DISEASE IN SWINE RESULTING FROM EXPERIMENTAL ADMINISTRATION OF MYCOBACTERIUM BOVIS, MYCOBACTERIUM AVIUM, OR GROUP-III MYCOBACTERIA

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James A. Ray

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This is to certify that the

thesis entitled

DISEASE IN SWINE RESULTING FROM EXPERIMENTAL ADMINISTRATION OF MYCOBACTERIUM BOVIS, MYCOBACTERIUM AVIUM, OR GROUP-III MYCOBACTERIA

presented by

James A. Ray

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of the requirements for

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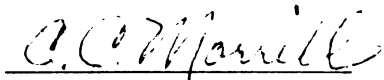

Major professor

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ABSTRACT

DISEASE IN SWINE RESULTING FROM EXPERIMENTAL ADMINISTRATION OF MYCOBACTERIUM BOVIS, MYCOBACTERIUM AVIUM, OR GROUP-III MYCOBACTERIA

by James A. Ray

To each of 39 Yorkshire-Berkshire-Landrace crossbred pigs, approximately 8 to 11 weeks old, 2 mg. (wet weight) of cells of 1 of 9 mycobacterial strains were administered orally or by intradermal injection of the left foreleg. The pigs were housed in isolation by groups 1) according to the route of administration and 2) so as to prevent cross-infection. Twenty-one additional pigs (litter-mates) were housed with the above-mentioned pigs to detect transmission by contact. Fifteen other litter-mates were housed in groups under similar conditions to constitute uninoculated controls.

The strains of organisms administered were as follows: 1 Mycobacterium bovis of porcine origin; 4 Group-III mycobacteria of porcine origin; 2 Group-III mycobacteria of pen origin; 1 M. avium of porcine origin; and 1 M. avium, laboratory strain.

The pigs were observed for signs of disease and tested with both avian and mammalian tuberculins during the course of the experiment. At various intervals during the experiment pigs were selected arbitrarily and killed. Pathologic and bacteriologic examinations were conducted on the tissues.

No signs of disease other than swelling or swelling and ulceration of the intradermal inoculation site were observed. In general, the lesions developing at the injection site were in decreasing order of severity following injection of M. bovis, Group-III mycobacteria of porcine origin, M. avium of porcine origin and M. avium, laboratory strain. Almost no change was produced by Group-III mycobacteria of pen origin. Following ulceration, which occurred in most cases where lesions developed, the lesions appeared to heal.

All pigs to which M. bovis was administered developed greater response to mammalian tuberculin than to avian tuberculin. When M. avium was administered, the responses to avian tuberculin exceeded those to mammalian tuberculin except in 2 pigs tested 62 days after inoculation. One was killed shortly after this test; the other responded in greater degree to avian tuberculin on a subsequent test. All pigs receiving Group-III mycobacteria of porcine origin had greater response to avian tuberculin than to mammalian tuberculin. There was only 1 measurable response (1 mm. swelling to avian tuberculin) in the pigs receiving Group-III mycobacteria of pen origin.

Bacteriologic data indicate that acid-fast organisms were widespread in all pigs except those inoculated with Group-III mycobacteria of pen origin and the uninoculated control pigs. With only 4 exceptions, acid-fast organisms were isolated from all tissues where lesions were found. In 83 pools of tissue out of 280 when no lesions were found, acid-fast organisms were isolated. In no instance was a mycobacterium isolated from a noncontact uninoculated control pig.

Lesions were found in most animals receiving M. bovis, either M. avium strain, or any of the Group-III mycobacteria of porcine origin and

each strain produced lesions in at least 1 pig when administered by either route. None were found when Group-III mycobacteria of pen origin were injected. The Group-III mycobacteria of porcine origin produced more extensive lesions than did M. avium and less extensive lesions than did M. bovis. In certain instances, the extent of disease caused by M. avium or Group-III mycobacteria of porcine origin appeared to lessen with the passage of time. The lesions caused by M. avium, M. bovis or Group-III mycobacteria of porcine origin could not be differentiated macroscopically or microscopically.

Lesions were found in the tissues of the pen-mates of pigs receiving M. avium (laboratory strain) intradermally, M. avium (porcine origin) orally, M. bovis orally, or Group-III mycobacteria (porcine origin) orally. (There were no pen-mates with those receiving M. bovis intradermally.) Acid-fast organisms were isolated from all pen-mates except 1 killed 52 days after M. avium, laboratory strain, was administered orally to other pigs housed with it.

No tuberculin hypersensitivity, lesions or acid-fast organisms were found in the uninoculated control pigs.

DISEASE IN SWINE RESULTING FROM EXPERIMENTAL ADMINISTRATION OF *MYCOBACTERIUM BOVIS*, *MYCOBACTERIUM AVIUM*, OR GROUP-III MYCOBACTERIA

By

James A. Ray

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To

Nancy, Tommy and Carolyn

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INTRODUCTION

Probably no affliction has caused more death, suffering and economic loss throughout the years than tuberculosis. It is undoubtedly the most studied of all bacterial diseases. Yet with all this study tuberculosis is not fully understood and is still the greatest killer of man in the world (Smith et al., 1964). Even in the United States where it is well controlled it remains the most prevalent microbial cause of death (Dubos, 1954).

The incidence of tuberculosis in man has dropped markedly in the United States during the 20th century. This is probably due to improved living conditions and sanitation as well as to an effective control program. This program was designed to stop the spread of the organism by the detection, isolation and treatment of persons shedding tubercle bacilli.

Because of its economic and public health importance, bovine tuberculosis has been subjected to extensive and relatively effective control programs. These programs have greatly reduced the incidence of the disease but have not eliminated it.

No such control program has been applied to porcine tuberculosis. This is true in spite of substantial economic losses and a potential, if not real, public health hazard afforded by this disease. While the infection rate in the United States as indicated by meat inspection reports

appears low (1.42%) (U. S. Department of Agriculture, 1965) it is 855 times that found in cattle during the same period. Since swine can be affected by Mycobacterium tuberculosis, M. bovis, or M. avium, tuberculous pigs are potential hazards for man, cattle, or fowl as well as other pigs.

When Group-III mycobacteria were isolated from tissues of more than 100 pigs (Mallmann, Mallmann and Ray, 1962), the question of the importance of pigs in the transmission of this disease was raised. Similar organisms had been incriminated as the source of disease in human beings (Runyon, 1959) and cattle (Mallmann, 1961). Epidemiologic studies of such cases in human beings indicated that the disease was not spread from man to man (Edwards and Palmer, 1959). While no positive source was found, Edwards and Palmer (1959) had found high rates of reaction (40%, 15%, 15%, respectively, of persons from 3 of 5 farms to a purified-protein-derived (PPD) tuberculin made from a Group-III mycobacterium of human origin.

Subsequently, McGavin (1964) was able to produce a primary complex (lesions at intradermal inoculation site and lymph node draining it) in only 1 of 11 calves when each of these animals was inoculated intradermally with 1.0 mg. (wet weight) of 1 or 6 Group-III mycobacteria of swine origin isolated by Mallmann et al. (1962). In general, the Group-III mycobacteria used in his study were less virulent for calves than were the Group-III mycobacteria of bovine origin.

The purposes of this study were 1) to reproduce the disease in swine with Group-III mycobacteria of swine origin and to reisolate the organisms from the tissues of the infected animals, thereby fulfilling Koch's

postulates, 2) determine if transmission occurred from pig to pig, and 3) compare the disease caused by these organisms to that produced by M. bovis and M. avium under similar conditions. The organisms were administered intradermally and orally as these routes seemed the most likely under natural conditions.

REVIEW OF LITERATURE

Since before recorded history, tuberculosis has afflicted man. Lesions resembling those of tuberculosis have been found in the bones of Egyptian mummies (Webb, 1936). Tuberculosis has killed more men than any other affliction. It is still the most common cause of death in man (Smith et al., 1964).

Many species of animal, including many birds and cold-blooded animals, have been found to be tuberculous (Francis, 1958). Tuberculosis of cattle, swine and birds is the most important from the economic and public health standpoint.

Bovine tuberculosis has been reduced in the United States by a rigorous eradication program. The incidence of tuberculin reactors was reduced from 5% in 1917 to 0.11% in 1954 (Johnson, Baisden, and Frank, 1961). However, Mallmann, Mallmann and Ray (1964) noted that the incidence of bovine tuberculosis has changed little since 1940. Hence the program has become a control program instead of an eradication program. Even as a control program, it is of great value. In Europe, when inadequate control methods were used, the incidence of tuberculosis was about 35% in cattle 5 years old (Francis, 1958).

The incidence of avian tuberculosis has been greatly reduced by the increased practice of having only pullet flocks. Schalk et al. (1935) found pathogenic M. avium in chicken litter 4 years after it was voided. They felt that disease could not be controlled by the slaughter of

tuberculin-positive birds because of soil contamination, and recommended eliminating all birds more than 1 year old from the flocks. Lee (1955) found 0.24% tuberculin-positive chickens when testing 33,769 birds from all-pullet flocks, while 11.52% of 5,382 hens 2 or more years old were tuberculin-positive. Pickard (1952) reported that 14% of 119 pullet flocks tested contained tuberculin-positive birds, while 57% of 95 hen flocks tested contained birds that were positive. Therefore, much of the tuberculosis in chickens can be eliminated by having only all-pullet flocks.

Following an extensive review of European and American literature, Francis (1958) stated that the incidence of porcine tuberculosis varied from 1 to 5% early in the 20th century. In the United States, the incidence was 2% in 1908 (Francis, 1958). It increased to a high 16.38% in 1922 (Karlson, 1964). This was thought to be due to M. avium infections transmitted to the pig from tuberculous poultry. The incidence of fowl tuberculosis decreased markedly with the change to all-pullet flocks (Francis, 1958; Karlson, 1964). During the same period the incidence of porcine tuberculosis dropped, reaching a level of 1.42% in 1965 (U. S. Department of Agriculture, 1965).

Bacteriologic studies of 23 workers, summarized by Francis (1958), indicate that porcine tuberculosis was caused by M. tuberculosis, M. bovis and M. avium. From this review and a similar review by Karlson (1964), it is clear that M. avium is the most common isolant of the 3 aforementioned species. Griffith established that subcutaneous inoculations of M. bovis (1907), M. avium and M. tuberculosis (1911) could cause tuberculosis in swine.

Porcine tuberculosis is mainly associated with the exposure of pigs to products, excreta, or diseased tissue of tuberculous cattle, fowls or human beings. Mohler and Washburn (1908 and 1917), Thornton (1961) and Anthony (1950) reported finding higher incidences of tuberculosis in pigs from areas where bovine tuberculosis existed. Dairy products, returned to farms to be used as swine feed, were recognized early as a source of infection. This is evidenced by early laws requiring the pasteurization of dairy products used as animal feeds in Denmark (Bang, 1908) and the United States (Mohler and Washburn, 1908). The latter preceded the requirement of pasteurization of dairy products for human use.

Mycobacterium tuberculosis has been isolated from tuberculous lesions of swine from Europe (Francis, 1958), the Philippines (Topacio, 1933), South Africa (Robinson, 1958), Japan (Hatokeyama et al., 1961) and the United States (Feldman, 1939). In all instances, the pigs had had direct or indirect contact with tuberculous human beings.

While early workers had shown that porcine tuberculosis could be caused by M. avium (Weber and Bofinger, 1907; Mohler and Washburn, 1908; Griffith, 1911), it was thought that most of it was due to M. bovis and that the control of bovine tuberculosis would also control porcine tuberculosis (Mohler and Washburn, 1908). When the incidence of tuberculosis in swine increased after the start of the bovine tuberculosis eradication project (Feldman, 1938; Karlson, 1964), attempts were made to determine the cause.

Van Es and Martin (1925) found M. avium in 74.6% and mammalian tubercle bacilli in 4.4% of the 248 tuberculous swine lesions examined. Mixed infections involving both avian and mammalian tubercle bacilli were found in 5.6% of the specimens. The swine from which these lesions were obtained were from Nebraska. In addition to these, they examined 14 lesions from Michigan swine and found M. avium in 92.9% and mixed infections in 7.1%. Similarly, high incidences of M. avium infection were found in lesions of pigs from Illinois (Graham and Tunnicliff, 1926), Minnesota (Feldman, 1938a; Feldman, 1939; Feldman and Karlson, 1940) and the North Central states (Crawford, 1938).

Mohler and Washburn (1908) produced tuberculosis in swine experimentally by feeding them viscera of tuberculous chickens. Schalk et al. (1935) successfully transmitted tuberculosis to swine by contaminating their pens with droppings of tuberculous chickens and by feeding pigs tuberculous viscera from chickens. They also were able to infect swine by maintaining them on ground on which tuberculous chickens had been kept 2 years previously.

The change of opinion regarding the main cause of porcine tuberculosis is well illustrated by quotations from a U. S. Department of Agriculture bulletin "Tuberculosis of Hogs" written in 1917 by Mohler and Washburn, and from a revision of the same bulletin (Mohler, 1938). From the 1917 circular we read: "Tuberculous cattle are the main source of tuberculosis in swine"; in 1938 it was revised as follows: "Tuberculous fowls are the main source of tuberculosis in hogs in the United States."

For many years, porcine tuberculosis was thought to be caused only by M. tuberculosis, M. bovis and M. avium (Francis, 1958; Merchant and Packer, 1961; Jubb and Kennedy, 1963; Karlson, 1964). Karlson and Feldman (1940) had reported isolating rapidly growing, nonchromogenic, acid-fast bacilli with characteristics dissimilar to those of the aforementioned species. Because of their lack of virulence for laboratory animals and calves and because of the lack of lesions in the animals from which they were isolated, little significance was attached to these reports.

However, Mallmann, Mallmann and Ray (1962) reported isolating nonchromogenic acid-fast bacilli from tissues of approximately 100 tuberculin-positive swine from a single herd. In most cases, these tissues contained gross tuberculoid lesions. These strains were classified as Group-III mycobacteria according to the classification scheme of Runyon (1959) by the methods of Mallmann, Mallmann and Robinson (1964).

In an effort to determine whether atypical mycobacteria were responsible for tuberculosis-like lesions in swine from other geographic areas, such lesions were obtained from pigs originating from Michigan, Illinois, Missouri, Ohio, and Indiana. These tissues were examined bacteriologically. Eighty-five per cent of the acid-fast organisms isolated were M. avium and 15% were Group-III mycobacteria (Mallmann et al., 1962).

Scammon, Froman and Will (1963) also isolated nonchromogenic acid-fast bacilli from tuberculous swine. They compared 43 stains isolated to 10 strains of the Battey bacillus (Group-III, human origin) and to 10 strains of M. avium. They concluded that there was a close relationship among swine and human Group-III organisms and M. avium.

Karlson (1964) pointed out that in 11 reports of bacteriologic studies conducted on tuberculous lymph nodes of swine, the authors had not been able to identify a causative agent in from 11 to 61.5% of the samples examined. He says that this may be due to: 1) inadequate technique, 2) the healing of the lesion, or 3) that the lesion may not be due to infection with tubercle bacilli. He drew attention to the reports of European and American workers who isolated Corynebacterium equi repeatedly from such lesions, sometimes alone and sometimes concurrently with M. avium. He says, "... localized lesions (of C. equi infection) ... cannot be easily differentiated from tuberculous processes either macroscopically or histologically."

Upon reviewing the works cited by Karlson (1964), i.e., the works of Van Es and Martin (1925), Graham and Tunnicliff (1926), Mitchell, Walker and Humphreys (1934), McCarter, Beach and Hastings (1935), Crawford (1938), Feldman (1938a, 1939), Feldman and Karlson (1940), Pullin (1946) and Bunkier (1946), one wonders whether the methods used would have demonstrated atypical mycobacteria, if present. All of the workers relied heavily upon animal inoculation for primary isolation. Even where artificial media were used, atypical colonies may not have been thought significant. In commenting about his failure to isolate tubercle bacilli from 4 specimens, Feldman (1938a) says:

> "Is it possible that unidentified acid-fast bacilli exist which are responsible for a tuberculous disease in swine? The failure of bacteria to grow on a culture medium which is suitable for ordinary forms of tubercle bacilli and the failure of emulsions made from morbid tissue to induce lesions in rabbits or guinea pigs can hardly be attributed to the infective agents' being

> nonviable or avirulent. The lesions in the swine were of a generalized and progressive nature suggestive of marked virulence or exceptional susceptibility on the part of the respective swine."

The isolation of atypical mycobacteria is not new. Shortly after Koch (1882) discovered the tubercle bacillus to be the causative agent of tuberculosis, Alvarez and Tavel (1885), Nocard and Roux (1888), and Ferran (1897) reported isolating acid-fast organisms with characteristics atypical of those of Koch's bacillus. Similar reports are repeatedly found (Brem, 1909; Frey and Hagan, 1931; Pinner, 1935; Baldwin, 1942; Buhler and Pollak, 1953; Runyon, 1959; Atwell and Pratt, 1960; McCusker and Green, 1962; Mallmann, Mallmann, and Ray, 1962; Corpe, Runyon, and Lester, 1963; Mallmann, Mallmann and Robinson, 1964). Some of these organisms can be classified as one of the species of mycobacteria as set forth in the 7th edition of Bergey's Manual of Determinative Bacteriology (Breed, Murray, and Smith, 1957). Others cannot be so classified and have been called atypical (Pinner, 1935), anonymous (Runyon, 1959), or unclassified (Corpe et al., 1963).

Classification of mycobacteria is difficult because of their variable characteristics. Xalaburder (1961), writing on the subject of classification of mycobacteria, said:

> "The only thing about them (mycobacteria) that is absolutely typical is their great plasticity and adaptability to an extreme variety of environments. ...
>
> Bacteriologists have established their systems of classification on the basis of assigned, fixed, precise and immutable characteristics to each group of organisms. This was necessary for didactic purposes in the purely descriptive stage of bacteriologic research. However, as the agreement was unilateral, the

> result that the bacteria, not aware of this convenient human arrangement, continued their normal biologic processes, which consisted of a steady evolution and adaptation to the greatest variety of environmental conditions, so that now there is great discrepancy between the real state of affairs and that envisaged by our well-intentioned blueprints."

The same can be said about any biologic classification. Classification is essential for communication about and study of biology, but one must remember that evolutionary and adaptive changes are constantly taking place.

Acid-fast organisms, both classified and unclassified types, are almost ubiquitous. Brem (1909) reported their frequent occurrence in samples from many inanimate sources. Frey and Hagan (1931) were successful in isolating acid-fast organisms from soil samples from many areas of the United States even though their techniques precluded the growth of many mycobacterial species. Pinner (1935) reported isolating acid-fast organisms from water taps and tap water. He wrote, "Acid-fast organisms have been isolated from almost any material properly scrutinized." This was confirmed by the work of Karlson (1958).

Traum (1916) reported observing acid-fast organisms in the lesions of "skin lesion tuberculosis" of cattle and later (Traum, 1919) isolated atypical acid-fast organisms from such lesions. Frey and Hagan (1931) hypothesized that acid-fast bacteria from the soil might be the cause of these "skin lesions" as well as being the sensitizing agent causing the no-gross-lesion tuberculin reactions. Daines and Austin (1932) also isolated atypical acid-fast organisms from "skin lesions". Pinner (1935) isolated atypical mycobacteria in about 1% of the cultural work then being done at the Desert Sanitarium and Institute of Research in Tucson, Arizona.

In some, but not all, cases, M. tuberculosis was also isolated from these patients. Baldwin (1942) reported the clinical, bacteriologic, and tuberculin-sensitivity findings of a case of "pulmonary tuberculosis-like disease" associated with a "nonpathogenic acid-fast bacillus". Buhler and Pollak (1953) also reported 2 cases in human beings due to infection with atypical acid-fast organisms. Following this, reports of infections in human beings with atypical mycobacteria increased in frequency (Timpe and Runyon, 1954; Wood et al., 1956; Crow et al., 1957).

Little or no significance was attached to the role of atypical acid-fast organisms in disease of man and other animals until the late 1950s and early 1960s. This was probably mainly due to 3 factors: 1) the high incidence of disease due to M. tuberculosis, M. bovis, or M. avium which masked the relatively low occurrence of atypical mycobacterial infections; 2) atypical mycobacteria either are not pathogenic or vary in their pathogenicity for laboratory animals, such as guinea pigs, rabbits and chickens (Pinner, 1935; Baldwin, 1942; Buhler and Pollak, 1953; Pollak and Buhler, 1955; Wolinsky et al., 1957; Runyon, 1959; Mallmann, Mallmann and Robinson, 1964). These animals were used extensively for classification and determining the pathogenicity of mycobacteria (Breed et al., 1957; Runyon, 1959); 3) mycobacteria are so widespread in nature that only the classical pathogenic species were considered capable of causing disease.

Karlson (1958) pointed out the difficulties in the classification of atypical mycobacteria with classification schemes existing at that time. Runyon (1959) studied atypical mycobacteria which were associated with

disease in human beings and proposed a system of classification. Based on colonial morphology, pigmentation and growth rate, he divided the atypical mycobacteria into 4 groups: viz., Group I (photochromogens), Group II (scotochromogens), Group III (nonphotochromogens) and Group IV (rapid growers).

Although Runyon's system of classification has been widely accepted and used, it has the major shortcoming of not separating pathogenic strains from nonpathogenic strains as was pointed out by Runyon (1959). He says that the guinea pig can no longer be used alone for determining the pathogenicity of strains for man and that other species must be found for the purpose.

Based on the work of Lester (1939) and Kite, Patnode and Reed (1952), Kubica et al. (1960) devised a method for virulence determination. With their method, 0.1 mg., 0.01 mg. or 0.001 mg. of the culture was injected intracutaneously in the abdominal region of guinea pigs. Cultures producing ulcers at the inoculation sites were considered to be pathogenic. Pollak and Buhler (1955), Durr et al. (1959), and Feldman (1963) were able to produce disease in hamsters with atypical mycobacteria suggesting the possible use of this animal either alone or in combination with other species to determine pathogenicity.

The problem of trying to associate pathogenicity in primary hosts with that in laboratory animals is difficult. Because of isolations of acid-fast organisms from apparently healthy human beings (Edwards and Palmer, 1959; Atwell and Pratt, 1960), and from normal animals (Mallmann, 1963), and because of the existence of apparently nonpathogenic atypical

mycobacteria (Mallmann et al., 1963; McGavin, 1964), one can never be certain that the organism isolated was actually the cause of a lesion. Also, as pointed out by Mallmann, Mallmann and Robinson (1964), repeated transfer on media or inoculation into animals can change the virulence of a mycobacterial strain.

A statement by Pinner (1935) regarding this matter is still applicable:

> "Classification as to pathogenicity is hampered by the fact that the term 'pathogenic' is nearly meaningless unless strictly defined in terms of animal species, dosage, time interval between infection and pathological examination; and, most important of all, there is no general agreement on what constitutes disease in infected animals. If any demonstrable tissue alterations be called disease, and accordingly any organism that causes them is considered pathogenic, then there are no apathogenic acid-fast organisms. To stipulate a minimal dosage that must produce disease, in order to admit the organism into the classification 'pathogenic' is totally arbitrary. To assign the term pathogenic only to those microorganisms that cause progressive disease, and to exclude all those that produce self-healing lesions, would exclude a major portion of all so-called pathogenic (non-acid-fast) organisms. But such proposals are on record. A fairly clear-cut distinction can be made by establishing whether a given microorganism causes lesions, progressively or retrogressive, in serial transfers from animal to animal. If this criterion is used to differentiate between pathogenic and saprophytic acid-fast bacilli it is apparent that all non-mammalian acid-fasts belong in the saprophytic group, although it has probably never been settled whether or not some acid-fast isolated from cold-blooded animals are, in the sense specified, pathogenic for the respective species."

Just as applicable is the statement of Kubica et al. (1960):

> "The fact that it has been impossible consistently and repeatedly to fulfill Koch's postulates as regards the anonymous and isoniazid-resistant acid-fast bacilli is no reason why we should negate their importance in human disease."

The lesions produced by atypical mycobacteria appear to be impossible to differentiate grossly or histologically from those caused by M. tuberculosis. Corpe and Stergus (1963) conducted a study wherein 27 pathologists who were interested in tuberculosis studied duplicate sets of 25 sections from human surgical specimens. Either M. tuberculosis or Group-III mycobacteria had been isolated from each of these specimens as well as from the sputum of the patient. On each slide they could choose one of the following classifications: 1) the histologic picture was compatible with tuberculosis due to M. tuberculosis; 2) the histologic picture was compatible with tuberculosis due to the Battey strain, Group III, nonphotochromogens; 3) the histologic picture was compatible with tuberculosis but that it was impossible to determine the strain; 4) nontuberculous.

The pathologists indicated that they could not tell which strain was the causative agent in 53% of the choices. Twenty-nine per cent of the choices indicated that the lesions were due to M. tuberculosis infection. Only 38% of these were correct. Nontuberculous disease was indicated in 6% of the instances, all of which were in error. The results confirmed the authors' previous impression it is impossible to accurately differentiate histologically between lesions caused by M. tuberculosis and those caused by Group-III mycobacteria.

Corpe, Runyon and Lester (1963) reported their inability to differentiate, histologically, lesions caused by infections with any of the 4 groups of atypical mycobacteria. These lesions were similar to those caused by M. tuberculosis. Crow et al. (1957) reported similar findings.

McGavin (1964) reported that, while atypical mycobacteria varied in their ability to produce lesions when injected intradermally into calves, when lesions were produced it was impossible to differentiate them histologically from those caused by M. bovis infections.

Feldman (1960a) noted difficulty in differentiating the lesions of tuberculosis from other infectious granulomas. He pointed out the importance of suitable bacteriologic studies to determine the cause of a lesion. He stated (Feldman, 1938):

> "The hog occupies an unfavorable position biologically as it is susceptible to all three forms of the tubercle bacillus, and provided that the opportunity for exposure exists, one can safely assume that the frequency of tuberculosis in hogs and the type of tubercle bacillus responsible for the lesions constitute a rather accurate index of the amount of tuberculosis in fowl, mammals, and human beings in any particular locality."

Consonant with this statement, one cannot help but wonder about the significance of the finding of atypical mycobacteria in the tuberculous lesions of swine and of similar findings in man and cattle. It is possible that the infections come from a common source yet unidentified or that interspecies transmission may be occurring.

MATERIALS AND METHODS

Experimental Animals

Pigs were obtained at various times from 1 tuberculin-negative herd. They were Yorkshire-Berkshire-Landrace crossbred animals approximately 6 to 8 weeks of age when purchased. They were fed a standard growing ration obtained from the Department of Animal Husbandry, Michigan State University, and water *ad libitum*.

The animals were housed in heated isolation rooms, several animals per room (Table 1). They were maintained without bedding on concrete floors, their refuse being washed into floor drains daily. All persons entering the rooms wore rubber suits and boots which were washed in disinfectant* after each use and left in the anteroom immediately adjacent to each animal room. All personnel wore disposable gloves, caps and masks which were used only once and in only 1 room.

Cultures

The cultures were from the collection of the Tuberculosis Research Project, Michigan State University, and were as follows:

1. *Mycobacterium bovis*, swine origin: 81-0.
2. Group-III mycobacteria, swine origin: $167C_1$-1, $172C_1$-1, 186C-1, and $193C_2$-1.
3. Group-III mycobacteria, pen origin: 15D, 19_2W.

*Torsite, The Dow Chemical Company, Midland, Michigan

4. Mycobacterium avium, swine origin: 206-2

5. Mycobacterium avium, laboratory strain

The atypical mycobacteria were classified according to the system of Mallmann, Mallmann and Robinson (1964). The Group-III cultures of swine origin had produced ulcers at the site of intradermal inoculation of guinea pigs while those of pen origin did not. The M. bovis, M. avium (swine origin), and the Group-III mycobacteria (swine origin) had been isolated from tissues of tuberculous swine. The Group-III mycobacteria of pen origin were from pens where swine infected with Group-III mycobacteria had been housed, isolated a week after the pens had been cleaned and disinfected* using a high-pressure apparatus. The M. avium (laboratory strain) had been maintained for many years on artificial media as a stock culture. It was, however, still virulent for chickens.

The culture numbers are those of the Tuberculosis Research Project and were used here so that easy reference can be made to their work. The derivation of 1 of these numbers ($167C_1$-1) will be explained so that they can be better understood. Culture $167C_1$-1 was isolated from tissues of the 167th animal examined by the project in 1961 (case number 167-1). The "C_1" indicates that it was the 1st of at least 2 colony types (which appeared to be different on primary isolation) from the pool of lymph nodes from the peritoneal cavity (designed as "C pool" by the Project personnel). The letters "D" and "W" used in the identification of the Group-III mycobacteria of pen origin refer to whether the swab used to collect the sample was wet or dry.

*Torsite, The Dow Chemical Company, Midland, Michigan

Administration of Mycobacteria

The pigs were maintained in the isolation rooms 2 to 3 weeks before the administration of mycobacteria. Each inoculated pig was given 2 mg. wet weight (approximately 2×10^8) cells suspended in 0.2 ml. Dubos broth base without Tween 80* but with 0.5% dextrose added. Intradermal inoculations were made on the lateral surface of the left foreleg, just distal to the carpus, using a 3/8-inch, 26-gauge hypodermic needle and syringe. The oral administrations were made after withholding feed from the animals for 1 day. Each pig was fed from a sterile pan a cohesive mixture of the ration and sterile molasses; 2 mg. wet weight of mycobacteria were incorporated into this mixture. Each animal was seen to eat all of the mixture.

Uninoculated pigs were housed with pigs to which mycobacteria were administered to determine if the organisms were transmitted by contact.

Uninoculated negative control animals which served as controls were maintained like the inoculated pigs in adjacent isolation rooms. In some instances, if these pigs had shown no signs of disease and were tuberculin negative, they were sold for slaughter in order to salvage their meat value. In this event they were inspected by careful and detailed examination for gross lesions.

Clinical Examinations

The pigs were observed daily for signs of disease. The sites of intradermal inoculation were carefully examined and blood samples were

*Difco Laboratories, Inc., Detroit, Michigan

drawn periodically from the anterior vena cava during the course of the experiment. Serum obtained from these samples were used for serologic studies being conducted concurrently.

Pathologic Techniques

The animals were anesthetized with intravenous injections of pentobarbital sodium* and transported to a necropsy room. There they were killed by exsanguination. Blood samples were collected for hematologic and serologic studies.

The necropsy technique was based on that of Jones and Gleiser (1954) as modified by McGavin (1964).

Sections of the liver, heart, lung, both kidneys, spleen, and ileum were put in 10% buffered, neutral formalin and Zenker's fluid (without acetic acid) while the necropsy was being performed.

The following lymph nodes and the skin inoculation site, where applicable, were dissected free, identified and put into plastic bags for transportation to the laboratory: right and left submaxillary, right and left parotid, right and left lateral retropharyngeal (atlantal), right and left medial retropharyngeal (parapharyngeal), anterior mediastinal, posterior mediastinal, right and left bronchial, hepatic, gastric, mesenteric, right and left prescapular, right and left prefemoral, right and left popliteal, internal iliac, right and left deep inguinal, right and left external inguinal and any others found affected. These tissues were cut in the laboratory under a bacteriologic hood and observed for gross lesions. Sections, 3 to 4 mm. thick, were fixed in 10% buffered, neutral formalin.

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*Halatal Solution, Jensen-Salsbery Laboratories, Kansas City, Mo.

The tissues were infiltrated and embedded in a tissue embedding medium* by techniques suggested in the U. S. Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics (1957), with chloroform being used as the clearing agent. Sections were cut at 6 microns' thickness and stained with the new fuchsin-hematoxylin-eosin method of Willigan, Garric and Trosko (1961).

The tissues were evaluated on the basis of the presence and characteristics of the gross and microscopic lesions and the location and number of lesions found.

Bacteriologic Techniques

Tissues to be examined bacteriologically were carefully handled so as to minimize contamination. They were transported in plastic bags to the laboratory, where the attached adipose tissue was trimmed from them. They were washed 5 times for 5 minutes each time in 500 p.p.m. sodium hypochlorite solution. The tissues were sectioned aseptically and samples taken for histopathologic and bacteriologic examinations.

The lymph nodes were pooled for bacteriologic examination as follows:

A. Lymph nodes of the head and neck region

B. Anterior and posterior mediastinal and bronchial lymph nodes

C. Hepatic, gastric, mesenteric and colic lymph nodes

D. Left prescapular lymph node

E. Lung

F. Skin inoculation site

I. Other tissues (cultured separately and identified)

*Paraplast, Arthur H. Thomas Co., Philadelphia, Pennsylvania

Following sectioning, the tissues for bacteriologic examination were ground in nutrient broth* with a Waring Blendor or mortar and pestle. Ten milliliters of the resulting homogenate were mixed with 10 ml. 4% NaOH, shaken and allowed to stand for approximately 15 minutes. The mixture was neutralized with 2% HCl, centrifuged and the supernatant fluid decanted. The sediment was used to seed 6 tubes each of Lowenstein-Jensen Medium,* Middlebrook 7H10 agar,* and Dubos Oleic agar.* The media were incubated at 35 C and observed biweekly for growth for 3 months.

Tuberculin Test Techniques

The animals were tuberculin-tested approximately 1 week before necropsy and, in certain cases, during the course of the experiment. The tests were conducted as follows: the thickness of the ear was measured by means of a vernier caliper** and the thickness recorded. Then, 0.1 ml. avian tuberculin*** was injected intradermally on the right ear and 0.1 ml. mammalian tuberculin# was injected on the left ear. The injection sites were examined approximately 48 hours later. The thickness of any detectable response was measured and recorded.

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*Difco Laboratories, Inc., Detroit, Michigan
**Scientific Products, Evanston, Illinois
***Tuberculin, avian, intradermic, produced for the Agricultural Research Service (ARS), USDA
#Tuberculin, mammalian, intradermic, produced forthe ARS, USDA

Hematologic Technique

A blood sample was collected during exsanguination into a tube containing dipotassium ethylenediamine tetraacetate (EDTA). This was used for hemoglobin, hematocrit and total leukocyte determinations.

TABLE 1. Index of pig number, housing schedule, route of administration, culture number and source, culture classification, duration of experiment, and page number in results.

Pig No.	Group[1] No.	Route	Culture No. and Origin	Culture Classification	Duration[2] in Days	Page No.
2-2	1	ID[3]	81-0;Swine	M. bovis	57	29
2-1	1	ID	81-0;Swine	M. bovis	66	32
2-4	1	ID	81-0;Swine	M. bovis	112	35
3-4	2	ID	$167C_1$-1;Swine	Group III[4]	59	37
1-3	2	ID	$167C_1$-1;Swine	Group III	107	42
3-2	2	ID	186C-1;Swine	Group III	66	43
3-1	2	ID	186C-1;Swine	Group III	107	44
3-3	2	Contact[5]			59	44
1-4	3	ID	$172C_1$-1;Swine	Group III	65	45
1-1	3	ID	$172C_1$-1;Swine	Group III	113	46
2-3	3	ID	$193C_2$-1;Swine	Group III	65	47
1-6	3	ID	$193C_2$-1;Swine	Group III	113	48
1-5	3	Contact			113	49
5-3	4	ID	Lab. strain	M. avium	82	49
5-4	4	Contact			82	50
5-2	4	ID	Lab. strain	M. avium	138	51
5-5	4	Contact			138	52

[1]All pigs of each group housed in a single and separate isolation room.
[2]Days after administration of the organism when necropsied.
[3]Intradermal inoculation.
[4]Runyon Group-III mycobacteria.
[5]Contact pen-mate which was neither inoculated with nor fed the culture.

TABLE 1--continued

Pig No.	Group No.	Route	Culture No. and Origin	Culture Classification	Duration in Days	Page No.
5-1	4	ID	Lab. strain	M. avium	215	53
5-6	4	Contact			215	54
10-3	5	ID	206-2;Swine	M. avium	43	54
10-1	5	ID	206-2;Swine	M. avium	57	56
10-4	5	ID	206-2;Swine	M. avium	113	59
10-2	5	ID	206-2;Swine	M. avium	113	59
10-5	5	ID	206-2;Swine	M. avium	175	60
10-6	5	ID	206-2;Swine	M. avium	175	61
10-7	5	Contact			182	61
10-8	5	Contact			182	62
161-3	6	ID	15D;Pen	Group III	138	62
161-4	6	ID	19_2W;Pen	Group III	138	63
161-6	6	ID	15D;Pen	Group III	NGL[6]	62
161-2	6	ID	19_2W;Pen	Group III	NGL	63
161-7	6	Contact			NGL	63
161-8	6	Contact			NGL	63
161-9	6	Contact			NGL	64
7-1	7	Oral	81-0;Swine	M. bovis	79	64
7-6	7	Contact			79	66
7-2	7	Oral	81-0;Swine	M. bovis	134	67
7-5	7	Contact			134	68

[6]No gross lesions when examined at slaughter.

TABLE 1--continued

Pig No.	Group No.	Route	Culture No. and Origin	Culture Classification	Duration in Days	Page No.
7-3	7	Oral	81-0;Swine	M. bovis	203	69
7-4	7	Contact			203	70
6-1	8	Oral	$172C_1$-1;Swine	Group III	78	71
6-6	8	Contact			78	72
6-2	8	Oral	$172C_1$-1;Swine	Group III	136	72
6-5	8	Contact			136	73
6-3	8	Oral	$172C_1$-1;Swine	Group III	206	73
6-4	8	Contact			206	74
8-2	9	Oral	Lab. strain	M. avium	52	76
8-1	9	Contact			52	76
8-3	9	Oral	Lab. strain	M. avium	108	76
8-5	9	Contact			108	76
8-4	9	Oral	Lab. strain	M. avium	169	77
8-6	9	Contact			169	77
9-2	10	Oral	206-2;Swine	M. avium	57	77
9-1	10	Oral	206-2;Swine	M. avium	57	78
9-3	10	Oral	206-2;Swine	M. avium	115	78
9-4	10	Oral	206-2;Swine	M. avium	115	79
9-5	10	Oral	206-2;Swine	M. avium	171	79
9-6	10	Oral	206-2;Swine	M. avium	171	80
9-7	10	Contact			177	81
9-8	10	Contact			177	80
1-2	11	Control[7]			108	81

[7]Negative control to which no culture was administered and which was not in contact with exposed pigs.

TABLE 1--continued

Pig No.	Group No.	Route	Culture No. and Origin	Culture Classification	Duration in Days	Page No.
2-6	11	Control			108	81
2-5	11	Control			108	81
4-1	12	Control			NGL	81
4-2	12	Control			NGL	81
11-1	13	Control			NGL	81
11-2	13	Control			NGL	81
11-3	13	Control			NGL	81
11-4	13	Control			NGL	81
11-5	13	Control			NGL	81
11-6	13	Control			NGL	81
11-7	13	Control			NGL	81
11-8	13	Control			NGL	81
11-9	13	Control			NGL	81
11-10	13	Control			NGL	81

RESULTS

Seventy-five pigs were used in this study. Mycobacteria (M. bovis, M. avium, or Group III) were administered to 39 pigs either intradermally or orally, while 20 were uninoculated pen-mates of these animals. The remaining 16 pigs were non-contact uninoculated control animals.

Unless otherwise noted, all pigs were apparently healthy throughout the experiment and were in a good state of nutrition at the time of necropsy.

Immediately following is a description of the findings from each pig organized according to culture and route of administration. The findings from the uninoculated pen-mates are included with those of their pen-mates. Bacterial isolations and lesions are summarized in Table 2, which follows the detailed descriptions. In all cases the organisms isolated resembled culturally the organisms administered.

The dimensions of the lesions found at the inoculation site upon clinical examination were obtained by measuring across the circular to oval lesions and, therefore, refer only to the area of the lesion. The 3rd dimensions are lacking since the height or thickness of the lesions could not be determined accurately during life because of their location over the carpus, their limited elevation, and the impossibility of determining at that time how deeply the lesion extended into the underlying tissue.

A. Intradermal Inoculations

Culture 81-0, *M. bovis*, swine origin

Pig 2-2

Clinical observations. Eight days after inoculation the inoculation site was swollen (25 x 30 mm.) and covered with an eschar. On the 15th day after inoculation the swelling at the site was 20 x 30 mm., ulcerated and a purulent exudate was draining from it. Twenty-three days after inoculation the lesion was swollen and hard (25 x 40 mm.) and still discharging a purulent exudate. By the 35th day the swollen area was 20 x 25 mm., denuded and dry. Thirty-eight days after inoculation the lesion was 20 mm. in diameter and dry. On the 44th day there was a very slight swelling (20 x 25 mm.) and a dry ulcer at the inoculation site. By 52 days the lesion consisted of a slightly swollen area (20 x 25 mm.) with a central, dry eschar.

Necropsy findings (57 days after inoculation). Generalized lesions of tuberculosis were found, with the following lymph nodes containing gross lesions: anterior cervical, left submaxillary, lateral retropharyngeal, middle cervical, anterior and posterior mediastinal, bronchial, mesenteric, hepatic, left prescapular and nodes of the lumbar chain. With the exception of the left submaxillary and anterior cervical lymph nodes, the lesions were multiple, white, 1- to 2-mm. foci scattered throughout the nodes. In the left submaxillary lymph node, multiple white caseous foci up to 5 mm. in diameter were found. The center of the anterior cervical lymph node was filled with yellowish caseous material.

The lesions in the hepatic lymph nodes were calcified. Multiple white foci, 1 to 2 mm. in diameter, were scattered throughout the parenchyma of the liver. An ulcerated area 25 mm. in diameter with a granulating base, was found in the skin at the site of inoculation.

Histopathologic findings. Microscopic granulomas were found in all lymph nodes which had gross lesions. These granulomas were similar, having central areas of caseation necrosis surrounded by macrophages. Many of these foci had become confluent and at times the caseation necrosis filled the center of the lymph node. In the left prescapular node, the granulomas were unencapsulated groups of macrophages, some with central caseation necrosis (Figures 1 and 2). The left parotid lymph node, in which no gross lesions were found, contained a few scattered unencapsulated granulomas consisting predominantly of macrophages and giant cells. The lumbar chain of lymph nodes contained numerous scattered focal lesions varying from accumulations of a few macrophages to granulomas with central caseation necrosis surrounded by macrophages and small amounts of connective tissue. Numerous small granulomas made up of small groups of macrophages were found scattered throughout the right prescapular lymph node. No necrosis or encapsulation was found. The supramammary lymph nodes contained numerous scattered discrete granulomas consisting of small groups of macrophages and occasional giant cells. In addition, there were a few larger granulomas with caseous centers surrounded by macrophages and some connective tissue. The degree of encapsulation of the granulomas varied from slight to marked in the anterior cervical, middle cervical, left submaxillary, right and left medial retropharyngeal, left

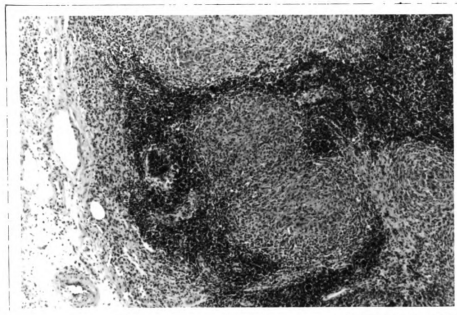


Figure 1. An unencapsulated, noncaseous granuloma found in the left prescapular lymph node of Pig 2-2, inoculated intradermally with Culture 81-0, M. bovis, swine origin. New Fuchsin - H & E. x75.

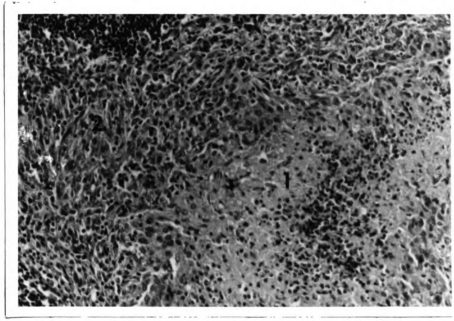


Figure 2. Left prescapular lymph node of Pig 2-2, inoculated intradermally with Culture 81-0, M. bovis, swine origin. Note area of coagulation necrosis and necrotic cells (1), surrounded by macrophages (2), and the absence of encapsulation. New Fuchsin - H & E. x187.

parotid, right bronchial, and hepatic lymph nodes. The skin ulcer from the site of inoculation contained much connective tissue, among which were granulomas composed of macrophages and having, in some instances, centers of caseation necrosis. Multiple scattered discrete or confluent granulomatous foci were found in the lungs. Some of these areas were caseous or caseocalcareous. Early attempts at encapsulation were present. Granulomas with caseocalcareous centers were found in the liver. Some areas of necrosis were surrounded by macrophages and lymphocytes. These lesions were slightly encapsulated. In the spleen, a few small granulomas with caseous centers were found. Early encapsulation attempts were in evidence.

Pig 2-1

Clinical observations. Eight days after inoculation the inoculation site was reddened (35 x 40 mm.) and ulcerated with a purulent material exuding from it. By 15 days after inoculation, there was a 40 x 50-mm. swelling with a purulent ulcer. There was a 40 x 55-mm. swelling with a 20 x 20-mm. suppurating ulcer present 23 days after inoculation. When examined 35 days after inoculation, there was a 50 x 55-mm. swelling with a dry ulcer found at the inoculation site. Three days later, the size of the lesion was 45 x 50 mm. Forty-four days after inoculation, the skin lesion was a 30 x 40-mm. swelling with a central dry ulcer. Fifty-two days after inoculation, a 50-mm.-diameter swelling with a central eschar was found.

Necropsy findings (66 days after inoculation). One- to 2-mm. white foci were found in the following lymph nodes: left parotid, left submaxillary, one node of the lumbar chain, anterior and posterior mediastinal, right bronchial and hepatic lymph nodes. Multiple white foci varying in size from 1 mm. in diameter to 1- x 4-mm. areas were found in the left prescapular lymph node. The liver and spleen contained white foci, 2 to 3 mm. in diameter, throughout the parenchyma. The skin lesion consisted of a swollen area 20 mm. in diameter with a central, 10-mm.-diameter ulcer. On cross section, the ulcer appeared shallow.

Histopathologic findings. Discrete and confluent granulomas were found in the parotid, retropharyngeal, left submaxillary, anterior and posterior mediastinal, right bronchial, lumbar, hepatic, mesenteric, and prescapular lymph nodes. Caseocalcareous lesions were found in all lymph nodes except the right prescapular. The degree of encapsulation varied somewhat, but unencapsulated lesions were found in all of these tissues. The cellular components were macrophages and occasionally giant cells. Hyperkeratosis, acanthosis, and a small ulcer were found in the skin at the site of inoculation. Numerous well-encapsulated granulomas were found in the dermis. Caseation necrosis and calcification were found in the center of 1 of these granulomas. Groups of lymphocytes and neutrophils were found in the lesion. Unencapsulated granulomas with caseocalcareous centers surrounded by macrophages and lymphocytes were found in the liver. Well-encapsulated granulomas with caseocalcareous centers were found in the spleen (Figure 3). In the lung, numerous scattered

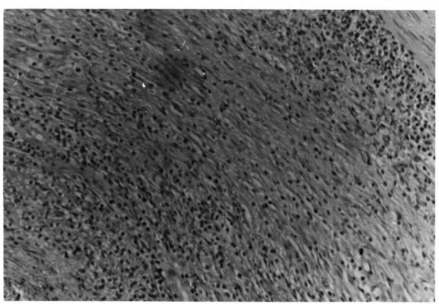


Figure 3. Capsule of a well-encapsulated granuloma found in the spleen of Pig 2-1, which was inoculated intradermally with Culture 81-0, M. bovis, swine origin. New Fuchsin - H & E. x187.

granulomatous foci, some with caseocalcareous centers, were found (Figures 4 and 5). These contained macrophages, eosinophils and lymphocytes. Little encapsulation was found.

Pig 2-4

Clinical observations. Eight days after inoculation, the inoculation site contained a circular lesion 25 mm. in diameter which was reddened with a necrotic center. By 15 days after inoculation the lesion consisted of a 25 x 35-mm. swelling with purulent material draining from a central ulcer. Twenty-three days after inoculation the skin lesion was a 25 x 40-mm. swelling with a 15 x 20-mm. draining central ulcer. The lesion was 30 x 45 mm. in size and reddened 35 days after inoculation. At 38 days after inoculation the lesion was 30 x 35 mm. in size. At 44 days, the lesion was a 30 x 40-mm. swelling with a dry, central ulcer. When examined at 52 days, the lesion was slightly swollen, 30 x 30 mm. in size, dry and covered with an eschar. Following this the lesion healed.

Necropsy findings (112 days after inoculation). Scattered 1- to 3-mm., yellowish foci were found in the supramammary, right submaxillary, right parotid, and medial and lateral retropharyngeal lymph nodes. The left submaxillary lymph node was enlarged and filled with a reddish-yellow caseous mass. The gastric, anterior and posterior mediastinal, and the bronchial lymph nodes were enlarged and contained both discrete and confluent caseocalcareous foci. The left prescapular lymph node was 40 x 30 x 20 mm. and filled with discrete and confluent caseocalcareous foci. Almost all mesenteric nodes contained caseocalcareous lesions 1 mm. in

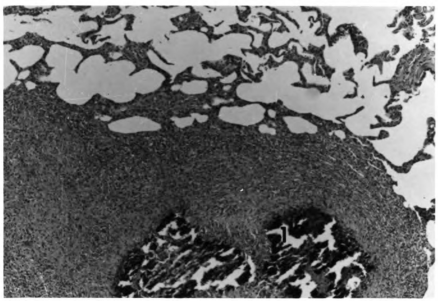


Figure 4. A granuloma with a caseocalcareous center (1) found in the lung of Pig 2-1, which was inoculated intradermally with Culture 81-0, M. bovis, swine origin. New Fuchsin - H & E. x75.

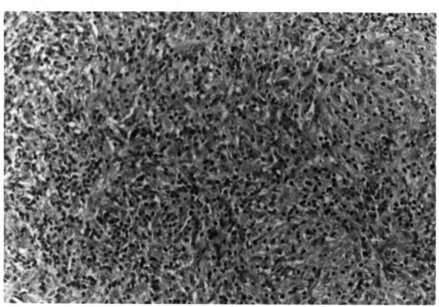


Figure 5. Noncaseating, unencapsulated granuloma found in the lung of Pig 2-1, which was inoculated intradermally with Culture 81-0, M. bovis, swine origin. New Fuchsin - H & E. x187.

diameter or larger. The hepatic lymph nodes were enlarged and filled with discrete and confluent caseocalcareous foci. The skin lesion was 60 x 30 mm. in area and 15 mm. thick and contained yellowish caseous foci distributed throughout. The lung, liver, and spleen contained many yellowish caseous foci, 1 to 6 mm. in diameter.

Histopathologic findings. Numerous granulomas were found in the following lymph nodes: submaxillary, medial and lateral retropharyngeal, anterior and posterior mediastinal, bronchial, hepatic, gastric, mesenteric, supramammary, left prescapular and right parotid. These lesions ranged from small, unencapsulated groups of macrophages to large, well-encapsulated granulomas with caseocalcareous centers. The caseous material almost filled the left submaxillary, supramammary, and left prescapular lymph nodes (Figure 6). Well-encapsulated granulomas with necrotic centers were found in the right prefemoral and left popliteal lymph nodes. Numerous scattered granulomas were found in the lungs, liver (Figure 7) and spleen. These varied from small groups of macrophages, lymphocytes, and a few giant cells to large, well-encapsulated granulomas with caseocalcareous centers. The dermis was thickened and contained numerous scattered granulomas, many with caseous or caseocalcareous centers. Cellular response included primarily macrophages and lymphocytes.

Culture $167C_1$-1, Group III, swine origin

Pig 3-4

Clinical observations. On the 8th day after inoculation there was a hard circumscribed lesion 20 mm. in diameter with a central eschar.

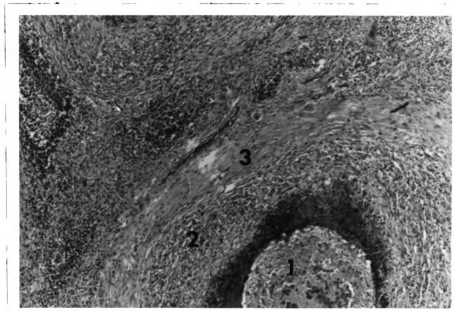


Figure 6. Well-encapsulated granulomas found in the left prescapular lymph node of Pig 2-4, which was inoculated intradermally with Culture 81-0, M. bovis, swine origin. Note necrotic center (1) surrounded by macrophages and lymphocytes (2) and dense connective tissue capsule (3). New Fuchsin - H & E. x75.

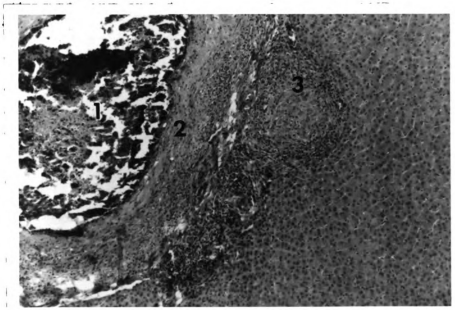


Figure 7. Granuloma with "daughter" tubercle found in the liver of Pig 2-4, which was inoculated intradermally with Culture 81-0, M. bovis, swine origin. Note caseocalcareous center (1), connective tissue capsule (2), and unencapsulated "daughter" tubercle consisting of macrophages and lymphocytes (3). New Fuchsin - H & E. x75.

Fifteen days after inoculation a 20 x 30-mm. swelling was found which had a central ulcer with purulent exudate exuding from it. By 23 days after inoculation the lesion was 30 x 40 mm. in size with caseous material in the ulcerated area. On the 35th day the lesion was 30 mm. in diameter and moist. The lesion was a soft swelling, dry and scabbed, and was 35 x 40 mm. by the 44th day. Fifty-two days after inoculation a 25 x 40-mm. swelling was present which had a small central eschar.

Necropsy findings (59 days after inoculation). Multiple white foci, 1 to 1.5 mm. in diameter, were found scattered throughout the left prescapular lymph node. They were more numerous in the middle portion of the node and under the subcapsular sinus. There was a raised lesion in the dermis which was yellowish and 10 mm. in diameter. In the underlying subcutis a localized reddened area was found.

Histopathologic findings. Several scattered small granulomas consisting of small groups of Langhans' giant cells and a few macrophages were found in the right submaxillary lymph node. In the left prescapular lymph node numerous granulomas were found which in some areas were becoming confluent. In many of the larger granulomas caseation necrosis and some calcification were found in the center (Figure 8). The necrotic areas were surrounded by macrophages, lymphocytes, some neutrophils, and eosinophils. Unencapsulated small granulomas and daughter tubercles were also found (Figure 9). Multiple scattered focal granulomas consisting of groups of macrophages and giant cells were found in the bronchial lymph nodes and posterior mediastinal lymph nodes (Figure 10). Focal granulomas consisting of groups of macrophages were found scattered

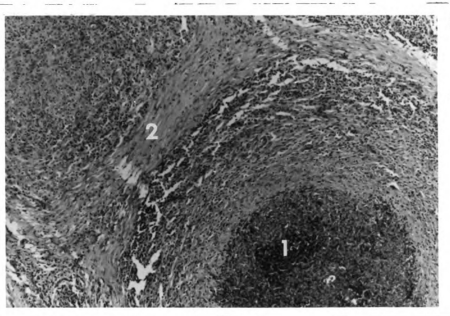


Figure 8. Contiguous granulomas found in the left prescapular lymph node of Pig 3-4, inoculated intradermally with Culture $167C_1$-1, Group-III mycobacteria, swine origin. Note necrotic areas (1) and dense connective tissue encapsulation (2). New Fuchsin - H & E. x75.

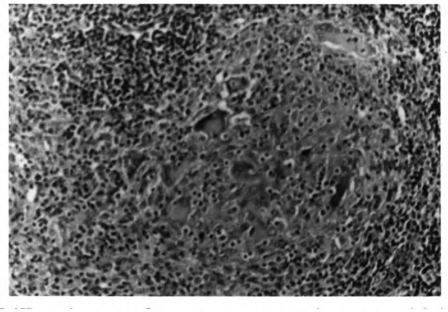


Figure 9. Unencapsulated, noncaseous granulomas containing macrophages, giant cells and lymphocytes found in the left prescapular lymph node of Pig 3-4. New Fuchsin - H & E. x187.

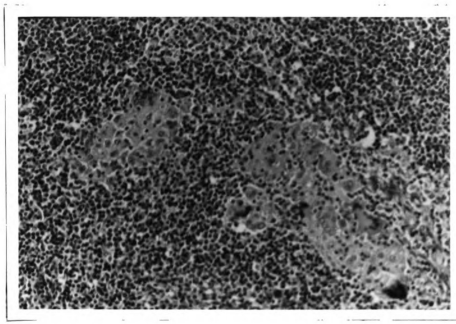


Figure 10. Unencapsulated, noncaseous granulomas consisting of groups of macrophages and giant cells found in the right bronchial lymph node of Pig 3-4, which was inoculated intradermally with Culture $167C_1$-1, Group-III mycobacteria, swine origin. New Fuchsin - H & E. x187.

throughout the spleen parenchyma. In the skin lesion, well-encapsulated granulomas made up of macrophages and lymphocytes and sometimes central areas of caseation necrosis were found. Areas of lymphocytic infiltration sometimes containing macrophages and/or giant cells were found throughout the parenchyma of the liver.

Pig 1-3

Clinical observations. Eight days after inoculation the lesion at the site of inoculation was swollen, soft, 15 x 20 mm. in size and had a central eschar. By 15 days the lesion was 20 mm. in diameter. A swelling 25 mm. in diameter was found at the inoculation site on the 23rd day. Thirty-five days after inoculation the lesion was 20 mm. in diameter, necrotic, and dry. At 44 days it was a soft 20 x 25-mm. swelling. The lesion was 20 mm. in diameter and covered with a dry eschar on the 52nd day, after which it healed.

Necropsy findings (107 days after inoculation). One of the submaxillary lymph nodes was filled with a yellow caseous material. The skin was reddened and the dermis slightly thickened at the inoculation site. No other lesions were found.

Histopathologic findings. One of the submaxillary lymph nodes contained numerous scattered granulomas; some were well-encapsulated and had caseocalcareous centers while others consisted of small groups of macrophages and/or numerous giant cells. Occasional isolated groups of 2 or 3 giant cells accompanied by a few macrophages were found in the left prescapular lymph node. The only lesion found at the inoculation site was slight dermal thickening.

Culture 186C-1, Group III, swine origin

Pig 3-2

Clinical observations. Eight days after inoculation a hard, 20 x 25-mm. swelling was found at the inoculation site. On the 15th and 23rd days the swelling was edematous and 25 mm. in diameter. On the 35th and 44th days after inoculation the skin lesion was edematous and measured 25 x 40 mm. Fifty-two days after inoculation the lesion consisted of a swollen area 25 mm. in diameter which was ulcerated with purulent material exuding from it.

Necropsy findings (66 days after inoculation). The prescapular lymph node contained numerous pale yellow foci 1 to 2 mm. in diameter scattered uniformly throughout the tissue. In the skin an ulcer 2 mm. in diameter was found. On section, this was reddened and appeared to be healing.

Histopathologic findings. Many scattered granulomas, consisting of macrophages and a few giant cells, were found in the left prescapular lymph node. Many of these granulomas had caseous or caseocalcareous centers. Sometimes the centers contained only a few degenerating neutrophils. Encapsulation varied from slight to moderate. In the skin, hyperkeratosis, acanthosis, and dermal thickening were found. Discrete and confluent granulomas were found in the dermis. These granulomas contained macrophages, lymphocytes, eosinophils, and neutrophils.

Pig 3-1

Clinical observations. A hard and edematous swelling 15 x 20 mm. in area was found at the inoculation site 8 days after inoculation. By 15 days after inoculation, the swelling was 20 x 50 mm. in area. Eight days later the swelling was soft and measured 25 x 50 mm. Thirty-five days after inoculation the lesion was a 20 x 35-mm., soft swelling. Little change was noted at 44 days. By 52 days the lesion had ulcerated and was 10 x 10 mm. in area. Following this it healed.

Necropsy findings (107 days after inoculation). The left prescapular lymph node was 20 x 25 x 16 mm. and was filled with discrete and confluent, whitish, caseous areas 1 mm. in diameter or larger. The skin at the inoculation site was possibly somewhat thickened.

Histopathologic findings. The left prescapular lymph node contained numerous lesions ranging from unencapsulated groups of macrophages to well-encapsulated granulomas with caseous or caseocalcareous centers surrounded by macrophages and lymphocytes. The larger, older lesions were becoming confluent. Some dermal thickening was noted in the skin at the site of inoculation.

Pig 3-3 (Uninoculated pen-mate of animals inoculated with Culture 167C_1-1 or culture 186C-1)

Clinical observations. No signs of disease were noted.

Necropsy findings (59 days after inoculation of pen-mates). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Culture $172C_1$-1, Group III, swine origin

Pig 1-4

Clinical observations. At 8 and again at 15 days after inoculation the lesion at the inoculation site was a 20 x 20-mm. swelling. Twenty-three days after inoculation the lesion was 25 x 30 mm. and soft and had a central eschar. At 35 days after inoculation the lesion was a 20 x 30-mm. swelling. By 44 days after inoculation the lesion was ulcerated, bleeding, and soft and 25 x 30 mm. in size. Fifty-two days after inoculation there was a 25 x 30-mm. swelling with a central eschar at the site of inoculation.

Necropsy findings (65 days after inoculation). Numerous, scattered, 1 to 3-mm. foci which appeared to be coalescing into lesions up to 10 mm. or more in diameter were found in the left prescapular lymph node. The skin was thickened (30 mm. in diameter) but did not appear to be ulcerated. One caseous area about 3 mm. in diameter was found in the thickened area.

Histopathologic findings. Numerous scattered granulomas were found throughout the hepatic lymph node. These ranged from small groups of macrophages to relatively large areas consisting of macrophages and giant cells surrounding a central area of caseation necrosis. Several scattered granulomas consisting of small groups of macrophages with a few giant cells were found in the nodes of the lumbar chain. There were a few scattered granulomas consisting of small groups of macrophages in

the posterior mediastinal lymph nodes. Numerous lesions were found in the left prescapular lymph node. These ranged from unencapsulated groups of macrophages and/or numerous giant cells to well-encapsulated granulomas with caseous or caseocalcareous centers surrounded by macrophages and lymphocytes. The dermis was thickened by an increase in connective tissue.

Pig 1-1

Clinical observations. Eight days after inoculation the skin lesion was swollen and hard and 15 x 20 mm. in area. At 15 days a 20 x 30-mm. swelling with a central eschar was found. By 23 days after inoculation the lesion was 25 x 30 mm. in area, ulcerated, and there was a purulent material exuding from it. Thirty-five days after inoculation there was a 25 x 25-mm. swelling and serous exudation. By 38 days the lesion was 25 x 30 mm. in area. Forty-four days after inoculation the lesion was 15 mm. in diameter and had a dry, central eschar. By 52 days the lesion was healed.

Necropsy findings (113 days after inoculation). Multiple discrete and confluent caseocalcareous foci were present in 1 of the submaxillary lymph nodes. There was a 30 x 25-mm. area which was 12 mm. thick in the dermis at the site of inoculation, but no caseous foci were found.

Histopathologic findings. Numerous lesions ranging from small, slightly encapsulated groups of macrophages to large, well-encapsulated granulomas with caseous centers surrounded by macrophages and lymphocytes were found in a submaxillary lymph node. Many giant cells were found in

the left prescapular lymph node. Cells of the Langhans type were more numerous but foreign-body giant cells were also present. In some places the giant cells were surrounded by macrophages. Acanthosis, some hyperkeratosis, a healing ulcer, and dermal thickening were found in the skin. There were a few scattered microscopic granulomas consisting of macrophages, lymphocytes, and eosinophils surrounded by connective tissue.

Culture $193C_2$-1, Group III, swine origin

Pig 2-3

Clinical observations. Eight days after inoculation there was a hard 30 x 40-mm. swelling at the inoculation site. By 15 days after inoculation the swelling was 30 x 40 mm. in area. At 23 days it was 30 x 60 mm. By 35 days the swelling was edematous, 30 x 50 mm., exuding serous fluid or weeping. Nine days later the swelling was 30 x 50 mm. and soft. The lesion was an ulcerated, 30 x 45-mm. swelling and was exuding purulent material by 52 days.

Necropsy findings (65 days after inoculation). The left prescapular lymph node contained numerous 1- to 4-mm., yellowish foci. A thickened area approximately 40 mm. in diameter containing numerous yellowish caseous areas was found in the skin. This lesion contained an 11 x 15-mm. ulcer.

Histopathologic findings. The left prescapular lymph node was almost filled with lesions ranging from unencapsulated groups of macrophages and giant cells to large, well-encapsulated granulomas with caseous or caseocalcareous centers. Also a few isolated giant cells were found.

Hyperkeratosis, acanthosis, dermal thickening, few granulomas, and an ulcer were found in the skin at the inoculation site.

Pig 1-6

Clinical observations. Eight days after inoculation a hard, 20 x 30-mm. swelling was present at the inoculation site. Seven days later the swelling was 25 x 35 mm. At 23 days after inoculation there was a 25 x 35-mm. swollen area with a central eschar. By 35 days the lesion was ulcerated, dry, and measured 20 x 40 mm. Three days later the swelling was 20 x 25 mm. At 44 days, it still was 20 x 25 mm. but was dry and had a central eschar. Fifty-two days after inoculation there was an eschar 10 mm. in diameter at the site, but no swelling. After this the lesion healed.

Necropsy findings (113 days after inoculation). A yellowish caseous lesion 5 x 5 x 8 mm. and 3 lesions 2 mm. in diameter were found in 1 medial retropharyngeal lymph node. One submaxillary lymph node contained many yellow, caseous, confluent or discrete lesions. The opposite node was filled with yellowish, caseous material. A slight thickening of the dermis was found at the inoculation site, but no caseous foci were detected.

Histopathologic findings. Numerous scattered granulomas consisting of small groups of macrophages and/or giant cells were found in the medial retropharyngeal lymph nodes. These were sometimes associated with small accumulations of necrotic neutrophils. Numerous giant cells

were found scattered throughout the submaxillary lymph nodes. These were occasionally surrounded by macrophages. Numerous, scattered granulomas were found in the left prescapular lymph node. These ranged from small unencapsulated granulomas consisting of 1 or more giant cells or a group of macrophages and/or giant cells to well-encapsulated granulomas with caseocalcareous centers surrounded by macrophages and giant cells. Only hyperkeratosis and dermal thickening due to increased collagen were seen in the skin.

Pig 1-5 (Pen-mate of swine inoculated with Culture $172C_1$-1 or $193C_2$-1)

Clinical observations. No clinical abnormalities were seen.

Necropsy findings (113 days after inoculation of pen-mates). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

M. avium, laboratory strain

Pig 5-3

Clinical observations. Eight days after inoculation there was a slight swelling, 10 mm. in diameter, at the inoculation site. At 14 days after inoculation there was a 15 x 17-mm., soft swelling with a chapped, weeping surface. At 19 days, the swelling was 10 mm. in diameter and had a central ulcer 2 mm. in diameter from which purulent material was draining. This swelling was still 10 mm. in diameter with central dry eschar at both 32 days and 43 days after inoculation. By 62 days after inoculation the swelling had disappeared and the lesion had healed.

Necropsy findings (82 days after inoculation). No gross lesions were found.

Histopathologic findings. A granuloma with central caseation necrosis surrounded by a few lymphocytes and macrophages and a moderate amount of connective tissue was found in the left prescapular lymph node (Figure 11). Also scattered, unencapsulated granulomas consisting of macrophages and giant cells were found. Only acanthosis, hyperkeratosis and dermal thickening with connective tissue were found in the skin at the site of inoculation.

Pig 5-4 (Pen-mate of animals inoculated with M. avium, laboratory strain)

Clinical observations. No abnormalities were detected.

Necropsy findings (82 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 5-2

Clinical observations. Eight days after inoculation there was a swelling 14 mm. in diameter at the site of inoculation. Fourteen days after inoculation a 16 x 20-mm., soft swelling was found. No change was noted when the lesion was examined 19 days after inoculation. At 32 days after inoculation the swelling was soft and 15 mm. in diameter with a central, 2-mm. ulcer covered by an eschar. Forty-three days after inoculation the swelling was 20 mm. in diameter and the ulcer 7 mm. in diameter. Sixty-two days after inoculation the lesion was a 5 x 6-mm.,

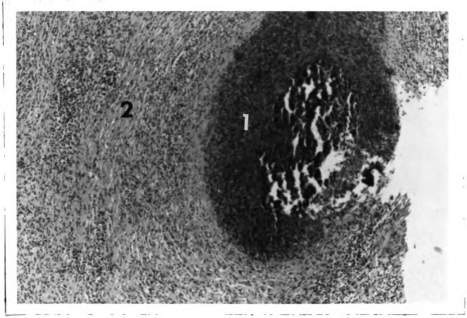


Figure 11. A granuloma found in the left prescapular lymph node of Pig 5-3 which was inoculated intradermally with M. avium, laboratory strain. Note central caseation necrosis (1) surrounded by macrophages, lymphocytes and a moderate amount of connective tissue (2). New Fuchsin - H & E. x75.

healing ulcer. Seventy-one days after inoculation the lesion was healed.

Necropsy findings (138 days after inoculation). A 15 x 15-mm., slightly thickened area was found in the skin. The distal 60 mm. of the spleen was bluish-red. A tumorous mass approximately 30 x 20 x 10 mm. was attached to the tip of the spleen. The central part of this mass was deep red.

Histopathologic findings. A granuloma consisting of a group of macrophages and lymphocytes was found in the left prescapular lymph node. The lesion was poorly encapsulated. Hyperkeratosis, parakeratosis, acanthosis and dermal thickening with connective tissue were found in the skin at the site of inoculation. Two large hematocysts filled with ghost cells and in process of organization were found in the section of the spleen.

Pig 5-5 (Pen-mate of animals inoculated with M. avium, laboratory strain)

Clinical observations. No abnormalities were detected.

Necropsy findings (138 days after inoculation of pen-mates). One yellow, caseous lesion approximately 10 mm. in diameter and a similar lesion approximately 5 mm. in diameter were found in the mesenteric lymph nodes.

Histopathologic findings. Two areas containing numerous granulomas consisting of macrophages and/or giant cells were found in the parotid lymph nodes. A few, similar granulomas were found in the submaxillary

lymph nodes. Two areas containing numerous scattered lesions ranging from unencapsulated granulomas made up of a few macrophages to large, coalescing, and well-encapsulated granulomas with caseous or caseocalcareous centers surrounded by macrophages were found in the sections of the mesenteric lymph nodes.

Pig 5-1

Clinical observations. Eight days after inoculation there was a swelling 10 mm. in diameter at the site of inoculation. Fourteen days after inoculation the swelling was soft and 13 x17 mm. At 19 days the lesion was a 15-x 18-mm., soft swelling. No change was noted at 32 days. Forty-three days after inoculation the swelling was 15 mm. in diameter and contained a central ulcer 10 mm. in diameter. Sixty-two days after inoculation there was a slight swelling, 10 mm. in diameter, with an 8-mm.-diameter, healing ulcer in the center. Seventy-one days after inoculation the ulcer was almost healed.

Necropsy findings (215 days after inoculation). No gross lesions were found.

Histopathologic findings. One granuloma with a caseocalcareous center surrounded by macrophages was found in the mesenteric lymph node. This was well-encapsulated. Hyperkeratosis, some acanthosis, and dermal thickening with connective tissue were noted in the area of inoculation.

Pig 5-6 (Pen-mate of animals inoculated with M. avium, laboratory strain)

Clinical observations. No abnormalities were detected.

Necropsy findings (215 days after inoculation). Three caseous lesions, approximately 2 mm. in diameter, were found in 1 mesenteric lymph node.

Histopathologic findings. One unencapsulated granuloma consisting of a few giant cells and macrophages was found in the retropharyngeal lymph node. In the mesenteric lymph node, several granulomas with caseo-calcareous centers surrounded by a few macrophages were found (Figure 12). These granulomas were well-encapsulated. Two granulomas were found in the section of the gastric lymph node. Each was made up of a few giant cells and macrophages and was unencapsulated.

Culture 206-2, M. avium, swine origin

Pig 10-3

Clinical observations. Seven days after inoculation there was a hard swelling 15 mm. in diameter at the inoculation site. At 14 days after inoculation the area was unchanged but the swelling was red and fluctuating. Twenty-two days after inoculation the lesion was soft, fluctuating, denuded and 24 mm. in diameter. Twenty-nine days after inoculation a soft, ulcerated, 30 x 40-mm. swelling was found. Thirty-six days after inoculation a soft, 30 x 35-mm. swelling with a 10-mm.-diameter ulcer was found.

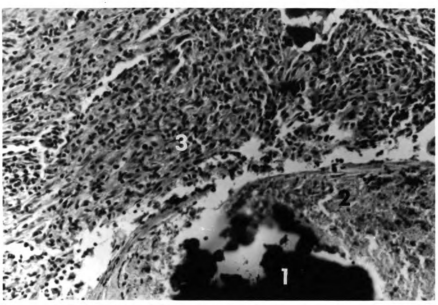


Figure 12. Mesenteric lymph node from Pig 5-6, inoculated intradermally with M. avium, laboratory strain. Note calcification (1), caseation (2), and area of macrophages and lymphocytes (3). New Fuchsin - H & E. x187.

Necropsy findings (43 days after inoculation). A swelling approximately 25 mm. in diameter with a central ulcer approximately 8 mm. in diameter was found in the skin at the inoculation site. Caseous foci, 1 mm. in diameter, were found in the left prescapular lymph node. Caseous foci, 1 to 10 mm. in diameter, were found in the anterior mediastinal lymph nodes. Caseous foci up to 15 mm. in diameter were found in the lungs.

Histopathologic findings. Numerous granulomas were found scattered throughout the left prescapular lymph node. These ranged from small, unencapsulated groups of macrophages and/or giant cells to well-encapsulated granulomas with caseocalcareous centers surrounded by macrophages. One area containing a few unencapsulated granulomas was found in the mesenteric lymph node. These granulomas consisted of small groups of giant cells and macrophages. Numerous large granulomas were found in the anterior mediastinal lymph nodes. In the liver numerous scattered granulomas with caseocalcareous centers surrounded by giant cells and macrophages were found. These lesions were well-encapsulated. In the lungs granulomas with caseocalcareous centers surrounded by macrophages, giant cells, and lymphocytes were found. Daughter tubercles were present; however, all were well-encapsulated.

Pig 10-1

Clinical observations. Seven days after inoculation there was a hard, 10 x 15-mm. swelling at the site of inoculation. No change was noted by 14 days after inoculation. By 22 days after inoculation the

swelling was soft, fluctuating, and measured 14 x 18 mm. Twenty-nine days after inoculation there was a 15 x 20-mm., soft, fluctuating swelling at the inoculation site. Thirty-six days after inoculation the lesion was 10 x 30 mm., soft, and fluctuating. Forty-nine days after inoculation there was a soft, fluctuating, ulcerated swelling 15 x 30 mm. at the inoculation site.

Necropsy findings (57 days after inoculation). One of the lateral retropharyngeal lymph nodes contained multiple, grayish-yellow foci 0.5 to 2 mm. in diameter scattered uniformly throughout the node. A 7-mm. red area was found at the inoculation site. There was no ulcer.

Histopathologic findings. One unencapsulated granuloma consisting of a few giant cells and macrophages was found in 1 section of a mesenteric lymph node. A few unencapsulated granulomas consisting of a few macrophages surrounding a small group of necrotic cells were found in the bronchial lymph nodes. Numerous scattered lesions ranging from rather large, unencapsulated groups of macrophages to well-encapsulated granulomas with caseocalcareous centers surrounded by macrophages were found in the lateral retropharyngeal lymph nodes (Figures 13 and 14). Granulomas made up of small, unencapsulated groups of macrophages and/or giant cells were found in the left prescapular lymph node. The dermis was greatly thickened with connective tissue at the inoculation site. A few granulomas with caseous centers surrounded by macrophages and lymphocytes were found in the dermis.

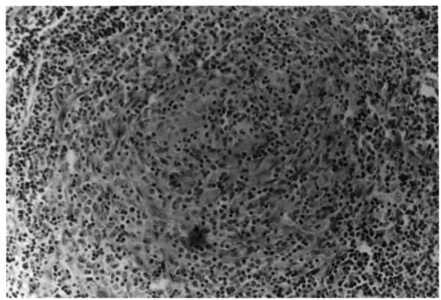


Figure 13. Unencapsulated group of macrophages found in the lateral retropharyngeal lymph node of Pig 10-1, which was inoculated intradermally with Culture 206-2, M. avium, swine origin. New Fuchsin - H & E. x187.

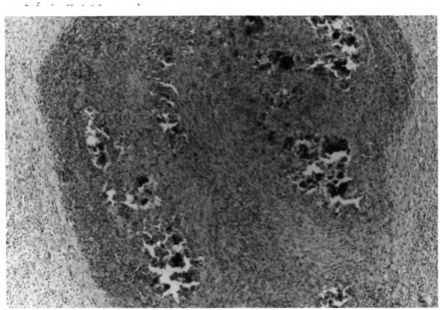


Figure 14. Well-encapsulated granulomas with caseocalcareous centers found in the lateral retropharyngeal lymph node of Pig 10-1, which was inoculated intradermally with Culture 206-2, M. avium, swine origin. New Fuchsin - H & E. x75.

Pig 10-4

Clinical observations. Seven days after inoculation a hard, 10-mm.-diameter swelling was found at the inoculation site. Fourteen days after inoculation the swelling was similarly hard and 7 mm. in diameter. At 22 days after inoculation there was a 10-mm.-diameter swelling with a central 5-mm. ulcer present. Twenty-nine days after inoculation there was a 10-mm.-diameter swelling while the ulcer appeared to be healing. By 49 days the swelling was 5 mm. in diameter and the ulcer was healed.

Necropsy findings (113 days after inoculation). No gross lesions were found.

Histopathologic findings. Scattered lesions ranging from small groups of macrophages with no encapsulation to moderately encapsulated granulomas with caseous centers surrounded by macrophages were found in the mesenteric lymph nodes.

Pig 10-2

Clinical observations. Seven days after inoculation there was a slight swelling 5 mm. in diameter found at the inoculation site. Fourteen days after inoculation, no swelling was detected. By 22 days, there was a hard, 14-mm.-diameter swelling at the site. The lesion was a soft, fluctuating swelling, 10 mm. in diameter, 29 days after inoculation. No change was noted at 36 or 49 days after inoculation.

Necropsy findings (113 days after inoculation). A 6-mm.-diameter caseocalcareous focus was found in the center of the left prescapular

lymph node. At the inoculation site a 15 x 2-mm. scar was found on the surface. On cross section, a 4 x 4 x 8-mm. lesion containing yellowish-green pus was found in the dermis.

Histopathologic findings. Numerous, scattered granulomas were found in the left prescapular lymph node. One was well-encapsulated and had a caseocalcareous center surrounded by macrophages and a few lymphocytes. This was surrounded by numerous daughter tubercles, some with necrotic centers and others made up of giant cells and/or macrophages only. These daughter tubercles were either nonencapsulated or only slightly encapsulated. One large granuloma with a caseocalcareous center which was surrounded by a few smaller granulomas was found in the dermis. Lymphocytes and macrophages were found in these granulomas. All were well-encapsulated.

Pig 10-5

Clinical observations. Seven days after inoculation the lesion at the inoculation site was a slight swelling 5 mm. in diameter. Fourteen days after inoculation there was a hard swelling, 10 mm. in diameter, found at the inoculation site. By 22 days after inoculation the lesion was a hard swelling, 18 mm. in diameter. A similar lesion, 14 mm. in diameter, was found 29 days after inoculation. Thirty-six days after inoculation a soft swelling 10 mm. in diameter was found. By 49 days after inoculation a 10-mm.-diameter, hard swelling was found which contained an ulcer 3 mm. in diameter.

Necropsy findings (175 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 10-6

Clinical observations. No swelling was found at the inoculation site until 36 days after inoculation when a hard 10-mm.-diameter swelling was found. Forty-nine days after inoculation the lesion was a soft, fluctuating swelling, 7 mm. in diameter.

Necropsy findings (175 days after inoculation). Two caseocalcareous lesions, 1 approximately 10 x 10 x 2 mm. and the other approximately 3 mm. in diameter, were found in 1 submaxillary lymph node.

Histopathologic findings. Numerous scattered lesions were found throughout a submaxillary lymph node. These ranged from small, unencapsulated groups of macrophages and/or giant cells to well-encapsulated granulomas with caseocalcareous centers. In these lesions, many macrophages and few giant cells were found.

Pig 10-7 (Pen-mate of animals inoculated with M. avium, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (182 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 10-8 (Pen-mate of animals inoculated with M. avium, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (182 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Culture 15D, Group III, pen origin

Pig 161-3

Clinical observations. No lesions were detected at the inoculation site when examined at 7, 12, 19, 33, and 48 days after inoculation.

Necropsy findings (138 days after inoculation). A slight thickening approximately 10 mm. in diameter was found at the inoculation site.

Histopathologic findings. There was some acanthosis, hyperkeratosis and slight dermal thickening with connective tissue at the inoculation site.

Pig 161-6

Clinical observations. No abnormalities were detected.

Necropsy findings. This animal was not necropsied. When slaughtered approximately 145 days after inoculation, no gross lesions were detected.

Culture 19_2W, Group III, pen origin

Pig 161-4

Clinical observations. A swelling 10 x 15 mm. was found at the inoculation site 7 days after inoculation. No abnormalities were detected when the lesion was examined at 12, 19, 33, and 48 days after inoculation.

Necropsy findings (138 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 161-2

Clinical observations. A 6-mm.-diameter swelling was noted 7 days after inoculation at the inoculation site. No abnormalities were found when the site was examined at 12, 19, 33, and 48 days after inoculation.

Necropsy findings. This animal was not necropsied. No gross lesions were observed when slaughtered approximately 145 days after inoculation.

Pig 161-7 (Pen-mate of animals inoculated with Culture 15D or 19_2W)

Clinical observations. No abnormalities were detected.

Necropsy findings (approximately 145 days after inoculation). This animal was not necropsied. No gross lesions were observed at slaughter.

Pig 161-8 (Pen-mate of animals inoculated with Culture 15D or 19_2W)

Clinical observations. No abnormalities were detected.

Necropsy findings (approximately 145 days after inoculation). This animal was not necropsied. No gross lesions were observed at slaughter.

Pig 161-9 (Pen-mate of animals inoculated with Culture 15D or 19_2W)

Clinical observations. No abnormalities were detected.

Necropsy findings (approximately 145 days after inoculation). This animal was not necropsied. No gross lesions were observed at slaughter.

B. Oral Administration

Culture 81-0, M. bovis, swine origin

Pig 7-1

Clinical observations. No abnormalities were detected.

Necropsy findings (79 days after administration of culture). The right lateral retropharyngeal lymph node was approximately 1/3 filled with yellowish caseous material. The right submaxillary lymph node was enlarged (70 x 50 x 30 mm.) and filled with yellow caseous material. Two lymph nodes were found in the area normally occupied by the left submaxillary lymph node. One of these nodes was 80 x 80 x 40 mm. and the other was 30 x 20 x 15 mm. The nodes were filled with a greenish, tenacious material and had some firm areas filled with yellow caseous material. The anterior and posterior mediastinal and the bronchial lymph nodes were enlarged and contained 1- to 2-mm.-diameter yellow foci scattered throughout. Several nodes from the gastric and hepatic group contained yellowish-white, caseous foci. Most of the mesenteric lymph nodes were filled with

yellowish-white, caseous foci. A 10-mm.-diameter, dull, whitish area was found in 1 kidney.

Histopathologic findings. The submaxillary lymph nodes were almost filled with lesions. In general, these were large granulomas with caseous or caseocalcareous centers surrounded by macrophages and were well-encapsulated. Also numerous small granulomas made up of small groups of macrophages or macrophages and giant cells were found. Several small granulomas consisting of a few macrophages and/or giant cells were found in the left medial retropharyngeal lymph node. Numerous scattered and confluent lesions ranging from small groups of macrophages and giant cells with no encapsulation to large, well-encapsulated granulomas with caseocalcareous centers surrounded by macrophages were found in the gastric, hepatic, and right lateral retropharyngeal lymph nodes. Similar lesions were found in the mesenteric lymph nodes except that they were still more numerous. In some nodes the granulomas had coalesced until the resulting lesion almost filled the node. Several focal granulomas, some of which were becoming confluent, were found in the left popliteal lymph node. Early, central areas of caseation necrosis surrounded by macrophages were found in these lesions. They were unencapsulated to moderately encapsulated. Lesions similar to those found in the submaxillary lymph node were also found in the right prescapular lymph node. In the kidney a few small, scattered, unencapsulated granulomas consisting of a few macrophages surrounded by lymphocytes were found. Also there were numerous small areas of lymphocytic infiltration. One granuloma with early central caseation necrosis surrounded by macrophages and lymphocytes

was found in an interlobular area of the liver. Also, isolated areas of leukocytic infiltration were found within and beside the lobules.

Pig 7-6 (Pen-mate of animals fed Culture 81-0, M. bovis, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (79 days after administration of culture to pen-mates). Several 1- to 2-mm.-diameter, yellowish foci were found in the gastric lymph nodes. A caseous, yellow lesion approximately 5 x 10 x 10 mm. was found in 1 mesenteric lymph node.

Histopathologic findings. A few granulomas were found near the capsule of the left parotid lymph node. These consisted of areas of early central caseation necrosis surrounded by macrophages and a few giant cells. Occasionally noncaseous granulomas were found. Scattered focal or diffuse, unencapsulated lesions consisting of large groups of macrophages and giant cells were found in the right submaxillary lymph node. Early caseation necrosis was sometimes found. Several unencapsulated to moderately encapsulated granulomas were found in the bronchial lymph nodes. Some had small caseous centers, while others consisted of small groups of macrophages. Numerous scattered granulomas with little or no encapsulation were found in the gastric and hepatic lymph nodes. Some had caseous or caseocalcareous centers, but the lesions were mainly groups of macrophages and few giant cells. In the area in which the gross lesion of the mesenteric lymph node was found numerous, discrete or confluent granulomas, some with a moderate amount of central, caseous or caseocalcareous material were found. The macrophage was the predominant

cell in these lesions. Some of the granulomas were moderately encapsulated while others were not. Other areas of the mesenteric lymph nodes were normal. In the lung, 1 unencapsulated peribronchial granuloma consisting of macrophages, lymphocytes, and plasma cells was found. Many of the bronchi contained macrophages. The alveolar walls were thickened.

Pig 7-2

Clinical observations. No abnormalities were detected.

Necropsy findings (134 days after administration of the culture). The left submaxillary lymph node was enlarged (70 x 40 x 30 mm.) and filled with yellow caseous or caseocalcareous, discrete or confluent lesions. The right submaxillary lymph node was enlarged (50 x 40 x 20 mm.) and contained similar lesions. The right bronchial lymph node was enlarged (50 x 20 x 25 mm.) and filled with yellowish, caseocalcareous lesions from 1 to 5 mm. in diameter. The left bronchial lymph node contained a few scattered, yellowish, caseous lesions 1 to 3 mm. in diameter. One of the anterior mediastinal lymph nodes contained a yellowish, caseous lesion approximately 5 mm. in diameter. Yellowish, caseous lesions from 3 to 5 mm. in diameter were found in the gastric and hepatic lymph nodes. Scattered, caseous lesions 1 to 3 mm. in diameter were found throughout the mesenteric lymph nodes. Scattered, yellowish-white foci up to approximately 0.5 mm. in diameter were found in the liver.

Histopathologic findings. Numerous, scattered granulomas were found throughout the bronchial and the anterior mediastinal lymph nodes. Many of these granulomas appeared to be coalescing. Most had caseous centers;

some also contained calcium. Small granulomas without necrotic centers were also found. These granulomas were made up of macrophages, giant cells, and lymphocytes. In general, the lesions were well-encapsulated, but some had little or no encapsulation. Similar lesions were found in the submaxillary lymph nodes, except that more coalescence, necrosis, calcification, and encapsulation were present. Daughter tubercles were also seen. In an area of the left parotid lymph node, several scattered granulomas were found. Most had caseous or caseocalcareous centers surrounded by macrophages. Early attempts at encapsulation were found. Numerous granulomas were found in the mesenteric lymph nodes. Most had caseous or caseocalcareous centers surrounded by macrophages. Encapsulation varied from none to extensive. The lesions in the gastric lymph nodes were numerous and scattered, ranging from small granulomas without necrotic centers to moderate-sized granulomas with caseous or caseocalcareous centers. Macrophages and a few lymphocytes were found in these lesions. The encapsulation varied from none to extensive. Similar lesions were found in the hepatic lymph nodes except that, in general, there was less encapsulation present. In the liver, occasional areas of lymphocytic infiltration were found. These were not granulomas. Numerous granulomas consisting of groups of macrophages and lymphocytes were found in the kidneys. In the lung, occasional granulomas consisting of accumulations of lymphocytes, macrophages, and giant cells were found.

Pig 7-5 (Pen-mate of animals fed Culture 81-0, *M. bovis*, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (134 days after administration of cultures to pen-mates). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 7-3

Clinical observations. No abnormalities were detected.

Necropsy findings (203 days after administration of organisms). Several scattered calcareous foci, 1 to 10 mm. in diameter, were found in the left parotid, right submaxillary, right medial retropharyngeal, bronchial, anterior mediastinal, gastric, and mesenteric lymph nodes. A 1-mm-diameter caseous nodule was found in the cortex of the left prescapular lymph node. A few 1- to 3-mm.-diameter, whitish foci were found scattered throughout the spleen.

Histopathologic findings. The submaxillary lymph nodes were almost filled with discrete or confluent granulomas. Most had extensive caseocalcareous centers surrounded by a few macrophages. In addition, small, unencapsulated granulomas consisting of groups of macrophages were found. Similar lesions were found in the right medial retropharyngeal and left parotid lymph nodes. Numerous granulomas were found almost filling the right bronchial lymph node. Many were becoming confluent. Numerous daughter tubercles were found. Encapsulation varied from slight to extensive. Similar lesions were found in the left bronchial and anterior mediastinal lymph nodes, except that the encapsulation was more pronounced. Numerous scattered lesions ranging from small groups of macrophages to

large confluent granulomas with caseocalcareous centers were found in the mesenteric lymph nodes. Encapsulation ranged from none to extensive. Similar lesions were found in the gastric lymph nodes. Occasional granulomas consisting of 1 to 2 giant cells and a few macrophages were found in the left prescapular lymph node. In the spleen, a large granuloma with caseocalcareous center and extensive encapsulation was found.

Pig 7-4 (Pen-mate of animals fed M. bovis, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (203 days after organisms were administered to pen-mates). The submaxillary lymph nodes were greatly enlarged and filled with confluent or discrete yellow caseocalcareous lesions. The bronchial, anterior and posterior mediastinal, and gastric lymph nodes contained numerous, scattered, discrete or confluent, yellowish foci 1 to 4 mm. in diameter. In the liver, scattered white areas 4 to 5 mm. in diameter were found lying just under the capsule.

Histopathologic findings. The submaxillary lymph nodes were almost filled with lesions ranging from small, unencapsulated granulomas made up of groups of macrophages to very large, confluent, well-encapsulated granulomas with caseocalcareous centers. In the parotid lymph nodes, numerous, scattered, unencapsulated granulomas made up of groups of giant cells and macrophages were found. Numerous, small granulomas, some having caseocalcareous centers and being slightly encapsulated and others having little or no encapsulation nor necrosis, were found in the lateral retropharyngeal lymph nodes. Numerous scattered lesions ranging from small

unencapsulated groups of macrophages to encapsulated granulomas with caseocalcareous centers were found in the medial retropharyngeal and anterior mediastinal lymph nodes. Similar lesions were found in the bronchial and posterior mediastinal lymph nodes, except that giant cells were more prevalent in these lesions. One area containing several granulomas was found in the mesenteric lymph nodes. These granulomas ranged from unencapsulated granulomas made up of small groups of giant cells and macrophages to slightly encapsulated groups of macrophages with early, central caseation necrosis. Similar lesions were found almost filling the gastric lymph nodes. A noncaseating granuloma consisting of a small group of giant cells and macrophages was found in the left prescapular lymph node. A group of granulomas, some with early caseation necrosis in their centers, were found in the liver. Also, scattered throughout the parenchyma of the liver were small areas of lymphocytes and/or macrophages. In the lung, 1 small granuloma was found which consisted only of a few macrophages. There was also alveolar hemorrhage and thickening of the alveolar wall.

Culture $172C_1$-1, Group III, swine origin

Pig 6-1

Clinical observations. No abnormalities were detected.

Necropsy findings (78 days after administration of the organisms). Whitish foci, 2 to 3 mm. in diameter, were found scattered near the surface of the liver.

Histopathologic findings. Many giant cells were found scattered throughout the mesenteric lymph nodes. These usually occurred singly, lying in groups of reticulum cells, but occasionally they were surrounded by groups of macrophages. Also granulomas with caseous or caseocalcareous centers surrounded by macrophages were found. One was well-encapsulated while the others were not encapsulated.

Pig 6-6 (Pen-mate of animals fed Culture $172C_1$-1, Group III, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (78 days after administration of the organisms). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 6-2

Clinical observations. No abnormalities were detected.

Necropsy findings (136 days after administration of the organisms). Several yellowish, caseous or caseocalcareous lesions were found in 2 of the mesenteric lymph nodes.

Histopathologic findings. Numerous, scattered granulomas were found in the mesenteric lymph nodes. Most of these granulomas were well-encapsulated and had caseocalcareous centers surrounded by macrophages and lymphocytes. A few had little or no necrotic material or encapsulation and were made up of macrophages and lymphocytes.

Pig 6-5 (Pen-mate of animals fed Culture $172C_1$-1, Group III, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (136 days after administration of the organisms). No gross lesions were found.

Histopathologic findings. One well-encapsulated granuloma, consisting of macrophages surrounding a few necrotic neutrophils, was found in the right submaxillary lymph node. Several lesions ranging from unencapsulated groups of macrophages to well-encapsulated granulomas with caseocalcareous centers were found in the mesenteric lymph nodes. Numerous scattered granulomas, consisting of groups of giant cells and macrophages, were found in the medial retropharyngeal lymph nodes.

Pig 6-3

Clinical observations. No abnormalities were detected.

Necropsy findings (206 days after administration of culture). Numerous caseous foci, 2 to 10 mm. in diameter, were present throughout the length of the chain of mesenteric lymph nodes. Similar lesions were found in the gastric and hepatic lymph nodes. Numerous caseous foci, 0.5 to 4 mm. in diameter, were found in the submaxillary lymph nodes.

Histopathologic findings. Several scattered granulomas with encapsulation ranging from moderate to none were found in the submaxillary lymph nodes. Some of these granulomas had caseocalcareous centers, while others were made up of macrophages only (Figure 15). Numerous scattered granulomas

were found throughout the mesenteric, gastric and hepatic lymph nodes (Figure 16). These ranged from unencapsulated groups of macrophages to large, well-encapsulated granulomas with caseocalcareous centers. Giant cells were prevalent in these lesions. Similar lesions were found in the colic lymph nodes.

Pig 6-4 (Pen-mate of animals fed Culture $172C_1$-1, Group III, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (206 days after administration of the culture). Two calcified foci, 1 mm. in diameter, were found in the mesenteric lymph nodes.

Histopathologic findings. One granuloma consisting of an area of coagulation necrosis surrounded by macrophages was found in the left submaxillary lymph node. Four unencapsulated granulomas were found in the right bronchial lymph node. Three of these consisted of small groups of macrophages and/or giant cells. The other had a small caseous center surrounded by macrophages. Numerous scattered lesions ranging from small groups of macrophages and/or giant cells to granulomas with caseocalcareous centers were found in the mesenteric lymph nodes. Encapsulation was slight to absent. Numerous, scattered granulomas consisting of groups of macrophages and giant cells were found in the left prescapular lymph node. These lesions were unencapsulated.

Figure 15. Two contiguous granulomas found in the left submaxillary lymph node of Pig 6-3 to which Culture $172C_1$-1, Group-III mycobacteria, swine origin were administered orally. Note the moderately encapsulated granuloma with caseocalcareous center (1) and the adjacent unencapsulated, noncaseous granulomas (2). New Fuchsin - H & E. x75.

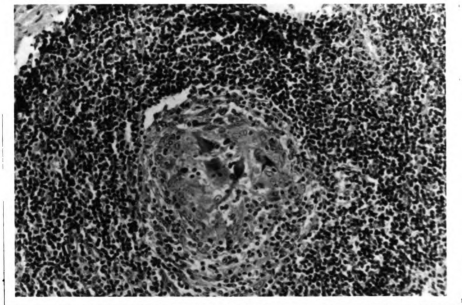


Figure 16. An unencapsulated, noncaseating granuloma found in a mesenteric lymph node of Pig 6-3 to which Culture $172C_1$-1, Group-III mycobacteria, swine origin was administered orally. New Fuchsin - H & E. x187.

M. avium, laboratory strain

Pig 8-2

Clinical observations. No abnormalities were detected.

Necropsy findings (52 days after administration of organisms). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 8-1 (Pen-mate of animals fed M. avium, laboratory strain)

Clinical observations. No abnormalities were detected.

Necropsy findings (52 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 8-3

Clinical observations. No abnormalities were detected.

Necropsy findings (108 days after inoculation). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 8-5 (Pen-mate of animals fed M. avium, laboratory strain)

Clinical observations. No abnormalities were detected.

Necropsy findings (108 days after administration of the culture). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Pig 8-4

Clinical observations. No abnormalities were detected.

Necropsy findings (169 days after administration of organisms). No gross lesions were found.

Histopathologic findings. Two granulomas were found in the submaxillary lymph nodes. Both had caseocalcareous centers surrounded by macrophages and were only slightly encapsulated.

Pig 8-6 (Pen-mate of animals fed M. avium, laboratory strain)

Clinical observations. No abnormalities were detected.

Necropsy findings (169 days after administration of organisms). No gross lesions were found.

Histopathologic findings. No microscopic lesions were found.

Culture 206-1, M. avium, swine origin

Pig 9-2

Clinical observations. No abnormalities were detected.

Necropsy findings (57 days after administration of organisms). No gross lesions were found.

Histopathologic findings. Numerous scattered granulomas were found in the mesenteric lymph nodes. These granulomas consisted of groups of

giant cells and/or macrophages. In some, there was early, central caseation necrosis. None was encapsulated.

Pig 9-1

Clinical observations. No abnormalities were detected.

Necropsy findings (57 days after administration of culture). No gross lesions were found.

Histopathologic findings. Numerous, scattered granulomas consisting of small groups of giant cells and macrophages were found in the submaxillary lymph nodes. These lesions were not encapsulated. Numerous granulomas were scattered throughout most of the mesenteric lymph nodes. For the most part, these consisted of accumulations of giant cells and macrophages with no encapsulations. However, some had caseous centers and were slightly encapsulated.

Pig 9-3

Clinical observations. No abnormalities were detected.

Necropsy findings (115 days after administration of culture). Circumscribed, yellowish-green, caseous foci, 1 to 7 mm. in diameter, were found throughout the mesenteric, hepatic, and gastric lymph nodes.

Histopathologic findings. Numerous scattered lesions, ranging from nonencapsulated groups of macrophages to well-encapsulated granulomas with caseocalcareous centers surrounded by macrophages were found in the mesenteric, hepatic, and gastric lymph nodes.

Pig 9-4

Clinical observations. No abnormalities were detected.

Necropsy findings (115 days after administration of culture). Throughout the length of the mesenteric lymph nodes there were whitish-yellow, circumscribed, caseocalcareous foci, 1 to 2 mm. in diameter. Similar lesions were found in the gastric and hepatic lymph nodes.

Histopathologic findings. Numerous, well-encapsulated granulomas with caseocalcareous centers were found in the gastric and hepatic lymph nodes. In addition, slightly encapsulated, daughter tubercles with or without caseous centers were found. Numerous, scattered lesions were found in all sections of the mesenteric lymph nodes. These ranged from unencapsulated groups of giant cells and/or macrophages to large, well-encapsulated granulomas with caseocalcareous centers surrounded by macrophages which almost filled the node. Numerous, scattered granulomas consisting of a few giant cells and/or macrophages and no encapsulation were found in the right submaxillary lymph node. In the liver, a few, scattered, unencapsulated granulomas were found in the parenchyma. These were made up of a few giant cells and macrophages.

Pig 9-5

Clinical observations. No abnormalities were detected.

Necropsy findings (171 days after administration of culture). About 30% of the mesenteric lymph nodes contained circumscribed yellowish caseocalcareous foci, approximately 2 to 5 mm. in diameter.

Histopathologic findings. A few unencapsulated granulomas consisting of a few giant cells and/or macrophages were found in the parotid lymph nodes. In the mesenteric lymph nodes, numerous, scattered lesions, ranging from unencapsulated groups of macrophages to large coalescing, well-encapsulated granulomas with central caseation necrosis were found. In general, the encapsulation was not extensive.

Pig 9-6

Clinical observations. No abnormalities were detected.

Necropsy findings (171 days after administration of the culture). Approximately 15% of the mesenteric lymph nodes contained yellowish, caseous foci, 2 to 4 mm. in diameter, in the cortical area.

Histopathologic findings. One well-encapsulated granuloma with caseous center surrounded by a few macrophages was found in a submaxillary lymph node. Numerous, scattered lesions were found throughout the mesenteric lymph nodes. They ranged from nonencapsulated groups of macrophages to well-encapsulated granulomas made up of almost entirely central caseocalcareous material. In general, the lesions were not well-encapsulated.

Pig 9-8 (Pen-mate of animals fed Culture 206-1, M. avium, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (177 days after administration of culture). A few circumscribed, yellowish, caseous foci, 2 to 4 mm. in diameter, were found in the submaxillary lymph nodes adjacent to the capsule. In the

liver, small, yellowish-white, solid foci, approximately 3 mm. in diameter, were found immediately beneath the capsule.

Histopathologic findings. Numerous, scattered granulomas were found in the submaxillary lymph nodes. These ranged from groups of macrophages with no necrosis or encapsulation to granulomas with caseocalcareous centers surrounded by macrophages and a moderate amount of connective tissue. In the liver, areas containing several lymph follicles each were found extending into the parenchyma from the capsule. These lesions were not considered granulomatous.

Pig 9-7 (Pen-mate of animals fed Culture 206-1, M. avium, swine origin)

Clinical observations. No abnormalities were detected.

Necropsy findings (177 days after administration of the culture). Approximately 3% of the mesenteric lymph nodes contained circumscribed, yellowish, caseous foci, 2 to 10 mm. in diameter.

Histopathologic findings. Numerous, scattered lesions were found in the mesenteric lymph nodes. These ranged from a few which were made up of groups of macrophages and/or giant cells and were not encapsulated to well-encapsulated granulomas with caseocalcareous centers which appeared to be coalescing.

Uninoculated Control Animals

Uninoculated animals served as controls for each phase of this experiment. In all, 15 animals were used for this purpose.

No clinical signs of disease were observed in any of the animals. None of the animals had any response to avian or mammalian tuberculin. Post-mortem examinations were performed on 3 of these animals and no gross or microscopic lesions were found. The remaining animals were slaughtered in order to salvage their meat value. Upon inspection, no gross lesions were found.

TABLE 2. Summary of lesions and bacteriologic isolations from pigs to which mycobacteria were administered.

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated*
2-2	81-0 M. bovis Swine	ID**	57	Ant. cervical, l. submaxillary, r. & l. lateral retropharyngeal, mid. cervical, ant. & post. mediastinal, r. & l. bronchial, mesenteric, hepatic, l. prescapular, lumbar, l. parotid, r. prescapular, & supramammary lymph nodes, lungs, liver, spleen & skin inoc. site.	A,B,C,D,E,F, liver - spleen
2-1	81-0 M. bovis Swine	ID	66	R. & l. parotid, r. & l. medial retropharyngeal, l. submaxillary, ant. & post. mediastinal, r. bronchial, lumbar, hepatic, mesenteric & r. & l. prescapular lymph nodes, liver, spleen, lung & skin inoc. site.	A,B,C,D,E,F, liver - spleen

*A - Lymph nodes of the head and neck region
B - Anterior and posterior mediastinal and bronchial lymph nodes
C - Hepatic, gastric, mesenteric and colic lymph nodes
D - Left prescapular lymph node
E - Lung
F - Skin inoculation site
**Intradermal inoculation

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
2-4	81-0 M. bovis Swine	ID	112	R. & l. submaxillary, r. & l. medial retropharyngeal, r. & l. lateral retropharyngeal, ant. & post. mediastinal, r. & l. bronchial, hepatic, gastric, mesenteric, supramammary, l. prescapular, r. parotid, r. prefemoral, and l. popliteal lymph nodes, lungs, liver, spleen & skin inoc. site.	B,C,D,E,F, liver - spleen
3-4	$167C_1$-1 Group III Swine	ID	59	R. submaxillary, l. prescapular, r. & l. bronchial, & post. mediastinal lymph nodes & spleen.	A,B,C,D,E,F, liver - spleen
1-3	$167C_1$-1 Group III Swine	ID	107	Submaxillary & l. prescapular lymph nodes.	A,B,C,D
3-2	186C-1 Group III Swine	ID	65	L. prescapular lymph node & skin inoc. site.	B,C,D,F
3-1	186C-1 Group III Swine	ID	107	L. prescapular lymph node.	B,C,D,F
3-3	$167C_1$-1, 186C-1 Group III Swine	Contact	59	None	B,C, liver - spleen

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
1-4	$172C_1$-1 Group III Swine	ID	65	L. prescapular, hepatic, lumbar & post. mediastinal lymph nodes & skin inoc. site.	B,C,D, liver - spleen
1-1	$172C_1$-1 Group III Swine	ID	113	Submaxillary & l. prescapular lymph nodes & skin inoc. site.	A,B,C,D,E,F
2-3	$193C_2$-1 Group III Swine	ID	65	L. prescapular lymph node & skin inoc. site.	B,C,D,F
1-6	$193C_2$-1 Group III Swine	ID	113	R. & l. medial retropharyngeal, r. & l. submaxillary & l. prescapular lymph nodes.	A,C,D,F
1-5	$172C_1$-1, $193C_2$-1 Group III Swine	Contact	113	None	C,D,F
5-3	M. avium Lab. strain	ID	82	L. prescapular lymph node.	B,C,D
5-4	M. avium Lab. strain	Contact	82	None	C, liver - spleen
5-2	M. avium Lab. strain	ID	138	L. prescapular lymph node.	C

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
5-5	M. avium Lab. strain	Contact	138	R. & l. parotid, submaxillary, mesenteric.	A,B,C
5-1	M. avium Lab. strain	ID	215	Mesenteric lymph node.	None
5-6	M. avium Lab. strain	Contact	215	Submaxillary, gastric & mesenteric lymph nodes.	A,C
10-3	206-2 M. avium Swine	ID	43	L. prescapular, anterior mediastinal & mesenteric lymph nodes & liver & lung.	A,B,C,E,F, liver - spleen
10-1	206-2 M. avium Swine	ID	57	Lateral retropharyngeal, left prescapular, r. & l. bronchial & mesenteric lymph nodes & skin inoc. site.	A,B,C,D,F
10-4	206-2 M. avium Swine	ID	113	Mesenteric lymph nodes.	A,B,C,D, liver - spleen
10-2	206-2 M. avium Swine	ID	113	L. prescapular lymph node & skin inoc. site.	D,E,F, liver - spleen
10-5	206-2 M. avium Swine	ID	175	None	Liver - spleen

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
10-6	206-2 *M. avium* Swine	ID	175	Submaxillary lymph node.	A,C,D
10-7	206-2 *M. avium* Swine	Contact	182	None	A
10-8	206-2 *M. avium* Swine	Contact	182	None	A,B,C
161-3	15D Group III Pen origin	ID	138	None	Observed B and D
161-6	15D Group III Pen origin	ID	138	None	Not examined
161-4	19_2W Group III Pen origin	ID	138	None	Observed B
161-2	19_2W Group III Pen origin	ID	138	None	Not examined

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
7-1	81-0 *M. bovis* Swine	Oral	79	R. & l. submaxillary, l. medial retropharyngeal, r. lateral retropharyngeal, gastric, hepatic, mesenteric, r. & l. bronchial, ant. & post. mediastinal, l. popliteal & r. prescapular lymph nodes, kidney & liver.	A,B,C,E
7-6	81-0 *M. bovis* Swine	Contact	79	L. parotid, r. submaxillary, r. & l. bronchial, gastric, hepatic & mesenteric lymph nodes & lungs.	B,C, liver - spleen
7-2	81-0 *M. bovis* Swine	Oral	134	R. & l. submaxillary, ant. & post. mediastinal, r. & l. bronchial, gastric, hepatic, mesenteric & l. parotid lymph nodes & liver & lungs.	A,B,C
7-5	81-0 *M. bovis* Swine	Contact	134	None	B,C
7-3	81-0 *M. bovis* Swine	Oral	203	R. & l. submaxillary, r. medial retropharyngeal, l. parotid, ant. mediastinal, r. & l. bronchial, gastric, mesenteric & l. prescapular lymph nodes & spleen.	A,B,C,D,E, liver - spleen

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
7-4	81-0 *M. bovis* Swine	Contact	203	R. & l. submaxillary, r. & l. parotid, r. & l. lateral retropharyngeal, r. & l. medial retropharyngeal, r. & l. bronchial, ant. & post. mediastinal, mesenteric, gastric & l. prescapular lymph nodes & liver & lungs.	A,B,C,D
6-1	$172C_1$-1 Group III Swine	Oral	78	Mesenteric lymph nodes.	B,C
6-6	$172C_1$-1 Group III Swine	Contact	78	None	C
6-2	$172C_1$-1 Group III Swine	Oral	136	Mesenteric lymph nodes.	A,C
6-5	$172C_1$-1 Group III Swine	Contact	136	R. submaxillary, mesenteric, & medial retropharyngeal lymph nodes.	A,C
6-3	$172C_1$-1 Group III Swine	Oral	206	Submaxillary, mesenteric, gastric, hepatic & colic lymph nodes.	C
6-4	$172C_1$-1 Group III Swine	Contact	206	L. submaxillary, r. bronchial, mesenteric & l. prescapular lymph nodes.	A,B,C

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
8-2	M. avium Lab. strain	Oral	52	None	A,D
8-1	M. avium Lab. strain	Contact	52	None	None
8-3	M. avium Lab. strain	Oral	108	None	A,E,I
8-5	M. avium Lab. strain	Contact	108	None	A,B,C,D,E
8-4	M. avium Lab. strain	Oral	169	Submaxillary lymph nodes.	A,E, liver - spleen
8-6	M. avium Lab. strain	Contact	169	None	A
9-2	206-2 M. avium Swine	Oral	57	Mesenteric lymph nodes.	A,B,C,E, liver - spleen
9-1	206-2 M. avium Swine	Oral	57	Submaxillary & mesenteric lymph nodes.	A,B,C, liver - spleen
9-3	206-2 M. avium Swine	Oral	115	Gastric, mesenteric & hepatic lymph nodes.	A,B,C,D, liver - spleen

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
9-4	206-2 *M. avium* Swine	Oral	115	R. submaxillary, gastric, hepatic & mesenteric lymph nodes.	A,B,C,D, liver - spleen
9-5	206-2 *M. avium* Swine	Oral	171	Parotid & mesenteric lymph nodes.	A,B,C,D,E
9-6	206-2 *M. avium* Swine	Oral	171	Submaxillary & mesenteric lymph nodes.	B,C,E, liver - spleen
9-8	206-2 *M. avium* Swine	Contact	177	Submaxillary lymph nodes.	A
9-7	206-2 *M. avium* Swine	Contact	177	Mesenteric lymph nodes.	A,C
1-2	Uninoculated control		108	None	None
2-6	Uninoculated control		108	None	None
2-5	Uninoculated control		112	None	None
4-1	Uninoculated control			None	Not examined

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
4-2	Uninoculated control			None	Not examined
11-1	Uninoculated control			None	Not examined
11-2	Uninoculated control			None	Not examined
11-3	Uninoculated control			None	Not examined
11-4	Uninoculated control			None	Not examined
11-5	Uninoculated control			None	Not examined
11-6	Uninoculated control			None	Not examined
11-7	Uninoculated control			None	Not examined
11-8	Uninoculated control			None	Not examined

TABLE 2--continued

Pig No.	Culture no., Classification, Origin	Manner of Exposure	Killed Days After Inoc.	Location of Lesions	Tissue pools from which acid-fast bacilli were isolated
11-9	Uninoculated control			None	Not examined
11-10	Uninoculated control			None	Not examined

TABLE 3. Increase in dermal thickness 48 hours after injection of avian and mammalian tuberculins at various intervals following exposure to selected mycobacteria.

Pig No.	Organism			Manner of Exposure	Days After Admin.	Increased dermal thickness (in mm.) following tuberculin	
	Cult. No.	Classif.	Origin			Avian	Mammalian
2-2	81-0	*M. bovis*	Swine	ID	35	4.0	10.0
2-1	81-0	*M. bovis*	Swine	ID	35	1.0	8.0
2-4	81-0	*M. bovis*	Swine	ID	35	4.5	5.0
					99	3.5	5.5
3-0	$167C_1$-1	Group III	Swine	ID	35	6.0	0.0
1-3	$167C_1$-1	Group III	Swine	ID	35	3.0	0.0
					99	7.0	4.5
3-2	186C-1	Group III	Swine	ID	35	5.0	3.5
3-1	186C-1	Group III	Swine	ID	35	7.0	4.0
					99	2.5	5.0
3-3	$167C_1$-1, 186C-1	Group III	Swine	Contact	35	0.0	0.0
1-4	$172C_1$-1	Group III	Swine	ID	35	2.0	0.0
1-1	$172C_1$-1	Group III	Swine	ID	35	0.0	0.0
					99	4.5	2.5
2-3	$193C_2$-1	Group III	Swine	ID	35	6.5	0.0
1-6	$193C_2$-1	Group III	Swine	ID	35	7.0	2.0
					99	9.0	4.0
1-5	$172C_1$-1, $193C_2$-1	Group III	Swine	Contact	35	0.0	0.0
					99	1.5	1.5
5-3		*M. avium*	Lab. Strain	ID	62	2.0	2.0

TABLE 3--continued

Pig No.	Organism: Cult. No.	Organism: Classif.	Organism: Origin	Manner of Exposure	Days After Admin.	Increased dermal thickness (in mm.) following tuberculin: Avian	Mammalian
5-4		M. avium	Lab. Strain	Contact	62	0.0	0.0
5-2		M. avium	Lab. Strain	ID	62	2.5	5.0
					133	24.0	14.0
5-5		M. avium	Lab. Strain	Contact	62	0.0	0.0
					133	23.0	2.0
5-1		M. avium	Lab. Strain	ID	62	3.0	1.5
					133	5.0	1.0
					208	3.5	0.0
5-6		M. avium	Lab. Strain	Contact	62	0.0	0.0
					133	1.0	0.0
					208	2.0	1.5
10-3	206-2	M. avium	Swine	ID	Not tested due to death after obtaining blood samples		
10-1	206-2	M. avium	Swine	ID	42	3.5	1.0
10-4	206-2	M. avium	Swine	ID	42	2.5NEC*	3.8
					98	5.5	1.5
10-2	206-2	M. avium	Swine	ID	42	4.0	2.0
					98	7.5	3.0

*NEC - necrotic

TABLE 3--continued

Pig No.	Organism: Cult. No.	Organism: Classif.	Organism: Origin	Manner of Exposure	Days After Admin.	Increased dermal thickness (in mm.) following tuberculin: Avian	Mammalian
10-5	206-2	M. avium	Swine	ID	42	4.0NEC	3.0
					98	6.0	2.0
					154	2.5	0.5
10-6	206-2	M. avium	Swine	ID	42	4.0NEC	4.0
					98	5.5	2.5
					154	2.0	1.0
10-7	206-2	M. avium	Swine	Contact	42	1.0	0.0
					98	5.0	4.0
					154	2.0	1.0
10-8	206-2	M. avium	Swine	Contact	42	0.0	0.0
					98	5.0	4.0
					154	1.0	0.0
161-3	15D	Group III	Pen	ID	48	0.0	0.0
161-4	19_2W	Group III	Pen	ID	48	1.0	0.0
161-2	19_2W	Group III	Pen	ID	48	0.0	0.0
161-6	15D	Group III	Pen	ID	48	0.0	0.0
7-1	81-0	M. bovis	Swine	Oral	71	1.0	2.5
7-6	81-0	M. bovis	Swine	Contact	71	3.0	4.0
7-2	81-0	M. bovis	Swine	Oral	71	3.0	6.0
					132	2.0	6.0
7-5	81-0	M. bovis	Swine	Contact	71	0.0	0.0
					132	0.0	0.0

TABLE 3--continued

Pig No.	Organism: Cult. No.	Organism: Classif.	Organism: Origin	Manner of Exposure	Days After Admin.	Increased dermal thickness (in mm.) following tuberculin: Avian	Mammalian
7-3	81-0	M. bovis	Swine	Oral	71	2.0	6.0
					132	2.0	7.5
					195	2.0	8.0
7-4	81-0	M. bovis	Swine	Contact	71	2.0	3.0
					132	3.0	8.0
					195	3.0	11.0
6-1	$172C_1$-1	Group III	Swine	Oral	72	4.0	2.0
6-6	$172C_1$-1	Group III	Swine	Contact	72	0.0	0.0
6-2	$172C_1$-1	Group III	Swine	Oral	72	3.0	2.0
					123	4.0	3.0
6-5	$172C_1$-1	Group III	Swine	Contact	72	1.0	0.0
					123	3.0	1.5
6-3	$172C_1$-1	Group III	Swine	Oral	72	5.0	4.0
					123	6.0	5.0
					186	4.5	4.0
6-4	$172C_1$-1	Group III	Swine	Contact	72	1.5	1.0
					123	4.0	2.5
					186	4.0	3.0
8-2		M. avium	Lab. Strain	Oral	42	0.0	0.0
8-1		M. avium	Lab. Strain	Contact	42	0.0	0.0
8-3		M. avium	Lab. Strain	Oral	42	0.0	0.0

TABLE 3--continued

Pig No.	Organism: Cult. No.	Organism: Classif.	Organism: Origin	Manner of Exposure	Days After Admin.	Increased dermal thickness (in mm.) following tuberculin: Avian	Mammalian
					98	2.0	0.0
8-5		M. avium	Lab. Strain	Contact	42	0.0	0.0
					98	0.0	0.0
8-4		M. avium	Lab. Strain	Oral	42	0.0	0.0
					98	1.0	0.0
					154	2.0	0.0
8-6		M. avium	Lab. Strain	Contact	42	0.0	0.0
					98	0.0	0.0
					154	0.0	0.0
9-2	206-2	M. avium	Swine	Oral	42	1.0	0.0
9-1	206-2	M. avium	Swine	Oral	42	2.0	1.5
9-3	206-2	M. avium	Swine	Oral	42	1.0	0.0
					98	5.0	2.0
9-4	206-2	M. avium	Swine	Oral	42	0.0	0.0
					98	4.0	3.0
9-5	206-2	M. avium	Swine	Oral	42	0.0	0.0
					98	10.0	4.5
					154	4.0	1.0
9-6	206-2	M. avium	Swine	Oral	42	0.0	0.0
					98	10.0	3.0
					154	1.5	1.0

TABLE 3--continued

Pig No.	Organism: Cult. No.	Organism: Classif.	Organism: Origin	Manner of Exposure	Days After Admin.	Increased dermal thickness (in mm.) following tuberculin: Avian	Mammalian
9-8	206-2	M. avium	Swine	Contact	42	0.0	0.0
					98	6.0	2.0
					154	2.0	2.0
9-7	206-2	M. avium	Swine	Contact	42	0.0	0.0
					98	8.5	4.5
					154	2.0	1.0

TABLE 4. Results of hematologic examinations of blood samples taken immediately prior to necropsy.

Pig No.	Hemoglobin (Gm./100 ml.)	Hematocrit (vol.%)	Leukocytes (per mm^3)
2-2	12.2	40	14,000
2-1	11.5	37	10,600
2-4	10.3	35	13,250
3-3	11.8	35	9,400
3-4	12.2	35	6,000
1-3	10.6	29	11,000
3-2	11.5	36	11,500
3-1	11.3	35	15,750
1-4	Not examined		
1-1	10.3	32	11,500
2-3	11.5	36	3,500
1-6	14.0	37	11,000
1-5	14.2	39	13,000
5-3	10.4	32	14,400
5-4	13.4	40	17,700
5-2	12.4	37	21,700
5-5	12.4	36	18,500
5-1	17.0	47	13,600
5-6	12.1	39	6,050
10-3	Not examined		
10-1	12.7	39	17,900
10-4	13.4	40	12,300
10-2	14.0	41	15,300

TABLE 4--continued

Pig No.	Hemoglobin (Gm./100 ml.)	Hematocrit (vol.%)	Leukocytes (per mm^3)
10-5	12.4	38	12,300
10-6	13.0	40	12,900
10-7	37.0	45	15,200
10-8	12.7	42	11,000
161-3	15.3	48	14,250
161-4	13.4	41	11,900
7-1	11.7	34	19,600
7-6	13.0	40	21,700
7-2	15.0	45	21,600
7-5	15.7	45	19,500
7-3	9.8	Not examined	7,250
7-4	15.0	44	12,100
6-1	10.4	31	14,700
6-6	11.4	34	18,100
6-2	14.0	43	15,300
6-5	17.0	49	16,000
6-3	12.1	35	13,500
6-4	11.7	35	11,500
8-2	10.8	38	11,200
8-1	10.8	37	11,900
8-3	13.4	40	14,700
8-5	12.1	36	11,700
8-4	15.0	43	19,550
8-6	15.0	44	12,800

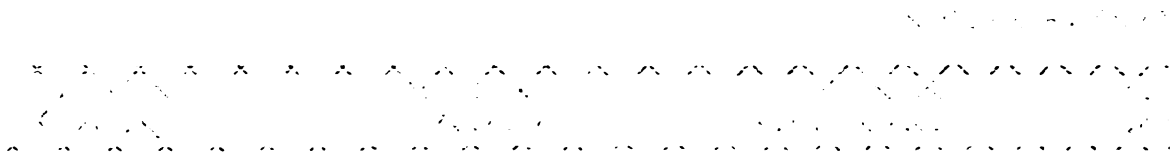
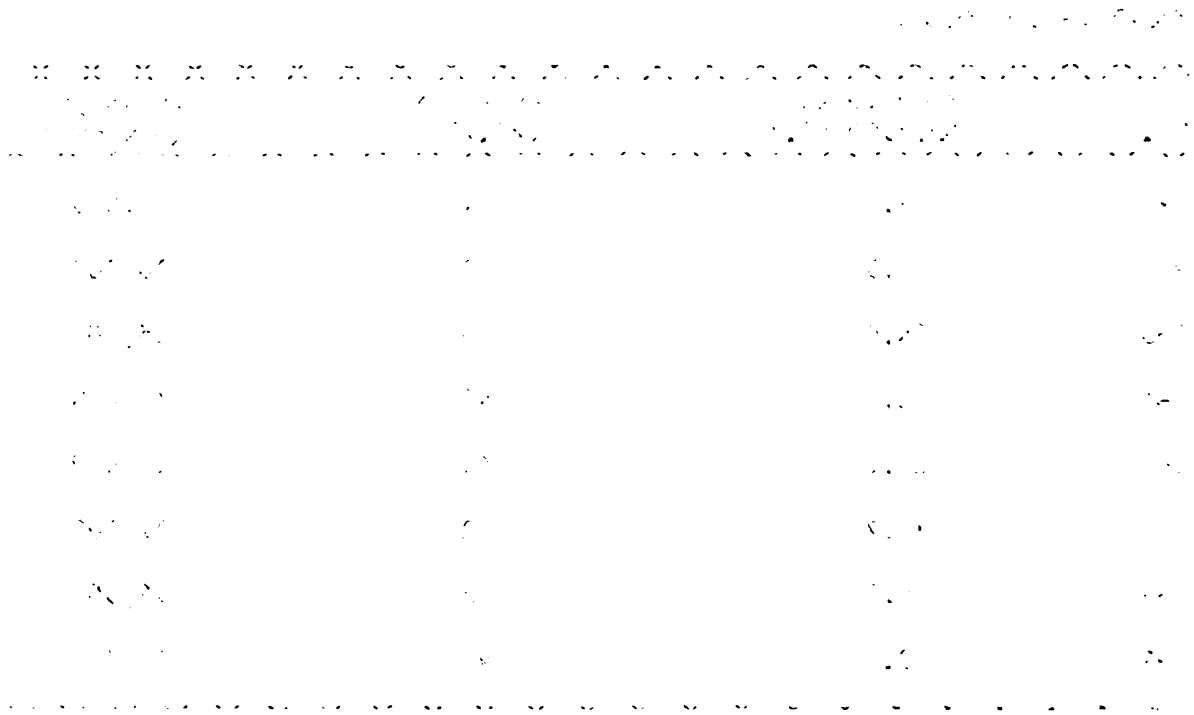


TABLE 4--continued

Pig No.	Hemoglobin (Gm./100 ml.)	Hematocrit (vol.%)	Leukocytes (per mm^3)
9-2	8.5	27	6,000
9-1	9.8	31	16,500
9-3	14.0	43	15,800
9-4	12.7	41	16,400
9-5	15.0	45	16,600
9-6	13.7	42	11,500
9-8	15.0	45	12,500
9-7	13.7	41	14,100



DISCUSSION

Clinical Findings

The only clinical abnormalities found were at the site of the intradermal inoculations.

In the 3 pigs inoculated with Culture 81-0, M. bovis of swine origin, swellings with ulcers were found at 8, 15, and 15 days, respectively, following inoculation. All lesions appeared to be healing when examined at 52 days. The swellings induced at the sites were, in general, the largest caused by any of the cultures. The maximum was a 50 x 50-mm. swelling found in Pig 2-1, 35 days after inoculation. McGavin (1964) reported ulceration followed by granulation of the intradermal inoculation site of calves inoculated with 1 mg. of the same culture. The maximum diameter of the swellings found were 20 mm. in 1 calf and 30 mm. in another. While many factors may have caused the larger swellings found in the pigs of this study, it should be noted that 2 mg. of the culture were injected into each pig.

Ulcers were found at the inoculation site of the 3 pigs inoculated with M. avium, laboratory strain. In 1 pig the lesion ulcerated between 14 and 19 days and was healed by the 62nd day. In the 2nd pig, an ulcer was found 32 days following inoculation. This lesion was healed by 71 days. In the 3rd pig, ulceration occurred between 32 and 43 days after inoculation. This lesion was almost healed by 71 days. The swellings were about half the size of those caused by M. bovis. McGavin (1964)

found no ulceration or swelling at the inoculation site when 1 mg. of this culture was injected intradermally into calves. This may have been due to the lower dosage, but more probably was due to a lower susceptibility of cattle to M. avium (Feldman, 1938).

The lesions produced at the inoculation site in the 6 pigs inoculated with Culture 206-2, M. avium of swine origin, varied considerably. At one extreme were Pigs 10-2, in which the lesion did not ulcerate and the size of the swelling was relatively small (maximum size was 14 mm. diameter at 22 days), and 10-6, in which no swelling was found until 36 days after inoculation. At the other extreme were Pigs 10-3, in which the swellings were almost as large as those caused by M. bovis and which ulcerated at 29 days, and 10-4, in which ulceration occurred between 14 and 22 days after inoculation. The severity of the lesion at the inoculation site in swine does not always correlate with the extent of the other pathologic changes found. These intradermal lesions were found to heal after ulceration, when sufficient time was allowed before the pig was killed. In general, the intradermal lesions caused by M. avium of swine origin were slightly more severe than those produced by the laboratory strain.

The 4 cultures of Group-III mycobacteria of swine origin were each injected into 2 pigs. Considerable variation in the time of ulceration was found in the case of the 2 pigs inoculated with Culture $167C_1$-1. An ulcer was found at the inoculation site in Pig 3-4, 15 days after inoculation while, in Pig 1-3, ulceration occurred between the 44th and 52nd day. In general, when pigs were inoculated with Group-III mycobacteria of swine origin, ulceration occurred between the 20th and 52nd day. The

lesions were larger in size than those produced by M. avium, ranging almost to the size of those found in some animals inoculated with M. bovis.

When McGavin (1964) inoculated these same Group-III cultures intradermally into calves, ulceration occurred 50% of the time, and the maximum diameter of the lesion was 5 mm. Granulomas were found at the inoculation site (and only there) in 5 of the 8 animals inoculated. This may have been due to the smaller numbers of organisms introduced or, more likely, to the higher resistance of calves to these organisms. In his study, Group-III mycobacteria of bovine origin produced ulcers in 6 of 10 animals.

Cultures 15D and 19_2W were each inoculated into 2 pigs. No lesions were found at the inoculation sites of Culture 15D. Culture 19_2W produced a 10 x 15-mm. swelling in 1 animal at 7 days and a 6 x 6-mm. swelling at 7 days in the other animal. Following this, no lesions were found.

Pathologic Findings

Characteristics of the Lesions

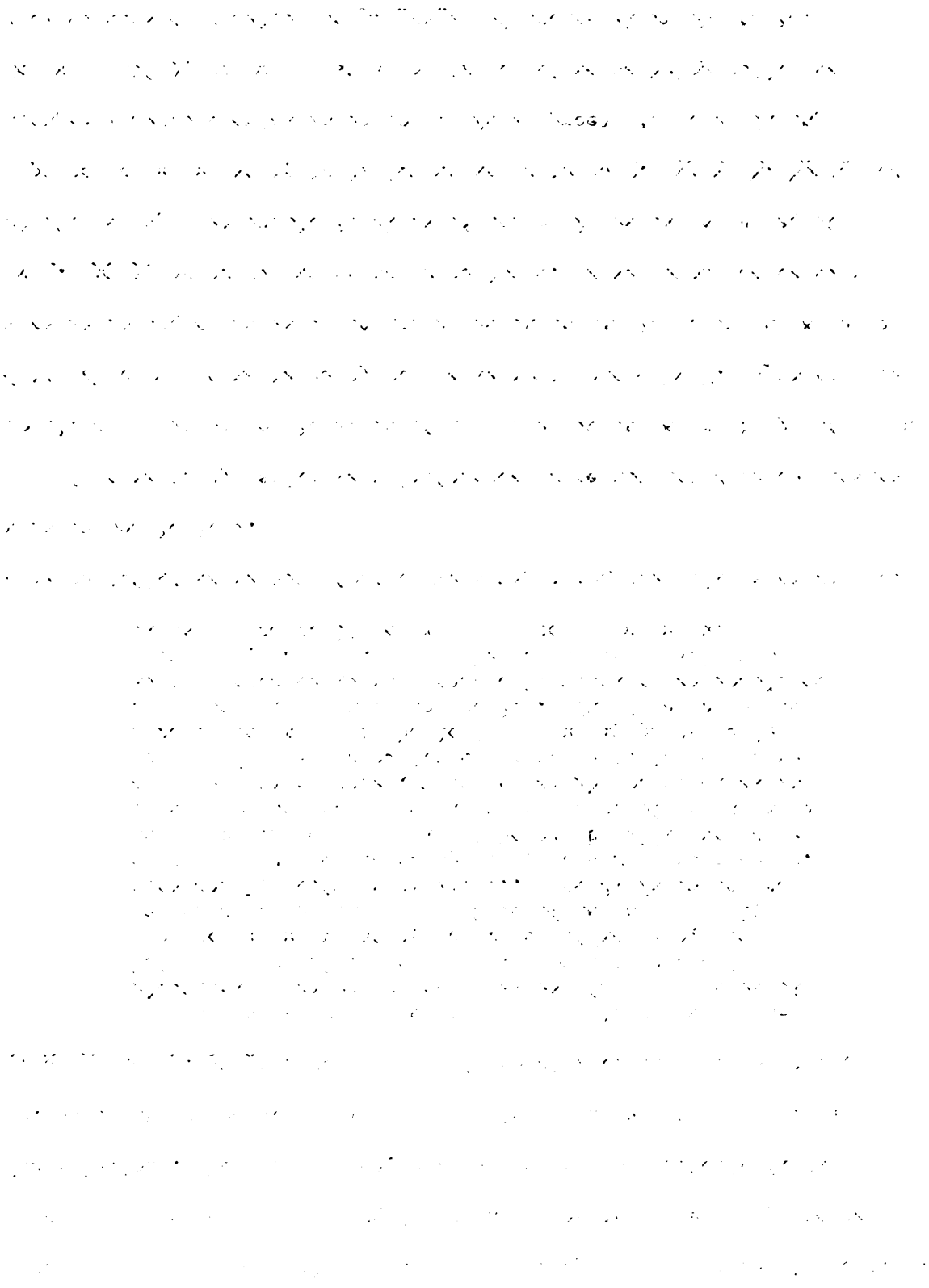
Controversy exists as to whether the type of tubercle bacillus can be determined by the character of the lesion it produces in swine. Day (1918) found the histologic structure of lesions due to M. avium, M. bovis and M. tuberculosis to be similar. Griffith and Griffith (1911) concluded that lesions caused by M. tuberculosis, M. bovis or M. avium could not be differentiated macroscopically. Cornell and Griffith (1929)

reported it was impossible to determine the type of the infecting organism when the nodes were not enlarged and when they contained only discrete focal lesions. Feldman (1938), citing the work of Nieberle (1931), Pallaske (1931) and Junack (1934) and his own findings, thought that M. bovis and M. avium infections could be differentiated. He said,

> "In the avian type infection, the infiltrative, neoplastic-like nature of the lesions, few or no tubercles being discernible, the minimal amount of caseation, and the slight tendency toward calcification, are features at variance with those of lesions usually produced by bovine bacilli. ... The differences are even more marked microscopically than macroscopically. In infections due to avian tubercle bacilli, the diffuse, progressive, nonencapsulated foci of epithelioid cells and histiocytes, with but slight or no caseation or calcification constitute a picture strikingly different from that of the lesions usually produced in swine by bovine tubercle bacilli. The disease in the latter instance is represented by nodular, encapsulated tubercles, which have a marked tendency to soften or caseate, and calcification is often pronounced."

Karlson (1964) expresses similar views regarding the differentiation of tuberculous lesions.

In this study, sufficient differences were not noted in the lesions to allow the accurate classification of the causative agent by this means. While there was some tendency of the lesions caused by M. avium to have more macrophages and less caseation and encapsulation than those caused by M. bovis, numerous exceptions were found. Based upon the results of this study, bacteriologic classification of the causative agent appears to be necessary to differentiate between M. avium, M. bovis and Group-III mycobacterial infections. This agrees with the findings of McGavin (1964), who was unable to differentiate pathologically the lesions caused in calves by M. bovis and Group-III mycobacteria.



Similarly, Corpe and Stergus (1963) concluded that lesions caused by Group-III organisms could not be differentiated from those caused by M. tuberculosis in man.

As pointed out by Rich (1950), solid epithelioid cell tubercles form before necrosis occurs. Therefore, early lesions of M. bovis infections would surely resemble the lesions of M. avium as described by Feldman (1938) and Karlson (1964). Moreover, in subsequent studies conducted by the tuberculosis research group at Michigan State University, lesions resembling those described by Feldman (1938) have been demonstrated in pigs inoculated with 2 other M. bovis strains. In these studies, 2 such M. bovis strains were injected into pigs intradermally. Two months after inoculation these animals were killed and necropsied. Lesions from these animals could not be differentiated from some caused by M. avium or from those reported by Feldman (1938).

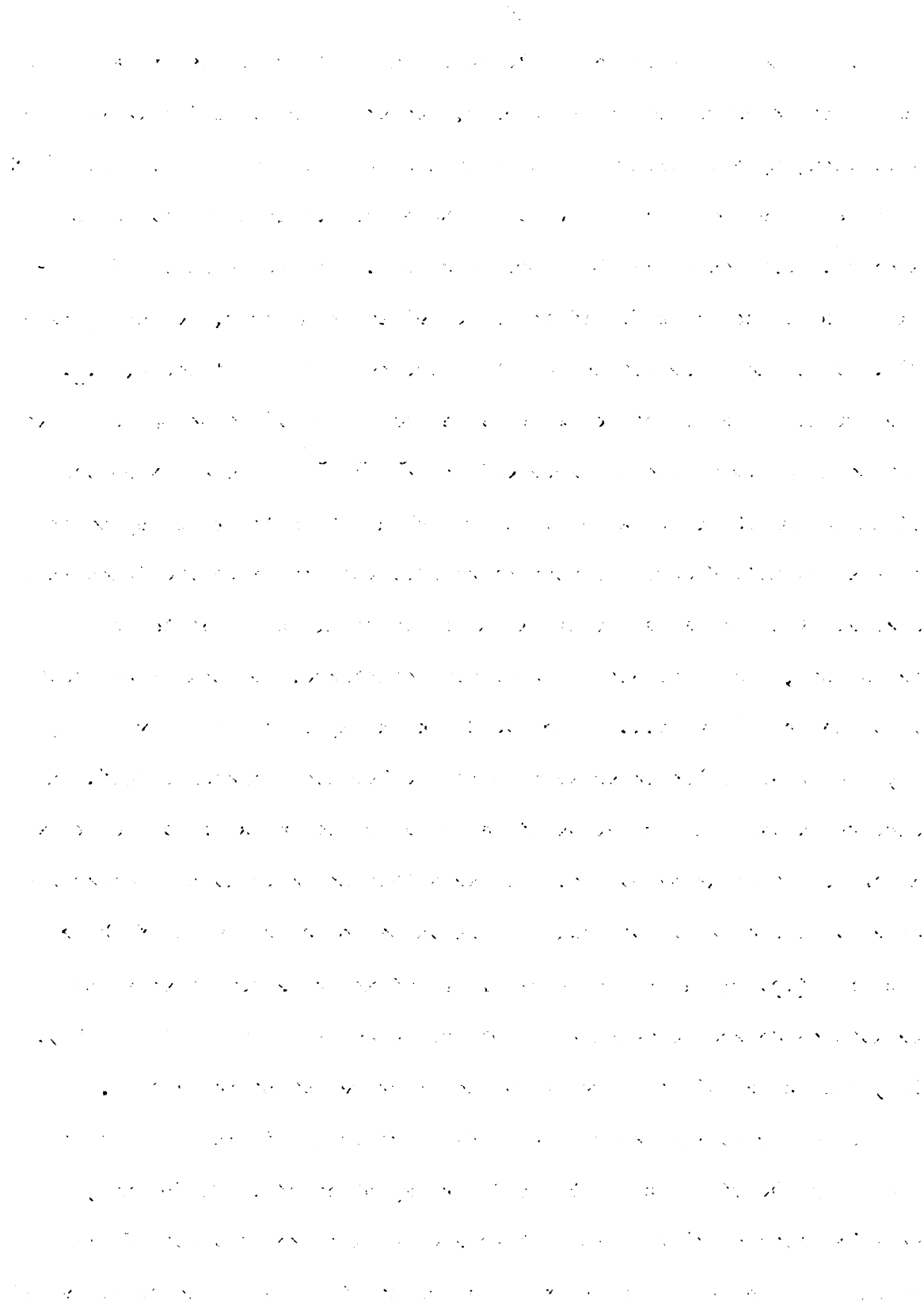
Perhaps the disagreement on this point is more apparent than real for Karlson (1964) and Feldman (1960, 1960a) both pointed out the danger of diagnosing tuberculosis from pathologic examination only. They say that tuberculosis cannot even be differentiated from other infectious, granulomatous diseases without suitable bacteriologic examinations. Also it should be noted that Feldman (1938) says,

> "Occasionally lesions due to avian tubercle bacilli occur which cannot be distinguished with certainty from those produced by bovine tubercle bacilli. In such instances resort must be had to laboratory procedures to establish the type of the infective agent."

He attributes this to lesions of long duration. In reading carefully his work, one notes that he says that the lesions usually can be differentiated and does not say that differentiation is always possible.

Histologic differences in lesions might better be used to get some idea about such factors as virulence, numbers of bacilli initiating the infection, hypersensitivity and resistance of the individual. Rich (1950) discusses the interrelationships of these factors as they relate to lesions. His ideas will be summarized here. For the complete discussion, the reader is referred to his excellent monograph, beginning page 714. In it he expresses these interrelationships in a formula, i.e. the extent and destructiveness of a lesion which will develop in a given tissue in a given time is expressed by $\frac{V \times N \times H}{NR + AR}$, wherein V equals virulence of the culture (defined by Rich as the ability to reproduce in the tissue), N equals number of bacilli that initiate the infection, H equals the hypersensitivity of the individual, NR equals the native resistance, and AR equals the acquired resistance. He points out that these factors "cannot...be expressed accurately or numerically in any way" and that it "is intended only as a schematic representation". He cautions against interpreting differences in lesions as caused by only 1 variable such as virulence. He feels that one must also consider the effects of the other factors. It should be noted that none of these factors, i.e. virulence, numbers of organisms, hypersensitivity, or resistance determine whether or not tubercles are formed (Rich, 1950). They influence only the extent and destructiveness of the lesion.

From a practical standpoint, this formula is difficult to use in comparing the virulence of 2 strains of mycobacteria. To do this, it must be assumed (1) that the native and acquired resistances of all animals in the study are constant (an assumption that would almost surely



be in error), (2) that the numbers of organisms initiating the infections are the same (this can be approximated only for the site of inoculation and only then if it is assumed that the rate of dissemination from the site is the same in all animals studied), and (3) one must know the degree of hypersensitivity that exists in the tissue at a particular time. It can be simplified somewhat by attempting to determine pathogenicity if pathogenicity is defined to include both virulence and ability to induce hypersensitivity. In order to minimize the effects of the variability inherent in "NR + AR" large numbers of animals could be used. Because of the problems, no attempt is made in this work to determine the degree of virulence or pathogenicity of the various organisms. Through necessity, such terms as "widespread disease" and "extent of disease produced" are used.

Acid-fast bacilli were not demonstrated in any of the sections stained by the new fuchsin-hematoxylin-eosin method of Willigan et al. (1961). Other workers have had similar problems when staining porcine tissues by this method (McGavin, 1963; Goyings, 1964; Schimmelpfennig, 1965). Subsequently, tissues containing gross and/or microscopic lesions from animals inoculated with cultures of M. bovis, M. avium and 2 swine-origin Group-III mycobacteria were sectioned and stained. Acid-fast bacilli had been isolated from these tissues. Duplicate sections were stained with the Ziehl-Neelsen stain for acid-fast bacteria (U. S. Armed Forces Institute of Pathology, 1960), by the same procedure except that staining time in the carbol fuchsin was increased to 30 minutes as suggested by Janack (1934) and with the MacCallum-Goodpasture stain for bacteria in tissue (U. S. Armed Forces Institute of Pathology, 1960).

No organisms were found in these tissues. Organisms in known positive slides stained well by all 4 methods. The reason for this finding was not determined. Perhaps the numbers of organisms were too few to be detected histologically. McGavin (1964) and Goyings (1965) were not always able to demonstrate acid-fast organisms in lesions of cattle caused by mycobacterial infections. Janack (1934) and Pallaske (1934) are quoted by Feldman (1938) as finding that acid-fast bacilli were more numerous in swine lesions caused by M. avium than in those caused by mammalian tubercle bacilli. Eastwood and Griffith (as quoted by Cornell and Griffith, 1929), Nieberle (1931) and Feldman (1938) reported that the number of organisms in lesions caused by M. avium varied from being exceedingly numerous to very few.

Extent of Disease

Culture 81-0, an M. bovis culture of swine origin, caused widespread tuberculosis in all of the test animals receiving it. This was true whether the culture was administered by intradermal injection or orally. This agrees with the reports of rapid generalization of porcine tuberculosis due to M. bovis reported by other workers (Schroeder and Mohler, 1906; Chausse, 1915; Griffith, 1907; Cornell and Griffith, 1929).

It should be noted that even with extensive disease present, these pigs gained weight rapidly and appeared normal. Schroeder and Mohler (1906) reported somewhat similar findings in a study wherein pigs were fed milk and bovine feces containing tubercle bacilli. They found the "general condition" of these pigs to be "excellent" despite extensive lesions of tuberculosis. Mohler and Washburn (1917) also noted that usually there are no signs of disease observed in the living animal.

The disease was somewhat more widespread in the pigs receiving the organisms by intradermal injection than in those to which they were fed. This was probably because of the loss of some of the bacteria in the digestive tract, either by death of the organism or its passage with the feces.

With the passage of time after inoculation, there was little difference found in the location of the lesions. However, the size of the lesions did increase. While practically all lesions contained unencapsulated granulomas, the amount of connective tissue encapsulation was greater in the lesions of the animals allowed to live the longest after administration of the culture.

In the swine inoculated intradermally the lesions of the lymph nodes draining the alimentary tract were probably due to the spread of organisms by 1 of the following ways: 1) hematogenous spread, 2) ingestion of organisms spread from the draining ulcers at the inoculation sites, or 3) from lung lesions, the organisms being coughed up and swallowed. The latter method of spread seems unlikely, since in Pig 2-2 the lesions in the nodes draining the alimentary tract appeared to have been established longer than those found in the lungs.

While either of the other 2 methods is feasible, hematogenous spread seems the most likely because lesions were also found in the spleen and right prescapular lymph node. Griffith (1907) reported finding tubercle bacilli in the blood of guinea pigs within 24 hours of subcutaneous inoculation. Muller and Isiwara (1914) found tubercle bacilli in 36.2% of 33 samples of heart blood from tuberculous cattle and swine, which indicates that a bacteremia may be fairly common in tuberculosis. Krause

(1920) reported the rapid dissemination of tubercle bacilli in guinea pigs. He isolated organisms from various lymph nodes, the spleen and lungs 48 hours after subcutaneous inoculation. Stimulated by these findings, Willis (1925) inoculated a group of guinea pigs intradermally with M. tuberculosis. He then removed the site of inoculation surgically from different guinea pigs at 1, 2, 3, 4, 5, 6, 10, 12, and 24 hours following inoculation and observed the animals for 43 to 75 days for signs and lesions of tuberculosis. He found generalized tuberculosis in some animals from which the inoculation site was removed at 1 hour, in most animals where it was removed in 2 hours, and in all animals whose lesions were not removed until 4 hours following inoculation. He felt that the spread was hematogenous. After isolating M. avium from normal appearing lymph nodes from swine which contained gross lesions of tuberculosis elsewhere, Feldman (1936) speculated that there might be hematogenous spread of the organisms in swine. Soltys and Jennings (1950) found that the blood of guinea pigs regularly contained tubercle bacilli the 1st few hours following the subcutaneous injection of 1 mg. of M. bovis. They felt "that the blood stream played an important part in the immediate distribution of tubercle bacilli in the body".

Two strains of M. avium were studied because the 1st strain used (laboratory strain), while still being pathogenic in chickens, caused only limited disease in swine. It was felt that the study could be justly criticized if only a strain which had been propagated for years on artificial media was used. Subsequently, Scammon et al. (1964) were unable to differentiate Group-III mycobacteria from M. avium by their

pathogenicity for chickens. A possible cause of their findings is suggested by the findings of the tuberculosis research group at Michigan State University. Mallmann (1965), using chickens of known high susceptibility to the virus of avian leukosis, found that in birds inoculated with the virus, lesions like those caused by M. avium were induced by injecting Group-III mycobacteria. In birds of the same genetic background that were not given the leukosis virus, tuberculosis was not produced by Group-III mycobacteria.

In 2 of 3 pigs inoculated intradermally with M. avium, laboratory strain, (Pigs 5-3 and 5-2) lesions were found only in the lymph node draining the inoculation site. It should be noted that the lesions found in the left prescapular lymph node of the pig killed at 82 days after inoculation were more extensive than those found in the pig killed at 138 days and that none were found in the prescapular node of the pig killed at 215 days. While this could be due to variations in sampling or in resistance, another possible cause will be suggested later in this work (page 123). By 82 days, when the 1st pig was killed, a lesion had appeared at the site of inoculation, had ulcerated and healed leaving only an area of acanthosis, hyperkeratosis and dermal thickening with connective tissue which contained no granulomas. In the 3rd pig (killed 215 days after inoculation) only 1 granuloma was found. It was well-encapsulated and was found in a mesenteric lymph node. This lesion may have been the result of either hematogenous spread or subsequent infection by the ingestion of organisms shed by one of the animals in the isolation room, most probably from the ulcerated lesions at the inoculation sites.

Feldman (1938) and Francis (1958) both state that avian tubercle bacilli have a predilection for the lymph nodes of the alimentary tract. This is true even when the organisms are injected subcutaneously.

Day (1918) found "necrotic foci" in the skin 4 months after injecting ground material from swine lesions, skin and body lymph nodes "into the skin" and subcutaneously into a 4-month-old hog. Subsequently, the organism was inoculated into chickens and classified as M. avium.

Lesions were found in only 1 pig (8-4) fed M. avium, laboratory strain. As with the M. bovis infections, this was probably due to fewer bacilli gaining entrance into the tissues. It also must be remembered that only 2 mg. of organisms were administered and that these were given all at one time. In a clinical situation, the likelihood of only 1 exposure is remote.

While lesions were found in none of the pen-mates of the pigs to which M. avium, laboratory strain, was administered orally, lesions were found in 2 of the 3 pen-mates of the swine inoculated intradermally. These probably resulted from the ingestion of organisms shed from the inoculation sites after ulceration. Supporting this is the fact that the lesions were found only in lymph nodes draining the alimentary tract. It is possible that these pigs ingested larger numbers of organisms than did those to which the culture was administered orally. Certainly their exposure was longer. Infection by some other route, hematogenous dissemination and localization in a tissue of predilection (lymph nodes draining alimentary tract) are other possible explanations.

In general, Culture 206-2, M. avium of swine origin, caused more extensive disease than did M. avium, laboratory strain. This is particularly evident when comparing the findings on the pigs to which the organisms were administered orally. It should be noted that with oral administration, lesions were found only in lymph nodes draining the alimentary tract. That Culture 206-2 appeared more pathogenic is not surprising when one recognizes that loss of virulence often occurs when mycobacteria are maintained on artificial media (Mallmann, Mallmann and Robinson, 1964; McGavin, 1964).

It is interesting that lesions were found in the lymph node draining the intradermal site (left prescapular) in the 1st 2 pigs killed (Pigs 10-3 and 10-1), while no lesions were found in this node in 1 pig (Pig 10-4) killed 113 days after inoculation or in either pig (10-5 and 10-6) killed 175 days after inoculation. Indeed, the extent of the disease in the animals receiving Culture 206-2, M. avium of swine origin, intradermally seemed to lessen with the passage of time. While this may well have been due to variations in the resistance of these pigs another possible explanation will be presented later (page 123).

No lesions were found in the pen-mates of the pigs inoculated intradermally with Culture 206-2, M. avium of swine origin. This was in spite of the facts 1) that ulcers at the inoculation site were observed in 4 pigs housed with them and 2) that they were housed with infected pigs for 175 days following inoculation. In comparison, lesions were found in 2 pen-mates of the animals inoculated with M. avium, laboratory strain, as compared with 6 which were inoculated with M. avium, swine origin.

Lesions were found in both pen-mates of the pigs to which M. avium of swine origin was administered orally. This indicates that the disease was transmitted.

When 4 cultures of Group-III mycobacteria of swine origin were injected intradermally, more extensive disease was produced than that caused by either M. avium culture introduced by the same route. Gross lesions were found in all pigs. However, the disease was less extensive than that produced by M. bovis.

The lesions were most extensive in the pig inoculated with Culture $167C_1$-1 and killed 59 days after inoculation; they were only slightly less widespread in the pig inoculated with Culture $172C_1$-1 and killed at 65 days after inoculation. The lesions of the pigs inoculated with Cultures $167C_1$-1, 186C-1, and $172C_1$-1 were more extensive in the pigs killed at 8 to 9 weeks after inoculation than in those killed 15 to 16 weeks after inoculation. The opposite was true in the pigs inoculated with Culture $193C_2$-1. These differences might well have been due to individual animal variations since there was only 1 animal per culture per time period. However, another possible explanation will be presented later (page 123).

It should also be noted that the pigs inoculated with Cultures $167C_1$-1 and 186C-1 were housed in the same isolation room. The same is true for those inoculated with Cultures $172C_1$-1 and $193C_2$-1. Since these cultures cannot be differentiated bacteriologically, it is impossible to rule out the possibility of cross-infection.

Although all of the inoculation sites of the Group-III mycobacteria of swine origin ulcerated, no lesions were found in the pen-mates.

One culture of Group-III mycobacteria of swine origin (Culture $172C_1$-1) was administered to 3 pigs orally and these had 3 contact pen-mates. The lesions developed more slowly in those receiving the organisms orally (no gross lesions 78 days after inoculation) than when the same culture was injected intradermally. However, they became more extensive with the passage of time as contrasted to the findings in the intradermal study. This was also true of their pen-mates. With the exception of early granulomas found in the bronchial lymph nodes of 1 pen-mate (killed 206 days after administration of the culture) the lesions of all 6 swine were confined to the lymph nodes draining the alimentary tract. In general, the lesions were slightly less widespread (but still extensive) in the animals administered the culture orally than in those inoculated intradermally. Again, it should be noted that this Group-III mycobacterium caused more extensive disease than M. avium but less than that produced by M. bovis.

The 2 Group-III mycobacteria of pen origin produced no disease. No ulceration and only slight swelling which disappeared by 12 days after inoculation was noted at the inoculation site. Almost no tuberculin hypersensitivity was produced (1-mm. increase in skin thickness at site of the injection of avian tuberculin in 1 animal only). Acid-fast bacilli were observed in but not grown from the tissues of these pigs. In light of these findings, these cultures are considered saprophytes and are of interest only for the confusion that they might cause in epidemiologic studies. They will not be discussed further in this work.

Bacteriologic Findings

Acid-fast bacilli were isolated from tissues containing lesions in all but 4 instances when these tissues were examined bacteriologically. Three of the lesions, in which no viable organisms were found, were well-encapsulated, nonprogressive lesions. The 4th lesion was a small group of macrophages in the left prescapular lymph node (Pig 5-2). It is entirely possible that no lesions were present in that portion of the tissue examined bacteriologically.

Bacteriologic data indicate that acid-fast organisms were widespread in most of the animals to which mycobacteria were administered and in their pen-mates. Acid-fast organisms were isolated from 12 animals in which no lesions were found. In only 6 pigs were organisms isolated from a single tissue pool and 4 of these animals had no detected lesions. Excepting the uninoculated controls and the pigs receiving Group-III mycobacteria of pen origin, only 2 animals were negative upon bacteriologic examination.

It is possible that lesions were present in the tissues, but not in the sections examined histologically. A section of tissue examined was 6 microns thick, which constitutes an exceedingly small sample. Rievel (1909) pointed out the difficulty in finding microscopic lesions. He examined 160 sections from 1 lymph node before finding 1 granuloma. In addition, lesions may have been present only in the material examined bacteriologically. It is also possible that a bacteremia was present or that organisms were in the tissue, either intracellularly or extracellularly, without causing detectable lesions.

The isolation of acid-fast organisms from tissues in which no lesions were found has been frequently reported (Joest, Noack and Liebrecht, 1907; Rievel, 1909; Nieberle, 1913; Muller and Isiwara, 1914; Cormio, 1933; Feldman, 1936; Soltys and Jennings, 1950; Mallmann, 1963; McGavin, 1964; Goyings, 1965). It should be noted, however, that acid-fast organisms were not found in the uninoculated control animals even though their tissues were handled and examined like those of the infected animals. This fact adds considerably to the significance of the isolations accomplished, and emphasizes the long survival of the organisms in vivo.

From a bacteriologic standpoint, the disease was widespread (organisms isolated from more than 1 tissue pool) in all animals except pigs 5-2 (M. avium, laboratory strain, ID*, killed at 138 days after inoculation), 5-1 (M. avium, laboratory strain, ID, 215 days after inoculation), 10-5 (M. avium, swine origin, ID, 175 days), 10-7 (pen-mate of M. avium, swine origin), 6-6 (pen-mate, Group-III mycobacteria, swine origin, oral,** 78 days), 6-3 (Group-III mycobacteria, oral, 206 days), 8-6 (pen-mate, M. avium, laboratory strain, oral, 169 days) and 9-8 (pen-mate, M. avium, swine origin, oral).

*Intradermal injection
**Oral administration

Tuberculin Tests

The results of tuberculin tests are difficult to interpret. Rich (1950) says,

> "A positive tuberculin test means only that the individual has been rendered hypersensitive through infection or through vaccination with attenuated or dead bacilli, and does not provide any certain evidence regarding the extent of the infection or the degree of its activity."

He points out that in the human being, "even a strong reaction can never be more than supportive evidence" of progressive tuberculosis. He further says that only a negative response is significant regarding the determination of the disease state and that its significance is limited by desensitization that occurs in the terminal stages of progressive disease and nonspecific depression of the reactivity of the skin. Tuberculin sensitivity drops when the disease becomes arrested (Willis, 1928; Sewall, de Savitsch and Butler, 1934) and fluctuates during the course of progressive disease (Willis, 1928; Rich, 1950).

Van Es (1926) noted that swine infected with M. avium reacted more to avian tuberculin than to mammalian tuberculin. When infected with M. bovis the mammalian response was greater. Hatokeyama et al. (1961) reported similar findings.

In this study, the responses to mammalian tuberculin were greater than to avian tuberculin in all pigs to which M. bovis was administered and in their pen-mates, except Pig 7-5. This animal, a pen-mate of animals given the culture orally, was negative to both tuberculins when tested at 71 and 132 days after administration of the culture. This correlates

well with the pathologic findings since no lesions were found. However, acid-fast bacilli were isolated from tissue pools B and C. This may have been due to a recently acquired infection.

Of the pigs that received M. avium, laboratory strain, intradermally, all responded to both tuberculins when tested at 62 days after inoculation. The responses were of the same magnitude in Pig 5-3. In Pig 5-2 the response was greater to mammalian than to avian tuberculin. When this animal was tested at 133 days after inoculation both tuberculins caused large responses but the avian response was larger than the mammalian. In Pig 5-1, the avian response was larger when it was tested at 62, 133, and 208 days after inoculation. Lesions were found in all of these pigs and acid-fast bacilli were isolated from the first 2.

None of the pen-mates of the pigs given M. avium, laboratory strain, intradermally, responded to either tuberculin when tested at 62 days. No lesions were found in Pig 5-4 which was killed shortly after this test, but acid-fast organisms were isolated from the pool containing the mesenteric lymph nodes and the pool of the liver and spleen. Both of the remaining pen-mates responded more to avian than to mammalian tuberculin when tested 133 days after inoculation, 1 (5-5) having a 23-mm. increase in thickness at the site of the avian injection and the other (5-6) having only a 1-mm. increase. When Pig 5-6 was tested at 208 days after inoculation, the avian response (2 mm.) was 0.5 mm. greater than the mammalian response. Lesions were found and acid-fast organisms isolated from both of these animals.

In pigs to which M. avium, laboratory strain, was fed, the finding of lesions correlates with the response to avian tuberculin in all but

1 pig (8-3); in this animal there was a 2-mm. increase to avian tuberculin but no lesions were found. None of these animals responded to mammalian tuberculin.

In all tests where responses were found, the avian response was greater than the mammalian response in the pigs receiving M. avium, swine origin, by either route. While 3 of the pigs fed the organisms failed to respond on the 1st test (42 days after administration of the culture) they responded in subsequent tests. Lesions were found in all but 1 of these animals and acid-fast bacilli were isolated from all of them. One of the 2 pen-mates of the intradermally inoculated pigs and both of the pen-mates of the group fed M. avium of swine origin were negative when tested 42 days after administration of the organisms. All of these pigs were positive on subsequent tests with the avian response always being larger. Lesions were not found in the pen-mates of the intradermally inoculated pigs while lesions were found in the pen-mates of the other group. Acid-fast organisms were isolated from all of these pigs.

Where responses were found, the avian response exceeded the mammalian response in the pigs receiving Group-III mycobacteria of swine origin and their pen-mates. This means that they were more consistent in this respect than the pigs receiving M. avium, laboratory strain. All but 1 of the pigs to which the organisms were administered responded the 1st time tested and this pig responded on subsequent tests. Lesions and acid-fast organisms were also found in all of these swine. No tuberculin hypersensitivity and no lesions were found in the pen-mates from both groups killed the earliest (59 days after inoculation in the intradermal

group and 78 days after administration of the organisms orally). Tuberculin hypersensitivity and acid-fast organisms, but no lesions, were found in the pen-mate of the intradermally inoculated group, whereas tuberculin hypersensitivity, acid-fast organisms and lesions were found in those of the other group.

In summary, greater response was found to mammalian tuberculin than to avian tuberculin in all pigs receiving M. bovis. The hypersensitivity induced by M. avium was greater for avian tuberculin than for mammalian tuberculin in all but 2 tests, while that induced by Group-III mycobacteria of swine origin was always greater for avian tuberculin. These findings agree with those of Van Es (1926) regarding M. bovis and M. avium infections and with Mallmann (1963) regarding the Group-III mycobacteria.

The Possible Resolution of Lesions

As was noted earlier, in certain instances fewer lesions were found in animals killed longer after inoculation than in those killed earlier. More specifically, this was found to be the case in the following swine: (a) Pigs 5-3, 5-2 and 5-1 which were inoculated intradermally with M. avium, laboratory strain. The lesions in the left prescapular lymph node, through which drained the lymph from the inoculation site, were more extensive in the pig killed at 82 days (Pig 5-3) than in that killed at 138 days (Pig 5-2), while no lesions were found in this lymph node in the pig killed at 215 days after inoculation (Pig 5-1); (b) Pigs 10-3, 10-1, 10-4, 10-2, 10-5, and 10-6, which were the animals inoculated intradermally with Culture 206-2, M. avium, swine origin. Lesions were

found in the left prescapular lymph nodes of both pigs killed soonest after inoculation (Pigs 10-3 and 10-1), while none were found in this lymph node in 1 pig (10-4) killed 113 days after inoculation or in either pig (Pigs 10-5 and 10-6) killed 175 days after inoculation; and (c) Pigs 3-4, 1-3, 3-2, 3-1, 1-4 and 1-1, which were the pigs inoculated intradermally with 3 of the 4 cultures of Group-III mycobacteria of swine origin. The disease was more extensive in the pigs killed sooner (59 or 65 days) after inoculation (Pigs 3-4, 3-2 and 1-4) than in those killed at 107 or 113 days after inoculation (Pigs 1-3, 3-1 and 1-1). While these findings could logically be attributed to variations in the resistance or even to sampling errors, the possibility of resolution of the lesions should also be considered. These studies were not conducted in such a way that resolution of lesions could be proved or disproved. However, the data do not rule out the possibility that resolution of some lesions may have occurred.

It is well accepted that tubercles can, under proper conditions, resolve into nodules of fibrous tissue (Rich, 1950; Smith and Jones, 1961; Jubb and Kennedy, 1963). The complete resolution and disappearance of tubercles from livers of rabbits (Soper, 1917; Rich and McCordock, 1929) and from rabbit bone marrow (Doan and Sabin, 1927) has been reported. Gardner (1922) found that in guinea pigs large tubercles with caseous centers can be absorbed and that "no trace remains at the site of the former lesion." This was confirmed by Rich (1950). Luke (1951 and 1953) reported that swine can overcome experimental infection with M. avium and M. bovis.

If similar complete resolution of tubercles is possible in the lymph nodes of swine, it is entirely possible that in the animals noted above tubercles were formed and then were resolved. This would seem particularly feasible when considering the lessening of the extent of the disease in the left prescapular lymph nodes. The existence of lesions in other tissues can be explained by difference in native local tissue resistance (Rich, 1950) that may exist. Indeed, native local tissue immunity probably explains why resolution of lesions occurred in the animals studied by Soper (1917), Dean and Sabin (1927), and Rich and McCordock (1929). It should be noted that the descriptions of the presumably resolving, nonencapsulated granulomas by these workers indicate that they could not be differentiated histologically from early granulomas. The main change was a reduction in the number of granulomas found with the passing of time. Perhaps, in the light of these findings, it would be better to note the degree of encapsulation and the presence or absence of "daughter" tubercles rather than to classify the lesions as "progressive" or "nonprogressive" as suggested by Feldman (1943). For emphasis it is repeated that the data do not prove that resolution occurred; they suggest it may have occurred. However, due to the importance that such resolution would have in the diagnosis and study of the pathogenesis and epidemiology of tuberculosis, it was adjudged worthy of mention.

SUMMARY

To each of 39 crossbred pigs approximately 8 to 11 weeks old, 2 mg. (wet weight) of cells of 1 of 9 mycobacterial strains, including M. bovis, M. avium and Group-III mycobacteria, were administered orally or by intradermal injection of the left foreleg. With the exception of the animals inoculated intradermally with M. bovis, litter-mates (21) were housed in contact with the above-mentioned pigs to detect transmission. Fifteen litter-mates were also housed under similar isolation conditions to provide uninoculated control pigs for the study. All pigs were observed for signs of disease and the development of tuberculin hypersensitivity.

The pigs were killed at various times and their tissues were examined bacteriologically and pathologically.

1) Lesions were observed in tissues from which acid-fast organisms were isolated following the administration of Group-III mycobacteria of swine origin.

2) Transmission occurred after Group-III mycobacteria of swine origin were administered per orum.

3) Transmission occurred after M. avium or M. bovis was administered per orum and after M. avium was administered intradermally.

4) No disease was found following the intradermal injection of Group-III mycobacteria of pen origin.

5) Group-III mycobacteria of swine origin produced more extensive disease than M. avium and less extensive disease than M. bovis.

6) Lesions caused by M. bovis, M. avium or Group-III mycobacteria of swine origin could not be differentiated pathologically.

7) There were no mycobacterial isolations from the negative control pigs.

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