THE LESIONS RESULTING FROM INOCULATION OF CALVES WITH ATYPICAL MYCOBACTERIA

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

M. D. McGavin

1964

This is to certify that the

thesis entitled

THE LESIONS RESULTING FROM INOCULATION OF

CALVES WITH ATYPICAL MYCOBACTERIA

presented by

M. D. McGavin

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Pathology

C. C. Norrill
Major professor

Date May 22, 1964

O-169

ABSTRACT

THE LESIONS RESULTING FROM INOCULATION OF CALVES WITH ATYPICAL MYCOBACTERIA

By M. D. McGavin

Fifty-five calves of various breeds and both sexes, and between six and ten months of age, were inoculated with mycobacteria. Each calf received a single culture. Unless otherwise specified, calves were inoculated with either 1 mg. or 2.2 mg., wet weight, of organisms intradermally on the legs. They were tuberculin-tested on the caudal fold with mammalian tuberculin, by the comparative cervical method using avian and mammalian tuberculins and johnin, and examined by necropsy 8-12 weeks after inoculation.

Thirty-seven calves were inoculated with Runyon Group III mycobacteria, 11 with Group IV mycobacteria, 2 with M. bovis, 1 with M. avium and 1 each with killed cultures of M. bovis, M. avium, a Group III of bovine origin and a Group IV mycobacterium.

Four of seven Group III cultures of bovine origin, inoculated into 14 calves, produced either a primary complex or generalized disease in 8 calves. Two other cultures failed to produce a primary complex and another produced this in only one of three calves.

The six different Group III cultures of swine origin were all isolated from swine mesenteric lymph nodes and were inoculated into 11 calves. Only one culture produced a primary complex with granulomas at the inoculation site and in the left prescapular lymph node.

Of seven calves inoculated with a total of four cultures of "pseudochromes", five had no lesions and two had only small granulomas at the inoculation sites. Four of these calves had each received a total of 10 mg., wet weight, of organisms, 2 mg. being administered by each of the peroral, subcutaneous, intradermal, intramuscular and intraperitoneal routes.

Five calves inoculated with three cultures of Group III mycobacteria of soil or cattle feed origin did not develop primary complexes.

Six cultures of Group IV mycobacteria were injected into 11 calves, 4 of which received a total of 10 mg., administered by the peroral, subcutaneous, intradermal, intraperitoneal and intramuscular routes. Four of these cultures were isolated from bovine "skin lesions". Two of them caused no lesions in the experimental calves. One culture produced a small intradermal granuloma in one calf, but no lesions in a second calf receiving the 10 mg. dose. The fourth culture produced no lesions in one calf, but the calf which received 10 mg. had encapsulated nonprogressive granulomas at the skin injection site and in the lymph nodes draining the intradermal, subcutaneous, and intramuscular injection sites.

Both calves infected with M. bovis developed generalized disease. The calf injected with M. avium developed only a nodule at the injection site. None of the calves injected with killed cultures developed lesions.

In every case the comparative cervical test differentiated between those calves with progressive disease, as determined by histological examination, and animals with either no lesions or localized nonprogressive lesions. Four animals inoculated with live mycobacteria and with no lesions, and one with a nonprogressive lesion, gave positive caudal fold tests. All

calves inoculated with killed mycobacteria gave negative comparative cervical results, but one gave a positive caudal fold reaction.

THE LESIONS RESULTING FROM INOCULATION OF CALVES WITH ATYPICAL MYCOBACTERIA

By

M. D. McGavin

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pathology

1964

ACKNOWLEDGEMENTS

This work was carried out as part of a cooperative project between the Department of Microbiology and Public Health and the Department of Pathology. It has been a pleasure to work with this group, and the writer wishes to record his indebtedness to Dr. W. L. Mallmann for his stimulating discussions and advice and to Dr. V. H. Mallmann for her cooperation and supplying of the cultures used in the work. To Dr. C. C. Morrill, I would express my gratitude for offering me the opportunity to do this work and for his constructive criticisms of the manuscript. The tuberculin testing was carried out solely by Dr. James A. Ray and his readiness to come to my help when the work load became especially heavy will always be appreciated.

Preparation of the histologic slides was the work of Mrs. B. Kay Trosko.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION AND OBJECTIVES

This study was authorized under contract No. 12-14-100-4511 (45) between the United States Department of Agriculture and Michigan State University, for investigations of the cause or causes of no-gross-lesion tuberculin reactors and to improve methods of diagnosis of bovine tuberculosis. Item VI of amendment No. 1 of this contract states that the contractor shall

> (a) Artificially expose susceptible non-tuberculin reacting cattle from a tuberculosis-free herd or herds to virulent M. tuberculosis and/or antigenically related agents
>
> (b) Follow the development of the disease in artificially exposed cattle through responses, such as, ... serologic and allergic response, post-mortem bacteriological examination of tissues as well as gross and microscopic tissue changes resulting from bacterial invasion.

Various atypical mycobacteria had been isolated from bovine and swine specimens and from inanimate sources. Experiments were designed to shed light on the following problems: (a) the infectivity for calves of 0.1 and 1.0 mg., wet weight, of atypical mycobacteria and M. bovis. (b) the pathogenicity of Group III mycobacteria of bovine, swine and feed and soil origins, of pseudochrome mycobacteria and Group IV mycobacteria for calves. (c) the ability of killed mycobacteria to induce sensitivity to tuberculosis. (d) the ability of pseudochrome and Group IV mycobacteria to induce pathogenic effects in calves using large doses by several routes of administration.

II. REVIEW OF LITERATURE

Mycobacteria which did not conform to the description of the classical mycobacteria have been known for many years. Xalabarder (1961) wrote:

> Scarcely had the echoes of the report of Koch's discovery of the tubercle bacillus died away than other mycobacteria were discovered that were unclassified in relation to morphology or because they were non-pathogenic when tested in laboratory animals (Alverez and Tavel, 1885; Nocard and Roux, 1885).

Karlson (1958) has drawn attention to the taxonomic difficulties in classifying the mycobacteria and states that up to 1945 there were 163 numbered strains. Mycobacteria are widespread in nature and Frey and Hagan (1931) were able to demonstrate acid-fast bacteria in hundreds of soil samples from various parts of the U.S.A. Because they are common in soil, mycobacteria are frequently found in vegetation and in the gastro-intestinal tracts of herbivorous animals. Karlson (1958) points out that acid-fast bacteria have been found in laboratories, in distilled water tanks, table-top dust, rubber hoses attached to water taps and even in the water bottles used for preparing the Ziehl-Neelsen stain. In fact, Pinner (1935) has stated that "acid-fast bacilli have been isolated from almost any material that was properly scrutinized." It can be appreciated, then, that contamination of biological specimens by such widespread mycobacteria could lead to confusion and such nonpathogenic organisms would have to be distinguished from pathogenic mycobacteria when acid-fast organisms are isolated from animal tissues. For many years those acid-fast bacteria which grew rapidly and which did not produce progressive disease in the guinea pig were classified as "saprophytes" and discarded

(Runyon, 1959). However, the repeated isolation from sputa and resected lung tissue of acid-fast bacteria which were nonpathogenic for guinea pigs has led to a change in attitude and further attempts to evaluate the significance of these unnamed mycobacteria.

The term "atypical" as applied to acid-fast organisms was first used by Pinner (1935). He described in detail the isolation of 15 strains from such sources as the sputum of a man with fibrotic tuberculosis, urine samples, gastric lavage, stools, blood and from pus from a tuberculous hip joint. Colonies were colored, being lemon-yellow to dark orange. When injected into guinea pigs in small doses, no demonstrable lesions were produced. However, 10 mg. doses injected subcutaneously into guinea pigs and intravenously into rabbits and chickens gave far from uniform results. Guinea pigs injected subcutaneously developed subcutaneous nodules 3-9 days later which soon became abscesses. Lesions in the regional lymph node were sometimes present. The subcutaneous lesion reached its maximum size in 2-3 weeks and then receded so that it was not palpable at 5-7 weeks after injection. In some cases the abscess eroded through the abdominal wall to produce a serous peritonitis. These atypical mycobacteria fell into one of three classes on the basis of guinea pig inoculation studies, viz., (a) those which always produced peritoneal lesions, (b) those which sometimes produced peritoneal lesions, (c) those which produced only localized lesions at the inoculation site.

Wolinsky, Smith, Mitchell and Steenken (1957), in reviewing the history of disease caused by the atypical mycobacteria, considered that the papers of Buhler and Pollack (1953) and Timpe and Runyon (1954) "marked the beginning of the recognition of such cases with increasing frequency--of human disease apparently caused by acid-fast bacilli which were nonpathogenic for guinea pigs."

Buhler and Timpe (1953) described two human cases of disease from each of which was isolated an acid-fast bacillus which grew a yellow colony. This was later identified by Runyon (1959) as a Group I mycobacterium. In the first case, the lung was resected and was found to contain a cavity lined by chronic granulation tissue heavily infiltrated with lymphocytes, plasma cells and occasional foreign-body giant cells. The lumen of the cavity contained necrotic debris in which there were polymorphonuclear leukocytes and monocytes. Another small nodule had an area of central caseous necrosis surrounded by epithelioid cells and giant cells. There was an outer zone of dense fibrous tissue in which there were plasma cells. The second case was that of a 21-year-old man who died. At autopsy, excess serosanguineous fluid was present in the abdomen, thorax and pericardium. Minute shot-like nodules were present in the lungs. The hilar lymph nodes were calcified and partly caseous. There was a caseous and liquefied lesion 1 cm. in diameter in the pancreas. The spleen was enlarged and contained numerous, well circumscribed white caseous and calcified areas up to 2 cm. in diameter. Histologically, the spleen and mesenteric lymph nodes contained large caseous masses with areas of calcification and enormous numbers of acid-fast bacilli. In the lung there were sharply defined nodules of confluent areas of bronchopneumonia which consisted of mononuclear and plasma cells. Fibrosis, but no caseation, was present. No tubercles or Langhans' giant cells were seen in any tissue.

Xalabarder (1961) spoke of the "rediscovery in the last few years of the unclassified so-called 'atypical' mycobacteria". He pointed out that the attitude was

> to agree tacitly to regard as unclassified those acid-fast bacilli, isolated from human specimens, but whose properties are similar to those of saprophytic mycobacteria or closely related organisms.

> Such a group of bacilli, pathogenic in man, but not the guinea pig, has already been described by many workers. In a review of the literature up to the end of 1950 ... the writer has noted that 66 investigators have reported the isolation of one or more of such organisms.

Runyon (1959) proposed the name "anonymous" for the unclassified mycobacteria and described a classification based on the effect of light on colonial pigment production and their rate of growth, as follows:

Group I. Photochromogens: little or no pigment when grown in the dark, pigmented (bright yellow) after brief exposure to light.

Later Runyon (1960) suggested that Group I photochromogens which represented a relatively homogeneous group be named M. kansasii.

Group II. Scotochromogens: yellow to pale orange when grown in the dark, more reddish if grown continuously in light.

Group III. Nonphotochromogens: little or no pigment whether grown in the dark or light; if pigment present this is not as in Group I or II organisms.

Group IV. Rapid growers: little or no pigment.

Runyon (1959) described in detail the relationship between the atypical mycobacteria in his collection and the patient's clinical and pathological findings. Group I mycobacteria had been recovered repeatedly from many sputa and from 34 resected lumps. Only 9 of the 122 patients had had infection with M. tuberculosis. The significance of Group II organisms was not clear. His collection included 26 strains recovered from cases also infected with true tubercle bacilli. There were also 96 other strains, but probably only in a very small number of patients had Group II strains established more than a transient residence. Of the 143 strains of Group III mycobacteria, each had been isolated at least twice from different sputum samples from each patient, and most of these

patients had serious pulmonary disease. Later, Runyon (1960) said that he thought that Group I was significant in human disease but Group II was not, and that both Groups III and IV may or may not have been associated with human disease. Corpe, Runyon and Lester (1963) stated that the pathological picture of disease associated with Group IV mycobacteria has not been clearly delineated but the evidence suggested that they do not produce the characteristic features of caseating granulomatous disease.

There is still not unanimity as to whether these unclassified mycobacteria should be called "atypical" or "anonymous". Karlson (1958) challenged the use of the term "atypical" on the grounds that they should be called Mycobacterium spp., as this classification existed in the 6th edition of Bergey's Manual (Breed, Murray & Hitchens, 1948). The 7th edition (Breed, Murray & Smith, 1957) makes no reference to Mycobacterium spp. and therefore, presumably this is no longer a legitimate term. Recently, Corpe et al. (1963) recommended that the term "unclassified" rather than the "atypical" or "anonymous" be used.

One of the biggest drawbacks to the determination of the pathogenicity of atypical mycobacteria is the lack of a suitable experimental animal. Runyon (1959) described this thusly:

> The guinea pig no longer sits alone on the throne of decision as to pathogenicity of acid-fast bacilli for man; there is the mouse, and also an empty chair. Mice have been found to be more susceptible than guinea pigs to some of the anonymous mycobacteria. The 'empty chair' pertains to the lack of any known animal host, in which certain strains can establish progressive infection, although these bacteria evidently have been involved in human disease.

Kubica, Beam, Vestal and Pool (1960) advocated the use of intracutaneous injection of acid-fast organisms in order to determine the virulence of mycobacteria. This was based on the observations of Kite, Patnode and

Read (1952) and Lester (1939). Kite *et al.* (1952) injected known pathogenic mycobacteria - *M. tuberculosis* ($H_{37}RV$), *M. bovis* (Ravenel), *M. avium* (Kirchberg)- and *M. phlei* in doses of 0.01 mg. in 0.1 ml. intracutaneously into the shaved abdomens of guinea pigs. In the case of the pathogens, a persistent ulcer always appeared in less than 10 days. Kubica *et al.* (1960) injected 0.1, 0.01 and 0.001 mg., each in 0.1 ml. saline, into the shaved abdominal skin of a guinea pig. An ulcer at the injection site was considered to be a positive result and usually appeared in 8-14 days, and only rarely did it take 21 days. All avian, human and bovine tubercle bacilli produced ulcers. One of five saprophytes also produced an ulcer. The Groups I and III mycobacteria were the most consistent in producing ulceration. Eleven of twelve strains of Group I and seven of eleven cultures of Group III mycobacteria caused ulcers. Only two of four Group II strains and two of nine Group IV mycobacteria gave positive results. These workers found that only those guinea pigs inoculated by the intracutaneous route with human and bovine tubercle bacilli produced progressive tuberculosis. Durr, Smith and Altman (1959) investigated the pathogenicity of some of the classical pathogenic mycobacteria, photochromogens and nonphotochromogens for laboratory animals. Chickens were injected subcutaneously or intravenously. Guinea pigs were injected subcutaneously, hamsters intraperitoneally and mice intravenously. Saprophytes did not produce any demonstrable disease in the animals. The photochromogens regularly caused progressive disease in hamsters and mice. Photochromogens and nonphotochromogens produced very little disease in the chicken, whereas avian tubercle bacilli produced extensive disease. Nonphotochromogens were much less virulent for any of the test animals. Pollack and Buhler (1955) described an atypical acid-fast organism which

they called a "yellow bacillus" and which Runyon (1959) later confirmed as a Group I mycobacterium. This organism produced progressive disease in the guinea pig, chicken and rabbit, produced variable amounts of disease in the mouse and rat and consistently produced progressive fatal disease in the Syrian golden hamster.

Perhaps the whole problem of animal inoculation experiments and their evaluation could not be stated more clearly than has been done by Pinner (1935):

> 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 criterium 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-fasts isolated from cold-blooded animals are, in the sense specified, pathogenic for the respective species.

It may be felt that the character of the inflammatory response may give some idea as to the species of the mycobacterium involved. However, Ippen (1956) found that in domestic and laboratory animals infected with the classical pathogenic mycobacteria, the type of organism did not alter the structure of the giant cell, but this depended on the host.

Feldman (1960b) has stressed the difficulties associated with making a histologic diagnosis of tuberculosis. He states that tubercles may be present in

> lymphogranuloma inguinale, tularemia, brucellosis, tuberculoid leprosy, syphilis, typhoid fever, infection with Schistosoma mansonia, so-called 'skin tuberculosis' of cattle, sarcoidosis, berylliosis, the intracutaneous tuberculin reaction, silicosis, and certain fungous infections such as nocardiosis.

The ability of the pathologist to diagnose tuberculosis was put to the test by Corpe and Stergus (1963). They sent duplicate sets of 25 slides to 27 pathologists (including one veterinary pathologist) in the U.S.A. All the slides had been made from surgically resected human specimens. From some M. tuberculosis had been isolated and from others the Battey strain, Group III, nonphotochromogenic mycobacteria. Only specimens from which the same strain was cultured, as was recovered from the sputum of the patient before surgery, were used.

> Pathologists were asked to check one of four choices on the questionnaire: (1) the histopathologic picture was compatible with tuberculosis due to M. tuberculosis (2) the histopathologic picture was compatible with tuberculosis due to the Battey strain, Group III, nonphotochromogens (3) the histopathologic picture was compatible with tuberculosis but the pathologist was unable to differentiate as to the causative organism (4) if the pathologist thought the histopathology was not due to tuberculosis he could mark it non-tuberculosis.

Twenty-seven pathologists voting on 25 slides each gave a total of 675 choices. Fifty-three per cent of the votes indicated that the pathologist could not differentiate between the causative organisms. Six per cent of the votes were cast for nontuberculosis disease of which there were no cases, giving a total of 59% which could not be differentiated. Of the 29% of the votes for M. tuberculosis, 62% of these were incorrect. In the words of the authors,

> This study confirms the writers' impression that the histopathologic picture of infections caused by the Battey strain, Group III, nonphotochromogenic mycobacteria is not distinguishable with any degree of accuracy from that caused by M. tuberculosis.

Corpe, Runyon and Lester (1963) in a review on the status of disease due to unclassified mycobacteria stated that

> The spectrum of gross and microscopic pathology produced in human tissues by infection with either Group I, II, or III mycobacteria is almost identical with that produced by M. tuberculosis. Thus, histopathologic findings cannot be relied upon to differentiate Group I, II or III infections or to distinguish them from lesions caused by M. tuberculosis. Present evidence suggests that the Group IV mycobacteria do not produce the characteristic features of caseating granulomatous disease.

Merckx et al. (1963) also agreed that he was not able to tell which of the 4 groups of mycobacteria had caused the lesions, and in most cases these were similar to those due to M. tuberculosis. Crow, King, Smith, Corpe and Stergus (1957) found that the gross and histopathologic findings in 7 surgical specimens they examined were identical with those caused by M. tuberculosis.

Perhaps the words of Feldman (1960a) summarise the present situation most lucidly:

> With few exceptions, the diseases produced naturally by mycobacteria can be classified properly as infectious. This designation is often fortunate and convenient for the hard-pressed pathologist who cannot always determine, by examination of the morbid tissue, the precise nature of the causative agent. Structural changes or patterns in the tissue that represent the host's response to mycobacteria and to many of the fungous infections have several cellular components in common. The diagnosis of such material based on histopathology alone is always unwise and often difficult, if not impossible. In dealing with infectious granulomas, the pathologist must recognise the diagnostic limitations of tissue microscopy. This is particularly important because the causative agent may be present in small numbers or may be difficult to recognise unless suitable bacteriologic studies are done.

A national program of tuberculin testing was established in the U.S. A. in 1917 (Steele and Ramsey, 1958). The percentage of reactors dropped from 5% that year to 0.11% in 1954 (Johnson, Baisden and Frank, 1961). The 48th state was accredited in 1940, this meaning that less than 0.5% of the cattle in each state were reactors to the tuberculin test (U. S. Dept. of Agriculture, 1960). This same author states that in 1955 the number of reactors started to climb and increased every year until in 1959, when a total of 0.23% of cattle tested were classified as reactors. Wilder (1962) points out that in 1914, 49,000 bovine carcasses were condemned or passed for cooking only, but in 1961 only 88 were in this category. Of the 14,000 reactors reported in 1961, less than 200 had lesions sufficiently extensive to be placed in this category, and 73% of reactor cattle during the 1961-62 fiscal year did not have gross lesions of tuberculosis (Wilder, 1962).

Cattle may be sensitised by other mycobacteria, such as M. paratuberculosis, Johne's bacillus, M. avium, M. tuberculosis and atypical acid-fast mycobacteria (Johnson et al., 1961). The presence of acid-fast bacilli in subcutaneous lymphangitis in cattle, frequently called "skin lesions" or "skin tuberculosis" has been known since 1916 (Traum, 1916). These lesions have been described by Karlson (1962) as being

> characterized by the occurrence, in the subcutis, of firm or fluctuant nodules, some of which ulcerate. The lesions usually are seen on the legs, but in some cases the nodules appear to coincide with the lymphatic vessels along the shoulders and neck, or were rarely, the flank or back. They vary from small, almost imperceptible elevations to large masses 10 cm. or more in diameter. Microscopically, the lesions are typical granulomas with varying degrees of necrosis, caseation, calcification and giant cells. Acid-fast bacilli are seen in most skin lesions. The histopathologic picture is typical of tuberculosis.

A considerable amount of work was done on this condition particularly by Daines and Austin (1932, 1934) and Daines (1938), who isolated a variety of acid-fast and nonacid-fast organisms from these lesions. In reporting the results of work done in England, Hole and Hulse (1939) stated that,

> We have to admit failure of our attempts both to cultivate a causal agent of the lesions and to transmit the condition by experimental animal inoculation.

The exact nature of the acid-fast organisms in "skin lesions" has yet to be determined. Other cases of the isolation of atypical acid-fast bacteria from cattle have been reported. In 1930, Hastings, Beach and Thompson reported isolating acid-fast bacteria from the lymph nodes of cattle which had reacted to the tuberculin test but which did not show evidence of tuberculosis at necropsy. Hagan(1931) also recovered acid-fast bacteria from normal mesenteric lymph nodes of cattle. These were classed as saprophytes and he postulated that as acid-fast organisms were common in the intestine, the presence of saprophytes in the mesenteric lymph nodes was not surprising. He also conjectured that these acid-fast bacteria may be able to cause transitory sensitivity to tuberculin. In 1940, Karlson and Feldman reported recovery of nonchromogenic acid-fast microorganisms from 25% of 94 swine tonsils. These cultures did not produce any recognizable disease in chickens, mice or calves. Smith (1954, 1958) reported the isolation of atypical mycobacteria from the lymph nodes of apparently normal cattle in England. Scammon, Pickett, Froman and Will (1963) discussed the cultural characteristics of 43 cultures of acid-fast microorganisms freshly isolated from organs of tuberculous swine obtained from a federally inspected abattoir. Most of the swine showed only caseous lymph nodes. Mallmann, Mallmann and Robinson (1964) reported the isolation of "a relatively large number of acid-fast organisms, not the classical

pathogens, from bovine and swine tissues." They selected 40 cultures at random from approximately 300 isolants from animals and classified them as: M. bovis, 3; Group I, 1; Group II, 1; Group III, 12; pseudochromes, 10; Group IV, 11; and saprophytes, 2.

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

should be equally applicable to the situation in the field of animal mycobacteriosis.

III. MATERIALS AND METHODS

Experimental Animals

Calves were obtained by Dr. R. M. Scott, Animal Disease Eradication Division, U. S. Department of Agriculture, Lansing, from various Michigan herds which had histories of negative caudal-fold tuberculin tests. Calves were tested by the cervical method using 0.1 ml. avian tuberculin, 0.1 ml. mammalian tuberculin and 0.2 ml. johnin. Only those that had no response to any of these three tests were selected. Calves were of various breeds - Holstein, Holstein-Angus crossbreeds, Guernsey, and Holstein-Guernsey crossbreeds - and were between 6 and 10 months old at the time of inoculation with mycobacteria. Details of the sex, breed and age at necropsy are given in the protocols.

Calves were housed in an excellent isolation unit, but as it had only 10 rooms, generally several animals were placed in each room. Calves 1 to 6 were housed in individual rooms, but calves 7 to 58 were accommodated 3 to 5 to a room. They were kept in strict isolation until necropsied at approximately 60 days after inoculation. Their rooms were cleaned daily and by personnel who wore rubber suits, boots and disposable gloves, caps and masks. Calves were fed a balanced ration and water ad lib., and the site of inoculation was examined closely and other clinical observations made once per week. Approximately 50 days after injection with the organisms the calves were tuberculin-tested.

Inoculations

Calves were inoculated with cultures of M. bovis, M. avium, and atypical mycobacteria. The atypicals were classified as Runyon Group III mycobacteria of bovine origin, swine origin or feed or soil origin, "pseudochrome" Group III mycobacteria or Group IV mycobacteria.

The cultures, supplied by Dr. V. Mallmann, were as follows:

Group III bovine origin: 51C-0, 68C-0, 50B-0, 62D-0, 107E-0, 71C-0 and 78B-0.

Group III swine origin: 93C-0, $172C_1$-1, 173C-1, $193C_2$-1, 186C-1 and $167C_1$-1.

Group III feed or soil origin.

Group III pseudochrome mycobacteria: 52H-1, 61B-0, 112B-0, 128F-0.

Group IV mycobacteria: 124F-0, 4F-1, 87H-0, 117B-0, 7F-1, 254F-1.

These atypicals were classified on the basis of the classification of Runyon (1959). Group III mycobacteria differ from the classical pathogenic mycobacteria, i.e., M. bovis, M. tuberculosis and M. avium, in that they do not initially produce progressive disease in guinea pigs, rabbits or chickens. The colonies are nonpigmented whether they are grown in light or in the dark. The optimum temperature is 35 C. to 37 C., but they may grow at 22, 30 and even 45 C. They are niacin-negative, generally neutral red-negative, produce a catalase which is inactivated at pH 7 and pH 5 in 20 or 25 minutes at a temperature of 60 C., and do not form serpentine cords. They may be uniform or long beaded acid-fast rods. All those of bovine or swine origin produced an ulcer at the site of inoculation when 0.1 mg. was injected intradermally into guinea pigs. Those mycobacteria of feed or soil origin produced no intradermal ulcer. Mycobacteria producing an ulcer also induced delayed sensitivity. In most cases the sensitivity was greater to avian tuberculin than to mammalian. Two cultures of bovine origin induced a

sensitivity greater to PPD-B, a purified protein derivative prepared from a human Battey strain isolant.

Pseudochromes

The pseudochromes are similar to Group III except: (a) while no pigment is produced by young cultures, on continued incubation, particularly on a moist slant or in a broth, a light yellow pigment is produced (these may be identical to organisms which have been classified by others as "pigmented Group III mycobacteria" or "Group IIIb"); (b) most of them do not produce an ulcer at the site of intradermal inoculation in guinea pigs. Generally sensitivity is induced.

Group IV Mycobacteria

These differ from the classical pathogenic mycobacteria, Group III mycobacteria and pseudochromes in their rate of growth, requiring only 2-5 days for isolated colonies to appear on Lowenstein-Jensen (Difco) slants at 35-37 C. They differ from the saprophytic mycobacteria such as M. phlei in that they generally do not grow at 44 C. and generally are not as strongly arylsulfatase-positive. They may or may not be pigmented and generally do not produce ulcers at the site of intradermal inoculation in the guinea pig using 0.1 mg. wet weight of organisms.

Calves 1 to 6 received a total of 2.2 mg. wet weight of the organisms intradermally. One milligram was given intradermally proximal to the carpus on the medial aspect of the left foreleg and another 1 mg. was given intradermally just distal to the hock on the medial aspect of the right hindleg. One-tenth milligram was also injected intradermally distal to the carpus on the medial aspect of the left foreleg and a similar amount proximal to the hock on the medial aspect of

the right hind leg. Except for calves 47 and 49, which received 10 mg., wet weight, of organisms, calves 7 to 50 received 1 mg., wet weight, of organisms intradermally on the lateral aspect of the left foreleg just proximal to the carpus. Calves 51 to 58 received a total of 10 mg., wet weight, of organisms each, 2 mg. being given by each of the following routes: oral, intradermal at the same site as calves 7 to 50, subcutaneous, behind the right elbow, intramuscular, into the right gluteal mass and intraperitoneal, into the right paralumbar fossa.

Necropsy Technique

The technique used was based on that of Jones and Gleiser (1954) and was designed to allow maximum examination of the cadaver under the existing facilities. All lymph nodes were removed, placed in plastic sacs with appropriate labels and brought to the laboratory where they were examined minutely in a bacteriological hood by slicing them transversely at approximately 3 mm. intervals. Prior to killing the animal 2 x 50 ml. tubes of blood were collected for serum, and for hematological examination blood was taken from the jugular vein into a tube containing EDTA.* The animal was killed by exsanguination after being anesthetized with chloral hydrate given intravenously. Fifteen milliliters of 40% chloral hydrate were injected per 100 lbs. of body weight. After anesthesia was induced, both the left and right common carotid arteries were dissected free and incised to give maximum exsanguination. The head of the animal was raised to prevent the accidental inhalation of blood. The ear tags were removed, numbers recorded, and both the tags were placed in the bottle with the corresponding specimens. The right foreleg and right hind leg were removed and the following lymph nodes dissected free, then placed in plastic sacs: right prescapular, right

*Ethylenediaminetetraacetic acid.

axillary, right prefemoral, right popliteal, right ischiatic, and left and right superficial inguinal lymph nodes. The right humerus was isolated and 3 inches of it was cut from the proximal half for later removal of the bone marrow. The skin over the lateral surface of the body was reflected dorsally. The abdomen was opened from the dorsal end of the costal arch to the xiphoid process and then along the linea alba to the pubis, and the freed abdominal wall was reflected dorsally. This method allowed access to the deep inguinal lymph node. The costal attachments of the diaphragm were cut and the dorsal ends of the ribs were severed with rib shears. Then the distal ends were similarly cut and the right chest wall was removed in toto. The diaphragmatic lobe of the right lung and a portion of the ventral lobe of the liver were removed aseptically for bacteriological examination. The skin was reflected dorsally from the midline incision from the symphysis mandible to the thoracic opening. The tongue was freed from the mandible, and the left and right submaxillary lymph nodes were identified and removed. The soft palate was transected, the greater cornua of the hyoid bones were disarticulated from the larynx, and the neck organs were dissected free. These and the lungs were removed in toto and placed on the table. The left and right lateral retropharyngeal lymph nodes and the left and right medial retropharyngeal lymph nodes were removed from the head and neck.

From the thorax the posterior mediastinal, left and right bronchial and anterior mediastinal lymph nodes, including the node at the bronchus to the right apical lobe, were removed with the minimum of contamination and placed in separate sacs for bacteriological examination. The esophagus and trachea were opened throughout their lengths, and the lungs were sliced transversely at approximately 2 cm. intervals.

Meanwhile, the assistant had removed the small intestine, as described by Jones and Gleiser (1954), as this method left all the mesenteric lymph nodes together attached to the mesentery. These were removed *in toto* and the lymph nodes then dissected free. The kidneys were isolated and then the forestomachs and spleen together. The latter was freed and half of it was collected for bacteriological examination. The lymph nodes of the forestomachs were removed and pooled. The liver and diaphragm were removed together by cutting through the diaphragm's attachments. The hepatic lymph nodes were dissected free. Then the left and right deep inguinal lymph nodes and internal iliac lymph nodes were removed. The head was removed and the left and right parotid lymph nodes were collected. The brain was removed. The carcass was turned onto its right side, and the left prefemoral, left popliteal, left axillary and left-prescapular lymph nodes were removed. The left prescapular node and the skin at the site of inoculation were taken for bacteriological examination. The left humerus was collected in a manner similar to that used for the right.

Histopathologic Technique

Lymph nodes were sliced transversely at 2-3 mm. intervals, and slices from the following nodes were fixed in 10% buffered neutral formalin: left and right parotid, left and right submaxillary (mandibular), left and right medial retropharyngeal (suprapharyngeal), left and right lateral retropharyngeal (atlantal), left and right prescapular, left and right axillary, anterior mediastinal, posterior mediastinal, left and right bronchial, hepatic (portal), mesenteric, colic and "forestomach", internal iliac, left and right deep inguinal, lumbar chain (if affected), left and right external inguinal (superficial

inguinal), left and right prefemoral, left and right ischiatic and left and right popliteal lymph nodes. Also fixed in formalin were sections of liver, lung, heart (right ventricle), both kidneys, spleen, brain, ileum and skin from the site of inoculation.

Identification of the lymph nodes was based on the classification of Baum (1912). The relationship between the terminology of Sisson and Grossman (1953) and Baum (1912) has been discussed by Webb (1944). Also duplicate blocks of heart, lung, spleen, liver and ileum were fixed in Zenker's fluid (without the addition of the acetic acid). Each brain was sliced transversely at approximately 0.5 cm. intervals by the technique described by McGavin, Ramby and Tammemagi (1962), and blocks were cut from the following sites: medulla oblongata at the level of the pyramidal decussation, medulla oblongata at the level of the obex, medulla oblongata in the middle of the fourth ventricle, cerebellum through the middle cerebellar peduncles, midbrain at the level of the rostral colliculi, corpus striatum at the level of the trigone olfactorium, cerebral cortex from the frontal and occipital poles and cerebral cortex (parietal) at the same level as the corpus striatum.

All tissues were embedded in paraffin, cut at 6 microns and stained with new fuchsin-hematoxylin and eosin (Williqan, Garric and Trosko, 1961). Selected lesions were stained by a Crossman's modification of the trichrome stain (U. S. Armed Forces Institute of Pathology, 1960) and for reticulum by the method of Nassar and Shanklin (1961).

In evaluating the results, the lesions have been classified in one of the following four categories:

(1) no gross or microscopic lesions

(2) lesions at the skin inoculation site only

(3) lesions in the regional lymph node draining the skin inoculation site with or without detected lesions at the skin inoculation site, i.e., a primary complex

(4) generalized lesions, i.e., lesions more extensive than the primary complex.

Attempts were also made to determine whether the lesions were progressive or not on the basis of the criteria laid down by Feldman (1943) for guinea pigs. He stated that the signs of progressive lesions were:

(1) peripheral extension of the disease with daughter tubercle formation

(2) confluence of morbid tissue

(3) conglomerate tubercles

(4) slight to extensive necrosis

Nonprogressive lesions were characterized by:

(1) peripheral encapsulation without daughter tubercles in the peripheral zone of the capsule

(2) presence of noncaseating tubercles

(3) evidence of the transition of epithelioid cells to fibroblasts

(4) fibrosis

(5) calcification or bone where there are no signs of progressive tuberculosis

Bacteriologic Technique

Tissues removed at necropsy with a minimum of contamination were taken to the bacteriological laboratory in plastic sacs. Here they were trimmed free of excess adipose tissue and soaked in 5% sodium hypochlorite solution, 5 changes of 5 minutes each. Then they were sliced under a bacteriological hood, using aseptic technique and fresh sterile instruments for each pool of tissues. The tissues were cultured separately or in pools as follows:

(1) left prescapular lymph node

(2) skin at the site of inoculation

(3) anterior mediastinal and left and right bronchial lymph nodes

(4) posterior mediastinal lymph nodes

(5) liver and spleen

(6) lung (diaphragmatic lobe of the right lung)

(7) hepatic lymph nodes (if lesions)

(8) bone marrow from both the left and right humeri

Tissues were blended with nutrient broth in a Waring blendor and a 10 ml. amount was transferred to a sterile tube. An equal volume of 4% NaOH was added to the tissue broth homogenate. After shaking, it was allowed to stand for 15 minutes, and then the pH of the solution was adjusted to 7 with 2% HCl. After centrifugation, the supernatant was removed and the sediment was seeded onto slants of each of Lowenstein-Jensen medium, Middlebrook 7H10 agar and Dubos Oleic agar.

Tuberculin Test Technique

Approximately 50 days after inoculation, calves were tuberculin-tested in the caudal fold with 0.1 cc. mammalian tuberculin and in the cervical region with 0.1 cc. avian tuberculin, 0.1 cc. mammalian

tuberculin and 0.2 cc. johnin. The mammalian and avian tuberculin used was "heat concentrated synthetic medium tuberculin" made to the specifications of the Agricultural Research Service, U. S. Department of Agriculture (Anon., 1962). The skin thickness was read with calipers at each site immediately before injection and at 24, 48 and 72 hours after injection. In the cervical region the avian site was the most anterior, the mammalian in the middle and the johnin the most posterior on the lateral surface of the neck. These techniques and their interpretations were based on the recommendations of Boddie (1962) and Anon. (1962).

Hematologic Technique

A blood sample was taken from the jugular vein immediately prior to necropsy into a bottle containing EDTA as an anticoagulant. Hemoglobin concentration, hematocrit and a total leukocyte count were determined. The cyanmethemoglobin and the microhematocrit methods were used (Benjamin, 1961). The leukocyte count was done using a Neubauer hemocytometer and Turk's diluting fluid.

TABLE I. Index to inoculum, group, calf no. and page no. in results.

Calf No.	Inoculum	Group	Page No.	Calf No.	Inoculum	Group	Page No.
1	50B-0	III	49	18	7F-1	IV	87
2	51C-0	III	26	20	81-0	*M. bovis*	100
3	62D-0	III	50	21		*M. avium*	96
4	68C-0	III	39	22	87H-0	IV	84
5	124F-0	IV	82	23	$172C_1$-1	III	61
6	130I-0	*M. bovis*	98	24	173C-1	III	64
7	71C-0	III	83	25	254F-1	IV	93
9	4F-1	IV	83	26	81-0	*M. bovis*	102
10	78B-0	III	59	27		*M. avium*	97
11	52H-1	Pseudochrome	76	28	117B-0	IV	85
12	87H-0	IV	83	29	51C-0	III	28
13	107E-0	III	55	30	$172C_1$-1	III	62
14	61B-0	Pseudochrome	79	31	$172C_1$-1	III	63
15	117B-0	IV	84	32	$193C_2$-1	III	65
16	93C-0	III	60	33	$193C_2$-1	III	66
17	112B-0	Pseudochrome	81	34	186C-1	III	69

TABLE I--Continued

Calf No.	Inoculum	Group	Page No.	Calf No.	Inoculum	Group	Page No.
35	$167C_1$-1	III	69	48	Soil orig.	III	74
36	186C-1	III	68	49	Soil orig.	III	74
37	$167C_1$-1	III	70	50	Soil orig.	III	75
38	71C-0	III	57	51	7F-1	IV	89
39	71C-0	III	58	52	117B-0	IV	85
40	107E-0	III	55	53	254F-1	IV	93
42	51C-0	III	29	54	254F-1	IV	94
43	51C-0	III	31	55	61B-0	Pseudochrome	80
44	62D-0	III	52	56	128F-0	Pseudochrome	81
45	68C-0	III	40	57	52H-1	Pseudochrome	77
46	Feed orig.	III	72	58	52H-1	Pseudochrome	78
47	Feed orig.	III	73				

IV. RESULTS

Fifty-five calves were injected with mycobacteria, these being atypical mycobacteria of bovine origin, swine origin and feed and soil origin, as well as M. bovis and M. avium. The results are subdivided according to the cultures used.

Group III Mycobacteria of Bovine Origin

Fifteen calves were inoculated with cultures of Group III mycobacteria of bovine origin, as follows:

Culture Number	Calf Number
51C-0	Calves 2, 29, 42, 43
68C-0	Calves 4, 45
50B-0	Calf 1
62D-0	Calves 3, 44
107E-0	Calves 13, 40
71C-0	Calves 7, 38, 39
78B-0	Calf 10

Culture 51C-0

Calf 2 - 2.2 mg. inoculum intradermally.

Clinical observations. None available.

Necropsy findings. (72 days after inoculation)

Holstein heifer, 8 months old.

Lesions were produced at both sites of inoculation in the left foreleg. The upper lesion consisted of a 1 cm. diameter encapsulated focus of yellow caseous material. The lower lesion also showed no ulceration to the surface and was well encapsulated and contained a thin yellowish-white material. No calcification was seen. There were also two

inoculation sites on the right hindleg. At the upper site, a 15 mm. diameter encapsulated lesion contained creamy yellowish-white material. The lesion at the lower inoculation site on the right hindleg consisted of a 5 mm. diameter nodule.

The anterior and posterior mediastinal and left and right bronchial lymph nodes contained numerous discrete and confluent yellowish-white foci, some of which were calcified. One of the mesenteric lymph nodes also contained a discrete yellowish-white focus.

Histopathologic findings.

Skin, upper foreleg. In the dermis there was an encapsulated granuloma which consisted of a peripheral fibrous capsule about a layer of lymphocytes. Internal to this was a layer of macrophages which enclosed a central core of caseous material in which there was some early calcification.

Skin, upper hind leg. An intradermal granuloma of epithelioid cells and numerous giant cells of the Langhans' type was present. Caseation was minimal, but, where present, calcification was well advanced. Acid-fast bacteria were frequently visible in the epithelioid cells.

Skin, lower hind leg. This lesion showed only a small encapsulated epithelioid-cell granuloma, without necrosis, caseation, calcification, acid-fast bacteria or giant cells.

Left prescapular lymph node. Nonprogressive granulomas with central caseation, calcification and well developed epithelioid and giant cells were seen. Numerous acid-fast organisms were present at the periphery of the caseous debris.

Anterior and posterior mediastinal and left and right bronchial lymph nodes. Multiple progressive granulomas, apparently early in development, were seen. These consisted chiefly of epithelioid cells with a few heterophils lying in the centers where necrosis was commencing. Caseation and calcification were absent. Acid-fast organisms were infrequently seen in the centers of the granulomas.

Mesenteric lymph nodes. Granulomas in varying stages of development were seen in these lymph nodes. A few heterophils were present in the centers of early lesions and older lesions showed caseation and calcification. Acid-fast organisms were rare and, when seen, lay in the necrotic material.

Right popliteal lymph node. Early granulomas consisting of epithelioid cells with occasional heterophils were seen. No giant cells, caseation or calcification were present. Acid-fast bacteria were rarely seen in the centers of the granulomas.

Bacteriologic findings. Acid-fast bacteria were recovered from the pool of the head and neck lymph nodes, the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes, lung, mesenteric lymph nodes, right internal iliac, right prefemoral lymph node, right popliteal, left prefemoral lymph node and the skin inoculation sites.

Calf 29 - 1 mg. inoculum intradermally (killed).

Clinical observations. The skin inoculation site never ulcerated. Swelling reached a maximum diameter of 10 mm. on the 21st day after inoculation and slowly regressed so that a 5 mm. diameter nodule was all that remained at necropsy.

Necropsy findings. (54 days after inoculation)

Guernsey heifer, 8 months old, good condition, 350 lb. weight.

The only lesion detected was the 5 mm. intradermal nodule on the cut surface of the skin at the site of inoculation.

Histopathologic findings. A diffusely infiltrating granulomatous inflammation was seen in the reticular layer of the skin at the site of inoculation. It consisted of macrophages, lymphocytes and some plasma cells and was not encapsulated.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the pool of the anterior and posterior mediastinal and the left and right bronchial lymph nodes, and from the bone marrow of the humeri.

Calf 42 - 1 mg. inoculum intradermally. This differed from that used in Calf 1 in that it had been stored in the refrigerator for a further 10 months.

Clinical observations. A diffuse hard swelling 15 mm. in diameter was present at the skin inoculation site seven days after inoculation. Ten days later this had reached a diameter of 25 mm. and was still hard, but the central area showed a 10 mm. diameter hairless area without ulceration. On the 24th day after inoculation, the swelling was reduced to 10 mm. in diameter and 5 mm. in elevation, and the hairless area was now 7 mm. in diameter. By the 33rd day after inoculation, the lesion had ulcerated and the swelling now occupied a 15 mm. diameter area with an elevation of 5 mm. Granulation was taking place around the periphery of the ulcer. On the 49th day after inoculation the injection site was represented by a nodule 10 mm. in diameter with an elevation of 5 mm.

The ulcer had healed so that the lesion was now completely closed, the central part of it being occupied by a hairless area 3 mm. in diameter.

Necropsy findings. (66 days after inoculation)

Holstein heifer, 9 months old, good condition, 450 lb. weight.

Gross lesions were confined to the left prescapular lymph node and to the skin at the site of inoculation. A cross section through the skin inoculation site showed a pink nodule 3 mm. in diameter lying in the dermis. The left prescapular lymph node contained a caseous lesion 20 mm. in diameter.

Histopathologic findings. In the skin at the site of injection, a granuloma was found lying in the reticular layer. This consisted chiefly of epithelioid cells with a few giant cells and some scattered lymphocytes. A fibrous capsule enclosed the granuloma and no acid-fast bacteria were seen.

Left prescapular lymph node. Multiple, sometimes confluent foci of granulomatous inflammatory tissue were seen in the section. These were frequently encapsulated and often contained a central caseo-calcareous focus. Epithelioid cells and giant cells were numerous, and acid-fast bacilli were frequently seen lying in the cells adjacent to the caseous material.

Bacteriologic findings. Acid-fast bacteria were recovered from the anterior mediastinal and left and right bronchial lymph nodes, the posterior mediastinal lymph nodes, the left prescapular lymph node, the skin inoculation site, the right diaphragmatic lobe of the lung, the hepatic lymph nodes and the pool of the liver and spleen.

Calf 43 - 1 mg. inoculum intradermally. This organism was the isolant from the left prescapular lymph node of calf 2.

Clinical observations. On the 7th day after inoculation the skin at the injection site had a swelling 15 mm. in diameter. Ten days later the swelling had reached a diameter of 30 mm., was very hard, and a hairless area 10 mm. in diameter which had not ulcerated lay in its center. On the 24th day after inoculation, the swelling had reduced to 15 mm. in diameter with an elevation of 5 mm., but had ulcerated through an orifice 10 mm. in diameter which was already granulating. The left prescapular lymph node was swollen. Nine days later the swelling and the ulcer were little changed, but the prescapular lymph node was more markedly swollen. By the 39th day after inoculation the ulcer had reached a diameter of 15 mm. in the swelling which was now 25 mm. in diameter. On the 49th day the ulcer had changed little and was 15 mm. in diameter and still open. The total swelling was 25 mm. in diameter with an elevation of 5 mm., and the prescapular lymph node was grossly enlarged. Throughout this period the animal's temperature did not exceed 102.3 and no pyrexia was noted.

Necropsy findings. (68 days after inoculation)

Holstein heifer, 8 months old, fair condition, 500 lb. weight.

Widespread caseo-calcareous lesions were found in the left and right bronchial lymph nodes, in the anterior and posterior mediastinal lymph nodes, in the left and right prescapular lymph nodes, hepatic lymph nodes, the mesenteric lymph nodes and in the liver, lungs and the skin at the site of inoculation.

Skin at the site of inoculation. The ulcer measured 10 mm. in diameter and lay in the middle of an area of swelling 20 mm. in diameter. On cross section, the swollen area of skin was seen to be deeply reddened and contained yellowish caseous areas.

Left prescapular lymph node. The left node was very difficult to cut, due apparently to fibrous tissue, and contained multiple large areas of yellow caseous material.

Right prescapular lymph node. Several yellow foci 2 mm. in diameter were seen in the cortex at one pole.

Anterior mediastinal lymph nodes. These consisted of four lymph nodes, two of which showed no lesions. The other two were filled with both discrete and confluent caseous foci.

Posterior mediastinal lymph nodes. There were five lymph nodes, all of which contained both discrete and confluent caseous foci.

Left and right bronchial lymph nodes. These were both filled throughout with yellow caseous material.

Mesenteric lymph nodes. Three yellow foci, 2 to 3 mm. in diameter were found scattered throughout the whole length of the mesenteric lymph nodes.

Hepatic lymph nodes. All four hepatic lymph nodes contained numerous caseous foci varying in diameter up to 4 mm.

Liver. The only lesion found was a white area approximately 3 mm. in diameter lying subcapsularly.

Lung. Numerous clear glistening foci 1 to 2 mm. in diameter were scattered throughout the lung, lying both in the substance and subpleurally.

Histopathologic findings. Besides the gross lesions, microscopic evidence of tuberculous inflammation was found in the left and right medial retropharyngeal, colic and left and right deep inguinal lymph nodes.

Skin at the inoculation site. The section of skin showed numerous focal or circumscribed granulomas lying in the reticular layer. These varied greatly in size and probably represented part of a multiloculated granuloma. The larger granulomas had caseous or caseo-calcareous centers and giant cells were very numerous. The fibrous tissue about the lesions appeared to be part of the reticular layer of the skin rather than a specifically developed capsule.

Left prescapular lymph node. The lesions in this lymph node were also similar to those seen in the posterior mediastinal lymph node, the granulomas being of the two types - those with central caseation and calcification and a peripheral fibrous capsule (Figures 1 and 2) and the unencapsulated noncaseous granuloma. Heterophils were present only about the calcified centers. No acid-fast bacteria were seen.

Right prescapular lymph node. Lesions in this lymph node were similar to those seen in the posterior mediastinal lymph node. They were either focal encapsulated granulomas with caseo-calcareous centers or noncaseous granulomas without encapsulation. Heterophils were seen only about the central calcified material. Acid-fast bacteria were rare and, when seen, lay in giant cells.

Left and right medial retropharyngeal lymph nodes. Tuberculous noncaseating microscopic granulomas were found in the cortex (Figure 3). These consisted of epithelioid cells and a few giant cells; caseation and calcification were absent. Acid-fast bacteria were rare and were seen only in a giant cell.

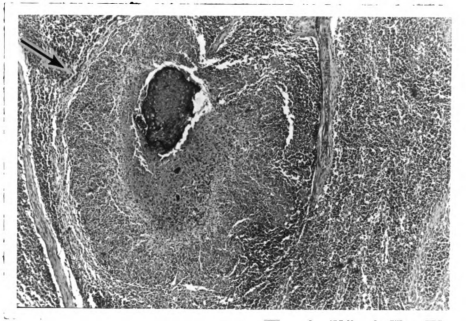

Figure 1. Left prescapular lymph node from calf 43, inoculated with 51C-0, a Group III mycobacterium. A caseocalcareous granuloma has a thin fibrous capsule (arrow) developing around it. New Fuchsin - H & E. x50.

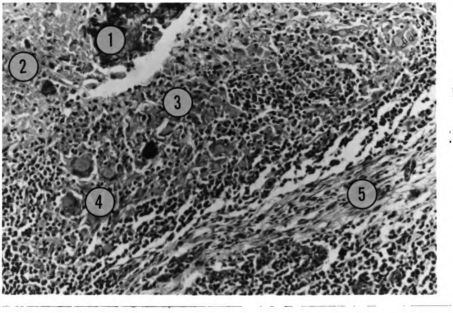

Figure 2. Left prescapular lymph node from calf 43, inoculated with 51C-0, a Group III mycobacterium. Note the calcified center (1), adjacent caseous material (2), epithelioid cells (3), Langhans' giant cells (4) and the thin capsule (5). New Fuchsin - H & E. x187.

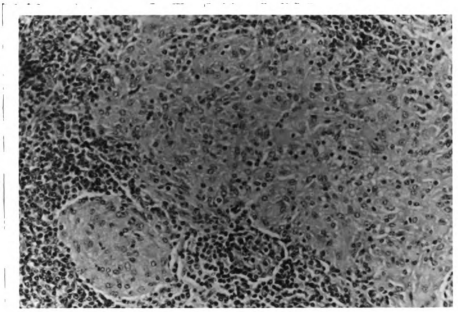

Figure 3. Medial retropharyngeal lymph node from calf 43, inoculated with 51C-0, a Group III mycobacterium. Most of this field is occupied by a noncaseous granuloma consisting of epithelioid cells. New Fuchsin - H & E. x187.

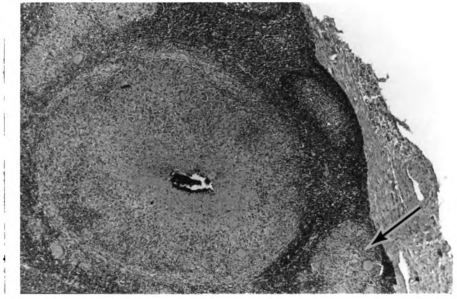

Figure 4. Mesenteric lymph node from calf 43, inoculated with 51C-0, a Group III mycobacterium. An encapsulated caseo-calcareous granuloma lies under the lymph node capsule adjacent to a small noncaseous granuloma (arrow). New Fuchsin - H & E. x50.

Anterior mediastinal lymph node. There was extensive involvement of this lymph node by tuberculoid granulomas, which were present in both the cortex and the medulla. Lesions were focal but often confluent. Epithelioid cells, giant cells, caseation and calcification were common. No acid-fast bacteria were seen.

Posterior mediastinal lymph node. Lesions in this lymph node were of two types - either the encapsulated focal caseous granuloma or the diffuse noncaseous granuloma. Acid-fast bacteria were very rare, and, when located, were seen in giant cells.

Left and right bronchial lymph nodes. The lesions in these nodes were similar also to those seen in the posterior mediastinal lymph node. Acid-fast bacilli, however, were not seen.

Hepatic lymph nodes. Again, the two types of granuloma as seen in the posterior mediastinal lymph node were visible. No acid-fast bacteria were detected.

Mesenteric lymph nodes. Two types of granuloma, the encapsulated focal caseous granuloma (Figure 4) and the noncaseous granuloma, as seen in the posterior mediastinal lymph node were detected. Acid-fast bacteria were frequent and were located in giant cells. The lesions in this lymph node appeared to be progressive, since daughter tubercles had formed about the periphery of some of the larger tubercles.

Colic lymph nodes. The lesions in the colic lymph nodes were similar to those seen in the mesenteric lymph nodes - the two types of granuloma being present but the caseous granulomas far outnumbered the noncaseous variety. Acid-fast bacteria were rare, being seen only in giant cells.

Left and right deep inguinal lymph nodes. Again the two types of granulomas were seen, i.e., the noncaseous form with daughter tubercles,

and the encapsulated granuloma with caseo-calcareous centers. Heterophils were adjacent to the central deposit of calcium. No acid-fast bacteria were seen.

Lung. The lung contained numerous microscopic noncaseous tubercles. These were frequently surrounded by lymphatic tissue, but were not encapsulated. Acid-fast bacteria and giant cells were relatively infrequent. These noncaseous granulomas usually occurred in the peribronchial lymphatic tissue, and in one case the tubercle had ulcerated through a bronchial wall to allow epithelioid cells and debris to fall into the lumen.

Liver. There was an apparent activation of the Kupffer cells, as these were slightly swollen and appeared to be increased in numbers. There was also an increase in the number of lymphocytes in the portal tracts.

Brain and meninges. The leptomeninges and some of the perivascular spaces in the brain adjacent to the pia mater were infiltrated with macrophages and lymphocytes (Figures 5 and 6). These changes were seen in the medulla oblongata, cerebellum, midbrain, corpus striatum and cortex. However, the lesions were most marked in the midbrain, in the molecular layer of the cerebellum and in the leptomeninges over the medulla at the pyramidal decussation. Some of these cuffs were three cells thick, but acid-fast bacilli were never seen.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the skin inoculation site, the pool of the anterior mediastinal and left and right bronchial lymph nodes, the posterior mediastinal lymph node, the hepatic lymph node, the pool of the liver and spleen and from the right diaphragmatic lobe of the lung.

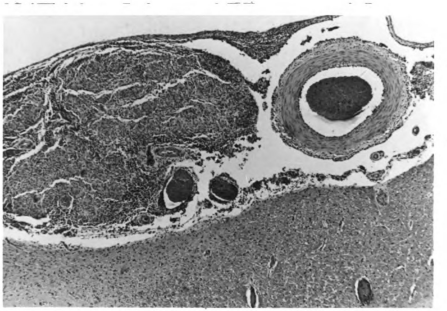

Figure 5. Leptomeninges adjacent to corpus striatum from calf 43, inoculated with 51C-0, a Group III mycobacterium. Note the well developed noncaseous granuloma distending the pia-arachnoid. New Fuchsin - H & E. x50.

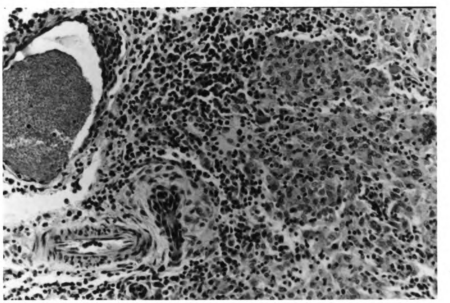

Figure 6. Leptomeninges adjacent to corpus striatum from calf 43, inoculated with 51C-0, a Group III mycobacterium. The pia-arachnoid is heavily infiltrated by epithelioid cells and lymphocytes. New Fuchsin - H & E. x187.

Culture 68C-0

Calf 4 - 2.2 mg. inoculum intradermally.

Clinical observations. None available.

Necropsy findings. (82 days after inoculation)

Holstein heifer, 7 months old.

The only lesions detected on necropsy were those at the left upper foreleg inoculation sites and in two of the lymph nodes of the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes. The skin lesion on the left foreleg of the upper inoculation site showed a well encapsulated focus 10 mm. in diameter which contained brownish-yellow caseous material. The lower inoculation site on the left foreleg was similar to that above but was only 5 mm. in diameter. Two of the lymph nodes of the anterior and posterior mediastinal and left and right bronchial lymph node pool contained yellow caseo-calcareous foci 2 mm. in diameter.

Histopathologic findings. The lesions in the thoracic lymph nodes were multiple granulomas of two types: (a) small noncaseous granulomas consisting of epithelioid and giant cells, and (b) encapsulated granulomas with central caseation, calcification and peripheral fibrous encapsulation. Acid-fast bacteria were rarely seen in the encapsulated lesions, but were more frequent in the noncaseous granulomas. Histological preparations were made only from the upper and lower inoculation sites of the foreleg. At the lower inoculation site there was a noncaseous granuloma lying in the reticular layer of the skin. This consisted of epithelioid cells and lymphocytes, and acid-fast bacteria were readily visible, particularly in the epithelioid cells. The upper inoculation site had a granuloma with central caseation, but without any peripheral

encapsulation lying in the reticular layer. Acid-fast bacteria were plentiful, particularly in the necrotic center, but were also found scattered throughout the granuloma.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the right popliteal lymph node, the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes, the right internal iliac node, the left popliteal node and the skin inoculation sites, both upper and lower, on the foreleg.

Calf 45 - 1 mg. inoculum intradermally. This organism was the isolant of culture 68C-0 from the left prescapular lymph node of calf 4.

Clinical observations. On the seventh day after inoculation the swelling at the inoculation site had reached a diameter of 50 mm. and was hard. The left prescapular lymph node was enlarged. Ten days later the swelling had reached a diameter of 30 mm. and was still hard and somewhat diffuse, but in its center was a hairless area 10 mm. in diameter which as yet had not ulcerated. On the 24th day after inoculation, the swelling now covered an oblong area 50 mm. by 30 mm., with an elevation of 10 mm. The central hairless area still measured 10 mm. in diameter and was still closed. The left prescapular lymph node was swollen. On the 32nd day the lesion had ulcerated to the surface through an orifice 10 mm. in diameter and the swelling now measured 30 mm. by 25 mm., with a 10 mm. elevation. On the 39th day after inoculation there was little change, but on the 49th day the swelling was 30 mm. in diameter with an elevation of 10 mm., and the ulcer, which was still 10 mm. in diameter, was red and granulating. The left prescapular lymph node was still swollen.

Necropsy findings. (65 days after inoculation)

Holstein heifer, 8 months old, fair condition, 350 lb. weight.

Lesions were found in the left axillary, mesenteric, hepatic, anterior mediastinal, posterior mediastinal, left and right bronchial and left prescapular lymph nodes, in the lungs and at the site of inoculation in the skin of the left foreleg.

Skin, at the inoculation site. A granulating ulcer 10 mm. in diameter was found lying in the middle of the area of thickened skin which measured 35 mm. in diameter. On cross section this skin was found to contain patchy caseo-calcareous material similar to that seen in the left prescapular lymph node.

Left prescapular lymph node. This measured 110 x 65 x 55 mm. and was affected throughout with multiple caseo-calcareous foci many of which had become confluent (Figure 7).

Left axillary lymph node. The cut surface showed numerous yellowish foci 0.5 to 1 mm. in diameter throughout the cortex.

Anterior mediastinal lymph nodes. Of all the 6 lymph nodes, 5 showed no gross lesions. The largest node was filled with multiple yellow caseo-calcareous foci.

Posterior mediastinal lymph node. All 4 nodes were filled with numerous discrete or confluent, yellow caseo-calcareous foci.

Left and right bronchial lymph nodes. Both these nodes contained numerous discrete or confluent caseo-calcareous foci.

Hepatic lymph node. Scattered 1 to 4 mm. in diameter caseous foci were seen throughout all these nodes.

Mesenteric lymph nodes. In the whole chain of mesenteric lymph nodes only 2 foci were found. Each of these had originally consisted of several yellowish-white foci 0.5 to 1 mm. in diameter which had now

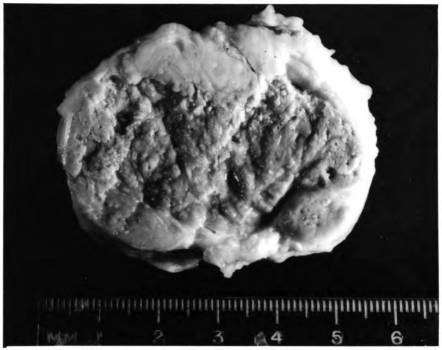

Figure 7. Left prescapular lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. Note the obliteration of the lymphoid tissue by granulomatous tissue.

Figure 8. Lung from calf 45, inoculated with 68C-0, a Group III mycobacterium. Note the pale subpleural granulomas.

become confluent so that each now occupied an area approximately 5 mm. in diameter subcapsularly.

Spleen. Several yellow foci approximately 4 mm. in diameter were found throughout the parenchyma.

Liver. Yellow foci 4 mm. in diameter, as seen in the spleen, were scattered throughout the liver parenchyma.

Lung. Numerous grey foci 4 mm. in diameter were seen scattered throughout the parenchyma and subpleurally in the lungs (Figure 8). These were uniform in distribution, although they were slightly more concentrated in the apical lobes of both lungs. Surrounding each of these grey foci, which often resembled bullae, was a rim of dark red lung tissue which was presumably atelectatic.

Histopathologic findings. Lesions were present in the colic lymph nodes as well as in those listed above under necropsy findings. In all lymph nodes, multiple foci of tuberculous granulomatous inflammatory tissue were seen. Giant cells were extremely numerous and caseation and calcification were present. Despite the extensive pathological changes, acid-fast bacteria were not seen in any lymph node.

Left prescapular lymph node. Both discrete and confluent caseo-calcareous granulomas (Figures 9 and 10) were present. Giant cells and epithelioid cells were numerous (Figure 10), but no acid-fast bacilli were detected. Noncaseous granulomas were present in the thickened capsule of the lymph node (Figure 11).

Left axillary lymph node. The microscopic lesions here were similar to those seen in the left prescapular lymph node. No acid-fast bacteria were detected.

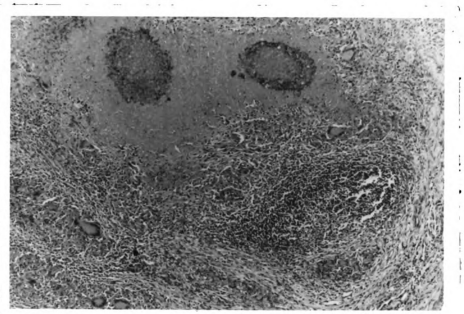

Figure 9. Left prescapular lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. Two confluent caseo-calcareous granulomas. New Fuchsin - H & E. x75.

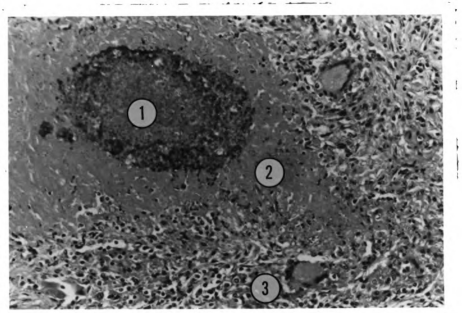

Figure 10. Left prescapular lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. A higher magnification of portion of Figure 9, to show the central calcified area (1), the surrounding caseous material (2), and the granulomatous tissue (3). New Fuchsin - H & E. x187.

Left and right bronchial lymph nodes. Progressive tuberculous granulomas consisting of focal and confluent areas were widespread throughout the section. Giant cells were numerous and caseation and calcification were extensive. No acid-fast bacteria were seen.

Anterior and posterior mediastinal lymph nodes. Again the lesions were similar to those seen in the bronchial lymph nodes. In none was there any attempt at encapsulation (Figures 12, 13 and 14). Daughter tubercles were readily visible in the sections of the posterior mediastinal lymph node.

Left and right medial retropharyngeal lymph nodes. Lesions in these lymph nodes were confined to two focal noncaseous granulomas. Giant cells were seen, but caseation, calcification and acid-fast bacteria were absent.

Mesenteric lymph nodes. Progressive tuberculous granulomas were seen in these lymph nodes. Many granulomas had enlarged until they had coalesced with adjacent ones. There was no attempt at fibrous encapsulation, and daughter tubercles were frequent. Many of the granulomas showed caseation and calcification and heterophils were often associated with the central area of calcification. No acid-fast bacteria were seen.

The colic lymph nodes. Two focal caseo-calcareous granulomas were seen in these lymph nodes. These were of microscopic size and only a few early attempts to form small giant cells were visible. No acid-fast bacteria were detected.

Liver. Tubercles consisting of both noncaseous and caseous granulomas were seen in the liver. The noncaseous granulomas contained giant cells and epithelioid cells and were surrounded by a peripheral rim of lymphocytes. The caseous granulomas were enclosed by fibrous capsules and contained epithelioid and giant cells, caseation and calcification (Figure 15).

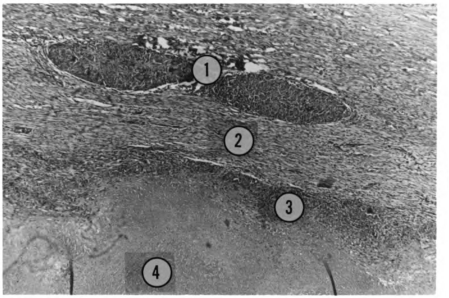

Figure 11. Left prescapular lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. Two noncaseous granulomas (1) lie in the thickened capsule of the lymph node (2). Under this a thin rim of granulomatous tissue (3) surrounds the caseous area (4). New Fuchsin - H & E. x 50.

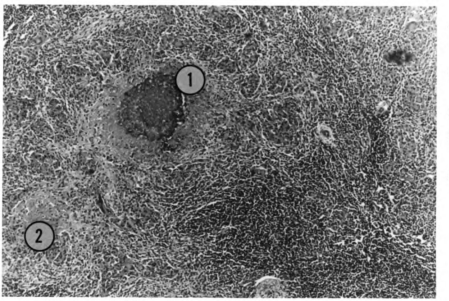

Figure 12. Posterior mediastinal lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. A caseo-calcareous granuloma (1) and a caseous granuloma (2) lie close to one another. New Fuchsin - H & E. x75.

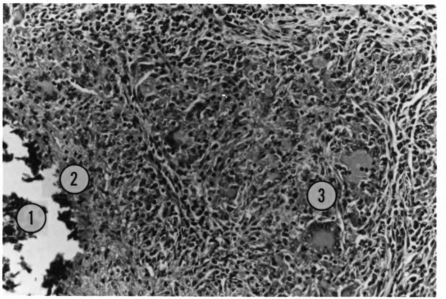

Figure 13. Posterior mediastinal lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. Note the central calcified area (1) surrounded by a small rim of caseous material (2), which is encompassed by the granulomatous tissue (3). There is no evidence of the formation of a fibrous capsule. New Fuchsin - H.& E. x187.

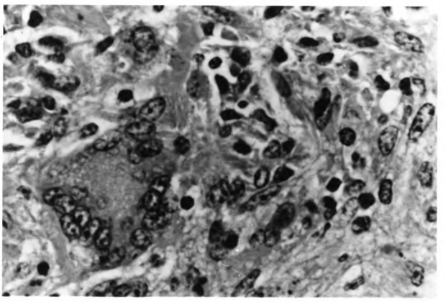

Figure 14. Posterior mediastinal lymph node from calf 45, inoculated with 68C-0, a Group III mycobacterium. Portion of the same field as Figure 13, enlarged to show the Langhans' giant cell, epithelioid cells and lymphocytes. New Fuchsin - H & E. x750.

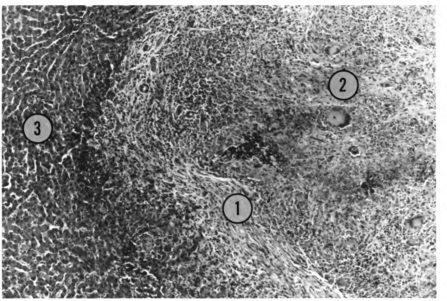

Figure 15. Liver from calf 45, inoculated with 68C-0, a Group III mycobacterium. A fibrous capsule (1) is being laid down around the granuloma (2), separating it from the liver parenchyma (3). Crossman's Trichrome. x75.

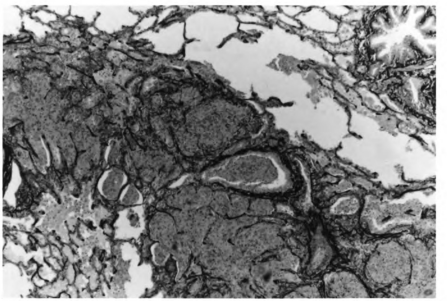

Figure 16. Lung from calf 45, inoculated with 68C-0, a Group III mycobacterium. Note the well developed non-caseous granuloma. H & E - reticulum. x75.

Lung. Seven sections of lung were examined. These showed multiple tuberculous granulomas which were frequently adjacent to the bronchioles (Figure 16). In one case the granuloma had eroded through the bronchiole wall to empty its contents into the lumen. These granulomas were lobular in distribution and frequently showed central caseation and calcification and there was some attempt at a peripheral fibrosis. Daughter tubercles had been formed by some granulomas. Acid-fast bacteria were not detected. Some subpleural tuberculous granulomas exhibited central calcification and caseation and had produced daughter tubercles which had penetrated through the pleura.

Bacteriologic findings. Acid-fast bacteria were recovered from the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node, the hepatic lymph nodes, the pool of the liver and spleen, from the bone marrow of both humeri, from the left prescapular lymph node, the skin inoculation site and the right diaphragmatic lobe of the lung.

Culture 50B-0

Calf 1 - 2.2 mg. inoculum intradermally.

Clinical observations. None available.

Necropsy findings. (59 days after inoculation)

Guernsey heifer, 7 months old.

Numerous yellow caseous foci without calcification were present throughout the anterior and posterior mediastinal and left and right bronchial lymph nodes. No other lesions were detected.

Histopathologic findings. Numerous encapsulated granulomas with central caseous areas throughout which there was fine calcification were seen in the anterior and posterior mediastinal and left and right bronchial lymph nodes. Acid-fast bacilli were seen at the periphery of the caseous material. No other lesions were detected.

Bacteriologic findings. Acid-fast organisms were recovered from the right prefemoral lymph node, the right popliteal, left internal iliac, the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes and the left prescapular lymph node.

Culture 62D-0

Calf 3 - 2.2 mg. inoculum intradermally.

Clinical observations. Immediately prior to necropsy the animal was depressed and was neither eating nor drinking. It was in very poor condition and could rise only with difficulty.

Necropsy findings. (55 days after inoculation)

Guernsey heifer, 7 months old, poor condition.

The right and left apical and cardiac lobes of the lung showed a patchy red and gray hepatization. An abscess 20 mm. in diameter, which contained green pus, was found in the left apical lobe. In the kidneys, grey foci 1 to 10 mm. in diameter were found throughout. Lesions in the lymph nodes were confined to the right popliteal, right internal iliac and left prescapular. These lymph nodes contained "abscesses" which were filled with yellowish "pus". The four skin inoculation sites were still visible. Three of these consisted of encapsulated intradermal "abscesses". The upper one on the left forelimb was 25 x 35 mm. and the lower one 15 x 25 mm. The lower lesion

on the right hind leg was 30 mm. in diameter and the upper inoculation site consisted of a hard nodule only 5 mm. in diameter.

Histopathologic findings.

Left prescapular lymph node. This contained an encapsulated granuloma which had a peripheral fibrous capsule, surrounding a layer of lymphocytes. Inside this was a layer of macrophages which surrounded the central necrotic mass. No well developed sheets of epithelioid cells were seen. Few scattered Langhans' giant cells were present. Acid-fast bacteria were seen in the central necrotic mass and the macrophages bordering it.

Right popliteal lymph node. The medulla of the lymph node had been obliterated by the encapsulated lesion which consisted of a fibrous wall inside of which was a wall of macrophages arranged as a palisade. No sheets of epithelioid cells were seen. Scattered fine granules of calcium were present in the capsule wall. Acid-fast bacilli were present in those macrophages adjacent to the necrotic debris.

Right internal iliac lymph node. The histological changes were similar to those seen in the right popliteal lymph node. Acid-fast bacteria were seen in the periphery of the necrotic debris.

Skin, lower foreleg, upper foreleg, lower hind leg. In each case, a focal granuloma consisting chiefly of epithelioid cells with some giant cells surrounding a central caseous mass, was present in the reticular layer. Acid-fast bacteria were seen in the macrophages lining the central cavity. In the skin of the hind leg, at the upper inoculation site, a focal collection of lymphocytes and macrophages was present in the papillary layer. Neither giant cells, caseation nor acid-fast bacteria were detected.

Bacteriologic findings. Acid-fast bacteria were recovered from the lung, left prescapular lymph node, right popliteal lymph node, the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes, the right internal iliac lymph node, the left popliteal lymph node, the upper foreleg skin lesion and the upper hind leg skin lesion.

Calf 44 - 1 mg. inoculum intradermally. This organism was the isolant from the left prescapular lymph node of calf 3.

Clinical observations. On the seventh day after inoculation, the skin at the injection site showed a diffuse hard swelling 50 mm. in diameter with an elevation of 10 mm. The left prescapular lymph node was swollen. Ten days later the swelling had reduced in diameter to 30 mm., with an elevation of 10 mm., but a central area, 15 mm. in diameter, was hairless. On the 23rd day after inoculation, the animal had lost condition and its temperature was 106 F. The swelling at the inoculation site was 10 mm. in diameter with an elevation of 5 mm. and had ulcerated through an orifice 10 mm. in diameter. The left prescapular lymph node was markedly swollen. On the 33rd day the lesion was little changed, but by the 39th day the ulcer was granulating and had reduced in diameter to 8 mm. The prescapular lymph node was still markedly swollen. Ten days later there was no change.

Necropsy findings. (67 days after inoculation)

Holstein heifer, 8 months old, fair condition, 400 lb. weight.

On gross examination, lesions were found in the left prescapular lymph node, in the skin at the site of inoculation, and in the left and right bronchial lymph nodes and the anterior and posterior mediastinal lymph nodes.

Left prescapular lymph node. This measured 100 x 50 x 40 mm. and cut with difficulty. At one pole there was a yellow caseous focus measuring 20 mm. in diameter. Two other caseous foci measuring 55 x 35 x 25 mm. were found, one in the center of the node and one at the other pole.

Anterior mediastinal and left and right bronchial lymph nodes. All 8 lymph nodes contained scattered yellow foci similar to those seen in the left prescapular lymph node.

Skin. The surface of the ulcer was covered by a scab measuring 10 x 5 mm. The swollen areaabout the scab measured 15 mm. in diameter and on cross section the skin was found to contain a yellow focus 3 mm. in diameter.

Histopathologic findings.

Left prescapular lymph node. The granulomas varied from small discrete microscopic to large confluent masses of granulomatous tissue. Many of the smaller granulomas were either partially or completely encapsulated. The large granulomas showed extensive caseation and some degree of calcification. Giant cells were frequent. Acid-fast bacteria, which were seen rarely, lay in the cytoplasm of the Langhans' giant cells.

Anterior mediastinal lymph node. Only very small granulomas were seen, and these were either noncaseous or encapsulated and caseo-calcareous. Giant cells were frequent but acid-fast bacteria were not seen.

Posterior mediastinal lymph node. Lesions in the posterior mediastinal lymph node were similar to those seen in the anterior mediastinal lymph node, numerous microscopic granulomas being scattered throughout

the parenchyma. Most of these were of the encapsulated caseo-calcareous type, but a few noncaseous granulomas consisting of epithelioid cells with a few giant cells were also seen. The central calcification of the caseous granulomas was quite marked. Acid-fast bacteria were not detected.

Left and right bronchial lymph nodes. The changes in these lymph nodes were similar to that seen in the anterior mediastinal lymph node. Scattered throughout the parenchyma of the lymph node were numerous granulomas 1 to 2 mm. in diameter which were encapsulated, and in their centers were calcified areas which apparently had been previously caseated. Giant cells were frequent but no acid-fast bacteria were seen in either of these lymph nodes.

Skin inoculation site. Scattered throughout the reticular layer of the skin were numerous granulomas which varied in size up to 2 mm. in diameter. These may have been discrete granulomas or they may have been part of a multiloculated granuloma. Many of them were noncaseous, consisting of epithelioid and giant cells. The larger ones frequently had caseous centers in which some calcification had taken place. Giant cells were exceedingly numerous, but acid-fast bacteria were not detected. There was little change in the overlying epidermis.

Liver. Scattered microscopic foci of mononuclear cells were seen throughout the lobules and the portal tracts.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the skin inoculation site, the pool of the anterior mediastinal and the left and right bronchial lymph nodes, from the posterior mediastinal lymph node, the pool of the liver and spleen and from the pool of the bone marrow from the left and right humeri.

Culture 107E-0

Calf 13 - 1 mg. inoculum intradermally.

Clinical observations. The skin lesion at the inoculation site did not ulcerate. The swelling reached a maximum diameter of 10 mm. on the 7th day after inoculation, maintained this size until the 23rd day and then regressed so that at necropsy the inoculation site was represented by a nodule 5 mm. in diameter.

Necropsy findings. (77 days after inoculation)

Holstein heifer, 9 months old, good condition, 400 lb. weight.

The only lesions detected were a red nodule 6 mm. in diameter visible on the cut surface of the skin at the inoculation site and a few fibrous adhesions between the parietal and visceral pleurae.

Histopathologic findings. A noncaseous granuloma was found lying in the reticular layer of the skin at the inoculation site. There was no necrosis and no attempt at encapsulation. Acid-fast bacteria were readily visible scattered throughout the granuloma.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node and from the skin at the inoculation site.

Calf 40 - 1 mg. inoculum intradermally. This organism was the isolant from the left prescapular lymph node of calf 13.

Clinical observations. On the eighth day after inoculation, the swelling at the skin inoculation site reached a diameter of 10 mm. and was soft and fluctuating. A week later the swelling was still 10 mm. in diameter but centrally there was a hairless area 3 mm. in diameter. On the 22nd and 29th days after inoculation the skin lesion was unchanged

from that seen on the 15th day and the lesion was still closed. By the 38th day after inoculation the skin inoculation site was represented by an intradermal nodule 5 mm. in diameter. Ulceration did not occur.

Necropsy findings. (70 days after inoculation)

Holstein-Angus bull, 10 months old, good condition, 500 lb. weight.

No gross lesions were detected except for a hairless area 15 mm. in diameter at the site of inoculation. On cross section of this area the skin was not seen to be thickened and no abnormality could be seen.

Histopathologic findings. No microscopic changes were seen. The skin section taken through the hairless area at the site of inoculation showed no abnormality.

Bacteriologic findings. Acid-fast bacteria were recovered only from the left prescapular lymph node.

Culture 71C-0

Calf 7 - 1 mg. inoculum intradermally.

Clinical observations. The inoculation site had swollen to 20 mm. in diameter on the seventh day following inoculation. Seven days later the swelling was 25 mm. in diameter and had ulcerated. On the 21st day it was 20 mm. in diameter and a scab had formed over the surface. The lesion slowly regressed in size so that, on the 58th day, a hairless area 5 mm. in diameter remained.

Necropsy findings. (71 days after inoculation)

Holstein steer, 9 months old, good condition, 500 lb. weight.

The only lesions detected were a nodule 5 mm. in diameter in the dermis at the site of inoculation and two abscess-like lesions,

25 and 35 mm. in diameter, respectively, in the cortex of the left prescapular lymph node. These "abscesses" were filled with a yellow flocculent "pus".

Histopathologic findings. Microscopic examination showed that the two abscess-like structures seen grossly were encapsulated granulomas in which there was central necrosis. This central necrotic area was surrounded by a wall of macrophages and epithelioid cells throughout which lymphoid cells were scattered (Figure 17). Acid-fast bacteria were very rare and were located either at the periphery of the caseous debris or in those macrophages bordering this debris.

Skin inoculation site. A granuloma with an area of central caseation and calcification surrounded by epithelioid cells and giant cells was lying in the reticular layer. No acid-fast bacteria were seen.

Bacteriologic findings. Acid-fast bacteria were recovered from the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes, from the left prescapular lymph node and from the skin inoculation site.

Calf 38 - 1 mg. inoculum intradermally. This inoculum differed from that used in calf 7 in that it had been stored in the refrigerator.

Clinical observations. Eight days after inoculation, the swelling at the inoculation site had reached a diameter of 40 mm. and was 20 mm. thick. Seven days later there was an ulcer 10 mm. in diameter in the center of the swelling. On the 22nd day the swelling had reduced to 15 mm. in diameter and was 3 mm. thick. The ulcer had closed so that the center of the swelling was now occupied by a hairless area 6 mm. in diameter. On the 29th day after inoculation the swelling was 10 mm. in

diameter and raised only 1 mm., but the hairless area was 7 mm. in diameter. By the 38th day the swelling had completely resolved and the lesion was represented by a hairless area 10 mm. in diameter. From the 8th to the 15th day after inoculation the animal had a temperature greater than 103 F., the maximum being 103.8 on the 8th and 14th days.

Necropsy findings. (63 days after inoculation)

Holstein steer, 10 months old, good condition, 500 lb. weight.

No gross lesions were detected. A cross section of skin at the site of inoculation failed to reveal any macroscopic changes.

Histopathologic findings. No microscopic changes were detected in the section of skin taken from the inoculation site or in any other organ or lymph node.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the skin at the site of inoculation, from the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node, from the lung and from the pool of the liver and spleen.

Calf 39 - 1 mg. inoculum intradermally. This organism was the reisolant of 71C-0 from the pool of the bronchial and mediastinal lymph nodes of calf 7.

Clinical observations. Eight days after inoculation, the injection site showed an actively inflamed area 20 mm. in diameter. Seven days later this had reached a diameter of 20 mm. and was 7 mm. thick. In the center was a hairless area 3 mm. in diameter. By the 22nd day after inoculation, the swelling had reduced the 10 mm. in diameter and 3 mm.

elevation. The central hairless area was now 5 mm. in diameter and the lesion was still closed. On the 29th and 38th days after inoculation the lesions still appeared the same as on the 22nd day.

Necropsy findings. (67 days after inoculation)

Holstein heifer, 10 months old, good condition, 500 lb. weight.

The only lesion detected was in the skin at the inoculation site. On cross section the skin showed a 7 x 5 mm. reddened area in the dermis.

Histopathologic findings. Microscopically the granuloma at the inoculation site was composed chiefly of epithelioid cells as well as some giant cells. Caseation and calcification were absent, but there was some attempt at peripheral encapsulation. Acid-fast bacteria were very numerous throughout the granuloma and were most concentrated in the Langhans' giant cells and the epithelioid cells.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the skin inoculation site, the pool of the anterior mediastinal and left and right bronchial lymph nodes and from the posterior mediastinal lymph node.

Culture 78B-0

Calf 10 - 1 mg. inoculum intradermally.

Clinical observations. At no stage did the skin inoculation site ulcerate to the surface. The swelling reached a maximum diameter of 20 mm. on the 7th day after inoculation and then regressed so that at necropsy a nodule only 5 mm. in diameter remained.

Necropsy findings. (73 days after inoculation).

Holstein steer, 9 months old, good condition, 350 lb. weight.

On cross section of the skin inoculation site, a nodule 10 x 5 mm. in diameter was seen in the dermis. Fibrous adhesions between the visceral and parietal pleurae over the diaphragmatic lobes were present. Several encapsulated abscesses containing yellow-green pus and varying in diameter from 10 mm. to 20 mm. lay in the left and right diaphragmatic lobes of the lungs.

Histopathologic findings. Microscopic examination of the skin at the inoculation site showed a large focus of lymphocytes but no granuloma was seen.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node and the skin inoculation site.

Group III Mycobacteria of Swine Origin

Eleven calves were inoculated with six cultures of Group III mycobacteria of swine origin, as follows:

Culture Number	Calf Number
93C-0	Calf 16
$172C_1$-1	Calves 23, 30, 31
173C-1	Calf 24
$193C_2$-1	Calves 32, 33
186C-1	Calves 34, 36
$167C_1$-1	Calves 35, 37

Culture 93C-0

Calf 16 - 1 mg. inoculum intradermally.

Clinical observations. The skin at the injection site on the left foreleg never ulcerated. Swelling here reached a maximum diameter of 27 mm. on the 23rd day after inoculation. It then slowly subsided so

that at necropsy the nodule was 10 mm. in diameter.

Necropsy findings. (86 days after inoculation)

Holstein steer, 8 months old, good condition, 450 lb. weight.

The only lesions detected were in the left prescapular lymph node and at the skin inoculation site. An encapsulated "abscess" 35 x 15 x 10 mm. was found in the dermis and on section was seen to be filled with yellow, creamy "pus". A pink focus 3 mm. in diameter was seen in the cortex of the left prescapular lymph node.

Histopathologic findings. The skin inoculation site showed an inflammatory change similar to that seen in the left prescapular lymph node. This was a granulomatous response in which there was central caseation with a little calcification, surrounded by epithelioid cells in which there were a few acid-fast bacteria. The left prescapular lymph node contained two granulomas. These showed central caseation and calcification, but giant cells were not common. Acid-fast bacteria were rarely seen; they were located in the epithelioid cells.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the skin inoculation site and the pool of anterior and posterior mediastinal and left and right bronchial lymph nodes.

Culture $172C_1$-1

Calf 23 - 1 mg. inoculum intradermally.

Clinical observations. The skin inoculation site never ulcerated. There was only a slight inflammatory swelling which was 2-3 mm. in diameter on the 17th day after inoculation. The lesion regressed and could not be detected on the 24th day after inoculation.

Necropsy findings. (70 days after inoculation)

Holstein bull, 6 months old, good condition, 350 lb. weight.

The only lesion detected was in the lung, the right apical lobe of which was completely consolidated. No lesions were found at the skin inoculation site or in the left prescapular lymph node.

Histopathologic findings. The only lesions detected were in the lung. A purulent bronchopneumonia with subacute bronchitis, purulent exudate in the alveoli and marked hyperplasia of the peribronchial lymphoid tissue was seen.

Bacteriologic findings. Acid-fast bacteria were not recovered.

Calf 30 - 1 mg. inoculum intradermally.

Clinical observations. Seven days after inoculation the skin inoculation site showed a circumscribed intradermal swelling 25 mm. in diameter. A week later the swelling had not changed in size, but a red hairless area 3 mm. in diameter was now visible in its center. On the 21st day after inoculation, an ulcer 5 mm. in diameter was seen in the middle of the swelling. Twenty-eight days after inoculation the injection site showed little swelling, being only 10 mm. in diameter and with only a slight intradermal thickening; but an ulcer 5 mm. in diameter was still visible. On the 35th day, swelling was absent and only a hairless area 6 mm. in diameter now lay in the center of the injection site. This hairless area had reduced to 5 mm. on the 42nd day and was still visible when the animal came to necropsy 56 days after inoculation.

Necropsy findings. (56 days after inoculation)

Holstein-Hereford steer, 8 months old, good condition, 400 lb. weight.

The only lesion seen was the hairless area at the site of inoculation into the skin of the left foreleg. On incision, a red nodule 3 mm. in diameter was visible in the dermis.

Histopathologic findings. The only changes found were at the skin inoculation site. A granuloma here exhibited epithelioid cells, lymphocytes, numerous giant cells and a little caseation and calcification. Acid-fast bacteria were not seen and there was no attempt at fibrous encapsulation.

Bacteriologic findings. Acid-fast bacteria were isolated from the left prescapular lymph node, the skin inoculation site, the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes, and a pool of liver and spleen.

Calf 31 - 1 mg. inoculum intradermally.

Clinical observations. The changes at the skin inoculation site were very similar to those of calf 30, which was also inoculated with the same organism, the skin lesion ulcerating on the 21st day after inoculation. On the 7th day after inoculation the swelling had reached a diameter of 20 mm. Seven days later it had reached a diameter of 25 mm., and in the center was a red hairless area 7 mm. in diameter. Unlike calf 30, after the lesion had ulcerated to the surface the swelling did not subside. It reached a size of 40 x 20 x 10 mm. on the 28th day after inoculation, at which time it was fluctuating and the ulcer had healed. On the 35th day after inoculation the swelling remained unaltered in size but now drained to the surface through a new sinus. By the 42nd

day the swelling had reduced to 40 x 15 x 5 mm. It continued to reduce thereafter, but the inoculation site was still detectable at necropsy.

Necropsy findings. (56 days after inoculation)

Holstein-Hereford steer, 9 months old, good condition, 400 lb. weight.

The only lesion detected was at the site of inoculation. This consisted of a red nodule 5 mm. in diameter lying in the dermis and, adjacent to it, an abscess-like lesion 2 mm. in diameter. These lay under the hairless area still visible but reduced to 5 mm. in diameter.

Histopathologic findings. Microscopic changes were limited to those seen at the skin injection site. The section through the abscess-like lesion showed a granuloma consisting of epithelioid and giant cells in which there was some central caseation and calcification. Acid-fast bacteria were rare. About the periphery of the granuloma some attempt had been made at encapsulation.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the skin inoculation site, the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node and from the pool of the liver and spleen.

Culture 173C-1

Calf 24 - 1 mg. inoculum intradermally.

Clinical observations. The skin at the injection site never ulcerated. The swelling reached a maximum diameter of 30 mm. on the 11th day after inoculation but regressed until on the 33rd day only a very slight swelling was detectable.

Necropsy findings. (71 days after inoculation)

Holstein heifer, 7 months old, good condition, 350 lb. weight.

No gross changes were seen in the animal. The skin inoculation site was not detected.

Histopathologic findings. No microscopic changes were found.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node and the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes.

Culture $193C_2$-1

Calf 32 - 1 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation a circumscribed firm swelling 25 mm. in diameter was seen at the site of inoculation. A week later this had reached a diameter of 26 mm., in which there was a central hairless zone 5 mm. in diameter. On the 21st day after inoculation the swelling had receded to 15 mm. in diameter. The central hairless area was still visible, but the lesion had ulcerated through this zone to the surface and was now covered by a scab. By the 28th day after inoculation, the lesion had receded to a healing ulcer 3 mm. in diameter. By the 35th day after inoculation there was little swelling, but a central hairless area 4 mm. in diameter was still clearly visible. On the 42nd day after inoculation the lesion was represented by only a hairless area 10 mm. in diameter.

Necropsy findings. (54 days after inoculation)

Holstein heifer, 8 months old, good condition, 400 lb. weight.

No significant gross lesions were seen. The skin inoculation site lesion had disappeared except for the area of hairlessness, and,

on section, no lesion was visible intradermally.

Histopathologic findings. A section of skin through a hairless area at what was presumed to be the inoculation site showed a diffuse granulomatous inflammatory response in the papillary layer and extending into the reticular layer. This consisted chiefly of macrophages and epithelioid cells which had made some attempt to form giant cells. A few heterophils and some lymphocytes were visible throughout the granuloma. No caseation or calcification was seen. Acid-fast bacteria were rare and confined to the epithelioid cells.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the skin inoculation site, from the pool of the anterior mediastinal and left and right bronchial lymph nodes and from the posterior mediastinal lymph node.

Calf 33 - 1 mg. inoculum intradermally.

Clinical observations. The inflammatory response at the skin inoculation site was similar to that seen with calf 32, inoculated with the same organism. On the 7th day after inoculation, the swelling had reached a diameter of 25 mm., was circumscribed but little elevated. A week later the swelling had reduced to 15 mm. in diameter, again was little elevated but had a central hairless red area 7 mm. in diameter which had not ulcerated. On the 21st day after inoculation, the swelling had subsided but a central ulcer 5 mm. in diameter was visible. This was unchanged on the 28th day after inoculation, but on the 35th day only a hairless area 9 mm. in diameter remained. This was the only lesion seen also on the 42nd day.

Necropsy findings. (54 days after inoculation)

Holstein-Angus heifer, 9 months old, good condition, 400 lb. weight.

No gross lesions were seen at necropsy.

Histopathologic findings. No lesions were detected as the skin inoculation site could not be determined at necropsy and thus microscopic examination of the area was not feasible.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the skin inoculation site, from the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node and from the pool of the liver and spleen.

Culture 186C-1

Calf 34 - 1 mg. inoculum intradermally.

Clinical observations. Calf 34 was killed in extremis on the 43rd day after inoculation because of what was later found to be bilateral pyelonephritis. The skin at the inoculation site never ulcerated. A diffuse intradermal swelling 10 mm. in diameter developed at the site of inoculation on the 7th day after inoculation. This became more circumscribed by the 14th day and remained as a nodule 10 mm. in diameter until the time of death. On the 43rd day after inoculation the animal was noticed to be sick. It could not rise, was obviously becoming comatose, and was killed in extremis.

Necropsy findings. (43 days after inoculation)

Holstein steer, 9 months old, fair condition, 350 lb. weight.

The gross lesions were confined to the kidneys, urinary bladder and the skin at the site of inoculation. The kidneys were

grossly swollen and, on the cut surface were mottled yellow and red with numerous streaks extending from the cortex to the medulla. The bladder was moderately distended with cloudy urine. The skin at the inoculation site showed a focus of yellow pus-like material 6 mm. in diameter x 3 mm. thick.

Histopathologic findings. At the skin inoculation site a granulomatous focus was present in the reticular layer of the skin. This consisted chiefly of epithelioid cells which showed some attempt to form giant cells. No caseation nor calcification was seen, but an attempt had been made to encapsulate the lesion. Acid-fast bacteria were not seen. Both kidneys were involved with acute pyelonephritis, characterized by extensive cellular and hyaline casts and an interstitial infiltration of leukocytes.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the skin inoculation site, from the pool of the anterior mediastinal and left and right bronchial lymph nodes and from the pool of the liver and spleen.

Calf 36 - 1 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation a diffusely thickened intradermal swelling 10 mm. in diameter was seen at the site of inoculation. A week later this was still 10 mm. in diameter but was more circumscribed. On the 21st day after inoculation the swelling had reduced to a nodule 5 mm. in diameter. A soft intradermal nodule 8 mm. in diameter was present on the 30th day after inoculation, and a week later a hard nodule 10 mm. in diameter was there, which remained until necropsy on the 56th day.

Necropsy findings. (56 days after inoculation)

Holstein steer, 9 months old, good condition, 600 lb. weight.

The lesion at the skin inoculation site was the only one seen. This consisted of an abscess-like lesion 6 mm. in diameter lying in the dermis and filled with yellow pus-like material.

Histopathologic findings. Microscopic examination of the skin inoculation site revealed an intradermal granuloma lying in the reticular layer. This was circumscribed, with a central area of caseation and calcification which was surrounded by an epithelioid cell zone. The periphery of this zone was infiltrated by lymphocytes. No acid-fast bacteria were detected.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the skin inoculation site, from the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node and from the pool of the liver and spleen.

Culture 167C$_1$-1

Calf 35 - 1 mg. inoculum intradermally.

Clinical observations. Seven days after inoculation the swelling at the inoculation site reached a diameter of 25 mm. with a diffuse thickening of the skin. A week later the swelling was still 25 mm. in diameter but was now 8 mm. in thickness, and a 5 mm. ulcer had formed in the center. On the 21st day the swelling had receded to 15 mm. in diameter and 5 mm. in thickness; the ulcer was healing and had receded to 4 mm. in diameter. On the 30th day after inoculation the skin inoculation site was indicated by a hairless area 7 mm. in diameter with

little swelling, but on the 37th day after inoculation the swelling recurred, reaching 35 mm. in diameter and 5 mm. in thickness with a central hairless area 10 mm. in diameter. By the 47th day after inoculation an 8 x 5 mm. nodule had formed at the inoculation site, and lay under the hairless area of the skin, then 5 mm. in diameter.

Necropsy findings. (54 days after inoculation)

Holstein steer, 9 months old, fair condition, 650 lb. weight.

The only lesion detected was that at the skin inoculation site, and this consisted of a somewhat diffuse 15 mm. intradermal thickening.

Histopathologic findings. Microscopic changes were seen only at the skin inoculation site. This showed small foci of granulomatous inflammatory cells in the papillary and reticular layers of the skin. Macrophages with some lymphocytes were involved, but giant cells and acid-fast bacteria were not seen. There was no attempt at encapsulation.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, from the skin inoculation site, from the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node and from the bone marrow from the left and right humeri.

Calf 37 - 1 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation the skin at the inoculation site showed a diffusely reddened and thickened area 15 mm. in diameter with a central soft focus which had not ulcerated to the surface. Seven days later the swelling was 20 mm. in diameter with a central hairless area 5 mm. in diameter. By the 21st day an

ulcer had developed which was 3 mm. in diameter and extruded a yellow caseous material. The swelling was 15 mm. in diameter and 5 mm. thick. On the 30th day after inoculation there was no swelling, only a central hairless area 4 mm. in diameter. The lesion was unchanged on the 37th day after inoculation, but by the 47th day the hairless area had been reduced to 7 mm. in diameter.

Necropsy findings. (56 days after inoculation)

Holstein steer, 9 months old, good condition, 550 lb. weight.

Apart from the small hairless area at the site of injection, no gross lesions were detected. A cross section of the skin at the inoculation site failed to reveal any gross abnormality.

Histopathologic findings. Microscopic examination of the skin at the site of inoculation showed several small microscopic foci of granulomatous inflammation lying in the reticular layer of the skin. These granulomatous foci consisted of macrophages and lymphocytes. Giant cells, caseation, calcification and acid-fast bacilli were not seen.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph node and from the pool of the liver and spleen.

Group III Mycobacteria of Feed and Soil Origin

Five calves were inoculated with three cultures of Group III mycobacteria of feed or soil origin as follows:

Culture Number	Calf Number
feed origin	Calves 46, 47
soil origin	
X31	Calf 48
X41	Calves 49, 50

A Culture of Feed Origin

Calf 46 - 1 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation the swelling about the inoculation site had reached a diameter of 10 mm. with an elevation of 5 mm., and in the middle of the elevation was a hairless area 5 mm. in diameter. On the 14th day the swelling was almost unchanged, being 10 mm. in diameter and 4 mm. in elevation. The central hairless area had shrunk to 2 mm. in diameter. By the 22nd day after inoculation the injection site was represented by a nodule 5 mm. in diameter, of which the central 3 mm. was hairless. On the 39th day after inoculation this nodule had receded to 2 mm. in diameter.

Necropsy findings. (65 days after inoculation)

Angus-Holstein crossbred bull, 11 months old, good condition, 500 lb. weight.

No gross lesions were found. The exact skin inoculation site could not be detected with certainty.

Histopathologic findings. No lesions were detected.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node, the pool of the anterior mediastinal and left and right bronchial lymph nodes and from the posterior mediastinal lymph node.

Calf 47 - 10 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation the swelling at the skin inoculation site had reached a diameter of 14 mm. with a 10 mm. elevation. There was no ulceration and no hair loss. By the 14th day the swelling was 25 x 20 mm. in area with an elevation of 3 mm. and with a 2 x 7 mm. hairless area in the middle. Eight days later the swelling covered an area of 35 x 25 mm. with an elevation of 7 mm., and in it lay two 5 mm. in diameter ulcers. On the 28th day after inoculation the two ulcers, now 7 mm. in diameter, were still present. No swelling remained on the 39th day after inoculation, but two hairless areas 5 mm. in diameter were visible.

Necropsy findings. (64 days after inoculation)

Angus-Holstein crossbred heifer, 9 months old, good condition, 400 lb. weight.

The only gross lesion seen was in the skin at the site of inoculation. Two areas, each approximately 10 mm. in diameter, were slightly swollen. On cross section one of these was found to have a reddened area 5 mm. in diameter and 2 mm. high in the reticular layer of the skin, but no lesion was detected on cross section of the other.

Histopathologic findings. The only microscopic change found was in the skin at the site of injection. In the reticular layer there was a patchy mononuclear cell infiltrate about the large blood vessels. No true granuloma was found.

Bacteriologic findings. Acid-fast bacteria were recovered from the skin inoculation site, from the left prescapular lymph node, the pool of the anterior mediastinal and the left and right bronchial lymph nodes,

the posterior mediastinal node, the pool of the liver and spleen and from the bone marrow from each humerus.

Cultures of Soil Origin

X31

Calf 48 - 1 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation, a 5 mm. nodule was found at the site of injection. Seven days later no lesion was detectable at the inoculation site, but on the 22nd day after inoculation there was a slight intradermal thickening but no hair loss. On the 28th and 39th days after inoculation no abnormality was found at the injection site.

Necropsy findings. (63 days after inoculation)

Angus-Holstein crossbred bull, 10 months old, good condition, 600 lb. weight.

No abnormality was detected.

Histopathologic findings. No microscopic changes were seen in any organs or lymph nodes.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node and the pool of the anterior mediastinal and left and right bronchial lymph nodes.

X41

Calf 49 - 10 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation, the swelling at the site of the injection had reached a diameter of 35 mm. with a 5 mm. elevation. No hair loss had occurred. Seven days later the

swelling had receded to 10 mm. in diameter with an elevation of 2 mm., but the central portion of the swelling had a hairless area 3 mm. in diameter. By the 22nd day after inoculation the swelling had completely resolved and the lesion consisted only of the area 5 mm. in diameter which had lost hair. On the 39th day the lesion was similar to that seen on the 22nd day after inoculation, in that the lesion showed only a hairless area 5 mm. in diameter.

Necropsy findings. (73 days after inoculation)

Holstein-Angus heifer, 10 months old, good condition, 600 lb. weight.

No gross lesions were found.

Histopathologic findings. No microscopic lesions were detected in any organs or lymph nodes.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph nodes, from the pool of the anterior mediastinal and left and right bronchial lymph nodes and from the posterior mediastinal lymph node.

Calf 50 - 1 mg. inoculum intradermally.

Clinical observations. On the 7th day after inoculation the swelling at the injection site reached a diameter of 10 mm. It was hard but had little elevation. Seven days later the injection site was represented by an intradermal nodule 5 mm. in diameter with an elevation of 2 mm. There was no hair loss. On the 22nd and 29th days after inoculation, the lesion was unchanged from that seen at 14 days.

Necropsy findings. (73 days after inoculation)

Holstein bull, 11 months old, good condition, 550 lb. weight.

No gross lesions were found.

Histopathologic findings. No microscopic changes were detected in any organs or lymph nodes.

Bacteriologic findings. Acid-fast bacteria were recovered from the lung and the posterior mediastinal lymph node.

Pseudochromes

Seven calves were inoculated with four cultures of pseudochrome mycobacteria as follows:

Culture Number	Calf Number
52H-1	Calves 11, 57, 58
61B-0	Calves 14, 55
112B-0	Calf 17
128F-0	Calf 56

Culture 52H-1

Calf 11 - 1 mg. inoculum intradermally.

Clinical observations. The site of inoculation became swollen and reached a maximum diameter of 12 mm. on the 14th day after inoculation. It then subsided to be replaced by a hard nodule 5 mm. in diameter. No other clinical signs were noted.

Necropsy findings. (72 days after inoculation).

Holstein steer, 9 months old, good condition, 350 lb. weight.

Gross lesions consisted of an intradermal nodule 10 x 5 mm. at the inoculation site, fibrous adhesions between the visceral and parietal pleurae over the diaphragmatic lobes of the lungs, and several encapsulated abscesses containing yellow-green pus in the substance of

the two diaphragmatic lobes. These thoracic lesions were undoubtedly associated with calf pneumonia present in this group of experimental animals before they were infected with acid-fast bacteria.

Histopathologic findings. Microscopic examination of the skin inoculation site showed a large focus of lymphocytes in the reticular layer of the skin, but no granuloma was seen. The diaphragmatic lobes exhibited an acute bronchopneumonia with pus cells lying in the alveoli and distention at the alveolar septa by inflammatory exudate, including serum, fibrin and some heterophils. No pathologic changes were detected elsewhere.

Bacteriologic findings. Acid-fast bacteria were recovered from only the skin inoculation site.

Calf 57 - 10 mg. inoculum: 2 mg. each by the intradermal, subcutaneous, intraperitoneal, intramuscular and peroral routes.

Clinical observations. The swelling at the site of the intradermal inoculation reached a maximum size of 10 mm. diameter on the 15th day after inoculation. At the same time, a nodule 2 mm. in diameter developed at the intramuscular injection site and a diffuse swelling approximately 20 mm. in diameter around the site of the subcutaneous injection. At necropsy all swellings had disappeared except for a very small nodule at the intradermal injection site. None of these lesions ulcerated. Intermittent attacks of coughing were noted, presumably due to the inhalation of an aerosol of ET-383, which occurred on the day of inoculation.

Necropsy findings. (58 days after inoculation)

Holstein steer, 8 months old, good condition, 450 lb. weight.

Generalized pulmonary atelectasis was present in the right apical lobe and scattered linear areas of atelectasis were present in the right cardiac and intermediate lobes. The intradermal injection site was represented by a red area 1 mm. in diameter in the dermis.

Histopathologic findings.

Skin at the inoculation site. A noncaseous granuloma was found in the reticular layer of the skin. This consisted of epithelioid cells and giant cells; acid-fast bacteria appeared to be absent. Although the granuloma was sharply delineated by the surrounding fibrous tissue of the reticular layer, there was no true attempt at encapsulation.

Lung. An extensive chronic bronchiolitis was present throughout the section. Extensive fibrosis had taken place in the lamina propria of the bronchioles and bronchi and the adjacent alveoli had become atelectatic.

Bacteriologic findings. Acid-fast organisms were isolated from posterior mediastinal nodes only.

Calf 58 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular, and intraperitoneal routes. This organism was the reisolant recovered from the skin inoculation site of calf 11.

Clinical observations. An intradermal nodule 10 mm. in diameter developed at the site of intradermal inoculation. This nodule never ulcerated, regressed slowly and had completely disappeared at the time of necropsy. Some slight thickening of the skin and subcutis was

noticed about the subcutaneous and intramuscular injection sites 21 days after inoculation. Intermittent attacks of coughing were present throughout the period of the experiment, induced, presumably, by the inhalation of an ET-383 aerosol on the day of inoculation.

Necropsy findings. (57 days after inoculation)

Holstein steer, 7 months old, good condition, 500 lb. weight.

No abnormality was detected. The inoculation sites could not be found.

Histopathologic findings. No lesions were seen.

Bacteriologic findings. Acid-fast organisms were isolated on a single tube each from the posterior mediastinal nodes and the pool of the left and right prescapular lymph nodes.

Culture 61B-0

Calf 14 - 1 mg. inoculum intradermally.

Clinical observations. The site of inoculation reached a maximum swelling of 25 mm. in diameter 11 days after inoculation, then subsided and never ulcerated. No other clinical changes were seen.

Necropsy findings. (78 days after inoculation)

Holstein heifer, 8 months old, good condition, 400 lb. weight.

No gross lesions were seen.

Histopathologic findings. Sections of the diaphragmatic lobes of the lung had a peribronchial follicular lymphoid hyperplasia and a purulent bronchopneumonia in some of the alveoli.

Bacteriologic findings. Acid-fast organisms were recovered from the left prescapular lymph node and the pool of the anterior and posterior mediastinal, and left and right bronchial lymph nodes.

Calf 55 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular and intraperitoneal routes.

Clinical observations. The swelling at the intradermal inoculation site reached a maximum diameter of 15 mm. 10 days after inoculation. It never ulcerated, and the swelling slowly subsided. Nodules 2-3 mm. in diameter were formed at the sites of the intramuscular and subcutaneoous injection, being most prominent 16 days after inoculation. These lesions completely resolved. On the day of inoculation the animal was exposed to an aerosol of ET-383 and developed a moist cough which persisted throughout the experiment.

Necropsy findings. (57 days after inoculation)

Holstein-Angus crossbred steer, 8 months old, good condition, 900 lb. weight.

Gross lesions were seen only at the site of the intradermal injection. This consisted of an intradermal red focus 4 mm. in diameter.

Histopathologic findings. A tuberculous granuloma was present at the site of the intradermal inoculation. Giant cells, epithelioid cells, lymphocytes and numerous acid-fast organisms were present.

Bacteriologic findings. Acid-fast organisms were isolated from the left and right prescapular, posterior mediastinal and thoracic nodes and liver-spleen pool.

Culture 112B-0

Calf 17 - 1 mg. inoculum intradermally.

Clinical observations. The intradermal inoculation site reached a maximum swelling of 22 mm. diameter on the 22nd day after inoculation, regressed and finally disappeared.

Necropsy findings. (80 days after inoculation)

Holstein heifer, 9 months old, good condition, 400 lb. weight.

No gross lesions were seen.

Histopathologic findings. No microscopic changes were detected in any organs or lymph nodes.

Bacteriologic findings. No acid-fast organisms were recovered.

Culture 128F-0

Calf 56 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular and intraperitoneal routes.

Clinical observations. A nodule 7 mm. in diameter developed at the site of intradermal inoculation. There was no response to the subcutaneous injection, but a nodule 2 mm. in diameter developed at the intramuscular injection site. None of these lesions ulcerated, and at necropsy no nodules remained. The steer had occasional attacks of coughing associated with the inhalation of an aerosol of ET-383 to which it was exposed on the day of inoculation.

Necropsy findings. (56 days after inoculation)

Holstein steer, 7 months old, good condition, 500 lb. weight.

No gross lesions were found. The exact sites of injection were not ascertained.

Histopathologic findings. Follicular hyperplasia of the peribronchial lymph nodes was present. There was a subacute tracheitis with heterophils in the epithelium, epithelial hyperplasia and subepithelial mononuclear infiltration.

Bacteriologic findings. Acid-fast organisms were isolated on one tube each from posterior mediastinal nodes and lung, and two tubes from the liver-spleen pool.

Group IV Mycobacteria

Twelve calves were inoculated with six cultures of Group IV mycobacteria as follows:

Culture Number	Calf Number
124F-0	Calf 5
4F-1	Calf 9
87H-0	Calves 12, 22
117B-0	Calves 15, 28, 52
7F-1	Calves 18, 51
254F-1	Calves 25, 53, 54

Culture 124F-0

Calf 5 - 2.2 mg. inoculum intradermally.

Clinical observations. None available.

Necropsy findings. (37 days after inoculation)

Holstein heifer, 8 months old.

No gross lesions were detected.

Histopathologic findings. A few scattered lung lobules showed atelectasis.

Bacteriologic findings. Acid-fast bacteria were recovered from the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes.

Culture 4F-1

Calf 9 - 1 mg. inoculum intradermally.

Clinical observations. The intradermal inoculation site showed a maximum swelling of 18 mm. diameter 14 days after inoculation. It did not ulcerate; rather, it subsided and at necropsy could not be detected.

Necropsy findings. (69 days after inoculation)

Holstein steer, 9 months old, good condition, 500 lb. weight.

No gross lesions were detected.

Histopathologic findings. No abnormality was detected.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node.

Culture 87H-0

Calf 12 - 1 mg. inoculum intradermally.

Clinical observations. One week after inoculation the intradermal inoculation site showed a swelling 5 mm. in diameter. The animal was recumbent and unable to rise, and it died the next day.

Necropsy findings. (8 days after inoculation)

Breed and sex not recorded, 7 months old, fair condition, weight unknown.

The cause of death was not determined.

Histopathologic findings. Only the skin inoculation site and the left prescapular lymph node were examined. No significant change was seen in the lymph node, but a small granuloma was found in the reticular layer of the skin. This consisted of macrophages and lymphocytes; giant cells were not seen. Acid-fast bacteria were very numerous and especially so at the periphery of the granuloma.

Bacteriologic findings. Due to the post-mortem decomposition no bacteriological examination was attempted.

Calf 22 - 1 mg. inoculum intradermally.

Clinical observations. No inflammation was seen at the site of the intradermal injection. Twenty-eight days after inoculation the animal was found dead in its stall.

Necropsy findings. (28 days after inoculation)

Holstein steer, fair condition.

Early pneumonic changes were present throughout the lungs. The skin inoculation site could not be detected.

Histopathologic findings. Only the left prescapular lymph node, liver, intestine, kidneys and lungs were examined. The liver showed a generalized centrolobular degeneration with hemorrhage into these areas. Early purulent bronchopneumonic changes were seen in the lungs, viz.: hyperemia, pus cells and debris in the bronchioles and alveoli and inflammatory edema and heterophils in the interlobular septa.

Bacteriologic findings. No acid-fast bacteria were isolated.

Culture 117B-0

Calf 15 - 1 mg. inoculum intradermally.

Clinical observations. A swelling 20 mm. in diameter was present at the site of inoculation 7 days after inoculation. This subsided over the next fortnight and then completely disappeared so that the inoculation site was not detectable at necropsy.

Necropsy findings. (78 days after inoculation)

Holstein heifer, 8 months old, good condition, 400 lb. weight.

No gross lesions were detected.

Histopathologic findings. No lesions were seen in any organ or lymph node.

Bacteriologic findings. No acid-fast bacteria were recovered.

Calf 28 - 1 mg. inoculum (killed) intradermally.

Clinical observations. Fourteen days after inoculation an intradermal nodule 2 mm. in diameter developed at the site of inoculation. This disappeared in the following week.

Necropsy findings. (70 days after inoculation)

Holstein-Angus crossbred heifer, 7 months old, good condition, 450 lb. weight.

No gross lesions were detected.

Histopathologic findings. No lesions were detected.

Bacteriologic findings. No acid-fast bacteria were recovered.

Calf 52 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular and intraperitoneal routes.

Clinical observations. The swelling about the intradermal inoculation site reached a maximum diameter of 10 mm. nine days after inoculation. This slowly subsided and at necropsy the injection site could not be found. No inflammatory response was seen at any period about the sites of intramuscular, intraperitoneal and subcutaneous inoculation. This

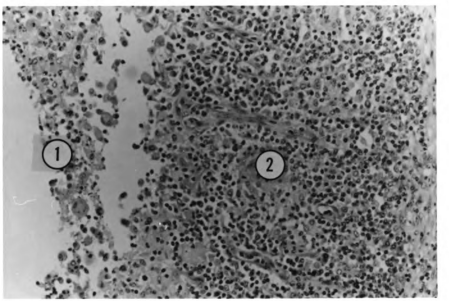

Figure 17. Left prescapular lymph node from calf 7, inoculated with 71C-0, a Group III mycobacterium. Note the necrotic cell debris (1) and the epithelioid cells (2) among lymphocytes lining the internal surface of the capsule. New Fuchsin - H & E. x187.

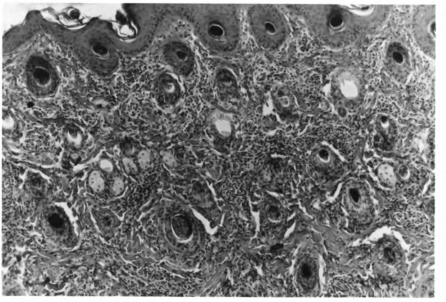

Figure 18. Skin inoculation site from calf 52, inoculated with 117B-0, a Group IV mycobacterium. Note the diffuse mononuclear clear cell infiltrate in both the reticular and papillary layers of the skin. New Fuchsin - H & E. x75.

animal was also exposed to an aerosol of ET-383 on the day of inoculation and subsequently showed intermittent attacks of coughing.

Necropsy findings. (52 days after inoculation)

Holstein heifer, 7 months old, fair condition, 550 lb. weight.

No gross lesions were detected.

Histopathologic findings. A section through what was thought to be the inoculation site showed mononuclear cells infiltrating diffusely through the fibrous bundles of the reticular layer of the skin (Figures 18 and 19). In the lung the bronchioles were affected with a subacute inflammation with excess goblet cells, infiltration of mononuclears into the lamina propria, epithelial hyperplasia and heterophil infiltration into the epithelium. Similar inflammatory changes were present in the trachea.

Bacteriologic findings. Acid-fast bacteria were recovered only from the posterior mediastinal lymph nodes.

Culture 7F-1

Calf 18 - 1 mg. inoculum intradermally.

Clinical observations. No inflammatory response was seen at the inoculation site.

Necropsy findings. (86 days after inoculation)

Holstein steer, 10 months, good condition, 450 lb. weight.

No gross lesions were detected.

Histopathologic findings. No abnormality was detected in any organs or lymph nodes.

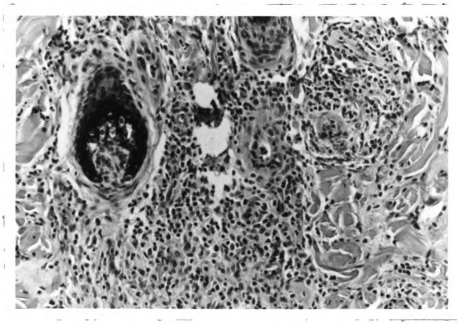

Figure 19. Skin inoculation site from calf 52, inoculated with 117B-0, a Group IV mycobacterium. A higher magnification to show the mononuclear cells in the reticular layer of the skin and the absence of giant cells and caseation. New Fuchsin - H & E. x187.

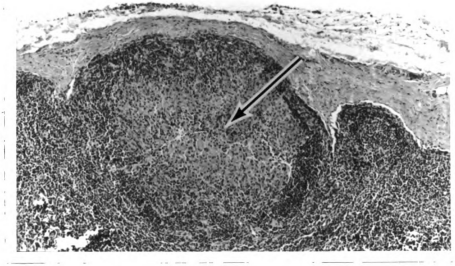

Figure 20. Right axillary lymph node from calf 51, inoculated with 7F-1, a Group IV mycobacterium. This contains a single noncaseous granuloma (arrow). New Fuchsin - H & E. x75.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular lymph node.

Calf 51 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular and intraperitoneal routes.

Clinical observations. The swelling about the intradermal inoculation site reached a diameter of 20 mm. nine days after inoculation. Six days later it was unchanged, but the subcutaneous injection site contained a nodule 30 mm. in diameter and the intramuscular site a swelling 20 mm. in diameter. These slowly regressed and at necropsy only the intradermal site could be detected. On the day of inoculation the animal was exposed to an aerosol of ET-383, and five days later it was coughing continuously with the expulsion of mucus. This clinical sign continued for approximately three weeks.

Necropsy findings. (52 days after inoculation)

Holstein steer, 8 months old, poor condition, 450 lb. weight.

The right apical and cardiac lobes were red and firm due to atelectasis, and their bronchioles had thickened walls.

Skin inoculation site. An encapsulated "abscess" 10 mm. in diameter which contained creamy yellow pus was present in the dermis.

Left prescapular lymph node. Several caseous foci 1-2 mm. in diameter were visible.

Internal iliac lymph nodes. Numerous yellow foci varying in diameter from 0.5 to 4 mm. were seen.

Histopathologic findings.

Skin inoculation site. Several granulomas (perhaps part of one granuloma) had central caseation in which were accumulations of heterophils.

These were surrounded by epithelioid cells in which were numerous giant cells. No acid-fast bacteria were found. There was no attempt at capsule formation.

Left prescapular lymph node. A noncaseous granuloma and another encapsulated granuloma with central caseation and calcification were seen. Epithelioid cells and giant cells were numerous. No acid-fast bacteria were detected.

Right axillary lymph node. Two noncaseous granulomas lay subcapsularly and consisted of epithelioid cells and few giant cells (Figures 20 and 21). No acid-fast bacteria were seen.

Internal iliac lymph node. Multiple nonprogressive encapsulated granulomas with central caseation and calcification were seen (Figures 22 and 23). No acid-fast bacteria were detected.

Right ischiatic lymph node. One noncaseous granuloma of microscopic size was present and consisted of epithelioid cells and a few giant cells (Figure 24). A giant cell contained a few acid-fast bacteria.

Lung, right apical lobe. Chronic bronchiolitis was present, with the epithelium showing hyperplasia and infiltration with heterophils and the lamina propria heavily fibrosed. Many of the alveoli were atelectatic.

Trachea. A subacute tracheitis was present, with epithelial hyperplasia, excess goblet cells and infiltrating heterophils in the epithelium and lamina propria.

Bacteriologic findings. Acid-fast bacteria were recovered from the skin inoculation site, the left prescapular lymph node, the pool of the anterior mediastinal and left and right bronchial lymph nodes, from the posterior mediastinal lymph nodes, the pool of the liver and spleen and from the internal iliac lymph nodes.

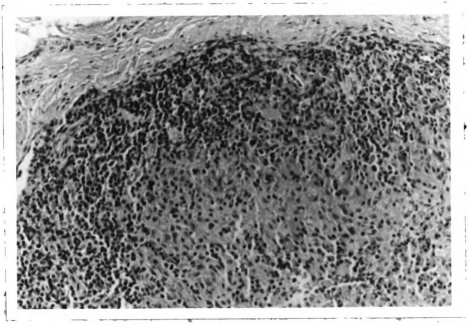

Figure 21. Right axillary lymph node from calf 51, inoculated with 7F-1, a Group IV mycobacterium. This higher magnification of a portion of Figure 20 shows the epithelioid cells in the center of the noncaseous granuloma. New Fuchsin - H & E. x187.

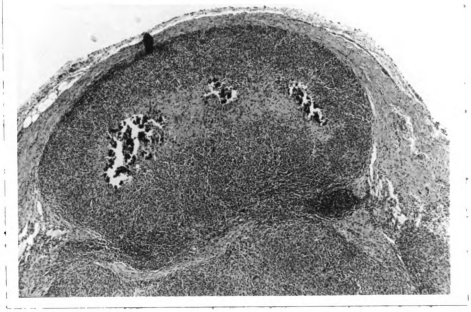

Figure 22. Internal iliac lymph node from calf 51, inoculated with 7F-1, a Group IV mycobacterium. An encapsulated caseo-calcareous granuloma lies under the capsule. New Fuchsin - H & E. x50.

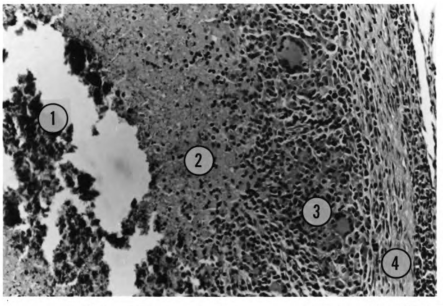

Figure 23. Internal iliac lymph node from calf 51, inoculated with 7F-1, a Group IV mycobacterium. Granuloma is seen with central calcification (1), a layer of caseation (2), a layer of epithelioid cells and giant cells (3), all enclosed within a fibrous capsule (4). New Fuchsin - H & E. x187.

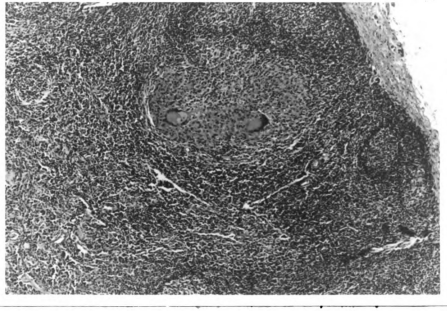

Figure 24. Right ischiatic lymph node from calf 51, inoculated with 7F-1, a Group IV mycobacterium. A solitary noncaseous granuloma with two giant cells lies in the field. New Fuchsin - H & E. x75.

Culture 254F-1

Calf 25 - 1 mg. inoculum intradermally.

Clinical observations. Sixteen days after inoculation the injection site opened and discharged a yellow pus-like material and then healed over completely in 10 days.

Necropsy findings. (70 days after inoculation)

Holstein bull, 6 months old, good condition, 400 lb. weight.

The only gross lesions detected were a hard red nodule 1 mm. in diameter in the dermis at the site of inoculation and scattered lobules of red consolidation on the ventral border of the right intermediate lobe of the lung.

Histopathologic findings. Because of the small size of the skin lesion this material was used solely for bacteriological examination.

Bacteriologic findings. Acid-fast bacteria were recovered from the left prescapular and the pool of the anterior and posterior mediastinal and left and right bronchial lymph nodes.

Calf 53 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular and intraperitoneal routes.

Clinical observations. The swelling about the intradermal inoculation site reached a maximum diameter of 10 mm. at nine days after inoculation, remained at this size for 20 days and then slowly subsided so that the inoculation site was not detected at necropsy. No inflammatory response to the intraperitoneal and intramuscular injections was seen. At the subcutaneous inoculation site a closed-in nodule 20 mm. in diameter had developed by the 15th day after inoculation and then subsided. No

ulceration occurred at any of the inoculation sites. This animal was also exposed to an aerosol of ET-383 on the day of inoculation but was never seen to cough.

Necropsy findings. (57 days after inoculation)

Hereford-Holstein crossbred steer, 8 months old, good condition, 800 lb. weight.

The only gross lesions seen were extensive atelectasis of both the apical and cardiac lobes of the right lung.

Histopathologic findings. The lesions seen were confined to the respiratory system and consisted of a subacute tracheitis, bronchitis and bronchiolitis, and patchy atelectasis. The inflammatory changes in the respiratory passages were characterized by epithelial hyperplasia, excess goblet cells, mononuclear infiltration of the lamina propria and the migration of heterophils through the epithelium.

Bacteriologic findings. Acid-fast organisms were isolated from the liver-spleen pool, lung, and from the pool of the anterior mediastinal and left and right bronchial lymph nodes, the posterior mediastinal nodes and the pool of the left and right prescapular lymph nodes.

Calf 54 - 10 mg. inoculum: 2 mg. each by the peroral, intradermal, subcutaneous, intramuscular and intraperitoneal routes. This organism was reisolated from calf 25.

Clinical observations. Nine days after inoculation the intradermal inoculation site presented a closed nodule 10 mm. in diameter. This reached a maximum diameter of 20 mm. 15 days after inoculation, remained unchanged for a further week and thereafter slowly receded to 15 mm. in

diameter at time of necropsy. The subcutaneous and intramuscular inoculations caused diffuse acute inflammatory responses 30 mm. in diameter 15 days after inoculation. A week later these had receded to 20 mm. in diameter; 28 days after inoculation the subcutaneous inoculation site had only a scar and the intramuscular site had a slightly elevated swelling 10 mm. in diameter. No response to the intraperitoneal injection was seen. No ulceration occurred at any site of inoculation. This animal was exposed to an aerosol of ET-383 on the day of inoculation and subsequently showed only an occasional attack of coughing, although a rapid respiration rate was seen three to four weeks after inoculation.

Necropsy findings. (53 days after inoculation)

Hereford-Holstein heifer, 7 months old, good condition, 800 lb. weight.

The skin at the site of intradermal inoculation contained an encapsulated abscess 10 mm. in diameter, in which was a yellowish granular "pus". No other lesion was seen.

Histopathologic findings. The skin contained one discrete granuloma about which some attempt at encapsulation had been made. Acid-fast bacteria were readily visible in a giant cell.

Kidney. Several microscopic foci of interstitial nephritis consisting of periglomerular fibrosis and interstitial infiltrations of mononuclear cells were seen.

Liver. Activation of the Kupffer cells had occurred, as judged by their prominence due to swelling. Several scattered microscopic foci of mononuclear cells lay in many of the lobules. Mononuclears and heterophils were present around the components of many of the portal triads.

Trachea, bronchi, lungs. Subacute inflammatory changes of the air passages and some atelectasis were seen, the lesions being similar to those in calf 51.

Bacteriologic findings. Acid-fast bacteria were recovered from the skin inoculation site, the left prescapular lymph node, the pool of the anterior mediastinal and left and right bronchial lymph nodes, the posterior mediastinal, the lung and the pool of the liver and spleen.

M. avium

One calf was injected with live M. avium and another with killed M. avium, 1 mg. organisms being injected intradermally into the skin of the lateral surface of the left foreleg just above the carpus.

Calf 21 - 1 mg. inoculum (M. avium) intradermally.

Clinical observations. Seven days after inoculation a swelling 30 mm. in diameter had developed at the site of injection. This had receded to 25 mm. on the 14th day after inoculation and remained unchanged on the 23rd day. On the 31st day the swelling was 33 mm. in diameter; six days later it reached the diameter of 45 mm., was soft and fluctuating and had an elevation of 10 mm. By the 52nd day after inoculation the swelling had returned to the diameter of 33 mm. and remained this size as fluctuating swelling until necropsy.

Necropsy findings. (84 days after inoculation)

Holstein heifer, 9 months old, good condition, 450 lb. weight.

The only gross lesions found were in the skin and the subcutis adjacent to the site of inoculation. On cross section of the skin a 40 x 30 x 20 mm. abscess, filled with a creamy pus, was found lying in

the dermis. In the subcutis under this lesion was another abscess which measured 35 x 25 x 15 mm. and was also enclosed by fibrous capsule.

Histopathologic findings. The only lesions detected were those in the skin and the subcutis at the site of inoculation. These consisted of a central caseous mass surrounded by a layer of epithelioid cells. Lymphocytes were scattered between the macrophages and there were occasional foci of heterophils also. Acid-fast bacteria were readily visible within the epithelioid cells. Early calcification had taken place in the caseous mass. Lymphocytes were most concentrated at the periphery of the granuloma directly under the fibrous capsule which completely enclosed the lesion.

Bacteriologic findings. Acid-fast organisms were reisolated from the pool of the anterior and posterior mediastinal and the left and right bronchial lymph nodes and from the left prescapular lymph node.

Calf 27 - 1 mg. inoculum (killed M. avium) intradermally.

Clinical observations. The only response to the intradermal inoculation was the development of a firm nodule. On the 14th day after inoculation a circumscribed intradermal nodule 10 mm. in diameter had formed. Seven days later this had reached a diameter of 15 mm., but by the 28th day it had regressed to a diameter of 8 mm. A diffuse intradermal thickening 5 mm. in diameter was the only evidence at the injection site on the 35th day after inoculation, and this persisted until necropsy on the 56th day.

Necropsy findings. (56 days after inoculation)

Hereford-Holstein heifer, 8 months old, good condition, 400 lb. weight.

Apart from a diffuse thickening of the skin at the site of the intradermal injection, no gross lesions were detected.

Histopathologic findings. The only microscopic changes seen were in the skin at the site of the inoculation. Microscopic noncaseous granulomas were found lying in the papillary layer of the skin. These consisted of macrophages with some fibroblasts, eosinophils and lymphocytes. There had been no attempt at encapsulation and no acid-fast bacteria were seen.

Bacteriologic findings. No acid-fast bacteria were recovered on bacteriological examination.

M. bovis

Two calves, 6 and 20, were inoculated with live M. bovis. Number 6 received 2.2 mg. intradermally, and number 20 received 1 mg. intradermally. Calf 26 was inoculated with 1 mg. of killed M. bovis. Calves 6 and 20 developed acute tuberculosis and were killed when they were in extremis. Neither was tuberculin-tested. At necropsy both animals had widespread lesions of tuberculosis involving most of the lymph nodes and parenchymatous organs in the body. These lesions were highly progressive and showed marked central caseation with some slight degree of calcification and with no effective development of peripheral capsules.

Calf 6 - 2.2 mg. inoculum (130I-0, M. bovis) intradermally.

Clinical observations. The animal was killed in extremis on the 37th day after inoculation.

Necropsy findings. (37 days after inoculation)

Holstein heifer, 6 months old, poor condition.

Gross lesions were found in the following lymph nodes:

mesenteric, internal iliac, right deep inguinal, right prescapular, left prescapular, right popliteal, anterior and posterior mediastinal and left and right bronchial. Miliary lesions were visible throughout the lungs. There were four sites of skin inoculation - two on the left foreleg and two on the right hindleg, 1 mg. and 0.1 mg. being injected into separate sites on both these legs.

Upper skin inoculation site, left foreleg. A swelling 40 mm. in diameter with a 15 mm. ulcer was visible. The ulcer had eroded through the epidermis and well into the dermis and was covered by a greenish necrotic exudate. There was a moderately developed fibrous capsule around the lesion.

Lower skin inoculation site, left foreleg. The swelling was 10 mm. in diameter, and a 5 mm. ulcer lay at its center. This was poorly encapsulated and contained a greenish-yellow, thick, necrotic debris.

Lower skin inoculation site, right hind leg. An ulcer 20 mm. in diameter, which was fairly well encapsulated and covered by a reddish-yellow exudate lay in the epidermis and dermis.

Upper skin inoculation site, right hind leg. An ulcer 30 mm. in diameter and covered with a scab was enclosed by a firm fibrous capsule and had a caseous necrotic center.

Histopathologic findings. On microscopic examination, all lymph nodes with gross lesions were found to have extensive areas of caseation necrosis, which were commonly confluent. There was some degree of calcification. Peripheral to the caseo-calcareous lesions was the granulomatous inflammatory reaction. This had numerous epithelioid and Langhans' giant cells and acid-fast bacteria were common. There was no effective encapsulation by fibrous tissue.

Lung. The section examined showed generalised consolidation with very few alveoli containing air. Macrophages, heterophils and acid-fast bacteria were visible in the bronchioles, whose walls showed epithelial hyperplasia and infiltration of the lamina propria with numerous mononuclears. The alveolar spaces were obliterated by edema fluid, congested alveolar walls, macrophages and alveolar septal cells. Extensive coagulation necrosis of some areas had taken place and acid-fast bacteria were evident at the periphery of these lesions.

Kidney. No abnormality was detected.

Spleen. On examination under the lower power objective no abnormality was seen. However, closer examination showed that in a few of the germinal centers there were focal collections of macrophages and adjacent to these were a few acid-fast bacteria. Another one of these noncaseous granulomas had a Langhans' giant cell which contained acid-fast bacteria.

Bacteriologic findings. Acid-fast organisms were isolated from all tissues.

Calf 20 - 1 mg. inoculum (8I-0 swine origin M. bovis) intradermally.

Clinical observations. Seven days after inoculation a swelling 40 mm. in diameter had developed at the site. Seven days later this had reached 45 mm. in diameter and was commencing to ulcerate. The swelling had reached a diameter of 55 mm. on the 23rd day after inoculation and by the 31st day was 50 mm. in diameter with an open ulcer 23 mm. in diameter. By the 37th day after inoculation the swelling had further increased to a diameter of 60 mm. and had an ulcer 25 mm. in diameter and was healing and granulating. The left prescapular lymph node was still grossly enlarged. The swelling had increased to 70 mm. in diameter on the 53rd day after inoculation and the ulcer was then 30 mm. in

diameter. The left prescapular lymph node had further increased in size and now measured approximately 130 x 250 mm. Six days later the ulcer had granulated so that it was only 7 mm. in diameter. The left prescapular and the posterior cervical lymph nodes were grossly enlarged. The animal was rapidly losing condition, was coughing frequently and had rapid abdominal breathing.

Necropsy findings. (65 days after inoculation)

Holstein steer, 9 months old, poor condition, 400 lb. weight.

Generalized lesions of tuberculosis were evident, the following lymph nodes showing gross lesions: anterior and posterior mediastinal, left and right bronchials, medial retropharyngeal, posterior cervical, left prescapular, left axillary, hepatic, and the mesenterics. An ulcer 15 mm. in diameter was evident at the skin inoculation site. Subcutaneous tissue underlying it was thickened up to 20 mm. in thickness and contained numerous foci of caseous yellowish material 2-3 mm. in diameter.

Liver. Numerous white foci measuring 3-4 mm. in diameter were scattered subcapsularly and throughout the parenchyma.

Lung. Numerous miliary tubercles were scattered throughout the whole of the lungs and were most evident toward the dorsal borders of the diaphragmatic surfaces of both the diaphragmatic lobes and in the left apical lobe. The typical focus, when cut in cross section, showed a central white area surrounded by a red periphery.

Histopathologic findings. Extensive lesions of progressive tuberculosis were found in all the lymph nodes listed above and were essentially the same in all lymph nodes. The inflammatory change was characterized

by multiple foci of caseation (Figure 25) which had often become confluent. In many lymph nodes the normal architecture had been obliterated by caseous necrosis (Figure 26), and in the necrotic areas only the blood vessels and a few lymphoid cells immediately adjacent to them remained visible (Figure 27).

Skin. The surface of the lesion was covered by necrotic debris (Figure 28), but multiple granulomas with central caseation and early calcification were present in the reticular layer. The caseous areas were surrounded by granulomatous tissue in which there were numerous Langhans' giant cells (Figure 29). No acid-fast bacteria were detected.

Liver. While no macroscopically visible granulomas were present, there were numerous microscopic ones scattered throughout the section. These varied from foci of several macrophages to larger granulomas containing giant cells and with early caseation. No acid-fast bacteria were seen.

Bacteriologic findings. Acid-fast organisms were isolated from all tissues.

Calf 26 - 1 mg. M. bovis (killed) intradermally.

Clinical observations. By 14 days after inoculation a firm intradermal nodule 3 mm. in diameter had developed at the site of inoculation. Seven days later this had increased to a diameter of 10 mm., was nonfluctuant and closed. By the 28th day it had reduced to 8 mm. in diameter and remained this size until necropsy.

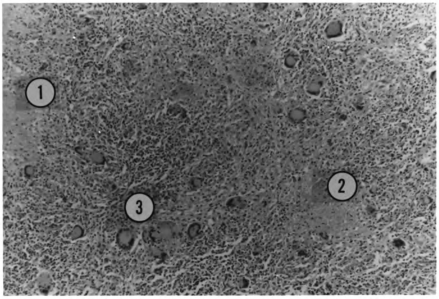

Figure 25. Anterior mediastinal lymph node from calf 20, inoculated with M. bovis. Note the areas of caseation (1, 2) and the granulomatous inflammatory tissue in which there are numerous giant cells (3). New Fuchsin - H & E. x75.

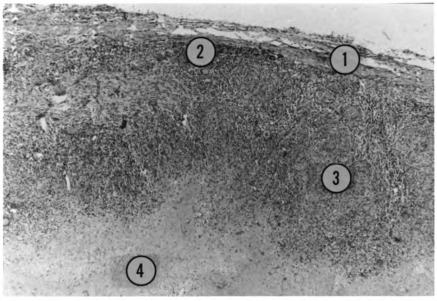

Figure 26. Left prescapular lymph node from calf 20, inoculated with M. bovis. Under the lymph node capsule (1), there remains a thin rim of lymphoid tissue (2), heavily infiltrated by granulomatous tissue (3) which surrounds the massive area of caseous necrosis (4). New Fuchsin - H & E. x50.

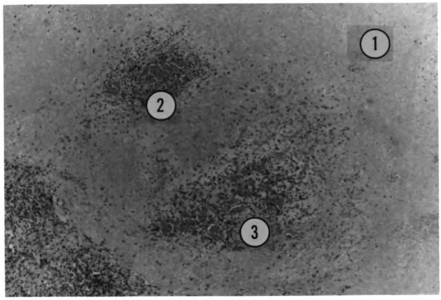

Figure 27. Left prescapular lymph node from calf 20, inoculated with M. bovis. Extensive areas of caseation (1), in which there are scattered islands (2, 3) of lymphoid tissue around blood vessels. New Fuchsin - H & E. x75.

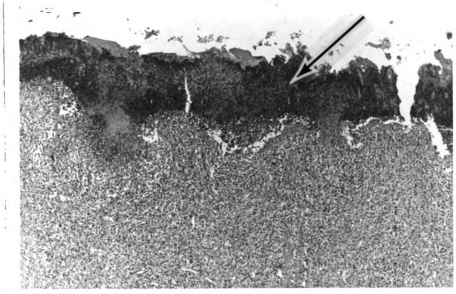

Figure 28. Skin inoculation site from calf 20, inoculated with M. bovis. The surface of the ulcer is covered by necrotic debris (arrow), and under this is granulomatous tissue which extends through the skin into the subcutis. New Fuchsin - H & E. x50.

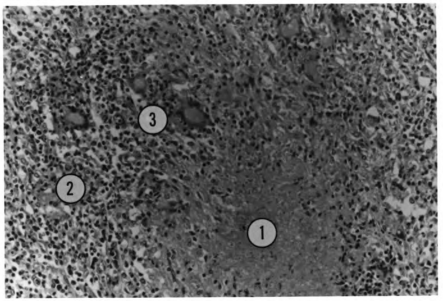

Figure 29. Skin inoculation site from calf 20, inoculated with M. bovis. Note the caseous area (1) surrounded by granulomatous tissue (2) in which there are Langhans' giant cells (3) and scattered lymphocytes. New Fuchsin - H & E. x187.

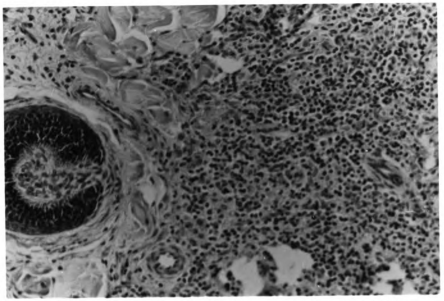

Figure 30. Skin inoculation site from calf 26, inoculated with killed M. bovis. Note the extensive infiltration of mononuclear cells in the reticular layer. New Fuchsin - H & E. x187.

Necropsy findings. (54 days after inoculation)

Holstein steer, 6 months old, good condition, 450 lb. weight.

The only lesion detected was a pale yellow focus 8 mm. in diameter lying in the dermis, seen on cross section of the skin inoculation site.

Histopathologic findings. Microscopic changes were confined to the skin inoculation site. A focal granuloma consisting of macrophages and some lymphoid cells lay in the reticular layer (Figure 30). Acid-fast bacteria, giant cells, caseation and calcification were absent.

Bacteriologic findings. No acid-fast bacteria were recovered on bacteriological examination.

TABLE II. Summary of lesions and bacteriologic isolations from calves inoculated with mycobacteria.

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.*	Isolations of Acid-fast Bacteria
1	50 B-0 Bovine III	2.2	70	Pool of the ant. & post. mediastinal & l. & r. bronchial lymph nodes.	-	R. prefemoral, r. popliteal, l. internal iliac, ant. & post. mediastinal & l. & r. bronchial pool, l. prescapular lymph node.
2	51 C-0 Bovine III	2.2	72	Head & neck pool, l. prescapular, mesenteric, r. popliteal, ant. & post. mediastinal & l. & r. bronchial lymph node pool.	+	Head & neck pool, ant. & post. mediastinal & r. & l. bronchial pool, mesenterics, r. internal iliac, l. prefemoral, r. prefemoral, r. popliteal lymph node & skin inoc. site.
3	62 D-0 Bovine III	2.2	55	R. popliteal, r. internal inguinal, l. prescapular lymph node, & skin inoc. site.	-	Ant. & post. mediastinal & r. bronchial pool, l. prescapular, r. popliteal, r. internal iliac, l. popliteal lymph node, lung, skin inoc. site.
4	68 C-0 Bovine III	2.2	82	Ant. & post. mediastinal & l. & r. bronchial lymph node pool, skin inoc. site.	-	Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular, r. popliteal, r. internal iliac, l. popliteal lymph node, skin inoc. site.
5	124 F-0 Bovine IV	2.2	37	No gross or microscopic lesions.	-	Pool of ant. & post. mediastinal & r. & l. bronchial lymph nodes.

*Progressive disease as determined by histopathologic examination.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
6	130 I-0 *M. bovis*	2.2	37	L. & r. prescapular, ant. & post. mediastinal, l. & r. bronchial, mesenteric, int. iliac, deep inguinal, r. popliteal, lung, skin inoc. site, spleen.	+	All tissues cultured.
7	71 C-0 Bovine III	1.0	71	L. prescapular lymph node, skin inoc. site.	-	Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular lymph node, skin inoc. site.
9	4 F-1 Bovine IV	1.0	69	No gross or microscopic lesions.	-	L. prescapular lymph node.
10	78 B-0 Bovine III	1.0	73	Skin inoc. site only.	-	L. prescapular lymph node, skin inoc. site.
11	52 H-I Bovine Pseudo-chrome	1.0	72	Skin inoc. site only.	-	Skin inoc. site.
12	87 H-0 Bovine IV	1.0	8	Skin inoc. site only.		Not attempted.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
13	107 E-0 Bovine III	1.0	77	Skin inoc. site only.	-	L. prescapular lymph node, skin inoc. site.
14	61 B-I Bovine Pseudo-chrome	1.0	78	No gross or microscopic lesion.	-	Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular lymph node.
15	117 B-0 Bovine IV	1.0	78	No gross or microscopic lesion.	-	None.
16	93 C-0 Swine III	1.0	86	L. prescapular lymph node & skin inoc. site.	-	Ant. & post. & r. & l. bronchial pool, l. prescapular lymph node & skin inoc. site.
17	112 B-0 Bovine Pseudo-chrome	1.0	80	No gross or microscopic lesions.	-	None.
18	7 F-1 Bovine IV	1.0	86	No gross or microscopic lesions.	-	L. prescapular lymph node.
20	81-0 Swine origin *M. bovis*	1.0	65	L. prescapular, l. axillary, ant. & post. mediastinal, l. & r. bronchial, medial retro-pharyngeal, post. cervical, hepatic, mesenterics, skin inoc. site.	+	All tissues cultured.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
21	M. avium laboratory strain	1.0	84	Skin inoc. site only.	-	L. prescapular lymph node & pool of the ant. & post. mediastinal & r. & l. bronchial lymph nodes.
22	87 H-0 Bovine IV	1.0	28	Bronchopneumonia only.	-	None.
23	172 C_1-1 Swine III	1.0	70	No gross or microscopic lesions.	-	No acid-fast bacteria were isolated.
24	173 C-1 Swine III	1.0	71	No gross or microscopic lesions.	-	Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular lymph nodes.
25	254 F-1 Bovine IV	1.0	70	Skin inoc. site only.	-	Pool of ant. mediastinal & r. & l. bronchial lymph nodes, l. prescapular lymph node.
26	81-0 M. bovis (killed)	1.0	54	Skin inoc. site only.	-	None.
27	M. avium laboratory strain (killed)	1.0	56	Skin inoc. site only.	-	None.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
28	117 B-0 Bovine IV (killed)	1.0	56	No gross or microscopic lesions.	-	None.
29	51 C-0 Bovine III (killed)	1.0	54	Skin inoc. site only.	-	Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular lymph node, bone marrow.
30	172 C_1-1 Swine III	1.0	56	Skin inoc. site only.	-	Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular lymph node, liver-spleen pool, skin inoc. site.
31	172 C_1-1 Swine III	1.0	56	Skin inoc. site only.		Ant. & post. mediastinal & r. & l. bronchial pool, l. prescapular lymph node, liver-spleen pool, skin inoc. site.
32	193 C_2-1 Swine III	1.0	54	No gross lesions detected but microscopic granuloma at inoc. site.	-	Ant. mediastinal & r. & l. bronchial pool, l. prescapular, post. mediastinal lymph node, skin inoc. site.
33	193 C_2-1 Swine III	1.0	54	No gross or microscopic lesions.	-	Ant. mediastinal, r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, liver-spleen pool, skin inoc. site.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
34	186 C-1 Swine III	1.0	43	Pyelonephritis & skin inoc. site.	-	Ant. mediastinal & r. & l. bronchial pool, l. prescapular lymph node, liver-spleen pool, skin inoc. site.
35	186 C_1-1 Swine III	1.0	54	Skin inoc. site only.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, bone marrow, skin inoc. site.
36	186 C-1 Swine III	1.0	56	Skin inoc. site only.	-	Ant. mediastinal & r. & l. bronchial pool, l. prescapular, post. mediastinal, liver-spleen pool, skin inoc. site.
37	167 C_1-1 Swine III	1.0	56	Skin inoc. site only.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, liver-spleen pool.
38	71 C-0 Bovine III	1.0	63	No gross or microscopic lesion.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, lung, liver-spleen pool, skin inoc. site.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
39	71 C-0 Bovine III Reisolant ex** Calf 7	1.0	67	Skin inoc. site only.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, skin inoc. site.
40	107 E-0 Bovine III Reisolant ex Calf 13	1.0	70	No gross or microscopic lesions.	-	L. prescapular lymph node.
42	51 C-0 Bovine III	1.0	66	L. prescapular lymph node & skin inoc. site.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, lung, skin inoc. site.
43	51 C-0 Bovine III Reisolant ex Calf 2	1.0	68	Ant. & post. mediastinal, l. & r. bronchial, l. prescapular, hepatics, mesenteric, l. & r. medial retropharyngeal, colics, l. & r. deep inguinal lymph nodes, liver, & skin inoc. site.	+	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, hepatics, l. prescapular lymph node, lung, liver-spleen pool, skin inoc. site.

**ex - from.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
44	62 D-0 Bovine III Reisolant ex Calf 3	1.0	67	Ant. & post. mediastinal, l. & r. bronchial, l. prescapular lymph nodes & skin inoc. site.	±	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph nodes, liver-spleen pool, bone marrow, skin inoc. site.
45	68 C-0 Bovine III Reisolant ex Calf 4	1.0	65	L. & r. bronchial, ant. & post. mediastinal, l. axillary, mesenterics, hepatic, l. prescapular, colic lymph nodes, liver, lung & skin inoc. site.	+	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, hepatics, l. prescapular lymph nodes, lung, liver-spleen pool, bone marrow, skin inoc. site.
46	Feed origin III	1.0	65	No gross or microscopic lesions.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node.
47	Feed origin III	10.0	64	Skin inoc. site only.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node, liver-spleen pool, bone marrow, skin inoc. site.
48	X31 Soil origin III	1.0		No gross or microscopic lesions.	-	Ant. mediastinal & r. & l. bronchial pool, l. prescapular lymph node.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
49	X41 Soil origin III	10.0	73	No gross or microscopic lesions.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, l. prescapular lymph node.
50	X41 Soil origin III	1.0	73	No gross or microscopic lesions.	-	Post. mediastinal lymph node, lung.
51	7 F-1 Bovine IV	10.0	52	Bronchitis & atelectasis of the r. apical & cardiac lobes; tuberculoid lesions in l. prescapular, r. axillary, internal iliacs, r. ischiatic & skin inoc. site.	-	Ant. mediastinal & r. & l. bronchial pool, post. mediastinal, internal iliac, l. prescapular lymph node, liver-spleen pool, skin inoc. site.
52	117 B-0 Bovine IV	10.0	52	Subacute bronchiolitis	-	Post. mediastinal lymph node.
53	254 F-1 Bovine IV	10.0	57	Atelectasis of r. apical & cardiac lobes; tracheitis & bronchiolitis.	-	Liver & spleen, lung, ant. mediastinal & r. and l. bronchial pool, the post. mediastinal and the l. prescapular lymph nodes.
54	254 F-1 Bovine IV Reisolant ex Calf 25	10.0	53	Skin inoc. site. Atelectasis of r. apical & cardiac lobes; tracheitis & bronchiolitis.	-	Liver & spleen, lung, ant. mediastinal & r. & l. bronchial pool, the post. mediastinal and the l. prescapular lymph nodes.

TABLE II--Continued

Calf No.	Inoculum No., Origin, Group	Inoculum mg.	Killed Days After Inoc.	Location of Lesions	Prog. Dis.	Isolations of Acid-fast Bacteria
55	61 B-1 Bovine Pseudo-chrome	10.0	57	Skin inoc. site & bronchitis.	-	Ant. mediastinal & r. & l. bronchial pool; post. mediastinal lymph node.
56	128 F-0 Bovine Pseudo-chrome	10.0	56	Bronchitis only.	-	Post. mediastinal lymph node, liver-spleen pool.
57	52 H-1 Bovine Pseudo-chrome	10.0	58	Skin inoc. site, bronchitis & atelectasis of r. cardiac & intermediate lobes of lung.	-	Post. mediastinal lymph node.
58	52 H-1 Bovine Pseudo-chrome Reisolant ex Calf 11	10.0	57	Bronchitis only.	-	L. prescapular lymph node, post. mediastinal lymph node.

TABLE III. Tuberculin test results of calves infected with mycobacteria*

Animal No.	Cervical												Caudal Fold			
	Anterior 0.1 ml. Avian Tuberculin				Middle 0.1 ml. Mam. Tuberculin				Posterior 0.2 ml. Johnin				0.1 ml. Mam. Tuberculin			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
7	4.5	8	20	21	4	10	9.5	10	4	13	23	25	5	5	10	11
		3.5	15.5	16.5		6	5.5	6		9	19	21		0	5	6
9	5	10	13	12.5	5	8.5	9.5	9	5	8.5	11	12	4	4	10	11
		5	8	7.5		3.5	4.5	4		3.5	6	7		0	6	7
10	5.5	11	14	15	4.5	9.5	11	11	3.5	6.5	9	10	5	6	8	8
		5.5	8.5	9.5		5	6.5	6.5		3	5.5	6.5		1	3	3
11	8	8	13	15	4	6	4	5.5	3.5	8	8	9.5	5	6.5	5	6
		0	5	7		2	0	1.5		4.5	4.5	6		1.5	0	1
12	Animal died suddenly 8 days after inoculation, not tuberculin-tested															
13	4.5	6	5	6.5	4	5.25	4	4	3	6	3	3	4	4	4	4
		1.5	0.5	2.0		1.25	0	0		3	0	0		0	0	0
14	6	11.5	13	13	4.5	4.5	4.5	4.5	3.5	3.5	3.5	3.5	6	6	6	6
		5.5	7	7		0	0	0		0	0	0		0	0	0
15	9	9	9	9	6	6	6	6	5	5	5	5	4	4	4	4
		0	0	0		0	0	0		0	0	0		0	0	0
16	5	14.5	21	20	4	8.5	9	8	3.5	9.5	14	10	3.5	4	4	4
		9.5	16	15		4.5	5	4		6	10.5	6.5		0.5	0.5	0.5

*Upper line for each animal indicates skin thickness in mm.; lower line for each animal indicates the increase in skin thickness in mm.

TABLE III--Continued

Animal No.	Cervical												Caudal Fold			
	Anterior 0.1 ml. Avian Tuberculin				Middle 0.1 ml. Mam. Tuberculin				Posterior 0.2 ml. Johnin				0.1 ml. Mam. Tuberculin			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
17	6	8.5	7.5	7.5	4.5	6.5	7	7	3.5	6.5	6.5	6	4	3.5	4.5	4
		2.5	1.5	1.5		2.0	2.5	2.5		3	3	2.5		0.5	0.5	0
18	5	13	18.5	14.5	4	10	9	7	3.5	10	11	10	4	4	4.5	4
		8	13.5	9.5		6	5	3		6.5	7.5	6.5		0	0.5	0
20	Not tested due to impending death															
21**		2.5x	2.9x	3.3x		2x	3.7x	1x		3.1x	4x	3.4x		1x	1.2x	1.3x
22	Animal died unexpectedly, 28 days after inoculation, not tested															
23	5	6	5	6	4	7	7	5.5	4	5.5	5	4	2	4	3	2
		1	0	1		3	3	1.5		1.5	1	0		2	1	0
24	4	8.5	10	10	5	11	9	9	4	6	7	7	5	5	5	5
		4.5	6	6		6	4	4		2	3	3		0	0	0
25	4	12	16	23	4	7	6	9	4	10	15	16	3	4	3.5	4
		8	12	19		3	2	5		6	11	12		1	0.5	1
26	10	15	14	12	10	13	14	14	8	13	11	10	5	7	7	6
		5	4	2		8	4	4		5	3	2		2	2	1
27	8.5	11	12	12	8	12	12	11.5	6.5	11	17	13.5	4	7	6.5	9
		2.5	3.5	3.5		4	4	3.5		4.5	10.5	7		3	2.5	5

**Only figures for times increase on skin thickness available.

TABLE III--Continued

Animal No.	Cervical												Caudal Fold			
	Anterior 0.1 ml. Avian Tuberculin				Middle 0.1 ml. Mam. Tuberculin				Posterior 0.2 ml. Johnin				0.1 ml. Mam. Tuberculin			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
28	10	11	10	10	6	7.5	7	8	4.5	8	6	7	4.5	5.5	4.5	6
		1	0	0		1.5	1	2		3.5	1.5	2.5		1	1	1.5
29	10	11.5	11	11	8	10	10	9.5	6	8	9	8	5.5	6	5	5
		1.5	1	1		2	2	1.5		2	3	2		0.5	-0.5	-0.5
30	6	15	22	22	6	11	16.5	14	5	10	17	16	6	6	7	9
		9	16	16		5	10.5	8		5	12	11		0	1	3
31	9	16	17	18	9	11.5	14	9	7	14	17	13	5	6	7	7
		7	8	9		2.5	5	0		7	10	6		1	2	2
32	9.5	13	20	14.5	7	11	12	9	8	10	12	13	5.5	6	6	7
		3.5	10.5	5		4	5	2		2	4	5		0.5	0.5	1.5
33	5	12	15	18.5	3.5	9.5	8	6	3.5	9	10	7	3.5	5	6	6
		7	10	13.5		6	4.5	2.5		5.5	6.5	3.5		1.5	2.5	2.5
34	Not tested because of unexpected death															
35	9.5	14.5	20	21	7	10.5	10	10	7	9.5	14.5	13	5	7	9	9
		5	10.5	11.5		3.5	3	3		2.5	7.5	6		2	4	4
36	7.5	13	12.5	15	7	13	10	11	5.5	12.5	14.5	11.5	4	8	9	9
		5.5	5	7.5		6	3	4		7	9	6		4	5	5
37	6	17.5	29	27.5	5	12	15	14	4	11	15	13	3	9	9	9
		11.5	23	21.5		7	10	9		7	11	9		6	6	6

TABLE III--Continued

Animal No.	Cervical												Caudal Fold			
	Anterior 0.1 ml. Avian Tuberculin				Middle 0.1 ml. Mam. Tuberculin				Posterior 0.2 ml. Johnin				0.1 ml. Mam. Tuberculin			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
38	6	11	11	9.5	4	9	9	7	3.5	7	9	7	5	5	8	6
		5	5	3.5		5	5	3		3.5	5.5	3.5		0	3	1
39	7	13	13	12	6	12	10	9	4.5	10	10	9	4	7	5	6
		6	6	5		6	4	3		5.5	5.5	4.5		3	1	2
40	10	14	13.5	13.5	9	14	10	11	7	14	12	12	6	8	6	6.5
		4	3.5	3.5		5	1	2		7	5	5		2	0	0.5
42	8	15	20	20	7	11	10	9	6	10	14	14	4.5	6	6	6
		7	12	12		4	3	2		4	8	8		1.5	1.5	1.5
43	10	17	20.5	19	7	22	34	31	6	8.5	13	13	4	10	19	19
		7	10.5	9		15	27	24		2.5	7	7		6	15	15
44	10	17	25	19	9	21	31	35	8	11.5	18	17	4	9	17	32
		17	15	9		12	22	26		3.5	10	9		5	13	28
45	10	15	20	23	7	23	35	37	6	15	19	17	4	12	20	27
		5	10	13		16	28	30		9	13	11		8	16	23
46	7	17	21.5	21	6.5	14.5	14	12	6	14	16	14	6	10	9	7
		10	14.5	14		8	7.5	5.5		8	10	8		4	3	1
47	7	15	20	18	6.5	12	12	10	6	11	15	12.5	6	8.5	8	7.5
		8	13	11		5.5	5.5	3.5		5	9	6.5		2.5	2	1.5
48	12	18	17	17	12	13	12.5	13	9	13	13	15	6	7	7	7
		6	5	5		1	0.5	1		4	4	6		1	1	1

TABLE III--Continued

Animal No.	Cervical												Caudal Fold			
	Anterior 0.1 ml. Avian Tuberculin				Middle 0.1 ml. Mam. Tuberculin				Posterior 0.2 ml. Johnin				0.1 ml. Mam. Tuberculin			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
49	7	11	11	11	6	10	9	8	5	10	11	10	3	5	4.5	4.5
		4	4	4		4	3	2		5	6	5		2	1.5	1.5
50	7	13.5	17.5	16	6	15	16	12	4	11	13	13	4	7	7	7
		6.5	10.5	9		9	10	6		7	9	9		3	3	3
51	6	19	22.5	23	6	13.5	13.5	11.5	4.5	12	18	16.5	3	7	9	6.5
		13	16.5	7		7.5	7.5	5.5		7.5	13.5	12		4	6	3.5
52	8.5	15	11	11	6	10	9	8.5	5.5	8	9	8.5	4	10	6	6
		6.5	2.5	2.5		4	3	2.5		2.5	3.5	3		6	2	2
53	10	19	17	16	9	15	12.5	11	7	13	12.5	11.5	8	17	13	10.5
		9	7	6		6	3.5	2		6	5.5	4.5		9	5	2.5
54	11	22.5	22	19	9	13	12.5	10	7	13	12.5	13	7	9	13	7
		11.5	11	8		4	3.5	1		6	5.5	6		2	5	0
55	10	19	20	21.5	8.5	18	17	17	8.5	15.5	15	16	7	13	13	13
		9	10	11.5		9.5	8.5	8.5		7	6.5	7.5		6	6	6
56	7	12	11	10	6	13.5	11	9	4	9.5	8	7.5	5.5	7	5.5	5.5
		5	4	3		7.5	5	3		5.5	4	3.5		1.5	0	0
57	5.5	7	8.5	10	4	6	7	7	3.5	6	7	7.5	6	5.5	6	6
		1.5	3	4.5		2	3	3		2.5	3.5	4		0	0	0
58	7	11	13	11	5	10.5	10	8	4	7	7	6.5	6	9	7	6
		4	6	4		5.5	5	3		3	3	2.5		3	1	0

TABLE IV. Results of hematologic examination of blood samples taken immediately prior to necropsy.

Calf No.	Hemoglobin (Gm./100 ml.)	Hematocrit	Leukocytes per mm^3
26	16.4	46	6,500
27	12.2	not done	not done
28	11.7	not done	not done
29	20.2	59	8,000
30	17.3	52	11,500
31	16.7	57	6,150
32	19.6	50	9,200
33	19.3	62	5,100
34	16.7	49	5,750
35	17.3	50	8,500
36	17.2	55	6,900
37	15.3	49	8,450
38	15.3	49	10,600
39	16.3	48	8,500
40	17.6	52	8,350
41	9.7	30	10,200
42	15.3	46	10,500
44	17.9	52	12,650
45	17.6	55	11,750
46	16.3	52	11,100
47	19.6	56	5,550

TABLE IV--Continued

Calf No.	Hemoglobin (Gm./100 ml.)	Hematocrit	Leukocytes per mm^3
48	17.9	53	7,400
49	17.0	48	8,000
50	18.3	50	11,200
51	14.3	45	16,000
52	12.7	38	7,800
54	14.3	41	8,300

TABLE V. Summary of the development of skin lesions at the inoculation site in calves inoculated with Group III mycobacteria of bovine origin.

Culture No.	Calf No.	Skin Ulcer	Days after inoc. ulcer appeared	Max. Dimensions	Granuloma at inoc. site	Lesions
51C-0	2	N/A*	...	...	yes	progressive generalized
	(killed)29	no	...	...	yes	i/d only**
	42	yes	33	7 mm.	yes	primary complex
	43	yes	24	15 mm.	yes	generalized
68C-0	4	N/A	...	...	yes	generalized
	45	yes	32	10 mm.	yes	generalized
50B-0	1	N/A	...	...	N/A	generalized
62D-0	3	N/A	...	...	yes	primary complex
	44	yes	23	10 mm.	yes	generalized
107E-0	13	no	...	...	yes	i/d only
	40	no	...	...	no	NML***
71C-0	7	yes	14	25 mm.	yes	primary complex
	38	yes	15	10 mm.	no	NML
	39	no	...	...	yes	i/d only
78B-0	10	no	...	...	yes	i/d only

*N/A - data not available
**NML - no microscopic lesions
***i/d - intradermal lesion

TABLE VI. Summary of the development of skin lesions at the inoculation site in calves inoculated with Group III mycobacteria of swine origin.

Culture No.	Calf No.	Skin Ulcer	Days after inoc. ulcer appeared	Max. Dimensions	Granuloma at inoc. site	Lesions
93C-0	16	no	...	...	yes	primary complex
$176C_1$-1	23	no	...	...	no	NML*
	30	yes	21	5 mm.	yes	i/d only**
	31	yes	21	5 mm.	yes	i/d only
173C-1	24	no	...	...	no	NML
$193C_2$-1	32	yes	21	5 mm.	yes	i/d
	33	yes	21	5 mm.	no	NML
186C-1	34	no	...	...	yes	i/d
	36	no	...	...	yes	i/d
$167C_1$-1	35	yes	14	5 mm.	yes	i/d
	37	yes	21	3 mm.	yes	i/d

*NML - no microscopic lesions
**i/d - intradermal lesion

TABLE VII. Summary of the development of skin lesions at the inoculation site in calves inoculated with Group III mycobacteria of feed and soil origin.

Culture No.	Calf No.	Skin Ulcer	Days after inoc. ulcer appeared	Max. Dimensions	Granuloma at inoc. site	Lesions
Feed origin	46	no	...	...	no	NML*
	47	yes	22	5 mm.	no	i/d**
Soil origin	48	no	...	...	no	NML
	49	no	...	...	no	NML
	50	no	...	...	no	NML

*NML - no microscopic lesions
**i/d - intradermal lesion

TABLE VIII. Summary of the development of skin lesions at the inoculation site in calves inoculated with pseudochromes.

Culture No.	Calf No.	Skin Ulcer	Days after inoc. ulcer appeared	Max. Dimensions	Granuloma at inoc. site	Lesions
52H-1	11	no	...	...	no	NML*
	57	no	...	...	yes	NML
	58	no	...	...	no	NML
61B-0	14	no	...	...	no	NML
	55	no	...	...	yes	i/d**
112B-0	17	no	...	...	no	NML
128F-0	56	no	...	...	no	NML

*NML - no microscopic lesions
**i/d - intradermal lesion

TABLE IX. Summary of the development of skin lesions at the inoculation site in calves inoculated with Group IV mycobacteria.

Culture No.	Calf No.	Skin Ulcer	Days after inoc. ulcer appeared	Max. Dimensions	Granuloma at inoc. site	Lesions
124F-0	5	N/A*	...	...	no	NML**
2F-1	9	no	...	...	no	NML
87H-0	12	no	...	...	yes	i/d***
	22	no	...	...	no	NML
117B-0	15	no	...	...	no	NML
	28	no	...	...	no	NML
	52	no	...	...	no	NML
7F-1	18	no	...	...	no	NML
	51	no	...	...	yes	primary complex
254F-1	25	yes	16	3 mm.	yes	i/d
	53	no	...	...	no	NML
	54	no	...	...	yes	i/d

*N/A - data not available
**NML - no microscopic lesions
***i/d - intradermal lesion

TABLE X. Summary of the development of skin lesions at the inoculation site in calves inoculated with *M. bovis* and *M. avium*

Culture Name	Calf No.	Skin Ulcer	Days after inoc. ulcer appeared	Max. Dimensions	Granuloma at inoc. site	Lesions
M. bovis	6	yes	N/A*	20 mm.	yes	generalized
M. bovis	20	yes	14	30 mm.	yes	generalized
(killed)	26	no	...	...	yes	i/d**
M. avium	21	no	...	...	yes	i/d
	27	no	...	...	yes	i/d

*N/A - data not available
**i/d - intradermal lesion

TABLE XI. Correlation of caudal fold tuberculin test results with lesions.

Caudal Fold Response	Lesions	Group III: Bov. origin +	Bov. origin -	Porc. origin +	Porc. origin -	Feed or soil origin +	Feed or soil origin -	Group IV: Pseudochr. +	Pseudochr. -	Totals
Response at 72 hours										
> 4 mm.		4	0	0	3	0	0	0	1	9
2-4 mm.		1	0	0	5	0	5	0	0	14
none		0	5	1	1	0	0	0	6	17
	Totals	10		10		5		7		40

TABLE XII. Correlation of comparative cervical tuberculin test results with lesions.

Caudal Fold Response	Lesions	Bov. origin +	Bov. origin -	Porc. origin +	Porc. origin -	Feed or soil origin +	Feed or soil origin -	Group IV: Pseudochr. +	Pseudochr. -	Totals
Response at 48 hours										
Positive*		3	5	0	0	0	0	0	0	8
		2	0	1	9	0	5	0	7	32
	Totals	10		10		5		7		40

*Increase in skin thickness at the site of mammalian tuberculin injection which was at least 5 mm. greater than that at the site of avian tuberculin injection.

TABLE XIII. Summary of results of tuberculin tests on calves injected with killed mycobacteria

Calf No.	Organism	Caudal Fold Test Increase in mm. at 48 hours	Max. Response on Cervical Test
26	*M. bovis*	2	Mammalian and avian equal at 48 hours
27	*M. avium*	5	Johnin at 48 hours
28	117B-0 Group IV	1.5	Johnin at 24 hours
29	51C-0 Group III	0	Johnin at 48 hours

V. DISCUSSION

Evaluation of Pathogenicity of Atypical Mycobacteria

The data on animals inoculated with Group III mycobacteria are presented in TABLE II.

Group III Mycobacteria of Bovine Origin

Analysis of the results shows that Group III mycobacteria of bovine origin produced the whole range of lesions from small, barely visible granulomatous foci at the skin inoculation site to generalized progressive lesions. Seven cultures, or their reisolants, were inoculated into 14 calves - Nos. 1, 2, 3, 4, 7, 10, 13, 29, 38, 39, 40, and 42 through 50. Six calves (Nos. 1, 2, 4, 43, 44, 45) had generalized disease, three (Nos. 3, 7, 42) had lesions in the lymph nodes draining the inoculation sites, three had lesions at the inoculation site and two had no gross or microscopic lesions. Calf 29 was injected with a killed culture. Even though generalized disease was produced in 6 calves, the lesions were not as progressive as those seen in calves 6 and 20, inoculated with M. bovis. Only three of the six (Nos. 2, 3, and 45) were classed as progressive; Nos. 43 and 44 were classified as doubtfully progressive and No. 1 was called nonprogressive. Comparison of the lesions due to M. bovis and those due to Group III mycobacteria shows that the disease caused by Group III organisms tended to be self-limiting.* Calcification and fibrous encapsulation were minimal in the M. bovis infected animals but were prominent in the Group III infected calves. No Group III infected animals died; both M. bovis calves were killed in extremis. The histological

*However, subsequent unpublished data indicate that Group III mycobacteria of bovine origin can kill calves.

indications are that in many of the Group III infected calves the lesions may have become nonprogressive with the chance of sterilization later. Even those animals with progressive lesions, such as calf 45, had many encapsulated caseo-calcareous lesions which had already become nonprogressive.

It is interesting to compare the pathological changes caused by the one culture when inoculated into more than one calf. Thus, when 2.2 mg. of Culture 51C-0 was inoculated into calf 2, generalized disease was produced, but some months later when 1 mg. of an inoculum from the same culture, which had been stored in a refrigerator, was injected into calf 42, only the regional lymph node was affected. The reisolant from calf 2 produced generalized disease when 1 mg. was injected into calf 43. Culture 62D-0 produced lesions only in the regional lymph nodes when 2.2 mg. were injected intradermally into calf 3. However, when a reisolant of this organism from calf 3 was injected at the dosage of 1 mg. intradermally into calf 44, generalized disease resulted. Culture 68C-0 produced generalized disease when 2.2 mg. were injected intradermally into calf 4 and 1 mg. of a reisolant of that organism produced generalized disease also in calf 45. Culture 107E-0 produced only an intradermal lesion at the site of inoculation in calf 13 but the reisolant from this calf produced no microscopic lesions in calf 40. When 71C-0 was injected into calf 7, it produced a primary complex. However, later, when the same organism was injected into calf 38, no microscopic lesions were seen. At the same time, a reisolant from calf 7 produced only a granuloma at the site of injection in calf 39. Thus, only two (107E-0 and 78B-0) of the 7 Group III cultures of bovine origin never produced significant lesions in calves. Thus, the Group III mycobacteria of bovine origin ranged from nonpathogenic to a pathogenicity approaching that of M. bovis.

Group III Mycobacteria of Swine Origin

Seven of these cultures or their reisolants were injected into 11 calves (Nos. 16, 23, 24, 30, 31, 32, 33, 34, 35, 36, and 37). Only one culture, 93C-0, which was injected into only one calf (No. 16), produced any significant lesions. These were a caseo-calcareous granuloma 3 mm. in diameter in the left prescapular lymph node and a 35 x 15 x 10 mm. abscess in the dermis at the site of injection.

Culture 172C_1-1 produced no microscopic lesions in calf 23 and only lesions at the intradermal inoculation sites in calves 30 and 31. Culture 193C_2-1 produced an intradermal inoculation site lesion only in calf 32, but in calf 33 no microscopic lesion was detected. Culture 186C-1 produced only intradermal inoculation site lesions in calves 34 and 36. Culture 167C_1-1 produced only an intradermal inoculation site lesion in calf 35 and no gross or microscopic lesions in calf 37.

Thus, in this series, the Group III mycobacteria of swine origin had little pathogenicity for calves.

Group III Mycobacteria of Feed and Soil Origin

Of the five calves (Nos. 46 through 50) inoculated with 3 cultures of these organisms, only one (No. 47) had any lesions. This was a small granuloma at the site of injection and was the response to an inoculum of 10 mg. (approximately 10^9 to 10^{10} organisms). Thus, the Group III mycobacteria of feed and soil origin used had no significant pathogenicity for calves.

Pseudochrome Mycobacteria

After the inoculation of calves 11, 14, and 17 had produced no evidence of pathogenicity, it was decided to inject four calves, Nos.

55-58, with larger doses. Two milligrams each were injected intradermally, subcutaneously, intramuscularly, and intraperitoneally, and 2 mg. were fed in a bolus of the ration. All these calves were stressed accidentally by exposure to an aerosol of ET-383, an orthophenyl phenol which is very irritant to the respiratory tract. All showed symptoms of bronchitis and all had a chronic bronchitis at necropsy. Nevertheless, calves 55-58 showed no evidence that these pseudochrome mycobacteria were pathogenic for them.

Group IV Mycobacteria

Because of the lack of pathogenicity, as in the case of the pseudochromes, four calves (Nos. 51-54) received 10 mg. of culture, and these calves were also unintentionally exposed to the ET-383 aerosol.

Eleven calves were inoculated with live Group IV mycobacteria and one (No. 28) was inoculated with killed Culture 117B-0. Only one calf (No. 51) showed any significant lesions, and these consisted of mostly nonprogressive encapsulated lesions in the regional lymph nodes. Calf 51 was considerably debilitated due to the inhalation of an aerosol of ET-383 and this had no doubt reduced his resistance to infection. Thus, it would appear that some Group IV mycobacteria can provoke primary complexes in calves, especially if the dosage is high and the animal is debilitated. However, even under such adverse conditions as these, calf 51 was able to render the infection generally nonprogressive and may have been able in time to have eliminated it completely. This ability of a Group IV mycobacterium to form caseo-calcareous granulomas is interesting in the light of the statement by Corpe, Runyon and Lester(1963) that "present evidence suggests that the Group IV mycobacteria do not produce the characteristic features of caseating granulomatous disease."

Histopathologic Differentiation

Histologically, it was not possible to differentiate between lesions produced by M. bovis and those by atypical mycobacteria. In this series the lesions due to the atypicals were mostly nonprogressive, and calcification, caseation and encapsulation were frequently seen. These features cannot be used to differentiate infection due to atypical mycobacteria from M. bovis infection - as similar changes may be seen in cattle infected naturally with M. bovis.

Acid-fast bacilli were not always detected by the new fuchsin-hematoxylin and eosin stain, even in advanced lesions such as those in calf 45. However, Braunstein and Adriano (1961) have shown that fluorescence microscopy can detect many more acid-fast bacilli in lesions than the standard Ziehl-Neelsen method. Also, Hanks (1956) found that unless special precautions are taken, caseous material which frequently contains the mycobacteria can be lost from the section during processing, either during the preparation of the block or the staining of the section.

Relationship Between Ulceration at the Skin Inoculation Site and Extent of Lesions

These data are summarized in TABLES V through X.

Group III Mycobacteria of Bovine Origin

At necropsy, twelve of fourteen calves had granulomas at the skin inoculation site and the remaining two had no observed lesions. Four of the twelve calves had no lesions other than at the skin inoculation site. The mere presence of a granuloma at the site of injection is not necessarily an indication of pathogenicity, since it is recognized that many foreign substances may stimulate a localized granulomatous response. However, ulceration at the injection site is a far better indication of

pathogenicity. For those calves on which data are available, of the six which had ulcers, three developed generalized disease, two had primary complexes and in one (calf 38) the skin ulcer healed completely.

Group III Mycobacteria of Swine Origin

Six of the eleven calves developed ulcers, but none developed even a primary complex. Calf 16, which did have a primary complex, did not develop an ulcer.

Group III Mycobacteria of Feed and Soil Origin

One (calf 47) of these five calves developed an ulcer although none of them had primary complexes. However, calf 47 had been injected with 10 mg. of inoculum.

Pseudochrome Mycobacteria

None of the seven calves developed skin ulcers or primary complexes.

Group IV Mycobacteria

Only one calf (No. 25) of the twelve developed an ulcer, but this had no primary complex. Calf 51, which did develop a primary complex from the intradermal injection, never had a skin ulcer. These results indicate that ulceration at the skin inoculation site was a good indication of pathogenicity in the case of the Group III mycobacteria of bovine origin, feed and soil origin, pseudochrome mycobacteria and Group IV mycobacteria. However, ulceration due to swine origin Group III mycobacteria did not correlate well with the pathogenicity. Six of these eleven calves had ulcers, but none of these had significant lesions; and the one calf with lesions (No. 16) did not have an ulcer at the inoculation site.

The first six calves were inoculated with 0.1 mg. and 1.0 mg., wet weight, of culture in separate locations, and both these doses were able

to induce ulcers if the mycobacterium was pathogenic. Most of the subsequent cattle were injected with 1.0 mg. except where low or no pathogenicity was expected, and then the dose was sometimes increased to 10 mg. It must be emphasized that this is a small dose compared to those previously reported in the literature. Francis (1958) records the use of 50 mg. M. bovis subcutaneously, 4 mg. M. bovis intravenously, 50 mg. M. tuberculosis subcutaneously, 10-500 mg. M. avium subcutaneously, 10-200 mg. M. avium intravenously and 1 gm. M. avium perorally.

The fate of organisms injected intradermally and the subsequent development of lesions in other organs is not simple. The lymphatic drainage from the hind leg is via the popliteal lymph node to the superficial inguinal lymph nodes and thence to the deep inguinals and internal iliacs, up the lumbar chain and finally into the thoracic duct. However, only one lymph node, the left prescapular, stands between the inoculation site on the lateral aspect of the left foreleg and the general circulation. After passing the left prescapular, acid-fast bacteria would be carried into the cervical duct, into the anterior vena cava and then immediately distributed to the lungs and the general circulation. It would seem, then, that inoculation into the skin of the foreleg would give the organisms a better chance to cause a generalized infection. Intradermal inoculation also allows the organisms to lie in the animal body without the danger of immediate complete phagocytosis; thus, these organisms would be able to sensitize the animal, as has been postulated by Rich (1951). He believes that the necrosis and inflammation which accompany a tuberculous infection are caused by the body's becoming sensitized to the tubercule-protein. However, time is required to do this, and if the organism is injected by routes other than the intradermal, they may be rapidly phagocytized and

broken down before sensitization can take place. This is particularly likely to occur if they are in small numbers and/or of low virulence.

Soltys and Jennings (1950) showed that after the subcutaneous injection of M. tuberculosis in guinea pigs in doses of 0.3 mg. and 1.0 mg., bacilli reach the lymph nodes within an hour. In those guinea pigs receiving the smaller dose, the spleen was not positive for tubercle bacilli for 48-96 hours. However, tubercle bacilli were recovered from the spleens of the guinea pigs receiving the larger dose within an hour of injection. Bacilli were consistently in the blood only in the first few hours. No similar data for intracutaneous injection have been found.

TABLE II shows that acid-fast bacilli were frequently recovered from organs and lymph nodes in which no lesions were detected either grossly or microscopically. It is possible that microscopic lesions were present in some of these lymph nodes, but doubtless a bacteremia was present in some animals. Horowitz and Gorelick (1951) recommend the bone marrow for the isolation and detection of tubercle bacilli in a bacillemia, but this did not give good results in our cases.

Evaluation of Tuberculin Sensitivity in Relation to Lesions Produced

The Group IV, Pseudochromes, Swine Origin Group III and Feed and Soil Group III mycobacteria produced either no lesions or nonprogressive lesions which could resolve in many cases. It would seem desirable, therefore, to analyze the tuberculin test results to determine if any of these animals would have been removed by the caudal fold test, and whether the comparative cervical test would have helped to differentiate between those animals with progressive disease and those with either no lesions or only localized ones. These data are summarized in TABLES XI and XII.

Interpretation of the tests was based on the 72 hour reading at the caudal fold site. The comparative cervical test results were computed from the 48 hour reading. An increase in skin thickness at the site of the mammalian injection which was greater than the increase at the avian site by 5 mm. was called a positive.

Group III Mycobacteria of Bovine Origin

Group III mycobacteria of bovine origin were by far the most pathogenic for cattle of the atypical mycobacteria. Of the ten animals infected with these organisms on which comparative cervical tuberculin tests were conducted, there were three generalized cases (Nos. 43, 44, 45), two cases (Nos. 7, 42) with lesions in the regional lymph nodes, three cases (Nos. 10, 13, 39) with lesions at the skin inoculation site only and two cases (Nos. 38, 40) with no gross or microscopic lesions.

All three generalized cases gave positive caudal fold tests (the minimum increase being 15 mm.) and positive comparative cervical tests. Calves 7 and 42 with only primary complexes gave different responses. Calf 7 had a 6 mm. caudal fold increase at 72 hours, but at 48 hours the avian increase was 10 mm. greater than that at the mammalian site. Thus, the two tests gave conflicting results, viz., a positive caudal fold and a negative comparative cervical test. Calf 42 had only a 1.5 mm. increase at the caudal fold site at 72 hours. The avian response was 9 mm. greater than the mammalian at 48 hours in the cervical test and thus negative. The remaining five calves all had negative caudal fold and cervical tests, although two of them (Nos. 10 and 39) had 3 mm. and 2 mm. increases, respectively, in the caudal folds.

In no case would the use of the comparative cervical test have removed nonprogressive cases from the herd. The caudal fold test would

have undoubtedly caused the removal of calf 7. If it is felt that the tuberculin test should remove all cattle with lesions, then the comparative cervical test failed to do this in two cases and the caudal fold test chose one of these and left the other.

Group III Mycobacteria of Swine Origin

Only one animal (No. 16) had lesions, which consisted of an abscess in the skin at the inoculation site and a granuloma in the left prescapular lymph node. The caudal fold test of No. 16 was negative, and the cervical test showed a maximal sensitivity to avian tuberculin. Thus, if the comparative cervical test were applied with the mammalian response exceeding the avian by at least 5 mm. before the animal was called positive, this animal would have been declared negative. On pathological grounds, it is possible that the infection could have been overcome and even eliminated. As isolations of mycobacteria were made only from the left prescapular lymph node and the skin inoculation site, this would tend to support the theory of only localized infection.

Of the remaining nine animals tested, only three gave caudal fold test increases greater than 3 mm. 72 hours after injection. However, on the comparative cervical test, nine calves had maximal reaction to avian tuberculin, and only one (No. 23) had a maximal response to mammalian tuberculin. This calf 23 had no gross or microscopic lesions and, as the mammalian tuberculin response was only 2 mm. greater than that of avian tuberculin, it would have been declared negative by the comparative test and by the criteria set up.

Thus, in this group of calves, the caudal fold test would have resulted in the condemnation of three animals without lesions and failed to identify one (No. 16) with localized lesions. The comparative cervical test would not have designated any animals as positive.

Group III Mycobacteria of Feed and Soil Origin

None of the five animals infected with Group III mycobacteria of feed or soil origin had lesions and none was positive to the caudal fold test. The largest increase was 3 mm. in the case of calf 50. In all cases the comparative cervical test showed greater sensitivity to avian tuberculin, and thus, all calves were considered negative.

Pseudochrome Mycobacteria

None of these seven calves had significant lesions, but one (calf 55) was positive to the caudal fold test with a 6 mm. increase. However, it and all others gave negative comparative cervical tests.

Group IV Mycobacteria

Only one (No. 51) of the eleven animals had significant lesions, and these were mostly encapsulated nonprogressive lesions in the regional lymph nodes. This animal, which had received the large dose of 10 mg., had an increase in its caudal fold of 3.5 mm., but the comparative cervical test was negative. The only other significant increase in the caudal fold was in calf 9, which had a 7-mm. increase, although it had neither gross nor microscopic lesions. In all cases the comparative cervical tests were negative.

Evaluation of Tuberculin Sensitivity Produced by Killed Mycobacteria

TABLE XIII summarizes the data on the results of the tuberculin tests on calves inoculated with killed mycobacteria. All calves gave negative tests to the comparative cervical tests, but calf 26, injected with killed M. bovis, had a 5 mm. increase in the thickness of its caudal fold at 48 hours. Calves 27 and 28 had small increases in the thickness of their caudal folds, but calf 29, injected with killed Group III mycobacteria, had no response. These findings indicate that killed mycobacteria can induce sensitivity but the comparative cervical test showed a greater sensitivity to avian tuberculin rather than mammalian.

VI. SUMMARY

Fifty-five calves of various breeds and both sexes, and between six and ten months of age, were inoculated with mycobacteria. Each calf received a single culture. Unless otherwise specified, calves were inoculated with either 1 mg. or 2.2 mg., wet weight, of organisms intradermally on the legs. They were tuberculin-tested on the caudal fold with mammalian tuberculin, by the comparative cervical method using avian and mammalian tuberculins and johnin, and examined by necropsy 8-12 weeks after inoculation.

Thirty-seven calves were inoculated with Runyon Group III mycobacteria, 11 with Group IV mycobacteria, 2 with M. bovis, 1 with M. avium and 1 each with killed cultures of M. bovis, M. avium, a Group III of bovine origin and a Group IV mycobacterium.

Four of seven Group III cultures of bovine origin, inoculated into 14 calves, produced either a primary complex or generalized disease in 8 calves. Two other cultures failed to produce a primary complex and another produced this in only one of three calves.

The six different Group III cultures of swine origin were all isolated from swine mesenteric lymph nodes and were inoculated into 11 calves. Only one culture produced a primary complex with granulomas at the inoculation site and in the left prescapular lymph node.

Of seven calves inoculated with a total of four cultures of "pseudochromes", five had no lesions and two had only small granulomas at the inoculation sites. Four of these calves had each received a total of 10 mg., wet weight, of organisms, 2 mg. being administered by each of the

peroral, subcutaneous, intradermal, intramuscular and intraperitoneal routes.

Five calves inoculated with three cultures of Group III mycobacteria of soil or cattle feed origin did not develop primary complexes.

Six cultures of Group IV mycobacteria were injected into 11 calves, 4 of which received a total of 10 mg., administered by the peroral, subcutaneous, intradermal, intraperitoneal and intramuscular routes. Four of these cultures were isolated from bovine "skin lesions". Two of them caused no lesions in the experimental calves. One culture produced a small intradermal granuloma in one calf, but no lesions in a second calf receiving the 10 mg. dose. The fourth culture produced no lesions in one calf, but the calf which received 10 mg., had encapsulated nonprogressive granulomas at the skin injection site and in the lymph nodes draining the intradermal, subcutaneous and intramuscular injection sites.

Both calves infected with M. bovis developed generalized disease. The calf injected with M. avium developed only a nodule at the injection site. None of the calves injected with killed cultures developed lesions.

In every case the comparative cervical test differentiated between those calves with progressive disease, as determined by histological examination, and animals with either no lesions or localized nonprogressive lesions. Four animals inoculated with live mycobacteria and with no lesions, and one with a nonprogressive lesion, gave positive caudal fold tests. All calves inoculated with killed mycobacteria gave negative comparative cervical results, but one gave a positive caudal fold reaction.

LIST OF REFERENCES

Alvarez and Tavel. 1885. Recherches sur le bacille de lustgarten. Arch. Physiol. Path., 6:303. Cited by Xalabarder, 1961.

Anonymous. 1962. Tuberculosis procedures manual. Mich. Dept. Agric., Livestock Disease Control Division, and U. S. Dept. Agric., Animal Disease Eradication Division, Lewis Cass Building, Lansing, Michigan.

Baum, H. 1912. Das Lymphgefasssystem des Rindes. A. Hieschwald, Berlin.

Benjamin, M. M. 1961. Outline of veterinary clinical pathology. Iowa State Univ. Press, Ames, Iowa.

Boddie, G. F. 1962. Diagnostic methods in veterinary medicine, 5th ed. Lippincott, Philadelphia, Pa.:289.

Braunstein, H. and Adriano, S. M. 1961. Fluorescent stain for tubercle bacilli in histologic sections. I. Diagnostic efficiency in granulomatous lesions of lymph nodes. Am. J. Clin. Path., 36:37.

Breed, R. S., Murray, E. G. D. and Hitchens, A. P. 1948. Bergey's manual of determinative bacteriology, 6th ed. Williams & Wilkins Co., Baltimore, Md.

Breed, R. S., Murray, E. G. D. and Smith, N. R. 1957. Bergey's manual of determinative bacteriology, 7th ed. Williams & Wilkins Co., Baltimore, Md.

Buhler, V. B. and Pollak, A. 1953. A human infection with atypical acid-fast organisms. Am. J. Clin. Path., 23:363.

Corpe, R. F., Runyon, E. H. and Lester, W. 1963. Status of disease due to unclassified mycobacteria. Am. Rev. Resp. Dis., 87:459.

Corpe, R. F. and Stergus, I. 1963. Is the histopathology of nonphotochromogenic mycobacterial infections distinguishable from that caused by Mycobacterium tuberculosis? Am. Rev. Resp. Dis., 87:289.

Crow, H. E., King, C. T., Smith, C. E., Corpe, R. F. and Stergus, I. 1957. A limited clinical, pathologic, and epidemiologic study of patients with pulmonary lesions associated with atypical acid-fast bacilli in sputum. Am. Rev. Tuberc., 75:199.

Daines, L. L. 1938. On certain acid-fast bacteria as the probable cause of skin lesions in tuberculin-reacting nontuberculous cattle. In Moulton, F. R. Tuberculosis and leprosy: The mycobacterial diseases. Am. Assoc. Advanc. Sci., Washington, D. C.

Daines, L. L. and Austin, H. 1932. A study of so-called skin-lesion and no-visible-lesion tuberculin-reacting cattle. J. Am. Vet. Med. Assoc., 80:414.

Daines, L. L. and Austin, H. 1934. Acid-fast and non-acid-fast micro-organisms in skin nodules of tuberculin-reacting cattle. Am. Rev. Tuberc., 30:209.

Durr, F. E., Smith, D. W. and Altman, D. P. 1959. A comparison of the virulence of various known and atypical mycobacteria for chickens, guinea pigs, hamsters and mice. Amer. Rev. Resp. Dis., 80:876.

Feldman, W. H. 1943. A scheme for numerically recording of tuberculous changes in experimentally infected guinea pigs. Am. Rev. Tuberc., 48:248.

Feldman, W. H. 1960, a. Avian tubercle bacilli and other mycobacteria. Am. Rev. Resp. Dis., 81:666.

Feldman, W. H. 1960, b. The fallacy of diagnosing tuberculosis without proof of etiology. Am. Rev. Resp. Dis., 82:112.

Francis, J. 1958. Tuberculosis in animals and man; a study in comparative pathology. Cassell, London.

Frey, C. A. and Hagan, W. A. 1931. The distribution of acid-fast bacteria in soils. J. Inf. Dis., 49:497.

Hagan, W. A. 1931. The no-lesion case problem in the tuberculosis eradication campaign. Cornell Vet., 21:163.

Hanks, J. H. 1956. Retention and differentiation of mycobacteria in tissue sections. Am. Rev. Tuberc., 74:608.

Hastings, E. G., Beach, B. A. and Thompson, I. 1930. The sensitization of cattle to tuberculin by other than tubercle bacilli. Am. Rev. Tuberc., 22:218.

Hole, N. H. and Hulse, E. C. 1939. The skin lesions of bovine tuberculin reactors, second report. J. Comp. Pathol. Therap., 52:201.

Horowitz, I. and Gorelick, D. F. 1951. Tubercle bacilli in bone marrow. Am. Rev. Tuberc., 63:346.

Ippen, R. 1956. Zur vergleichenden Pathologie der Langhans'schen Riesenzelle im Tuberkulosen Granulationsgewebe. Mh. Vet. Med., 11:217.

Johnson, H. W., Baisden, L. A. and Frank, A. H. 1961. Recent research findings on bovine tuberculosis. J. Am. Vet. Med. Assoc., 138:239.

Jones, T. C. and Gleiser, C. A. (ed.). 1954. Veterinary necropsy procedures. Lippincott, Philadelphia, Pa.

Karlson, A. G. 1958. Acid-fast bacilli. Importance of their proper classification. Minnesota Med., 41:721.

Karlson, A. G. 1962. Nonspecific or gross-sensitivity reactions to tuberculin in cattle. Adv. in Vet. Sci., 7:147. Academic Press, New York.

Karlson, A. G. and Feldman, W. H. 1940. Studies on an acid-fast bacterium frequently present in tonsillar tissue of swine. J. Bact., 39: 461.

Kite, J. H., Patnode, R. A. and Read, T. 1952. Distinguishing saprophytic from virulent mycobacteria by intracutaneous inoculation of guinea pigs. Am. J. Clin. Path., 22:250.

Kubica, G. P., Beam, R. E., Vestal, A. L. and Pool, G. L. 1960. The intracutaneous inoculation of guinea pigs as a means of determining the virulence of acid-fast bacilli with special emphasis on the anonymous (atypical) acid-fast bacilli and isoniazid-resistant tubercle bacilli.

Lester, V. 1939. Saprophytic acid-fast bacilli as a source of error in diagnostic work. Acta. Tuberc. Scand., 13:251.

Mallmann, V. H., Mallmann, W. L. and Robinson, P. 1964. Relationship of atypical bovine and porcine mycobacteria to those of human origin. Health Lab. Sci., 1:11.

McGavin, M. D., Ranby, P. D. and Tammemagi, L. 1962. Demyelination associated with low liver copper levels in swine. Aust. Vet. J., 38:8.

Merckx, J. J., Karlson, A. G. and Carr, D. T. 1963. Disease in man associated with unclassified acid-fast bacteria. Proc. Staff Meet., Mayo Clin., 38:271.

Nassar, T. K. and Shanklin, W. M. 1961. Simplified procedure for staining reticulum. A. M. A. Archiv. Path., 71:21.

Nocard and Roux. 1888. Sur la cultur du bacille de la tuberculose. Ann. Inst. Pasteur (Paris), 2:285. Cited by Xalabarder, 1961.

Pinner, M. 1935. Atypical acid-fast microorganisms. III. Chromogenic acid-fast bacilli from human beings. Am. Rev. Tuberc., 32:424.

Pollak, A. and Buhler, V. B. 1955. The cultural characteristics and animal pathogenicity of an atypical acid-fast organism which causes human disease. Am. Rev. Tuberc., 71:74.

Rich, A. B. 1944. The pathogenesis of tuberculosis. Charles C. Thomas, Springfield, Ill.

Runyon, E. H. 1959. Anonymous mycobacteria in pulmonary disease. M. Clin. North Am., 43:273.

Runyon, E. H. 1960. Anonymous mycobacteria in human disease in "The anonymous mycobacteria in human disease," edited by J. S. Chapman. Charles C. Thomas, Springfield, Ill.:3.

Scammon, L. A., Pickett, M. J., Froman, S. and Will, D. W. 1963. Non-chromogenic acid-fast bacilli isolated from tuberculous swine. Am. Rev. Resp. Dis., 87:97.

Sisson, S. and Grossman, J. D. 1953. The anatomy of the domestic animals, 4th ed. Saunders, Philadelphia, Pa.

Smith, H. W. 1954. The isolation of mycobacteria from the mesenteric lymph nodes of domestic animals. J. Path. Bact., 68:367.

Smith, H. W. 1958. The isolation of Mycobacterium johnei and other acid-fast bacilli from the retropharyngeal and ileocaecal lymph glands and spleen of apparently normal cattle. J. Path. Bact., 76:201.

Soltys, M. A. and Jennings, A. R. 1950. The dissemination of tubercle bacilli in experimental tuberculosis in the guinea pig. Am. Rev. Tuberc., 61:399.

Steele, J. H. and Ranney, A. F. 1958. Animal tuberculosis. Am. Rev. Tuberc., 77:908.

Timpe, A. and Runyon, E. H. 1954. Relationship of "atypical" acid-fast bacteria to human disease. J. Lab. Clin. Med., 44:202.

Traum, J. 1916. Case reports of lymphangitis in cattle caused by an acid-alcohol fast organism. J. Am. Vet. Med. Assoc., 49:254.

U. S. Armed Forces Institute of Pathology. 1960. Manual of histologic and special staining technics, 2nd ed. The Blakiston Division, McGraw-Hill Book Co., Inc., New York.

U. S. Dept. of Agriculture, Animal Disease Eradication Division, Agricultural Research Service. 1960. Why tuberculosis in livestock is increasing. Publication ARS-91-21.

Webb, R. M. 1944. The lymph nodes of the head and neck in the domestic animals. Aust. Vet. J., 20:181.

Wilder, C. W. 1962. No gross lesions and the tuberculosis eradication program. J. Am. Vet. Med. Assoc., 140:41.

Willigan, D. A., Garric, V. A. and Trosko, B. K. 1961. New Fuchsin-hematoxylin-eosin; A stain for acid-fast bacilli and surrounding tissue. Stain Technol., 36:319.

Wolinsky, E., Smith, M. M., Mitchell, R. S. and Steenken, W., Jr. 1957. Atypical chromogenic mycobacteria associated with pulmonary disease. Am. Rev. Tuberc., 75:180.

Xalabarder, C. 1961. The so-called problem of unclassified mycobacteria. Am. Rev. Resp. Dis., 83:1.