These schizonts mature and rupture, each liberating some hundreds of merozoites (first generation) These tend to localize in epithelial cells deep in the glands where they become another generation of schizonts The cells containing these forms are crowded into the subepithelial tissues where the parasites proceed to grow into huge schizonts which finally break up into two or three hundred or more of second generation merozoites These are much larger than those of the first generation. About the time the schizonts rupture, freeing the merozoites, multiple hemorrhages into the subepithelial tissues occur, the mucosa is undermined and sloughs away thus releasing the parasites. These invade remaining epithelial cells and, if the host continues to live, develop into a third generation of schizonts which is the last, the next generation developing as gametocytes.

The oocysts of *E. tenella* are broadly ovoid, there being little difference between the two ends. They measure 19 5 to 26 by 16 5 to 22.8 microns with an average measurement of 19 by 22.6 microns. The cytoplasm of the oocyst occupies only a fraction of the space within the shell. Sporulation is

complete in 48 hours at room temperature. After experimental feeding, oöcysts are found in the droppings on the 7th day.

EIMERIA NECATRIX

Next to the preceding species, this coccidium is the most pathogenic for chickens. The damage caused by this species is more apt to occur in older birds, and immediate death losses are not so frequent, the disease taking a longer course, the so-called chronic coccidiosis.



Fig. 104 Cecal Coccidiosis, Chicken Normal ceca are shown on the right On the left are the diseased The affected organs are swollen and filled with clotted blood, shown through the incision The color of the bloody content shows through the thin walls of the organs giving them a dark red color (Courtesy of E L Brunett)

The developmental phases of this species are quite similar to those of *E tenella*, except that its localization is different. The sporozoites invade the epithelial cells of the small intestine, rather than those of the ceca The schizonts liberate the first generation of merozoites into the lumen of the intestinal glands, these invade cells of the deeper parts of the glands and reach the subepithelial tissues in the same manner as *E tenella*. The second generation merozoites escape into the lumen of the bowel with the help of hemorrhages and denudation of the mucous membrane. This occurs on the fourth day of the infection. Instead of invading other nearby cells, the second generation merozoites allow themselves to be carried with the intestinal content to the lower bowel from whence they enter the ceca. Here another schizogonic generation occurs and probably several more, but gametes begin to form in the third generation and sporogony thereafter replaces the schizogony. Oocysts do not appear in the droppings until rather late in the course of the infection but oöcyst formation continues for a considerable time after it begins.

The oöcysts are broadly oval. Some are somewhat egg-shaped, with one end more pointed than the other. They are somewhat smaller than those of *E. tenella,* measuring 11.3 to 18.3 by 13.2 to 22.7 microns, averaging 14.2 by 16.7 microns. Sporulation is completed in 48 hours at room temperature. The shedding of oocysts begins on the 7th day after infection.

Massive infections with this species, especially in younger birds may result fatally within a week. The usual form is more prolonged. Symptoms begin on the 4th day. The bird stands with dejected attitude, there is roughening of the feathers and it does not eat. If heavy infections do not destroy the bird by the 7th day, it usually survives although it may be emaciated and worthless

The small intestines of birds suffering from this parasite present a characteristic appearance Showing through the muscular and serous coats, clearly visible on the exterior of the unopened intestines, are many whitish spots which are conspicuous against the dark background made by the bloody content They are the masses or colonies of the large second-generation schizonts. The wall also generally shows punctate hemorrhages The intestinal content may be a blood-stained mucus or it may be almost wholly of blood.

EIMERIA MAXIMA

This species receives its name from the size of its oocysts which are larger than any of the other species of chickens. They measure 21 5 to 42 5 by 16.5 to 29.8 microns, averaging 22.6 by 29 3 microns They have a yellowish color, and the shells often have a roughened surface. Sporulation is complete in 48 hours at room temperature. Oocysts appear in the droppings on the sixth day after experimental infections

The schizonts are found in the epithelium of the small intestine particularly of the middle portion. They are smaller than those of the other species found in the chicken and usually are located above the nuclei of the parasitized cells Schizogony does not continue beyond the 5th day. The gametocytes develop below the nuclei of the epithelial cells and apparently are more injurious to the host than the schizogonic forms.

The affected portions of the intestine may be somewhat thickened and the mucosa usually is covered with mucus in which blood flecks are found. There are no extensive hemorrhages such as occur in infections with the two species described above.

Heavy doses of pure cultures of this species will cause fatal infections, however under usual conditions its pathogenicity is not great. It usually is

associated with other species and in particular with *E. necatrix* in the so-called chronic infections of older birds.

EIMERIA HAGANI

This species was separated from a "culture" of *E. maxima* by Levine who used a micro-pipette to pick out the oocysts. The oocysts are broadly oval with both ends of equal breadth The measurements are 15 8 to 20 9 by 14 3 to 19 5 microns, averaging 17.6 by 19.1 microns The protoplasm almost completely fills the oocyst shell Sporulation is complete in 48 hours, the four elongated sporocysts taking up most of the space within the shell. After an infective feeding, oocysts appear in the feces toward the end of the 6th and on the 7th day.

When large numbers of sporulated oocysts are fed to young birds, many hemorrhagic spots about 1 mm in diameter appear in the wall of the duodenum and the first half of the small intestine. The lower end of the small intestine is free from lesions On the 6th day the mucous membrane appears greatly inflamed The content of the bowel is thin and watery and sometimes mucous casts of the lumen of the bowel are present. The birds generally recover

EIMERIA ACERVULINA

This species, like *E hagani*, *E praecox*, and *E mitis*, inhabits principally the upper half of the small intestine, although some forms may be found in the lower half and a few in the ceca near their outlets In the intestine the schizonts tend to concentrate in limited areas, and since they localize largely in the epithelium of the villi they form grayish areas which are visible to the naked eye when the bowel has been opened and the content washed away The schizonts develop very superficially, above the nuclei of the epithelial cells. The infections have little effect upon the health of the bird

The oocysts are egg-shaped They sporulate in less than 24 hours, in which respect they differ from the other species found in chickens They measure 17 7 to 20 2 by 13 7 to 16 3 microns, averaging 14 3 by 19.5 microns. After experimental feeding, oocysts appear in the droppings on the 4th day

EIMERIA PRAECOX

This species is found in the upper third of the small intestine like the preceding but colonization with the development of grossly visible lesions does not occur The species is practically non-pathogenic, there being little evidence of change in the intestinal wall except that an excess of mucus appears and mucous plugs often are formed.

The oöcysts are ovoidal in form and measure 19.8 to 24.7 by 15.7 to 19.8 microns, averaging 17.0 by 21.3 microns. Sporulation is completed in 48 hours under favorable conditions. The name *praecox* was given to this species by Johnson to indicate the precocity which it shows in eliminating oöcysts in the droppings on the 4th day after infection. This precocity is shared with *E acervulina* The schizonts are formed in the epithelium of the sides of the villi and appear below the nuclei of the host cells.

EIMERIA MITIS

This species is only slightly pathogenic Large and repeated doses of sporulated oocysts produce mild symptoms It develops in the epithelial cells of the upper part of the small intestine, to a lesser extent in the lower part, and sometimes even in the ceca.

The oocysts are nearly spherical, averaging 15 5 by 16 2 microns in size. The protoplasm practically fills the shell The sporocysts are elongated, measuring 6 by 10 microns. Sporulation is completed within 48 hours Oocysts appear in the droppings on the 5th day after experimental feeding

Schizonts not only develop in the epithelium of the villi but also in the glands Generally they occur below the nuclei of the host cells but sometimes they are above Unlike all of the other species of the chicken in which the cycles of development are regular, resulting in the occurrence of certain phases at certain times, all phases of the life cycle often can be found in a single section of intestine infected with *Eimeria mitis*

TREATMENT OF THE COCCIDIAL INFECTIONS OF CHICKENS

Coccidiosis in chickens can be handled best by instituting prophylactic measures These are simple sanitary precautions designed to prevent the birds from obtaining massive infections These have already been discussed

For treating cecal coccidiosis of young chicks, Beach and Davis (1), in 1925, recommended the inclusion of 20 per cent of milk sugar or of 40 per cent of dried milk in the diet. They found that on such a diet the lactic acid released in the ceca created a decidedly acid medium which seemed to favorably influence the course of the infection with *Eimeria tenella.* They supposed that this effect was exerted upon the sporozoites and merozoites, although they recognized that oocysts were discharged from affected birds in spite of the milk treatment. This treatment is widely used at present and is an effective one. At the onset of the disease all of the birds are given a mash containing 40 per cent dried milk. This is kept before the birds as their sole feed for one day, at which time it generally can be withdrawn, temporarily or permanently During this period the houses and premises should

TABLE XVI

THE COCCIDIA AFFECTING CHICKENS

	E tenella	*E necatrix*	*E maxima*	*E hagani*	*E acervulina*	*E praecox*	*E mitis*
Oöcysts — Average size (microns)	19 × 22 6	14 2 × 16 7	22 6 × 29 3	17 6 × 19.1	14 3 × 19 5	17 1 × 21 3	15 5 × 16 2
Oöcysts — Sporulation time	48 hrs.	48 hrs	48 hrs	48 hrs.	21 hrs	48 hrs.	48 hrs.
Oöcysts — *Prepatent period	7 days	7 days	6 days	7 days	4 days	4 days	5 days
Region of intestine affected	Schizonts and oöcysts in the ceca	Schizonts in small intestine, oocysts in ceca	Post half small intestine	Ant half small intestine	Ant half small intestine	Ant third small intestine	Ant half small intestine
Gross lesions	Gross hemorrhage into ceca	Hem exudate small intestine Petechia and whitish opacities	Intestinal wall thickened Blood flecks in exudate	Petechial hemorrhages seen thru serosa Mucous exudate in intestine	None	None	None
Degree of Severity of the disease	++++	++++	++	++	+	+	+

* The prepatent period is the time which elapses between the feeding of the sporulated oöcysts and the appearance of a fresh crop of oöcysts in the feces

be thoroughly cleaned. Chemical disinfection alone is practically worthless in controlling coccidial infection.

Herrick and Holmes (2), in 1936, introduced the use of flowers of sulphur for the control of cecal coccidiosis. Many others have studied this treatment and it is firmly established that it is of value in protecting uninfected birds in outbreaks. The continued feeding of 5 per cent sulphur has serious disadvantages, however, one of which is the production of the so-called "sulphur rickets." Also, sulphur has little effect upon the coccidia which develop wholly in the small intestine (6). Recently Levine (7) has shown that one of the new "sulfa" drugs, sulfaguanidine, when incorporated in the feed in 0 5 per cent concentration, will prevent development of the species which inhabit the small intestine, and in a somewhat higher concentration will markedly reduce the severity of infections with *E. tenella* and *E. necatrix*.

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The Coccidia of Birds Other Than the Chicken

A few years ago it was commonly believed that coccidiosis of domestic birds was spread from flock to flock by wild birds and particularly by sparrows. This belief was disproved by Smith and Smillie (2) who showed that sparrows commonly carried coccidia but that they belonged to the genus *Isospora*. The common species of sparrows and other wild birds is now known as *Isospora lacazii*. It is not infective for any of the domesticated birds except some of the cage pets such as canaries and other members of the finch family.

THE COCCIDIA OF TURKEYS

Turkeys are occasionally affected with coccidia of the genus *Eimeria* but the diseases produced by them have little practical importance. Turkeys can-

not be infected with any of the coccidia of chickens, nor can chickens be infected with species isolated from turkeys. Apparently the coccidia of turkeys are host specific *Eimeria meleagridis*, described by Tyzzer (3) in 1927, produces a cecal coccidiosis, and *E meleagrimitis*, described by the same author (4) in 1929, affects the small intestine. The latter is similar in many respects to *E mitis* of the chicken but cannot be transmitted to young chickens experimentally, hence it is regarded as a distinct species.

THE COCCIDIA OF DUCKS

Coccidiosis of ducks has been described in Europe but there is little evidence that it is of economic importance The species concerned have not been thoroughly described or dignified by specific names

THE COCCIDIA OF GEESE

In geese three species of intestinal coccidia have been described in Europe These have been named *Eimeria anseris*, *E nocens*, and *E parvula* Rather severe outbreaks have been ascribed to the first named.

The most important species occurring in geese is *Eimeria truncata*, which produces a severe form of renal coccidiosis This disease has been known in several European countries for many years Only a single outbreak has been described in this country, an outbreak occurring in northern Iowa and studied by McNutt (1) The disease affects ducklings from three weeks to three months of age When heavy infections occur the ducklings die within two or three days after the symptoms are first seen The mortality often is very severe and may be 100 per cent Milder infections occur, however, in which case no symptoms may be observed Yellowish-white spots are seen under the capsule of the kidney Sections show large numbers of developmental forms in the epithelial cells of the uriniferous tubules. The oocysts are ovoid in shape and average 16 3 by 23 5 microns in size There is a protuberance at the smaller and flattened end on which the micropyle is located

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The Coccidia of Cattle

For many years cattle were thought to harbor but a single species of coccidium. In 1918 Smith and Graybill (8) showed that there were two kinds

of oöcysts in the feces of calves which they studied in New Jersey, one being very much larger than the other. Yakimoff and Galouzo (11), working in Europe, decided that the one with the smaller oocyst was identical with the form which had long been regarded as the causative agent of bloody dysentery in Switzerland and which had been called *Eimeria zurnii* Therefore they proposed the name *E smithi* for the other. It is quite certain that these two species are the most prevalent in cattle, however, six additional species have been described. Careful work on the individual pathogenicities of these species has not been done, however it is generally believed that *E zurnii* is much more pathogenic than the others and is primarily responsible for most outbreaks of clinical coccidiosis in cattle There is evidence to indicate that some of the other species are not wholly harmless, however.

Practically all adult cattle harbor coccidia. The oocysts usually are not numerous in the feces and methods of concentration must be used to demonstrate them Marsh (7) found that *E smithi* and *E. ellipsoidalis* were found most often in apparently normal cattle and *E zurnii* when clinical evidence of coccidiosis in the herd existed He found small numbers of *E zurnii* in healthy cattle, however, and therefore believes that outbreaks of coccidiosis in cattle are not due to the entrance of new infection in the herd but to the release of dormant infection already there Baker (1) studied a herd of 63 dairy heifers in New York for a period of 18 months and found four species of coccidia regularly present although no evidence of clinical coccidiosis, except possibly an occasional case of mild diarrhea, appeared The species most frequently encountered were *E zurnii* and *E smithi* In dairy districts where cattle are kept in many small units fairly well isolated from each other, coccidiosis occurs very sporadically, that is, isolated cases occur here and there with no physical connection between them Occasionally outbreaks involving a considerable number of calves and heifers are seen, but these outbreaks are sporadic also. The evidence indicates that the parasite is widespread and that clinical disease depends upon conditions which permit massive infections. It is significant that in Switzerland where the disease is frequent and serious during the summer months, the cattle are then on mountain pastures and drinking from shallow pools where conditions for sporulation of oöcysts are excellent In the eastern part of the United States the disease is found throughout the year but mostly during the warmer months and particularly during the early fall months. In Montana, according to Marsh, the disease occurs mostly during the winter months often at times when the temperature is very low. This situation must be due to the fact that the young animals are concentrated on small areas of ground at that time of year where opportunity for massive infection is better than during the grazing season.

Acute coccidiosis of cattle occurs principally among young animals from two months to two years of age, however sporadic cases are not infrequent in much older animals The affected animals lose their appetites and rapidly lose condition as the disease progresses Dysentery associated with fetid, watery discharges streaked with blood is characteristic. In many cases the amount of blood discharged is very considerable, sometimes the discharge appears to consist largely of blood clots which, as a rule, are bright red in color, indicating that the hemorrhage originated in the lower bowel and, as a consequence, has not been blackened by action of the intestinal enzymes. The animals often show tenesmus As a result of the loss of blood, affected animals often show severe anemia, and because of the loss of fluids, severe dehydration of their tissues

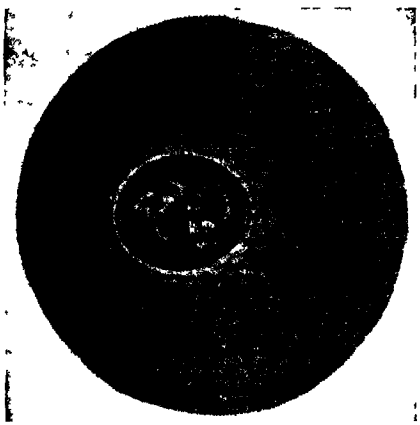


FIG. 105 *Eimeria zurnii* Sporulated oocyst x 1100

The lesions in acute coccidiosis of cattle are located in the cecum, large intestine, and rectum These organs are greatly thickened and edematous The mucosa is highly hemorrhagic and thrown into thick folds In the rectum these folds run longitudinally with large ecchymotic hemorrhages which tend to appear principally along the crests of the folds The thickening is so great as to be easily recognized by manual examination In some cases the tenesmus results in prolapses, the exposed rectal tissue presenting a visual picture of the lesions present Clots of blood of considerable size often are found, at autopsy, attached to the mucosal surface of the cecum and large intestine The mortality rate is considerable, probably averaging from 30 to 50 per cent in severe outbreaks. No specific treatments are known The usual treatment is to give protectives such as mineral oil and milk containing astringents, and intestinal antiseptics.

EIMERIA ZÜRNII

This species is most often associated with outbreaks of clinical coccidiosis and is believed to be the most important species affecting cattle. The developmental forms are located wholly in the cecum and lower bowels. They are never found in the small intestine. Schizonts are found largely near the

base of the glands and they cause considerable denudation of epithelium which is the cause, of course, of the extensive hemorrhages. The oocysts are nearly spherical and measure from 14 to 18 microns in diameter, averaging about 16 microns No micropyle is visible. The color is slightly greenish. Sporulation under favorable conditions takes place in from 48 to 72 hours The prepatent period is not known.

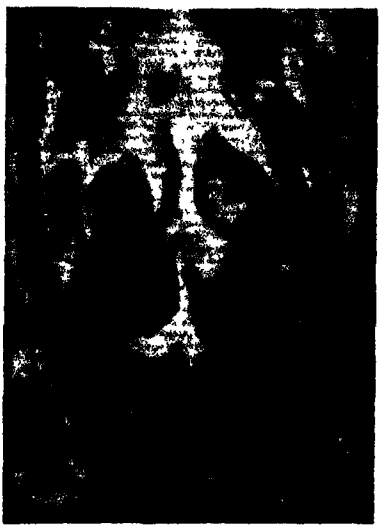


Fig 106 Bovine Coccidiosis (*Eimeria zurnii*) Developmental forms in epithelial cells in the base of an intestinal gland x 600

EIMERIA SMITHI

In spite of the fact that this species is very common in cattle, there is little information available on its pathogenicity. It is found in clinical cases of coccidiosis but always, apparently, in association with *E zurnii* which, doubtless, is the principal pathogenic agent. In some of their cases which were mixed infection of *E. zurnii* and *E smithi*, Smith and Graybill (8) mention developing forms in the villi of the lower small intestine Since other authors deny that *E. zurnii* develops in the small intestine, it may be assumed that the forms seen in the ileum were *E smithi* Becker (2) quotes Wilson as saying that in pure infection of *E smithi*, oocysts were as numerous in the content of the ileum as in that of the large intestine, another indication that this species multiplies in the small intestine

The oocysts are much larger than those of *E. zurnii* and are egg-shaped. At the smaller end a micropyle is easily visible They are quite uniform in size, measuring about 21 by 29 microns The oocyst wall is slightly tinged with brown. The sporulation time, under favorable conditions varies from three to five days

EIMERIA ELLIPSOIDALIS

Becker and Frye (3) found the oocysts of this species in the feces of a healthy calf. They were also encountered by Baker (1) in a herd of calves in which clinical disease was absent The specific name is derived from the

shape of the oocysts which are ellipsoidal, occasionally ovoidal. They measure about 15 by 22 microns, thus being considerably smaller than those of *E. smithi* The protoplasm of the oocyst practically fills the shell when freshly passed, but contracts later into a spherical mass, leaving open spaces at the ends A micropyle can be seen at one end where the shell is slightly thinned. The oocyst is colorless. Its sporulation time is rather long and variable—two to ten days, according to Baker Nothing is known of the developmental forms of this species, and nothing of its pathogenicity.

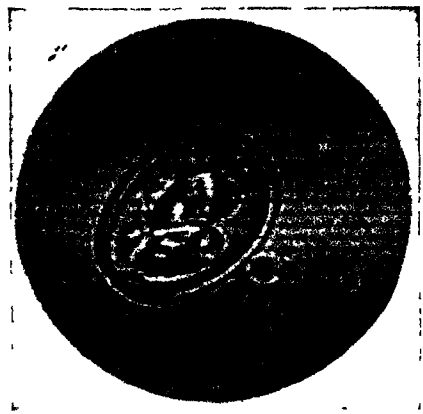


FIG 107 *Eimeria smithi* Sporulated oocyst × 110

EIMERIA BUKIDNONENSIS

This species was described by Tubangui (9) in the Philippine Islands in 1931 Only the oocysts were observed and nothing is known about the developmental forms of its pathogenicity Subsequently it has been seen by the Russian worker, Yakimoff, and by Baker (1), in New York, whose identification was confirmed by Christensen (4) The oocysts have very thick walls (2 microns) which are dark brown in color and show radial striations They are distinctly egg-shaped and have a conspicuous micropyle at the smaller end Baker's measurements indicate that the average size is about 30 by 42 microns This is somewhat smaller than was reported by Tubangui The sporulation time is long—from 24 to 27 days, according to Baker, but only 5 to 7, according to Christensen and Porter

EIMERIA CYLINDRICA

Little is known about the incidence of this species Wilson (10) described its oöcysts from Virginia cattle in 1931 It has not been reported elsewhere * The shape is quite elongated, the ratio between breadth and length being greater than in any other bovine species Its average size is 14.4 by 23.7 microns Sporulation occurs very rapidly being complete in 48 hours under favorable conditions. Wilson reports artificial infection of one calf Blood streaked feces were noted on the 4th day Oocysts were not eliminated until the 7th day. Nothing is known about its developmental stages.

* See footnote on page 449

EIMERIA ZURNABADENSIS

This species was described from oocysts found in Russian cattle by Yakimoff. It has not been reported from the United States.* The species also occurs in the zebu and the buffalo The oocysts are cylindrical, measuring about 25 by 34 microns with considerable variation noted. They are yellowish in color.

EIMERIA AUBURNENSIS

This species was described by Christensen and Porter (5) in 1939. Oocysts were found in a calf at Auburn, Alabama Later it was found in other animals there, and the authors think that the large oocysts mentioned by Smith and Graybill in New Jersey, by Marsh in Montana, and by Wilson and Morley in Virginia were undoubtedly those of this species

The oocysts are larger than those of any other bovine species except *E thianethi*, measuring 23 1 by 38 4 microns (average) They are the shape of an elongated egg The oocyst wall usually is clear and smooth and moderately thick (1 to 1 5 microns). Sometimes, however, they have rough, mammilated walls The color is noticeably brownish but they are not so deeply stained as the oocysts of *E bukidnonensis* A small micropyle is located on the smaller end All variations from the heavily mammilated type to the perfectly smooth types are found, but the smooth types greatly outnumber the others. Sporulation is complete in about 48 hours under favorable conditions

A calf which was free of all coccidia was fed a dose of 8,000 sporulated oocysts A profuse, watery, greenish, diarrheal discharge was noticed from the 9th to the 13th day accompanied by slight apathy on the part of the host. Oocysts were not found until the 24th day when large numbers were encountered. The oocyst count rapidly diminished during the next three days but they were discharged in small numbers for several weeks.

Nothing is known about the developmental stages.

EIMERIA THIANETHI

This species was described by Gwelessiany (6) in 1935 on the basis of oocysts found in Russian cattle This species has not been described in America The oocysts are even larger than those of *E auburnensis* They are oval, slightly yellowish, and measure 28.6 by 42.6 microns on an average. The wall is very thick and consists of two layers. The outer is thin and smooth;

* NOTE Without mentioning specific names, Christensen and Porter (5) state that they have encountered all known species of bovine coccidia, except *E thianethi*, in the vicinity of Auburn, Alabama This means, presumably, that they have encountered both *E cylindrica* and *E zurnabadensis*

the inner thick and shows transverse striations. The author made no studies on sporulation time or on pathogenicity. Since he found it in only five out of more than 300 cattle examined, it is presumed to be of little importance.

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The Coccidia of Sheep and Goats

Coccidiosis of sheep often assumes serious proportions At least seven species exist, but as in the case of the bovine coccidia, it is probable that one species, *Eimeria ahsata*, is concerned with most of the clinical disease whereas the others are only slightly pathogenic Also, as in the bovine group, mixed infections with two or more species is the rule rather than the exception It appears too that most adult sheep harbor coccidia (1, 3) and that the clinical disease develops only when conditions favoring massive invasion exist As a rule the clinical disease is seen in lambs, seldom in the old animals. In the western states of this country where lambs are raised on the range and brought in to be fattened in the feed lot, the disease is seen most often within the first month they are on feed This has been noted by Newsom and Cross (4), and by Deem and Thorp (2), in Colorado in a number of successive years, even when sanitary conditions are well maintained. They believe, therefore, that the change of feed plays an important influence in favoring the development of the parasites; that this may be more important than the increased opportunity, usually presented in the feed lot, of picking up massive doses of oocysts It has been noted, for instance, that lambs fed on beet tops are more likely to develop severe clinical cases of coccidiosis than those that are fed

alfalfa or other roughage. Newsom and Cross (4) report that the incidence of clinical coccidiosis seen by them in a number of large bands of feeder lambs in the 1929 and 1930 seasons averaged 24 per cent, and deaths from the disease, about 2 8 per cent. In addition to the actual mortality, however, a considerable number of lambs survived but failed to feed out satisfactorily as a result of the disease. Deem and Thorp (2) noted that as a rule the lambs arriving at the feed lots are already infected During the first month there is a rapid increase in the oocyst count, the high count is maintained for one to three weeks, then it declines sharply until very few can be found. Occasionally they have noted secondary rises in the count in the 3rd or 4th month They attribute this to infection with new species against which the lambs have not developed immunity They also noted that *Eimeria parva* usually is responsible for the initial high counts and that *Eimeria arloingi* later prevails in the same animals All clinical cases were associated with the *E arloingi* infection

The clinical disease is also seen in lambs at pasture, and even in very young ones that are confined before being turned to pasture The affected animals suffer from diarrhea and the feces usually are streaked with blood

An indication of the relative frequency of the various species of coccidia of sheep is given in the study by Christensen (1) who surveyed 100 animals originating in Idaho, Wyoming, and Maryland, with a single animal from New York Analysis of the types of oocysts indicated that mixed infections predominated There were only four negative animals in the series, 62 showed mixed infections (two or more species) and 34 showed pure infections (one species) Oocysts of *E arloingi* were found in 28 of the pure infections, those of *E parva* in 4, and those of *E granulosa* and *E nina-kohl-yakimovi* in one each In total incidence *E. arloingi* occurred 90 times, *E. parva* 50, *E intricata* 14, *E faurei* 11, *E pallida* 10, *E granulosa* 10, and *E nina-kohl-yakimovi* 3 times In none of these cases was there any evidence of clinical coccidiosis

It is presumed that all of the species which affect sheep will also infect goats, but this presumption may not be correct *E arloingi* occurs in goats, in fact was first described in goats, and in this animal is capable of causing the same type of disease as in sheep.

EIMERIA ARLOINGI

The developmental stages of this species are found in the small intestine, particularly in the middle portions, although it may involve the greater part from the duodenum to the ileum The schizonts are found in the epithelial

cells usually above their nuclei. The macrogametocytes tend to collect in patches from 0.5 to 6 mm in diameter and these show to the naked eye as yellowish-white areas which are best seen when the bowel is opened but may also be detected from the serous surface These areas tend to stand up above the level of the mucous membrane and often are very conspicuous. The content of the bowel is thin, watery, mucoid, brownish, and often streaked with blood.

The oocysts vary considerably in shape and size. Usually they are ellipsoidal, and are conspicuous because of a mucoid cap which covers the operculum at one end They measure 13 to 27 microns in width and 17 to 42 microns in length (average is 18 by 27 microns) The cap apparently is a tough structure but it is often dislodged and even absent, having been lost The micropyle, covered by the cap, is rather wide, averaging 5 microns The smaller oocysts have little color but the larger are yellowish or brownish Sporulation occurs between 24 and 48 hours under favorable conditions.

EIMERIA PARVA

Except for *E arloingi*, this seems to be the most prevalent species of coccidium of the sheep There is little precise information about the pathogenicity of this species but it evidently is low, judging by the reports of the Colorado workers referred to above The developmental stages in the sheep have not been described The oocysts are small and only a little longer than broad (sub-spherical) They measure 10 to 18 microns broad by 12 to 22 microns long (average 14 1 by 16 5 microns) There is no perceptible micropyle. The color is very faintly yellow or yellowish-green A heavy diffraction line along the inner surface of the capsule gives this oocyst a distinctive "double contour." In concentrated solutions, used for concentrating the oocysts, they frequently collapse if left for long Sporulation occurs between 24 and 48 hours These oocysts may be confused most readily with those of *E pallida* but they may be distinguished by being more nearly round, and by the double contoured appearance

EIMERIA PALLIDA

This species was differentiated from *E parva* by Christensen (1) in 1938. Only the oocyst is known This is more elongated than that of *E. parva* Measurements average 10 by 14 2 microns. The shape is ellipsoidal A micropyle is not apparent The wall is thin, transparent, and slightly yellowish-green in color Sporulation is nearly complete in 24 hours under favorable conditions

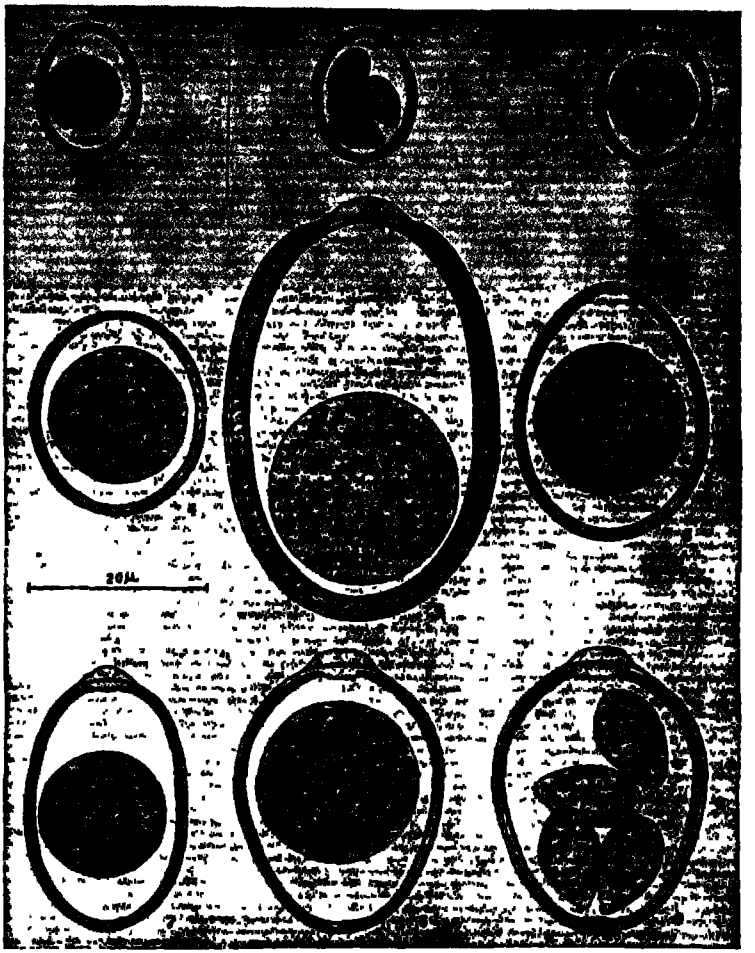


FIG 108 The Coccidia of Sheep Camera lucida drawings of oocysts. Scale indicated in illustration (1) *Eimeria pallida* Unsporulated (2) *E pallida* Sporulated (3) *E parva* Unsporulated (4) *E nina-kohl yakimovi* Unsporulated (5) *E intricata* Unsporulated (6) *E faurei* Unsporulated (7) *E arloingi* Unsporulated (8) *E granulosa* Unsporulated (9) *E granulosa* Sporulated (From Christensen Courtesy *Jour Parasitology*)

EIMERIA FAUREI

At one time all coccidia of sheep were considered to belong to this species. It is now known that it is relatively uncommon and it is believed that it has little pathogenicity.

The oocysts are distinctly egg-shaped with a small micropyle located at the smaller end and without a polar cap. They average 21 microns in width and 28.9 microns in breadth. The wall is clear and discontinuous at the micropylar end. It is of a pale yellowish-brown color. Sporulation occurs between 24 and 48 hours The shape of this oocyst serves to distinguish it from all others occurring in sheep.

EIMERIA INTRICATA

The oocysts of this species are very large, ellipsoidal, dark brown in color, and opaque. They average 32 by 47 microns in size. The micropyle is seen as a wide gap (averaging 8 microns) in a heavy wall, and it is covered by a prominent, transparent, yellowish-green cap The wall averages 2.5 microns in thickness and consists of two layers, the inner being twice as thick as the outer, more deeply colored, and more prominently striated The outer surface of the outer layer is irregularly corrugated Sporulation occurs in from 72 to 120 hours.

EIMERIA GRANULOSA

This species was described on the basis of the oöcysts alone by Christensen (1) in 1930. The oocysts are egg-shaped but differ from most forms in that the micropyle is located on the broad end. This suggests the form of a broad urn. A rather prominent polar cap covers the micropyle This cap is easily displaced and is often lost. The wall is of moderate thickness, transparent, smooth, and of a yellowish-brown color They average 20 9 microns in breadth at the widest point and about 29.4 microns in length. The sporulation time is from 72 to 96 hours.

EIMERIA NINA-KOHL-YAKIMOVI

This species was described on the basis of its oöcysts from Russian goats. Christensen (1) found them in three sheep in his series of 100, two animals coming from Idaho, the other from Maryland.

The oocysts resemble those of *E. parva* but are distinguished, according to Christensen, by their faint, brownish-yellow tint, somewhat larger size, a thin and double contoured wall at the micropyle end, and a single heavy refraction line marking the inner surface of the wall.

The shape of the oocysts usually is ellipsoidal, but they are rather broader than most ellipsoidal forms. The micropyle is inconspicuous but can be found by careful examination. There is no polar cap. The wall is thin, pale, and almost imperceptibly brownish-yellow. Their average measurement is 18.3 by 23.1 microns Sporulation is completed in the interval between 24 and 48 hours.

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The Coccidia of Swine

Four species of *Eimeria* and one of *Isospora* have been described in swine. Of these it is probable that only one, *Eimeria debliecki*, is of much practical importance, although more work will have to be done on this group before one can be sure of this Coccidial infection of swine has been reported from Russia, Germany, Holland, France, and the United States (Iowa and California) There is little doubt but that coccidia of swine occur wherever swine are raised in considerable numbers.

Many of the earlier authors contended that coccidial infections of swine were benign Feeding trials by Noller and Frenz (6) in Germany and by Biester and Murray (2) in the United States disproved this idea, although they did show that the swine strains are probably less pathogenic for their hosts than those of any of the other species Both groups showed that large doses are capable of producing severe diarrhea and an occasional death among young pigs Constipation frequently follows the diarrheal period. The pigs that recover from the acute infections frequently show severe emaciation, pot-bellies, and general unthriftiness which persists long after all evidences of the coccidia have disappeared Older swine usually are resistant to infection, probably because they have passed through minor infections early in life and have developed a considerable degree of immunity.

EIMERIA DEBLIECKI

This appears to be the most common and most pathogenic of the species affecting swine. Developmental forms are found in the small intestine, and to a lesser extent in the cecum and colon. They occur in the epithelial cells.

The oöcysts vary considerably in size and shape This suggests that there may be more than one species included in present descriptions of this one. The shape varies from sub-spherical to ovoid. Measurements given by various observers vary from 12 to 33 microns in length by 10 to 32 microns in width. A micropyle is not present. The protoplasm of the unsegmented oocyst practically fills the shell At one end of each sporocyst a knob-like protuberance occurs The sporulation time varies from 7 to 9 days, and oocysts may be recovered from the feces in from 6 to 7 days after feeding sporulated oocysts When reinfection is prevented, oocyst-shedding continues for 10 to 15 days, then ceases. The infection is self-limiting Second infections can be induced, according to Biester and Schwarte (3), but the animal shows evidence of considerable resistance Nearly absolute immunity to reinfection can be induced by continued day to day feeding of small numbers of oocysts but this immunity is short lived, since it is possible to set up a new infection with the same species after a lapse of about three weeks Chronic coccidiosis of swine, according to these authors, is a result of continued reinfections

EIMERIA SCABRA

This species was described by Henry (5) in California in 1931 Apparently it has not been seen elsewhere Developmental forms occur in the villi of the small intestine The oocysts are characterized by a thick (1 5 to 2 0 microns) shell with a rough outer surface and a brown color The size varies from 16 to 25 microns in width by 22 5 by 35 5 microns in length The shape is ellipsoidal or slightly ovoidal At one end, the small end of the ovoidal forms, the shell wall is considerably thinner than elsewhere but a definite micropyle is not present The non-segmented oocyst presents a shell nearly filled with the cytoplasm when freshly passed but later the cytoplasm contracts into a spherical form before dividing Sporulation is completed in from 9 to 12 days under favorable conditions

EIMERIA PERMINUTA

This species was described by Henry (5) in California in 1931 and apparently has not been encountered elsewhere. Nothing is known about it except the oocysts which resemble those of *E. scabra* except that they are much smaller They resemble the oocysts of *E. debliecki* in form and shape, but the latter does not have the roughened surface of this species. The color is yellowish. They measure from 9.6 to 12 8 microns in width by 11.2 to 16 microns in length. The sporulation time is about 12 days.

EIMERIA SPINOSA

This species was discovered and described by Henry (5) in 1931 and has not been reported elsewhere Only the oocysts are known. These are characterized by small spiny structures which arise in the shell and project about 1 micron beyond its surface They are spaced about 1 micron apart over the entire surface. The color is brown The form is ellipsoidal and no micropyle is visible The measurements vary from 12 8 to 16 microns in breadth by 16 to 22.4 microns in length The sporulation time is about 12 days

ISOSPORA SUIS

In 1934, Biester and Murray (3) published a brief note on their finding of a coccidium belonging to the *Isospora* in swine A few additional details are given by Biester in a note appearing in Becker's (1) book on the coccidia No other references to this organism have been found.

The oocysts are thick-walled (1 5 microns), yellowish-brown in color, and sub-spherical in shape A micropyle was not seen In breadth they average about 19 5 microns, in length about 22 5 microns Sporulation is completed in about 4 days Oocysts are eliminated in from 6 to 8 days after a single infective feeding and this continues for about 8 days

The developmental forms occur in the epithelium of the villi of the small intestine Diarrhea occurs on the 6th or 7th day after feeding There is no blood in the diarrheal discharge Constipation follows the period of diarrhea The species appears to be host specific Dogs, guinea pigs, and rats did not become infected when fed sporulated oocysts, and conversely, pigs failed to develop infection when fed sporulated oocysts of *Isospora bigemina* from the dog

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CHAPTER XXXVII

THE SPOROZOA (*continued*)

The Hemosporidia

The hemosporidia are blood-inhabiting protozoa which are closely related to the coccidia, and may possibly have originated as coccidia The stages in the life cycles are very much alike as will be seen by comparing them In both cases infection of the vertebrate host occurs as the result of the invasion of suitable cells by sporozoites coming in from the outside These sporozoites, developing inside of cells of the host, become schizonts which produce a crop of merozoites These give rise to a second generation of schizonts In the case of the coccidia all of this goes on in epithelial cells, usually of the intestinal tract. In the case of the hemosporidia the cells are those of the vascular system In both cases the schizogonic multiplication finally ceases, an alternation of generation occurs, and gametocytes are formed At this stage there is divergence. In the group of coccidia fertilization occurs in the original host, zygotes are formed which are protected by oocysts and these leave the host to undergo sporulation as free bodies in moist soil, or in water In the process of sporulation sporozoites are formed which are then ready to infect another host if chance causes them to be ingested. In the group of hemosporidia, the gametocytes are not fertilized in the vertebrate host but must wait until they reach the stomach of a blood-sucking arthropod The zygotes resulting from the fertilization in the stomach of the arthropod, instead of being motionless bodies as in the coccidia, are worm-like organisms (*ookinetes*) which bore their way through the stomach wall to form oocysts in the tissues These increase in size and their cytoplasm divides into many small cells each of which eventually becomes a highly motile sporozoite. These sporozoites migrate to the mouth parts of the arthropod and are ready to infect another vertebrate host at the time of the next blood meal.

The hemosporidia are divided into two groups of families which are known as the *Hemoproteidae* and the *Plasmodiidae* Members of the *Hemoproteidae* multiply by schizogony in fixed cells of the circulatory system (usually endothelial cells of capillaries) and only gametocytes are found in the circulating blood. The *Plasmodiidae*, on the other hand, develop wholly in the circulating erythrocytes, hence both schizonts and gametocytes are found

in the circulating blood. Because of the absence of the schizogonic form of the *Hemoproteidae* in the circulating blood, diseases caused by such forms cannot be transmitted directly from one vertebrate host to another by the inoculation of blood This can be done, however, in the plasmodial diseases. Sporogony, in diseases caused by both groups, occurs only in blood-sucking invertebrates

The *Hemoproteidae* are further divided into two genera, *Hemoproteus* and *Leucocytozoon* Members of the genus *Hemoproteus* occur only in birds. A great many species of wild birds harbor them. The gametocytes are found in the erythrocytes, the fully developed forms being horse-shoe shaped, forming a sort of collar or halter around the nuclei. This suggested the name *Halteridium* by which these forms are commonly known.

The Hemoproteus Group

Members of the genus *Hemoproteus* occur in many wild birds but so far as is known do not affect any of the domesticated species except the pigeon. *Hemoproteus columbae* of the pigeon occurs in the Mediterranean region of Europe and Africa, in India, and in South America It has not been reported in North America. The transmitting agent is a blood-sucking fly, and possibly other arthropods.

The Leucocytozoa

Members of this group of hemosporidia are found only in birds. The name was given by earlier workers who believed that the greatly distorted cells seen in the circulating blood which harbored the gametocytes were leucocytes. These cells are devoid of hemoglobin but it is now believed that they are immature erythrocytes rather than leucocytes.

Leucocytozoa occur in the blood of many species of wild birds They have been reported also in the domestic duck and turkey, in which they cause serious losses There have been reports also of infections of chickens and geese but little is known about them Heavy losses of wild ducks, wild turkeys, and wild grouse have been attributed to members of the group. The transmitting agents, in the cases in which the life cycle has been worked out, have been gnats or black flies of the genus *Simuliidae*.

LEUCOCYTOZOON SMITHI

In connection with his studies on enterohepatitis (blackhead) in turkeys, Theobald Smith (4) in 1895 mentioned certain structures in the blood which

undoubtedly were the gametocytes of this organism, hence it has been named in his honor Laveran and Lucet (2) saw the parasite in France in 1905 It was described again by Volkmar (6) in this country in 1930 Skidmore (3) described a serious outbreak of the disease in a turkey flock in Nebraska in 1932. Johnson and co-workers (1) in Virginia published a number of papers between 1935 and 1938 on the parasite and the disease which apparently is rather serious in many parts of the southeastern United States Travis, Goodwin, and Gambrell (5) described the disease in the same area in 1939

The disease affects young birds, principally those under twelve weeks of age The mortality rate may be very high. In the flock described by Skidmore there were only 40 poults remaining at the end of the summer out of an original flock of 385 birds Symptoms in the young birds may be seen for only a day or two before death intervenes The sick birds lose their appetites, appear depressed, and have a tendency to lie down When disturbed these birds move with difficulty. Often they fall over, gasp a few times, and are dead Infections of older birds usually are not so severe and many show no symptoms Such birds may be carriers for several months, and they constitute the means by which the infection is propagated from one year to the next. Johnson mentions a common symptom of moist tracheal rales which he believes is a part of this disease Older birds may become severely emaciated and anemic The liver generally is enlarged and the gall-bladder distended The lungs, liver, and spleen usually are congested The proventriculus and the first portions of the small intestine are often congested and edematous The pericardial sac may be filled with fluid.

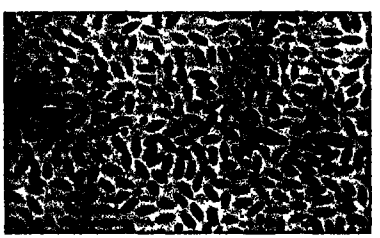


FIG 109 *Leucocytozoon smithi* A stained blood film of a turkey showing two macrogametocytes and one microgametocyte. Each body has two nuclei, one being that of the parasite and the other that of the parasitized erythrocyte x 200 (Courtesy of E P Johnson)

Morphology and Staining Properties. As they occur in the blood, the gametocytes are elongated structures of such size that they are quite conspicuous. Some authors refer to them as "cigar-shaped." They stain rather darkly with Wright's and Giemsa's stains The cells in which they are located have pointed ends This form is seen only when the blood-films are quickly made, for shortly after blood is drawn from the bird, the protoplasm of these cells tends to assume a spherical form. Two nuclei are found in most of these

forms, one presumably being the nucleus of the parasitized cell. Skidmore does not give measurements but his photomicrographs indicate that the parasites are from three to four times as long as the greatest diameter of the normal erythrocytes Johnson and his co-workers, and West and Starr (7) point out that differentiation of the microgametocytes from the macrogametocytes is not difficult, the male cells being much more lightly stained than the female. The latter give the average measurements as 10 5 by 20 microns.

Transmission. The disease cannot be transmitted by the inoculation of blood. A few gametocytes may be found in the blood after such inoculations but they tend to disappear, and the bird shows no symptoms of the infection.

In Virginia the natural transmitting agent, according to Johnson, Underhill, Cox, and Threlkeld (1), is *Simulium nigroparvum*, a black fly which breeds abundantly in the small streams of the western part of that state. In Nebraska, Skidmore showed that the transmitting agent was a black-fly but one of a different species, *Simulium occidentale* Both of these flies are active blood-suckers, and turkeys are attacked vigorously especially in the region of the head. The small insects act as true hosts, the life cycle in them being very much like that of the other hemosporidia Zygotes and ookinetes have been identified in the stomach contents of infected flies. Oocysts have not been definitely identified but sporozoites have been recognized in the salivary glands in from 3 to 5 days after infective feedings In experimentally infected turkeys gametocytes have been found in the blood on the 9th day.

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LEUCOCYTOZOON SIMONDI

Synonym. *Leucocytozoon anatis*

Attention was drawn to the probable importance of this species by Wickware (4) in 1915 who investigated a serious outbreak of disease of ducks near Ottawa, Canada, which he believed was caused by this parasite. Thinking that

the parasite had not been described previously, he proposed the name *Leucocytozoon anatis* by which it is best known. In 1938, however, Herman (1) called attention to the fact that Mathis and Leger had described what seems to have been the same organism in 1910. This being the case, the name proposed by the latter takes precedence over that of Wickware. Most of the details of the incidence of this species and its life cycle were clarified by O'Roke (2) (3), working in Michigan.

Morphology. The microgametes are spindle-shaped structures oriented parallel to the cell nucleus, which becomes very elongated. The host cells average 46 6 microns in length, the parasite 3 1 to 4 3 by 14.7 to 18 9 microns. The cytoplasm stains a pale-blue with Giemsa's stain. The nucleus is centrally located, oval, pale pink in color, and it shows no karyosome The macrogametes occur in the same kind of host cell but these cells become more elongated, measuring 55.4 microns in length on an average The spindle-shaped parasite measures 3 2 to 4 4 by 14 5 to 22 microns The cytoplasm is stained dark blue with Giemsa's stain, and it is granular The nucleus is spherical, centrally located, and it contains a distinct karyosome.

Pathogenicity. This parasite attacks both wild and domestic ducks The mortality rate in young ducks, according to O'Roke, amounts to about 35 per cent. Adult birds carry the parasite with no evident effect on their health. The infected ducklings refuse their food and show evidence of stupor. When aroused they often show violent paroxysms The head is frequently carried in a peculiar position and sometimes is waved in a peculiar fashion Often there is difficulty in maintaining equilibrium, the wings frequently being called into play in the effort to stand Wickware mentions a purulent ophthalmitis as a frequent symptom Death usually occurs within a day or two after symptoms are first observed The only gross lesions, aside from anemia in the more chronic cases, is a hemorrhagic enteritis

Transmission. At the place where he worked in Michigan, O'Roke found that all ducks that were not protected from the bites of the black-fly, *Simulium venustum*, which was very prevalent, became infected with the protozoon. Those that were kept under screens escaped The developmental forms in the fly were traced.

Life Cycle. Schizonts are found in the capillaries of the lungs, liver, spleen, and kidney but only gametocytes occur in the blood Following the bite of infected black-flies, gametocytes are first found on the 7th day By the 10th day these have matured. The bird shows symptoms and may die at this time although more often it dies on the 12th day.

O'Roke was able to follow the entire life cycle in the fly. Gametogenesis and ookinete-formation takes place in the stomach. The oocysts form in the outer layers of the stomach wall and the sporozoites escape to the salivary glands. O'Roke estimated that the entire cycle in the black-fly requires about 5 days, and since that in the duck requires about 10, the whole life-cycle of the parasite can be completed in about 15 days.

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LEUCOCYTOZOON BONASAE

This parasite was discovered by Clarke (1) in 1934 in ruffed grouse which were dying in large numbers in Algonquin Park, Ontario The mortality was largely among the young birds of which about 60 per cent had died by mid-summer All dead birds showed this parasite in their blood and no other cause for the mortality could be found, hence he was inclined to attribute the deaths to it. Since it has long been known that grouse mortality is periodic in the northern countries, the population rising and falling in cycles, the author (2) suggests that this parasite may possibly be the causative agent

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The Plasmodia

Malaria is one of the most widespread and most serious diseases of man. Three principal types of malaria are known, these corresponding to three different species of plasmodia which are the causative agents. Both schizonts and gametes occur in the erythrocytes of the circulating blood As a result of the incomplete destruction of hemoglobin by the parasites, a brownish-black pigment known as melanin or hemozoin is formed and appears as granules in the parasitized erythrocytes. The diseases can be transmitted from man to man by the injection of infected blood but they are transmitted naturally by anopheline mosquitoes in which the sexual cycle of the parasite occurs Among animals, plasmodial infections occur in monkeys, a number of species of oriental mam-

mals, and in certain reptiles, but they occur most frequently in birds. In general the species are rather host specific but this does not always apply in the bird malarias.

According to Herman (3) at least 30 North American species of birds harbor malaria parasites. A great many bird-infecting species of plasmodia have been described but it appears that many of these are not valid and that actually the known valid species do not number more than a dozen. Herman (3) limits them to 8, and Manwell (5) to 12.

A great deal of work has been done on the malarias of birds, partly because of their intrinsic interest, but more especially because they are easily transmitted and handled in the laboratory and have yielded much information which has led to important advances in our knowledge of human malaria All but 3 of the known avian species are infective for canaries, and since this is a convenient bird with which to work, it has been commonly used. Acute and fatal infections, or chronic infections, can be produced experimentally at will. The parasites can be readily studied in the blood of these birds In a general way, they resemble the forms which occur in the blood of human infections.

The malaria infections of birds are transmitted by mosquitoes, but unlike the human infections, they are transmitted, with one exception, by culicine species. The exception is *Plasmodium gallinaceum*, which will be referred to below

Three species of bird-malaria parasites, *Plasmodium praecox, P circumflexum*, and *P. cathemerium* are relatively common in passerine birds in North America Ordinarily they do not appear to do a great deal of damage to their hosts but all three are capable, experimentally, of causing fatal infections. Manwell (4) has demonstrated that all three of the species mentioned are capable of developing in chickens but the developmental level in this abnormal host is so low that parasites could not, ordinarily, be demonstrated in their blood by microscopic examination but had to be detected by massive inoculations of blood into more susceptible birds. Infections in no cases lasted more than 10 days Taliaferro and Taliaferro (6), working with another species (*P lophurae*) succeeded in producing an acute rise in blood parasites by inoculating chickens with large doses, but the numbers disappeared rapidly and this was followed by a period of latency which lasted as long as four months

The only species of bird malaria which is of any economic importance in domesticated birds is *P gallinaceum* which, as has been pointed out above, is unlike all other known species in that it is transmitted by anopheline mosquitoes. This species does not occur in North America, except in certain laboratories, where it is being studied because of its closer relation to human

malaria than the common bird types. *P. gallinaceum* was described and named by the French parasitologist, Brumpt (1), who obtained it from Ceylon. According to Brumpt (2) this species develops rapidly in all of the yellow fever mosquitoes and other anopheline types but not in the culicine species. It is highly pathogenic for chickens, particularly for young birds. Older birds are more resistant and often become chronic carriers Geese are also readily infected, but ducks, pigeons, and canaries are refractory.

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The Piroplasmata

Members of the sub-order *Piroplasmidea* inhabit the red blood corpuscles of their hosts but do not form the blood pigments from hemoglobin (melanins) which characterize the members of the *Hemoproteus* and *Plasmodium* groups Each parasite consists of a small bit of cytoplasm and a nucleus With Giemsa's stain the cytoplasm stains bluish and the nucleus red They divide (schizogony) into 2 or 4 daughter elements and never into large numbers as in the malaria group

The sub-order *Piroplasmidea* is divided into two groups, the families *Babesidae* and *Theileridae* The members of the first family develop wholly in the red blood cells, whereas the schizogonic forms of the latter are found exclusively in endothelial cells of the internal organs and only the gametocytes occur in the erythrocytes of the circulating blood

There is considerable confusion with respect to the nomenclature of members of the family *Babesidae* Some authors recognize as many as six genera in this group while others prefer to group all into a single genus, *Babesia*. The single generic name will be used here, the other generic names being given as synonyms.

The Babesiae of Cattle

In 1893, Smith and Kilborne (6) described the blood parasite of the so-called Texas fever of cattle and gave it the name *Pyrosoma bigemina* Since this

generic name was found to have been pre-empted for another organism it was shortly changed to *Piroplasma* under which it is best known. The group of diseases which later were found to be caused by this and similar organisms are frequently called the *piroplasmoses*. It appears, however, that Babes (1) had seen and described this organism in Roumanian cattle in 1888, although he was mistaken as to its nature, and that the generic name *Babesia* had been suggested for it in his honor before Smith and Kilborne had applied their name. The name *Babesia* accordingly has priority.

Three types of piroplasmoses of cattle are recognized The diseases are similar but the parasites are morphologically and immunologically different and the transmitting agents differ American students are particularly interested only in one of these, *Babesia bigemina,* since it is the only one that occurs in the western world

BABESIA BIGEMINA

Synonym *Piroplasma bigemina.*

This organism was first accurately described by Smith and Kilborne (6) in the United States in 1893 These workers also made the discovery that the infection was transmitted by an arthropod, a discovery of epochal importance since this was the first protozoan disease shown to be so transmitted.

Morphology. *Babesia bigemina* occurs in the erythrocytes as pear-shaped forms, principally, although round and irregular forms are not uncommon Generally the pear-shaped forms are seen in pairs and this accounts for its specific name The two individuals of the pair lie side by side with their pointed ends in contact with each other. They are about 4 microns in length and lie crosswise of the erythrocytes Sometimes only one form appears and in other cases there may be 4, arranged in fan-shaped formation Multiplication is by cell division, 2 individuals resulting from division of each of the mature forms. The chromatic material is divided equally between the newly formed individuals, taking its place as a distinct granule located near the small end of the pear-shaped cells Details of the growth process at this stage are rather meager. Apparently the host cells are destroyed since hemoglobinemia is a prominent part of the pathological picture It is believed that the pear-shaped elements, freed from one cell, proceed to invade others, repeating the process in the second cell. In acute piroplasmosis large numbers of infected cells are seen in blood films. In chronic cases it is difficult and often impossible to demonstrate parasites in blood films, but the infection can be shown to be present by inoculating young susceptible calves with the blood. In Giemsa-stained blood

films the cytoplasm of the parasites stains bluish and the chromatic material red. The erythrocytes take a yellowish-pink color, although many of them are low in hemoglobin and take a pale straw color as a consequence.

The Transmitting Agent. In the United States the sole transmitting agent is the Southern blue tick, frequently called the Texas fever tick, or *Margaropus annulatus* (*Boophilus annulatus*) At one time this tick was prevalent in the southern half of the United States but the eradication campaign which has been waged against it since 1906 has eliminated it from all but eight counties in southeastern Texas. More than 99 per cent of the territory in the United States originally infected with this tick (and Texas fever) has been freed from the tick and the disease transmitted by it The island of Puerto Rico and some of the Virgin Islands have also been freed. The Chief of the Bureau of Animal Industry (4), U. S Department of Agriculture, reported in 1941 that no cases of Texas fever had been reported in the United States for more than two years

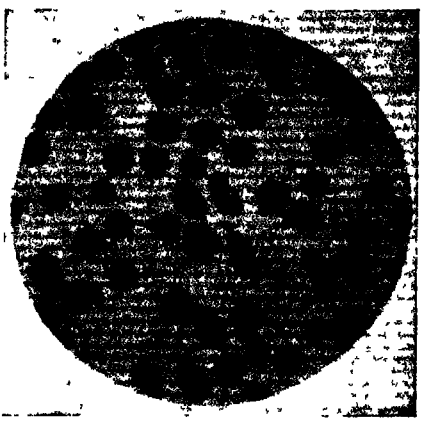


FIG 110 *Babesia bigemina.* Stained film of blood from a cow suffering from Texas fever One parasitized erythrocyte is located near the center of the field This cell contains two parasites x 900

In other parts of the world other species of *Margaropus* are responsible for transmitting this parasite In southern Europe it is *M. calcaratus*, in central Africa, *M. decoloratus*, in Australia, Brazil, Venezuela, and the Caribbean Islands, *M australis* *

Since these ticks are the only means by which piroplasmosis of the Texas fever type is spread, the disease can be eradicated by their elimination. This is done by systematic dipping of all cattle, horses, and mules in an arsenical solution which is poisonous to the ticks In areas where the tropical tick (*M. australis*) is the transmitting agent, it is often necessary to dip sheep and goats as well.

The life cycle of the protozoon in the tick is unknown. Certain developmental forms in the stomach of the arthropod have been followed but nothing further is known about it except that some form of the parasite is

* This species instead of *M Annulatus* formerly was the transmitting agent in a limited area of southern Florida

carried from the adult female tick through her eggs to the larval form, which, when feeding upon the blood of another bovine, introduces the infection into the second host

Pathogenicity. Texas fever ordinarily affects only cattle Cases in deer have been reported Other animals harbor the ticks which transmit the disease and may keep the disease alive in a community by propagating the transmitting agent but they are not affected by the disease, nor do they harbor the protozoon, so far as is known

Calves are much less susceptible to the disease than older animals Where the disease is prevalent most calves contract the disease early, recover from it, and become chronic carriers throughout the remainder of their lives Such animals frequently are unthrifty and anemic but they show no acute symptoms and are able to resist severe exposure indefinitely When disease-free cattle are placed with such animals, acute Texas fever appears only if the transmitting tick is present. These facts explain why Texas fever used to appear among northern cattle which had been shipped into the southern part of the United States, and why it appeared in the northern states among native cattle when apparently normal southern cattle were placed among them

The early history of Texas fever in the United States is rather vague In the early summer of 1868, however, attention of the people was focussed on this disease because of large losses in northern stock originating in a shipment of Texas cattle sent by Mississippi boat to Cairo, Illinois, and from there by rail to points in Illinois and Indiana. Cattle shipped from these states to other northern points carried the disease with them Little progress was made in determining the nature of the disease at that time D E Salmon, Chief of the Bureau of Animal Industry, U S Department of Agriculture, surveyed the country to determine the areas where the disease was enzootic and defined this area by what came to be known as the Texas fever line This line ran across the country a little north of the 35th parallel of latitude corresponding roughly with the Mason and Dixon line. Although it was not known at the time, this line marked the northern limit of the distribution of *Margaropus annulatus.* Work on the causation of the disease was begun in the Bureau laboratories under Salmon's direction, the actual work being carried on by Theobald Smith, F. L. Kilborne, and Cooper Curtice, assisted by V. A Moore. Smith (5) published preliminary observations upon the blood protozoon in 1889, and Smith and Kilborne (6) the completed study in 1893 Curtice (2) published his studies on the biology of the Texas fever tick in 1891. Its relationship to Texas fever was clearly exposed in the paper by Smith and Kilborne.

Susceptible cattle placed among the chronically infected will, in the presence of the transmitting tick, develop the first symptoms in not less than 10

days. A few instances are on record in which the disease has been transmitted by surgical operations, such as dehorning, when the instruments used have not been sterilized between animals. In these cases, the disease should make itself evident in from 8 to 10 days The onset is marked by a high fever, sometimes reaching 107° F. Hemoglobinuria frequently occurs although the brownish-red urine may not be noticed during life but is found at autopsy in the urinary bladder. The blood is pale because of severe anemia. The mucous membranes are pale and jaundiced. The animals do not eat, are depressed and weak, and usually die within 10 days, often by the 5th to 8th day. Less severe cases occur, the animals recovering after 10 days Undoubtedly still milder forms occur in which few or no symptoms are seen

Pathological Changes. Except for the usual changes which accompany severe anemia and icterus, the principal lesions are found in the spleen, liver, and kidneys

Edematous areas often occur in the subcutaneous tissue of the ventral part of the body and in the fatty tissue around the kidneys. Hemorrhages are frequent in the subcutaneous tissue, on the walls of the heart, and on the mucosa of the urinary bladder The most conspicuous changes are in the spleen and these are responsible for the old name for this disease, *splenetic fever* The organ is much enlarged—from 2 to 4 times its normal size The color is reddish-brown, it is often much softer than normal, and the normal structure, the Malpighian bodies and trabeculae, are obscured. The organ is engorged with blood cells and with phagocytic cells filled with blood pigment. A great deal of free blood pigment, in the form of granules, may be seen.

The liver is swollen, and pale in color because of fatty degeneration The surface and cut sections are yellowish and mottled The bile ducts are engorged and the gall bladder is distended with thick, viscid, dark-colored bile containing flocculi in suspension The kidneys often are swollen and dark in color The urine in the bladder frequently, but not always, is tinged with blood pigment

The changes are those associated with extensive blood-cell destruction. The count of erythrocytes which normally is about seven millions per cubic millimeter often sinks to less than one million at the time of death. Regeneration of blood cells occurs in the disease as is manifested by the presence of nucleated erythroblasts and other juvenile types in the blood stream. Extreme poikilocytosis, associated with the severe anemia, generally occurs.

Immunity. A number of methods have been tried for immunizing susceptible cattle which are to be shipped to areas where they will come in contact with piroplasmosis. Small doses of virulent blood usually will accomplish the

purpose although the method is, of course, somewhat hazardous in itself. If given to calves from two to six weeks of age, as was advocated by Francis and Connaway (3) in Texas, the reactions usually are not severe. In older animals they are likely to be severe but non-fatal. Another method of immunizing young calves, practiced in Texas and elsewhere, was to deliberately liberate one or two infected ticks on the animal before it was turned out on pastures where heavy tick infestations would be picked up. Within two weeks the animal will have passed through a mild acute attack which will protect it thereafter from more severe exposure.

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BABESIA BOVIS

Synonyms *Babesiella bovis, Microbabesia bovis, Piroplasma bovis*

The disease caused by this species is essentially like Texas fever It occurs in all parts of the continent of Europe The principal transmitting agent is *Ixodes ricinus*, other ticks occasionally are vectors Like the piroplasm of Texas fever, *Babesia bovis* passes through the egg of the adult female to the larvae and these, feeding upon other animals, transmit the disease to them In morphology, this species resembles *Babesia bigemina*, but they are much smaller. The largest diameter does not exceed 1 5 microns. They are pyriform and commonly occur in pairs with their pointed ends together. Some of the forms are so small that they appear to consist entirely of chromatic material without cytoplasm.

Stockman and Wragg demonstrated that cattle which had survived infection with *B. bovis* were still susceptible to *B bigemina*. This seems to indicate that these forms are different species, however there is much confusion with regard to cross-immunization between the piroplasms, and some authors hold that immunity tests do not indicate relationships in this group.

BABESIA MUTANS

Synonyms· *Piroplasma mutans, Gonderia mutans.*

This parasite was differentiated from other blood protozoa by Theiler in 1906. It occurs in the blood corpuscles of cattle as very small bodies of various

shapes. There is difference of opinion as to whether it really is a piroplasm, some regarding it as a form of *Theileria parva*. Infections have been recognized in southern Europe, Africa, Asia, and Australia. The infection is quite benign Those who believe in the piroplasmic nature of this parasite base their belief on the fact that schizonts are not ordinarily found in the spleen, bone marrow, and lymph glands, as is the case in *Theileria* infections. Some, however, have claimed that in unusually intense infections with this parasite schizonts are demonstrable in the internal organs

Theiler was unable to transmit this parasite through the agency of the types of ticks which transmit the other piroplasmoses. He did succeed, however, with *Rhipicephalus evertsi* and *R appendiculatus*. The parasite was not transmitted through the egg, as in the other piroplasms.

The Babesiae of Sheep and Goats

Piroplasmosis in sheep was first noted by Babes in Roumania who found the parasites in the blood of animals suffering from a disease known as "carceag." He believed the parasite to be the same as the one which he had described in hemoglobinuria of cattle Later studies have shown that there are three types of piroplasmosis in sheep, the parasites corresponding in morphology to the three which occur in cattle Cross inoculations do not succeed, however, hence they are regarded as distinct species There is a relatively large form, to which Wenyon gave the name *Babesia motasi*, which is comparable to *B. bigemina* of cattle The disease produced often is severe, there being high temperatures, much blood-cell destruction, icterus, and hemoglobinuria. This is the "carceag" of eastern and southeastern Europe. Transmission is by means of a single species of tick, *Rhipicephalus bursa*.

The parasite of intermediate size, corresponding to *B. bovis* of cattle, is *Babesia ovis* The disease produced is much milder than that caused by the preceding species. Fever, jaundice, and anemia are produced but recoveries generally occur The transmitting agent is not known. The small species is known as *Babesia sergenti* (Wenyon), *Gonderia ovis*, and *Theileria ovis* Its morphology and such facts of its life cycle as are known relate to *B mutans*. Like the latter, it is relatively harmless to its host. The transmitting agent is not known.

The Babesiae of Horses

Piroplasmosis of horses was recognized in central Europe quite early. In 1910 Nuttall and Strickland recognized two kinds of parasites of horses, one of which they named *Piroplasma caballi*, the other *Nuttallia equi*.

BABESIA CABALLI

Synonym · *Piroplasma caballi.*

This species resembles *Babesia bigemina* in size and morphology The disease also is similar to Texas fever, there being high fever, icterus, anemia, and hemoglobinuria. It occurs in southern and southeastern Europe and in the Caucasus region of southern Russia Darling has reported the existence of this disease in Panama, this being the only region in which it is known to occur in the western hemisphere. It is transmitted by the tick, *Dermacentor reticulatus.*

BABESIA EQUI

Synonym *Nuttallia equi.*

This species is smaller than *B caballi* It is pyriform in shape. In the process of division it regularly forms four individuals, these being attached by their pointed ends forming a sort of maltese cross Later they break apart, each cell escaping from the parasitized erythrocyte to enter other cells in which the process is repeated The disease is highly virulent for adult horses and other species of the horse family, but is mild in young animals. Inoculation of colts as a means of protection later in life is commonly practiced. The disease has been reported in southern Europe, Africa, southern Asia, and South America The transmitting agent in South Africa is *Rhipicephalus evertsi*

The Babesiae of Dogs

Piroplasmosis of dogs occurs in southern Europe, in various parts of Africa, and in Asia It has been reported in Puerto Rico and in Brazil It was seen by Clark in Panama in imported hunting dogs It was recognized in the United States for the first time in 1934 In the Old World the only species occurring in dogs is *Babesia canis* The type occurring in South America is somewhat different and has been placed in a different species, *Babesia vitali.* Wenyon questions whether it should be considered as a separate species.

BABESIA CANIS

Synonym · *Piroplasma canis.*

The disease caused by this parasite in dogs is essentially like Texas fever in cattle. Young animals are fairly resistant, hence dogs which are raised in an infected environment usually suffer only from the mild, chronic form which may be practically symptomless, yet the parasites in their blood are exceedingly virulent for animals which have never been in contact with the disease earlier in life. Imported dogs usually suffer from the acute form of the disease,

for this reason. In the acute disease there is high fever, progressive anemia, icterus, and hemoglobinuria, and the disease frequently is fatal. The lesions consist of splenic enlargement, fatty degeneration of the liver lobules with distention of its bile ducts, nephritis, and icterus

The parasites are relatively large. Typical pear-shaped forms occur in pairs as in the cattle disease but large amoeboid forms with large vacuoles are also seen The cycle of development was studied and described by Nuttall and Graham-Smith (3) (4) (5) (6) Often parasitized cells are much more numerous in the capillaries of the internal organs than in the peripheral circulation, in fact it is often difficult to find infected blood-cells in the blood of acutely ill patients In these cases a diagnosis can be made by the inoculation of blood into non-immune animals Such animals react promptly with a febrile reaction and the other symptoms of piroplasmosis but in these animals it may be impossible to find the parasites. Sanders (7) recommends, as a more satisfactory procedure, the inoculation of young, splenectomized dogs, since in these it usually is easy to find parasites in the peripheral blood during the febrile period, 2 or 3 days after inoculation

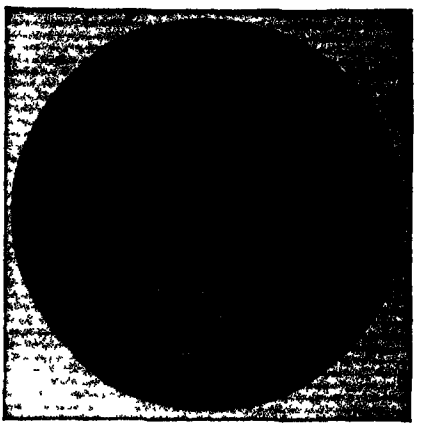


FIG 111 *Babesia canis* Stained blood film of a dog suffering from canine babesiosis showing one parasitized erythrocyte near the middle of the field This cell contains two parasites having the shape of apple seeds and lying characteristically with the pointed ends together (Specimen from which photograph was made furnished through the courtesy of D A Sanders) x 900

Canine piroplasmosis was first recognized in the United States by Eaton (1) in 1934 Since then it has become evident that the disease is not uncommon in Florida, and there is evidence that the disease occurs in Texas It is not unlikely that it occurs in other of the southern states where the brown dog tick, *Rhipicephalus sanguineus,* the transmitting agent, is common. Inasmuch as this tick is becoming common in the more northerly states, the disease should be watched for in areas where it is known to occur (2).

Canine piroplasmosis is naturally transmitted by several different ticks. In Europe the principle vector is *Dermacentor reticulatus.* In India and in the United States, it is *Rhipicephalus sanguineus,* and in South Africa, *Hemaphy-*

salis leachi. The parasite passes through the eggs of the ticks to the next generation. Christopher, in India, claims to have followed the developmental cycle in *Rhipicephalus sanguineus* and found it to be not unlike that of the plasmodia.

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The Theileriidae

It will be recalled that the Theileria differ from the Babesia in that schizogony occurs in endothelial cells of the internal organs and only certain forms enter the erythrocytes and thus appear in the peripheral blood stream. Multiplication within the blood cells does not occur, and the blood ordinarily is not infective by inoculation Occasionally some of the endothelial cells containing schizonts appear in the circulation and this explains why *Theileria* infections can sometimes be transmitted by blood injection. *Theileria parva*, the cause of East Coast Fever in cattle is the most important representative

THEILERIA PARVA

Synonym. *Theileria kochi*.

This parasite is the cause of East Coast Fever of cattle, a disease which causes severe losses in the countries along the greater part of the eastern coast of Africa It also occurs in Transcaucasia and India The disease was long confused with piroplasmosis since both diseases occurred in the same area and the same animals often were infected with both. It is now recognized that chronic carriers of piroplasms often suffer from acute attacks because of the stimulation offered by the *Theileria*. In East Coast Fever the anemia, icterus, and jaundice, so characteristic of piroplasmosis, is absent. In from 10 to 20 days after infection, fever begins and there is swelling of the superficial lymph nodes. There is difficulty in respiration, emaciation and weakness, and the passing of dry, tar-like, bloody feces At the height of the fever as many as 90 per cent of the red blood cells contain the characteristic parasites, however the blood cells are not destroyed by them, as in piroplasmosis. The disease is or-

dinarily of short duration and the majority of affected animals die Milder forms of the disease are known Animals which recover do not continue to harbor the parasite for ticks cannot be infected from them. In this respect this disease differs from all the piroplasmoses.

Animals which die of this disease present petechial hemorrhages on the serous membranes and in the subcutaneous tissue All of the lymph nodes are enlarged but the spleen is essentially normal in size and color. Hemorrhagic and sometimes ulcerative enteritis is seen The kidneys sometimes show wedge-shaped infarcts

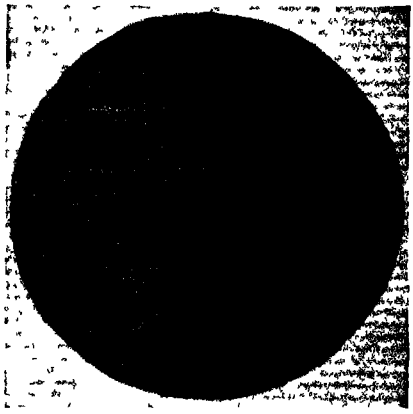


FIG. 112 *Theileria parva* Forms of the parasite found in the circulating erythrocytes during the febrile period of East Coast Fever Unlike the piroplasms, which these bodies resemble, the protozoon of this disease does not ordinarily multiply in the circulating blood, and blood usually is non infective When more than one body is found in a red corpuscle it is believed that a multiple infection has occurred The schizogonic phases of this parasite occur in the tissues in the form of "Koch's blue bodies" x 900

Most characteristic of this disease are the structures commonly called "Koch's blue bodies" (2) which are found in the lymph nodes, the spleen, and in smaller numbers in other organs They are from 3 to 10 microns in diameter, are roughly spherical, and occur within endothelial cells, especially of the capillaries In films they sometimes appear to be free but it is believed that this is because the mother cells have broken down. In films or sections stained with Giemsa's stain they appear as masses of bluish-stained cytoplasm in which numbers of red-staining chromatin dots varying from 1 to 2 to 30 or more are seen These are the schizonts of the parasite. When fully mature these break down, releasing minute elements, each containing one of the chromatin granules These either enter other endothelial cells to repeat the multiplicative process, or they enter red blood cells and circulate in the blood. In blood films these are seen as round bodies, 1 to 2 microns in diameter, or they may be ovoid, pear-shaped, or elongated, rod-like forms. Most of the erythrocytes contain only 1 element but 2 or even 4 may be found in a single cell. It is believed that the multiple forms represent multiple invasions rather than multiplication within the cell. Occasionally, in very severe infections,

Koch's blue bodies may be found in the blood, and such blood is infectious when injected into other animals. This is the exception rather than the rule.

Theileria parva is transmitted most commonly by the tick, *Rhipicephalus appendiculatus R. simus, R evertsi, R. nitens,* and *R. capensis* are also capable of transmitting it Nuttall and Hindle (3) who studied this disease in England, using infective ticks brought in from Africa, noted that animals did not become infected unless the tick was allowed to feed for at least two days. As in the case of the piroplasms, knowledge of the cycle in the tick is rather vague. Gonder (1) claims to have traced a cycle not unlike that of the malaria parasites.

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The Anaplasmata

In the course of their classical work on piroplasmosis or Texas fever of cattle, Smith and Kilborne observed and described small coccus-like bodies located near the periphery of many of the red blood cells in animals suffering from the disease They interpreted these bodies as a stage in the life cycle of the Texas fever parasite It is clear today that these small bodies were not piroplasms and that they were dealing with animals which suffered from two diseases simultaneously, anaplasmosis and piroplasmosis In 1910, Theiler (7) in South Africa, differentiated the two diseases, but since both occurred in the same regions, there were many who disagreed with Theiler and continued to look upon the small marginal bodies either as artifacts or as piroplasms. The matter was not fully cleared up until recent years when anaplasmosis has been found to be prevalent in most of the southern and some of the northern states of the United States, regions that are now free of piroplasmosis (6) and in some cases regions in which piroplasmosis has never been known to occur Anaplasmosis has been definitely diagnosed in at least twenty-two states, including, in general, all of the states of the southern half of the country and a few of the more northerly It has not been diagnosed in the New England states and the northern tier of states with the exception of Idaho and Montana All doubts about its infectious nature and its separate identity have been removed by these experiences.

ANAPLASMA MARGINALE

This name was given to this organism by Theiler. The word anaplasma means without plasma (cytoplasm) and refers to the fact that the parasite seems to consist of nothing but a small bit of chromatic material without any evidence of cytoplasm The specific name is derived from the fact that these bodies are located, characteristically, near the periphery of the red blood cells and thus appear, in smears, as if on the margin of the cells

Morphology. The parasites of anaplasmosis are seen in the red blood corpuscles as minute, deeply staining points usually located near the margin of the cell If stained with the Giemsa stain they are deep red in color, with other stains they are apt to be so dark that color in them is not distinguished They are spherical in form and ordinarily there is but one organism per cell, although two or more may be seen in some cells In the early stages of the disease before the temperature rise occurs, few or no parasites can be found. When the febrile period begins the percentage of infected cells may reach 25 per cent or even more than 50 per cent If the animal recovers the number of cells containing the marginal bodies diminishes rapidly until none can be found microscopically The blood of recovered animals is infectious for many years and probably for life, hence it is probable that a few bodies are present indefinitely, so few as to make the finding of them impossible.



FIG 113 *Anaplasma marginale* Stained blood film from a cow suffering from acute anaplasmosis and showing many organisms in her blood x 900

Life History. The life history of the parasite of anaplasmosis is wholly unknown and therefore its proper classification is problematical. It is included here with the piroplasms, because of the similarity of the diseases caused by them, but it should be pointed out that our knowledge of the *Anaplasma marginale* is much less complete than that of *Babesia bigemina*. It is known, for example, how the piroplasms multiply; this is not known for the anaplasms.

It cannot be said, in fact, that it has been proven that the bodies which are found in the red cells are protozoa and the cause of the disease in which they are found.

The Natural Disease. Anaplasmosis occurs only in cattle As in piroplasmosis, young animals are quite resistant. Cases in calves under one year of age are rare, although in infected territories many calves pass through the infection and become immune carriers. The natural resistance of young calves is removed by splenectomy. The marginal bodies appear in great numbers in such animals, hence, they are suitable for diagnostic purposes

In older animals the disease may be acute or chronic Those affected with the acute form may die within two or three days after the appearance of the first symptoms. The disease begins with a high temperature, 105° to 107° F After a day or two, signs of anemia and icterus appear and about this time the temperature falls to normal and even sub-normal as death approaches The mucous membranes are pale and yellowish The yellow color is evident also in the thin-skinned parts of the body Urination is frequent but the urine is not blood-tinged, as it often is in piroplasmosis. The animal usually is constipated, the feces being dark, often blood stained, and covered with mucus

In the more chronic cases the animals live longer, are weak, progressively emaciate, show icterus and anemia The red blood cell count may fall from a normal of about 7 million to less than 1 million per cu mm , the hemoglobin may be less than 10 per cent

The mortality is quite variable. It may be greater than 50 per cent, or less than 5 per cent Losses are greatest in hot weather, and in older animals

In natural infections the period of incubation varies from 20 to 40 days Experimentally symptoms may be produced much earlier by inoculation with large doses of acutely infected blood.

The diagnosis is made conclusive, of course, by the finding of the characteristic marginal bodies in the red blood cells In this connection it is well to warn the examiner to be cautious in his identification, so as not to confuse such structures as basophilic stippling and the "Jolly" bodies, seen in severe anemias, as anaplasmata. Artifacts resembling these bodies often are seen, also. The examinations should be made only on good films which are well stained.

Schmidt (5) says that he often has encountered typical cases of anaplasmosis in which he was unable to demonstrate blood parasites. These occur in animals which have been inoculated for diagnostic purposes and sometimes lead to embarrassment. It is necessary in such instances to inoculate another animal known to be susceptible. The chances of finding the parasite in the blood in inoculated animals is much better if mature rather than young animals are used

Boynton and Woods (3) have reported a simple test which seems to have some value in the diagnosis of anaplasmosis. The blood is allowed to clot and a little clear serum is obtained. Two drops are added to two cc. of distilled water in a tube. The serum of normal animals does not cloud the water; that of animals affected with anaplasmosis causes an immediate clouding and after standing overnight, a white precipitate covers the bottom of the tube. Animals affected with acute anaplasmosis and recently recovered carriers give this reaction. The test depends upon the precipitation of euglobulin which appears to be present in increased amount in this disease

The principal lesions are those associated with blood destruction, anemia, and icterus. The spleen is enlarged and the pulp is dark and soft The blood appears as if diluted with water A catarrhal enteritis is common. There may be a few petechial hemorrhages on the heart-wall and on the mucosa of the urinary bladder The lymph nodes are swollen and edematous. The liver shows marked icterus with the bile channels engorged and the gall bladder distended with dark green, mucilaginous bile.

Transmission. At least 17 species of ticks have been shown to be capable of transmitting anaplasmosis Most of these probably are mechanical rather than biological carriers but it is known that several species are biological carriers (1) (2) Among the latter are *Margaropus annulatus, Dermacentor occidentalis,* and *Dermacentor andersoni* which occur in the United States. In these species the infective agent passed through the egg into the next generation of the species. Nothing is known about the life cycle of the anaplasma in the invertebrate biological vectors

In addition, at least seven species of horse-flies (*Tabanidae*) have been shown to be mechanical carriers and certain mosquitoes have also been incriminated The stable fly (*Stomoxys calcitrans*) and the horn fly (*Hematobia serrata*) apparently seldom, if ever, act as transmitters.

An important means of transmission is through the use of common surgical instruments which have not been thoroughly disinfected after being used on one animal and before being used on another. Reese (4) has shown that anaplasmosis can very easily be carried on a lancet which is used for drawing blood if it is merely wiped after being used on an infected animal. Outbreaks have occurred in herds after dehorning operations, the drawing of blood samples, castrations, and minor operations. In areas where anaplasmosis occurs, veterinarians should be exceedingly cautious in carrying out these mass operations to avoid serious consequences resulting from the spreading of this disease to many animals from a few, or even one, carrier animal.

Immunity. Animals which recover from anaplasmosis remain carriers for long periods and probably for life. Such animals are resistant to additional in-

fection. Young calves are relatively resistant to the infection. Symptoms are seldom seen in calves less than one year of age. Such animals can be inoculated with virulent blood, especially in the winter months when the disease is not so severe naturally as it is in the hot months, and made immune thereafter. Because of the permanent carrier situation which is created, and because of the multiplicity of vectors which exist, such animals are a source of danger to any non-immune animals with which they may come in contact at any time later in life.

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ANAPLASMA CENTRALE

Theiler (2), in his studies which led to differentiation of the anaplasms from the piroplasms, distinguished two kinds of anaplasms, the marginal bodies which have already been described, and which are the only ones found in anaplasmosis in the United States, and central bodies which are identical in appearance with the marginal bodies but are located characteristically in the central part of the red blood cells. They have come to be called under the name which heads this paragraph

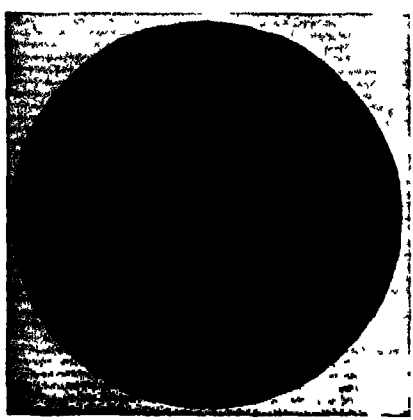


FIG. 114 *Anaplasma centrale* Two typical parasites are seen as sharply staining dots in red blood cells near the center of the illustration Poikilocytosis and anisocytosis are present as a result of anemia Blood, bovine x 900

Theiler showed that animals which had recovered from an infection with blood containing the central bodies could still be infected with blood containing the marginal bodies. He also noted that the disease caused by the *A centrale* was much

milder than the other. In South Africa, according to Schmidt (1), adult cattle coming from Europe are first injected with blood containing *Anaplasma centrale* and later with *A. marginale*, the first and milder infection giving some protection against a severe second

Apparently the *A. centrale* infection produces symptoms similar to mild infections with *A marginale*

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CHAPTER XXXVIII

THE PATHOGENIC CILIATES

Protozoa belonging to the Class *Ciliata* are exceedingly numerous in nature They occur as free-living organisms in stagnant water everywhere, and "cultures" can easily be obtained by placing straw, grass, or almost any kind of vegetable material in ordinary tap water and keeping the dilute infusions at room temperature for a few days. Pathogenic species belonging to this group are not numerous. All of the known pathogens belong to a single genus, *Balantidium* These are intestinal parasites *Balantidium coli* occurs commonly in pigs and occasionally in man A smaller species, *Balantidium suis* is found in pigs, and this species appears to be specific for swine. Members of this genus have been reported in various types of monkeys, cattle, sheep, and horses. It is not known whether these are separate species, or whether they are *B. coli*. It is quite certain that many of the monkey types are *B coli* for many cross infections have been secured experimentally (1).

BALANTIDIUM COLI

This organism apparently occurs in swine everywhere In the greater part of the infections no apparent damage is done, however in animals that are debilitated for other reasons severe lesions result The same situation exists with respect to the human infections. Most of these have been seen in persons who have had close contact with swine, hence it is believed that human infections are incidental. The organism occurs in the intestines, particularly in the large intestines Occasionally it is found in the lower end of the small intestines as well.

Morphology. *Balantidium coli* is a relatively large protozoon It is ovoid or pear-shaped and generally measures from 50 to 80 microns in length and about two-thirds as broad. Some individuals, however, are very much larger, measuring as much as 150 microns in length. The surface presents longitudinal ridges which run slightly spirally In the grooves between these ridges are rows of cilia which vary from 4 to 10 or 12 microns in length. These cilia occur on all parts of the body. In active individuals they are in constant motion.

At the anterior end of the organism there is a slightly oblique depression, the *peristome*, at the bottom of which is an opening, the *cytostome*, which

leads into a tubular structure, analogous to an esophagus, which ends blindly in the cytoplasm of the cell. The peristome contracts into a slit-like structure at times and at others relaxes into a broad funnel. Large cilia surround the opening of the cytostome and line a considerable part of the wall of the tube which extends from it into the interior of the organism At the posterior end of the organism a small opening in the body wall, the *anal aperture*, occurs. The nucleus is a large sausage-shaped structure lying somewhat diagonally across the long axis of the organism Vacuoles, red blood cells, leucocytes, and other foreign bodies are often seen in various parts of the cytoplasm.

Reproduction is by transverse division The nucleus divides prior to the division of the cytoplasm, one half going into each of the new individuals. The cytostome remains in the new individual constituted by the anterior half of the parent cell The other daughter cell forms a new cytostome.

Round or oval cysts are formed protected by thick walls consisting of two distinct layers. These cysts usually contain but a single individual but sometimes two individuals appear to fuse (conjugation) within the cyst wall. The rounded parasites may be seen within the cyst, the cilia moving slowly These cysts are the means by which the species is propagated They are passed to the ground in the feces, and by fecally contaminated food into a new host

Pathogenicity. As has been stated above, *B coli* occurs in many swine which show no symptoms and apparently little harm is done by it Occasionally, however, in stunted, heavily parasitized pigs, especially young pigs, the organism is found in association with an ulcerative colitis and severe bloody diarrhea Two such cases of this type have been seen by the author, and others have been reported from nearly all parts of the world. These cases apparently are not numerous, and it is not clear whether the malnutrition is caused by the *Balantidium* or whether the parasite takes advantage of a host whose resistance has been depressed by other factors. The ulcers are not unlike those seen in amebiasis of man Sections show the parasite both on the surface and deeply embedded in the intestinal wall around the ulcers They multiply in the tissues and it is supposed that the ulcers are formed by the rupture of abscesses formed in the submucosa by organisms which have forced their way into this location by purely mechanical means.

Treatment. This organism is very refractory to treatment and no successful methods have been developed.

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CHAPTER XXXIX

PATHOGENIC PROTOZOA OF UNDETERMINED CLASSIFICATION

Several organisms believed to be protozoa but which do not fit in with established classifications are considered under this heading When sufficient information about them has been acquired to make it possible, they will, of course, be placed in their proper relationship with other forms They are grouped here for convenience only and not because they have features in common

The Toxoplasmata

The genus *Toxoplasma* was created in 1909 by Nicolle and Manceaux (4) for a parasite which they found in a small mammal, the gondi, which lives around the borders of the Sahara desert in north Africa They gave it the name *Toxoplasma gondii* A little later Splendore (9) described what appears to be the same organism from a Brazilian rabbit under the name *Toxoplasma cuniculi* Both of these parasites were readily inoculable into pigeons, and Splendore's also infected a dog The identity of these two forms is further suggested by the fact that spontaneous canine infections have since been reported from both Brazil and Tunis In later years parasites morphologically indistinguishable from *T gondii* have been found in a number of species of wild birds, mice, rats, guinea pigs, rabbits, dogs, cats, cold-blooded animals, sheep, and man Many of these have been confused with stages of the life cycle of coccidia, leishmania, and plasmodia Some have been given specific names according to the hosts in which they were found. In many instances transmission experiments were not made, the identification being made on the basis of morphology alone However, it has been found by transmission experiments that the parasite is not host specific, thus Sabin and Olitsky (8) have recently found that a strain isolated from guinea pigs and another from a human infection could be inoculated successfully into mice, guinea pigs, rabbits, chickens, and *rhesus* monkeys, producing fatal infections in all cases except in the monkeys Also, these authors were unable to find any serological differences between the two strains. These facts suggest that many and

possibly all toxoplasmas, irrespective of host, are identical. If this is the case, they should be known under the name *Toxoplasma gondii*.

Into this confused picture the parasite known under the name *Encephalitozoon cuniculi* must also be injected. This name was given by Levaditi, Nicolau, and Schoen (1), in 1923, to an organism which several had noted previously in the brains of rabbits suffering from encephalitis. This organism proved to be inoculable into mice, and subsequently spontaneous mouse infections have been described. It seems likely that this organism is a toxoplasm.

TOXOPLASMA GONDII

This organism was named by Nicolle and Manceaux (4), in 1909. It was found by them the previous year in a small rodent at the Pasteur Institute of Tunis in northern Africa. It is assumed that all forms found subsequently in many kinds of birds and mammals, including man, belong to this genus, although proof of this is lacking

Morphology. *As seen in fresh preparations the parasite is somewhat elongated, one or both ends being somewhat pointed.* In stained films it is ovoid or pyriform In tissue sections it is ovoid or round It varies somewhat in size but averages 2 by 4 microns A solid staining nucleus is located centrally or toward the blunter end of the ovoid forms. In fresh preparations it is non-motile, and flagella have not been demonstrated

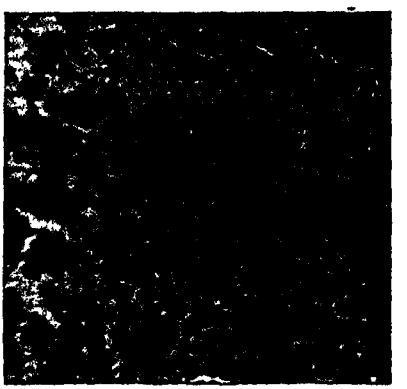


FIG. 115 Toxoplasmata in a Capillary Wall, Lymph Node, Cat x 450 (Courtesy of Olafson and Monlux, Cornell Veterinarian)

In tissues the organisms may be found singly or in compact masses in cyst-like structures containing as many as fifty individuals It is believed that they multiply only intracellularly, particularly in mononuclear and endothelial cells, but also in cells of the parenchyma of the lungs, liver, brain, kidneys, heart muscle, and smooth muscle. They are found in all of these cells frequently lying in cyst-like vacuoles of the cytoplasm Reproduction is by means of longitudinal division. The individuals are released when the host cell disintegrates.

Artificial Cultivation. Sabin and Olitsky (8) succeeded in propagating an organism derived from guinea pigs through six generations without loss of

virulence. The medium used consisted of minced chick embryo suspended in Tyrode's solution (Rivers-Li medium). Development was wholly within cells. They did not succeed in obtaining growth in cell-free media.

Pathogenicity. Experimental animals are readily infected with most strains by intraperitoneal, intravenous or intracranial inoculation Subcutaneous inoculation is less likely to succeed and the disease is more chronic when initiated in this way. Sabin and Olitsky succeeded in infecting mice by introducing the inoculum by mouth and nose The course of the disease depends upon many factors; the dosage, route of inoculation, species of animal, and particularly, upon how well adapted to the species of animal the strain may be as a result of few or many passages in it Death usually results in from 1 to 3 weeks but may occur as early as the third day and chronic infections may result in death after several months, or recoveries may occur.

After intraperitoneal inoculation, a large amount of viscid exudate usually collects in the cavity and often in the pleural and pericardial cavities as well. Many organisms occur in these exudates both free and intracellularly After subcutaneous inoculation, local inflammation and necrosis occurs with or without generalization Intravenous inoculation causes a generalized disease in the more highly susceptible species Intracranial injection produces encephalitis, the type depending upon the species of animal used In the rabbit the brain usually is not affected unless the inoculum is introduced directly into it In the mouse, according to Sabin and Olitsky, brain lesions invariably occur no matter how the inoculation is made The brain lesions of mice inoculated intracerebrally are found near the ventricles and in the mid-brain, the organisms apparently spreading by means of the cerebrospinal fluid When the inoculum is introduced otherwise, the brain is infected by way of the blood stream and lesions are found around the blood vessels.

The rabbit and guinea pig usually show small areas of necrosis in the liver, spleen, adrenals, intestines, and lungs The parasites are found in abundance in these areas. The parasites are also found abundantly in the same organs of mice but, rather oddly, gross lesions are not usually present in mice.

The Spontaneous Disease

IN DOGS. The first case described in dogs was that of Mello (3) in Italy in 1910. A dog at the Pasteur Institute of Tunis contracted the infection in 1916, probably from the gondi disease which was being studied there at the time. Another case was reported from Brazil In 1939, Machettie (2) described two cases seen in Baghdad In 1942 Olafson and Monlux (5) reported the first cases in dogs in the United States. It seems likely that this disease is more common in dogs than the reported cases would indicate.

The affected animals present symptoms and pathological findings which vary considerably but there are certain things which all cases have in common. There is gradual emaciation with enlargement of the lymph nodes in all cases. The abdomen may be tender upon palpation Dyspnea is common and a bloody diarrhea sometimes occurs.

The lesions consist of enlarged lymph nodes which often are acutely inflamed, the surrounding tissue frequently being edematous and hemorrhagic. Sections of these nodes usually show necrotic areas. Small nodules may occur in the lung tissue and this organ often is edematous Nodules and ulceration of the intestinal wall frequently occurs Focal necrotic areas may occur in the liver, spleen, and other organs The spleen may be moderately enlarged When extensive involvement of abdominal organs occurs, a bloody exudate may be found in the peritoneal cavity

In the necrotic areas, wherever found, *Toxoplasmata* may readily be found usually in large numbers Eosinophilic infiltration of these areas is usual The disease in dogs is usually, if not always, fatal.

IN CATS One case of toxoplasmosis in a cat has been described This was reported by Olafson and Monlux (5) in New York. The lesions were not unlike those seen in dogs and there was a fatal outcome The pathogenicity of the organism was not proved, the diagnosis being based upon the character of the lesions and the demonstration of typical organisms in the lesions An interesting lesion seen in this animal but not in the three canine cases was a proliferation of epithelial cells in the lung This adenomatous-like lesion has been reported in human cases

IN SHEEP Only one case of toxoplasmosis in sheep has been reported, that of Olafson and Monlux (5) in New York. This was a case of toxoplasma encephalitis The animal showed nervous symptoms for about two weeks and finally became comatose. There was marked dyspnea with some nasal discharge. It was destroyed for autopsy examination

There were no gross lesions. Examination of the brain showed a diffuse encephalitis with slight meningitis. The cervical and thoracic regions of the cord showed lesions more severe than those in the brain These consisted of pronounced monocytic perivascular infiltration, focal areas of cell infiltration, and vacuolization of the white matter. Cyst-like structures filled with typical toxoplasmata were present in and around the inflamed areas. Lesions were present in sections from all levels of the cord They occurred most frequently in the white matter about the ventral gray columns. Unfortunately other tissues of this animal were not examined microscopically and no inoculation experiments were done since toxoplasma infection was not suspected at the time

of the autopsy. It is known that in man the infection often is limited to the nervous system, and sometimes this is true also in rabbits. This case in a sheep apparently was of such a type.

IN MAN. Wolfe, Cowan and Paige (10) described the first human cases of toxoplasmosis. Their cases occurred in new-born infants and took the form of fatal encephalitis. The infections must have occurred *in utero* although the mothers showed no evidence of the disease. Sabin (7), and Pinkerton and Henderson (6) recently have described two cases in children and one in an adult man All suffered from encephalitis and the diagnosis was made by finding the organism in the cerebro-spinal fluid. One of the children recovered; the other cases proved fatal

Transmission. The mode of transmission of toxoplasmosis is unknown Sabin and Olitsky (8) found it possible to infect mice by feeding them with contaminated material, and they also found cases traceable to cannibalism in half-starved mice At the Pasteur Institute in Tunis, the disease spread through a large group of rodents (the gondi) kept in captivity The present of intestinal ulcers in dogs suggests that the intestinal tract may be the point of entry Olafson (5) found that the disease spread rather quickly in a litter of puppies one of which had been inoculated intraperitoneally Levaditi and co-workers (1) found that the organism in rabbits suffering from encephalitis occurred in the kidneys and was eliminated in the urine. It may be said, therefore, that whatever the mode of transmission it is associated with intimate contact and that spreading occurs readily and quickly. There is no evidence that invertebrate carriers play any role in this disease.

Immunity. Inasmuch as most cases in domestic animals are fatal there has been little chance to study immunity Sabin and Olitsky, working with monkeys which frequently recover, were able to show that they were immune to reinoculation They also showed that the serum from recovered monkeys had the power of protecting other monkeys. The tests were done in the manner usually used in virus research, that is, by mixing the organismal suspensions with serum from recovered animals and incubating for a short time before injecting the mixture into the test animal. For test animals they used rabbits and mice. The test in mice involved life and death of the animal; in rabbits, however, they injected the mixtures intradermally and judged immunity by the presence or absence of the local lesions. It was possible to run a number of such tests simultaneously on a single animal. Since the parasites in the mixtures with immune serum seemed not to be changed in any way, they separated them from the immune serum by centrifuging and found they had re-

tained full virulence. In some manner, not understood, the serum inhibits infectivity of the organism without directly damaging it, *in vitro*.

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The Sarcosporidia

The parasites included in this group make up a single genus *Sarcocystis*. They are found in the striated muscular fibres, as intracellular parasites, of all of the domestic animals but are especially common in horses, cattle, sheep, and swine They are not common in dogs and cats. They occur frequently in wild herbivorous animals and in a number of species of birds. Ducks are more frequently affected than other birds. A few cases in man have been reported. They are not often seen in young animals.

The literature contains the names of many species of *Sarcocystis*, most of them having been named with respect to the animals in which they were found It has been demonstrated, however, that these organisms are not always host specific, since Erdmann (2) has shown that mice may be infected by feeding them infected sheep muscle and Darling (1) infected guinea pigs with an organism found in an opossum Furthermore there is no substantial morphological differences between the sacrosporidia of different animals except that in some the cysts in which they occur become larger than in others. Since it is known that this is determined, in part at least, by their age, there are no criteria for establishing different species within the group except the host relationship It is possible that all represent a single species This question cannot be answered at present.

SARCOCYSTIS MIESCHERIANA

Synonyms. From the discussion above it will be seen that the question of whether all sarcocysts belong to one or to many species cannot be answered

at present. If they are regarded as a single species, the name given above has priority. If it is shown that there are many species this name will be valid only for the parasite of the pig, to which it was applied by Kuhn in 1865. The specific names often used for the forms found in domestic animals are as follows:

Horse—*Sarcocystis bertrami*	Goat—*S moulei*
Cow—*S hirsuta* and *S. cruzi*	Mouse—*S. muris*
Pig—*S miescheriana*	Rabbit—*S cuniculi* and *S leporum*
Sheep—*S. tenella*	Duck—*S. rileyi*

Morphology. Sarcosporidia, in whatever host they may be found, have the same appearance. They are seen in striated muscles as elongated structures, the long axis of which is parallel to the muscle fibres In some instances the structures are large enough to be seen with the naked eye, in which case they appear as white streaks. If great numbers are present the muscles may become grayish-white instead of the normal color. These bodies were first seen in muscular tissue by Miescher in 1843 and have become known as *Miescher's tubes* In most instances the number of these structures is so few and their size so small that they are not detected except in microscopic sections Sections of muscles of old horses, cattle, and sheep, nearly always show a few of these structures

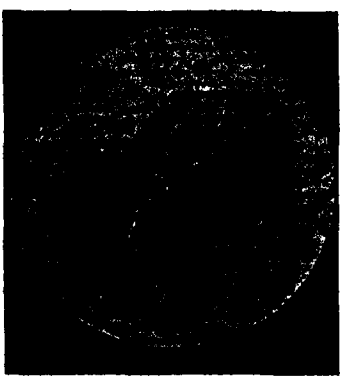


Fig 116 Sarcocyst, Heart Muscle, Horse. The photograph is of a stained cross section The sickle-shaped spores are shown crowding a capsule which is lined with germinal cells x 600

The sarcocysts develop inside of muscle fibres but as they become larger the fibres containing them disintegrate so the larger forms are embedded in connective tissue. All sizes may be seen from forms about 25 microns to others four or five centimeters in length. The elongated structure is surrounded by a definite capsule from which fine trabeculae or septa extend into the interior dividing it into chambers which are filled with the characteristic banana-shaped spores varying from 3 to 7 microns in length. These spores, in hematoxylin stained sections, take the blue dye, staining intensely. The trabeculae usually are not easily seen, thus the parasite appears like a sac filled with spores. In cross-sections these appear spherical, being many times as large as the normal muscle cells; in longitudinal section, they are, of course, elongate.

The spores apparently are formed from a germinal layer around the periphery and this layer continues to function for long periods of time. The very old sarcocysts not infrequently show nothing but debris and degenerated spores in their interiors with normal spores around the periphery. A fully developed parasite often contains many thousands of spores which escape only when the organism ruptures There is no evidence that this happens during the life of the animal.

Transmission. Theobald Smith (6) demonstrated in 1901 that mice could be infected by feeding them spores contained in the muscles of other mice. Erdmann (2) infected mice by feeding infected sheep muscle It seems evident that meat-eating animals can acquire the infection by ingestion, but this does not serve to explain the infections in herbivorous animals. The mode of infection in these animals is unknown It has been suggested that blood-sucking flies may be responsible Several observers have found what they believed to be spores in blood films

Scott (5) who studied the infection in Wyoming sheep decided that infections occurred only during the summer months He was not successful in showing that the ingestion of insects, or insect excreta, had anything to do with the infection Approximately 100 per cent of the older sheep became infected on the range, but experimental sheep, kept on a dry lot, watered with deep-well water and fed only on dry feed, also became infected.

Life Cycle. Erdmann (3) studied the infection in mice She found that the cyst walls disintegrated in the intestines releasing the spores which, after assuming an amoeboid form, penetrated the intestinal epithelium Here the trail was lost. It was picked up again only after about 40 days when the developing sarcocysts in the muscles were found.

Pathogenicity. There is little evidence that sarcosporidia seriously injure their hosts, even when heavy infections occur Deaths of sheep have been attributed to sarcocysts but the evidence is not convincing Occasionally in sheep and swine, which have appeared to be normal during life, there are so many sarcocysts in the muscular tissue as to lead to the condemnation of the carcass by meat inspectors principally because of the altered appearance of the meat. It is true, however, that mice may be killed by large doses of infected material and this suggests that the same thing may possibly be true occasionally in the larger animals

Pfeiffer (4), in 1890, made the discovery that extracts of the sarcosporidia of sheep were markedly poisonous for mice, rabbits, and sheep. Small animals may be killed within a few hours by injecting them with aqueous or glycerin extracts of sarcocysts. The dosage required is small. The poisonous property

is a toxin to which the name *sarcocystin* has been given. Teichmann and Braun (7) produced an antitoxin with which they succeeded in passively immunizing other animals. The toxin is contained in the spores and apparently little of it is released from the encapsulated parasite in the naturally infected hosts.

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The Globidia

Closely related to the sarcosporidia, in appearance and probably zoologically as well, is a group of parasites which are found in the mucosa of the alimentary tract of herbivorous animals These have been given various names but Wenyon groups them together in a single genus, *Globidium* Some of these forms are found also in the skin and in the fasciae of muscles. The relationship of these parasites to sarcosporidia and to rhinosporidium, described below, is not clear.

Globidia are common in sheep in England This form which is known as *Globidium gilruthi* was found by Triffit (2) in more than nine-tenths of the series which he examined. Marsh and Tunnicliff (1) have described it in Montana sheep where it was associated with severe diarrhea.

G. gilruthi is usually found in the wall of the abomasum but Marsh and Tunnicliff found it in the intestines. It appears as spherical cysts which vary from 200 to 500 microns in diameter. The cysts consist of a rather thick wall which encloses enormous numbers of spores not unlike those of sarcocysts. They are sickle-shaped and measure 1 5 by 10 microns The cysts may be seen with the naked eye as minute, opalescent nodules beneath the mucosa They evidently are formed within cells, for often the cell nucleus can be seen along one border of the cyst. These forms rupture into the lumen of the bowel, and often cause hemorrhages. In heavy infections, symptoms may be produced but in most instances the numbers are so few as to cause little damage.

Globidium leuckarti occurs in the intestine of the horse and resembles the form in the sheep except that the cysts are oval instead of spherical.

Globidium besnoiti of cattle has been found in the skin and connective tissue of muscles. Cystic bodies containing spores are often found in small numbers in the intestinal mucosa of cattle but whether or not they belong to this group is not known.

Some authors regard the globidia as fungi rather than protozoa. Others believe that some of the forms occurring in the intestinal epithelium are stages in the life cycle of coccidia. Until they have been studied in greater detail, their nature will not be known. Practically all studies in the past have been purely morphological. Nothing is known about the life cycles and mode of infection.

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The Rhinosporidia

Similar to the sarcosporidia and globidia, the rhinosporidia are found in large cyst-like structures which give rise to, and are found in, nasal polyps in man and horses. The human disease has been seen in Argentina and in southern Asiatic countries. A case in a horse was described by Zschokke (2) in South Africa. It is believed that the organism of the horse and of man are identical. The cysts become as large as 300 microns in diameter and are filled with small, spherical, spore-like bodies which escape from a pore in the cyst wall. These structures have long been regarded as protozoa but Ashworth (1) who studied the human disease in detail arrived at the conclusion that they were fungi.

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PART VI

THE VIRUSES

CHAPTER XL

THE VIRUSES

General Considerations

A large and increasing number of diseases of man, animals, and plants are known which, although infectious, are not caused by protozoa, bacteria, or other fungi, or, in fact, by any agent which can be seen with the microscope. The infectiousness can be demonstrated by inoculating suitable hosts with diseased tissues or even with cell-free filtrates of such tissues. Some of the first-studied agents of this type were separated from bacteria by passing the suspensions through clay (Pasteur) filters, which retained the bacteria but allowed the other agents to pass, hence they came to be called *filterable viruses* The qualifying adjective is now generally dropped since filter-passing is no longer regarded as a highly important characteristic Some viruses are so closely bound to the cells of the host, or are so large, that they do not readily pass filters, furthermore, many small bacteria will pass filters readily and even the larger bacteria can be made to pass by arranging the conditions suitably The word virus is used to connote a series of characters of which filter-passing is only one, and not one of the more decisive characters.

The first known virus was that of the tobacco mosaic disease. It was demonstrated by the Russian, Iwanowsky (9), in 1892 In 1898 Loeffler and Frosch (11), in Germany, demonstrated that foot and mouth disease of cattle was caused by an agent which readily passed bacteria-proof filters and could not be seen with the microscope It was the first animal virus discovered. In the same year Sanarelli (18) proved that a highly contagious rabbit tumor (myxomatosis) was caused by a virus. In the years which have elapsed since these early discoveries, many viruses and virus diseases have been found or differentiated.

Little was learned of the nature of the viruses until about 1920 when d'Herelle (8) described the agent which became known as bacteriophage. This agent possesses many of the properties of a virus, a virus in this case pathogenic for bacteria. The ease with which it could be studied greatly stimulated interest in the subject and this interest extended to the other viruses. Another stimulus was given in 1935 when Stanley (19) announced his success in extracting from tobacco plants affected with mosaic disease a highly purified

crystalline nucleo-protein which had the properties of the mosaic disease virus. The virus research field has been very productive during the last ten years, a highly specialized technic having been developed by a group of workers well-trained in modern physico-chemical concepts The word *virologist* occasionally is used to designate such workers.

THE BIOLOGIC NATURE OF VIRUSES

Viruses are best known for what they do rather than for what they are. The only criterion for their identification is their ability to induce recognizable changes in living cells resulting in abnormalities in form and function which constitute disease Many have speculated upon the possibility of the existence of free-living agents comparable in structure and function to the disease-producing viruses, and several such agents have been described, but it is clear that they are not comparable, since all evidence points to the fact that true viruses are always highly parasitic

Whether viruses are living or non-living agents has been the subject of controversy almost from the beginning, it having been introduced by Beijerinck (4) in 1899 with his idea of a *"contagium vivum fluidum,"* an inanimate, fluid, disease-producing agent This idea was not very seriously considered by the majority of pathologists, however, the general belief being that viruses were minute living agents, comparable to bacteria, but smaller Beijerinck's idea was revived, however, by Stanley's work, referred to above Extended discussions of the present concepts of the nature of viruses can be found in the papers of Rivers (15), Findlay (7), Laidlaw (10) and others, to which the reader is referred. These may be reduced to two primary concepts, as follows (a) That they are infinitesimally small, living things, obligatorally parasitic and dependent upon the host cells in which they live for their nutrition and perhaps also for nearly all other metabolic necessities except the power of reproduction This conception would make them the most highly developed types of parasitic life known. (b) That they are autocatalytic agents, inanimate and incapable of autonomous increase or multiplication but able to instigate abnormal metabolic activities in the host cells, one of the end products of which is more of the same material which instigated the abnormal activity. This material then is available, through destruction of the host cells, for repeating the same process in other cells

Which of these concepts is the true one cannot, of course, be proved. The isolation of a crystalline protein, by Stanley (19), which through repeated recrystallizations retained the properties of the tobacco mosaic disease virus, jarred the complacency of those who had discarded the autocatalytic theory

in favor of the parasitic, for it is difficult to believe that a living agent could be made to behave in this manner On the other hand it should be pointed out that no animal disease virus has been isolated or purified by Stanley's methods, or various others which have been tried since 1935, except by differential centrifugation which is, of course, merely a physical means of concentrating or separating out from complex mixtures bodies of certain size and specific gravity Rivers (15) has repeatedly pointed out that it is probable that all viruses are not of the same nature, that it is possible that some are autocatalytic agents fabricated by the host cells and others, living parasitic organisms of minute size

PROPERTIES OF VIRUSES

Filterability. Since it is known that pore size of silica filters (Pasteur, Berkefeld) is not the sole factor which determines whether or not particles in suspension will be passed by them, such filters have practically been discarded in virus research as a means of determining the size of virus elements The early experiments with such filters demonstrated, however, that viruses must be of particulate nature, rather than fluid, since all viruses can be filtered out of suspension if sufficiently fine filters are used It is possible of course that fluid virus is completely absorbed by particulate material derived from parasitized cells, that it always is filtered out of suspensions when the "carrier" elements are retained

Bechhold (3), in 1907, first used collodion membranes as virus filters Much later Elford (6) (1931), and Bauer and Hughes (1) (1934) standardized such filters (Gradocol membranes) so they might be used to determine the approximate size of virus particles By the use of high speed centrifuges of the Svedborg type the sedimentation rate of many virus particles have been studied by Bauer and Pickels (2) and others, and by this means it has been possible to calculate their approximate size The agreement between the results of the two methods has been very good It is known from experiments conducted by these methods that the particle size of viruses varies from some that are only a little larger than the molecules of some ordinary proteins to others that are nearly as large as the smaller bacteria The size of some animal viruses, in comparison with bacteria and ordinary proteins, is indicated in the following table It is customary in measuring such small objects to use the millimicron as the unit of size, this unit being $\frac{1}{1000}$ micron. By such a scale, the "elementary body" of psittacosis, for example, measures about 300 millimicrons In the table microns are used as the unit to avoid confusion.

TABLE XVII

THE APPROXIMATE SIZE OF VIRUS UNITS IN COMPARISON WITH OTHER WELL-KNOWN MOLECULES, AND BACTERIA. (Measurements given in microns)

Staphylococcus aureus	0.8 to 1.0	St. Louis encephalitis	0.025
Psittacosis	0.3	Louping ill	0.025
Vaccinia	0.15	Foot and Mouth Disease	0.010
Pseudo-rabies	0.12	Poliomyelitis	0.010
Vesicular stomatitis	0.085	Serum globulin	0.0063
Fowl plague	0.075	Serum albumin	0.0056
Rift Valley Fever	0.029	Egg albumin	0.004

Inclusion Bodies. In many virus diseases, round or ovoid bodies may be found in the cytoplasm, occasionally in the nucleus, of the diseased cells. These have long been known under the name *inclusion bodies*. The nature of these bodies has been a matter of much controversy and it cannot be said that the matter has been settled, however it is known that such bodies are formed only in virus diseases, thus they are regarded as indicators of the presence of virus. Some of these bodies are so characteristic that diagnoses may be based upon them with little chance for error. The Negri body, found in nerve cells affected with the virus of rabies, is a good example. Inclusion bodies have not been found in all virus infections.

Some of the earlier workers looked upon inclusion bodies as the infective agent. Some regarded them as protozoa, others thought them to be aggregations of minute parasites embedded in capsular material. When filtration experiments demonstrated that the viruses of many of these infections obviously were very much smaller than the inclusion bodies, there was a tendency to regard the intracellular bodies as products of degeneration of the cell. More recently, however, there has been an increasing tendency to regard them as aggregates of virus elements, embedded in degenerate cytoplasm or in capsular material.

Borrel (5) early studied the inclusion bodies found in fowl pox and which are known as *Bollenger* bodies. These are rather large structures found in the cytoplasm of diseased epithelial cells. Microscopically, minute spherical corpuscles were detected within the larger body and when the mass was crushed the smaller bodies were released. These corpuscles are known as *Borrel* bodies. They are capable of causing pox when transferred to the skin of susceptible birds. In human small-pox a similar situation exists. The inclusion bodies (*Guarnieri* bodies) in this disease contain smaller structures known as *Paschen* (13) bodies. Similar bodies are found in psittacosis infections. These structures are believed to represent the viruses of these diseases. They are very minute but are on the border of microscopic visibility. All of them are grouped under the name of "elementary bodies." Suspensions of such bodies can be obtained by crushing tissue rich in them and separating them from debris by

differential centrifugation. Such suspensions are virulent. Also they may be agglutinated specifically by the serum of patients recently recovered from these diseases.

The inclusion bodies found in different virus diseases vary in morphology, size, and staining properties. Some of them are hyaline in structure, others are granular Generally they are acidophilic, but some are basophilic.

Artificial Cultivation. Many animal viruses have been cultivated artificially but only in the presence of living susceptible cells. Cultural methods have demonstrated clearly that true viruses develop only intracellularly, and only in cells that are living and carrying on metabolic activities.

There are two general methods of cultivating viruses artificially and basically these methods are not very different The earlier consisted of making suspensions of finely minced living tissue, generally embryonic tissue, in saline or serum-saline solutions The first success with a medium of this type was achieved by Parker and Nye (12) in 1925 who cultivated vaccine virus in a medium consisting of minced rabbit testicle suspended in plasma The virus develops and Paschen bodies are produced in abundance but this goes on only so long as the epithelial tissue of the testicle retains its vitality Such cultures *usually are grown in shallow layers in small Erlenmeyer flasks*

The second method was introduced by Woodruff and Goodpasture (20) in 1931. These workers incubated fertile hen's eggs until the embryos were 10 to 12 days old, cut openings in the shells, and introduced virus-containing materials, placing them on the chorio-allantoic membrane which lies immediately beneath the inner shell membrane and thus is easily reached Most of the viruses which develop in epithelial cells have been cultivated in this way, and also a considerable number of others which do not usually develop naturally on epithelial tissues. The epithelial viruses usually do not invade the embryo, but produce opaque plaques on the membrane. The embryo in such cases may continue to develop and may actually hatch In other instances the embryo is invaded by the virus, with or without the development of lesions on the membrane, in which case embryonic death usually ensues within one to 3 or 4 days It is possible also to inoculate the chick embryo intravenously by injecting some of the large blood vessels of the chorion, or the inoculum can be introduced directly into the yolk sac. By the use of these methods it is possible to produce large amounts of some viruses very economically, such as, for instance, the virus of equine encephalomyelitis Successful vaccines have been made from cultured virus

Specificity of Viruses. *Various degrees of specificity for host and for certain tissues are exhibited by viruses.* Many viruses are strictly host-specific. Thus

the virus of hog cholera affects only swine, and that of rinderpest only animals of the ruminant family. On the other hand there are viruses, like that of rabies, which affect a large variety of animal species. Between these extremes we find most viruses which are limited to a small number of host-species.

Definite tissue affinities are also exhibited by most viruses. One group affects principally epithelial cells, another has a definite affinity for nerve cells and another largely affects structures which contain cells of mesodermal origin. We also have *pantropic* viruses which affect various types of cells. It should be noted that in spite of a fairly characteristic affinity for certain types of cells, these affinities may be altered experimentally, and spontaneous alterations often appear which change the pathological picture entirely Changes in viruses which ordinarily have little or no effect upon the nervous system into types which are distinctly neurotropic seem to be particularly common.

Immunity in Virus Diseases. Immunity in a considerable proportion of virus diseases is absolute and lifelong. This is very different from immunity to bacterial diseases which usually is relative and not lifelong Such solid and lasting immunities do not occur in all viruses diseases, it should be pointed out, in fact some of them, such as herpes simplex infections in man seem to recur frequently in the same tissues which were affected but a short time previously, suggesting that the virus may not have wholly disappeared between the two periods when symptoms were evident.

Prolonged solid immunity is difficult to explain on the basis of accepted ideas of the nature of immunity. When antigens come in contact with tissues antibodies are produced and these usually can be recognized in the body fluids. If the antigen is contained in a parasitic organism which multiplies and retains its position in the tissues, antibodies continue to be formed over as long a period as the antigenic stimulus remains If it is a non-viable antigen, antibody formation ceases as soon as the antigen is destroyed and eliminated, and the antibody content of the body fluids rapidly falls and ultimately, in most cases, reaches practically zero The same thing happens when an animal recovers from an infection, unless the animal becomes a convalescent carrier. How then can continued virus neutralizing power be maintained, as it is in many virus diseases, long after all evidence of the disease has disappeared?

No definite answer can be given to this question It has been suggested, however, that it is possible that in these diseases all animals which have suffered from them become permanent virus carriers; not carriers in the usual sense of which we have examples among bacterial infections in which the carrier host is a source of infection for other individuals, but carriers in which the disease-producing agent remains permanently in the hosts cells in such a way as to provide constant antibody stimulation but does not escape to cause new

infections. Since viruses exist intracellularly, it is possible that they could continue to exist, even when there were considerable amounts of neutralizing antibodies present in the fluids which bathe these cells Sabin (17) has shown that neutralizing antibodies do not necessarily destroy virus, since he was able to recover vaccinia virus by ultra-centrifugalization of neutralized virus-serum mixtures, the virus particles in this case being thrown out of the mixture, retaining their full virulence after the experience Rivers, Haagen, and Muckenfuss (16) inoculated rabbit cornea with vaccine virus and then cultivated the corneal tissue in antivaccinal plasma, finding that corneal lesions developed in spite of the antibodies present. On the other hand if the virus was first mixed with the antibodies and then used as the culture medium for rabbit corneal tissue, the corneal tissue did not become infected. These experiments, and others of similar nature, indicate that the neutralizing antibodies in virus diseases act as a barrier between the virus and the susceptible cells, but if the virus reaches the cells before neutralization, antibodies in the body fluids are relatively useless These experiments serve to explain long standing clinical experience that serum therapy in virus infections is valuable prophylactically but relatively or completely useless therapeutically. In other words it appears that immunity in virus diseases consists in neutralizing the virus before it reaches the host cells, if the cells have been reached and entered, the virus is out of reach of the neutralizing agent

The first to use a virus for purposes of practical immunization was Pasteur (14) The Pasteur rabies vaccine was produced by passing canine virus repeatedly through rabbits in series until it had acquired a very high virulence for rabbits but had lost most of its original virulence for dogs and man. The virus in the spinal cords of rabbits was further weakened by drying them over caustic potash. The process of immunization of man consisted of injecting emulsified bits of the cords with the least virulence in the beginning and using more virulent material for the later doses Active but altered virus was used as vaccine In later years Semple of the British Army in India introduced the use of a so-called "phenol-killed" rabies vaccine, which apparently has been just as successful as the Pasteur, is more cheaply produced, and has better keeping properties Since it had been generally believed that immunity in virus diseases could be induced only through the use of active, even if altered, virus, the Semple vaccine raised the question of whether immunity really was being obtained through the use of "dead" virus. There were many who argued that the virus in this vaccine was not wholly inactivated, and that the immunity was the result of altered but active virus held in the material in a masked form. Inasmuch as we have no criteria of "life" or activity in viruses other than their ability to produce lesions in susceptible hosts, it is conceivable that

these persons were right; that "life" or activity might remain in a virus suspension which had been so attenuated as to rob it of its disease-producing power. Certainly we have many such examples among the bacteria. It might be said here that in virus terminology the word *inactivated* is used for a virus which has lost its disease-producing power, in order to avoid the use of the word *killed* which implies that viruses are endowed with life. It is obvious, from what has been said, that inactivated viruses may, or may not, be "dead."

Experience with a number of viruses in recent years has furnished evidence which is quite convincing that it is possible to immunize animals temporarily with inactivated or "dead" vaccines, but that the degree of the immunity which results is dependent upon the antigenic activity of the strain of virus used and upon the concentration of inactivated virus present in the vaccine. Successful vaccines of this type depend upon whether or not it is possible to obtain concentrated virus suspensions from which to make them. This subject will be discussed further under equine encephalomyelitis, which is an object lesson in this regard.

Resistance. Viruses seem to possess about the same degree of resistance to heat, drying, and chemical agents as the vegetative forms of most bacteria Most are destroyed by temperatures around 55 to 60° C in 30 minutes or less Drying is fatal to many viruses; others are quite resistant to it Even those viruses that were thought to be most susceptible to drying can, however, survive for long periods if the drying is carefully and completely done. This method is, in fact, one of the best methods of preserving a great many of the more labile viruses If the drying is thoroughly done and if moisture is rigorously excluded many viruses can be stored almost indefinitely, especially if kept in the cold.

Viruses usually are not injured by cold, even by extremely low temperatures. Their resistance to chemical disinfectants is not very great, but varies from type to type Usually the virus elements are mixed with cellular debris and coagulable proteins and these undoubtedly serve to protect them from the effects of coagulating poisons For many years creolin solutions and bichloride of mercury were commonly used for disinfecting premises after outbreaks of foot and mouth disease. Olitsky, Traum, and Schoening showed that such agents, being protein coagulants, were very inefficient and recommended the use of alkalies which are not materially affected by the presence of foreign proteins. Lye is now the standard disinfectant for this purpose. Most viruses are well preserved by placing them in strong solutions of glycerin (50 to 100%). Such solutions dehydrate tissues and prevent autolysis of cells.

CLASSIFICATION OF VIRUSES AND VIRUS DISEASES

Knowledge of the nature of viruses is too meager to make it possible to

prepare workable classifications on this basis. They can be classified on the basis of hosts affected into plant and animal viruses. Levaditi proposed that the animal viruses be divided into groups according to the germinal layers from which the affected host cells were derived and loose classifications of this sort are frequently used. It has to be recognized, however, that many viruses are easily modified and frequently those that ordinarily affect epithelium will suddenly become modified so they will attack the nervous system. There is no generally accepted classification of viruses

The loose grouping of viruses used by Topley and Wilson will be used here. These authors divided the animal disease viruses into four groups.

Group A Characterized by lesions of the skin
Group B. Characterized by lesions of the central nervous system
Group C. Characterized by catarrhal or generalized infections.
Group D. Characterized by tumor formation.

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CHAPTER XLI

VIRUS DISEASES CHARACTERIZED BY LESIONS OF THE SKIN

FOOT AND MOUTH DISEASE

This disease, also known as aphthous fever, is found throughout most of the world where cattle are kept except in certain areas, such as the United States and Canada, where drastic means of excluding the disease are taken. The disease is exceedingly contagious On a number of occasions it has managed to gain entrance into the United States but each time it has been stamped out by the method of slaughtering all affected and exposed animals

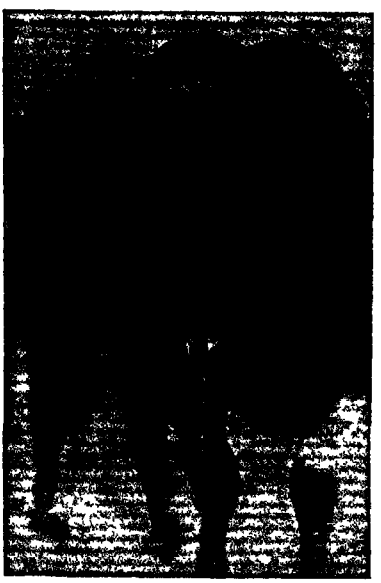


FIG. 117 Foot and Mouth Disease Showing the characteristic drooling of saliva Affected animals do not eat because of the soreness of their mouths They champ their jaws, making a smacking sound (Courtesy of L M Hurt)

The disease affects cloven-footed animals, especially cattle and swine Sheep are sometimes infected, and there have been outbreaks in deer and bison Human infections occasionally occur, but cases are rare and not very serious Carnivorous animals are resistant, but a few cases have been reported in dogs Solipeds are immune

Character of the Disease. In cattle the disease is characterized by the appearance of fever, and vesicles filled with a clear fluid The vesicles occur principally on the mucous membranes of the mouth, tongue, and lips, but are seen also on the skin of the muzzle, between the claws, and on the teats and udder. Affected cattle become lame because of the soreness of the foot lesions, and they drool from

the mouth and refuse to eat because of mouth soreness The vesicles rupture, leaving raw surfaces which become shallow ulcers.

Lameness is usually the most conspicuous symptom in foot and mouth disease in swine They may, however, develop mouth lesions and are particularly prone to develop large vesicles on the skin of the snout.

The mortality from foot and mouth disease is not high; generally not more than 1 to 3 per cent. Calves and young cattle are more severely affected than older animals. In some years more virulent forms of the disease appear in European countries in which case the mortality rate may be much higher



FIG 118 Foot and Mouth Disease Tongue of a cow showing extensive denudation caused by rupture of vesicles in the mucosa Loose flaps of mucous membrane indicate where the epithelium has been undermined (Courtesy of L M Hurt)

than usual. The greatest losses as a rule consist of the morbidity, the loss of flesh and of milk, rather than the loss of life Vesicles which form on the teats of cattle often become infected with bacteria and these frequently invade the udder producing acute mastitis Quite often this sequela is as serious as the primary disease.

The incubation period of the naturally acquired disease is seldom more than 4 days and frequently may not be greater than 48 hours The onset is marked by fever and depression. The vesicles appear in the mouth in from 12 to 36 hours after the onset of fever. Cases without complications caused by pyogenic bacteria usually recover within 2 or 3 weeks. Complications are frequent, however, and recoveries may be greatly delayed

The Inoculation Disease in Guinea Pigs. Research work on the disease has always been hampered by the facts that large expensive animals had to be used and that rather elaborate equipment was needed to prevent the disease from

spreading to all bovine animals in the establishment. Waldmann and Pape (11), in 1921, announced their discovery that guinea pigs could be used as test animals and since that time this animal has proved to be very useful in research work on foot and mouth disease. Not only are these animals very susceptible to the virus, and thus can be used to detect it in animal tissues and dejecta, and in materials which have been in contact with cases, but, curiously, the disease in guinea pigs is not naturally transmissible and thus the problem of keeping susceptible animals on the premises where the experimental work is being done was immediately solved.



FIG 119 Foot and Mouth Disease Lesions on the teat of a cow Beginning as papules which change to vesicles, the latter rupture leaving raw surfaces These become infected with bacteria and mastitis often develops as a result of extension of the infection into the teat canal The surface lesions finally heal under scabs The lesions here depicted are healing (Courtesy of L M Hurt)

Very young and very old guinea pigs are not satisfactory for work with this virus Half grown animals, weighing about 350 grams are best. Inoculation is done by introducing the virus intradermally into the foot pads either with a fine hypodermic needle or by scarification A primary vesicle usually appears at the point of inoculation within 24 hours, occasionally longer At the time the primary vesicle appears, virus can be demonstrated in the blood Within 18 to 36 hours, later, secondary vesicles appear in the mouth, and virus disappears from the blood Complete repair of the lesions requires several weeks Only an occasional animal dies of the disease There is no evidence that the virus multiplies elsewhere than at the site of the lesions. It is not known why the disease in this animal is not naturally transmissible

Rabbits may also be inoculated with foot and mouth disease virus, especially with virus which has become adapted to guinea pigs In this species too, the transmission may be accomplished by intradermal inoculation or scarification.

Transmission. The disease is transmitted by the direct or indirect transfer of the virus, usually that which is in the saliva as a result of admixture with vesicle fluid and with fragments of epithelium from the vesicle walls. The dis-

ease spreads rapidly in affected herds and practically every animal on the premises belonging to a susceptible species will contract the disease before the outbreak is over. Knowing this, it has long been the practice in countries where the disease is common for farmers to deliberately spread the disease by swabbing the mouths of unaffected cattle with a cloth which has been soaked in saliva of the affected in order that all animals will pass through the disease simultaneously A somewhat more certain method, often employed by veterinarians, is to lightly scarify the mucous membrane of one of the lips with a sharp instrument which has been dipped in vesicle fluid obtained by aspiration with a hypodermic syringe.

Stockman and Minett (5) studied the survival of virus in carcasses of animals slaughtered while suffering from the disease This is an important matter in countries where the disease does not exist, for many outbreaks of the disease have been traced to garbage containing meat trimmings. It was found that in muscular tissue the acidity which develops shortly after death usually destroys the virus within a few days even under refrigeration Virus was found in the marrow of the long bones, even when the meat was stored in brine, for more than 40 days and occasionally much longer The tissue of foot vesicles also retained virus for long periods

Long experience has made it clear that within a short time after an outbreak has subsided, or after all infected animals have been removed, susceptible animals brought on the premises do not usually contract foot and mouth disease This practice is not recommended, however, Jackson (1) states that it is the practice in Great Britain to allow animals to be brought back on farms on which foot and mouth disease has existed six weeks after thorough disinfection, or eight weeks after the slaughter of the last diseased animal, whichever period is the shorter Only half of the stock is permitted at first. If, after three weeks, no cases have developed, the remainder of the animals are brought in and all restrictions are removed He states that in the 20 years between 1909 and 1928, 5,659 premises had been infected with foot and mouth disease in England, and of this number the disease reappeared on 57 premises after disinfection.

The virus of foot and mouth disease ordinarily is not resistant but when carefully dried it may retain virulence for many months and even years. Without question new outbreaks occur now and then from animals which have become infected with such dried virus. In the United States, for example, none of the outbreaks which have occurred have been traced to live animals; all have originated in virus imported in or on contaminated objects In one case it was thought to have been on straw in which flower bulbs had been packed, in another it was shown to have been a contaminant of vaccine virus [Mohler and

Rosenau (2)], in others it is believed to have been in garbage containing meat scraps brought in from foreign ports by ships.

The question of whether virus may be carried by recovered animals has been a subject of considerable debate. It seems quite clear that such animals seldom carry the virus for long after symptoms have disappeared. The matter is reviewed by Schoening (4) who was unable to find virus in 20 recovered animals.

Transmission of foot and mouth disease by insects, birds, migratory animals, and human beings has often been suspected but not often proved

Properties of the Virus. The virus of foot and mouth disease is known to be of small particle size, probably less than 10 millimicrons in diameter It occurs in the vesicles of the disease and, transitorily during the febrile period, in the blood During the period of blood infection, it also appears in the urine and feces. It develops only in epithelial cells, producing a spreading necrosis of these cells with separation of the cornified layers, thus producing the vesicles. Several have reported the presence of bodies in affected cells but these are believed not to be specific, that is, they are not inclusion bodies The virus is readily destroyed by heat but cold has a preservative effect Alternate freezing and thawing does not seem to have a deleterious effect upon the virus Resistance to drying is variable As a rule drying is quickly destructive to it, but if the drying is quickly and thoroughly done, and if the dried material is kept in a thoroughly dry atmosphere, virus may be kept for long periods of time Virus enclosed in epithelial fragments is very much more resistant than that in vesicle fluid. Schoening (4) found that virus dried on hay and on soil particles might remain viable for about a month, and Trautwein (6) found infective epithelial fragments after they had been exposed to winter weather for more than two months Glycerol has a preservative effect upon the virus It is also resistant to alcohol, ether, and chloroform Coagulating disinfectants are not especially effective probably because the virus generally is protected by albuminous material. Olitsky, Traum, and Schoening (3) found bichloride of mercury and cresol solutions relatively ineffective but alkalies destroyed it quickly. Formalin also is effective.

Artificial Cultivation. Although there have been many attempts to cultivate the virus of foot and mouth disease most of them have been wholly unsuccessful. It seems likely that the virus has been cultivated on a limited scale in tissue cultures containing epithelial cells, but no practical use has so far been made of such cultures.

Immunity. Cattle which have recovered from foot and mouth disease generally have enough immunity to protect them from the same type of virus for

a year or more, but the resistance is not life long. Natural immunity in cattle and swine is negligible although some individuals have greater resistance than others.

PLURALITY OF VIRUSES. The French workers, Vallée and Carré (7) in 1922 reported that they had discovered the existence of two types of foot and mouth disease virus They were distinguished by the fact that while each immunized animals against itself the two viruses would not immunize against each other. Both produced typical disease and these could not be distinguished clinically with certainty One of these types was designated Type A, the A standing for Allemand (French word for Germany), the other was called Type O (for Oise). Waldmann and Trautwein (12) in 1926 announced their finding of three types of virus in Germany, their designations for them being A, B and C. These findings have been amply confirmed. The German A and B correspond to the French O and A, respectively To avoid confusion it has been generally agreed to retain the French designations, and thus we now have three types of viruses, designated as A, O and C. A number of workers have reported viruses which do not immediately conform to any of these three or have characters placing them midway between the accepted types. Generally, after a few generations of passage through experimental animals, these conform to one of the three recognized types.

Cattle, swine, and guinea pigs can be infected with all three of these viruses in quick succession since one does not protect against the others This explains numerous observations of foot and mouth disease striking twice in the same herd in a single season

Differentiation of types can be accomplished with the aid of susceptible animals, guinea pigs usually being employed. By having stocks of animals immune to each of the three types of virus, the type of the unknown virus is indicated by the group which proves to have protection against it

PASSIVE IMMUNIZATION Susceptible animal species can be protected against the ravages of the disease by the use of convalescent or hyper-immune serum. The protection is short-lived—only about two weeks—but this is often sufficient to protect herds which are in neighborhoods in which the disease has appeared. The German government for many years has produced a hyper-immune serum by injecting cattle with the three types of virus, and bleeding them out when the titre of serum is highest. The serum is unconcentrated. Other countries have used the serum of animals that have recently recovered from the disease, and claim that it gives results as good as those obtained with the hyper-immune serum. Such sera are, of course, monovalent and can be expected to protect only against the type from which the donor animals suffered.

Active Immunization. Waldmann (9) recommends a method of active immunization which is said to give good results. The method consists of giving a dose of hyper-immune serum and exposing the animals to the virus simultaneously. This method should be used only when the disease is in the immediate neighborhood and it is considered quite certain that the herd will become infected if not immunized.

Vallée, Carré and Rinjard (8) in France, and Stockman and Minett (5) in England have reported successful results in active immunization with formolized vaccines These methods, however, have not proved wholly successful in other hands Waldmann and Kobe (10) reported very successful results with a formalin treated virus which had been concentrated by absorption on an aluminum gel. They claimed to have successfully vaccinated 150,000 cattle.



FIG 120 Foot and Mouth Disease Eradication In the United States this disease has been eradicated on a number of occasions by the drastic method of immediate slaughter of all infected and exposed cattle Rigid quarantine methods are used in the infected areas The illustration shows the disinfection of a truck coming from the quarantined area (Courtesy of L M Hurt)

The immunity, it is said, is evident in four or five days and it lasts for at least five months

Methods of Control. Two general methods of control are used:

a Local quarantine with or without the use of serum or vaccines.

These methods are generally used in European countries, particularly by the continental countries where national border-lines are close together, where the disease practically always exists in one area or another, and where, as a

consequence of these conditions, it has been found impossible or uneconomical to use the more drastic method of slaughter of all infected and exposed animals. Even in these countries, the slaughter method is sometimes used in the beginning of outbreaks with the hope of curbing the disease before it becomes widespread. Generally speaking, the philosophy in these countries is that they must resign themselves to living with the disease.

b. The drastic slaughter method.

This method has been used in countries which are sufficiently isolated to make the method of control economically feasible. This is the method which



FIG 121 Foot and Mouth Disease Eradication in the United States Outbreaks of this disease in this country are stamped out by the drastic method of slaughter and burial on the premises of all infected and all exposed animals Great trenches are dug, the cattle are driven into them, slaughtered there, and covered with a deep layer of soil Before the soil is returned to the trench, the hides of the carcasses are slashed and they are covered with a layer of quick-lime The photograph shows the burial of a herd of infected cattle (Courtesy of L M Hurt)

has always been used in the United States, frequently in the British Isles, and occasionally elsewhere. As soon as the disease is diagnosed in a herd, the entire premises, all animals, and people, are placed under rigid quarantine and as rapidly as possible all susceptible animals are slaughtered and buried on the premises After thorough cleaning and disinfection, the quarantine is lifted but susceptible animals are barred for sufficient time to insure that no

active virus remains. All herds in the neighborhood are carefully and frequently inspected for signs of the disease The area quarantine is not lifted until 60 or 90 days after all evidence of the disease has disappeared, and during this period all animal traffic in the area ceases and human travel is reduced to a minimum. Milk is released only for immediate pasteurization which destroys any virus which might be present.

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VESICULAR STOMATITIS

This disease is primarily one of horses and mules but cattle are sometimes naturally affected Experimentally, sheep and swine can be infected but the spontaneous disease had not been reported in these species until 1943 when an outbreak in swine occurred in Kansas City In cattle and swine the disease resembles foot and mouth disease so closely that a differential diagnosis cannot be made on the basis of clinical findings only In the horse there is no difficulty on this score because horses are not susceptible to foot and mouth disease

Vesicular stomatitis is said to have occurred in South Africa during the latter part of the 19th century but little was known about the disease until it appeared in the mid-western part of the United States during World War I. It apparently was shipped in horses from the United States and Canada with the American expeditionary forces to the European battlefields where it occasioned considerable trouble. The disease was described in the United States by Mohler (3) in 1918 The virus was identified by Cotton (1) in 1926 Ex-

perimental infections of cattle and swine were reported by Olitsky, Traum, and Schoening (4) in 1926

The disease resembles foot and mouth disease in a great many ways. The mouth lesions cannot be distinguished from those of foot and mouth disease. Lesions around the feet and on the udders of cattle are not so frequent as in foot and mouth disease but they do occur, and of course such lesions may be absent in the latter disease as well Vesicular stomatitis is not so highly contagious as foot and mouth disease, thus outbreaks may be limited to one or a few farms, and they may disappear spontaneously. As in foot and mouth disease the virus escapes principally in the saliva which is infected with fluid and fragments from the ruptured vesicles Infection of other animals occurs from direct contact and through watering troughs and mangers Animals which have had mouth vesicles for six days usually are no longer capable of infecting others

Guinea pigs can be infected with this virus in the same way as with foot and mouth disease virus, and the lesions and course of the disease are identical.

Properties of the Virus. The virus particles of vesicular stomatitis are considerably larger than those of foot and mouth disease, being of the order of 70 to 100 millimicrons (2). Toward physical and chemical agents this virus behaves in about the same way as the virus of foot and mouth disease

According to Cotton (1), a plurality of viruses occurs in this disease He found that a virus obtained from New Jersey was not immunologically identical with another from Indiana.

Methods of Control. Vesicular stomatitis has been brought under control in many areas by strict quarantine of infected premises and a ban on shipment of animals for a period of at least thirty days after all evidence of the disease has disappeared. This method has operated satisfactorily, a clear indication that the disease is not nearly so contagious as foot and mouth disease.

Immunity. Horses and cattle that have recovered from vesicular stomatitis are solidly immune thereafter for at least one year and perhaps much longer. Guinea pigs can be passively immunized with serum from recovered animals and presumably this applies also to the other animals. Olitsky, Traum, and Schoening (4) have shown that no cross-immunity exists between the virus of this disease and that of the O and A viruses of foot and mouth diseases.

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VESICULAR EXANTHEMA

This is a disease primarily of swine, which resembles foot and mouth disease so closely as to make it impossible to differentiate it with certainty. By inoculation it usually is possible to infect horses, but the disease does not occur naturally in horses, so far as is known, and it does not infect cattle

The disease has been seen only in California. A disease appeared there in garbage fed swine in 1932 which was believed to be foot and mouth disease

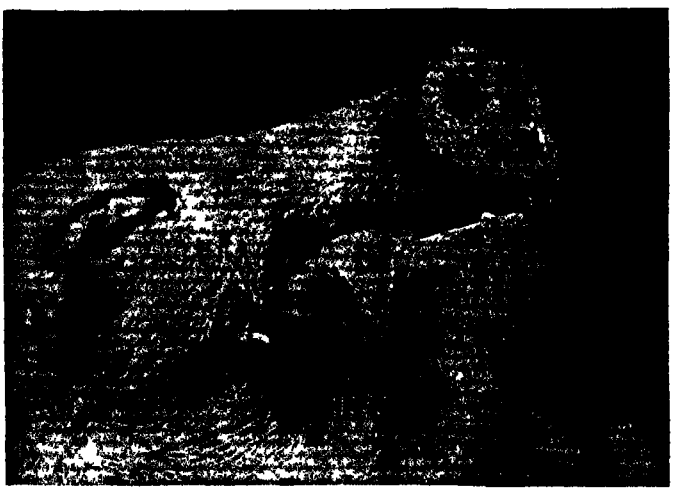


FIG 122 Vesicular Exanthema Showing ruptured vesicles on the tongue, snout and lips These lesions cannot be distinguished from those of foot and mouth disease (Courtesy of L M Hurt)

and was successfully handled as such. It recurred in 1933, at which time it was shown by Traum (1), that the disease was not foot and mouth disease but another entity for which the name vesicular exanthema was proposed. The disease occurred again in 1934 and in each succeeding year up to 1944 except in 1939 Almost without exception it occurs only in garbage-fed hogs.

In this disease, as in foot and mouth disease, vesicles of varying size occur on the snout, lips, tongue, and gums, or on the feet between the claws, or around the coronary band. Udder and teat lesions are seen in many nursing sows. These lesions are preceded by fever, as in the other two diseases mentioned above, but the animals are not usually so severely ill. Usually in

vesicular exanthema the pigs continue to eat, and various degrees of lameness appear. The disease does not spread so extensively as foot and mouth disease and sometimes only a part of the pigs in the same pen become affected. In general the disease is much milder than most outbreaks of foot and mouth disease. It is not of great importance except for its confusion with the more serious foot and mouth disease.

Traum was able to show that this disease is caused by a virus which is immunologically different from those of foot and mouth disease and vesicular stomatitis Certain differences in infectivity for animals were also found. After it had been established that the disease was not foot and mouth disease the slaughter method of control was abandoned and simple quarantine measures were substituted These proved to be satisfactory.

REFERENCE

1 TRAUM Jour Am Vet Med Assoc., 1936, *88*, 316

DIFFERENTIATION OF THE VIRUSES OF FOOT AND MOUTH DISEASE, VESICULAR STOMATITIS, AND VESICULAR EXANTHEMA

In countries where the slaughter method of eradicating foot and mouth disease is followed, it is of paramount importance to establish a positive diagnosis at the earliest possible date When the natural disease is in cattle, the question to be decided is whether the disease is foot and mouth disease or vesicular stomatitis, when it is in swine, it is whether the disease is foot and mouth disease or vesicular exanthema Vesicular stomatitis will infect swine by inoculation but no natural outbreaks have been reported To differentiate these diseases Traum (1) suggests the following procedures

1 Inoculate at least two cattle. One should be injected with fresh vesicular fluid intravenously or intramuscularly, the other should be injected into the mucosa of the tongue, lips, or dental pad, or it may be rubbed into scarified areas

If the virus is that of foot and mouth disease, both animals should develop the disease

If it is vesicular stomatitis the animal injected intravenously or intramuscularly will fail to develop the disease; the other should do so

If it is vesicular exanthema both animals will fail to develop the disease.

2. Inoculation of swine is useless since the animals usually will develop disease when injected as above if any of the three viruses are present.

3. Inoculation of horses is very helpful in differentiating between foot and mouth disease and vesicular stomatitis The horse is susceptible to the virus

of vesicular stomatitis but is entirely resistant to that of foot and mouth disease. The virus of vesicular exanthema is mildly pathogenic for horses. Some animals develop small vesicles near the point of inoculation, others do not. Horses should be injected into the mucous membrane of the dorsum of the tongue, or it may be introduced through scarification at this site.

4. Guinea pigs are helpful in differentiating between the virus of vesicular exanthema and the other two viruses, since the former does not cause infections when introduced through the foot pad, whereas foot and mouth disease and vesicular stomatitis quite regularly infects, as described

REFERENCE

1 TRAUM Jour Am Vet. Med Assoc., 1936, *88*, 316

COITAL EXANTHEMA

Synonyms Coital Vesicular Exanthema, Genital Horse Pox

This disease occurs in both horses and cattle It is characterized by the appearance of vesicles, which become pustules, on the mucous membranes of the genital tract and on the skin bordering on these membranes The disease appears during the breeding season and is transmitted by copulation Animals occasionally are infected by means of infected sponges, curry combs, and other infected objects Reisinger and Reimann (1) reported the recognition of a filterable virus in this disease in 1928 The naturally occurring disease does not ordinarily pass from horses to cattle, or vice-versa, but Zwick and Gminder (2), in 1913, reported success in transmitting the disease from cattle to horses, sheep, and goats These authors also believed that coital exanthema had some relation to the very common granular venereal disease of cattle They claim to have produced lesions of the latter with the virus of coital exanthema, but were not successful in producing the exanthema with secretions of animals affected with the granular venereal disease

Character of the Disease. In female animals the disease is manifested by the appearance of a purulent vaginitis The vulva becomes swollen and reddened, a discharge appears, and the affected parts itch The animal stands with an arched back, and urinates frequently Many vesicles from 1 to 3 mm in diameter appear on the mucous membranes and on the skin of the vulva These change to flat, yellowish pustules, then to superficial ulcers which finally heal. The skin lesions usually leave small unpigmented spots In male animals similar changes appear on the penis and prepuce The disease usually runs a course of several weeks.

Immunity. According to Zwick and Gminder the disease does not convey any appreciable immunity since recently recovered animals often develop the disease a second time.

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CONTAGIOUS ECTHYMA OF SHEEP

Synonyms Sore mouth, Contagious postular dermatitis.

Of the domestic animals this disease affects only sheep and goats Mild infections of man have been reported It is a common disease during the spring and summer months in the sheep-raising areas of the western states of the United States, and occurs sporadically in the eastern states It has also been reported from many other sheep-raising countries It probably is world-wide



FIG 123 Contagious Ecthyma (Sore Mouth), Sheep (Courtesy of Jen-Sal Laboratories, Inc)

Losses from fatalities are not great as a rule, and then only when the disease is complicated by other factors In the southwestern part of the United States enormous losses occur, according to Boughton and Hardy (1), as a result of invasion of the tissues through the lesions of sore mouth by larvae of the flesh-fly or screw-worm (*Cochliomyia americana*) In the more northerly states where the screw-worm does not exist, losses are usually due to secondary invasion of the lesions with the necrosis bacillus (*Actinomyces necrophorus*), according to Marsh and Tunnicliff (2).

It is usually the lambs and kids which are affected with the disease, the older animals having passed through the disease early in life and acquired an immunity to it. When it occurs without complications the disease runs a rather benign course but the animals are unable to eat for considerable periods and stunting

of growth occurs in the period when normal growth should be greatest. Permanent damage results from the experience.

The disease is characterized by the formation of papules and vesicles on the skin of the lips. These are transformed into pustules and finally heavy scabs are formed. The infected lambs or kids with their thickened, stiff, sensitive lips can neither suckle nor graze and rapid emaciation occurs. Healing occurs spontaneously in about one month, the scabs falling off on the ground leaving the lips smooth and without scars.

The dried scabs contain a virus which is remarkably resistant to drying. Such material kept thoroughly dry in the refrigerator will retain virulence for many months. In the soil it survives the winter very readily and it is in this way that the new crop of lambs of the following year are infected The disease recurs annually in infected pastures for indefinite periods

Animals which recover from the disease are immune thereafter for periods up to 28 months and perhaps longer, and this is long enough to carry most range sheep through their period of useful life

The virus is filterable through Berkefeld V candles So far as is known there is but a single type of virus immunologically Seddon and McGrath (3), who tested English and Australian strains, found them to be identical

Boughton and Hardy demonstrated that susceptible animals could be effectively immunized to this disease, and their method of protection is widely employed where sore mouth is prevalent The vaccine is made from dried scabs which are thoroughly ground and suspended in 50 per cent glycerin solution in the proportion of one part of scab to 100 parts of solution The vaccine is used in about the way that smallpox vaccine is used on man The point of vaccination is the skin of the inside of the flank. One or two superficial scratches are made, a drop of virus-suspension is placed on the area and rubbed in with a small stiff brush. "Takes" appear as vesicles which become pustules and then become covered with a scab An area as large as a dime or a quarter is sufficient to give a strong immunity Some of the lambs will nibble at the developing lesions and thus convey the infection to their lips but this occurs in not more than 10 per cent of the vaccinated animals and the disease in such animals is nearly always very mild.

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The Animal Poxes

Diseases characterized by the formation of pustules on the skin, with or without general manifestations of illness, occur in all the domesticated animals, and in man In man the disease is known as variola or smallpox. By inoculation man may be locally infected with cowpox and this trivial infection results in a solid immunity to smallpox. This discovery, made by Jenner, in England, in 1796 is the basis of our method of vaccination for smallpox. The discovery, moreover, indicated a close relationship between the human and the cow disease It is believed by some that all of the pox diseases originally came from a common stock, the basis for the belief being the similarity in the type of lesions produced, the fact that many of these viruses can be adapted to new hosts, and the fact that a certain degree of cross-immunization occurs between members of the group. Cowpox and fowlpox are widespread diseases. The others are more localized. The pox diseases of man, sheep, and fowl are severe and destructive The others are rather trivial when not complicated with other diseases

That the poxes are caused by filterable viruses has long been known. The pox viruses are among the larger ones, the particulate size being of the order of 150 millimicrons or larger. In all the pox diseases, epithelial tissue is primarily involved and the parasitized cells contain characteristic inclusion bodies which, in turn, contain the smaller "elementary bodies" which represent the virus itself, or at least contain virus

FOWLPOX

Synonyms Chicken Pox, Sore head, Contagious epithelioma.

Fowlpox attacks chickens, principally. Occasionally outbreaks in turkeys are seen, and there are reports of its having occurred in other species. Pigeons have a type of pox which differs somewhat in pathogenicity from that found in chickens, but the pigeon pox virus partially immunizes to chicken pox, indicating a close relationship. Virus which Brunett (3) isolated from a turkey proved to be identical in every respect to that of chickens, but one isolated by Brandly and Dunlap (2) was not typical Pheasants sometimes suffer from pox, and the virus usually is considered to be identical with that of fowlpox but Dobson (4) found one in a serious outbreak on a pheasant farm which seemed much more closely related to pigeon pox than to fowlpox It seems likely that all bird poxes are caused by a single type of virus which becomes modified according to the hosts in which it occurs That this modification

becomes considerable, however, is indicated by the fact that pigeon pox virus does not naturally pass from pigeons to chickens, and that fowlpox virus is transmitted to pigeons by inoculation only with great difficulty.

Pox in chickens is manifested by characteristic lesions on the head. They appear on the comb, wattles, around the corners of the mouth, around the nostrils and the eyes In some cases the lesions spread into the mouth and even into the trachea causing whitish lesions which ulcerate forming what is commonly called "cankers" This form of pox was considered to be a separate disease and was known under the name of avian diphtheria for many years. In such infections the infraorbital sinus is frequently involved, becomes greatly distended, and thus distorts the facial features The content is a yellowish or brownish caseous mass



FIG. 124 Fowlpox Showing characteristic dry scabs on the comb, around the eye, nostrils, and corner of the mouth

The skin lesions consist first of small pustules which soon dry and become transformed into warty epithelial crusts which may become quite thick The affected birds become very ill, refuse to eat, become emaciated, stop laying, and many of them die. The lesions are confined to the featherless part of the head, as a rule, but occasionally pox lesions are found around the vent, and even on the feet If the infection remains on the skin and does not involve the mucous membranes of the head, the effect on the bird is much less severe and recoveries more common In favorable cases the course of the disease is three or four weeks; in the presence of complications it may be much longer

Properties of the Virus Fowlpox was proved to be caused by a virus in 1902 by the German workers, Marx and Sticker (9) Previously various bacterial agents, fungi, and even protozoa had been regarded as having a part in the disease It is doubtless true that some of these agents do have a part in the etiology of the lesions, particularly of the lesions on the mucous membranes

The affected epithelial cells of the cornified layer of the skin are greatly enlarged and vacuolated Conspicuous in them are the inclusion bodies which Bollenger had seen as early in 1873 and mistaken for protozoa These often are larger than the nuclei of the cells in which they occur. Contained in these bodies are the elementary bodies first seen by, and named for, Borrel These

bodies were studied by Woodruff and Goodpasture (11) who found that they were resistant to tryptic digestion and could, therefore, be obtained free of tissue debris by digesting the latter with artificial pancreatic juice. The free Borrel bodies are about 0.25 microns in diameter, they stain readily with Giemsa's and fuchsin stains, and have the appearance of minute cocci. Suspensions of the washed Borrel bodies readily produce pox when inoculated into chickens. When the bodies are centrifuged out of suspension, the supernatent fluid is innocuous. This proves that the Borrel bodies contain



FIG. 125. Fowlpox. Swollen epithelial cells in an early lesion showing the Bollinger bodies. The inclusion bodies are spherical and prominent in the stained section. In several of the cells the nuclei may be seen crowded against the cell wall. × 350.

the virus. They are now looked upon quite generally as *being* the virus. Filtration experiments prove the virus to be of about the size of the Borrel bodies, an indication that these bodies are not merely aggregations of still smaller virus elements.

The virus of fowlpox is very resistant to drying. In the dried crusts removed from epithelial lesions, the virulence remains unimpaired for many months providing the drying has been well done. In soil, subject to usual conditions, the viability of the virus is not longer than several weeks as a rule. The disease tends to recur, year after year, on the same premises. It is not known how the virus is preserved in the intervening periods. The virus is readily destroyed by alkalies and by most disinfectants. It is preserved for long periods by 50 per cent glycerol.

Transmission. Fowlpox is believed to be transmitted principally by direct inoculation from bird to bird through fighting wounds, and by the birds picking one another. The disease may also be spread by the bites of mosquitoes

(Kligler, Muckenfuss and Rivers (8)), (Matheson, Brunett and Brody (10)), and possibly by other arthropods. Arthropod transmission is certainly not as important as direct contact in the spread of this disease through flocks, but it is likely that it may be the way by which the infection is spread from one flock to another Doyle and Minett (5) placed susceptible birds in cages which had just been occupied by infected birds Transmission did not occur except when the skin or mucous membranes of the susceptible birds had been scarified. Infection seems to depend, therefore, upon breaks in the continuity of the skin.



Fig 126 The Borrell or "Elementary" Bodies of Fowlpox These minute spherical bodies were obtained free of tissue debris by tryptic digestion of the Bollenger bodies contained in virus infected cells The bodies are here stained after admixture with a streptococcus to indicate comparative size (Courtesy of E W Goodpasture and *The American Journal of Pathology*)

Artificial Cultivation. Goodpasture, Woodruff and Buddingh (6) cultivated the virus of fowlpox on the chorio-allantoic membrane of the developing chick embryo in 1931 Brandly (1) and others have confirmed these findings, and such cultures are now being used for vaccine production.

Immunity. Birds which have recovered from fowlpox are solidly immune thereafter In flocks in which the disease has occurred for some years, it is only in the young birds that the disease is seen In previously uninfected flocks, birds of all ages develop the disease.

Practical immunization of poultry flocks is carried out in all regions where pox is prevalent. Two methods are in use. One consists in the use of virulent fowlpox virus; the other, of pigeon pox virus Both methods give satisfactory results.

The fowlpox vaccine is made from the dried epithelial growth which appears on the combs of cockerels after scarification and inoculation with virulent material, or from the growths obtained on the chorio-allantoic membrane of developing chick embryos following inoculation with bacteria-free virus The epithelial tissues are well dried in a desiccator and then ground to a fine powder. If this powder is kept thoroughly dry the virus will keep for months. At the time of use, the powder is placed in sterile water in the proportion of 1 gm. of powder to 100 cc. of water.

The pigeon pox vaccine is prepared by plucking feathers from the breast of a susceptible pigeon and rubbing pigeon pox virus into the denuded area. When the inflammation has subsided and the area is covered with crusts, these are removed, or the bird is destroyed and the entire piece of infected skin removed and dried. This material is made into a coarse powder by grinding and used as described above

There are two accepted ways of applying the vaccines, the "feather follicle" method and the "stick" method The method used depends upon the preference of the operator.

In the follicle method of vaccinating six or eight feathers are plucked preferably from the outside of the leg about the middle of the tibial region The virus suspension is then applied to the exposed follicles with a small, stiff brush The "stick" method, suggested by Johnson (7), is performed with a small, sharp-pointed scalpel which is wrapped with adhesive tape leaving only about one-eighth inch of the tip exposed This instrument is used to make several pricks in the skin, the blade having been dipped in the vaccine suspension just prior to use Some operators have made use of the under surface of one of the wings in vaccinating in this way, others use an area on the outside of the leg The latter is preferable because the bird is less likely to contaminate its head from surface virus in this case With either method a "take" is manifested by swelling of the feather follicles, the appearance of cheesy material and finally scabs which generally fall off leaving a fully healed lesion within 3 or 4 weeks

When pigeon pox virus is used the constitutional effect upon the birds is practically nil, and for this reason this material is recommended when the birds are not in first class physical condition for any reason, and when the birds are in full production Its only disadvantage is that the immunity conferred is not as complete and as lasting as that conferred by the fowlpox virus In field use it nevertheless appears to give excellent satisfaction.

Fowlpox vaccine gives satisfactory results when used properly and at the right time. The birds should be in good physical condition and about two months before coming into production. The resulting immunity is solid and lifelong The birds usually suffer a physical reaction from it. They go off feed and, when in production, stop laying for a considerable time It should be kept in mind that fowlpox vaccine is fully virulent material It should not be spilled, it should be kept away from the head region of the birds as they are handled, and utensils, empty bottles, and other contaminated objects should be destroyed or sterilized immediately after they are used.

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PIGEONPOX

Pigeonpox frequently causes considerable trouble in squab-raising plants. The squabs may become infected while still in the nest but more often the disease appears in well developed birds Cankers are found in the mouth, and the corners of the mouth are covered with crusts The eyelids may also become affected and the birds may be blinded The legs and toes may be involved The death rate may be high Pigeons may be protected against the disease by vaccination with pigeon pox vaccine, using it in the same way it is used on chickens

COWPOX

Synonym Vaccinia

Cowpox is a rather common disease in dairy cattle in the United States In Europe it appears that the disease was once much more common than at present, the explanation offered being that when smallpox was prevalent it very frequently was transmitted to cattle by milkers suffering from the disease. Frequent observations indicate that this was of rather common occurrence [Reece (5)] There are indications too that horsepox was not uncommonly transmitted on the hands of milkers to cattle The observations of Jenner on the transmission of cowpox to man and the immunization given thereby are well known Although smallpox, cowpox, and horsepox are regarded as distinct diseases it is clear from early experiences with these diseases that the viruses are closely related and that not infrequently they stray from their usual hosts to others where they produce diseases which often cannot be distinguished from those produced by the native viruses

Transmission of pox virus from one species to another probably does not frequently occur today but it occasionally happens. Boerner (2) has described a situation in which a herd of cattle became infected with vaccine virus from two small boys who helped in milking during a period in which they were undergoing a vaccine reaction The boys apparently contaminated their hands by scratching the vaccination site, and then transmitted the disease to the teats of the cattle that they milked Not only did many of the cattle become infected but nearly every unvaccinated person on the farm developed vaccinia lesions on their faces, arms, hands, and other parts of their bodies Christen has described a similar occurrence, and others have reported outbreaks of cowpox lesions on the teats and udder of cattle occurring after vaccination of milkers against smallpox It is evident that recently vaccinated persons should not be permitted to milk cattle

There is some evidence to suggest that there may be more than one disease of cattle diagnosed as cowpox This question has been discussed recently by Hester, Boley and Graham (3) It will be further discussed below under the consideration of the so called "milkers nodules"

Cowpox as seen today is a comparatively mild disease, the animals showing little or no evidence of a general reaction It is manifested by the appearance of small papules on the skin of the teats and of the udder, these being reddish and tender The papules change to vesicles and then to pustules which have a yellowish color and frequently a small pit in the center There may be only one lesion on a teat, or there may be many which may become confluent After about ten days, if undisturbed, the lesions dry and are covered with scabs which finally fall off leaving a smooth surface In milking animals, the early vesicles are broken by friction, leaving denuded, raw, inflamed areas which heal very slowly Bacterial infections usually occur and these may extend through the teat canal to the glandular structure, resulting in mastitis.



FIG 127 Cow Pox Showing well advanced lesions on the teats and udder. (Courtesy of Robert Graham)

Properties of the Virus. The virus occurs in epithelial cells in the lesions. These cells are greatly swollen and vacuolated They contain inclusion bodies which are morphologically identical with the Guarnieri bodies of smallpox.

Great numbers of elementary bodies (Paschen bodies) occur in these inclusions. The Paschen bodies are believed to constitute the actual virus of the disease. They are spherical in form and average between 150 and 200 millimicrons in diameter

Artificial Cultivation. The virus of vaccinia has been cultivated in a number of different types of tissue culture. Bacteria-free virus usually is easily obtained by inoculating rabbits intratesticularly Several passages usually are necessary to rid the material from the concomitant bacteria. Vaccinia virus can be cultivated readily on the chorio-allantoic membrane of developing chick embryos.

Transmission. Reference to the infection of cattle from persons suffering from vaccine reactions already has been made. There are also numerous reports of similar infections from persons suffering from mild cases of smallpox One surmises from early reports that this mode of infection of cattle formerly was quite common The disease is transmitted from cow to cow on the hands of milkers without doubt, but where the virus exists between outbreaks, and how it reaches new herds is not known.

The virus of vaccinia and also that of variola (smallpox) can readily be propagated on the cornea of the rabbit's eye Paschen (4) believes that the cornea test (Paul's test) is a reliable indicator of the presence of vaccine or variola virus. This test is done by placing a suspension of the suspected material on the cornea of the eye of a rabbit, introducing it very carefully in order to avoid mechanical irritation After 36 to 48 hours the animal is destroyed, the eye enucleated and placed for a few minutes in a sublimate-alcohol mixture. Pox lesions are indicated by grossly apparent opalescent, circular lesions which are not apparent before the chemical treatment

Immunity. After having recovered from an attack cattle are immune thereafter for a considerable time and probably for life. Second attacks have been reported but these may have been due to the pox-like disease which occurs in cattle. Experimentally, solid immunity is conveyed by one exposure. This suggests the vaccination of cattle in herds in which the cowpox is causing trouble. Hester, Boley, and Graham carried out some vaccinations in such a herd using commercial vaccine virus and introducing it into slight scarifications on the tail folds This was followed by cessation of the disease but the authors were not convinced that the naturally-occurring disease was true pox, or that the vaccination had any connection with the disappearance of the disease

Human Infections. The presence of pox-like lesions on the hands of milkers led Jenner to believe that the bovine disease was transmissible to man and that the mild human form protected the victims from smallpox. There can be

no doubt that cowpox can be transmitted to man in this way. From "milker's nodules" Schultz, Seifried, and Schaaf (6) succeeded in producing pox-like lesions on the skin of a calf, and from this calf the disease was transmitted to sheep, goats, rabbits, and guinea pigs. Typical corneal lesions were produced in the eyes of rabbits and Guarnieri bodies were demonstrated in the corneal lesions. These animals were refractory to a second inoculation of the milker's nodule virus and also to vaccine virus. The proof is adequate that the virus recovered from the human hand lesion was that of vaccinia On the other hand, other German workers, and Becker (1) in the United States were unable to prove the relationship of the virus of milker's nodules to that of vaccinia Also, Hester, Boley, and Graham (3) failed to show that a disease clinically diagnosed as cowpox was associated with vaccine virus. These workers were unable to obtain corneal reactions in rabbits and naturally infected animals and persons were not immunized to vaccine virus German workers for the most part regard milker's nodules as a virus disease contracted from bovine teat and udder lesions which simulate cowpox but which are due to a vaccine-like virus which they call *paravaccinia* Another explanation is that the paravaccinia virus is a highly attenuated but true vaccine virus

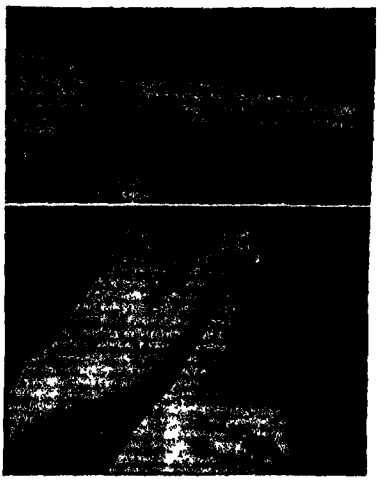


FIG. 128 Milker's nodules (Courtesy of F T. Becker, *Jour Am Med Assoc*)

Milker's nodules appear in abrasions on the hands and fingers of milkers in from 5 to 7 days after contact with the diseased cow. There may be only one but as many as 40 simultaneously developing lesions have been described. They begin as erythematous papules which gradually enlarge into firm, bluish-red nodules from 1 to 2 centimeters in diameter. Usually they are painless. The nodules are surrounded by inflammatory areolas. A depression commonly appears in the center of the lesion. Beneath the epithelial covering reddish granulation tissue is found. Occasionally the regional lymph nodes become enlarged and a few cases have been reported in which generalized infections have occurred. Healing occurs in from four to six weeks. The granulation tissue is slowly absorbed and usually no scar remains.

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HORSEPOX

Synonyms. Contagious pustular dermatitis, "Grease", "Sore heels"

The causative agent of horsepox apparently is the same as that of vaccinia of cattle. Virus removed from the horse lesions will infect cattle, and that of cattle will infect horses The cow and horse diseases reciprocally immunize against each other, and both will transmit to persons who have not been vaccinated, and not to those who have been

The disease in horses takes two forms. The less important is an infection of the pastern region of horses, apparently spread by the hands of horseshoers and hostlers This condition is known as grease or grease-heel It is manifested by the appearance of a papular eruption on the flexor surface of the joints in the lower part of the leg The papules change to vesicles, then to pustules which finally dry up, forming crusts The legs become somewhat painful but there is no general reaction as a rule

The other form is manifested by the appearance of multiple lesions on the inside of the lips and the opposing surfaces of the gums, on the frenum of the tongue, and the inside of the cheeks These begin as papules, change to vesicles, and then pustules The animal may have some fever, and young animals may become very sick and occasionally even die Food is refused, saliva drools from the corners of the mouth, and the animal likes to dip his mouth in water. Beginning with a few lesions, new crops occur and finally nearly all of the mucous membrane of the mouth will be involved In some cases the lesions are found also in the nasal passages

Recovery from the disease leaves a substantial immunity Since lesions on the skin are less severe than those on the mucosa of the mouth, some European authors have suggested vaccination of horses on the skin, claiming good results therefrom.

Horsepox has not been reported from the United States and it seems to be much less common in Europe than it was a half century ago.

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SWINEPOX

This disease has been reported in Europe, Japan, and the United States. It is very common in many of the swine-raising areas of the mid-western states of this country It has been studied by McNutt, Murray, and Purwin (1), by Schwarte and Biester (3), and by Shope (4) in the United States. For detailed descriptions of this disease the reader is referred to these papers. As it occurs in this country it is generally thought that the disease is not very important, however, there are some veterinarians who believe that the importance of the disease is being underestimated It affects principally the young growing animals, suckling pigs being especially affected McNutt and associates report that the lesions are usually found on the lower part of the abdomen and inside the thighs and arms, but in the outbreak described by Schwarte and Biester the lesions were located on the backs and sides Lesions are not located on the head nor on the lower parts of the legs, as a rule.

The lesions, as described by the Iowa workers, consist of red papules which appear 4 or 5 days after virus is placed on the scarified skin A slight fever and mild general reaction occurs at this time The lesions rapidly develop into raised, hard elevations which may be from 1 to 3 cm in diameter Hard crusts form on these areas, these drop off in a few days and the whole process is completed in 12 to 14 days Vesicles and pustules do not ordinarily appear in field cases but Schwarte and Biester found typical lesions which passed through the papule, vesicle, and pustule stages on the abdomen of artificially inoculated pigs

Properties of the Viruses. Two different diseases are known at present under the name of swinepox One of them is caused by a virus which is closely related to that of vaccinia, the other to an unrelated virus Manninger, Cosontos, and Salyi (2) who have seen both diseases in Europe, propose to designate as swinepox the one which is caused by the vaccinia-like virus and to call the other a pox-like disease of swine Schwarte and Biester who have dealt with the pox-like disease of Manninger prefer to call the disease swinepox So far as is known, all cases of swinepox occurring in the United States are caused by a virus which is unrelated to the pox viruses of other animals.

Japanese workers, and also Manninger and co-workers, have dealt with the swinepox caused by the vaccinia-like virus. The virus of this disease can be

readily transmitted to rabbits, and pigs can be immunized to the naturally occurring disease with cowpox vaccine. The pox-like disease of Manninger could not be transmitted to rabbits and was immunologically different from cowpox. Schwarte and Biester, working with this disease, found that it could be readily transmitted to swine but attempts to transmit it to the horse, calf, sheep, dog, cat, rabbit, fowls, rats, mice, and man, failed All of these animals, subjected a little later to vaccine virus, gave good reactions. This experiment included two pigs which had recovered from the pox-like disease and had been shown to be solidly immune to it.

Transmission. Swinepox does not ordinarily pass from one animal to another directly. The transmitting agent usually is the hog louse, *Hematopinus suis*. Since this parasite is found on the lower parts of the animal, on the belly, and in the arm-pits and on the inside of the thighs, this explains why pox lesions usually are found in these locations. In a large herd studied by Schwarte and Biester, the pigs were free of lice and the lesions were found largely on the back and sides This suggested that flies or other insects might be the transmitting agents, and this idea is supported by the fact that the disease disappeared as soon as cold weather eliminated them

Immunity. Pigs which have recovered from the disease appear to be solidly immune for life Practical immunization is not practiced, the disease not being important enough for that The elimination of lice probably would do a great deal to control the disease.

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SHEEPPOX

Of all the animal poxes, sheeppox is the most damaging Fortunately this disease does not exist in the Western Hemisphere. In past times it has done great damage in Europe but at present has been controlled or eliminated from the greater part of that area. It continues to exist in Southern and Eastern Europe and in North Africa.

A generalized pox eruption occurs on the skin and similar lesions often occur on the mucous membrane of the pharynx and trachea, sometimes even in the abomasum. Hemorrhagic inflammation of the respiratory passages and of the digestive tract occurs. Caseous nodules and areas of catarrhal pneu-

monia occur in the lungs The mortality varies from about 5 per cent to higher than 50 per cent.

Vaccination to protect against this disease is commonly practiced in areas where it is enzootic. Fully virulent lymph, collected from skin vesicles, is injected intradermally into the skin of the under surface of the tail, or virus may be rubbed into a small scarified area Generalization of the disease may occur as a result of vaccination but as a rule only a localized, rather mild disease results, and this gives complete protection thereafter. The use of virulent material for vaccination should not be permitted except in areas where the disease actually exists and the flock actually endangered, because vaccinated flocks are a source of danger to unvaccinated neighboring flocks. Some of the countries of western Europe were compelled to enact laws which forbade vaccination except by special permit which was issued only when the disease had been definitely diagnosed in the flock

CHAPTER XLII

VIRUS DISEASES CHARACTERIZED BY LESIONS OF THE CENTRAL NERVOUS SYSTEM

In this group only those viruses which always affect the nervous system are placed It should be borne in mind that many viruses, which are described in other groups because their effect is generally seen in other tissues, frequently exhibit neurotropic tendencies The nerve tissues are often attacked, for example, by the virus of canine distemper causing an encephalitis which is recognized as the "nervous form" of distemper The virus of hog cholera sometimes shows neurotropic properties and the common *herpes simplex* of man, the cause of "fever blisters" or "cold sores" on lips, may cause encephalitis

RABIES

Synonyms: Hydrophobia, Lyssa

The virus of rabies will produce disease by inoculation in all mammals and many if not all birds In the more highly populated parts of the earth it is seen principally in dogs, occasionally in cats It occurs in wolves, foxes, skunks, and other wild carnivora It also occurs occasionally in man The fear of the disease held by people is out of all proportion to its actual importance, but is instilled by the dreadful symptoms which precede death. Rabies is almost invariably fatal in whatever species it occurs

Nature of the Disease. Rabies is a specific encephalitis caused by a virus which has a strong affinity for nerve tissue and apparently develops only in nerve cells The disease is contracted only by the entrance of the specific virus into the tissues through a wound, generally one made by the bite of a rabid animal. Coming in contact with nerve endings in a wound the virus is transported along the nerve sheaths until it reaches the spinal cord and brain. Virus is found in the blood stream only rarely and not for long periods. In the central nerve organs a characteristic form of encephalitis is produced with disturbances of consciousness leading finally to motor paralysis and death.

The symptoms of rabies are similar irrespective of the type of animal affected. After prodromal symptoms consisting of vague changes in the animal's temperament, symptoms which may not be noticed, a period of excitation usually occurs In this stage, dogs, cats, and other carnivorous animals may become frenzied, utter strange cries or howls, and become ferocious. While in this stage they frequently travel considerable distances, biting other animals or people. If restrained they will often chew metal chains or cage bars, breaking their teeth in their frenzy They will often swallow stones, pieces of wood, or eat their own feces After a few hours the excitement gives way to the paralytic period which lasts not more than a day or two before death occurs

Rabies in horses is frequently first manifested by itching at the site of the infecting wound The victim rubs and bites the parts often tearing the flesh The animal is alert, his ears being erect and moving backwards and forwards as if trying to listen to sounds from all directions He often tries to break his halter rope and attacks the manger with his teeth with such force as to break his teeth or even his lower jaw He refuses food but often swallows wood, straw, and manure Genital excitement is common The first signs of paralysis are in the throat The animal tries but is unable to swallow water Paralysis of the legs soon follow

The symptoms in cattle are similar to the above. Cattle may bawl, paw the earth, and charge attendants if not held in restraint In the cattle of Trinidad which are infected with rabies through bites of the vampire bat, the disease never shows the stage of excitement, the symptoms being salivation, marked constipation, weakness of the hind quarters, staggering gait, paralysis, and death (15).

The period of excitement is known as the "furious stage"; the period of paralysis, the "dumb stage" In some cases the period of excitement is short, mild, or absent, and the first symptoms noticed are those of the paralytic stage A considerable part of all cases are of "dumb rabies." Owners of dogs frequently expose themselves to great hazard of infection by trying to extract with their bare hand the "bone in the throat" which they believe is causing the symptoms.

Period of Incubation. The incubation period in natural infections with rabies is relatively long and very variable It is seldom shorter than 3 weeks and longer than 6 weeks. In occasional animals it may be as long as 3 months, and authentic records indicate that periods of 4 and even 5 months occur, but these instances are rare Some reports of incubation periods extending for as long as a year should be received with skepticism

Bites which occur on the head and neck are apt to have a shorter period of incubation than those which occur on the extremities, presumably because

of greater proximity to the brain. Also such bites are more apt to result in rabies than those which occur in areas where nerves are less abundant.

Transmission. It has been known for centuries that rabies was transmitted by the bites of rabid animals. Early workers showed that the disease could be produced experimentally by the inoculation of saliva collected from rabid animals. It was Pasteur (18) who first recognized that the true seat of the disease was in the brain and that the causative agent existed in a pure state there. The manner by which the virus reaches the saliva from the nervous system is not certainly known but is believed to be through the nervous mechanism of the salivary glands and possibly also through the nerve endings in the tongue At any rate it is known that saliva usually becomes infective by the time the first symptoms occur, and sometimes for a day or two earlier, and that it remains infective until after death

The disease is transmitted almost wholly by carnivorous animals, especially dogs, because these animals naturally attack others by means of their bite Horses and cattle affected with rabies may become dangerous but they seldom transmit the disease because they attack by other means The same thing may be said of rabid human beings There is no authentic record of rabies having been transmitted from man to man The saliva of man and of all rabid animals contains the virus but unless this enters the tissues of another, either through a bite-wound or a wound of some other origin, the disease will not be transmitted It should be pointed out that cases of human rabies occasionally result from contamination of scratches and other wounds with saliva of rabid animals.

In sparsely settled parts of the country various wild animals often become important means of transmission of the disease In parts of South Africa the mongoose (22) is the main transmitting agent Rabid skunks have given considerable trouble in parts of the United States. Also ground squirrels, ordinary squirrels, foxes, wild cats, coyotes, and wolves (14) sometimes become involved In Trinidad and parts of South America, rabies is transmitted to animals and occasionally to man through the bite of the small blood-sucking mammal, the vampire bat (15) Since this animal is afield during the hours of darkness, rabies in these regions occurs principally in livestock which is exposed to its bite while on pasture at night. Animals which are kept in buildings during darkness are seldom affected

Bite-wounds made by animals suffering from rabies do not always cause the disease. As a matter of fact it is estimated that before the prophylactic treatment for the disease was developed, not more than one fourth of the persons thus exposed developed the disease Bites in the face and neck region are most dangerous, particularly if there is considerable laceration of tissue Bites

through clothing or thick hair coats are less dangerous than those in regions where the skin is exposed. Experimentally, it can be easily shown that introduction of virus by intracerebral inoculation is certain to produce rabies if the animal is susceptible and the virus is virulent. Virus introduced into the chamber of the eye is about as certain as introduction into the brain. Intramuscular inoculation is much less certain, probably not producing the disease in dogs in more than one half of the cases Subcutaneous inoculation is even less certain Another factor which has an important bearing on the incidence of rabies in animals bitten by dogs is the fact that virus is not constantly present in the saliva of these animals.

Properties of the Virus. The cause of rabies was long a mystery. It finally was shown to be a virus by Remlinger (19) in 1903, who demonstrated that diluted brain material remained infectious after being passed through rather coarse Berkefeld filters The virus will not pass the porcelain or Pasteur filters, thus indicating a rather large particle size The virus probably consists of spherical elementary bodies which can be discerned inside the characteristic inclusion bodies of the disease which were first described, and have been named for, the Italian pathologist, Negri

The virus of rabies is not particularly resistant to disinfectants and drying. Most of the common disinfectants are effective against the virus contained in saliva, and dried saliva loses virulence within a few hours. Virus contained in nerve substance is protected and consequently more resistant to all influences. Brain substance kept in the refrigerator and protected from drying will retain virulence for months Putrefaction destroys the virus rather slowly. Bits of nerve tissue will retain their virulence for months when kept in 50 per cent glycerol

Artificial Cultivation. The virus of rabies can be cultivated in tissue cultures containing viable nerve cells Noguchi (17) in 1913 believed that he had succeeded in cultivating the virus but his results were not confirmed and it is now quite evident that he merely preserved the original virus in his cultures. It is clear, however, that Webster and Clow (26) obtained multiplication of rabies virus in a medium consisting of embryonic mouse or chicken brain suspended in Tyrode's solution Kligler and Bernkopf (11), and Dawson (1) showed that the virus could be propagated in chick embryos The virus is placed upon the chorio-allantoic membrane. No lesions develop on this membrane but invasion of the embryo regularly occurs, the virus being present in large amount in the embryonic brain. Dawson reports that many Negri bodies are seen in such brains. The embryos continued to live and some of them hatched, according to Kligler and Bernkopf Ataxia and other symptoms were seen

soon after hatching but these symptoms disappeared and afterwards virus was not demonstrable in them.

Diagnosis. The symptoms of rabies are so characteristic in most instances that a diagnosis may be made from the clinical symptoms. This is not always true, however, especially in animals that are not commonly affected by the disease. In several instances, diseases that have been diagnosed as encephalitis, both in man and animals, have turned out eventually to be rabies The nature of the disease, when the clinical diagnosis is not clear, can be confirmed only by laboratory examinations. The most important of these are.

1. BY THE INOCULATION OF LABORATORY ANIMALS This is the oldest method of diagnosis, antedating the work of Pasteur For many years the rabbit was the animal of choice and it is still satisfactory for most diagnostic work The guinea pig may be used as a substitute, but in recent years white mice of the Swiss type have been used by preference The Swiss mouse apparently is more susceptible to the virus of rabies than larger animals and thus is preferred when testing vaccines or when searching for low concentrations of virus Also, such mice are cheaply produced and easily cared for

The test animals are inoculated intracerebrally In rabbits and guinea pigs a small hole must be drilled in the skull to admit a hypodermic needle which is thrust into one of the cerebral hemispheres In young mice the skull bones are sufficiently soft so a sharp needle may be thrust through the bones without making a preparatory opening Leach (13) suggests that a 27 gauge needle ¼ inch long be used and that this be forced directly into the brain at right angles with the external surface at a point a little off the median line and about half way between the eyes and the ears The mouse should be etherized before the injection is made The material for injection is prepared by grinding a part of the Ammon's horn in a sterile mortar, suspending it in sterile broth in a proportion of one part of brain to nine of broth, and then centrifuging the mixture at 2,000 revolutions per minute for five minutes The opalescent supernatent fluid is used for inoculation For the mouse the standard dose is 0 03 cc injected with a 0 25 cc. tuberculin syringe For guinea pigs and rabbits as much as 0 1 cc or even larger amounts may be given, but the larger doses are not necessary or desirable

Paralysis of the hind legs of inoculated mice occurs in from 8 to 10 days, and death one or two days after the onset of paralysis Convulsions may occur just before the paralysis becomes evident. Guinea pigs and rabbits generally require a few days longer to show symptoms, and occasionally the incubation period is much longer. Paralysis, especially of the hind quarters, is usually seen Occasionally these animals pass through a period of convulsions before

paralysis occurs Negri bodies can be found in the brains of animals inoculated with street virus by the time symptoms are evident Leach states that they may be found in white mice sometimes as early as the fifth day after inoculation.

If it has been demonstrated satisfactorily that an encephalitis-producing virus is concerned in a particular disease but it is not clear for any reason whether or not the virus is that of rabies, the specific identification can be made by conducting a virus-neutralization test Using an anti serum for rabies virus, tests may be conducted on Swiss mice to determine whether the questionable virus is, or is not neutralized by it *in vitro* Inasmuch as there is no plurality of viruses in this disease, so far as has been demonstrated, neutralization with a known anti-rabies serum indicates that the unknown virus is that of rabies

2. BY THE FINDING OF NEGRI BODIES In 1903 Negri (16) described the inclusion body of rabies which now bears his name The identification of Negri bodies makes it possible to diagnose rabies very quickly and with certainty If they are not found it is not permissible, however, to assume that the disease was not rabies, since a certain number of cases of rabies do not exhibit recognizable Negri bodies Webster (25) quotes others to the effect that about 10 per cent of cases of rabies in Alabama and Georgia are negative for Negri bodies but positive to animal inoculation tests This experience is quite different from that of a series of workers in the diagnostic laboratories of the New York State Veterinary College where dogs' heads have been examined for forty years During this period it always has been the practice to inoculate animals with all brains that fail to show Negri bodies and only in a very few cases have these animal inoculations been positive. Re-examination of the original material has in practically all of these cases shown Negri bodies which

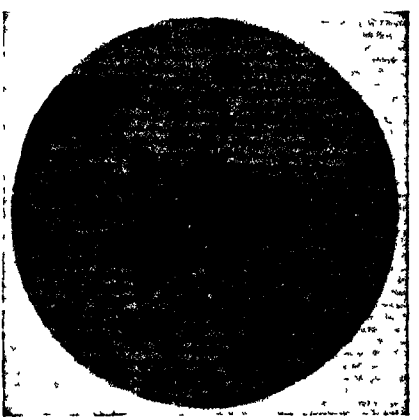


FIG 129 Rabies, Brain, Dog An impression preparation of a large motor cell of the Ammon's horn showing a Negri body The cytoplasm of the cell has been partially disrupted in making the impression The nucleus, containing a distinct nucleolus is seen near the center of the cell The Negri body is located in the cytoplasmic material well to the left of the nucleus x 900

previously had been overlooked. On the basis of this experience it is our custom to render a preliminary negative diagnosis when the brain is received in good condition and satisfactory smear preparations have been obtained, with a final diagnosis when the animal tests have been completed.

Negri bodies occur in the cytoplasm of nerve cells in all parts of the brain and spinal cord They are numerous in the hippocampal convolution or Ammon's horn of the cerebrum and this is the part that is subjected to examination. Smears, impression preparations, or sections may be made Near the periphery of the convolution large triangular motor nerve cells are found forming an almost continuous line It is in these cells that the Negri bodies are found Similar cells are found also in the cerebellum at the juncture between the white and gray matter and these cells usually contain Negri bodies

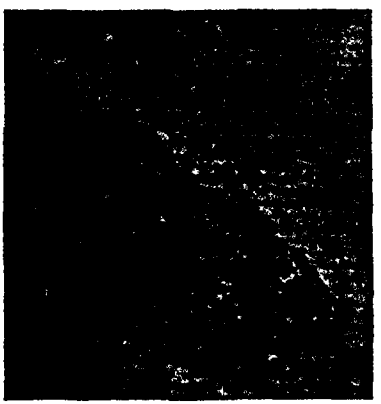


Fig. 130 Rabies Perivascular cuffing in the Ammon's horn Rabbit brain x 250 (Courtesy of S H McNutt)

Negri bodies usually take acidophilic stains They appear as round or oval bodies which vary in size from forms less than 1 micron to large ones more than 10 microns in diameter. Frequently there are several of various sizes and shapes in a single cell The smaller bodies usually have a hyaline appearance but the larger often show a number of minute, poorly staining, "inside" bodies Whether these are of the same nature as the elementary bodies of the pox diseases is not known They have not been freed from the larger inclusion bodies as have the elementary bodies of vaccinia and fowlpox

Immunity. Since recoveries from naturally acquired rabies are so rare, little is known about the resulting immunity. Experience with vaccines makes it quite certain, however, that such individuals possess a high degree of immunity for a year or more. No biological or other form of treatment has been discovered which is useful in treating cases of rabies in which symptoms have been exhibited Taking advantage of the fact that the period of incubation in human rabies is rather long, Pasteur devised a vaccine which can be given after the patient has been exposed to the disease and before symptoms have appeared. This vaccine and its modifications have reduced the human mor-

tality from this disease to a small fraction of what it used to be. The vaccine can also be used to immunize animals, in fact Pasteur proved that the vaccine would immunize dogs before he attempted to use it on man. The Pasteur vaccine has never been extensively used on animals principally because of its expense, but some of the newer vaccines have been so employed.

Vaccines for Rabies. Rabies vaccines are never used on man except on persons who are definitely known to have been exposed and who are presumed to be in the incubation period of the disease. Vaccines can be used on animals under the same circumstances but such use, on dogs, cats, and other animals which frequently transmit rabies, should be discouraged. Vaccines do not always succeed, the incubation period of the disease is variable and relatively long, and frequently too much is at stake to make it advisable to take the chance that the animal may develop the disease in spite of treatment and perhaps be the cause of another outbreak. If vaccines are used on very valuable dogs or cats which are known to have been exposed to rabies, the animals should be held in strict quarantine, confined securely, and observed for a period of not less than six months before being allowed to return to normal life.

Rabies vaccines are most often used on animals, particularly dogs, which are living in areas where rabies is prevalent, as a means of lessening their susceptibility to the disease. Such use is purely prophylactic.

All vaccines for rabies are related to the original one of Pasteur in that they are all made from Pasteur's *virus fixé*, or fixed virus. This will be described in the comments which follow on the Pasteur vaccine. A number of newer vaccines for use on man and animals have been developed, the multiplicity of them indicating in itself that the ideal vaccine has not yet been found. These vaccines fall into two general classes.

a. Those that contain active virus. The most important of these are the Pasteur, the Hogyes, and the Harris vaccines.

b. Those that contain virus which has been inactivated with chemicals, such as phenol, chloroform, formalin, or ether, or with ultra-violet light. These are the so-called "killed vaccines." They are much more commonly used today than those containing active virus.

Rabies Vaccines Containing Active Virus

THE PASTEUR VACCINE. The basis of this vaccine is a virus modified by long continued serial passage through rabbits (18). The first rabbits usually die in about 15 days after intracerebral inoculation with brain-virus from a naturally occurring disease in a dog (*street virus*). As the process of serial inoculations in rabbits continues, the period of incubation becomes shorter and shorter.

Finally the inoculated animals die regularly on the 6th or 7th day and no matter how long the serial inoculations are continued this incubation period cannot be shortened A stage of maximum and stable virulence for the rabbit has been reached Pasteur called such a virus, his *virus fixé*, and this term continues to be used for it. Whereas fixed virus is much more highly virulent for rabbits than street virus, its pathogenicity for dogs and man has greatly declined In his fixed virus, Pasteur and co-workers found a way to propagate an attenuated rabies virus, suitable for vaccine manufacture for man and dogs.

In making the Pasteur vaccine the fixed virus in the spinal cords of rabbits is further attenuated, or perhaps only reduced in amount, by drying the cords, suspended in the air in large bottles over caustic potash Fresh cords are obtained every day, so that tissues which have been dried for periods at daily intervals varying from 1 to 14 days are on hand Treatment of man is begun by injecting suspensions of such cords which have been dried for 14 days. Gradually, day by day, in successive doses, suspensions of cords which have been dried for shorter periods are used until finally those which have been dried only 2 or 3 days are used. In the older treatments, 21 days were required for the full course of treatments but more recently they have been completed in 14 days, two injections being made per day in the beginning, particularly if the bite happened to be on the face in which case it is desirable that immunity be built up as rapidly as possible because of the short incubation period of such cases

The Pasteur treatment can be applied to animals successfully, as was demonstrated first by Pasteur himself, however, it has seldom been used on animals because of its expense As a matter of fact, this form of vaccine is seldom used on man today, since cheaper, more stable vaccines are available which are probably just as efficient. Because the material for the Pasteur method has to be manufactured daily, so as to have fresh material on hand constantly, this method is applicable only in places where laboratories for its production are at hand For many years this was managed in Pasteur Institutes located in many parts of the world, to one of which the patient had to go for the treatment.

THE HOGYES VACCINE This is sometimes called the Hungarian vaccine, it having been developed in the Budapest Pasteur Institute It has been used there for human patients since 1895. Fresh spinal cords of rabbits dead of fixed virus infection are emulsified in saline solution and multiple dilutions are made. Treatment begins with highly diluted suspensions and as it proceeds more and more concentrated suspensions are used. In the beginning the treatments began with suspensions diluted as much as 1 5,000. Later much more

concentrated suspensions have been used with safety. This vaccine probably differs little from the original Pasteur since dilutions probably accomplish the same purpose as the drying process. The method has been successfully applied to animals

THE HARRIS VACCINE This differs little from the Hogyes vaccine except that it has good keeping qualities and thus can be shipped and administered in places remote from a laboratory The vaccine is made of the spinal cords of rabbits dead of fixed virus infection (6) The fresh cords are frozen with carbon dioxide snow, ground to a powder, and dried quickly while frozen. If kept dry and frozen the virulence remains constant for long periods. For use the powder is removed from the vacuum ampoules in which it is stored, suspended in saline solution, and injected Beginning with very small quantities of virus in the first injections, the dosage is increased as the injections proceed This vaccine is used in human practice only.

Rabies Vaccines Containing Inactivated Virus

THE PHENOL "KILLED" VACCINES Semple (21), working with the British Army in India, as early as 1911 used fixed virus inactivated with phenol as a vaccine for rabies in man In recent years the *Semple Vaccine* has been extensively used for human protection, especially in the United States It has given results which apparently are as good as those of the original method of Pasteur. Since the Semple vaccine can be made up in lots and kept for a considerable time before use and can be shipped long distances and administered by local physicians, its use is much simpler and more economical than the vaccines containing living virus Treatment with the Semple vaccine consists of multiple doses, each dose consisting of the same material as the preceding.

In 1921, Umeno and Doi (23) published the results of the immunization of dogs in Tokyo with a phenol-treated vaccine Their vaccine was made from the brains and spinal cords of rabbits dead of fixed virus infection. The nerve substance was finely triturated, then suspended in four times the quantity of fluid consisting of 60 per cent glycerol containing 1.25 per cent phenol. The suspensions were kept for 30 days in the refrigerator, or for two weeks at room temperature, before being released for use In use the suspension was injected subcutaneously in the proportion of 1 cc per 5 pounds of body weight. Only a single injection was given as a prophylactic dose. They reported that the vaccine had been used on about 215,000 dogs of which 175 later died of rabies (0.76 per cent) whereas in the same region 2,860 cases of rabies were seen in dogs not vaccinated.

The vaccine of Kondo was quite similar to that of Umeno and Doi and the results of its use were similarly gratifying, according to the author.

Phenol-treated vaccine was introduced into the United States by Eichhorn and Lyon (2) in 1922 The results in general have not been nearly as good as those achieved by the Japanese workers. The reports have been so conflicting that it is impossible to judge of the vaccine's effectiveness, but there have been enough adverse reports to indicate clearly that it frequently did not convey the protection that was expected of it. Recent developments, which will be referred to later, appear to have given the answer to the question of why the vaccine seemed effective at times and ineffective at others

In 1928, Kelser (9), of the U S Army Veterinary Corps, published preliminary results on a rabies vaccine for dogs in which the fixed virus was inactivated with chloroform Schoening (20), in 1930, compared Kelser's vaccine with a phenolized vaccine and concluded that the former was superior as an immunizing agent for dogs Kelser (10) gave details for the manufacture of his vaccine in 1930 The brain and spinal cord of fixed-virus rabbits are triturated thoroughly and suspended in 2 parts of physiological saline solution The mixture is filtered through gauze, chloroform is added to the extent of 1 per cent, the mixture is allowed to stand at room temperature for 24 hours and then stored in the refrigerator for 6 weeks

The original phenol and chloroform treated vaccines intended for the single-injection use on dogs were made from the brains and cords of rabbits Since the amount of vaccine material obtained from these species is relatively small, many manufacturers have used the brain of sheep and especially of horses, killed by intracerebral injection of fixed virus, for this purpose

Rabies vaccines have been made by treating fixed-virus suspensions with formalin, and with ether, but the reports do not indicate that either of these types have any advantages over the ones already described Recently Hodes, Lavin, and Webster (7) have described a new type of rabies vaccine prepared by inactivating fixed virus with ultra-violet light Later papers by Webster and associates (8) (27) have shown that this vaccine will protect mice and dogs The vaccine has not yet been used for field work but the experimental results are promising

Webster (24) in 1939 studied the immunization of white mice with various rabies vaccines, both specially prepared and commercial products, purchased on the open market It was found that living virus, administered intraperitoneally effectively protected these animals, the protection against intracerebral inoculation being established within 10 days and lasting for at least 9 months When fixed virus was given subcutaneously the protection was not so certain, the protecting dose being quite close to that which infected the animal. Commercial vaccines containing living virus behaved like that prepared experimentally in the laboratory The phenol-treated vaccines, however, both those

made for human and for canine use, generally failed to protect. When used in very large doses, one phenol-treated commercial product gave satisfactory protection Both laboratory-prepared and commercial vaccines inactivated by chloroform, according to Kelser's directions, gave protection when administered in doses from 2 to 5 times as great as that prescribed. The chloroform proved very irritating to the peritoneum, however

Following the suggestion provided by the work of Webster, referred to above, that commercial rabies vaccines varied in immunizing properties, Habel (3) of the U. S Public Health Service, published in 1940 the first of several papers dealing with the antigenicities of different rabies viruses. His method involves the use of 48 white (Swiss) mice for each lot tested. Thirty of these are given 6 doses at two day intervals of 0 25 cc of a 0.5 per cent brain emulsion of the vaccine, intraperitoneally Fourteen days after the first dose of vaccine was given, all vaccinated and 18 control (unvaccinated) mice are given doses of fixed virus, intracerebrally Both vaccinated and control groups are divided into smaller groups and are injected with varying dilutions of virus. The M. L D of the virus is considered to be that dilution which kills 3 out of 6 mice with rabies The immunity end-point in the vaccinated animals is the lowest dilution of the test dose of virus in which 3 of the 6 mice survive The number of M L Ds against which protection is given can be calculated from the number of times greater the concentration of virus must be to kill the vaccinated as compared with the controls. In using materials which he prepared himself, Habel found that the protection often is good against as many as 50,000 M L Ds He suggested that for commercial products, there should be a requirement that they protect against not less than 1,000 M L Ds This method of determining the potency of commercial rabies vaccines is known as the *Habel Test*

In studying commercial vaccines, using the method just described, Habel (4) found that only 12 of a group of 31 protected against as much as 100 M L Ds and some gave no protection whatsoever. Of these 31 strains, 25 originated from the old Pasteur fixed-virus strain, but had been propagated in different ways and by different persons Some of the potent strains had been derived from ones which proved to be impotent Because of the great irregularity found in the antigenicity of the different strains in use for commercial vaccine production, it was suggested that all manufacturers be required to test the antigenicity of each lot of commercial vaccine before its release, and this requirement has now been imposed on all manufacturers in the United States.

Another factor of importance in rabies vaccines, and apparently in all vaccines made from viruses, is the amount of virus present (5). This is especially true in inactivated viruses since there is no multiplication in the body of the

animal treated and thus all antigenic stimulation must come from antigens present in the vaccine. The amount of virus present in the nervous system of animals dead of rabies apparently varies from animal to animal A vaccine prepared from one which has little virus will not be so potent as another made from an animal with a greater virus concentration The Habel test will, of course, indicate whether there is enough virus to give good immunizing properties as well as show whether that which is present is sufficiently antigenic to make a useful vaccine.

Kligler and Bernkopf (11), and Dawson (1) have shown that rabies virus can be propagated in the brains of chick embryos and that it occurs in great concentration there. If greater concentrations of virus can be obtained in this or any other way, such material probably will make more effective vaccines than can be made from brain material Kligler and Bernkopf (12) have immunized successfully a small number of experimental dogs using a tissue-culture vaccine inactivated with formalin

It seems likely that much more effective vaccines for the prevention of rabies are now available than heretofore, and it will be interesting to note the results of field use on dogs of the newer vaccines of proven antigenicity Since the amount of virus contained in the inactivated vaccines is so important, several doses of vaccine will undoubtedly be more effective than a single dose It may be, however, that with a single large dose of high potency vaccine, dogs may be successfully protected from this disease Field use will have to provide the answer to this question.

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PSEUDO-RABIES

Synonyms *Aujeszky's Disease, Mad Itch, Infectious Bulbar Paralysis*

This disease has been observed in cattle, dogs, and cats in which marked symptoms occur and in which the mortality rate is high, and in swine in which the symptoms are mild and the mortality low It has also been reported in sheep and horses but infections in these animals appear to be rare By inoculation many other animal species may be infected Human infections have not been observed

The disease occurs sporadically in Europe, where it has been seen in most of the countries of the continent It has caused large losses in Hungary where it was first described by Aujeszky (1), whose name is attached to the disease. It has been seen in the United States where it occurs in the mid western states. In this country the disease has been seen only in cattle and in swine. In cattle the disease is sporadic In swine, according to the work of Shope (7) (8) the disease is evidently widespread in the cornbelt region where it occurs as an inapparent disease The cause is a filterable virus

Character of the Disease. In animals other than swine, the disease is manifested by an intolerable itching of the skin which crazes them and causes them to lick, rub, and bite certain areas, especially where abrasions occur and which represent the atrium of infection These animals may mutilate themselves badly in their attempts to allay the itching. *Dogs* and *cats* may chew and tear foreign materials and injure themselves badly by throwing themselves violently against the sides of cages They do *not* attack man, however, as such animals do when affected with rabies in the furious stage. In these animals pharyngeal paralysis occurs early and saliva flows from the mouth. Death

usually occurs within 24 to 36 hours after the appearance of the first symptoms. In *cattle* the disease usually begins by an itching of a localized area of the skin, frequently in the flank region The part is constantly licked by the animal until it becomes reddened and inflamed The animal may rub the part against fence posts or even against barbed wire in which case the affected area becomes a great ragged wound The animals often bellow loudly, sweat profusely, and stamp their feet The animals do not eat, paralysis of the pharynx occurs, they fall exhausted, and die within 48 hours There is no evidence of fever during the course of the disease The disease in *swine* is quite different from that in other animals It is quite apparent that the disease occurs in the mid-western part of the United States in many swine in which the sickness is never recognized It usually takes the form of a mild affection accompanied by several days of fever and perhaps vomiting but resulting generally in recovery. In exceptional cases only do fatalities occur In all animals occasional cases are seen in which the disease takes the form of an encephalitis without cutaneous itching These cases are almost invariably fatal even in swine.

The Inoculation Disease. The symptoms seen in naturally infected cases can be produced experimentally by the inoculation of animals with the virus A little of the edematous tissue from the lesion in cattle, injected subcutaneously in rabbits, results in typical mad itch symptoms These begin after an incubation period of about 2 days The animal first licks the point of inoculation, later becomes more frenzied and bites and tears the skin of this area This lasts for 4 to 6 hours by which time the animal is exhausted It then lies on its side, shows clonic spasms, labored respiration, and dies Material from cattle will not infect guinea pigs or mice when inoculated subcutaneously but, curiously, the virus which has been passed through a rabbit brain will then cause mad itch symptoms in these animals (6)

According to Hurst (2), who studied the distribution of the virus of pseudo rabies in the rabbit, whether the animal is inoculated subcutaneously, intradermally, or intramuscularly, the virus reaches the central nervous system by passage through the peripheral nerves in spite of the fact that virus occurs for a time in the blood After intracerebral inoculation virus passes centripetally from the nervous system to the lungs After intravenous inoculation, the virus rapidly disappears from the blood, forming multiple infective foci in the organs from which it passed through the nerves to the brain When subcutaneous inoculation is done in an area deprived of its nervous supply, symptoms are delayed because the virus must then pass from the local area through the blood to establish visceral foci from which the infection of the central nervous system occurs secondarily. He considers this a pantropic virus, that is, one which affects many cells derived from all of the embryonic layers.

When the virus is injected intracerebrally, it is uniformly fatal for rabbits, guinea pigs, rats, and mice. Pruritis of the skin does not occur in such cases. After an incubation period of 24 to 48 hours, symptoms of excitement are shown and blindness evidently occurs. The animals run about their cages wildly and injure themselves by running into the walls. Salivation and grinding of teeth frequently occur. Death occurs after a short period of coma.

The lesions in cattle dead of this disease are not extensive. At the site of the local lesion the skin is lacerated, denuded, and generally covered with dried bloody exudate The subcutaneous tissue is bloody and frequently extensively edematous. The lungs usually show congestion and edema. Except for hemorrhages on the heart wall, and fluid in the pericardial sac, there are no other characteristic lesions In inoculated rabbits the lesions are similar to those of cattle except that the pulmonary edema is much more severe The lung lesions occur in rabbits whether the inoculation is made subcutaneously or intracerebrally.

Properties of the Virus. The particulate size of the virus of pseudo-rabies is rather large It has been estimated by ultra-filtration to be about 120 millimicrons Shope (4) found that the virus in brain suspensions passed Berkefeld V, N and W filters He also reported that virus stored in 50 per cent glycerin in the refrigerator was only slightly attenuated after 154 days In affected animals the virus is found in the edematous subcutaneous tissue in the local skin lesion and in the lungs The blood, liver, spleen, and other organs ordinarily are free from virus When animals are inoculated intracerebrally, virus is regularly found in the brain and in the lungs, but not usually elsewhere. When infections are produced by intranasal instillation, virus occurs in the lungs and usually in the brain as well, but not elsewhere.

Transmission. The transmission of pseudo-rabies has been largely cleared up through the work of Koves and Hirt (3), and of Shope (9) The European workers were the first to recognize that the disease sometimes assumed epizootic proportions in swine and that it was definitely contagious in this species. They assumed that the virus escaped in the saliva and possibly in the urine. Shope also observed that the disease spread from animal to animal in swine but did not in any other species with which he worked. He was unable to find virus in the saliva, urine, and feces but demonstrated that the nasal secretion, beginning about the sixth day after inoculation, was infectious and that it was by means of this secretion that the disease was transmitted. The nasal secretion shows the presence of virus quite regularly about the time of the temperature rise and it continues to be eliminated for several days during the course of the symptoms but disappears shortly thereafter. Rabbits were readily

infected by rubbing a slightly scarified skin surface on the nose of pigs during the period of symptoms

The disease in other animal species is not contagious Since it is a general custom in the mid-western part of the United States to let swine run with cattle, it is Shope's belief (9) that swine represent the natural reservoir, and that cattle infections occur incidentally, probably through minor wounds of the skin which become contaminated with nasal secretions of swine which usually are not recognized to be sick Shope (7) (8) also showed that ordinary brown rats, which are frequent around corn cribs and animal quarters in the mid-western states, readily develop pseudo-rabies by ingestion, and he suggests that these rodents may be the means of carrying the infection from one farm to another.

Artificial Cultivation of the Virus. Traub (10) was the first to report success in cultivating the virus of this disease He succeeded in obtaining multiplication in media containing minced testicular tissue of rabbits and guinea pigs, and also in a minced chick embryo medium

Immunity. Practicable methods of immunization have not been developed Shope (9) has shown that swine which have recovered from the disease have neutralizing antibodies in their sera Using this technic he was able to show that the European disease and the one in America cross-immunized perfectly and thus could be considered to be identical (5) The same method showed that swine on a farm where cattle had been lost from this disease had immunizing antibodies in their sera, and also that many swine originating in the mid-western states and being used for the production of anti-hog-cholera serum possessed neutralizing antibodies, whereas similar animals raised in the eastern states lacked them (8)

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The Infectious Equine Encephalomyelitides

At least four immunologically distinct viruses have been isolated from horses suffering from encephalomyelitis The first of these was from a disease which has been reported only from Germany and which is commonly known as *Borna disease,* the name being derived from a village around which the disease was enzootic This disease can be differentiated from the others on the basis of a number of features which will be discussed below. The *Western* and *Eastern* types of encephalomyelitis in the United States, and the *Venezuelan* type of South America produce diseases which are similar All of these are destructive Similar diseases have been described in other parts of the world Whether they are caused by these or different viruses is not known. All of these viruses naturally occur in other species of animals and all but that of Borna disease have been found as the causative agents of serious diseases of man

BORNA DISEASE

This disease is an encephalomyelitis of horses, occasionally of sheep, which has occurred annually for a century or more in certain localities in Saxony. There are no characteristic gross lesions but microscopically the usual lesions of virus encephalitis and myelitis are exhibited These consist of perivascular infiltrations of lymphocytes (blood vessel "cuffing"), degeneration of ganglion cells, neuronophagia, and multiplication of neuroglia cells The lesions are most marked in the brain stem Lesions in the spinal cord are much less severe than those in the brain A characteristic feature of Borna disease is the intranuclear bodies, commonly called *Joest* bodies which were first described by Joest and Degen (1) in 1909 These inclusion bodies are seen in the ganglionic cells in the hippocampus and in the olfactory lobes, more rarely in other parts of the brain and cord With the Giemsa stain these bodies appear as reddish, round or oval bodies, varying in size, embedded in the nuclei which are stained a light blue color Each of the bodies is surrounded by an unstained halo These bodies can be found in nearly all cases of Borna disease In 1927, Zwick, Seifried, and Witte (2) isolated a virus from the brains of naturally infected horses and demonstrated its causal relationship to the disease.

Character of the Disease. The period of incubation of this disease is at least 4 weeks, in this respect differing from the other forms of virus encephalomyelitis in which it is very much shorter. The initial symptoms consist of a low fever, difficulty in swallowing, salivation, hyperesthesia, reflex irritability, spasms of the neck muscles, and other signs of cerebral irritation These ter-

minate in drowsiness and paralysis, either localized or general The symptoms vary greatly The course of the disease is also varied. Many cases die within 1 week after the appearance of the first symptoms; others may not die for 3 weeks The mortality averages 90 per cent.

Borna disease can be transmitted from horses to rabbits by intracerebral, intraocular, corneal, nasal, intravenous, intraperitoneal, or subcutaneous inoculation of brain material. Guinea pigs, rats, hens, and sheep can be infected by inoculation but these species are not so susceptible as rabbits The virus can be propagated indefinitely in rabbits by brain to brain inoculation The incubation period in rabbits is from 3 to 4 weeks and the period of symptoms is from 1 to 2 weeks. Death occurs in nearly all cases. A variety of nervous symptoms are exhibited by the animals and general paralysis occurs before death ensues Zwick and co-workers produced two cases of the disease in monkeys, which suggests that human infections might occur, however none have been recognized

Nature of the Virus. The virus of Borna disease is filterable with difficulty Elford and Galloway estimate its size as between 85 and 125 millimicrons It is much more resistant than the other encephalitis viruses In 50% glycerol brain virus can be kept for at least six months Zwick and Witte found that dried virus kept its virulence for more than three years In aqueous suspensions in which fermentation is prevented, the virus remains alive for long periods

Transmission. The mode of transmission of this disease is not known with certainty Unlike the other forms of virus encephalitis of horses which occur during the warm periods of the year almost wholly, Borna disease occurs throughout the year Most of the cases are seen from February to July About the time of year when the other forms of encephalitis appear, Borna disease cases become less frequent. The seasonal occurrence suggests that insects play no part in its transmission According to Joest and Degen, the virus is present in the salivary glands, in the saliva, and in the secretions of the naso-pharynx The fact that lesions may be demonstrated regularly in the olfactory tract is suggestive of an entry path here by way of the naso-pharynx Successful feeding experiments have also been reported, hence the digestive tract may be a portal of entry.

Artificial Cultivation. Successful cultivation of the virus of Borna disease in artificial media has not been reported.

Immunity. Zwick, Seifried, and Witte (3) report the successful immunization of horses with lapinized virus. The method has been used successfully in the field.

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NORTH AMERICAN EQUINE ENCEPHALOMYELITIS

It appears certain that an enzootic encephalomyelitis of horses of virus origin has occurred in the United States for many years In the late summer and early fall of 1912, large numbers of horses were lost in Kansas, Nebraska, Colorado, Oklahoma, and Missouri from what was most certainly virus encephalitis although it was not recognized as such at the time. The outbreak and the characteristic lesions were described by Udall (33). It is estimated that 35,000 horses died of the disease from mid-summer until heavy frosts in October put an end to the outbreak. In later years small outbreaks of the malady appeared in many of the western states.

In July 1930 the disease appeared among horses in the San Joaquin valley in California The outbreak continued through August, reached its peak in September, and disappeared with the advent of cool weather in November It was studied by Meyer, Haring, and Howitt (22) who estimated that 3,000 horses and mules perished from this disease, this number being about one-half the total of recognized cases These workers isolated and studied the virus of the disease The following year the disease reoccurred in the same area and appeared for the first time in several of the neighboring states. The disease reappeared in each successive summer spreading over a larger and larger area In 1937 the disease was recognized in every state west of the Mississippi river and in several east of it The peak in the disease incidence occurred in 1938 when 184,000 horses were estimated by the U S Bureau of Animal Industry to have died from it By this time every state lying west of the Appalachian mountains had had cases. The incidence of the disease in 1939 amounted to only about 4 per cent of that of 1938 and in more recent years it has remained at about this level.

In 1933 an isolated focus of the disease appeared along the coastal plains of Delaware, Maryland, Virginia, and southern New Jersey and it is certain that at least 1,000 horses died of the disease in that year. The symptoms of affected animals were much like those exhibited by horses in the western parts of the country but the mortality rate was much higher, approximating 90 per cent. It was generally believed at first that the disease was identical with that which prevailed in the country west of the Appalachian mountains but Ten Broeck and Merrill (31) pointed out that the virus was immunologically different, since animals which had been immunized to the virus of the eastern disease

were not protected against the virus of the western disease, and vice-versa. These results were quickly confirmed by others, and it became generally accepted that there were two types of the disease in the country, these being differentiated under the names of the *western type* and the *eastern type*, respectively. Both types produce encephalomyelitis in horses, the symptoms and pathological changes being practically identical, the principal differences being that the eastern type is much the more virulent for horses, most experimental animals, and man, and that there is little or no cross-immunity between the two types The eastern type of equine encephalomyelitis remained localized along the eastern seaboard in the general area where it was first recognized, reoccurring each summer and fall, until 1938 when it suddenly extended northward, appearing in Connecticut, Rhode Island, and eastern Massachusetts. In this year a considerable number of fatal cases were recognized in human infants, these cases occurring in the area where the horses were dying of it, principally in Massachusetts These will be discussed below. In the same year naturally-occurring fatal cases in ring-necked pheasants were recognized by Tyzzer, Sellards, and Bennett (32), and the virus was isolated from a common pigeon by Fothergill and Dingle (6). The avian infection occurred in eastern Massachusetts and Connecticut in areas where horses were dying The virus in each case proved to be of the eastern type In 1941 Randall and Eichhorn (23) reported a small outbreak of encephalomyelitis near Brownsville, Texas, near the seacoast in which the virus proved to be of the eastern type Except for this instance, the two types of the disease have not appeared in the same territory, the eastern type remaining on the eastern seaboard and the western, west of the Appalachian chain Many cases of the disease have occurred in the central and western provinces of Canada, the western type virus being the causative agent In 1939 a few cases occurred in eastern Ontario near the Great Lakes, the virus being of the eastern type.

Character of the Disease in Horses. The disease occurs almost exclusively in farm horses from June to November In the southern part of the country sporadic cases may occur during the winter Horses of all ages are equally susceptible Usually not more than 20 per cent of the horses on any one place become infected, and considerable periods may elapse between cases on the same premises. The mortality from the western type of the disease usually is not in excess of 50 per cent and may be considerably less; that of the eastern type of the disease is much greater, being 90 per cent or higher.

The incubation period is from 1 to 3 weeks. In this respect the American disease differs markedly from the German Borna disease in which it is from 4 to 7 or more weeks.

The symptoms are those of deranged consciousness. The affected animal

in the earlier stages of the disease may walk aimlessly in circles or may crash through fences and into obstacles of any kind. Later a sleepy attitude develops, the animal standing with depressed head Local paralysis may develop and later this becomes so complete that the animal goes down and is unable to rise. Death occurs within a day or two after symptoms appear. Animals which recover from severe affections frequently show permanent cerebral damage,



FIG 131 Equine encephalomyelitis (*Courtesy of Edward Records*)

manifested by loss of ability to react to normal stimuli. Such animals are often called "dummies" by horsemen.

There are no characteristic gross lesions in animals dying of this disease Hurst (14), who studied the histology of the lesions in the central nervous system, says that the grey matter is affected to a greater extent than the white, and that the lesions are most marked in the cerebral cortex, thalamus, and hypothalamic regions, the brain stem and spinal cord being less involved as a rule The lesions consist of degeneration of nerve cells with the appearance in the neurons of intranuclear inclusion bodies resembling those described by Joest in Borna disease, perivascular cuffing with mononuclear and polymorphonuclear cells in varying proportion, polymorphonuclear leucocyte infiltrations into the grey matter, and proliferation of glial cells. The lesions produced by the western type virus are, as a rule, less intense than those caused

by the eastern type. Meyer, Haring, and Howitt (22) were unable to find inclusion bodies and made a point of this fact in differentiating this disease from the Borna disease of Germany. During the Kansas horse plague of 1912 a number of workers sought unsuccessfully to demonstrate inclusion bodies similar to the Joest bodies of Borna disease.

The Disease in Animals other than Horses. That of the virus of equine encephalomyelitis might have a natural reservoir in animals other than horses was suggested by Ten Broeck, Hurst, and Traub (30) in 1935. The suggestion was based upon epizootiological evidence. Reference has already been made to the finding of naturally infected pheasants by Tyzzer, Sellards, and Bennett (32), and of a naturally infected pigeon by Fothergill and Dingle (6) during the 1938 outbreak in Massachusetts and Connecticut In 1941, Cox, Jellison, and Hughes (5) reported the finding of a naturally infected prairie chicken (*Tympanuchus cupido americanus*) in an area in North Dakota in which the disease was prevalent in horses and man In 1940 Gwatkin and Moore (9) examined the brains of a considerable number of ground squirrels (*Citellus richardsoni*), captured in the field, for virus In one lot of brains some agent produced febrile reactions in two guinea pigs The experimental animals recovered but later proved refractory to a large dose of western type encephalomyelitis virus, hence the authors believed that the virus existed in the brain of the ground squirrel used for inoculation

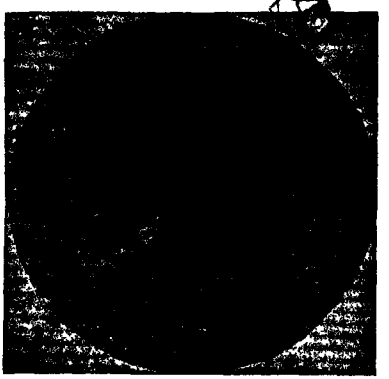


FIG 132 Equine Encephalomyelitis Brain of a guinea pig inoculated with the western type virus Cellular infiltrations of both gray and white matter, and perivascular cuffing x 100 (Courtesy of S H McNutt)

Animals Susceptible to Experimental Inoculation. Meyer, Haring, and Howitt found that guinea pigs were highly susceptible to intracerebral inoculation with virus of equine origin, and this animal is the most suitable for diagnostic work. Death occurs in four to six days, as a rule, being preceded by an early febrile reaction, followed by muscular tremors, flabbiness of the abdominal muscles, salivation, and trotting movements after the animal goes down. Rabbits are much less susceptible. A febrile reaction occurs and virus exists in the blood but symptoms are very mild or absent, and recovery

generally occurs White mice are very susceptible. They may be infected by intracerebral inoculation and also through the undamaged nasal mucosa. Calves can be infected by intracerebral inoculation. These animals display marked nervous symptoms beginning about the 5th day. By the 14th day recovery usually is complete, according to Giltner and Shahan (8). These authors found that sheep, dogs, and cats were refractory to inoculation. The common ground squirrel of the western states (*Citellus richardsoni*) may readily be infected by intracranial inoculation

Avian species known to be susceptible to inoculation are as follows: sparrows, quail, pheasants, juncos, thrushes, pigeons, young chicks, ducklings, and turkey poults Older domesticated birds are resistant to the inoculation and ordinarily do not die or even show symptoms, however virus can often be demonstrated in their blood for a day or two after inoculation.

Artificial Cultivation. In 1935, Higbie and Howitt (12) reported successful cultivation of both eastern and western types of equine encephalomyelitis virus in chick embryos Minute amounts of brain virus placed on the chorioallantoic membrane resulted in deaths of the embryos in from 15 to 24 hours, the embryonic tissues being saturated with virus of a very high titer The sensitivity of chick embryos to the virus of this disease is very great, frequently less than $\frac{1}{10}$ M L D for the guinea pig being required to infect The virus can be cultivated very successfully in minced chick-embryo tissue suspended in Tyrode's solution, but this method has been superseded by the simpler method detailed above

Nature of the Virus. According to Bauer, Cox, and Olitsky (1), the particle size of the virus of equine encephalomyelitis, as determined by ultra filtration studies, is about 25 to 35 millimicrons in diameter This places it among those of relatively small particulate size The virus remains viable for long periods when suspended in 50 per cent glycerin in a buffer mixture. It is destroyed rapidly in slightly acid solutions and disappears quickly from tissues after death of the affected animal, probably because of the developing acidity This is of importance with regard to diagnosis from tissues recovered at autopsy. The virus is readily destroyed by formalin but is rather resistant to phenol

Transmission of the Disease. Vawter and Records (34) showed in 1933 that horses could be readily infected by intranasal instillation of virus, and transmission in this way probably occurs at times. The epizootiology of the disease indicates, however, that this cannot be the usual mode of transmission. Transmission by blood-sucking insects and particularly by mosquitoes had previously been suspected, but Kelser (15) was the first to show, in 1933, that mosquitoes could be infected and could convey the disease from animal to

animal. Kelser used the yellow fever mosquito (*Aedes aegypti*) in his work, showing that 6 to 8 days after they had been allowed to feed on infected guinea pigs they were capable of infecting other guinea pigs and a horse. This finding was confirmed by several workers Merrill, Lacaillade, and Ten Broeck (19) in the following year showed that the ordinary salt marsh mosquito (*Aedes sollicitans*) was capable of transmitting both the eastern and western types of virus Another salt marsh mosquito (*Aedes cantator*) proved capable of transmitting the eastern type virus but not the western *Anopheles quadrimaculatus* and *Culex pipiens* proved incapable of transmitting either type. Madsen and Knowlton (18) in 1935 showed that local species of *Aedes* mosquitoes in Utah, *Aedes dorsalis* and *A nigromaculis* were capable of transmitting the western type virus Others have shown that *Aedes albopictus, A. taeniorynchus* and *A vexans* were capable of transmitting the western type virus Merrill and Ten Broeck (20) proved that the virus multiplies in the affected *A. aegypti* by feeding starved individuals upon previously infected mosquitoes which had been ground into a paste In this way they propagated the disease through ten lots of mosquitoes in each of which it was estimated that there had been a dilution of at least 1 100 They concluded that the results could be explained only upon the basis that the virus had increased in the insects.

In all of the mosquito experiments referred to above, the insects were infected by feeding them upon artificially infected animals or brain material It was not until the summer of 1941 that naturally infected wild mosquitoes were detected. In that year, Hammon, Reeves, Brookman, Zumi, and Gjullin (11) demonstrated the western type virus in one lot of mosquitoes (*Culex tarsalis*) caught in the Yakima valley in Washington during the course of an outbreak of the disease in horses This species is widely distributed in the states west of the Mississippi river It is known to feed upon man, horses, mules, cattle, and mallard ducks.

The accumulated evidence has convinced most workers that equine encephalomyelitis is transmitted principally by mosquitoes which have fed upon animals during the early stages of the disease when the virus is commonly present in the blood.

Syverton and Berry (28) showed, in 1937, that the spotted fever tick, *Dermacentor andersoni*, could serve as a vector for the western type of equine encephalomyelitis virus. Adult and nymphal stages of this tick were allowed to feed on recently infected guinea pigs At intervals varying from 32 to 80 days thereafter, successive stages in the developmental cycle of these ticks were allowed to feed on normal guinea pigs and ground squirrels. The disease was conveyed to these animals. Continuity of the virus through all stages, in-

cluding the eggs, was demonstrated At the time of the report virus had remained in these ticks for 130 days, a period sufficiently long to suggest that this might be one way by which the virus was preserved from one season to the next Gwatkin (9) confirmed the essential details of this work

In 1940, Kitselman and Grundmann (16) demonstrated the western type virus in a large blood-sucking insect known as the "assassin bug" (*Triatoma sanguisuga*) captured in a pasture in Kansas Since this insect is common in many parts of the west and is known to feed upon horses it is possible that it sometimes plays a part in the transmission of the virus

Immunity. Records and Vawter (24) showed, in 1934, that horses might be effectively immunized to the western type of virus by injecting them with fully virulent brain virus, subcutaneously Traub and Ten Broeck (29) showed that a virus strain which had been passed through many generations in pigeons became modified so that it had lost much of its infectivity for horses and could be used subcutaneously as a vaccine Live virus, as an immunizing agent, has obvious disadvantages since it circulates for a time in the blood and the hazard exists that blood-sucking insects might become infected and initiate outbreaks. The way was opened for a safer vaccine when Shahan and Giltner (26) demonstrated that horses could be effectively immunized by the injection of brain virus which had been inactivated with formalin The use of a formalinized brain virus met with only partial success in the field, however it was widely used until a better vaccine, made from chick embryo cultures was introduced by Beard, Finkelstein, Sealy, and Wyckoff (3) in 1938

The *chick embryo vaccine* is made by inoculating the chorio-allantoic membrane of developing chicks about 10 days old (incubation time) with virus. The virus multiplies very rapidly, reaching a very high titer in about 15 hours, and the embryo usually dies from the virus reaction in from 15 to 24 hours. The vaccine is made by harvesting the virus-containing embryos, grinding them into a paste, suspending them in a saline buffer solution in a 10 per cent concentration and treating the suspension with 0.4 formalin Undoubtedly the greater effectiveness of the chick-embryo vaccine over the horse brain or guinea pig brain vaccines lies in the fact that the concentration of virus is very much greater The virus content of the chick embryo is from 1,000 to 10,000 times as great as that of infected mammalian brains The eastern type virus regularly attains 3×10^9 mouse infective units per gram whereas the western attains 3×10^8 and sometimes 3×10^9 units. Two doses of 10 cc. of the vaccine are given, the second one week after the first Within two weeks thereafter the animal is solidly immune. Immunity, of course, is established only against the type of virus in the vaccine The immunity appears to last at least six months and probably much longer Annual immunization is prac-

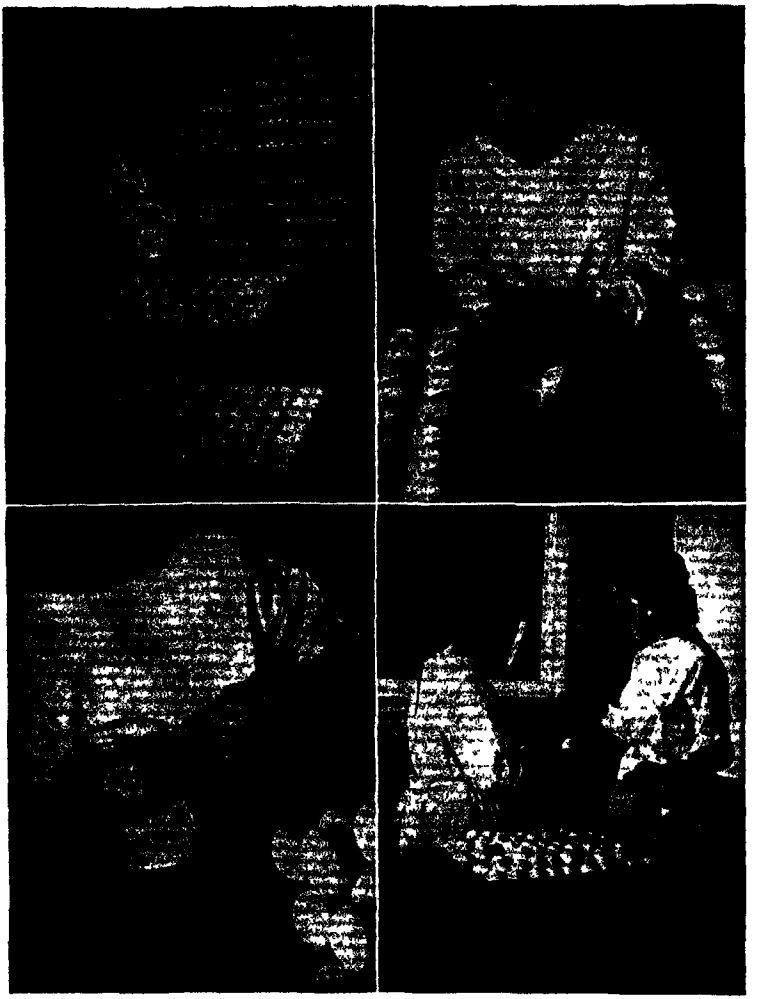


FIG 133 Commercial Manufacture of Equine Encephalomyelitis Vaccine from Egg-embryo Propagated Virus (1) Trays of fertile hen's eggs in an incubating room The eggs must be incubated until the embryos are about ten days old After inoculation they are incubated for an additional 24 hours at which time most of the embryos have died from the effects of the virus (2) Making holes in the shells to permit inoculation This is being done with a power driven dental drill (3) Inoculating the embryo with virus A small amount of virus is deposited on the chorio-allantoic membrane From this point it quickly invades the embryo (4) Harvesting the embryos. The girls wear shields to protect their faces from splattering material (Courtesy of the Lederle Laboratories Inc)

ticed on horses living in infected areas. Ordinarily serious reactions are not seen after vaccination, however there have been a few reports of a post-vaccination disease of rather serious nature. These were described by Shahan, Giltner, Davis, and Huffman (27) The nature of this malady, commonly called X disease, is not clear The disease is characterized by icterus, constipation, and nervous symptoms, and is highly fatal Parenchymatous degeneration of the liver and kidneys is commonly found No lesions are found in the nervous system and virus has not been demonstrated in the tissues There is some evidence to indicate that these reactions may have been due to autolytic changes in the vaccine, since many of the cases were seen in animals vaccinated with materials which had been made the previous season and had been held over

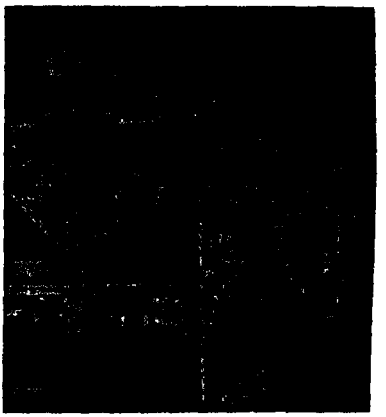


Fig. 134 Commercial Manufacture of Equine Encephalomyelitis Vaccine from Egg embryo Propagated Virus The propagation and harvesting of the virus is depicted in Fig 133 The virus-containing embryos are ground to a paste which is suspended in saline solution Formalin is added in 0 4 per cent concentration and the suspensions are placed in large bottles which are kept in an incubator room until the virus has been inactivated It is then packaged and stored in refrigerators until needed (Courtesy of the Lederle Laboratories, Inc)

The Disease in Man. Meyer (21), in 1932, reported three cases of human encephalitis in persons who had associated with horses suffering from the western type of encephalomyelitis Virus was not isolated from any of these cases One proved fatal The author suggested that these cases might have been human infections with the virus of the horse disease and advised workers to be on the lookout for such cases The first human cases proved to be caused by the equine encephalomyelitis virus were described by Fothergill, Dingle, Farber, and Connerley (7) in the late summer of 1938 Shortly afterwards others were reported by Wesselhoeft, Smith, and Branch (36). These cases occurred in eastern Massachusetts during the height of the outbreak in horses At least 40 human cases occurred and the nature of the virus was proven in 9 cases Webster and Wright (35) proved by neutralization tests on laboratory animals that the virus was of the eastern type, and Schoening, Giltner, and Shahan (25) showed that the human virus would kill unprotected horses as well as ones immunized to the western type virus but was innocuous to

horses immunized against the eastern type virus The persons affected were mostly children, there were no multiple cases in families, and none had had any contact with horses The season had been very wet, however, and mosquitoes were very common. It is believed that mosquitoes were the transmitting agents It was during this outbreak, it will be recalled, that infected pheasants and an infected pigeon were found, these probably being infected in the same way as the human cases.

The onset of the disease was sudden and was characterized by high fever, convulsions, vomiting, and drowsiness which rapidly progressed to a comatose condition Nearly all patients died. The high death rate distinguishes this illness from other forms of virus encephalitis in man which ordinarily have a much lower mortality rate.

In 1938, Howitt (13) reported the first proven case of equine encephalomyelitis virus infection in man caused by the western type of virus This was in a 20-month-old infant who died after an illness of five days In 1941 the most extensive outbreak of human encephalitis ever recorded was reported by Leake (17) in the north-central part of the United States Nearly 3,000 human cases were recognized In general the cases were mild, however 195 deaths occurred Cox, Jellison, and Hughes (5) isolated the western type of equine encephalomyelitis virus from eight fatal cases, and virus neutralizations with sera of recovered cases leave no doubt that the equine virus was the cause of the outbreak The disease in horses at the time of the human outbreak was not nearly so prevalent as it had been in several preceding years when human cases were not recognized It was a rather damp summer, however, and mosquitoes were unusually numerous An interesting feature of this outbreak was that cases were more than twice as numerous among males as in females, which means, presumably, that the males were more exposed to mosquitoes while working in the harvest fields than were the women

Interesting results were reported by a commission consisting of Hammon, Gray, Evans, Izumi, and Lundy (10) which worked in the Yakima valley of Washington in the summer of 1941 During the preceding summer both St. Louis encephalitis and equine encephalomyelitis had prevailed in this area. Using virus neutralization tests, it was shown that neutralizing antibodies against both of these viruses were surprisingly prevalent in the domestic animals of the region As a result of their work it was concluded that barnyard fowl, which were found in large numbers in the small towns of the region, probably had served as a reservoir for both of these viruses

Following the demonstration that an effective vaccine could be made for protecting horses from the virus propagated in developing chick embryos, a number of laboratories began manufacturing the vaccine in 1939 Very soon

several fatal human infections occurred in these laboratories. The manufacturers then began to immunize their workers with a somewhat refined vaccine of the type used on horses [See Beard, Beard, and Finkelstein (2)]. This has proved effective and has not caused unusual discomfort or resulted in undesirable sequelae. The vaccine is recommended for persons unusually exposed to danger of infection. Laboratory workers who are exposed to both western and eastern types of virus should use a mixture of both types of vaccine.

VENEZUELAN EQUINE ENCEPHALOMYELITIS

A virus encephalomyelitis of horses occurs in South America Beck and Wyckoff (4) studied the virus and compared it with the North American types They found it considerably more virulent for guinea pigs and chick embryos than either the eastern or western types of North America A vaccine made of formalinized tissue of the Venezuelan virus protected against the homologous virus but not against the North American types Animals immunized to the North American types succumbed to the Venezuelan virus, but those immunized with the eastern type virus showed evidence of partial protection

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INFECTIOUS PORCINE ENCEPHALOMYELITIS

An infectious encephalomyelitis of swine which causes serious losses has been reported by many workers in Germany in recent years The disease was first described by Klobouk in 1929 in what used to be Czecho-Slovakia The disease is commonly called *Teschen disease*, the name taking origin from the name of the community where it was first recognized It is caused by a filterable virus

Character of the Disease. After an incubation period of about 14 days the disease begins with weakness of the hind quarters Usually a progressive paralysis begins in the hind quarters and extends to the fore legs so the animal is unable to stand by the second or third day The animal is unable to lift its head, the tongue is often paralyzed and sometimes the lower jaw as well. Many, if not all, cases begin with a mild febrile reaction which disappears by the time the paralysis is evident Some animals die within two or three days with respiratory paralysis and brain impairment which makes them stuporous. Most cases last longer, for several days or even several weeks In severe outbreaks the mortality may approach 90 per cent; in milder ones it may not be greater than 50 per cent. Recovered animals usually show atrophy of the leg muscles and permanently impaired locomotion.

Gross lesions are absent Histological examination of the brain and cord, however, show lesions typical of virus induced encephalomyelitis; round cell infiltrations, neuron destruction, neuroglia proliferation, and perivascular cuffing The lesions are most marked in the gray matter of both brain and cord. Inclusion bodies have not been found

Nature of the Virus. Klobouk was the first to demonstrate the virus nature of the causative agent He showed that brain material free of microorganisms was capable of transmitting the disease to pigs and that the histological picture was typical of a virus infection According to many authors transmission is not certain even when the material is injected intracerebrally Diernhofer (1) says that he has succeeded uniformly when pigs weighing 16 to 20 pounds were used, but his results were erratic when larger animals were employed. Infection by intranasal instillation, and intravenous and subcutaneous inoculation, occurs only occasionally, but such animals often are immunized by such experiences The virus is not pathogenic for mice, rats, guinea pigs, sheep, and cattle, even by intracerebral inoculation.

Transmission. The mode of transmission of this disease is not known. Seldom do all animals in a herd become infected, hence the disease is not highly contagious by direct contact Several authors have noted the similarity between this disease and poliomyelitis of man, however there have been no reports of human infections in those who have been closely associated with the swine disease It has been suggested that this malady was Aujeszky's disease but if this were true the characteristic effect upon rabbits should be demonstrable It has been suggested that the disease was caused by the virus of hog cholera, but Diernhofer has found that animals immune to Teschen disease can be infected with cholera virus, and that cholera immune pigs can be infected with the virus of Teschen disease

Artificial Cultivation. There have been no reports of the artificial cultivation of the virus of porcine encephalomyelitis

Immunity. It has been shown that swine can be immunized by injecting them with a vaccine made from infected brain and cord material which has been treated with 1.5 per cent formalin for ten days In the field this vaccine has given doubtful results in many cases In the face of an outbreak of the disease the vaccine is useless since protection is developed too slowly.

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INFECTIOUS AVIAN ENCEPHALOMYELITIS

Synonym: Epidemic Tremor of Chicks.

This disease was first described by E. Elizabeth Jones (1) in Massachusetts in 1932. In a more complete description of the disease and the virus which causes it, Jones (2), in 1934, called the disease *epidemic tremor* because of the peculiar vibration of the head and neck which characterizes many cases. Because this symptom is not so frequently seen as others referable to damage of the nervous system, Van Roeckel, Bullis, and Clarke (6) proposed that it be named infectious avian encephalomyelitis. The distribution of this disease is unknown. It has been diagnosed in a number of the New England and adjoining states of the United States. It has not been reported elsewhere.

Character of the Disease. The disease usually makes its appearance in chicks when they are two or three weeks of age. Van Roeckel and associates say that they have observed cases in chicks upon their removal from the incubator within 24 to 48 hours after they were hatched, but such cases are certainly exceptional. The first symptom noted is an ataxia or inco-ordination of the muscles of the legs. The symptom becomes more evident as the disease progresses and finally the bird loses all control of its legs and cannot stand. Before this stage is reached the chicks are reluctant to move and may walk on their shanks. The vibration of the head and neck muscles usually appears well after the ataxic symptoms have been observed. The tremor is periodic, continuing for varying lengths of time. Finally the birds are unable to feed, become somnolent, and die. In some cases the course of the disease is very rapid, somnolescence appearing within a day after the first symptoms were noted. The losses from the disease may be rather high; in excess of 50 per cent in some cases.

Chicks dead of encephalomyelitis show no characteristic gross lesions. Microscopically, the islands of lymphatic tissue which, in birds, are scattered throughout the organs, show evidence of hyperplasia. Since such lesions are found in other conditions in birds it is not clear that they are actually caused by the virus of this disease. Characteristic lesions are found, however, in the central nervous system. In the brain the most marked lesions are the exceptionally well marked perivascular cuffs, consisting principally of lymphocytes and a few monocytes. Neuron degeneration occurs but is most marked in the anterior horn of the cord, in the medulla and the pons, whereas the blood vessel cuffing is widespread. In the degenerating neurons of the cord Olitsky speaks of brightly staining acidophilic bodies of about the size of red blood cells embedded in the cytoplasm. He does not refer to them as inclusion bodies, however.

Nature of the Virus. The virus is present regularly in the nervous system of affected chicks. It is sometimes present in other tissues, according to Van Roeckel and associates, but Olitsky (4) was unable to find virus in the blood at any stage of the disease. Olitsky found that decimal dilutions of brain material from 10^{-1} to 10^{-3} were regularly infective, that 10^{-4} and 10^{-5} infected most birds, and that 10^{-6} infected occasionally when injected intracerebrally into young chicks Virus suspensions pass V and N Berkefeld filters, and Seitz discs. Olitsky and Bauer (5) showed by filtration through Gradocol membranes that the virus particle size was about 20 to 30 millimicrons in diameter. Virus is preserved for at least 88 days by suspending brain material in 50 per cent glycerin Rapidly dried virus also has excellent keeping properties.

Thinking that this virus might be related to the virus of equine encephalomyelitis which was prevalent in the New England states, Olitsky conducted a number of comparative tests which indicated that there was no relationship between them. The chick virus proved innocuous for mice, guinea pigs, and monkeys which are susceptible to the horse virus, furthermore there was no cross immunization between the two viruses

Transmission. The mode of transmission is unknown Inasmuch as the virus cannot be propagated in chick embryos it appears that egg transmission does not occur

Artificial Cultivation. Kligler and Olitsky (3) found that they could not obtain multiplication of the virus in chick embryos in the shell, although they could readily infect newly hatched chicks This peculiar situation has not been explained Using minced chick embryos, and chick embryo brains suspended in Tyrode's solution they failed to obtain increase of virus but when serum was added to such cultures virus development occurred. The concentration of virus in such cultures was low.

Immunity. Chicks which have recovered from the disease are resistant to reinoculation Older birds also are resistant but whether this is from antibody formation or from some other sort of mechanism is not known Neutralizing antibodies can be demonstrated in birds after recovery Since the disease develops in very young chicks which could not be immunized profitably even if such an agent were available, little effort has been made to develop it.

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INFECTIOUS ENCEPHALOMYELITIS OF FOXES

Synonym Epizootic Fox Encephalitis

Fox encephalitis causes serious losses of red and silver foxes bred in captivity The causative agent is a virus which may be found in various tissues of the diseased animals but occurs most regularly in the nervous system and it is here that the principal lesions are found The virus affects dogs and wolves but it is harmless for ferrets and mink The disease has long been confused with canine distemper which affects foxes as well as dogs, but distemper virus is highly pathogenic for ferrets and mink Furthermore, Green and co-workers have pointed out a number of respects in which encephalitis virus differs from that of distemper It is now generally accepted that fox encephalitis virus is not related to that of canine distemper, even though the symptoms of the two diseases may often be quite similar The disease has been reported only from North America

Character of the Disease. This disease was first described by Green (2) in 1925 who attributed it to a bacterium of the Salmonella group Later Green and co-workers were inclined to the belief that it was caused by a streptococcus but finally, in 1930 (7), they published evidence which indicated that the causative agent was a filterable virus

The losses are seen principally on fur ranches but the disease also occurs among wild animals The losses may amount to 15 or 20 per cent of the population of the ranch The disease appears suddenly and the course in affected animals is very short Loss of appetite may be noted for a day or two before other symptoms appear In many cases animals are found dead without symptoms having been observed Violent convulsions often initiate the symptoms These are followed by a lethargic state in which the animal wanders about aimlessly and blindly This may be interrupted by other convulsions. Usually the affected animal dies within 48 hours or less of the time when the first symptom is seen Toward the end various paralytic symptoms may be noted; paralysis of one leg, or of the hind quarters, or of the entire body. A terminal coma may last for periods up to 24 hours At the onset of the disease a watery nasal discharge is common, and sometimes there is a similar discharge from the eyes The feces become soft and filled with mucus, sometimes there is a profuse diarrhea in which blood streaks are common

Gross lesions consist of hemorrhages in various parts of the body. These occur as rather large extravasations in some cases, and are small or absent in others. Large hemorrhages into the brain or into the cord serve to explain the paralytic symptoms often seen in this disease. Large hemorrhages into the lungs sometimes occur.

The most significant lesions are microscopic and are located in the nervous system. In some cases there are myriads of small hemorrhages in the nerve substance and in the meninges; in other cases these are not numerous or are absent. Typical virus-type perivascular cuffing is a constant feature of the disease The cells are of the lymphoid type for the most part but polymorphonuclear leucocytes also occur in considerable numbers Green, Kaiser, Shillinger, and Hanson (5) have described intranuclear inclusion bodies which they claim are characteristic of this disease These occur principally in the endothelial cells of the blood vessels of the brain and meninges but they are also found in some cases in hepatic cells They are found in most cases but apparently not always. In artificial infections produced by introducing the virus into muscles, peritoneal cavity, and testicles, the bodies appear in the endothelial cells of the capillaries of the nervous system only When the virus is introduced into the ventricles directly the bodies are found also in the ependymal cells lining the cavities These bodies always occur in the nuclei of the affected cells They stain readily with hematoxylin and eosin, taking a pink or purplish color. The affected nuclei show considerable swelling and the chromatic material generally becomes distributed around the nuclear margins.

Nature of the Virus. The virus of fox encephalitis readily passes the Berkefeld N filter It may be isolated by animal inoculation at any time during the course of the disease and for several days after death if putrefaction is prevented by refrigeration It may be stored in 50 per cent neutral glycerin solution for several years without great deterioration Data on particulate size of this virus is lacking

Artificial Cultivation. The cultivation of this virus artificially has not been reported.

Transmission. The means of natural transmission of fox encephalitis is not certainly known Green, Zeigler, Dewey, and Shillinger (8) found that the disease could be propagated experimentally by inoculation into the brain, by intramuscular or intraperitoneal injection, and by intranasal instillation. The virus usually was derived from nerve substance, but the spleen contained virus quite regularly, and the blood, sometimes Inoculation succeeded in about 80 per cent of young foxes, much less often in older animals Occasionally entire litters were found to be resistant to inoculation

Immunity. Foxes which have recovered from this disease are highly immune to reinfection Green, Ziegler, Green, and Dewey (7) failed in their attempts to actively immunize foxes by inoculating them with mixtures of virus and immune sera. Animals treated in this manner did not develop the immediate disease that appeared in the controls but after about five weeks the disease appeared and destroyed the animals. It appeared that the virus had remained and became effective as soon as the passive immunity, conveyed by the immune serum, had worn off Experimental animals treated with virus attenuated with sodium ricinoleate were partially immunized The method was tried on more than 8,000 foxes in 1928, and the mortality from the disease was less than one half the normally expected during that season More recently an altered active virus has been used on a large scale with satisfactory results.

Canine Infections with the Virus of Fox Encephalitis. Green and co-workers showed as early as 1927 that dogs could readily be infected with the virus of fox encephalitis The experimental disease in dogs was described in some detail by Green and Shillinger (6) in 1934 and by Beckman and Torrey (1) in 1940. These findings have raised the question of how often canine infections occur naturally The symptoms of fox encephalitis infection in dogs are similar to those of canine distemper and can readily be confused with them Green (4) says that the disease in dogs is of short duration, lasting, as a rule, less than one week, and that the mortality in experimental dogs is greater than 50 per cent. The symptoms resemble those of acute distemper, beginning as a rule with coryza and ending with convulsions and lethargy Symptoms of chorea, so frequent in dogs suffering from distemper, have not been seen in the fox encephalitis virus infections The microscopic lesions in the dog are similar to those in the fox Dogs which have been immunized against the canine distemper virus and are insusceptible to additional injections are susceptible to the fox virus, thus proving that there is no relationship between the two viruses

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LOUPING ILL OF SHEEP

Synonym: Infectious encephalomyelitis of sheep.

Louping ill is an encephalomyelitis of sheep which occurs in Scotland and the northern part of England It is not known to occur elsewhere It is a virus disease transmitted principally by a tick, *Ixodes ricinus* The disease can be transmitted to sheep and pigs by intracerebral inoculation, and a few human infections have occurred The disease gets its colloquial name from the peculiar leaping gait shown by the ataxic animals.

Character of the Disease. Louping ill has occurred for more than a century in certain districts in Scotland and the border countries of England, where it has caused enormous losses especially in the lambs and in imported animals. Sheep that have survived more than one year in these districts are generally immune

The disease was shown to be inoculable by intracerebral injection by Pool, Brownlee, and Wilson (7) in 1930 Greig, Brownlee, Wilson, and Gordon (4) proved, the following year, that the causative agent was a filterable virus

After an incubation period varying from 6 to 18 days, the infected animal refuses to eat and shows a high temperature At this stage virus is present in the blood The temperature frequently falls and the animal appears better but a second rise usually occurs about the fifth day and at this time the nervous symptoms appear Consciousness usually is not greatly impaired. Ataxia, muscular inco ordination, and tremors appear and these are usually followed by paralysis

There are two distinct phases in the course of the disease In the first there is a rapid multiplication of virus in the blood and this corresponds to the period of initial fever The second phase begins at the time of the secondary temperature rise, at about the fifth day of symptoms, when the cerebro-spinal barrier is invaded and infection of the nervous system occurs At about this time the virus disappears from the blood and the nervous symptoms begin In some animals only the first stage of the disease occurs, in which case they recover quickly and show no residual damage When the nervous system is invaded, the death rate is high and animals which recover often show permanent neuro-muscular damage The virus behaves, in many ways, like that of poliomyelitis in man

There are no gross lesions in animals dead of this disease. Microscopically evidences of a severe, diffuse, encephalomyelitis exist. Destruction of the Purkinje cells of the cerebellum is especially marked. Specific inclusion bodies have not been demonstrated.

Nature of the Virus. The brain virus is readily filterable. According to Elford and Galloway (1) who conducted ultrafiltration studies on it, the particle size is about 15 to 20 millimicrons Virus is preserved very well by glycerol, but in broth or saline solution it deteriorates very rapidly particularly if the suspension is dilute and the diluent somewhat acid. Alkaline broth preserves it very much better

Pathogenicity for Experimental Animals. By intracerebral inoculation the disease may be transmitted to pigs, horses, monkeys, and white mice. Guinea pigs and rabbits are not susceptible Monkeys and mice can readily be infected by placing a few drops of brain virus in the nostrils, according to Galloway and Perdrau (2). In monkeys the virus can spread directly to the central nervous system from the nasal mucous membrane without the blood becoming infective The incubation period in these cases varies from 13 to 22 days, averaging 17 days In the brain of infected mice, Hurst (5) found characteristic cytoplasmic inclusion bodies He could not find such bodies in the brains of monkeys, and others have not found them in other animals While working with the virus of louping ill imported into this country for purposes of study, three workers almost simultaneously developed illness of an influenzal nature, and after recovery showed neutralizing antibodies for the louping ill virus in their blood The cases were described by Rivers and Schwentker (9) who believe that they represent human infections

Transmission. It had long been thought that the castor bean tick, *Ixodes ricinus*, which is prevalent in the louping ill districts was instrumental in transmitting the infection This was proven by McLeod and Gordon (6) in 1932. The larval ticks, feeding on infected sheep, convey the infection to a new host when they next feed as nymphs, or if the tick becomes infected as a nymph, it conveys the disease when it feeds on a new host as an adult The disease appears in the early summer, subsides during mid-summer, and reappears in early fall These periods correspond to the periods of tick activity in the infected area

Artificial Cultivation. Rivers and Ward (8) were successful in obtaining artificial cultures of this virus on a minced chick-embryo medium Success has been attained also on the chorio allantoic membrane of developing chick embryos.

Immunity. Recovery from natural or artificial infections results always in a solid and enduring immunity According to Gordon (3) the subcutaneous injection of a vaccine prepared from formalinized nerve tissue (brain and spinal cord) produces antibodies which will effectively protect young lambs

by neutralizing virus which reaches their blood and thus prevents invasion of the nervous system Such vaccines do not immunize the nervous system but this is not necessary if virus multiplication in the blood can be prevented.

The disease can be controlled in another manner, that is, by a dipping routine which will destroy the ticks

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PERIODIC OPHTHALMIA OF HORSES

Synonym "Moon blindness" of horses.

This disease causes serious losses among horses in many parts of the world. Since it leads finally to total blindness in many cases, the disease is more important in racing and saddle horses than in the farm breeds where blindness is not such a serious handicap Errington who examined more than 2,000 horses in the vicinity of Lexington, Kentucky, where fine horses are raised, found that 8 3 per cent showed evidence of the disease

According to Errington (1) the disease is essentially an inflammation of the iris and ciliary body in its earlier stages, but as it progresses opacities of the cornea and lens develop and a destructive retinitis occurs Glaucoma frequently develops The disease tends to recur at intervals, that is, the acute symptoms associated with lacrimation and hypersensitivity to light. Each recurrence increases the permanent damage The disease is more frequent in some areas and on some farms than on others, but there is little evidence that the disease is directly contagious

Most veterinarians who have had experience with disease feel that its causation has not yet been established. The disease is included here because of the work of Woods and Chesney (2) who claim to have transmitted the disease to horses and to rabbits with bacteriologically sterile filtrates of the exudate in the anterior chamber of affected eyes Confirmation of their work has not been reported.

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SPONTANEOUS VIRUS DISEASES OF THE NERVOUS SYSTEM OF EXPERIMENTAL ANIMALS

No attempt will be made here to describe the spontaneous encephalitides which occur in animals commonly used for the isolation and study of viruses of man and animals. It is desired merely to call attention to the fact that such viruses exist and workers must be on their guard, when using such animals, not to confuse these diseases with ones which are believed to be in the material inoculated. Viruses causing spontaneous encephalitis have been found in rabbits, guinea pigs, and mice, and probably they occur occasionally in all species. These viruses often are latent, or masked, and become evident only when inoculations containing foreign material act as a local irritant to the nerve tissue. Having been activated in this way, such viruses may then be passed readily from animal to animal in series Romer (4) has described a virus of guinea pig paralysis, and Traub (5) demonstrated that the virus of lymphocytic chorio-meningitis may sometimes occur spontaneously in colonies of white mice. The virus of herpes of man has been found occurring spontaneously in rabbit colonies When viruses are recovered from experimental animals, it is important to make sure, either by a study of the specific histological changes, the study of the action of the virus on other animal species, or by immunological procedures, that the virus is not one that occurred spontaneously in the experimental animal

In the study of neurotropic viruses it sometimes happens that two or more viruses exist in the same material, or a virus may become contaminated with another which existed spontaneously in some animal through which the original material was passed While studying the etiology of St Louis encephalitis in man, Armstrong and Lillie (1) first encountered the virus which now is known as that of lymphocytic chorio-meningitis. In another instance Dalldorf, Douglass, and Robinson (3) produced a nervous disease in monkeys by injecting them with canine distemper virus. This surprising discovery was explained later when Dalldorf (2) found that the distemper virus had been contaminated with the virus of lymphocytic chorio-meningitis and that the symptoms were caused by the latter rather than by the virus of distemper.

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CHAPTER XLIII

VIRUS DISEASES CHARACTERIZED BY CATARRHAL OR GENERALIZED INFECTIONS

CANINE DISTEMPER

Distemper is a world-wide disease of young dogs, highly contagious, and manifested by fever, acute catarrh of the respiratory mucous membrane, catarrhal pneumonia, and sometimes by symptoms referable to damage of the central nervous system The causative agent is a virus, but many of the pathological changes and complications seen in naturally-occurring cases are due to bacterial agents which are secondary invaders In addition to dogs, the disease occurs naturally in wolves, foxes, and mink Ferrets are exceedingly susceptible and have been much used in experimental work with the disease on this account Weasels, ermine, and martens are said to be susceptible to inoculation. Cats are not susceptible, nor are other domestic animals and man

Character of the Disease. Canine distemper is so contagious and so widespread that few dogs escape the disease during the first year of life The disease begins by lassitude and inappetence followed shortly by high fever Catarrhal inflammation of the upper respiratory tract and of the conjunctival membranes appears early and the discharges soon become purulent The blood at this time is rich in virus, but later in the disease the virus content of the blood and organs is reduced and may wholly disappear The patchy, catarrhal pneumonia probably is initiated by the virus but is generally complicated by secondary bacterial invaders, especially streptococci and *Brucella bronchiseptica* Animals which escape pneumonia usually recover within a week, and some cases are so abortive that the owner does not notice that the animal is sick Many which have pneumonia recover after several weeks. Digestive disturbances are common. Vomiting is usually seen, the vomit consisting of mucoid material which frequently is bile colored. Nervous symptoms usually are present, even in the milder cases. In some cases the nervous symptoms are severe, consisting of muscular spasms, sometimes localized and sometimes generalized. Epileptiform seizures are common. The muscular

spasms may be followed by paralysis of groups of muscles, coma, and death. In other cases paralysis, or muscular spasms (chorea), persist after recovery from the acute symptoms and the animal must be destroyed. In the early stages of the disease leucopenia occurs but later leucocytosis usually appears.

The autopsy findings depend on the course that the disease has taken Animals which die during the acute attack usually present reddened mucous membranes covered with muco-purulent exudate Muco-purulent plugs usually can be expressed from the smaller bronchioles. The pneumonic process involves the anterior and lower lobes as a rule. These areas are reddish or brownish in color, and if the animal survives for some time, purulent foci are frequently seen in the involved portions The gastric and intestinal mucous membranes are reddened and covered with muco-purulent secretion The Peyer's patches are swollen and sometimes ulcerated Parenchymatous degeneration of the internal organs usually is seen The spleen may be normal or only slightly swollen

Gross changes in the nervous system consist only of meningeal inflammation Microscopically, however, diffuse encephalomyelitis of varying degrees of severity is seen Both grey and white matter are involved. Perivascular infiltrations, proliferation of glial cells, localized infiltrations of lymphocytes and monocytes, and degeneration of nerve cells in the more superficial parts of the cerebrum and of the Purkinje cells of the cerebellum are seen

The Experimental Disease in Dogs. Dunkin and Laidlaw (2) raised many dogs for experimental work in strict isolation so they were protected from the secondary bacterial contaminations which invariably complicate the disease picture under natural conditions When such animals were inoculated with distemper virus they found that the disease was much milder than that seen normally, and the mortality rate was comparatively low. The incubation period was remarkably constant Occasionally it was as short as three days and sometimes as long as six days, but in the vast majority of cases the animals sickened on the fourth day The disease picture was typical except that bronchitis and broncho-pneumonia never occurred This proves that the pneumonia which destroys many dogs under natural conditions is largely the result of bacterial complications.

Natural Infection in Animals other than Dogs

IN FERRETS. Ferrets are exceedingly susceptible to the virus of canine distemper (3) Natural outbreaks of the disease often occur and the mortality is very nearly 100 per cent The disease transmits readily through the air. Dunkin and Laidlaw (3) found that they could not keep normal ferrets in the same building with those infected with virus, no matter how much care

was used to prevent the spread of the virus. They concluded that the virus was air-borne.

The incubation period is about ten days as a rule but may occasionally be one or two days shorter. A watery discharge from the eyes and the nose indicates the onset of the disease. This quickly becomes purulent and the eyelids become swollen and pasted together. The chin becomes reddened and small vesicles form around the mouth where the hair meets the naked skin of the lips. The feet swell, the foot pads become red, and sometimes the skin of the abdomen reddens. On the third day the vesicles on the chin become pustules and the animal remains curled up in the cage, refusing all food. The ferret becomes weaker and generally dies on the fifth or sixth day Occasionally an animal lives longer, develops pneumonia or nervous symptoms but ultimately almost always dies

IN FOXES In foxes the disease is similar to that in dogs It occurs in the fall, as a rule, after the young foxes are placed on the fur ranges and the mortality may be as high as 60 per cent The disease must be distinguished from fox encephalitis with which it may readily be confused and with which it may occur concurrently Green (5) states that ferrets are immune to the virus of fox encephalitis and thus distemper may be recognized by the inoculation of ferrets. He also points out that the inclusion bodies in the epithelial cells of the air passages, urinary bladder, and other epithelial surfaces will serve to distinguish distemper from encephalitis since inclusion bodies are not found in these cells in fox encephalitis

Nature of the Virus. The nature of the causation of canine distemper long was a matter of dispute. As early as 1905 Carré (1) carried on studies which convinced him that a filterable virus was the essential cause and that the bacterial agents, held by others to be the cause, were only secondarily involved The question was finally settled through the work of Laidlaw and Dunkin in England who began work in 1923 on a rather elaborate basis with the financial support gathered by public subscription in a campaign fostered by a sportsman's magazine called "Field" The investigations carried on under the auspices of the "Field" Distemper Fund were continued from 1923 until about 1930. They not only proved that distemper was a virus disease, but introduced the use of ferrets as experimental animals, and developed several immunizing procedures which have formed the basis of present methods of preventing the disease References to the reports of their work are given at the end of this discussion (2) (3) (8) (9) (10) (11).

Virus is found in the blood of affected dogs during the first several days of the disease; later it disappears The spleen during the early period is regularly

infective, and spleen material is frequently used as virus in experimental work, and for immunization, as will later be described.

The virus is not readily filterable. It usually will pass the coarser diatomaceous earth filters but is retained by the finer clay filters. It is destroyed by heating at 58° C for 20 minutes and is readily destroyed by drying, although if dried in a frozen state it may be kept for considerable periods of time unimpaired.

Inclusion bodies occur in the vast majority of cases of canine distemper. These are located principally in the epithelial cells of the respiratory tract, urinary tract, intestine, bile ducts, and salivary ducts. They may also be seen in liver cells, and in cells of the reticulo-endothelial system of the spleen and lymph nodes. They are located for the most part in the cytoplasm but some are found intranuclearly They stain well with the ordinary hematoxylin-eosin stain and have a considerable resemblance to Negri bodies. They may be demonstrated in paraffin sections, or in smear preparations. Green and Evans (7) have described them and claim that distemper can readily be diagnosed by their recognition They suggest that smears be made from the mucosa of the urinary bladder where they are unusually numerous, and thus can easily be found and identified

Artificial Cultivation. Plummer (17) has reported partial success in cultivating the virus of canine distemper on the chorio-allantoic membrane of developing chicks He was successful on two occasions in carrying the virus through six generations but each time the strains died out later. Ferrets inoculated with 5th and 6th generations developed typical distemper; those inoculated with the 11th failed to develop the disease Small whitish opacities were noted on the membrane so long as viable virus was present. These disappeared as virus activity disappeared

Transmission. Virus is present in the exudate from the mucous membranes early in the course of the disease and transmission undoubtedly is through direct contact or by droplet infection. Arthropod carriers have not been found

Immunity. Animals which recover from an attack of canine distemper thereafter are solidly immune for life. The virus of distemper is so widespread that practically all dogs, except a few that have led extremely sheltered lives out of contact with all other dogs, have had contact with it before they are one year old, and are immune Puppies, born of immune mothers, apparently obtain an effective immunity from the mother but this is passive and disappears by the time the animal is six weeks old. Slanetz (19) found it impossible to infect such puppies with virus during the first month of life. Animals which had

been inoculated with virus when one and three weeks of age proved refractory to large doses of virus when they were three months old.

Artificial immunization to distemper is a matter of great interest to dog owners. Earlier efforts at immunization were based upon the idea that distemper was caused by *Brucella bronchiseptica*. Vaccines containing that organism, and serum containing antibodies for it, were rather extensively employed in the United States between 1915 and 1930, and many strongly believed that the course of the disease was favorably influenced by them As it has already been pointed out that the secondary bacterial infections in distemper do a great part of the damage, especially in the respiratory tract, it is reasonable to suppose that the bacterial immunizing products might affect the course of the disease in individual animals It is now clear, however, that they do nothing toward reducing the prevalence of the disease

Artificial immunization to distemper, using tissues containing virus, was first carried out by Puntoni (18) in Italy in 1923 and 1924. This work attracted little attention but furnished the basis upon which Laidlaw and Dunkin prepared their vaccine five years later The work of the latter attracted world-wide attention. Since about 1930 nearly all prophylactic products have been made from the virus

PASSIVE IMMUNIZATION The first to make a virucidal antiserum for canine distemper was Lockhart, Ray, and Barbee (14) in 1925 These workers hyperimmunized immune dogs by injections of virus-blood removed from susceptible dogs which had been injected with virus and were suffering a febrile reaction

Laidlaw and Dunkin (11) prepared an immune serum by hyperimmunizing dogs which had recovered from an injection of virus about one month previously The hyperimmunization was accomplished by making two subcutaneous injections, on successive days, of 20 cc of a 10 per cent emulsion of spleen and lymph node tissue, removed from distemper affected dogs early in the course of the disease when the virus content of these organs is highest

Distemper antiserum will protect susceptible dogs from the disease for a limited time It is used in most animal hospitals for protecting young canine patients, which may not have had distemper, from infection which they are likely, otherwise, to contract there. If the animal comes in contact with the virus while protected by the serum it may develop an active enduring immunity Usually one 10 cc. dose of serum is enough to protect for a few days' stay in the hospital or at a dog show If the sojourn is prolonged another dose should be given about the tenth day. Since active immunization of very young

dogs is not very satisfactory, immune serum often is used to protect valuable animals until they are from three to four months old at which time the active immunization can be carried out This procedure is rather expensive but it is the only way known to protect young animals that are exposed to the disease. Puppies raised in comparative isolation usually are untreated until old enough for active immunization.

As in all virus diseases specific biologic treatment after symptoms have appeared is not very satisfactory Some veterinarians regularly use antiserum at the rate of 1 cc per pound weight, repeated daily for several days, for treatment of early cases Late in the disease serum treatment probably is valueless. Some manufacturers immunize their serum-producing dogs with the bacteria commonly found as secondary invaders in distemper, claiming that the antiserum thereby is useful in combating the secondary bacterial invaders as well as the virus.

ACTIVE IMMUNIZATION It has already been said that Puntoni was the first to attempt active immunization of dogs Using a virus obtained by inoculating dogs intracerebrally Puntoni (18) prepared his vaccine by treating the brain virus suspensions with formalin Lebailly (12), in 1927 announced that he had made an efficient vaccine from dog spleens by treating the suspensions of spleen pulp with formalin. Laidlaw and Dunkin (10) announced their vaccine in 1928 These authors found that vaccines alone could not be depended upon to give a sufficiently solid and lasting protection, hence they administered their formalin-treated spleen virus, made from ferret spleens in the beginning but later from those of dogs, and followed this treatment in about 14 days with a small dose of active virus in the form of dried ferret spleen. After the injection of the living virus the animal usually shows some fever, inappetence, and sometimes more marked symptoms of listemper about the fifth day but in most cases the symptoms are mild This method of immunization has been extensively employed and generally with excellent success, but not all reports are favorable Apparently one fault of this method of immunization is that the dried virus does not always remain viable and the treated animal thus does not obtain the final reaction which results in the actively enduring immunity In other cases the virus reactions are severe and the animal may even die from distemper. Some veterinarians regularly give two doses of the formalin-inactivated virus at 14 day intervals before giving the active virus two weeks later Some give one dose of immune serum, followed by one or two doses of vaccine at two week intervals. If the animals are exposed to active infection in the meantime, they usually will develop an active immunity.

The Lockhart (13) method of treatment makes use of immune serum and blood virus, without vaccine Dogs may be treated in several different ways; by injecting serum and virus simultaneously, by injecting a dose of serum followed by serum and virus two weeks later, or by injecting serum at two week intervals and placing the dog in the presence of active infection in the meantime.

Recently a different method of active immunization has been introduced. This method is described by Green, Carlson, and Swale (6), who claim that by passing canine distemper virus through a series of ferrets they have obtained a strain which has been so modified as to have lost most of its virulence for dogs. Whereas this virus produced deaths from clinical distemper in 84 per cent of inoculated puppies originally, after the 53rd passage it caused deaths in only 8 per cent, and continued ferret passage has reduced this percentage still further Vaccination is accomplished by a single injection of dried, living virus. This method of immunization is frequently called the Fromm method, from the name of the commercial company which has undertaken its manufacture.

IMMUNIZATION OF FOXES AND FERRETS Laidlaw and Dunkin (9) found it possible to immunize ferrets much more easily than dogs The first part of the treatment is the injection of 2 cc subcutaneously of a vaccine prepared from spleens of ferrets The spleens are removed from the animals on the fourth or fifth day of illness, finely ground and made up into a 20 per cent suspension Formalin is added to a concentration of 0 1 per cent The vaccine is ready to use after the fourth day when no active virus can be demonstrated One dose of this material usually renders ferrets strongly immune after a few days The immunity is strengthened by the inoculation of a dose of virus two weeks after the administration of the vaccine Usually the animals show no reaction to the introduction of the virus and become permanently and solidly immune thereafter. Pinkerton (16) reported satisfactory results in immunizing mink by the use of a tissue vaccine made from the lungs. At the beginning of an outbreak on a ranch some of the first animals were used for making vaccine for the remainder of the stock The finely pulverized tissue was made up into a 10 per cent emulsion and treated with 0 3 per cent formalin. Several injections were given consisting of 2 to 4 cc at weekly intervals

For fox immunization the method of Laidlaw and Dunkin may be used. Ott (15) has reported success in using antiserum alone, this being given to all animals at the time the seasonal outbreak is expected, or has actually begun. Green and Carlson have reported the successful use of the mink-modified distemper virus of Green (4) for protecting foxes

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FELINE INFECTIOUS ENTERITIS

Synonyms Feline distemper; Feline agranulocytosis, Feline panleucopenia.

This disease is highly contagious and the mortality rate is high It apparently occurs in many parts of the world, since it has been described from France, England, United States, Canada, and India. A disease which may be the same but which is manifested a little differently has been described by Seifried and Krembs (7) in Germany The disease destroys many pet animals, especially of the finer breeds, and is a scourge in many catteries and in veterinary hospitals In the latter the disease is most apt to occur in animals which have undergone surgical operations, and frequently it is a safer procedure to send the animal home immediately after the operation rather than to risk the danger of its contracting this disease during convalescence

In the past the disease has been attributed to various bacterial agents but none of the bacteria which are commonly found associated with this disease are capable of reproducing it A filterable virus was first identified as the causative agent by Verge and Cristoforoni (8) in 1928. The findings of the French workers were confirmed by Hindle and Finlay (3) in England in 1933, and by Leasure, Lienhardt and Taberner (6) in this country in 1934. In 1938

Lawrence and Syverton (4) studied a disease spontaneously occurring in cats kept for laboratory purposes. The disease was manifested by symptoms which will be described below, but the most striking characteristic was the rapid disappearance of white blood corpuscles from the blood. It was shown to be caused by a virus. The following year Hammon and Enders (2) described the disease independently. It was they who gave the name panleucopenia to it, because of the almost total disappearance of all types of white corpuscles from the blood. There is no doubt that these workers were dealing with the disease known as infectious enteritis Prior to their work the disease had been well known but the severe leucopenia had not been recognized

Character of the Disease. The disease affects young cats especially, although older ones are susceptible if they have not had previous contact with it The disease begins with loss of appetite and fever, which sometimes is very high and other times quite moderate The animal shows depression, lies in a corner, dislikes to be disturbed, and pays little attention to its surroundings The coat is rough and frequently weight is lost rapidly Most of the cases, but not all, develop a profuse diarrhea, the discharge often being blood-tinged. Vomiting is common Some animals develop watery discharges from the nose and eyes

The incubation period is relatively short The temperature peak generally is reached on the 5th to 8th day after exposure, and death usually occurs one or two days after the peak Cats which live past the 9th day nearly always recover. The mortality generally is about 65 per cent.

The lesions are practically confined to the intestinal tract and its associated lymph nodes The liver, kidneys, and spleen may be somewhat swollen but often show nothing but the affect of the fever Rarely there may be some pneumonia. The heart often is petechiated The enteritis is not confined to any particular part of the intestine but usually the lesions are most severe in the ileum The severity varies from simple congestion of the intestinal wall to severe, hemorrhagic, pseudomembranous enteritis with necrosis of the mucosa.

The blood reaction in this disease is exceedingly interesting Lawrence, Syverton, Shaw, and Smith (5) divided their cases into two classes according to the manner in which the leucocytes disappeared from the circulation under the influence of the virus In the first group the leucocytes gradually diminished from the time of exposure until the time of the temperature peak In the other, there was little change in the leucocyte count for five to seven days, then during the febrile reaction there was a precipitous drop. From a normal of about 15,000 leucocytes per cu mm the count usually dropped to 1,000 or less In some instances no leucocytes could be found in

the circulation; in about 20 per cent of the cases the count varied from 0 to 200 per cu. mm. During this time there was only a slight fall in the red cell count and the hemoglobin diminished only slightly. The virus, it is evident, had a severely destructive effect upon the hemopoietic centers of the bone marrow and lymphatic organs.

Both Lawrence and his collaborators (5), and Hammon and Enders (2) observed characteristic inclusion bodies in the epithelial cells of the intestinal mucosa and in certain cells of the spleen, lymph nodes, and bone marrow. These occur intranuclearly They are acidophilic and are variable in size and shape. The chromatin of inclusion-containing nuclei "marginates," i.e., arranges itself around the periphery, creating the impression of a halo around the bodies The affected cells often become greatly hypertrophied.

Nature of the Virus. Virus is found in the blood, all vascular organs, nasal secretions, urine, and feces The virus is readily filterable, indicating that its particulate size is not great, however no studies on the size have been reported The virus keeps fairly well in 50 per cent glycerin solution, but does not resist drying very well.

The virus of feline enteritis, so far as is known, does not affect any animals other than cats Hindle and Finlay have reported cases in members of the wild cat family but others have disputed this point Dogs, rabbits, guinea pigs, ferrets, and mice are not susceptible No human infections have been recognized

Artificial Cultivation. There are no reports of successful cultivation of this virus on artificial media

Transmission. Since all of the secretions and excretions of affected animals contain virus, it is not surprising that the disease is highly contagious Animals are infected either by intimate contact with cases, or by contact with materials soiled with discharges

Immunity. Cats which have recovered from an attack of this disease are immune thereafter for life Hindle and Finlay (3) conducted limited experiments in which it was indicated that the serum of recovered animals had a protective effect upon susceptible kittens. Leasure, Lienhardt, and Taberner (6) produced a hyperimmune serum by giving multiple injections of virus to immune animals. The serum gave complete protection to susceptible kittens when virus was injected simultaneously. They also attempted to produce an heterologous immune serum by immunizing a mule with multiple virus injections This serum protected only partially; about one third of the

treated animals died when inoculated with virus. Enders and Hammon (1) also succeeded in producing a satisfactory immune serum by hyperimmunizing cats.

Leasure, Lienhardt, and Taberner prepared vaccine for active immunization from the brain, kidneys, and spleen of cats suffering from enteritis To a 10 per cent tissue suspension they added 0.2 per cent formalin After it had stood for three days, the virus appeared completely inactivated Doses of 2 cc were given to kittens, and in some instances the dose was repeated after four days. Ten days later the immunized and control kittens were inoculated with virus All of the controls sickened and the vaccinated remained well. This work was confirmed by Enders and Hammon (1), who used formolized spleen and liver emulsions as vaccine These workers observed that kittens from immune mothers received a fairly strong natal immunity By exposing these kittens to the disease before they were two months old, they found that they did not sicken, and later it was shown that all were actively immune This, apparently, is what usually happens in areas where this disease is enzootic.

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HOG CHOLERA

Synonym Swine Fever (English)

Hog cholera is an acute, highly contagious disease of swine characterized by degenerations in the walls of the smaller blood vessels which result in multiple hemorrhages, necrosis, and infarctions in the internal organs Affected animals are prone to suffer from the effects of bacterial agents which frequently accompany the virus, but these secondary agents are not necessary for the production of the disease The cause of the disease is a filterable virus

Hog cholera was first recognized as a separate disease entity by Salmon and Smith (10) in 1885 but it was erroneously believed to be caused by the bacterium which they called *Bacillus cholerae suis*, now known under the name *Bacterium choleraesuis* or *Bact suipestifer* The error was corrected in 1903 when deSchweinitz and Dorset (4) proved that the disease was caused

by a virus, and that the "hog cholera bacillus" played a secondary and non-essential role in the disease

Hog cholera is a tremendously destructive disease which, despite fairly satisfactory immunization procedures, continues to cause large losses in the United States and most other parts of the world where swine are raised. The disease seems to have been first seen in the State of Ohio in 1833 and from there spread to all parts of the United States through the shipment of stock. Its greatest prevalence in the United States is in the north-central states, the so-called corn belt, where the greatest concentration of the swine population occurs. Cholera was not seen in England until 1862, and on the continent of Europe not until 1887.

Character of the Disease. The blood, tissues, and all secretions and excretions of cholera-affected pigs contain the virus of the disease The disease is highly contagious since every susceptible pig in a lot where infection appears soon shows evidence of the disease The incubation period is short, about five or six days, as a rule. The disease is first manifested by the appearance of fever (106° F or higher), lassitude, inappetence, crowding together in the corner of the house or under a hay stack, constipation followed by diarrhea, a muco-purulent exudate from the conjunctiva, and vomiting In white skinned pigs, a livid coloring of the skin is apt to appear, this being most marked on the abdomen Cutaneous hemorrhages may also occur. If the course of the disease is prolonged beyond one week, bacterial complications may occur, these being manifested chiefly by pneumonia and by ulcerative enteritis Animals that die within one week usually show lesions that are largely of virus origin

The pure virus disease is best seen in inoculated animals held under good hygienic conditions Fever appears in such animals on the third or fourth day coincident with the symptoms mentioned above By the sixth or seventh day the temperature generally has reached its peak, the animal is very sick and frequently dies a day or two later. If destroyed when the temperature peak is reached, the lesions often are very scanty but the virus content of the blood and tissues is at its highest point The lesions consist of hemorrhages, usually petechial, beneath the capsule of the kidneys and in the mucosa of the urinary bladder, the larynx and trachea, and sometimes in the serous membranes. Larger hemorrhages are often found in the intestinal mucosa, in the lungs, under the epicardium, in the spleen, and especially in the cortex of the lymph nodes. These hemorrhages are caused by rupture of capillaries and small arteries in which retrogressive changes regularly occur as a result of the action of the virus The endothelial cells of these vessels commonly show swelling and proliferation and many of them are plugged with such cells. Degeneration of these cells then occur and the degenerative process extends

into other parts of the vessel walls. Many small vessels become degenerated, hyaline tubes which readily rupture. These changes have been minutely described by Seifried and Cain (12). Sometimes such hemorrhages are very few and one finds only swollen lymph nodes with reddened pulp, and nothing else.

Lewis and Shope (8) called attention to a reaction in hog cholera which apparently had first been noted by Dinwiddie (5) as early as 1914. This is the precipitous fall in the number of circulating leucocytes in the blood—a severe leucopenia. Within 48 hours after the inoculation of the hog cholera virus, the leucocytes which vary between 14,000 and 24,000 per cu. mm. in normal pigs fall to a level below 4,000 and sometimes no leucoytes can be found Leucopenia is a common reaction to virus activity in animals So far as is known, no other common disease of pigs exhibits this reaction Late in cholera, when secondary bacterial action plays a prominent part in the disease picture, the leucopenia is replaced by a leucocytosis

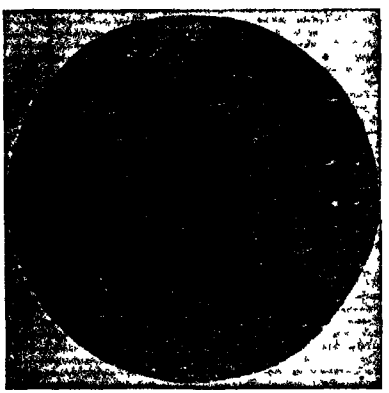


Fig 135 Hog Cholera Showing lesions of encephalitis which commonly occurs in this disease Perivascular cuffing and infiltrations of round cells into the nerve tissue are shown x 100 (Courtesy of S H McNutt)

In the cases which run a longer course, one finds fibrinous pneumonia, often with necrotic foci in the consolidated portions and fibrinopurulent enteritis with ulceration of the mucosa In chronic cases the "button" ulcers of the intestinal mucosa may be found, especially in the region of the ileo-cecal valve These are of varying size Because of the deposition of multiple layers of fibrin in concentric formation on the ulcerated areas, raised, button-like deposits are formed These ulcers are usually, if not always, caused by the activities of the hog cholera bacillus, usually in association with the necrosis bacillus (*Actinomyces necrophorus*).

Nervous symptoms occur quite commonly in hog cholera These may be manifested by grinding of the teeth, evidence of local paralyses, locomotor disturbances, and occasionally by lethargy and convulsions These are manifestations of an encephalomyelitis which apparently occurs in a large percentage of all cases. Seifried (11) found brain and cord changes in 33 out of 39 cases, although the animals in most cases had not manifested unusual

nervous symptoms. Macroscopically, hemorrhages are often found in the meninges and in the brain substance. Microscopically, besides the hemorrhages one finds the usual evidence of encephalomyelitis, i.e., perivascular "cuffing" with lymphocytes, mononuclear, and a few plasma and eosinophilic cells. The glial cells show proliferation both diffusely and in the form of compact nodes. There is degeneration of nerve cells and some neuronophagia Inclusion bodies have not been found Changes of this type are found in some pigs very early in the disease and before recognizable symptoms have occurred. They represent a true virus type of reaction

Nature of the Virus. The virus of hog cholera is readily filterable through both Berkefeld and Pasteur filters Its particulate size has been estimated as about 35 millimicrons It occurs in all organs, secretions, and excretions of the affected animal and is especially abundant at about the time the first temperature rise reaches its peak Very minute amounts of virus are capable of infecting swine which have not previously had contact with it Young pigs are most susceptible but older animals are readily infected The virus is not infective for any known species of animal other than swine

Hog cholera virus is readily destroyed by drying and for this reason infected premises usually become free of the infection within a few days after infected animals have been removed In the fluid state the virus is quite resistant, especially if kept cold Blood removed from pigs during the temperature reaction is commonly used in the simultaneous treatment for active immunization This material is partially protected against bacterial action by the addition of 0 5 per cent phenol which does not injure the virus Such virus blood will often retain full virulence for a month or more at room temperature, although this should not be depended upon Chapin, Powick, McBryde, and Cole (3) have shown that the virus is more stable in blood in which the pH is kept at about 5 5 than when it is kept at its normal level of 7 0 to 7 4, but the physical characters of the blood is changed at this pH level and it becomes gelatinous and hence is not very desirable for animal inoculation. Virus is preserved for many months by admixture with glycerin if stored in the refrigerator.

Artificial Cultivation. Ten Broeck (13) reported success in cultivating the virus of hog cholera artificially in 1941. Fresh minced testicular tissue from swine was suspended in Tyrode's solution, or it was spread on the chorio-allantoic membrane of developing chick embryos, or most simply of all, it was spread on the surface of agar slants containing sterile swine serum. In flasks the virus was passed through fourteen transfers, while on egg membrane it was grown for 13 transfers, followed by an equal number on slant agar, 26 in all. The final cultures were highly virulent for swine. They con-

tained at least as much virus per volume as fresh natural blood virus. Dilutions of 1×10^{-6} usually were infective for swine when injected in 1 cc. doses.

Transmission. Hog cholera is transmitted principally by intimate contact with sick animals and directly or indirectly with fresh secretions and excretions. It is not known precisely how the virus is passed from farm to farm in every case. Birds have been suspected of carrying virus on their bodies, and undoubtedly virus may be carried on the shoes and clothing of persons and animals if they travel rather directly from infected to non-infected premises. The disease may be carried to new premises by the careless handling of blood virus, which is used for immunization, or by the bottles which have contained such virus. In garbage-fed swine, the disease is often introduced by uncooked pork trimmings.

Immunity. Animals which have recovered from an attack of hog cholera are solidly immune for the remainder of their lives Because of the high mortality rate in this disease this is an expensive way to obtain protection

Young suckling pigs born of immune mothers are passively immune to this disease for about six weeks If they come in contact with the disease before the natal immunity disappears they develop an active immunity If this is not fortified by contacts with virus from time to time, this immunity may wear off and the animals may contract the disease when exposed at any time after they are eight months old. Natural immunization undoubtedly occurs in the field in this way, but reliance on it is hazardous and is not to be recommended. Unless the herd of swine is small, is kept in very isolated quarters, and is not fed upon offal of any kind, the danger of serious losses from cholera is too great to make it profitable to attempt the keeping of susceptible pigs in areas where the disease exists.

ARTIFICIAL IMMUNIZATION Several methods have been developed for the immunization of swine to cholera. Passive immunization is accomplished with a hyper-immune antiviral serum Active immunity can be conferred by the injection, simultaneously with the antiviral serum, of active virus Recently two vaccines have been developed, one by Boynton (1) and the other by McBryde and Cole (9). Although both have been used in the field they have had so little use that they must be regarded as experimental.

ANTI-HOG CHOLERA SERUM. The value of an antiviral serum was demonstrated by a group of workers connected with the Bureau of Animal Industry, of the U. S. Department of Agriculture. The first report was by Dorset, McBryde, and Niles (6) in 1908. The serum is prepared from pigs. These either have acquired immunity naturally or they are first given virus with sufficient anti-

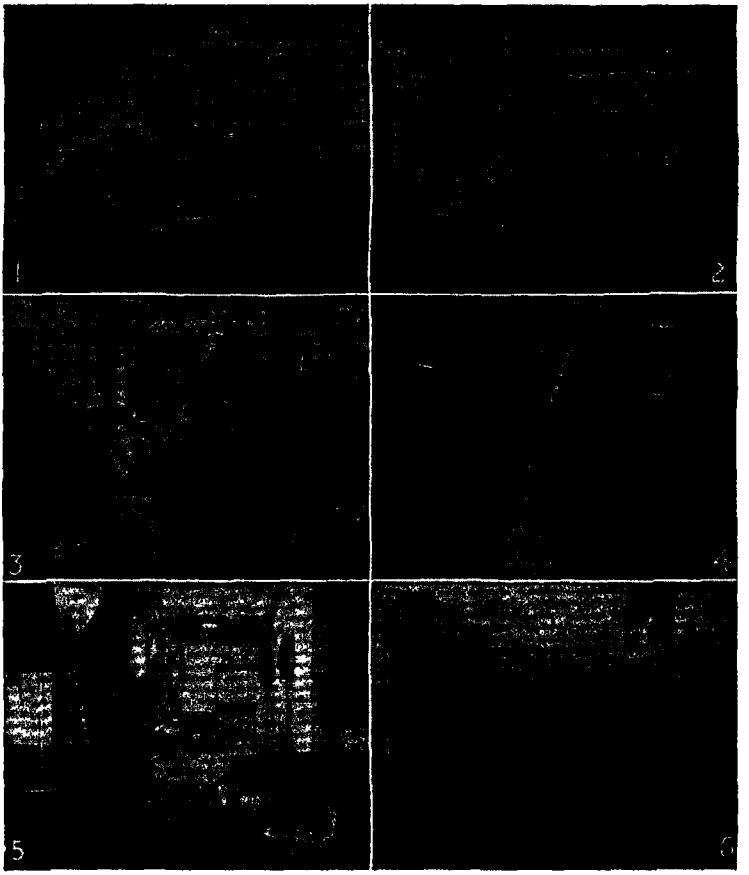


FIG 136 The Production of Anti-Hog-Cholera Serum (1) "Hypering" Large quantities of highly virulent blood are injected into the ear veins of immune pigs. (2) Preparing the hyperimmunized pigs for tail bleeding The animals are restrained in special crates which lift their feet off the floor Their tails are cleaned and shaved (3) Tail bleeding The tails of the pigs shown in the previous picture pass through small openings in the wall into the bleeding room. (4) Detail of the vacuum bleeding apparatus. After it has been thoroughly cleaned and disinfected, the end of the tail is amputated and the stump placed in a metal tube connected with the bleeding jar Suction created by the vacuum line seen at the right of the picture hastens bleeding and tends to prevent clotting in the caudal arteries A flexible shaft operates an agitator in the blood jar which defibrinates the blood as it is drawn (5) "Bleeding out" After the hyperimmunized pig has been tail-bled a number of times it is finally bled out This is done by thrusting a large canula through the base of the neck into the large blood vessels at the base of the heart (6) Bottling the serum The large tank is used for mixing the sera of many animals. (Courtesy of Pittman-Moore Co)

serum, simultaneously, to protect them. The immune animals are then given fresh, defibrinated blood taken at the height of the temperature reaction, from young pigs which have been infected with cholera. This blood is injected into one of the ear veins at the rate of 5 cc. per pound of body-weight. In about two weeks the pig is ready to be sacrificed for its blood serum, or blood may be taken from its tail at weekly intervals for three or four bleedings. It may then be given another dose of virus blood intravenously and again bled on several occasions at weekly intervals. The blood is drawn into sterile containers with provision for defibrinating it as it is drawn. The defibrinated blood is freed of cells by centrifugation, the serum is heated at 58° C. to obviate the possibility of its carrying the virus of foot and mouth disease, and it is then ready for use. Before it is sent out for use each lot of serum is tested for its ability to protect susceptible pigs against a test dose of virus.

Anti-hog cholera serum usually is used as a prophylactic measure but large doses are sometimes used for its curative effect. If given very early in the course of the disease, pigs sometimes are saved When the symptoms are well developed the value of the animals saved by it usually will not offset the cost of the serum used. Its principal use is at the beginning of an outbreak to protect exposed animals that have not yet developed symptoms of the disease. For this purpose it is very efficient, but it has the disadvantage, of course, that the immunity is short-lived.

THE SIMULTANEOUS TREATMENT The simultaneous injection of serum and virus has long been used for actively immunizing pigs to cholera. The method was developed by Dorset and co-workers of the U S Bureau of Animal Industry. It consists in injecting the pigs with a small dose of virulent blood, subcutaneously, at one site and a dose of antiserum at another If the pigs are thrifty, kept under good conditions and are well fed the method usually is very successful. Four or five days after the inoculation the animals usually show a mild reaction, manifested by lack of appetite and some fever. This ordinarily passes off after a day or two and the pig thereafter is strongly and permanently immune. If the pigs are parasitized, or unthrifty for other reasons, or if they have been recently shipped, or subjected to surgical operations, it is unsafe to administer the simultaneous treatment since many pigs will often develop cholera and die in spite of the antiserum which they have had. If the serum is lacking in potency, the same thing may happen in healthy pigs. These happenings are called "serum breaks" since they represent failure of the serum to provide the expected protection Serum breaks are always immediate. "Virus breaks" on the other hand are cases in which the pigs, sometime after receiving the simultaneous treatment, develop cholera. These failures occur when the virus lacks proper potency, since of course the pigs then

are only passively immunized. Both types of breaks occur occasionally. By means of such breaks hog cholera is perpetuated. The simultaneous method of immunization has given excellent service but it has many disadvantages which will mean its abandonment as soon as a safer method of active immunization has been discovered.

THE BOYNTON VACCINE. The Boynton (1) vaccine was developed by using methods which the originator had used successfully in the Philippine Islands in developing a vaccine for rinderpest. It is a tissue vaccine prepared by finely grinding the spleen, lymph glands, and bone marrow of pigs destroyed while at the height of the febrile period of cholera, and treating the suspension with eucalyptol until the virus has been modified so it will no longer produce cholera. The exact method of manufacture has not been described Boynton, Woods, and Wood (2) have used this vaccine successfully on large numbers of pigs and have reported excellent success with it They claim that in garbage feeding establishments where immunization with the simultaneous method often is unsatisfactory, they have had excellent success. The vaccine is not given when cholera exists on the premises until after all animals have previously been protected temporarily by a dose of antiserum. Full immunity develops in about three weeks Annual revaccination of all animals is recommended.

THE CRYSTAL-VIOLET VACCINE. This vaccine was described by McBryde and Cole (9) who give credit to Dorset for initiating the work on it. The procedure followed in making the vaccine has differed a little from time to time. Generally about 90 parts of fresh, defibrinated virus blood is first mixed with 10 parts of 1 per cent phenol solution and then 5 parts of a 1 per cent aqueous solution of crystal-violet is added. The mixture is shaken, then incubated at 37° C for 14 days, shaking it a little each day It is then refrigerated until needed The vaccine is quite stable if kept cool. A single dose of vaccine is enough, ordinarily, to protect pigs against subcutaneous inoculations of virus given at any time after a 14 day interval during which the immunity is becoming established. Some irregularities in the immunizing properties of the vaccine have been reported, and there is a question as to whether the immunity conferred is sufficiently durable to protect the pig for the remainder of his life. Edgington and Schalk (7) found that pigs were protected perfectly for about two months, but when virus was administered to a group from 75 to 90 days after being treated with the crystal-violet vaccine, about half of them developed cholera, and one quarter of the lot died of the disease.

PRACTICAL CONTROL OF HOG CHOLERA. Hog cholera is reasonably well controlled in the United States through active immunization with serum and

virus. This method of control leaves much to be desired because the use of active virus has served to keep the disease alive, and the expense of constant immunization of such short-lived animals is very great. The question has been raised whether it would not be more satisfactory in the long run, and more economical as well, to prohibit the use of virus and to depend upon serum alone, as the Dominion of Canada has been doing for many years There would then be hope of eventually eradicating the disease, a hope which cannot be entertained so long as present methods are used. The same result could be obtained, of course, if a practicable vaccine were developed for general use.

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RINDERPEST

Synonym: Cattle Plague.

Rinderpest is an acute, febrile disease of ruminants characterized by a rapid course and a high mortality rate. The disease is enzootic in parts of Asia and Africa and has spread on many occasions in the past, especially in times of war, to Europe where it has affected cattle, principally. The name by which it is best known is of German origin and it means, literally, cattle plague because its devastating effect in Europe has been principally in cattle. The disease causes great losses in sheep and goats. In Asia the greatest losses are in the carabao or water buffalo, a common beast of burden. The cause is a filterable virus.

Where rinderpest is enzootic the losses are serious but the greatest losses have occurred when the disease has invaded Europe where the cattle are highly susceptible. On some occasions in the past the disease has nearly com-

pletely destroyed the cattle population of Europe. The disease does not exist in the western hemisphere at the present time. Only once, in 1921, did the disease obtain a foot-hold in the western world. At that time the disease appeared in Brazil where it probably had been imported in zebu cattle. This outbreak was quickly stamped out after less than 1,000 cattle had died from it and about 2,000 other exposed cattle had been slaughtered. Rinderpest undoubtedly could do great damage in the range cattle of North and South America should it ever gain a substantial foothold. Veterinarians of these continents should constantly be vigilant with regard to it, since modern methods of swift travel by air have increased greatly the chances of its importation.

Character of the Disease. The incubation period in naturally-acquired rinderpest usually is short—from 3 to 8 or 9 days as a rule, occasionally somewhat longer. Fever usually precedes other symptoms by one or two days. This often rises as high as 106° F The febrile period is short. At the onset of diarrhea the temperature falls to normal or below. During the latter part of the febrile period, nervous symptoms often occur because of a virus induced encephalitis. Usually there is stupor, the animals standing with ears drooped and the head resting on the manger Some animals show signs of excitement. The appetite is lost and constipation is apt to occur during the febrile period. The conjunctiva and nares are reddened, show petechial hemorrhages, and exude a muco-purulent secretion which dries into brownish crusts. The mucous membrane of the mouth is reddened and shows whitish spots which evolve into shallow erosions covered with fibrinous deposits. The diarrhea which develops after a few days is very profuse for a time, then becomes scanty. Tenesmus is common, the dark red rectal mucosa often being everted through the rectum. The animals become emaciated, weakened, and usually death results within a week or less after the first symptoms are seen. Milder cases are sometimes seen in the hardier cattle, and in calves shortly after weaning when they still retain some of the natal immunity derived from their immune mothers. The symptoms in these cases may subside after several days and the animals thereafter are immune.

The principal lesions are found in the intestinal tract. The carcass shows great emaciation and dehydration. The lymph nodes are swollen, congested, and sometimes hemorrhagic. The spleen usually is normal. There may be some areas of consolidation in the lungs. Microscopically there is evidence of capillary damage similar to that seen in hog cholera, and there are lesions characteristic of diffuse virus-type encephalomyelitis. The mucosa of the digestive tract from the mouth to the anus is reddened, swollen, covered with fibrino-purulent exudate, and small, superficial erosions are common.

Nature of the Virus. The virus of rinderpest occurs in the blood, all tissues, and all secretions and excretions. It is present in the blood and tissues in greatest concentration during the febrile period. Nicolle and Adil-Bey (9), who discovered the virus of rinderpest in 1902, succeeded in passing it through Berkefeld and porcelain filters The virus is not easily or regularly filterable, however. Daubney (3) claims, in fact, that rinderpest virus contained in blood is almost wholly contained in or attached to the leucocytes He found that the filtrates of diluted blood from Berkefeld or Seitz filters would not produce infection when as much as 25,000 minimum infective doses, based on the virus content of the unfiltered blood, were inoculated into cattle. Plasma separated from highly infectious blood by centrifugation was not infectious unless large doses were used, whereas the sedimented cells were highly infectious. Finally by filtering the blood through non-absorbent cotton, which passes the erythrocytes but traps most of the leucocytes it was shown that most of the infectiousness of blood was removed Since it was not possible to wash the virus free from leucocyte suspensions Daubney was of the opinion that it was contained in these cells rather than merely attached to them. Similar observations were made by Hornby (4).

Artificial Cultivation. Success in cultivating the virus of rinderpest in artificial media has not been reported Because of its similarity to the virus of hog cholera, it would be of interest to attempt its cultivation by the simple method employed by Ten Broeck for the latter.

Transmission. Rinderpest can be transmitted to susceptible animals by feeding them with blood, urine, feces, nasal discharges, and perspiration. Natural transmission occurs by direct contact with diseased animals, or by the consumption of feed and water which has been contaminated by them. Virus is inactivated rather quickly (one or two days) in dried secretions, but in the presence of moisture it retains its activity somewhat longer. Boynton (2) found that virus could never be detected by placing susceptible animals in corrals which had contained infected animals longer than 36 hours previously, even when water was present and parts of the area were shaded from the sun. He concluded that rinderpest virus does not survive long in pastures after affected animals are removed from them.

Immunity. Animals which survive an attack of rinderpest are permanently immune thereafter. Methods of artificially immunizing animals both actively and passively are available.

RINDERPEST ANTISERUM Serum from animals which have recovered from rinderpest possesses considerable protective value for susceptible animals but

its value is greatly enhanced by hyper-immunization using the general method employed for the production of hog cholera antiserum. Such sera will protect animals for 10 to 14 days. They are used on recently infected herds to treat animals that are in the incubation period of the disease, and for protection against transient infection such as occurs during shipping. Immune serum has little value for treating animals in which symptoms have appeared.

SERUM AND VIRUS IMMUNIZATION. A method of immunization comparable to the simultaneous method used in hog cholera has been extensively used for rinderpest in the past but has largely been abandoned in favor of better methods. Immunized animals usually shed the virus during the process, hence the method was permitted only in areas where the disease was indigenous. Also, heavy losses sometimes occurred as a result of the development of rinderpest by the vaccinated animals

Another disadvantage in the use of serum and virus is that in areas where rinderpest occurs other blood-borne diseases are common and may be transmitted by the blood virus Anaplasmosis, piroplasmosis, and trypanosomiasis may be transmitted in this way.

BILE IMMUNIZATION. For many years bile of animals dead of rinderpest was used for protecting susceptible animals. The immunity was due to virus contained in the bile but apparently modified by the effect of the bile salts. The immunity was uncertain and rinderpest sometimes was produced by the treatment. The method has now been abandoned except as an emergency measure when other materials are not available

TISSUE VACCINES There are a number of different types of vaccines made from tissues Most of them are made by destroying the virus with chemicals. Pfaff (10), who worked in Burma, developed a vaccine by passing rinderpest virus through goats The goat adapted virus, according to his claim, lost most of its virulence for cattle, and dried spleen pulp from these animals satisfactorily immunized cattle and carabao.

Kakizaki, Nakanishi, and Ozumi (5) made a vaccine by treating spleen and lymphoid tissue pulp with glycerin, phenol, and eucalyptol. With this vaccine they successfully immunized thousands of cattle in Korea and Manchuria. Bennett (1) used a similar vaccine in the Sudan, in Africa, with excellent results. This vaccine did not work so well in India, according to Edwards.

Boynton (2) made a vaccine in the Philippines by treating organ pulp with phenol and glycerin. The mixture was heated in a water bath at 42° C. for three hours, then was kept in the refrigerator for several months until tests indicated that the virus had been destroyed. The vaccine then had to be used rather promptly since older vaccines did not immunize successfully. This vac-

cine gave excellent success, but its method of manufacture was tedious and the keeping qualities were poor.

Kelser (6) prepared a vaccine by making a pulp of the spleen and lymphoid tissue of cattle, diluting the pulp with an equal quantity of saline solution and adding 0.75 per cent of chloroform. The chloroform rapidly destroys the rinderpest virus. After 48 hours the vaccine is ready for use. Kelser, Youngberg and Topacio (7) used three doses given at weekly intervals Rodier (11) later found that a single but somewhat larger dose was satisfactory. Excellent results were reported from the use of this vaccine.

A formolized spleen vaccine was developed and used by Daubney (3) in Africa. This vaccine also was used successfully by Keylock (8) in China.

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AFRICAN HORSE SICKNESS

Synonyms· Equine Plague; Pestis equorum.

This disease occurs only on the African continent It is enzootic in parts of South Africa and has occurred in the central part of the continent. It affects horses, mules, and asses. It has been reported in zebras It has long been recognized as a devastating disease of horses kept on low-lying farms At times it has destroyed practically all horses in some of these areas The disease is not contagious, i. e., it does not spread from animal to animal. Apparently it is transmitted by blood-sucking insects. The cause is a filterable virus.

Character of the Disease. The disease occurs only during the warm, rainy season in low-lying marshy districts. Within one week after frosts have occurred no new cases appear. The disease does not occur, as a rule, in animals that are kept in buildings at night.

The incubation period usually is about 6 or 7 days, occasionally somewhat

longer. The disease is characterized by edematous swellings of the lymph nodes and subcutaneous tissues of various parts of the body, and edema of the lungs. The course of the disease is from one to two weeks and the mortality rate is high, especially in horses imported from regions which are free of the disease. The symptoms of the disease are somewhat suggestive of anthrax but it is easily differentiated from it by the absence of spleen swelling and of bacilli in the blood. Inclusion bodies have not been recognized in this disease.

Nature of the Virus. That the disease was caused by a filterable virus was first shown by McFadyean (5) in 1900. It passes through both Berkefeld and porcelain filters. In the blood the virus is in or attached to the cells and cannot be washed from them Horses can readily be infected by the injection parenterally of small amounts of blood, tissue emulsions, and bronchial secretions. The urine is infective only occasionally. By feeding, the disease can be produced only by the administration of large amounts of virus The virus contained in blood is remarkably resistant so long as drying is prevented. It withstands phenol and glycerin unusually well. Blood stored in a refrigerator without preservatives will retain infectivity for many months. It may be stored still longer if the blood is preserved by the addition of glycerin. Blood virus may also be preserved for long periods by the addition of 3 per cent phenol.

In addition to the animals which are naturally susceptible, disease can be produced by inoculation in dogs, guinea pigs, and mice Man is not susceptible.

Artificial Cultivation. Successful cultivation of the virus of horse sickness in artificial media has not been reported.

Transmission. Horse sickness is not directly transmissible from animal to animal Affected animals placed in stables with susceptible horses do not cause outbreaks of the disease. Outbreaks occur in warm, damp weather, on swampy, low-lying farms, and only in horses which are pastured at night. These facts indicate that night-flying insects are the probable vectors but, surprisingly, the carrier agent has not been positively identified in spite of much work on the subject. Certain types of mosquitoes may harbor the virus for a time and it is thought that some of these insects are likely to be the carriers. Mules, asses, and dogs, which are not so susceptible to the disease as are horses, have been suggested as possible reservoirs of virus.

Immunity. Animals which have recovered from horse sickness are not always permanently immune to the disease. Immune mares convey a passive immunity to their colts which protects them until after they are weaned. Cases have been reported of animals having the disease a second time. These have

been explained by the finding that there are at least six immunologically distinct types of virus and immunization against one type does not fully protect against another. Usually second attacks of the disease are mild.

An immune serum serves to provide passive protection from the disease for a limited time This serum is made by hyperimmunizing recovered horses by transfusing them directly from horses in the febrile stage of horse sickness. To obtain a lasting immunity, horses formerly were given simultaneous injections of large doses of immune serum and small doses of virus. About 85 per cent of such horses developed fever, in which case another dose of immune serum was given. A considerable number always developed severe disease and about a 4 per cent mortality was expected. The method has been abandoned lately in favor of vaccines.

duToit, Alexander, and Neitz (4) reported in 1933 on a vaccine for horse sickness made from formolized spleen pulp emulsion. Four doses were given, the first having been treated by formalin in a conçentration of $\frac{1}{1000}$, the second in $\frac{1}{2000}$, the third and fourth in $\frac{1}{3000}$ and $\frac{1}{4000}$, respectively The results were generally satisfactory but the resulting immunity was not permanent.

Alexander and duToit (2), Alexander (1), and Alexander, Neitz, and duToit (3) have reported very successful immunization of horses with a living vaccine made by modifying the virulence of the horse sickness virus by intracerebral passage through mice As the virus became adapted as a neurotropic strain for mice, its virulence for mice increased but that for horses decreased After it had been passed through more than 100 passages in mice, the virulence became fixed for this species, and was no longer capable of producing the disease in horses Because of the several immunological types it was necessary to make fixed virus from each type, and the field vaccine is manufactured from a mixture of these types A single dose of 100 mouse-infecting doses of each type is enough to give excellent protection.

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RIFT VALLEY FEVER

This disease takes its name from a geographic area in Kenya Colony, British East Africa where the disease occurs principally in sheep, especially lambs,

but also in goats, cattle, and man. The disease in man has occurred in England and in America in laboratory workers who have studied the virus. The disease was first described by Daubney and Hudson (1) in 1931.

Character of the Disease. The disease attacks young lambs especially, and the mortality may amount to 90 per cent of the lamb crop. In old ewes it is from 10 to 20 per cent. In cattle it is still lower and in man, negligible. Ewes which become infected before lambing time commonly abort. The disease appears not to be contagious but is spread by blood-sucking insects.

In lambs the disease is characterized by high fever and prostration which leads to death in many cases in less than 24 hours In less acute cases there is a nasal discharge and a bloody diarrhea. In man the symptoms are similar to those of dengue fever violent headache, nausea, pains in the joints, fever of a transient character, and prostration Recovery occurs within a few days Only one fatal case has been reported. This occurred in a laboratory worker in New York whose case has been described by Schwentker and Rivers (5). The fatality was caused by complications with thrombophlebitis

The chief lesion of the disease in lambs is a massive necrosis of the liver. In some cases the necrosis of liver cells is so complete that sections are hardly recognizable. In others there may be many foci of necrosis with fairly normal liver structure between them. Findlay (2) has described inclusion bodies of an intensely acidophilic character in the cytoplasm of the liver cells.

Nature of the Virus. The virus of Rift Valley Fever is found in the blood and organs of affected animals. It is readily filterable through Berkefeld and porcelain filters The particulate size has been estimated as about 25 to 30 millimicrons The disease can readily be induced in lambs by the injection of minute amounts of blood. The disease can readily be induced in mice, in which it is highly fatal, and in ferrets and monkeys in which recovery usually occurs. Most human infections have occurred in persons who have conducted autopsies on sheep or laboratory animals. The exact mode of infection is not known. The lesions in laboratory animals consist principally of liver necrosis. The blood virus is fairly resistant. Blood preserved with 0 5 per cent phenol remains infective for six months when stored in the refrigerator.

Artificial Cultivation. Mackenzie (4) reported success in cultivating this virus in a minced chick-embryo medium. It probably can be cultivated on the chorio-allantoic membrane of developing chicks, but no reports on such experiments have been seen.

Transmission. Daubney and Hudson (1) noted that the disease did not transmit from sick to healthy lambs that were kept together in the laboratory.

It is believed to be transmitted naturally by mosquitoes. Francis and Magill (3) believe that the disease may sometimes be transmitted through the respiratory tract.

Immunity. Animals which recover from Rift Valley Fever have virus-neutralizing antibodies in their serum and are refractory to artificial inoculation for some months but the immunity is not enduring. No methods of artificial immunization have been worked out.

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BLUE TONGUE OF SHEEP AND CATTLE

Synonym: Catarrhal Fever of Sheep.

This disease occurs in South Africa. In sheep the disease is malignant and sometimes causes large losses In cattle it usually is quite benign but is sometimes mistaken for foot and mouth disease It is caused by a virus The disease occurs on low-lying swampy farms in sheep which are pastured at night It does not occur on farms on high lands or in sheep which are kept in buildings The disease is not directly contagious It is believed to be transmitted by blood-sucking insects but this has not been proven.

Character of the Disease. The disease begins with fever and lassitude followed by a hemorrhagic inflammation of the buccal mucosa and especially of the tongue which becomes greatly swollen. Edema of the regions of the head and larynx regularly occurs Diarrhea and ulcerative keratitis are frequent symptoms.

The lesions consist of those already described, also splenic hypertrophy and cloudy swelling of the principal organs.

Nature of the Virus. Not much is known about the virus of this disease. It occurs in the blood and organs and filtrates from any of the internal organs will cause the disease. The disease may be transmitted to cattle from sheep, and from sheep to cattle. Goats are resistant. Human infections have not been reported. No information about the susceptibility of laboratory animals is available.

Transmission. Natural transmission of this disease is believed to be due to night-flying, blood-sucking insects but the transmitting agent has not been identified.

Immunity. Animals which recover from this disease are permanently immune thereafter but their blood is said to contain virus for a considerable time after clinical recovery. Serum of recovered animals and small doses of virus blood have been successfully used for immunization. Theiler found that when the disease was passed through a series of sheep by blood inoculation, the virulence gradually decreased. He made use of such blood for immunization Recently Curasson has reported successful immunization of sheep with a vaccine consisting of infective spleen pulp rendered avirulent with formalin.

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NAIROBI DISEASE OF SHEEP

This disease occurs only in a small district in British East Africa. It affects sheep which each year are brought down from the northern districts into Nairobi to be offered for sale. It has been described by Montgomery (1).

The disease is characterized by acute hemorrhagic gastro-enteritis. The mortality varies from 30 to 70 per cent The causative agent is a virus which is readily filterable. The blood and tissues are always infective during the temperature reaction. The urine is said to be infective at this stage, but the feces ordinarily contain no virus.

The disease is transmitted by the adult forms of a tick, *Rhipicephalus appendiculatus,* which have fed as nymphs upon infected sheep

Recovered animals possess a lasting immunity. Artificial immunization has been attempted only on a small scale. Control depends upon eradication of the transmitting agent. This tick also transmits East Coast Fever of cattle, hence dipping of both sheep and cattle should be done, with benefit to both species.

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SWINE INFLUENZA

Synonym: Hog "flu."

Swine influenza is an acute disease of the respiratory organs which occurs in the colder months of the year. The onset of the disease is sudden and

practically all animals in an affected herd show symptoms almost simultaneously. The symptoms are quite similar to those of epidemic influenza of man, and the virus of swine influenza is closely related to that of human influenza.

The disease was first recognized as an entity in the mid-western part of the United States in the fall of 1918 when a pandemic of human influenza was under way The similarity of the diseases in man and pigs was recognized. Koen is credited by Dorset, Niles, and McBryde (2) as being the one who suggested the name "flu" or influenza for the disease, since he was convinced that it had been contracted from human cases. Much later, when the etiological agents of the two diseases were better understood, the concept that swine may have become infected from man, thus giving rise to a new disease in the species, became much more plausible than before Two immunologically different viruses are now recognized as the causative agents of human influenza The virus of swine influenza is immunologically different from both of these, but the swine virus is more closely related to one of the human viruses than the two human viruses are to each other Many adult human beings carry antibodies which neutralize, in part at least, the virus of swine influenza This has been regarded by some as evidence that these persons have been infected at some time in the past with the same type of virus that exists in pigs, but other explanations are possible Since the influenza viruses were not known at the time of the last great pandemic in man when the swine virus first appeared, it is not possible now to know whether the human virus of that outbreak was identical with either of the two viruses that now exist in man It is possible, of course, that the 1918 virus in man was identical with that now current in swine, or the virus may have been modified by long continued residence in swine

Character of the Disease. Swine influenza is a disease of autumn and early winter In many of the mid-western states where swine raising is an important industry, the disease assumes epizootic proportions each fall, beginning usually in October when cold weather arrives. Cases continue to develop during the winter months but the disease is wholly absent during the warm parts of the year.

The disease usually appears suddenly in swine herds and whole herds commonly develop symptoms almost simultaneously. The development of the disease in many animals at almost the same time has commonly been attributed to an extreme degree of contagiousness, but Shope has put forward another conception very recently. This is that the disease-producing agent spreads widely without producing obvious disease and that a precipitating agent is

responsible for the simultaneous development of many cases. It was suggested that the precipitating agent in this case might be the advent of cold, wet weather with consequent chilling of the animals.

The disease begins with fever, anorexia, extreme weakness, and prostration. The animals crowd together, lying down, and are moved only with difficulty. When moved or handled the animals exhibit evidence of muscular stiffness and pain. In uncomplicated cases the disease runs a short course varying from 2 to 6 days, recovery occurring almost as suddenly as the disease began. Other cases develop edema of the lungs and broncho-pneumonia, and these usually die At the height of the disease the animals exhibit a jerky type of respiration, caused by spasms of the diaphragm, which is commonly known as "thumps" Bronchitis is indicated by coughing. When the animals are in good condition in the first place, and are kept during the course of the disease in a dry, fairly warm place, well bedded with straw, the principal loss from this disease usually is in the retardation of growth and weight loss which occurs. The mortality rate is low as a rule, generally amounting to not more than 4 per cent, but sometimes it may be as high as 10 per cent.

Animals killed at the height of the disease exhibit no significant lesions outside of the chest cavity The lung lesions are characteristic. They are limited, as a rule, to the cephalic, cardiac, and azygos lobes. Sometimes all five of these lobes are involved; sometimes only part of them Usually the involvement is bilateral, but in some cases it is unilateral, the lobes of the right side being involved somewhat more often than those of the left. The involved portions are collapsed, deep purplish-red in color, and do not crepitate. They are not pneumonic The condition is an atelectasis caused by a thick, mucilaginous exudate in the bronchioles and bronchi of the parts. The remainder of the lungs is usually pale because of interstitial emphysema The cervical, bronchial, and mediastinal lymph nodes are swollen and filled with fluid.

The spleen often is moderately enlarged. There is hyperemia of the mucosa of the stomach in most cases. The other abdominal organs generally are normal.

In the cases in which pneumonia occurs, the consolidated portions are the same as are atelectatic in the milder ones The non-pneumonic lung portions in these cases are congested and edematous.

Nature of the Virus. Shope (8) announced his discovery of the virus of swine influenza in 1931. Lewis and Shope (4) at the same time described a hemophilic bacillus (*Hemophilus suis*) which they found regularly in field cases of influenza The virus alone administered to normal pigs in an area where swine influenza did not exist, produced a very mild, almost symptom-

less disease which surely would be overlooked on the farm. The hemophilic bacillus on the other hand was virtually non-pathogenic for swine. When both agents were given simultaneously, however, typical influenza resulted. The disease, then, is a result of the concurrent action of two agents, a virus and a bacterium.

The virus of swine influenza is readily filterable Its particulate size has been estimated at about 120 millimicrons In affected pigs the virus is found in the nasal secretions, in the tracheal and bronchial exudate, in the lungs, and in the lymph glands draining the lungs. It is not ordinarily found in the blood, spleen, liver, kidneys, mesenteric lymph glands, and brain. [Orcutt and Shope (5)].

Andrewes, Laidlaw, and Smith (1) demonstrated that the virus of swine influenza was pathogenic for mice when introduced by a special technic into the nasal passages. When introduced by simple injection, "takes" are not ordinarily achieved. The technic of the English workers consisted of partially etherizing the mice by placing them in a jar with a pledget of cotton soaked in ether. When consciousness is lost and the animals are breathing convulsively, they are removed from the jar and their noses are immersed in the inoculating fluid. In this way fluid is aspirated into the smaller air passages, and this, apparently, is what is needed to bring about infection. Infections of the mice regularly occur The resulting pneumonia is caused by a pure virus infection, that is, the bacterial component is not needed in mice as it is in pigs. The disease can be transmitted indefinitely in mice by making emulsions of the pneumonic lungs and infecting other mice as described above. It is of interest to note that the virus of human influenza cannot be transmitted directly from the human being to mice but must first be adapted to ferrets. These viruses are harmless to mice when injected subcutaneously or intraperitoneally.

Mouse-passage virus retains its virulence for swine indefinitely. The virulence of the *Hemophilus suis* may decline, however, in which case new cultures are needed to supply the necessary bacterial factor for producing the swine disease.

Artificial Cultivation. Kobe and Fertig (3), and Scott (6) have reported the successful cultivation of the virus of swine influenza on the chorio-allantoic membrane of the developing chick. Scott reports that his cultures as far as the 50th generation were virulent for mice and swine, but that the 85th and later generations had lost their virulence.

Transmission. Swine influenza is recognized as a highly contagious disease since it occurs in wide epizootics. Inasmuch as the nasal and bronchial secre-

tions contain both the virus and the bacterial agent, it is from these that new infections are produced. A single infected pig placed in a pen with susceptible animals will quickly cause infections of all. The hemophilic agent is carried by many apparently normal pigs, and such animals need only the virus to precipitate the disease.

The question of how the disease is maintained through the non-epizootic portions of the year when the disease is not seen possibly has been answered by Shope (13) who has demonstrated that the lungworm and the earthworm can harbor the virus for long periods of time. Lungworms, living in the bronchi of affected pigs, ingest virus and the virus is carried through the eggs and into the larvae of the parasite. These, hatching out in the air passages of the pig, are coughed up and swallowed, eventually reaching the ground in the feces. Here they are ingested by earthworms in which the larvae lodge, most of them being found in the heart and calciferous glands. They may remain in the worms from one season to the next. If the earthworms are fed to swine, as was done by Shope, the pigs show no ill effects However, if the pigs are then given several intramuscular injections of the *H. suis* about one half of them will suddenly develop typical swine influenza and both virus and bacterium will be found in the lungs. Shope regards the injections of the bacterium merely as a precipitating or provoking agent, since he was able to provoke a similar effect in a few cases by injecting calcium chloride into the pleural cavity. He has repeated these experiments successfully many times in the late fall, winter, and spring months but never in the summer. He speaks of the virus in the earthworms as existing in a "masked" form.

Immunity. The immunity in swine influenza is not enduring, since the same pigs often contract the disease several times during their comparatively short lifetime. Neutralizing antibodies are present in the blood for some weeks and the animals cannot be reinfected artificially during this time.

Of the two components required to produce swine influenza, only the virus will effectively immunize. Shope (9) has shown that the filtrate disease (the mild, almost symptomless effect of virus without the organism) confers solid immunity to the complex of virus and organism. The organism, on the other hand, when used as vaccine is capable of modifying the course of the disease but will not prevent it (11). Swine which have recovered from the effects of an injection of a mixture of human influenza virus and the *H. suis* are usually immune to swine influenza. Those which recover from the effects of the human influenza virus alone usually are not immune to swine influenza. Those which have recovered from swine influenza are refractory to the human virus.

No practicable methods of immunizing pigs in the field against this disease

have been developed and none are urgently needed because of the comparatively low death rate.

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"FERKELGRIPPE"

Since the disease here considered has been reported only in German speaking countries it has only a German name The term means grippe of young pigs It is a disease quite similar in its manifestations to swine influenza except that it occurs generally in very young pigs, i e, those less than six weeks old. Occasional cases in older animals are very mild The loss in young pigs is very considerable, amounting to more than 50 per cent quite often According to German authors the disease causes large losses in their country

The disease is caused by a virus which is found only in the lungs and its lymph nodes, and in the upper air passages. It can readily be transmitted from animal to animal with these discharges or filtrates made from them. The filtrates will also produce a disease in mice similar to that of influenza. The principal features of the disease have been described by Waldmann (2) and others Kobe and Fertig (1) found that both this virus and that of influenza could be cultivated on the membranes of the developing chick embryo. After a number of generations on chick embryos, the ferkelgrippe virus lost its virulence for pigs whereas the influenza virus did not Waldmann suggests that the ferkelgrippe virus may be a modified form of influenza virus.

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EQUINE INFLUENZA

Synonyms: Epizootic catarrhal fever; Shipping fever; Stable pneumonia; Pink eye.

Influenza in horses is similar in its rapidity of spread, in symptoms and lesions to influenza in man and swine. The disease affects horses of all ages but occurs mostly in young animals which have been moved into new surroundings particularly where they come in contact with many other horses. The disease gives much trouble in sales' and dealers' stables, and in army remount stations In centers where fresh "green" horses are arriving from time to time, the newly arrived animals serve to keep the disease going, and in virulent form The disease occurs in farm horses but in such animals it usually is mild Like influenza of man, this disease has a history of great panzootics in which large areas are affected, nearly every horse in these areas falling victim to the disease. The last one of this kind in the United States occurred in the winter of 1872–1873 At that time traffic in some of the large cities of the country was very nearly stopped because of the lack of horses able to draw the horse cars, drays, and delivery wagons Like human influenza, the horse disease occurs every year between the epizootic periods.

Character of the Disease. The disease is highly contagious, practically every horse on the premises becoming infected whenever the disease appears. The mortality rate is low, seldom being as high as 10 per cent and usually amounting to less than 4 per cent. The chief economic loss from the disease is the inability of the affected animals to work for a period of from one to three weeks and sometimes longer.

The disease affects only horses, mules, and asses The onset is sudden and is manifested by a high temperature—106° F and higher The appetite is lost, and there is evidence of great depression. The fever continues high for three to six days and suddenly falls in uncomplicated cases There is great muscular weakness, tremors often occur, and sometimes paralytic signs appear especially in the hind legs. The stuporous condition and the paralytic signs point toward a virus encephalitis, but no reports of brain examinations have been found

Lachrymation and photophobia are always present and often the conjunctivae protrude because of infiltration of the tissues. A muco-purulent discharge usually occurs after a few days, the cornea often becomes clouded, and other inflammatory changes in the eye sometimes lead to blindness. Nasal catarrh is usually present and the lymph nodes of the head may become swollen and tender, but pneumonia occurs only in protracted cases. In some outbreaks edematous swellings of the subcutaneous tissue of the lower parts of the body

commonly occur. Inflammation of the tendon sheaths of the legs occur in some cases. Mild icterus is frequently seen Catarrhal and even hemorrhagic enteritis occurs in some cases and kidney damage is common. In the early stages of the disease leucopenia frequently occurs.

Except in cases in which pneumonia, severe enteritis, nephritis, and heart damage occurs, recovery is rapid and uneventful

Nature of the Virus. The virus of the disease occurs in the blood and all the tissues. Filtrates made from blood or tissue extracts readily produce the disease when injected subcutaneously. The virus has not been studied extensively but it is obvious that the particulate size is not large

There have been numerous reports of the transmission of the disease by stallions used for breeding purposes months after having had the disease. Several reports of the demonstration of virus in the semen of such animals for periods varying from 1 to 6 years are in the European literature Schofield (5), in Canada, observed two outbreaks apparently initiated by breeding stallions which had had the disease some months previously. The examination of the semen of one of these animals six months later failed to show the presence of virus. Infectivity of the blood several months after recovery also has been reported Blood virus, kept in the refrigerator, retains virulence for some months.

Inoculation experiments on animals other than horses have been negative, but no reports have been seen in which there have been attempts made by modern methods to induce adaptation of the virus to other animal species.

Artificial Cultivation. There are no reports of successful cultivation of the virus of equine influenza in artificial media.

Immunity. Animals which have recovered from an attack of equine influenza are generally regarded as permanently immune, however Todd and Soutar (6) cite rather extensive experiments conducted in India with Army horses in which about 10 per cent of the animals proved susceptible to the disease when exposed four years after recovery from the original outbreak. No practicable methods of artificially immunizing horses to this disease have been developed.

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CONTAGIOUS PNEUMONIA OF YOUNG CALVES

Calf pneumonia in many large breeding herds is one of the most serious of the disease problems. The disease occurs in animals as old as six months but generally is seen in calves from ten days to four months of age. The disease is not usually serious in small herds where the annual calf crop is small and many young calves are not kept in close association The larger establishments usually maintain special calf barns where the young calves are kept together. These often are models of elaborate construction, but it is in such units that the disease usually takes its greatest toll The disease is highly contagious and the fresh calves that are being constantly brought into such units serve to add fresh fuel to the fire.

It is generally believed that there is a direct connection between calf pneumonia and calf scours or diarrhea. The diarrheal disease usually precedes the pneumonia, since it usually is seen in animals from one or two days of age up to perhaps two or three weeks. The pneumonia may occur earlier but is most often seen in animals from three weeks to three or four months of age, that have been debilitated by the early enteritis Scours, however, occurs in calves that do not show pneumonia later, and pneumonia is seen in animals that did not suffer from scours.

A miscellaneous group of organisms is commonly found in the lung tissue of calves dead of pneumonia. Streptococci, *Corynebacterium pyogenes,* and *Pasteurella multocida* are the most frequent Since it has not been possible to reproduce the disease with the bacteria commonly found in the lungs, it has been assumed that they were secondary invaders. The fact that the disease transmitted so readily has caused many to believe that a respiratory tract virus was the initiating factor, but such a virus had not been demonstrated until very recently. Baker (1) in 1942, announced his discovery of a virus which, it seems likely, is the causative agent.

Character of the Disease. The disease is seen at all times of the year but is most prevalent during the winter months when animals are kept closely crowded in weather-proof buildings in which the ventilation often is very faulty. The disease is manifested by loss of appetite, high fever, unthriftiness, prostration, a scanty nasal discharge, and rapid breathing. The older the animal and the stronger it is originally, the better will it withstand the disease, the longer will the course of the disease be, and the more likely it is to eventually recover. The younger, weaker calves may die after a course of only a few

days; the larger, stronger animals may run a course of several weeks. If recovery from the acute phase of the disease occurs, permanent unthriftiness often remains because of the damage to the lung tissue from the bacterial agents which invariably accompany the virus.

The lesions are found most commonly in the anterior and ventral lobes. Usually the disease is bilateral. The pneumonic lung tissue is usually dark red, or dark red mottled with gray The affected tissue is firmer than normal, is not dry like hepatized tissue but is rather moist. Fibrinous exudate sometimes is found on the pleura but this is rather exceptional. Usually the pleura is smooth and glistening over the pneumonic areas The nonpneumonic portions of the lung sometimes show emphysematous changes If the disease has been protracted, small abscesses commonly are found in the pneumonic areas, and the bronchi are filled with thick muco-purulent exudate. The pneumonic tissue in these cases is practically always mottled with grayish areas made up of large collections of neutrophilic leucocytes

Nature of the Virus. Information about the virus of this disease is limited because only a preliminary report of his work has been issued by Baker. The virus readily passed a Berkefeld N filter The virus was recovered by introducing calflung filtrates into the air passages of etherized white mice in the manner by which the influenza viruses of man and swine are recovered

Pathogenicity. Baker found that a transmissible pneumonia of white mice could be produced by introducing calflung filtrates into their air passages. Filtrates of mouse lung virus readily produced disease in calves when it was introduced into their air passages, and on two occasions the disease was transmitted by pen contact from artificially inoculated calves to normal ones The disease, according to Baker, was typical in every way of that which naturally occurs

Artificially inoculated calves developed fever in from two to four days and this lasted for three to five days, the peak usually being from 104° to 106° F. Diarrhea usually appeared the day after fever began, and symptoms of pneumonia about the fifth day after inoculation The pneumonic symptoms usually were mild and the calves ordinarily recovered Destroyed animals exhibited evidence of catarrhal enteritis and pneumonic foci occurred in the ventral lobes of the lung Calves that had recovered from the disease were resistant to reinfection, and their serum developed neutralizing antibodies Neutralizing antibodies for the mouse virus also developed in calves which suffered from the naturally occurring disease.

Artificial Cultivation. No attempts to cultivate this virus on artificial media have been reported.

Transmission. It is quite clear that natural transmission of this disease commonly occurs through inhalation of infective droplets projected into the air of the calf quarters through coughing of affected animals.

Immunity. It is clear, both from ordinary clinical experience and from Baker's work that one attack of this disease confers immunity to reinfection.

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EQUINE CONTAGIOUS PLEURO-PNEUMONIA

Synonym: *Brustseuche*

The etiology of this disease has not been definitely proved but it seems likely that a virus is the primary agent It is clear that the principal damage is caused by *Streptococcus equi*, but this organism will not reproduce the essential features of the disease

The disease has been confused with equine influenza, but it does not have the great contagiousness of the latter, the incubation period of artificially induced cases is much longer, and virus can never be demonstrated in the blood or any part of the body other than the lungs and air passages.

Character of the Disease. Contagious pleuro-pneumonia of horses is characterized as an acute febrile disease in which necrotic pneumonia regularly occurs accompanied in almost all cases by marked fibrinous pleuritis According to McFadyean (3) the pneumonia begins in multiple centers in the lung but rapid coalescence of these foci forms large pneumonic areas which have the gross appearance but not the histology of croupous pneumonia The early lesions show the bronchi and air cells filled with neutrophilic leucocytes, a broncho-pneumonia. Softening of the consolidated tissue quickly occurs forming large masses of liquefied tissue, commonly called abscesses. The pneumonia may be unilateral or bilateral

It is evident that some cases of this disease are abortive and the animals recover after a febrile period lasting for 2 or 3 days without developing pneumonia. Such cases cannot be distinguished with certainty from influenza and other infections but they have been noted in large stables where frank cases of contagious pleuro-pneumonia were occurring.

The mortality from this disease, when the cases are carefully nursed, varies from 5 to 15 per cent, but when good care is not given it may be much higher.

The incubation period is relatively long, and for this reason it is often difficult to trace the source of infection. Gaffky and Lührs (2), who studied the disease on an extensive scale in Germany, found that it varied from 18 to 39

days. In large stables, according to McFadyean, the disease, unlike influenza, does not usually spread from horse to horse in adjoining stalls but is apt to appear erratically, first in one part of the stable and then in another, and there may be considerable periods between cases.

Nature of the Virus. As has been said above, a virus has not been definitely identified from cases of this disease, however it seems fairly certain that there is such an agent at work.

For many years it was accepted by many that the etiological agent was an hemolytic streptococcus (*Strep. equi*). This conception was based largely upon the work of Schutz (4) who produced pneumonia simulating that of the natural disease by injecting cultures of streptococci directly into the lungs through the chest wall The disease produced by Schutz differed from true *Brustseuche* however, in that it did not prove contagious A study of the disease was undertaken by Koch and Gaffky, but Koch died before they had progressed far and the work was continued by Gaffky (1) Little progress was made in reproducing the disease in a contagious form until Gaffky and Luhrs finally tried destroying affected animals during the first febrile reaction before definite pneumonia had occurred and using bronchial secretions as the inoculating agent. With such material smeared on the nostrils of susceptible horses, the disease was produced in a typical form in many horses. Since these secretions often were bacteriologically sterile, or nearly sterile, and seldom contained the streptococcus of Schutz, it has been concluded that the primary agent must be a virus Such, at least, were the conclusions of McFadyean who had had a large experience with the disease. The causative agent of the disease is never found in the blood or tissues other than those of the smaller air passages, and after the pneumonia has developed, bacteria, particularly the hemolytic streptococcus, are present in large numbers in the diseased tissue and the original agent, if it be a virus, can no longer be recognized.

Transmission. Transmission is believed to occur by droplet infection, and, as has been pointed out above, to occur in the very early stages of the disease rather than after the pneumonic symptoms have appeared.

Artificial Cultivation. There have been no reports of the artificial propagation of a virus of this disease.

Immunity. Animals which have recovered from the disease are immune for a considerable time thereafter, but how long is not known. Immune serum has been used but its value cannot easily be assessed. Vaccines made from the bacterial agents of the disease have also been extensively employed as prophylactic agents with some success. In more recent years, neoarsphenamine

has proved very useful and it is said that in World War I, its use became routine in the armies of all combatants, and that the disease was almost wholly suppressed by its use.

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PSITTACOSIS

Synonym Parrot Fever (of man).

Psittacosis is regarded as a disease primarily of birds, especially of those belonging to the parrot family (*Psittacidae*), and secondarily of man. In recent years it has become obvious that the disease frequently occurs naturally in birds other than the psittacine group. Pinkerton and Swank (16) recently found the disease in the common pigeon, and unpublished reports indicate that the disease may be relatively common in this species Haagen and Mauer (7) identified the virus of psittacosis in a type of sea-bird, the Fulmar petrel, which is used as food by the inhabitants of the Faroe Islands. Their suspicions were directed toward this bird through an outbreak of psittacosis which occurred in the human inhabitants of these islands. Java sparrows and reed birds (thrushes) contract the disease when naturally exposed. Infections in canaries have been recognized. By inoculation the disease may be transmitted to young chicks (but not older chickens), mice, guinea pigs, rabbits, and monkeys. Mice are highly susceptible and are commonly used for diagnostic purposes, as was first suggested by Rivers and Berry (17) The disease in guinea pigs, rabbits, and monkeys frequently is not fatal.

The disease has attracted wide attention from time to time because of epidemics in man. During one of these outbreaks which occurred in Paris in 1893, Nocard isolated a bacterium belonging to the *Salmonella* group which he regarded as the causative agent. It was commonly accepted until the pandemic which occurred in Europe and the United States in the winter of 1929–1930, during which workers showed that Nocard's organism was not commonly present but that a virus regularly could be isolated. The *Salmonella psittacosis* of Nocard is now looked upon as *Salmonella typhimurium*, a chance contaminant.

The outbreak in man which occurred in 1929–1930 was traced to Green

Amazon parrots imported from South America where the disease has long been enzootic in these birds Restrictions on the importation of these birds into the United States became effective in 1934, but these measures have not served to eliminate the disease In a survey made in 1932, Meyer, Eddie, and Stevens (14) discovered that the disease was well established in Southern California in shell parakeets (love birds) These workers found that more than 1,100 aviaries engaged in breeding of birds and containing more than 100,000 birds existed as a "back yard industry" in that part of the country, and that nearly one-half of these were infected The existence of wide-spread pigeon infection in this country makes it evident that a permanent problem has to be faced Fortunately it appears that the virus of the pigeon infection is not nearly so virulent for man as that which exists in the psittacine birds

Character of the Disease

IN BIRDS. In the older birds of the *psittacidae,* the disease often is inapparent (13) Some of these birds eliminate no virus, or perhaps eliminate it only intermittently, but the inoculation of susceptible animals with organ suspensions indicates that they carry virus which is fully virulent. The clinical manifestations of psittacosis are seen mainly in the younger birds and these are the principal spreaders of the virus The affected birds refuse food, their feathers become ruffled, and they become weak and emaciated before they die Such birds commonly suffer from a severe diarrhea, and they usually have nasal discharges. These discharges contain virus. Death frequently occurs after a few days, or after several weeks, but some individuals recover Fully recovered birds usually eliminate virus for long periods, and these birds often are the source of human infections

The most conspicuous lesions in birds which have died from psittacosis are focal necrosis of the liver and spleen swelling A sterile fibrinous or purulent pericarditis is sometimes found, and petechiae often occur on the heart. The necrotic foci in the liver are small and numerous The spleen swelling is largely due to infiltration with monocytic cells. Occasionally necrotic foci occur in the spleen as well as in the liver.

Numerous minute coccoid bodies may be regularly found intracellularly in the macrophages and endothelial cells of the spleen, in the epithelial cells of the intestine, bile ducts, and kidneys and in the Kupffer cells and hepatic cells of the liver. These will be discussed below.

IN MAMMALS. In mammals the general characters of the disease are similar to those in birds except that an atypical pneumonia occurs. The pneumonia is lobar in extent but lobular in character. The affected lobes are mottled, showing both red and gray hepatization in the same lobules Fibrin, erythrocytes,

and leucocytes are present in the earlier stages but later these elements are replaced by monocytes and desquamated epithelial cells. The alveolar epithelium undergoes fatty degeneration and often contains the tiny coccoid bodies mentioned above. In man there is swelling of the liver and spleen but focal necrosis is not so commonly seen as in birds. In mice, inoculated intraperitoneally, focal necrosis of the liver is generally well marked.

Nature of the Virus. That the disease could be produced by filtrates made from organs of diseased birds was shown by Krumwiede, McGrath, and Oldenbusch (8) in the United States and by Bedson, Western, and Simpson (4) in England, working independently, early in 1930

The virus of psittacosis is filterable only through rather coarse filters. Membrane studies indicate that the particulate size of the virus is between 200 and 300 millimicrons, a size sufficiently great that the elements should be visible microscopically Since it happens that the coccoid bodies found in the lesions of parrots, mice, and men, are of about this size, and since there is a general relation between the concentration of these bodies and virulence, it is generally believed that they are actually the virus These bodies were found at about the same time and were described independently in 1930 by Levinthal (9) in Germany, by Coles (5) in England, and by Lillie (10) in the United States. They are generally known as L.C.L. bodies, the letters representing the initials of the names of these workers Lillie at first regarded them as rickettsiae, but now they are regarded as elementary bodies similar to the Borrel bodies of fowlpox and the Paschen bodies of smallpox These bodies sometimes are grouped in dense masses forming larger bodies which lie inside affected cells and which correspond to the inclusion bodies of the other diseases More often they are seen as separate tiny bodies which stain bluish with the Giemsa stain, and may be stained differentially with a number of special staining methods. These bodies are diagnostic of psittacosis and are helpful in diagnosis. They may be seen in sections, or more rapidly, in stained films

Artificial Cultivation. The elementary bodies of psittacosis may be propagated rather easily by most of the methods which have succeeded in the cases of other viruses Yanamura and Meyer (20), who surveyed this matter in 1941, reported successful cultivation of the virus in tissue fragments suspended in fluids, in tissue fragments spread over the surface of an agar medium, and in the yolk sac of developing chick embryos when virus is introduced by a technic developed by Cox (6) for the propagation of rickettsiae. It will also develop on the chorio-allantoic membrane of the chick embryo.

Transmission of the Disease. Natural infections of man apparently occur only by inhalation. Rivers and Schwentker (19) found that monkeys could

not be infected by fully virulent virus injected intravenously and intramuscularly, and later they used the same method for immunizing a number of human volunteer laboratory workers who were exposed to the risk of contracting the infection in the course of their work with this disease.

A considerable number of laboratory workers have contracted psittacosis. Most of these persons were handling infective material but in some cases infections occurred in persons who worked in the same building but who had no contact with infected birds [See McCoy (12), and Badger (1)].

Diagnosis. The diagnosis of psittacosis often presents some difficulties. Rivers and Berry (17) called attention to the value of the white mouse for this purpose. Intraperitoneal injection of filtrates of human sputum, or of unfiltered sputum, in case organisms are not present which kill the animals prematurely, usually kill these animals in from 5 to 14 days, occasionally as late as 30 days. If any of the mice sicken after the 4th or 5th day, they should be destroyed and examined for the characteristic focal necrosis of the liver Smears are made from the liver tissue and a search is made for the elementary bodies of psittacosis. If the mice are still living after 30 days, it is well at that time to inject them with known psittacosis virus to determine whether or not they may have developed immunity from an infection which did not become apparent.

The method described apparently is efficient for detecting the disease in man or birds of the parrot family, but the pigeon virus usually does not kill mice or produce the liver lesions. Virus from these birds can usually be recovered, however, by inoculating the mice intracerebrally instead of intraperitoneally. The pigeon virus is more highly virulent for pigeons than that from parrots. The parrot virus will, however, usually produce inapparent infections in pigeons (15).

Immunity. It has been pointed out that after recovery from the active disease, birds usually harbor the virus for long periods of time. During this time they possess a marked resistance to reinfection as might be expected. The virus tends to remain in mammals also for considerable periods after clinical recovery, and it has been suggested that the immunity in this disease is always due to the harboring of latent infection. This has not been proved to be the case, however.

Unlike many other virus diseases, neutralizing antibodies are not always demonstrable in animals immune to the virus and seldom are present in great concentration. Nevertheless, animals which have received virus intramuscularly without harm, cannot be infected by intratracheal inoculations which produce pneumonia in unprepared animals. Bedson (3) has shown that mice

can be partially immunized by the use of formalin inactivated virus. Animals so treated could be infected but the disease was benign.

Bedson (2) has carried on a series of studies on the immunology of psittacosis. He has shown that fixation of complement can be obtained using extracts of the washed elementary bodies as antigen, and that these bodies can be agglutinated by immune serum. Also he has made extracts of these bodies which flocculate with immune serum but which do not fix complement. He concludes that the virus of psittacosis is a complex body; that it contains at least several different antigenic complexes.

Although human beings can be immunized by parenteral injection of virulent virus, this method is not advocated for general use. No practicable methods for general immunization of man or animals have been developed.

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EQUINE INFECTIOUS ANEMIA

Synonym: Swamp Fever.

This is a virus disease which occurs naturally only in members of the horse family. It is characterized by a great diversity of symptoms and an exceedingly

variable course. In the acute attacks there generally is rapid destruction of blood corpuscles with resulting anemia, fever, and weakness. It is caused by a virus which is readily filterable and with which the disease can be produced by inoculation.

Infectious anemia of horses has been recognized in practically all parts of the world where horses are raised The name "swamp fever" is derived from the fact that the disease is most frequent in animals pastured on low-lying areas, however Scott (7) says that the disease is sometimes seen in Wyoming on lands 9,000 feet above sea-level.

The disease does not spread from farm to farm readily or quickly, but tends to occur enzootically on certain farms or areas. During periods of war when horses are assembled from many areas, the disease is likely to spread, and the return of such horses to civilian life is likely to set up many new foci. This is said to have occurred following World War I in Germany. Great losses formerly occurred in many of the northern and western states of the United States but in recent years the disease appears not to be so common in these areas In the Mississippi delta region many mules are lost from it. In the eastern part of the country the disease is not often recognized on the farms but biological supply companies have encountered many cases in serum-producing horses in which, presumably, latent cases are caused to become active through provocation by the injections of antigenic substances Udall and Fitch (10) recognized a focus of the disease in northern New York in 1914 It is evident that the disease is more widely spread in the United States than clinical reports would lead one to believe.

The greater number of acute and subacute cases are seen during the late summer and early fall months, a circumstance which fits in well with the idea that most initial infections occur from the bites of blood-sucking flies and mosquitoes.

Character of the Disease. Infectious anemia may appear in an acute, subacute, or chronic form Many cases have been described in which horses have harbored the virus for periods upward of ten years, during which they have exhibited only mild recurrent symptoms, or perhaps no symptoms. Many of these cases eventually develop acute symptoms, as a result of provocation the nature of which may or may not be recognized During the long latent periods the blood remains infectious as can be demonstrated by inoculating other horses (6). Some authors believe that recovery from this disease never occurs.

The acute cases of infectious anemia vary in duration from several days to several weeks and the subacute may last two or three months. The more

acute forms may lead to death, or they may lapse into what appears to be recovery but which is actually only the chronic form. Such animals are apt to have recurrences of the acute symptoms at variable intervals during any of which death may occur.

The symptoms of the periods of exacerbation are marked weakness and prostration, mental depression, a high continuous fever, slight icterus, petechial hemorrhages on the mucosa of the mouth and conjunctiva, edematous swellings of the limbs, rapid emaciation, and rapidly developing anemia. A number of authors have reported cases in which the anemia was not marked, however these cases are the exceptions and not the rule

The general lesions resemble those of a septicemia. Hemorrhages occur in most of the parenchymatous organs, the spleen generally is enlarged and softened, and its capsule is hemorrhagic. The lymph nodes of the abdominal cavity are swollen and usually their peripheries are infiltrated with blood from hemorrhages in the organs which they drain. Hemorrhages occur on the serous membranes, and on the mucous membranes of the intestine The kidneys and heart muscle show parenchymatous degeneration If the long bones are split lengthwise it will be seen that the yellow marrow has been replaced rather completely with red marrow, an indication of tremendous stimulation of hematopoiesis, an effort on the part of the blood forming tissue to compensate for the loss of erythrocytes from the circulation. In late stages of the acute disease there is great stimulation of the endothelial cells of the liver sinusoids, many of which are loaded with brownish, iron-containing pigment granules (hemosiderin)

Nature of the Virus. The virus nature of the causative agent was determined by Carré and Vallée (1) in 1904, and has been amply confirmed by many others The blood is infectious in all stages of the disease, and filtrates of Berkefeld or porcelain filters are about equally infectious. Virus is also found in washed blood cells, all parenchymatous organs, milk, urine, saliva, and feces. Studies on the particulate size of the virus have not been reported, but undoubtedly it is small.

The persistence of the virus in the blood of chronically affected, apparently normal animals is astonishing. Schalk and Roderick (6) have published an account of one case which, after a number of acute attacks during a period of three months following inoculation with infected blood, lived in apparent health for 14 years then suddenly developed acute symptoms and died. During this apparently normal period 18 other horses were inoculated with his blood at intervals of about one year and all but one of these developed acute symptoms of infectious anemia. During the first three years after inoculation

there were occasional febrile periods but during the remainder of the time until the final illness there were only rare febrile periods which may or may not have been due to the infection.

The virus of this disease may infect swine by inoculation. The disease may be fatal after a few days or weeks, but frequently the animals appear to recover. The virus persists in recovered swine for several months and perhaps longer. The virus also multiplies in rabbits, chickens, and pigeons after inoculation, and some German authors have claimed to have found virus in naturally infected birds on farms where horses were infected. Kobe (4) has reported recently that small pigs which have been splenectomized are especially suitable subjects for the inoculation disease

The virus contained in blood is quite resistant to heat, chemicals, and putrefaction. Dried blood retains virulence for some months if protected from sunlight. Thin layers are quickly inactivated by sunlight and thus it is believed that infected secretions, such as urine, will not usually remain virulent on pastures for long

Transmission. Infectious anemia is believed to be spread principally through the agency of insects, particularly of blood-sucking flies *Stomoxys calcitrans,* the common stable fly, is capable of transmitting the disease and perhaps is the principal agent. Scott (8), who studied the disease in Wyoming, believed that this fly was the principal agent concerned and in this the Japanese Commission (3) concurred. Luhrs (5), in Germany, believes that mosquitoes, particularly *Anopheles maculipennis,* are the chief offenders. Other blood-sucking flies, particularly Tabanids, probably are involved at times. Scott showed that infections in horses could be produced by a single prick with a hypodermic needle which had been infected by pricking an infected horse. This finding suggests that any type of insect which feeds first upon one animal and then another at short intervals could carry the disease.

There seems to be sufficient evidence to indicate that the disease may spread from one animal to another by simple contact without the intervention of insects Fulton (2) believed that he had produced cases by injecting horses with water from swampy areas in pastures where infected horses were kept, and there are accounts of infections in horses kept within screened buildings with infected animals, all animals being watered out of common containers and all being fed from the floor so there would be ample opportunity for contamination of food and water with infectious secretions. Inasmuch as infections can be produced, somewhat irregularly, by feeding infectious material, it is reasonable to believe that natural infections can occur more or less directly.

Several authors have suggested that some of the round-worms parasitic in

horses might play a part in the transmission of the disease. Stein, Lucker, Osteen, and Gouchenour (9) tested this hypothesis by injecting emulsions of washed parasites from animals suffering from infectious anemia. Of a number of such experiments they succeeded only once in obtaining an infection. In this case the infecting material consisted of an extract prepared from strongyles.

Artificial Cultivation. There are no reports of successful cultivation of the virus of equine infectious anemia in artificial media.

Diagnosis. Clinical diagnosis of this disease offers many difficulties. It is now well recognized that many apparently normal animals are virus carriers, and the only way that these can be detected with reasonable certainty is by inoculation of their blood into other horses. This is expensive and in areas where the disease is enzootic there is danger, of course, that the animal selected for inoculation already carries the infection and therefore will not react. If the horse selected is susceptible, the inoculation with blood will give rise to a febrile reaction which occurs at some time within a period of 12 to 90 days, usually between 15 and 30 days. During the febrile reaction there usually is a rapid decrease in the number of erythrocytes in the blood, a fall in hemoglobin, an increase in the sedimentation rate, and an increase in the globulins of the plasma, but none of these changes are invariable and therefore cannot be depended upon for diagnosis.

The inoculation of swine, rabbits, and birds has been suggested as diagnostic means but it is apparent that these measures are not reliable.

Immunity. Means of immunization against this disease have not been developed. The control of the disease is an unsolved problem, especially because there are no means of accurately determining carrier animals which are foci of infection.

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EQUINE VIRUS ABORTION

Dimock and co-workers (2) (3) (4) in Kentucky believe that there is a form of epizootic abortion in mares caused by a virus. The belief is based upon the facts that the disease manifests itself somewhat differently from the bacterial abortion in this species, that no bacteria are to be found in the organs of the aborted fetuses, that the blood of the mare is negative to the agglutination test for *Bact. abortivo-equinus* infection, that immunization of the mares, which usually is quite successful against the bacterial abortions, does not prevent this disease, and finally that certain characteristic intranuclear inclusion bodies are found in cells of the liver and bronchial epithelium. Anderson and Goodpasture (1) have strengthened the case a great deal by demonstrating that new-born hamsters are susceptible to inoculation with the bacteriologically sterile tissues of aborted fetuses and that in the tissues of these animals inclusion bodies identical with those of the equine fetuses can be identified. Goodpasture and Anderson (5) also have succeeded in cultivating the virus on human amnion which had been grafted to the chorio-allantoic membrane of the developing chick embryo.

Character of the Disease. The virus abortion disease presents certain differences from that caused by bacteria The mare appears to suffer practically no injury; the genital tract returns to normal practically as soon as those of normal animals The fetuses show lesions which are not usually seen in bacterial abortions. These consist of multiple minute areas of focal necrosis in the liver, hemorrhages in the heart muscle, spleen, and liver, and an excessive amount of fluid in the chest cavity Not all of these are found in every fetus The liver necrosis, however, is a rather constant finding.

Nature of the Virus. The virus of this disease has not been extensively studied, hence little is known of its properties Dimock and Edwards (4) produced abortions in pregnant mares by inoculating them intravenously with Berkefeld filtrates of infected fetal tissue.

Pathogenicity for Experimental Animals. Dimock and Edwards (3) were not successful in causing infections of experimental animals, except possibly in the case of pregnant guinea pigs In several instances abortions were caused in this species by intraperitoneal injection of equine fetal material. The tissues of the aborted fetuses were bacteriologically sterile and no lesions were found. These experiments were done before the inclusion bodies had been recognized, hence they may have been present in the guinea pig fetuses. Anderson and Goodpasture attempted to cause abortions in pregnant hamsters without success. It is possible that the inoculations were made too late in the period of pregnancy. They then inoculated new-born hamsters intraperitoneally with

equine fetal liver tissue. Three days later one of the litter of four was missing, on the 9th day two partially eaten bodies of the litter were found and on the 12th day the fourth member was missing. The hamster mother has the habit of eating her young when they die. Sections showed areas of focal necrosis in the livers and heart muscle and in these intranuclear inclusion bodies like those of equine fetuses were found. In later experiments similar results were obtained Apparently new-born hamsters usually die in from three to five days after inoculation with material containing this virus. The virus was maintained for three generations in new-born hamsters.

Artificial Cultivation. Goodpasture and Anderson failed to obtain growth of the virus of this disease on the chorio-allantoic membrane of developing chick embryos, and they state that a personal communication from Dimock indicated that he had similarly failed They succeeded, however, in obtaining growth of the virus on human amnion which had been grafted on the chick membrane Here inclusions were found in some of the epithelial cells, necrosis of others occurred, and the infected areas were surrounded by zones of inflammation As a result of this experience, the authors warn that it is probable that this virus is pathogenic for man.

Inclusion Bodies. The inclusion bodies produced by this virus are always intranuclear. They are acidophilic and located near the center of the nucleus, the chromatic material of the nucleus being dispersed around the margins. Usually they are homogeneous but occasionally granular. These bodies are found most abundantly in liver cells near the margins of the areas of focal necrosis in the liver They also occur in the bronchial epithelium. In artificially infected hamsters they occur in liver cells and in heart muscle cells in the areas of focal necrosis.

Transmission of the Disease. Nothing is known about the mode of transmission of this disease. Lesions of the placenta have not been described, however it is probable that they occur, and that the discharges of the aborting mare are infectious.

Immunity. Dimock and his co-workers have been experimenting with formalinized emulsions of infected fetal liver as a vaccine for preventing this disease. No conclusions have been drawn as to the efficacy of the method.

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MALIGNANT CATARRHAL FEVER

Synonym: Malignant Head Catarrh of Cattle.

Malignant catarrhal fever affects cattle principally. In this country it has been diagnosed only in this species, but abroad it is said to affect buffalo, possibly sheep, and African wildebeest. In the latter, symptoms of disease do not occur but Mettam found that they carried virus and served as a reservoir of infection for cattle. It is probable that more than one disease entity is included under the name of this affection At least one form of the disease is caused by a filterable virus.

Character of the Disease. The disease usually occurs during the spring and fall months. Most cases are sporadic but it sometimes occurs as a herd disease. On some farms it occurs year after year and causes severe losses.

The disease is characterized by an initial febrile reaction The animal refuses food and water and is greatly depressed. Inflammation of the conjunctival and nasal mucous membranes appear early There is photophobia, lachrymation, injection of the sclera, cloudiness, and even ulceration of the cornea. The nasal mucosa becomes deep red in color, edematous, and covered with a fibrino-purulent exudate. Ulceration of the nasal mucosa occurs, the breath becomes fetid, and there may be nasal hemorrhages A similar process occurs in the mucosa of the mouth and often of the pharynx. Breathing may be difficult, and fibrino-purulent pneumonia may develop.

Nervous symptoms develop early in most cases Usually these take the form of stupor but sometimes there is excitement. The animals grind their teeth, bellow, and may charge against walls, mangers, and other objects The animals lose condition rapidly. Death ensues in most cases in from three to seven days.

The lesions, except those that are referable to the general effects of fever, are largely those of the mucous membranes of the head described above. The dark red, swollen, glassy membranes of the nasal passages and sinuses are covered with shreds of fibrin and a dirty purulent secretion. Ulceration is often severe. Similar lesions frequently occur in the larynx, trachea, and larger bronchi, and there may be areas of broncho-pneumonia in the anterior lobes of the lung. The mucous membrane of the abomasum usually is inflamed and sometimes is ulcerated and lined with fibrinous exudate. Similar lesions may also occur in the intestine. The meninges often are congested and may show hemorrhages but the brain itself ordinarily is normal in appearance.

Histologically, however, there is a non-purulent diffuse encephalitis of the virus type.

Nature of the Virus. The virus of malignant catarrhal fever is not readily filterable and some authors, Mettam (3) in South Africa for example, deny the filterability of the causative agent and yet believe it to be a virus. In blood the virus is closely attached to the blood corpuscles and cannot be washed off of them. Blood inoculation does not invariably produce the disease, in fact the German and French workers who have conducted inoculation experiments are in agreement that infections are produced in not more than one half of the animals inoculated. It is not clear in the literature whether blood for inoculation was taken from animals very early in the course of the disease, or later when the symptoms are more pronounced. Experience in recent years has shown that there are many virus diseases in which the filterable agent is present during the early, febrile period of the disease, and disappears by the time the symptoms are well developed This may be the case in this disease.

Transmission. The mode of transmission of the disease is not known. Attempts to transmit the disease with nasal and other secretions of affected animals have failed, and the sporadic character of the disease makes it clear that direct transmission does not occur readily. Several German workers believe the disease is transmitted to cattle from sheep, the sheep not being clinically diseased, however it often occurs in cattle which have not been in contact with sheep, hence such a mode of transmission cannot be the sole one if it occurs at all. Mettam believed that the South African "snotziekte," which is thought to be catarrhal fever, was transmitted to cattle from wildebeest, although the latter show no symptoms of this disease. Insect transmission is improbable because of the relatively low concentration of virus in the blood. It is possible, however, as has already been suggested, that larger concentrations occur very early in the course of the disease.

Artificial Cultivation. The virus of this disease has not been propagated artificially.

Immunity. Virtually nothing is known about immunity to malignant catarrhal fever. No practicable methods of specific treatment have been developed.

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FOWL PLAGUE

Synonym: Fowl Pest.

Fowl plague is an acute, highly fatal disease of chickens, turkeys, pheasants, and certain wild birds. Ducks, geese, and other waterfowl are less susceptible but develop the disease at times. Pigeons ordinarily are not susceptible, a point which is of importance in distinguishing fowl plague from Newcastle disease. The symptoms and lesions of fowl plague are similar to those of fowl cholera. It is caused by a virus which occurs in the blood and all organs and is readily filterable.

Fowl plague has been known since about 1880 when it was recognized in Italy as a separate disease entity. Early in the present century it spread throughout the greater part of Europe. The virus was brought into the United States illegally in 1923 by a laboratory worker. In the fall of 1924 the virus escaped from the laboratory into the New York poultry market where it has been estimated to have killed more than one-half million birds (4) From this market, it spread to a considerable number of eastern poultry farms, probably on contaminated crates of dealers, causing large losses. The disease was stamped out within one year by rigid quarantine methods

The disease has been reported in oriental countries but it is probable that the diagnosis was confused with the pseudo-fowl plague or Newcastle disease which is known to be prevalent there.

Character of the Disease. The period of incubation is rather short—3 to 5 days as a rule Inoculated birds may show symptoms within 24–36 hours. A high temperature rapidly develops (110–112° F), the appetite is lost, and the birds rapidly become lethargic The comb and wattle commonly become bluish-black in color. A mucoid nasal discharge appears, and often edema of the head and neck develops. The course of the disease is very rapid, death usually occurring within a few hours after the appearance of the first symptoms The temperature commonly falls to subnormal shortly before death. The mortality rate is close to 100 per cent.

The lesions generally are not numerous. They consist of petechial hemorrhages on the heart, on the fatty tissue around the gizzard, on the serosa of the body cavity, and on the mucous membranes of the proventriculus In some cases a sero-fibrinous exudate appears in the pericardial sac. The principal organs may show petechiae, and cloudy swelling. The nervous system appears normal but microscopic examination shows a diffuse encephalitis

with cuffing of the blood vessels, degeneration of nerve cells, and necrotic foci around which there is proliferation of glia cells.

Nature of the Virus. The filterability of the causative agent of fowl plague was first demonstrated by Centanni and Savonuzzi (2) in 1902. The virus is present in the blood during all stages of the disease, in all organs and tissues, and in all secretions and excretions. The concentration in the blood is great since as little as 0.000,001 cc. will regularly infect by inoculation parenterally. The virus readily passes both Berkefeld and porcelain filters Elford and Todd (3) by ultra-filtration studies estimated its particulate size as between 60 and 90 millimicrons.

The virus is unusually resistant. Infective blood may be kept in the refrigerator for many months, especially if it is mixed with glycerol. Dried blood or tissues remain virulent for a year or more in the refrigerator and for several weeks or months at room temperature if protected from sunlight.

Artificial Cultivation. Burnet and Ferry (1) were successful in cultivating the virus of fowl plague on the chorio-allantoic membrane of developing chick embryos. Plotz (5) cultivated the virus for more than 100 generations in this way and found that the virulence for chickens was not impaired.

Transmission of the Disease. Transmission of the virus is believed to occur through ingestion since the disease can easily be produced by the feeding of infected materials. Infections may also occur through contamination of slight wounds or of the conjunctiva. External parasites (mites and lice) may also play a part.

Immunity. Only a few birds recover from the disease. These birds are solidly immune for several months at least and possibly for life.

The serum of recovered birds will give a considerable degree of immunity to susceptible fowls but since the immunity is short-lived and the amount of such serum which can be obtained from immune birds is small, the method has no practical value.

There is a rather large European literature on vaccines for fowl plague. Attempts have been made to produce vaccines from blood and from tissues. Treatment with heat, phenol, glycerol, ether, and formaldehyde will weaken and finally destroy the virus. Vaccines in which active virus exists are apt to produce the disease, and those in which the virus has been wholly inactivated have conferred little immunity. Various authors have reported success with one or another of these vaccines but in the hands of others they have met with failure. The matter can be summed up by saying that no successful vaccines, useful on a large scale, have been developed.

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NEWCASTLE DISEASE

Synonym: Pseudo-plague of fowls.

This disease was first recognized by Doyle (2) in England in 1926. The first diseased flock studied was in Newcastle-on-Tyne and for this reason it received the name by which it is best known. In later years the disease has been recognized in other parts of the world, particularly in the orient, where it causes great losses. It has spread widely in the Philippine Islands, according to Coronel (1), since 1927 when it first appeared in Manila. The disease has been recognized in India, Java and Japan. Doyle and most other workers regard this disease as a separate entity from fowl plague but Manninger (4) regards it merely as an attenuated type of plague. All who have worked with it admit that the disease is very similar to plague, in symptoms, lesions, and character of the causative agent.

Some of the differences between the two diseases are pointed out by Doyle and they may be summarized as follows:

1. In about 70 per cent of birds affected with Newcastle disease, respiration is of a gasping type through the half opened beak. This is not seen in plague.
2. The period of incubation in Newcastle disease is longer than in plague. In artificially inoculated birds it is about five days in the former and only 24 to 48 hours in the latter. Furthermore the period of visible illness in the former is two to three days or longer, whereas in plague it is a matter of several hours at the most.
3. The lesions in Newcastle disease are even less marked than in plague. The petechial hemorrhages may occur, but usually they are few in number and often are wholly absent. A sero-fibrinous exudate is found, in many cases of plague, in the pericardial sac and sometimes in the body cavity. This is never found in Newcastle disease.
4. There is marked difference in the virus potency of the blood in the two diseases. Whereas that of plague is always highly virulent, as little as 0.000,001 cc. of defibrinated blood being regularly infective when injected subcutaneously into fowls, in Newcastle disease the highest potency en-

countered was 0.000,04 cc. and often blood removed at the height of the disease has failed to infect when inoculated in much larger doses.

5. Healthy birds placed in a cage with a plague-infected bird do not ordinarily contract plague from it. Healthy birds placed in contact with a bird infected with Newcastle disease invariably develop the disease. Also, birds placed in cages in which Newcastle disease has occurred within 48 hours usually contract the disease, whereas they seldom do under the same circumstances when the disease is plague.

6. Finally, and most convincingly, there is no cross immunization between the two diseases. Only a few birds recover from either of these diseases, but those that do are immune to the same disease but not to the other.

It has already been pointed out that fowl plague is not ordinarily transmissible to pigeons (except very young birds) and that Newcastle disease is regularly infective for this species.

In spite of the differences indicated, it seems most likely that Newcastle disease is produced by a virus which is a variant from the fowl plague virus, a variant which is less pathogenic for chickens. Manninger (4) in Austria, and Nakamura, Oyama, and Wagatsuma (5) in Japan claim to have succeeded in altering the Newcastle disease virus by passage through fowls so that its pathogenicity was greatly increased, that virus was regularly present in the blood in great concentration, and that immunological differences from plague virus largely disappeared.

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AVIAN INFECTIOUS LARYNGOTRACHEITIS

A variety of diseases of the respiratory tract of birds were, until a few years ago, grouped together under the name of "roup." These have been differentiated during recent years into nutritional, bacterial, parasitic, and virus disorders. Infectious laryngotracheitis was shown to be a specific virus disease by Beach (1) in 1930. First recognized in the United States it is now known to exist in nearly all parts of the world where poultry are kept. The disease affects chickens and occasionally pheasants. Other types of birds are wholly immune.

Character of the Disease. Infectious laryngotracheitis affects chickens of all ages. It is highly contagious and when it enters a susceptible flock the disease does not stop until practically every bird has been attacked. Infection occurs through the respiratory tract. The incubation period is short, less than 48 hours as a rule. The course of the disease is acute, some birds dying within 24 hours of the time the infection is first detected, others running a course as long as 5 or 6 days. Birds which do not die during the first 5 days of symptoms practically always recover Recovery generally is rapid. Although no single bird will show evidence of disease for as long as a week the disease may require three or four weeks to run through a flock. An important fact is that a considerable number of recovered birds continue to harbor the virus and such birds usually act as centers of new infections when transferred to new flocks, or when new birds are added, perhaps a season or more later [Gibbs (9)].

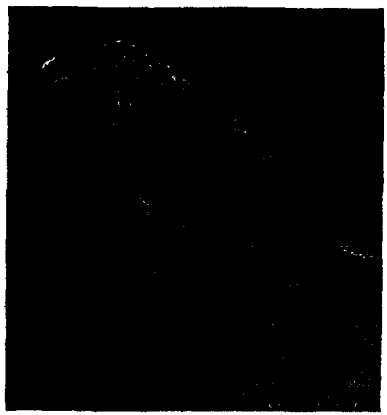


FIG 137 Infectious Laryngotracheitis Showing the characteristic gasping type of respiration (Courtesy of E L. Brunett)

The symptoms depend upon the age of the birds and the season of the year. Young birds during the warm months usually are less severely affected and the mortality rate is lower than in older birds during cold weather Affected birds show respiratory embarrassment varying in degree. In severe cases the chickens extend their heads, open their mouths, and inhale in a gasping manner. Gurgling and rattling sounds are often heard. Sometimes it is best described as whistling. These sounds are due to partial obstruction of the air passages by exudate. Not all birds show respiratory embarrassment, since the exudate sometimes is in the nasal passages, or nasal sinuses. In mature birds in heavy production the mortality rate may be as high as 70 per cent. Death appears to be due largely to suffocation.

The lesions are confined to the larynx, trachea, and bronchi. These are red dened, petechiated, and covered with a slimy exudate containing streaks of bright red blood. Sometimes the exudate is of a caseous nature, and plugs of such material may wholly block the trachea.

Nature of the Virus. Beach (1) who first identified the virus of this disease found that it could be filtered readily through Berkefeld V filters but not in

every case through Berkefeld N filters. Gibbs (8) estimated the size of the virus elements as between 45 and 85 millimicrons. The virus is present only in the air passages and is infective only by introduction in this way, except that rarely infections can be established by intravenous inoculation. Injections subcutaneously, intramuscularly, and intraperitoneally are harmless.

The virus is moderately resistant Premises do not retain effective quantities of virus for long after infected and carrier birds are removed, however. Beaudette and Hudson (4) reported that egg-propagated virus, when dried and kept in a refrigerator, retained its potency and immunizing properties for 421 days.

Artificial Cultivation. The virus of infectious laryngotracheitis was cultivated on the chorio-allantoic membrane of developing chick embryos by Burnet (5) in 1934, by Brandly (7), and many others shortly afterwards. The virus produces whitish plaques on the membrane. Two kinds of plaques have been described but immunologically the viruses appear to be identical. Diluted viruses, according to Burnet (6), can be roughly assayed for virulence by counting the number of plaques produced per volume of virus.

Transmission of the Disease. The disease is transmitted naturally through droplet infection.

Diagnosis. Ordinarily after the disease is well under way in a flock the diagnosis is easy on the basis of symptoms and lesions. In individual birds it may not be so simple If the question is sufficiently important to warrant the trouble the answer can be obtained by swabbing the larynx of one or two susceptible birds. These birds should develop symptoms sometime between 2 and 5 days later if the disease is laryngotracheitis. If immunized birds are included in the test, they should prove resistant while the others sicken.

Immunity. Birds which recover from this disease are solidly immune for the remainder of their lives. Many of these birds are virus carriers, hence when the disease has once occurred in a flock, annual recurrences of the disease must be expected in the young stock unless they are artificially immunized. In small flocks it is often simpler and less expensive to dispose of all old stock.

Beaudette and Hudson (3) developed a method of actively immunizing birds to infectious laryngotracheitis which has proven to be very successful. After trying modified viruses with unsatisfactory results, they hit upon the idea of using fully virulent material on the mucous membrane of the Bursa of Fabricius, which is an outpouching of the cloaca. In this location the virus sets up a harmless inflammatory reaction which immunizes solidly. This method has been extensively employed. Its principal objection is that it utilizes

a fully virulent disease-producing agent which, if carelessly used, may do much damage. It should not be used until it is absolutely certain that the flock already contains the infection. Full immunity is not developed until about nine days after treatment.

The virus for the cloacal method of vaccination may be obtained directly from the trachea of diseased birds, or it may be virus which has been propagated on egg-embryo membranes. Commercially the virus usually is dried and shipped in sealed vacuum tubes with a separate container of glycerol-water mixture in which to dissolve it prior to use. The virus mixture is applied with a small swab or brush directly on the mucous membrane of the bursa, taking care not to soil the feathers or to spill any vaccine. Five days later it is well to catch and examine a few birds to make sure that "takes" have occurred. If the vaccine has been potent and the work properly done most of the birds will show swelling, inflammation, and a small amount of exudate at the point of inoculation. If the vaccine has not produced a high percentage of "takes," the flock should immediately be revaccinated with fresh vaccine, since virus has been introduced into the flock and a serious outbreak of tracheal infections is sure to develop otherwise.

In flocks in which the disease has occurred in previous years, vaccination of the young stock should be done in the summer months after the birds are at least 6 weeks of age, preferably when they are about 4 months old. All birds on the premises must be vaccinated, of course.

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AVIAN INFECTIOUS BRONCHITIS

Synonym: Chick Bronchitis.

This disease was first described by Schalk and Hawn (4) in 1931. These authors did not determine its cause. In 1933 it was studied by Bushnell and Brandly (2) who carried on some filtration studies as a result of which they decided that a filterable agent was its cause. More extensive studies were re-

ported by Beach and Schalm (1) in 1936 which showed that the causative agent was a virus which differed in several respects from that of infectious laryngotracheitis. This disease has been reported only from North America, where it frequently causes serious losses.

Character of the Disease. This disease occurs in chicks from two days to three or four weeks of age. Older birds can be infected artificially but they are more resistant and seldom become infected naturally. The disease occurs principally in hatcheries, and from them is spread to flocks through the sale of young chicks. It causes large losses in establishments engaged in raising birds for the broiler trade, since in these plants large numbers of young birds are raised in greatly crowded conditions.

The disease is characterized by listlessness, depression, gasping, and by such rapidity of spread that nearly all exposed birds develop the disease at almost the same time. The outbreak runs a rapid course and the mortality rate is from 50 to 90 per cent. Exudate which may be mucoid or caseous is regularly found in the bronchi and sometimes in the nasal passages In chicks that die, plugs of fibrino-purulent exudate often are found in the lower part of the trachea, or in the larger bronchi Chicks that are inoculated into the trachea develop the disease after an incubation period of 24 to 48 hours as a rule, occasionally as long as 4 days, and death or recovery occurs in from 6 to 18 days.

Nature of the Virus. Beach and Schalm found that the virus was readily filterable. It passed readily through all grades of Berkefeld filters although with considerable loss of potency. It will be recalled that the virus of laryngotracheitis passes only through the coarser grades of these filters.

Virus can be recovered from the blood, spleen, liver, and kidneys, and typical infections can be obtained by inoculating birds subcutaneously, intramuscularly, intraperitoneally, and on the mucous membrane of the Bursa of Fabricius. In all of these respects it differs from laryngotracheitis virus. Various strains of chick bronchitis virus are neutralized by serum of recovered chicks, and such chicks cannot be reinfected with bronchitis virus but are susceptible to the virus of laryngotracheitis. Conversely, birds that are immune to laryngotracheitis virus can be infected with the virus of chick bronchitis.

Beach and Schalm found that dried virus, stored in the refrigerator, retained its potency for at least 180 days. Virus stored in 50 per cent glycerol became inactive after 80 days.

Artificial Cultivation. Delaplane and Stuart (3) reported success in cultivating this virus on embryonated eggs in 1941.

Transmission of the Disease. Infections can readily be induced by introducing minute amounts of virus into the respiratory apparatus. Natural infections, it seems certain, are contracted through inhalation of infective droplets. Virus carriers have not been described.

Immunity. No practicable methods of immunization to this disease have been developed. Since the disease begins so early in life, and the unit value of the stock is so low, it is not likely that such methods will be developed. Passive immunization was accomplished by Beach and Schalm experimentally. The disease is best controlled by disposing of all infected stock, disinfecting the premises thoroughly, and beginning again with uninfected stock.

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PULLET DISEASE

Synonyms: Blue Comb; X-disease

This disease has been recognized as an entity by poultry pathologists in the New England states for a number of years It has been diagnosed, also, in New York, Pennsylvania, Delaware, and Maryland It probably exists elsewhere in the eastern part of the United States. The best description of the disease is that of Jungherr and Levine (1) who, however, did not venture to decide as to its causation. Waller (2) claims to have isolated a virus which he has cultivated on the chorio-allantoic membrane of the developing chick embryo, and with which he has successfully produced many of the features of the disease. Since the inoculated birds did not die whereas the mortality in the natural disease is relatively high, there is a question as to whether Waller's virus is actually the cause of the disease.

Character of the Disease. The disease usually appears in the summer months among pullets which have been recently housed and are in egg production. It is manifested by a sudden decrease in food consumption and egg production, a diarrhea, a darkening of the comb, dehydration of the tissues, and distention of the crop with liquid or food which has a very sour-smelling odor. Some birds show high temperatures and others are normal or even subnormal.

The lesions consist of degenerative areas in the liver, pancreas, kidneys, and skeletal muscles. Punctiform hemorrhages usually are found on the serous surfaces. The muscular lesions consist of areas of Zenker's degeneration. The

organ lesions may or may not be visible to the unaided eye. Jungherr and Levine were inclined to regard the disease as one primarily of the liver and kidneys, and of a uremic nature. Few data are given to support this conclusion.

The disease is seen most often in birds 5 to 7 months of age in production. The mortality varies from negligible to more than 50 per cent in different flocks.

Transmission. The mode of transmission of the disease under natural conditions is not known. Quite commonly a large part of the birds of susceptible age develop the disease at about the same time, and this fact is of value in arriving at a diagnosis. Waller states that he was able to induce symptoms suggestive of pullet disease very readily with his egg propagated virus. In from 84 to 96 hours the inoculated birds become depressed and cyanotic. The inoculated birds do not die, but if they are sacrificed in from 96 to 120 hours the following gross lesions may be recognized: subcutaneous edema, icterus, hemorrhages into the skeletal muscles, congestion and swelling of the liver and kidneys, collection of urates in the ureters, petechiation of the serous membranes of the heart and duodenum, sub-periosteal hemorrhages of the flat bones, hemorrhages into the lungs, and catarrhal inflammation of the duodenum.

Immunity. Nothing is known with regard to immunization against this disease.

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CHAPTER XLIV

VIRUS DISEASES CHARACTERIZED BY TUMOR FORMATION

The Transmissible Chicken Tumors

In 1908 Ellermann and Bang (3) produced evidence to indicate that a leukemic condition of fowls could be transmitted to other fowls by the inoculation of cell-free filtrates. In 1910 Rous transmitted a spindle-celled sarcoma by inoculation and in the following year showed that cell-free filtrates carried the tumor-inducing agent. Up to 1933 when their paper was written, Claude and Murphy (1) reported that no less than twenty-seven different types of tumors of chickens had been proved to be transmissible Not all of these had been transmitted by filtrates but the authors listed 19 instances in which they felt that evidence of filtrate-transmission was satisfactory.

Earlier most pathologists were inclined to look upon these transmissible tumors not as true neoplasms but rather as types of granulomas This idea has now disappeared for there is nothing in the morphology and behavior in the host animal to distinguish many of the transmissible from the non-transmissible tumors

Most of the transmissible tumors of chickens may be regarded as laboratory curiosities rather than as economically important problems. Except for the leukemias which sometimes occur in unusual numbers in certain flocks, a fact which suggests that they are naturally transmissible, there is little evidence that these tumors behave like infectious diseases in flocks. Their principal importance is the light which their study has thrown on the etiology of tumor formation in general.

The filterable agents which induce the formation of tumors are generally looked upon as viruses. Considerable progress has been made in the purification of some of these viruses, particularly that of the Rous sarcoma which has been studied more extensively than the others. The evidence indicates that the causative agent of this tumor is a comparatively simple chemical substance which withstands more physical and chemical manipulation than most other animal viruses.

Discussion of all of these tumors will not be undertaken here. Those who wish to pursue the matter further will find much information about them

in the publications of Rous, Murphy, Furth, and their associates, the greater part of which are to be found in the Journal of Experimental Medicine.

FOWL LEUKOSIS

Synonyms: Fowl leucosis; Fowl leukemia.

Fowl leukosis is regarded as a neoplastic disease since it is characterized by unrestricted multiplication of certain cells of the hematopoietic tissues. These immature cells circulate in the blood stream and tend to stagnate in the vascular bed of the liver, spleen, and kidneys where they multiply and cause enlargement of these organs.

The disease is primarily one of the myeloid tissue of the bone marrow; the changes in the blood and visceral organs are secondary. The air spaces and fatty tissue of the normal marrow are replaced by grayish-red tissue consisting of proliferating myeloid tissue Sometimes the proliferating cells are precursors of the granulocytes in which case the disease is termed granuloblastic or myeloid leukosis; in other cases they are erythroblastic cells in which case the disease is called erythroblastic leukosis, or erythroleukosis. The filterable agent which acts as the stimulant may cause proliferation of either type, hence if a number of birds are inoculated with blood of a single bird suffering from either type, some of them are likely to develop one type and some the other. Thus Stubbs and Furth (8) inoculated 25 fowls with material from two chickens affected with leukosis, one of which was of the myeloid type and the other of the erythroid. Thirteen of these birds (52 per cent) developed leukosis. Both types were represented in the series, being distributed through both groups of birds irrespective of the type used for inoculation. Ellermann and Bang (3) earlier had found that a single virus might produce both types of the disease.

Character of the Disease. The affected bird becomes weak and anemic. The comb and wattles are pale and the bird nearly always dies. The liver, spleen, and kidneys are found to be moderately enlarged and pale in color, the blood is thin and watery, and petechial hemorrhages are usually found in the loose areolar tissue and in the mucosa of the intestines. In practically every case, the immature myeloid cells, previously mentioned, are found in large numbers in the circulating blood and the red cells are present in greatly reduced numbers.

Nature of the Virus. The filterable agent of fowl leukosis can be carried from bird to bird indefinitely by intravenous inoculation, but not all birds are susceptible. Ellermann and Bang found that from 20 to 40 per cent of chickens were susceptible to inoculation. The barred Plymouth Rock breed apparently is somewhat more susceptible than others. When the virus is passed from bird

to bird by blood inoculation, the virulence often increases as indicated by a shortening of the incubation period and sometimes by an increase in the percentage of birds in which "takes" are obtained. In the beginning of a series of passages the incubation period may be several months in length. This may be shortened to less than one month after a few serial passages.

According to Furth and Miller (6), the agent of leukosis passes all silicious filters. Collodion membrane filtration is uncertain but the authors thought that their results indicated that the particulate size of the virus was somewhat less than 100 millimicrons. In blood the virus is divided about equally between the plasma and the corpuscles Furth (4) found that as little as 0 000,001 cc. of plasma was sufficient to produce the disease by intravenous inoculation He also found that the infectious agent retained its potency for at least 54 days when dried, and for at least 104 days when preserved in glycerin.

Transmission. The mode of natural transmission is not known. It is likely that blood-sucking insects are responsible The disease does not ordinarily transmit naturally between chickens kept in experimental lots in the same or neighboring cages. Naturally the disease occurs sporadically, thus indicating that its contagiousness is low. Stubbs and Furth (9) reported on a certain farm, however, where the disease was fairly prevalent. Most of our knowledge of this disease has been acquired from the study of comparatively few strains of virus which have been propagated by artificial inoculation. The disease is unimportant economically.

Artificial Cultivation. There are no reports of the successful cultivation artificially of the virus of leukosis.

Immunity. Practically nothing is known about natural immunity to this disease since affected birds seldom recover. It is obvious, however, that many birds possess a natural resistance of rather a high order Ellermann (2) did not succeed in actively immunizing a group of birds which he inoculated subcutaneously with virulent blood.

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THE INFECTIOUS SARCOMATA OF CHICKENS

Rous (3) in 1910 described the first of a series of transplantable, malignant sarcomas of the chicken. In 1911 (4) he showed that the tumor could be induced with dried cells, with cells that had been destroyed with glycerol, and with cell-free filtrates The tumor is now known as the Rous sarcoma I. It is a spindle-cell sarcoma which metastatizes freely and usually destroys its host within one month. This tumor has been extensively studied. In 1912 Rous, Murphy, and Tytler (5) described another chicken tumor transmissible with filtrates, an osteochrondosarcoma, and a third, a spindle-cell angiosarcoma. A considerable number of additions have been made to the list in later years.

The active principle of these tumors, particularly of Tumor I, has been extensively studied by Murphy and co-workers (1) (2), and by Sittenfield, Johnson, and Jobling (6). The active agent has not been isolated in a pure state but relatively pure extracts have been made by chemical precipitation. It seems quite clear that the viruses of these tumors are relatively stable chemical agents. They possess the power of stimulating neutralizing antibodies when injected into rabbits and in other respects do not differ in behavior from viruses which cause infectious diseases.

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Papillomas of Animals (Verruca vulgaris)

Papillomas, or common warts, occur in all species of animals. They seem to be most frequent in man, cattle, dogs, and rabbits, and all of these tumors contain filterable agents with which the tumors may be transmitted to other individuals. Schultz (2) in 1908 claimed to have transmitted warts of cattle to man. Except for this report, no information has been found bearing on the question of whether or not warts of the different species are due to a single virus or whether there is a virus specific for each host. Warts occur in epizootic form at times in herds of cattle and in kennels of dogs. As a rule, they are con-

fined to the species in which they begin, hence it is clear that there is a considerable measure of host specificity in them. In man and all animals warts occur principally in the young. Adults are seldom affected.

BOVINE PAPILLOMATOSIS

Warts frequently occur in calves and young stock less than two years old. They appear most often in the winter months when the animals are closely housed. The head, especially the region about the eyes, is most often involved, but they appear on the sides of the neck and less often on other parts of the body. They do not often occur on the legs. They appear first as small nodular



FIG 138 Bovine Papillomatosis (Warts).

growths which develop slowly for a time and then often grow rapidly into dry, horny, whitish, cauliflower-like masses which finally fall off as a result of dry necrosis of their bases. Sometimes hundreds of these masses occur on a calf at the same time. The size varies from small ones no larger than a pea to confluent masses several inches in diameter. Such warts have been seen along the sides of the neck beginning at points where blood samples have been drawn from the jugular vein, an indication that an infected bleeding needle has been the transmitting agent. Warts have also been found in the nasal openings

of many animals in the same herd, apparently transmitted by the fingers of persons who have held the animal or by a bull-lead which has been used as a means of restraint. In adult animals warts are found most often on the teats and udder.

The losses from warts are considerable. In young animals affected with many of these tumors, growth may be retarded. The greatest losses are in damages to the hides of slaughtered animals. Frequently the owner is most concerned by the reduction in the sales value of warty animals, especially in pure-bred stock.

Nature of the Virus. Very little is known about the causative agent of bovine warts except that it is filterable. Creech (1) inoculated 11 calves with ground unfiltered wart material and 11 additional with filtrates of the same materials from Berkefeld N filters. The filtrates were bacteriologically sterile. Eight "takes" were secured with the unfiltered material and seven with the filtered. The inoculations were made by scarification and intradermal injections.

Artificial Cultivation. The cultivation of the causative agent of cattle warts has not been accomplished.

Transmission. The mode of transmission of warts is unknown. It has been pointed out above that there are indications that transmission may occur through the handling of the animals by people and by needles used for bleeding. Presumably they may be transmitted by friction between warty animals and others with which they come in contact. Often in the same pen, animals may be found with extensive crops of warts and others of the same age with few or none.

Immunity. Animals affected with warts always clear up spontaneously after some time. This time may be rather prolonged, however, and often there is a demand for curative treatment. There are indications that animals that have suffered from warts are resistant thereafter. It also has been observed that surgical removal of several large warts often is followed by dry necrosis and the falling off of the remaining ones. This has been interpreted as meaning that wart virus, escaping from the tumors during the operation and being absorbed through the wound produced, has resulted in immunization. This explanation has not been confirmed by experimental proof, and it should always be kept in mind that warts frequently disappear spontaneously, hence the value of all methods of treatment should be accepted with caution.

Active immunization of cattle with formalinized wart-tissue vaccine is

practiced and such vaccine is available commercially. The vaccine is made by grinding fresh wart tissue, trimmed free of the horny epithelial layers, into a fine suspension in saline solution. The suspension is treated with 0.4 formalin solution to destroy the virus. The vaccine is given subcutaneously or intracutaneously. It is claimed that a single dose of such vaccine will lead to rapid recovery in a great majority of all cases. A vaccine of this type was used by Stein (3) to control human warts.

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CANINE PAPILLOMATOSIS

Benign epithelial growths, commonly called warts, are not uncommon in young dogs. The tumors usually are found around the lips and in the mouths of the animals, where they may cause serious inconvenience. The condition is highly contagious, often spreading through all of the dogs in a kennel, according to Penberthy (3).

Character of the Disease. The warts begin around the lips, as a rule, as smooth whitish elevations which later develop a roughened surface and appear as typical papillomas. Usually following the first one or two tumors there is a secondary crop appearing on the insides of the cheeks, the hard palate, the tongue, and even on the walls of the pharynx. The tumors have the appearance of cauliflowers They may interfere considerably with mastication. After several months without any sort of treatment they disappear spontaneously.

Nature of the Virus. McFadyean and Hobday (2) showed that the warts were infectious by rubbing pieces of the tumors on the scarified mucous membranes of other dogs. DeMonbreun and Goodpasture (1) likewise found it easy to propagate the tumors in this way. McFadyean and Hobday state that the incubation period is from 4 to 6 weeks. DeMonbreun and Goodpasture found that it was about 30 to 32 days as a rule but it was somewhat longer in malnourished dogs. The latter workers passed tumor suspensions through Berkefeld N and W filters and found that the virus was present in abundance in the filtrates. Wart material was dried while frozen and kept in this state for 64 days. At the end of this time it readily produced tumors, the incubation period being 32 days, an indication that there had not been appreciable attenuation of the virus as a result. Wart tissue kept in glycerin

for the same period likewise kept its virulence relatively unimpaired. Wart tissue which had been heated at 45° C. for one hour retained its virulence but that which was heated at 58° C. and 80° C. proved inactive.

The attempts of DeMonbreun and Goodpasture to produce warts on the vaginal mucous membrane, on the mucous membrane of the conjunctiva, and on the skin of the abdomen proved unsuccessful, both with filtrates and

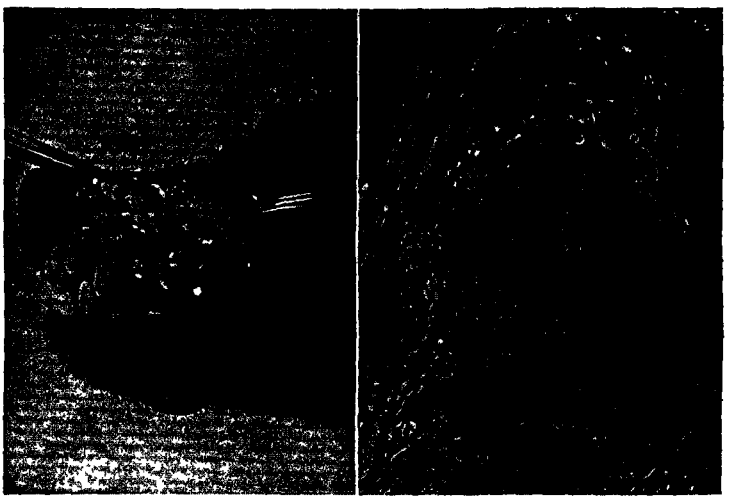


FIG 139 Canine Oral Papillomatosis *Left,* Puppy's mouth showing the warts as they appeared sixty-six days after injection of a Berkefeld filtrate of tumor emulsion. In this case the incubation period was thirty-three days *Right,* A transverse section of a papilla of an actively growing wart The inner core of Malpighian cells is approximately normal in size They are surrounded by the enlarged, vacuolated wart cells x 200. (Courtesy of DeMonbreun and Goodpasture, *Am Jour Pathology*)

with fresh unfiltered wart tissue. They also failed to infect the mouths of cats, rabbits, guinea pigs, and rats.

Immunity. Clinical experience indicates that dogs which recover from an attack of warts seldom or never are infected again. McFadyean and Hobday, and DeMonbreun and Goodpasture both found it impossible to reinfect, experimentally, dogs which had recovered.

Vaccines are sometimes used for treatment. These are made as in the case of those of cattle. Some veterinarians have reported good results from the use of these vaccines. Since the disease is self-curing, the results of the use of vaccines must be accepted with caution.

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PAPILLOMATOSIS OF RABBITS

Synonym: The Shope Papilloma.

In 1933, Shope (4) showed that the common wart of the western wild cottontail rabbit was infectious, and that the infectious agent was a virus. These warty growths are not uncommon among wild rabbits of the mid-western part of the United States. Usually there are from one to ten of these tumors on the infected animal but occasionally there may be hundreds of small tumors covering almost the entire body surface. Even when the tumors are numerous they have little effect upon the general health of the rabbit. They are of little economic importance.

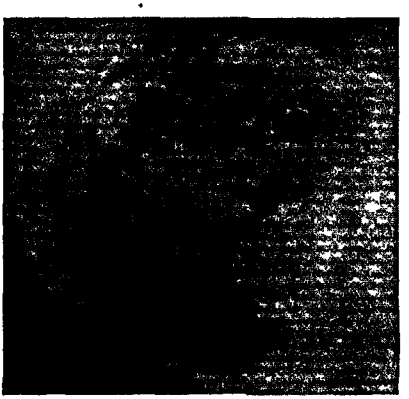


FIG 140 Rabbit Papillomatosis Warts on the scarified skin of the abdomen of a rabbit produced experimentally The inoculation had been carried out about one month previously (Courtesy of R E Shope, *Jour Exp Med.*)

Character of the Disease. The naturally occurring tumors appear as tall, thin, horny structures, usually grayish or even black in color. Hunters sometimes refer to such animals as "horned" rabbits, especially when the warts occur upon the head. The sides of the neck, the shoulders, the abdomen, and the inside of the thighs are the sites of predilection.

Experimentally the disease may be transmitted easily by inoculating scarified skin areas with filtered or unfiltered tumor tissue. These tumors can be transmitted in series in the cottontail rabbit, but those produced in domestic rabbits by such inoculations are not transmissible although otherwise typical. Rous and Beard (3) showed that if the tumor-bearing domesticated rabbits were kept for long periods (200 days or longer) a considerable number of the benign papillomas become transformed into malignant carcinomas. Kidd and Rous (2) studying the matter further showed that the same thing was true of

such tumors produced by inoculation in jack rabbits and snow-shoe rabbits. They believe that in rabbit species in which the virus is foreign there is virus variation which leads to malignancy. The malignant tumors arise from cells, which are already neoplastic as a result of virus action, by gradual change in character.

Nature of the Virus. Shope (4) found that the virus was readily filterable through Berkefeld filters of all grades of porosity, but not through Seitz filters, as a rule. The first detectable evidence of epithelial proliferation is seen from the 6th to the 12th day, averaging 8 days, after inoculation. Rather surprisingly, filtrates often produce tumors after incubation periods which are somewhat shorter than those of the same suspensions unfiltered. This is interpreted as meaning that some inhibiting agent had been removed by filtration. Inoculation of the scarified skin is the only way by which the disease can be transmitted with a high degree of regularity. Subcutaneous, intramuscular, intraperitoneal, and intravenous inoculations usually fail to infect. Shope infected two out of four animals, however, by scarifying a skin area and then injecting virus intravenously, the tumors appearing in the scarified areas.

The virus is unusually resistant to heat Virus suspensions heated at 67° C. for 30 minutes were not inactivated. Those heated at 70° C. failed to produce tumors. The virus keeps for long periods in glycerin.

Artificial Cultivation. Cultivation of this virus on artificial media has not been reported.

Transmission. In view of the fact that animals can easily be infected through superficial scarifications of the skin, it is presumed that natural infections occur through direct contacts between infected and susceptible animals.

Immunity. Shope (4) (5) has shown that rabbits carrying experimentally produced papillomas are partially or wholly immune to reinfection, and also that the sera of such animals is capable of partially or completely neutralizing the virus *in vitro*. Even though the tumors produced in domesticated rabbits contain no demonstrable virus, injections of suspensions of such tumors will actively immunize susceptible animals. Shope concluded that in the tumors of domesticated rabbits the virus is present but is masked in some unknown way. Bernheim and co-workers (1) recently claim to have obtained the virus from cottontail rabbit tumors in the form of a homogeneous protein, but they were unable to find this protein in the domesticated rabbit tumors. They did find in the latter, however, a non-infectious, antigenic protein which immunizes animals to the virus. They suggest that a factor exists, possibly enzymic in nature, in the domesticated rabbit which destroys the virus. They also speculate

about the possibility of the existence of such agents in other mammalian tumors, and the possibility that this may explain why viruses have not been recognized in most of them.

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INFECTIOUS MYXOMATOSIS OF RABBITS

This is a highly contagious and almost always fatal disease of domesticated rabbits which was first recognized in South America and later in Mexico and the United States (California). The disease often destroys whole rabbitries It was first described in 1898 by Sanarelli (7) who was working in Montevideo, Uruguay. Sanarelli ascribed the disease to a virus since he could not see or cultivate any organisms in the lesions It is of interest to note that Sanarelli's paper appeared in the same year as that of Loeffler and Frosch who determined the causative agent of foot and mouth disease of cattle to be a virus. The myxoma virus takes its place, historically, as the second animal disease virus to be recognized.

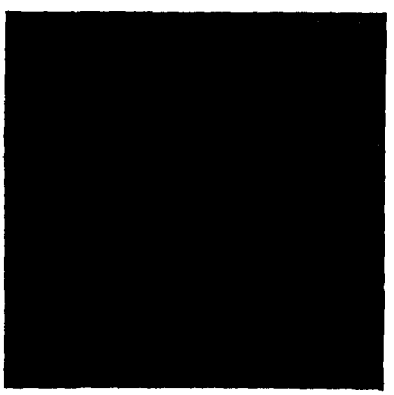


FIG 141. Virus Myxomatosis, Rabbit. Multiple primary tumors induced by virus on the freshly shaved skin. (Courtesy of Thos. M Rivers)

Myxomatosis affects ordinary domestic rabbits, Angora rabbits, Belgian hares, and Flemish Giants, but the wild rabbit of Brazil, the common cottontail, and the jack rabbit of the United States are almost wholly immune The virus does not affect any animal species other than certain rabbits, and man is also immune.

Character of the Disease. The disease begins with inflammation of the eyes. The eyelids swell and a copious discharge from the conjunctival mucous membrane appears. At first the discharge is serous but shortly it becomes purulent. Within 24 to 48 hours the eyes cannot be opened because of the swelling. A nasal discharge also appears, and swellings are

noted involving the skin of the face and ears. The head may become very misshapen, then similar swellings may be noted on other parts of the body. The genital openings become inflamed, and discharge a purulent exudate. Finally the tumorous masses may involve nearly the whole body, the affected animal appears very ill, and almost invariably dies in from 7 to 15 days after the first symptoms are noted.

The tumor-like masses consist of tissue having a rubbery, gelatinous consistency. Usually the lungs, liver, and kidneys are normal in appearance but

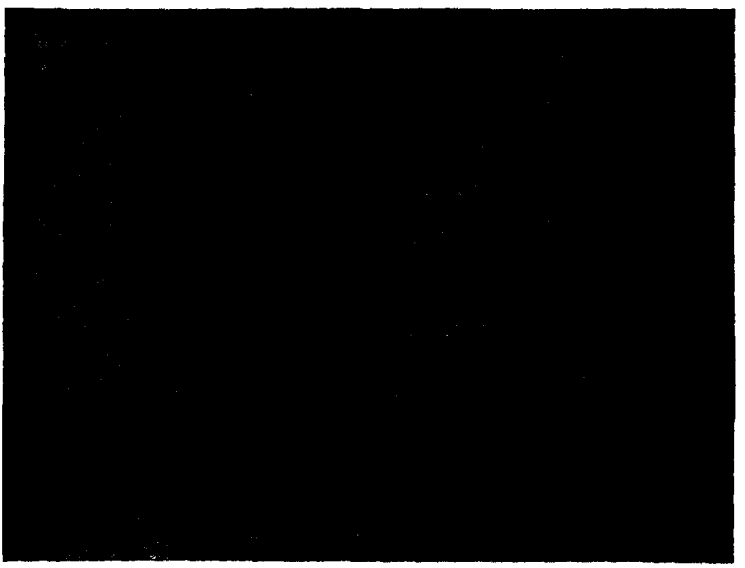


Fig 142. Virus Myxomatosis The virus attacks epidermal cells as well as those of the subcutaneous tissue In the lower part of the photograph the myxomatous tissue is seen In the upper part, a series of vesicles—the end result of infection of epithelial cells. x 100 (Courtesy of Thos M Rivers, *Jour Exp Med*)

the spleen is always swollen and the lymph nodes are swollen and hemorrhagic. Although the external genitalia are inflamed, the testicles, uterus, and ovaries usually are free of lesions. The testicular swelling mentioned by many authors is usually caused by changes in the scrotum rather than in the testicle.

Sections of the myxomata show tissue characteristic of that type of tumor, that is, large stellate cells embedded in a homogeneous, gelatinous substance which is largely if not wholly mucin. In addition, however, there is evidence

of inflammation manifested by engorgement and hemorrhages from the blood vessels and by collections of neutrophilic leucocytes. Rivers (5) was the first to call attention to another characteristic feature of these virus tumors, that is, a peculiar type of degeneration of the epithelial coverings. The epithelial cells are greatly swollen and vacuolated, and acidophilic bodies rapidly develop in their cytoplasm. These bodies contain blue-staining coccoid elements. The whole structure resembles the Bollenger bodies of fowlpox.

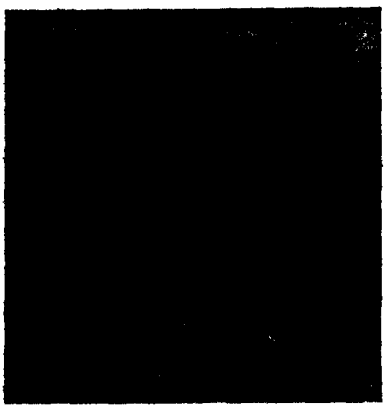


FIG. 143 Virus Myxomatosis. Epidermis of a tumor showing cytoplasmic inclusion bodies x 600 (Courtesy of Thos. M Rivers, *Jour Exp Med*)

Rivers and Ward (6) have found it possible to obtain suspensions of these elementary bodies, which they regard as the virus, in a relatively pure form. Not only are such suspensions highly pathogenic but the bodies are specifically agglutinated by serum of recovered or immunized animals

Nature of the Virus. Virus can be obtained from the tumors, internal organs, blood, and from the discharges from the body openings. It is filterable through Berkefeld filters. The particle size is rather large. It is believed that the elementary bodies, mentioned above, actually constitute the virus. Since Findlay (1), in England, could not find the elementary bodies of Rivers in his material, the question was raised whether Rivers had not been dealing with two viruses, one of which affected the fibroblasts and the other the epithelial cells. Attempts to separate two viruses, however, have failed. Hyde and Gardner (4) in this country have confirmed Rivers' findings and think that Findlay's results may be explained by the fact that he worked with a different type of rabbit. Rivers found that the virus, kept in glycerol for two years, retained its infectivity.

Artificial Cultivation. Hoffstadt and Pilcher (2) have reported successful cultivation of myxoma virus on the chorio-allantoic membrane of the developing chick embryo.

Transmission of the Disease. Natural transmission of myxomatosis occurs through contact with the infective discharges from the bodily openings. Whether the disease is initiated with a naturally or an artificially infected

animal, the disease readily spreads to other rabbits in the same and neighboring cages. The incubation period is about five days.

Immunity. Since nearly every rabbit affected with myxomatosis dies, there has been only limited opportunity to study natural immunity. It is known, however, that in a few that have recovered, a solid immunity has been established. Hyde (3) obtained some degree of resistance by injecting rabbits before inoculation with a heat inactivated (60° C. for 30 minutes) tissue vaccine. The disease was rendered less progressive, death was delayed, and some animals survived.

Shope (8) discovered that the virus of the Shope fibroma, which is a benign tumor, immunized rabbits to the virus of myxomatosis. This fact has aroused great interest but the method of immunization can hardly be called a practical one for protecting stocks of rabbits. There are no practical vaccines for this disease, hence outbreaks have to be controlled by the destruction of affected stock and the practice of rigid sanitary precautions.

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THE SHOPE FIBROMA OF RABBITS

Shope (5) in 1932 described a type of fibrous tumor of the cottontail rabbit which proved to be transmissible to other cottontail rabbits and to the domestic species by the injection of cellular suspensions and of Berkefeld filtrates. Although of interest on its own account, this virus has attracted much interest because of its relationship to the virus of myxomatosis.

Character of the Disease. The tumor occurs subcutaneously in naturally infected cases. There may be one or several in the same animal. They are firm, spherical masses which can be moved about under the skin, being only loosely attached. Sections show that the masses are made up of spindle-shaped, connective tissue cells, without evidence of inflammatory reaction. Filtrates of tumor tissue when injected into the testicles regularly cause

the formation of similar tumors. Subcutaneous and intramuscular inoculations frequently, but not always, succeed. Intraperitoneal and intracerebral inoculations fail.

Transmission of the tumors occurs equally well by inoculation in domestic and cottontail rabbits but the behavior of the tumors in these species differ. In the cottontail rabbit, growth is slow and it continues over a long period of time. In the domestic rabbit growth is rapid but after about ten days of active proliferation further growth does not occur and retrogression begins. The virus content of the cottontail tumors remains high for a long period (77 days at least), whereas in domestic rabbit tumors it is highest about 7 to 9 days after inoculation, and disappears as retrogression occurs (6) Guinea pigs, rats, mice, and chickens proved refractory to inoculation. So far as is known, no animal other than rabbits can be infected with this virus.



FIG 144 The Shope Virus Fibroma Tumor on the shaved skin of the abdomen of a rabbit produced by experimental inoculation 11 days previously (Courtesy of R E. Shope.)

Nature of the Virus. The virus in rabbit fibromatosis is found only in the tumors. It has not been demonstrated in the blood, visceral organs, or in any of the secretions or excretions. In susceptible animals it stimulates a proliferation of the connective tissue at the point where it is deposited. There is no evidence of inflammation or of necrosis in the lesions.

The virus is readily filterable through Berkefeld V and N filters. It remains viable in glycerol for long periods of time.

One strain of rabbit fibroma virus after 18 passages in domestic rabbits suddenly mutated and thereafter failed to produce tumors but instead caused inflammatory reactions in the injection sites. This change was detected by Andrewes (1) in England to whom Shope had sent the material. Shope (9) himself was able to confirm Andrewes' findings. Passage through a series of cottontail rabbits restored part of the tumor-producing power. Other strains have not so changed. The changed strain continued to immunize against the tumor producing strains.

Artificial Cultivation. Cultivation of the Shope fibroma virus on artificial media has not been reported.

Transmission. The mode of natural transmission of the virus is not known. It does not transmit from animal to animal by simple contact. Hyde and Gardner (4) found that it was not transmitted from mother to young either through the placenta or through the milk. Experimentally the disease has been produced only by inoculation.

Immunity. Shope (6) showed that domestic rabbits in which tumors had formed and retrogressed could not be reinfected with the same virus. To his

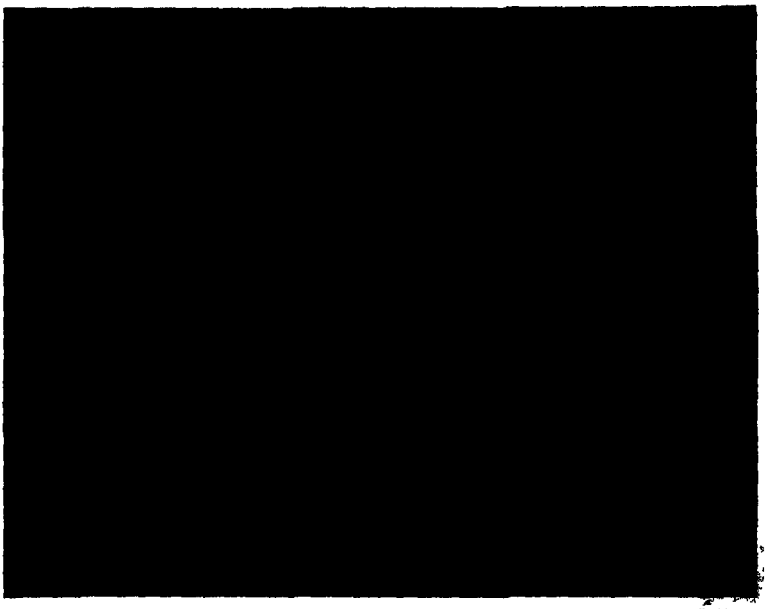


FIG 145 The Shope Virus Fibroma Section of a testicular tumor produced by experimental inoculation of a rabbit. x 300. (Courtesy of R E. Shope.)

surprise he found that such rabbits also had a high degree of resistance to the virus of myxomatosis. Whereas myxoma virus is almost always fatal to normal rabbits, it destroyed only one of a group of fifteen fibroma-recovered animals. These animals then proved to be highly immune to myxoma virus as well as to fibroma virus. One rabbit which recovered from myxoma without having previously been affected with fibroma, proved to be resistant to fibroma as well as myxoma.

Thinking that fibroma might be the natural reaction of cottontail rabbits to the virus of myxomatosis, Shope (8) attempted to pass the myxoma virus

serially through these animals. Only minimal reactions were induced and these had the character of neither fibroma nor myxoma.

The unexpected finding of the immunological relationship between fibromatosis and myxomatosis suggested, of course, that the benign fibroma was caused by an attenuated strain of the malignant myxoma, however Shope (7) expressed the opinion that such was not the case; that the viruses were qualitatively different.

Certain experiments conducted by Berry and Dedrick (3), and by Berry (2), in 1936 and 1937, indicate that fibroma virus probably is an attenuated form of myxoma. The virus of myxomatosis was heated to 75° C. which completely inactivated it. Mixed with fibroma virus, however, the mixture produced typical myxomatosis when injected into rabbits, and this disease could then be transmitted to other animals indefinitely. It was suggested that something in the heated myxoma virus had acted as a sort of hapten to lend greater virulence and malignancy to the fibroma virus.

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