MYCOBACTERIOSIS OF CATTLE INOCULATED WITH GROUP-III ORGANISMS OF BOVINE ORIGIN

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Lloyd Samuel Goyings
1965

This is to certify that the

thesis entitled

MYCOBACTERIOSIS OF CATTLE INOCULATED WITH GROUP-III ORGANISMS OF BOVINE ORIGIN

presented by

Lloyd Samuel Goyings

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Pathology

Robert F. Langham
Major professor

Date February 25, 1965

O-169

ROOM USE ONLY

ABSTRACT

MYCOBACTERIOSIS OF CATTLE INOCULATED WITH GROUP-III ORGANISMS OF BOVINE ORIGIN

by Lloyd Samuel Goyings

Two experiments were conducted to determine the relative pathogenicity of Group-III mycobacteria of bovine origin in cattle when inoculated by the intrauterine route and by aerosol.

The results were determined by tuberculin testing, serologic procedures, clinical observations and detailed macroscopic, microscopic and bacteriologic examination of tissues.

Intrauterine Experiment

Nine heifers between 21 and 23 months of age were divided into three groups of three heifers each. Each group was inseminated with semen containing a different strain (1 mg. wet weight) of bovine-origin Group-III mycobacteria after artificial production of estrus.

One week prior to each scheduled necropsy, all heifers were tuberculin tested in the caudal fold with mammalian tuberculin and in the cervical region with mammalian and avian tuberculins and johnin. Following the tuberculin tests, one

heifer from each group was examined post-mortem at 2, 4 and 6 months after inoculation.

The six heifers, three inoculated with culture 51C-O and three with culture 68C-O, had evidence of generalized disease. Three heifers inoculated with culture 50B-O had little evidence of disease.

The distribution of the lesions indicated that the infection spread from the uterus to lymph nodes via the lymphatics and to the peritoneal cavity through the opening of the fimbria of the Fallopian tube. Further spread of the infection occurred from the peritoneum to lymph nodes via the lymphatics.

The heifers examined post-mortem at 4 months or at 6 months after inoculation (except those inoculated with culture 50B-O) had evidence of both lymphatic and hematogenous spread of the infection to other organs and lymph nodes. The lesions found in the lymph nodes of the head region suggested oral reinfection.

There was a marked dissimilarity between the distribution of lesions observed in naturally acquired bovine tuberculosis and the lesions found in the heifers inoculated by the intrauterine route with cultures 51C-O and 68C-O.

Group-III mycobacteria indistinguishable from the strains inoculated were recovered from most of the lymph node pools which had lesions. Frequently isolations were made from lymph nodes with no detectable gross or microscopic lesions.

Heifers inoculated with culture 51C-0 had a progressive decrease in tuberculin sensitivity after an initial hypersensitivity. There was a negative correlation between the results of the tuberculin sensitivity and the presence of gross and microscopic lesions. In contrast, heifers inoculated with culture 68C-0 had a progressive increase in tuberculin sensitivity. The mammalian tuberculin sensitivity was greater than the avian tuberculin sensitivity.

The heifers inoculated with culture 50B-0 had no significant increase in sensitivity to mammalian tuberculin. One heifer had a slight increase in sensitivity to avian tuberculin.

Aerosol Experiment

Nine calves, between 3 and 6 months of age, were divided into three groups of three calves each. Each group was inoculated with a different strain of bovine-origin Group-III mycobacteria by aerosol containing approximately $1.5 \times 10^9 \pm 10^2$ organisms. The calves were tuberculin tested as described previously. The calves were euthana-

tized and a necropsy performed ahead of the scheduled 2, 4 and 6 months after inoculation due to anticipated death.

The six calves, three inoculated with culture 51C-O and three with culture 68C-O, had extensive lesions of pulmonary disease. The thoracic lymph nodes with afferent lymph vessels from the lungs also had extensive lesions. The microscopic lesions could not be distinguished from lesions found in cattle infected with Mycobacterium bovis. This was also true of lesions studied from the intrauterine experiment. The disease rapidly spread to most of the lymph nodes, the abdominal organs (liver, spleen, kidney), and throughout the body, principally by the hematogenous route.

The distribution of lesions in the six calves exposed by aerosol to cultures 51C-O and 68C-O was markedly similar to lesions found in naturally acquired bovine tuberculosis. Calves inoculated with culture 50B-O had no detectable gross or microscopic lesions.

Group-III mycobacteria, indistinguishable from the strains inoculated, were recovered from most of the lymph node pools in which lesions were found. Acid-fast organisms were recovered from the thoracic lymph nodes of the group of calves inoculated with culture 50B-O.

Five of the six calves, inoculated with culture 51C-O and 68C-O, had a marked mammalian tuberculin sensitivity.

Calves inoculated with 50B-O had a slight increase in sensitivity to avian tuberculin.

MYCOBACTERIOSIS OF CATTLE INOCULATED WITH GROUP-III ORGANISMS OF BOVINE ORIGIN

By

Lloyd Samuel Goyings

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Pathology

1965

ACKNOWLEDGEMENTS

The author is grateful to, and wishes to express appreciation to, the following people for their help in making this thesis possible.

To Dr. R. F. Langham for his guidance in stimulating me to do graduate work in pathology, his help in acquiring a postdoctoral fellowship for the first two years of my graduate program and, above all, his role in my pathology training.

To Dr. C. C. Morrill for my selection to participate as a pathologist on the tuberculosis project leading to these investigations and for his counsel and guidance of the research.

To Dr. W. L. Mallmann for his counsel and guidance, his enthusiatic motivation in research and his significant part in making this research possible.

To Dr. E. P. Reineke for his counsel and guidance in my graduate program, and particularly his philosophy as a scientific investigator.

To Dr. S. D. Sleight for his fellowship, patience and helpful suggestions in the writing of this thesis.

To. Dr. V. H. Mallmann for her assistance in preparation of the cultures; to Dr. J. A. Ray for conducting much of the tuberculin testing; and to both for their preliminary work leading to these investigations.

To the technical staff of the tuberculosis project for their cooperation and assistance in performing the bacteriological and histopathological procedures.

To the United States Department of Agriculture through which this research was supported with contract-cooperative agreements.

To Dr. R. M. Scott for his assistance in selection of the cattle for these investigations.

To Dr. D. J. Ellis for his technical assistance in the intrauterine experiment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The decrease in numbers of tuberculin reactors in the cattle population in this country is rather impressive when one compares the incidence of the disease from 1917 to 1963 (5.0% and .10% respectively). This was accomplished mainly by the diligent and persistent use of the intradermal tuberculin test as the diagnostic tool for removing tuberculin reactor animals from herds. Of greater significance, perhaps, from the public health standpoint has been the rapid decrease of animals with gross lesions. Only 1780 lesion reactors were reported in the United States Department of Agriculture (USDA) fiscal year 1961 (Wilder, 1962).

However the eradication program is not without problems. During the last 20 years the decrease in the number of reactors was not significant when compared to the previous 20 years. In fact there was an increase from 11 to 23 reactors of every 10,000 animals tested between 1952 and 1959 (Anon., 1960). This would suggest to the outside observer that the reaching of a plateau indicates in essence only a control program. Analysis of the post-mortem findings in reactors shows a marked increase in the percentage of "no gross lesion" (NGL) animals on necropsy.

By 1961 (Wilder, 1962), the NGL animals represented approximately 73% of the reactors slaughtered. If the positive tuberculin tests of the NGL animals are due to organisms other than pathogenic mycobacteria or to non-specific substances, then the livestock industry is suffering an unnecessary economic loss. This caused grave concern in the USDA which has the responsibility of conducting the tuberculosis eradication program. The entire eradication program needed reevaluation. A series of regional meetings was held beginning with a conference at Michigan State University in 1958 on tuberculosis eradication.

There are many problems that must be resolved. Such problems include: 1) the need for a reliable test for diagnosis; 2) the failure to demonstrate unequivocally the cause of the large percentage of NGL reactors; 3) the true relationship of the tuberculoid skin lesion to tuberculin sensitivity; and 4) the possible role that mycobacteria other than the classical organisms may have in causing tuberculin sensitivity (Hastings et al., 1930).

A research program on bovine tuberculosis was set up at Michigan State University by contract grants from the USDA. The research was to be conducted by a team composed of staff from the Departments of Pathology and Microbiology and Public Health.

Although there were scattered reports and brief mention of atypical mycobacteria in the human literature, no real emphasis was placed on these organisms until such reports as

those of Timpe and Runyon (1954), Crow et al. (1957) and the monumental work of Runyon (1959). More will be said about these reports later.

It was soon recognized by different investigators (Mallmann et al., 1961; Scammon et al., 1963; Yoder, 1964), that an analogous situation was present in the animal field. In our bacteriology laboratory, large numbers of acid-fast organisms were isolated from bovine tissues which appeared not to conform to the characteristics of classical mycobacteria. In order to evaluate the possible significance of these organisms, laboratory animals, swine and cattle have been inoculated primarily by the intradermal route. Some of the Group-III mycobacteria were found to cause disease and tuberculin hypersensitivity.

Through a contract (No. 12-14-100-6852 (45)) between the USDA and Michigan State University, investigations were conducted to determine the relative pathogenicity and significance of different routes of inoculation. The research described herein is limited to studies of cattle inoculated by the aerosol and intrauterine routes with bovine-origin Runyon Group-III isolants of low and high virulence.

Specific objectives included a study of the course of the disease in inoculated cattle by the use of tuberculin tests, serologic procedures and clinical observations. Further objectives included a detailed macroscopic, microscopic and bacteriologic examination.

REVIEW OF LITERATURE

Isolation of mycobacteria which did not conform to the characteristics established by Koch in 1881 were soon recognized (Nocard and Roux, 1888) as reported by Xalabarder (1961). Scattered reports in the literature (Baldwin, 1942; Feldman, 1943; Karlson and Feldman, 1953; Buhler and Pollak, 1953) described clinical and bacteriologic findings, tuberculin sensitivity and animal pathogenicity studies of disease associated with atypical mycobacteria. In most laboratory situations the atypical mycobacteria were considered as saprophytes or contaminants, and were often discarded. This was understandable since there were many reports (Frey and Hagan, 1931; Karlson, 1958; Mallmann, 1963) of acid-fast isolations from innumerable inanimate materials. These organisms were considered ubiquitous and thus easily contaminated material submitted for culture.

Not until sufficient clinical data had been accumulated and reported (Wood *et al.*, 1956; Crow *et al.*, 1957; Hall *et al.*, 1957), did it seem necessary to reassess the possible significance of the isolation of atypical mycobacteria.

The need for a working classification system for atypical mycobacteria was emphasized by Karlson (1958) when he reported that by 1945 there were 163 known strains. Such a

grouping was very ably devised by Runyon (1959). This classification was based on human isolants collected from different sources throughout the United States. Preparation for this work was started by other investigators, particularly Dr. William Feldman who circulated detailed outlines on how to study these organisms (Runyon, 1959). The features used in classification included: 1) pretherapy drug resistance; 2) strong catalase activity; 3) capacity to grow at room temperature; 4) failure to produce progressive disease in guinea pigs; 5) colonies strongly pigmented or smooth, easily dispersed in liquid and with rapid growth; 6) niacin, peroxidase neotetrazolium reduction and microcolonial pattern in neutral red.

The atypical mycobacteria were divided into four categories: Group I (photochromogens); Group II (scotochromogens); Group III (nonphotochromogens); and Group IV (rapid growers). The Group-III strains had features which overlapped with strains of M. avium, M. tuberculosis and M. bovis. Probably the most distinctly different feature was their pathogenicity for laboratory animals. Human isolants classified as belonging to Group III were not uniform in their pathogenicity. Some strains were found to be no more pathogenic than Group I (Runyon, 1959) which did not produce progressive disease when inoculated subcutaneously with 5 mg. (dry weight) of the organisms. The author further stated that when 1 mg. was inoculated intracardially, death often resulted in 4 to 5 weeks. When 0.01 mg. was inoculated

intravenously or 3 mg. intraperitoneally, disease was manifested by formation of lesions in lungs, liver, spleen and kidneys. Other Group-III strains produced no detectable lesions.

Later, Corpe et al. (1963) reported that as a result of the lack of pathogenicity for the recognized laboratory animals used in tuberculosis diagnostic laboratories, there was no standard test for mycobacterial pathogenicity. This was considered unfortunate since the relative significance of an isolant depended on the closeness and consistency of association of the recovered organisms with the clinical and pathologic findings of the disease since it was impossible to consistently fulfill Koch's postulates. The problem was compounded by the many isolations of acid-fast mycobacteria from apparently healthy individuals (Edwards et al., 1959; Atwell and Pratt, 1960).

Other workers investigated the pathogenicity of atypical mycobacteria for laboratory animals either by using different routes of inoculation or different laboratory animals in an attempt to find a suitable way to evaluate virulence. Pollak and Buhler (1955) described the pathogenicity of the so-called yellow bacillus (classified in Group I by Runyon, 1959) for the guinea pig, hamster, rat, mouse, rabbit and chicken. The organism did not produce progressive disease in guinea pigs although there was a lesion at the site of inoculation and microscopic lesions in some of the visceral organs 2 to 4 weeks after inoculation. Intramuscular and

intraperitoneal inoculation failed to produce either gross or microscopic lesions in chicks. Likewise no disease was noted in the white rat. The authors concluded that the hamster was the animal of choice, since consistently progressive disease was produced when large numbers of these organisms were inoculated intraperitoneally. Although mice were generally susceptible to the organisms, there appeared to be considerable variability in the results.

Similar results were reported by Feldman (1963) using Group-III mycobacteria. He inoculated hamsters in the substance of each testis (0.001 mg.) and under the dermis of the concha of each ear (0.005 mg.). Under the conditions of the experiment, irreversible pathologic changes occurred in the hamsters after intratesticular inoculation. Chickens and rabbits were also inoculated with the same mycobacteria. Although the rabbit was resistant, a formidable disease occurred in chickens when infection was induced by intracerebral or by intravenous inoculation.

Kite et al. (1952) used the intradermal and subcutaneous routes of inoculation in guinea pigs in an attempt to distinguish between saprophytic and virulent mycobacteria. A comparison between the two routes of inoculation was made with 266 unknown cultures. The guinea pig was inoculated into the right groin with 1 ml. (0.1 mg) and 0.1 ml. (0.01 mg.) intracutaneously in the axillary or inguinal region of the abdominal wall, with the same suspension. There was a close correlation between the subcutaneous and intracutaneous

routes of inoculation. A virulent organism produced an ulcer in 2 to 3 weeks. The saprophytic organism occasionally produced a nodule which seldom ulcerated and usually healed within six weeks. The test was considered positive if there was an ulcer at the site of inoculation and if acid-fast organisms could be demonstrated in the regional lymph node.

Kubica et al. (1960), using the same technique, studied the comparative virulence of atypical mycobacteria and classical mycobacteria. Guinea pigs were inoculated with 0.1 mg., 0.01 mg. and 0.001 mg. intracutaneously in the shaved abdominal skin. Of the atypical mycobacteria, Group-I and Group-III strains were the most consistent in producing ulceration. Approximately 50% of the scotochromes (Group II) and 30-40% of the rapid growers (Group IV) produced ulceration at the site of inoculation. The author concluded that the intracutaneous route of inoculation appeared to give a better measure of the virulence for man than the subcutaneous, intraperitoneal or intracardial route. No data were given on the condition of the lymph node draining the site of inoculation.

One report (Durr et al., 1959) described the use of saprophytes (M. phlei and M. smegmatis) as a control when studying the extent of pathology produced by the atypical and classical mycobacteria. Three-to-six-week-old chickens were inoculated subcutaneously (Experiment I) or intravenously (Experiment II) with 1.0 ml. and 0.4 ml. of diluted culture, respectively; adult golden hamsters were inoculated

intraperitoneally with 1.0 ml. of diluted culture, guinea pigs were inoculated subcutaneously with 1.0 ml. of diluted culture and mice were inoculated intravenously with 1.0 ml. of diluted culture. Eight weeks after inoculation the animals were euthanatized. The results are summarized in the following table:

	Chicken	Guinea Pig	Hamster	Mouse
M. tuberculosis	—	+++	+++	+
M. avium	++	—	+	+
Nonphotochromogens	±	—	±	—
Photochromogens	±	—	++	++
Saprophytes				
M. phlei	—	—	—	—
M. smegmatis	—	—	—	—

Progressive disease resulting in death of all of the animals in a particular group is indicated by +++; extensive gross disease without a fatal outcome by ++; microscopic disease only by +; and microscopic disease in some, but not all, of the animals in a particular group, by ±.

The disease caused by atypical mycobacteria in man commonly takes the form of a chronic systemic disease involving principally the lung but sometimes also lymph nodes, skin, meninges and joints (Wood *et al.*, 1956; Crow *et al.*, 1957; Weed *et al.*, 1956; Merckx *et al.*, 1963). Occasionally, the disease was generalized (Wood *et al.*, 1956; McCusker and

Green, 1962). The lesions were indistinguishable from lesions caused by classical tubercle bacilli as was well exemplified by Corpe and Stergus (1963) who submitted a group of representative tissue sections from Group-III and M. tuberculosis infected patients to 27 pathologists who had an interest in tuberculosis. Of this group, 53% did not distinguish between the causative organisms by studying the histopathologic material. This may imply that 47% could distinguish between the organisms from studying the histopathologic changes but, statistically, similar results could be obtained by merely tossing a coin (Dixon and Massey, 1957).

The disease was found more often in white males in the 3rd, 4th, 5th and 6th decades of life (Runyon, 1959; Merckx et al., 1963). There was little or no concrete evidence of transmission from one person to another (Merckx et al., 1963). In a rather high percentage of cases, there were complicating or contributing factors such as emphysema, anthracosis, silicosis, bronchiectasis, etc. (Runyon, 1959). Although the epidemiologic data were limited, there appeared to be a definite geographic distribution of the different groups of atypical mycobacteria (Merckx et al., 1963; Edwards et al., 1960).

There is ample evidence that the presence of atypical mycobacteria is not a new phenomenon in the animal kingdom. As early as 1930, Hastings et al. reported recovery of acid-fast bacteria from "no gross lesion" reactors. This was followed by a report by Hagan (1931), who isolated acid-fast

mycobacteria from mesenteric lymph nodes with no detectable gross and microscopic lesions. The isolants were considered to be saprophytes from the intestine which could cause tuberculin sensitivity.

Feldman (1938) described the isolation of M. avium-like mycobacteria from swine and chickens which did not manifest the usual pathogenicity for chickens. The organisms probably would be classified as nonchromogenic acid-fast bacilli under the current grouping of atypical mycobacteria.

In another report (Karlson and Feldman, 1940) nonchromogenic acid-fast microorganisms were recovered from 25% of 94 swine tonsils. The organisms failed to produce detectable disease in chickens, mice and calves. Also, little if any virulence was noted when they were inoculated into guinea pigs and rabbits. However, the organisms induced sensitivity to avian tuberculin and homologous culture filtrates, but not to mammalian tuberculin.

Unidentified mycobacteria were cultured from the retropharyngeal and ileocecal lymph nodes and spleens of 100 apparently healthy cattle (Smith, 1954, 1958).

An acid-fast bacterium identified as Mycobacterium lacticola was isolated from the milk and mammary tissue of cows with tuberculoid mastitis (Stuart and Harvey, 1951). The condition was only in those animals infused for mastitis with an oily vehicle. The affected animals did not react to either avian or mammalian tuberculin. Another report (Tucker, 1953) described the isolation of mycobacteria from

a herd which had an incidence of 21.7% infection. Previous to examination, there were 14 cows which reacted to the tuberculin test. The organism appeared to be identical to Mycobacterium fortuitum. The disease was characterized by multiple granulomas. The udders of these animals had also been infused with an oily suspension for mastitis treatment.

Nonchromogenic acid-fast bacilli were isolated from tuberculous swine (Scammon et al., 1963). Of 63 cultures studied, 43 strains were swine isolants, 10 were avian strains and 10 were Battey strains. Forty of the 43 strains of swine origin consistently produced death in chickens while all of the avian strains produced typical avian tuberculosis. The Battey strains did not produce death at three months in chickens. The author concluded that none of the strains could be distinguished from each other using the established criteria for Group-III organisms.

Approximately 100 strains of nonchromogenic acid-fast bacilli were isolated from a university swine herd (Mallmann et al., 1962). Isolations were made from swine which had gross lesions and from those with no gross lesions. This prompted a bacteriologic survey to determine the geographic distribution of these organisms. Tissues with lesions were collected from abattoirs processing swine from Illinois, Missouri, Ohio, Indiana, and Michigan. Eighty-five percent of the isolants were M. avium and the remainder were Group-III mycobacteria. The Group-III strains were inoculated intradermally into swine and guinea pigs and intraperitoneally

into chickens. No lesions were demonstrated in chickens although the organisms could be recovered. There was a skin lesion characterized by ulceration at the site of inoculation in all the swine and guinea pigs inoculated. At necropsy, lesions were found in some of the swine, involving lymph nodes of the head and the lymph node that drained the site of inoculation. No gross lesions were detected in the guinea pigs at necropsy.

Another report (Mallmann et al., 1964) described the bacteriologic, allergenic, and pathogenic investigations from a sample of 40 out of 300 isolants. These organisms were recovered from bovine and swine tissues. The organisms appeared to be highly heterogeneous and variable in their characteristics. Some resembled the isolants made from man and classified into Runyon's grouping. A few of the strains appeared to be between Group I and II and were called pseudochromes. With some strains, virulence was increased following reisolation and reinoculation. The intradermal route of inoculation was used to give presumptive evidence of virulence. A skin lesion at the site of inoculation confirmed the virulence of the organism.

McGavin (1964) and McGavin et al. (1964) found that when Group-III mycobacteria of bovine origin were inoculated intradermally into calves, there was considerable variability in pathogenicity of the organisms. Of 14 calves, using 7 cultures for inoculation, six calves had generalized disease, three had lesions at the site of inoculation and the lymph

node draining the inoculation sites (primary complex), three had lesions at the site of inoculation only, and two had no detected gross or microscopic lesions. There was considerable variability in the extent of the disease produced with the same culture. Of 10 of the 14 calves on which a comparative cervical tuberculin test was conducted; the three cases with generalized disease had positive tuberculin tests in the caudal fold and positive mammalian and avian tuberculin tests in the cervical region; the two cases with lesions sufficient to be called a primary complex had conflicting results. One calf had a positive tuberculin caudal fold test but a negative comparative test, although the avian response was 10 mm. larger than the mammalian response. The other calf had only a 1.5 mm. increase at the caudal fold. The remaining five calves were considered negative. It therefore appeared that the Group-III mycobacteria caused tuberculin sensitivity and sensitivity was dependent on the extent of disease occurring in the inoculated animal.

McGavin (1964) and McGavin et al. (1964) also found that Group-III isolants of swine origin had little pathogenicity for calves. Seven cultures were inoculated intradermally into calves. Only one culture produced significant lesions, which consisted of a caseo-calcareous granuloma in the lymph node draining the inoculation site and an abscess at the site of inoculation. The calf inoculated with this culture had a negative caudal fold test with mammalian tuberculin and was negative to avian tuberculin in the cervical region.

The Group-III mycobacteria of feed and soil origin and pseudochromes of bovine origin were not pathogenic for calves. Likewise the Group-IV mycobacteria of bovine origin produced no significant lesions in calves, except for one culture in one calf. This animal had lesions at the site of inoculation and the draining lymph node. Inasmuch as 10 mg. (wet weight) of culture was used and the animal was stressed by an unintentional exposure to disinfectant aerosol, little significance was attached to this finding. It was not possible to differentiate between lesions produced by M. bovis and by atypical mycobacteria. The calves inoculated with Group-III organisms of feed and soil origin, pseudochromes of bovine origin and Group-IV organisms produced little or no sensitivity. Two calves, one inoculated with a pseudochrome and the other with a Group-IV mycobacterium, had a caudal fold response of 6 mm. and 7 mm. respectively. Both of these animals were negative to the cervical test.

Cattle have been sensitized to tuberculin by mycobacteria other than M. bovis and atypical mycobacteria (McGavin, 1964). These included M. paratuberculosis, M. tuberculosis, and M. avium (Feldman, 1960; Johnson et al., 1961; Wilder, 1962).

A significant problem related to tuberculin sensitivity is the so-called "skin tuberculosis" of cattle. The first report was by Traum (1916), who described the clinical condition as a granulomatous process associated with acid-fast bacilli which could not be grown and identified. Hole and

Hulse (1939) grouped the lesions on a pathologic basis. Type-I lesions were cellular and had many giant cells and little necrosis; type II had multiple areas of necrosis and calcification; and type III had encapsulated foci of liquefaction which ulcerated and discharged pus. The lesions varied similarly in material studied by Daines and Austin (1932), Hedstrom (1949) and in this laboratory (Goyings, 1962). Hedstrom (1949) studied 606 specimens and found that 20% could be called type III, 73% type II and the remainder type I. In this laboratory (Mallmann, 1962; Goyings, 1962), out of 351 cases of "skin lesions" studied, acid-fast organisms were isolated from 42% of the cases. Two isolants were classified as Group I, 14 as Group II, 39 as Group III, 17 as pseudochromes, 34 as Group IV, and 42 were not classified. Most of the lesions were type II or III and at least some of the types I and II had undergone ulceration. Differences in the type of lesion appeared to be due to the stage of the disease and not to the mycobacteria isolated. The relationship of the isolation of acid-fast bacteria to the spontaneous lesions was not known.

Other workers (Beach and Hastings, 1924; Hastings et al., 1924; Carpenter and Goldberg, 1925; Mitchell, 1928; Day, 1928) had been either unsuccessful in culturing the acid-fast organisms or unsuccessful in reproducing the disease.

Although acid-fast organisms were isolated from skin lesions as a result of the extensive work of Daines (1938),

the production of sensitivity and lesions in cattle was not constant and the lesions were only examined clinically.

The only apparent reproduction of the condition was by Hedstrom (1949) who was able to produce typical lesions and tuberculin sensitivity in 3 of 25 cattle inoculated using a composite sample of 4 spontaneous lesions.

Of particular interest is the fact that the condition usually remains localized. Runnells (1932) did not find involvement of the regional lymph nodes in a series of skin-lesion cases studied. However, Krantz (1938) reported involvement of the regional lymph node in 12 of 32 cases. Others (Thomann, 1949; Hole and Hulse, 1939) have observed lesions in some regional lymph nodes. The lesions were generally seen only on histopathologic examination and were characterized by a granulomatous inflammatory process. Acid-fast bacilli were demonstrated in some of the microscopic lesions.

Williган (1961) studied histopathologically tissues from 56 tuberculin reactors and found 38 (67.9%) with microscopic granulomas in the carcass lymph nodes (primarily the right and left prescapular but occasionally the right and left prefemoral lymph nodes).

A later review (Goyings, 1962) revealed that, out of 179 tuberculin reactors in which carcass lymph nodes were studied and in which skin lesions were not found or submitted, microscopic granulomas were found in 79 (44%). Gross lesions were found in 3% of the carcass lymph nodes. Skin lesions, organs and lymph nodes from 35 tuberculin reactors

were studied histopathologically. Twenty-one (60%) of the carcass lymph node pools (one pool per animal) had microscopic granulomas. The lesions were characterized by few to numerous scattered foci of epithelioid cells in the cortices of the affected lymph nodes. In many cases giant cells were a component of the inflammatory reaction. Caseation and calcification were not salient features. Three reactors (9%) had gross lesions, acid-fast organisms were found in 7 (20%) and acid-fast organisms were isolated from 2 (6%).

According to Paterson (1956), much of the data concerning the skin lesion problem as a cause of tuberculin sensitivity has been biased, since a search for the lesions has been made only when anomalous tuberculin reactions were found. There are very few data on skin lesions in tuberculin-negative herds.

The problem of mycobacterial classification was difficult if not almost impossible due to the complexity of the organism (Xalabarder, 1961). The use of bacteriologic techniques for primary isolation was considered essential by Karlson (1964) with better laboratory techniques and the recognition of strains of M. tuberculosis less virulent for guinea pigs and of atypical mycobacteria as potential pathogens. Yet in another laboratory (Ekdahl and Macfarlane, 1963) it was pointed out that potential pathogens could be overlooked since primary isolation of mycobacteria by guinea

pig inoculation was considered more sensitive than bacteriologic techniques.

Feldman (Hull, 1963) considered bacteriologic findings to be subject to considerable variation and thus only sufficient for presumptive identification; therefore laboratory animals (guinea pigs, rabbits, chickens) were needed for final identification of M. bovis, M. avium and M. tuberculosis. Inasmuch as the atypical mycobacteria did not cause disease in guinea pigs, bacteriologic findings formed the basis of identification by Runyon (1959).

Karlson (1958) questioned the use of "atypical" or "anonymous" and stated that the 6th edition of Bergey's Manual (Breed et al., 1948) listed them as Mycobacterium spp. However, subsequent editions have not used this designation. Examples of conflicting mycobacterial identification illustrating this confusion can be found in many literature reports.

Karlson et al. (1962) described a yellow-pigmented strain of Mycobacterium avium isolated from tuberculous lesions in a trumpeter swan. The organism was classified by one laboratory as a skotochromogen even though virulence for chickens and guinea pigs was present. In another report, the same author in 1964 stated:

> "....certain in vitro features characterize M. bovis and may lead to a presumptive identification. The identification of M. bovis must be confirmed by animal pathogenicity tests."

In still another laboratory (Kubica, 1964) animal inoculations were avoided whenever possible due to their supposed unreliability.

From our laboratory, a culture was submitted to two different laboratories for identification (Mallmann, 1964). The culture had been typed as a Group III. Laboratory 1 identified the culture as a Group III and Laboratory 2 as M. bovis.

This problem was ably reviewed by Xalabarder (1961) who stated that:

> "....there is only one thing about them (mycobacteria) that is absolutely typical, and that is their great plasticity and adaptability to an extreme variety of environments. To suit those conditions, these organisms will change not only their metabolic patterns and morphologic characteristics, but even their mode of reproduction.....although there are a great many exceptions to this rule, the presence or absence of pigment has been one of the factors which has been used by Penso and associates (1949) and more recently by Runyon (1959) to classify mycobacteria. And yet, if the past history of bacteriology is studied, it must be noted what little value can be attributed to pigmentation as a reliable criterion for differentiation."

Even the relative pathogenicity of the saphrophytic and pathogenic strains has been considered unreliable. Xalabarder (1961) found that when guinea pigs were inoculated with saprophytic strains and kept for a much longer period than two months, histopathologic lesions developed which were characterized by epithelioid and giant cells with marked fibroblastic proliferation. Reinoculation into

guinea pigs caused caseation of the mediastinal lymph nodes and fibroblastic proliferation of all organs.

The cytochemical reactions appeared to have limited value in classification. Hauduroy (1955) found that 50% of the strains tested showed discrepancies. Possibly this problem could be at least partially alleviated if quantitative rather than qualitative tests were used. This was emphasized by Wayne (1964) who stated that:

> "....time complicates the problem of applying biochemical tests to classification of mycobacteria by virtue of the great range of rates of their metabolic reactions. One must take into account inoculum and substrate concentration, time of observation, and magnitude of reaction."

The trend to apply computer methods for bacterial classification was discussed by Floodgate (1962). His approach was based on comparison of reaction kinetics on a large group of organisms with a high agreement with one another. The author concluded that selection of characters for classification were based on natural frequency distributions of randomly selected organisms. Further computer analysis avoided the arbitrary selection of characters and thus reduced the bias that was introduced into a classification. The raw data dictated the boundaries of a grouping.

According to Wayne (1964), the development of an acceptable and useful taxonomic mycobacterial classification will await a group of research workers representing a number of disciplines with sufficient scope and competence to study the problem with computer methods. The author concluded

that the group of mycobacteria selected for study should be taken from the natural population of mycobacteria on a random basis.

MATERIALS AND METHODS

A. Intrauterine Experiment

1. Source of Animals

Nine heifers were obtained by Dr. R. M. Scott, Animal Disease Eradication Division (ADED), USDA, Lansing, Michigan from a Mississippi herd of dairy cattle. These animals had a history of no tuberculosis reactors and were essentially negative to the caudal fold test using 0.1 ml. mammalian tuberculin. They were also negative to a cervical test using avian tuberculin, mammalian tuberculin and johnin. The heifers were Holstein, Guernsey and Holstein-Guernsey crossbreds. The animals were infected at approximately 21 to 23 months of age.

2. Isolation Procedures

The heifers were divided into three groups and so kept, 3 animals to each isolation room, until necropsy. Separate boots, rubber aprons, jackets and disposable gloves were provided for personnel entering each room. In addition, the attire which was worn but not changed between rooms included disposable cap and mask and a pair of coveralls. Each time the personnel left the room, the boots, apron, jacket and any instruments removed were washed in disinfectant*.

---

*Torsite, The Dow Chemical Company, Midland, Michigan.

The heifers were fed a balanced ration consisting of coarsely ground alfalfa hay mixed with a mineral-supplemented concentrate. Water was supplied ad libitum.

Since no bedding was used on the floor, the feces were disposed of in the sewage system.

3. Inoculation of the Animals

Each group of 3 heifers was inoculated with a different strain of Mycobacterium. The strains were Runyon Group III's of bovine origin, selected on the basis of previous pathogenicity and sensitivity studies in laboratory animals and calves (McGavin, 1964).

One strain, culture 50B-0 appeared to be of low virulence and had greater sensitivity to PPD-B*. The other two strains were of higher virulence, culture 51C-0 and culture 68C-0.

The animals were injected with pregnant mare serum (PMS)** at a dosage of 20 ml.(10,000 units) subcutaneously, 2 days prior to inoculation, in an attempt to produce estrus. Bull semen*** diluted with buffered semen**** extender, but without antibiotics, was used to inseminate the heifers.

---

*Purified Protein Derivative-Battey strain (produced by Seibert's method), received from L. Edwards, Communicable Disease Center, Atlanta, Georgia.

**"Gonadin", Ashe Lockhart, Division of Haver-Lockhart Laboratories, Shawnee, Kansas.

***Obtained through courtesy of Michigan Animal Breeder's Cooperative, East Lansing, Michigan.

****Cornell University Extender (egg-yolk-citrate extender), courtesy of Michigan Animal Breeder's Cooperative, East Lansing, Michigan.

One mg. (wet weight) of mycobacteria (approximately 1 x 10^8 organisms) was placed in a disposable insemination tube for each of the nine heifers. The inoculum was sealed in the tube by introducing a plug of vaseline on each side of the culture.

The insemination tube was introduced by rectal manipulation into the body of the uterus through the external os of the cervix. A disposable 10-ml. syringe filled with 5 ml. of extended bovine semen was attached to the insemination tube. With gentle pressure the inoculum and the semen were forced into the uterus. The insemination tube and syringe were disposed of by incineration.

4. Clinical Examinations

The heifers were observed weekly for the first six weeks and semiweekly thereafter for signs of disease. Observations included rectal temperature, condition of the animal and rectal examination. Blood samples (8, 20-ml. tubes per heifer) were taken every 2 weeks throughout the experiment for serologic examination (hemagglutination test, etc.).

5. Tuberculin-Test Technique

All of the heifers were tuberculin-tested one week prior to each scheduled necropsy or one week prior to 2, 4 and 6 months after inoculation. The left caudal fold and the left cervical neck area were used for the sites of injection. After clipping the hair, the skin thickness at the

site of injection was measured before injecting tuberculin (zero hour reading) using a Hauptner dermal thickness gauge* (used for all tuberculin measurements).

The left anterior proximal third of the neck was injected intradermally with 0.1 ml. of avian tuberculin**; the left middle third of the neck with 0.1 ml. of mammalian tuberculin***; and the left posterior third with 0.2 ml. of johnin****.

The left caudal fold was injected intradermally with 0.1 ml. of mammalian tuberculin.

The skin thickness at the site of injection was measured at 24, 48 and 72 hours after injection of tuberculin. All measurements were recorded in millimeters. The comparative test results (subtraction of corrected mammalian tuberculin response from corrected avian tuberculin response) was also recorded. The interpretation of the tuberculin tests was based on recommendations of other workers (Boddie, 1962; Anon., 1962).

---

*H. Hauptner Co., Factory for Vet. Instruments, 565 Salingen, Germany.

**Tuberculin, avian, intradermic, produced for the Agricultural Research Service (ARS), USDA.

***Tuberculin, mammalian, intradermic, produced for the ARS, USDA.

****Johnin, intradermic, produced for the ARS, USDA.

6. Necropsy Technique

The necropsy technique was essentially based on that of Jones and Gleiser (1954) and modified by McGavin (1964) for the existing facilities.

Prior to euthanatizing the animal, approximately 32 (20-ml.) tubes of blood were removed from the jugular vein for serologic procedures. The animal was given sufficient chloral hydrate (approximately 15 ml. of 40% solution per 100 lbs. of body weight) to cause anesthesia. The animal was placed on its left side and the right foreleg was dissected free from the thoracic chest wall and reflected back. The right common carotid artery was then exposed and incised. Euthanasia was performed by rapid exsanguination.

Identification tags were removed and saved for future reference. The right foreleg was dissected free from the animal and the right prescapular and axillary lymph nodes removed. All lymph nodes removed from the carcass were either individually labeled and placed in individual plastic sacks or pooled and placed in plastic sacks for transportation to the bacteriological laboratory. The lymph nodes and organs saved for bacteriologic examination were kept separate from those lymph nodes and organs saved only for histopathologic examination. At all times, attention was given to careful dissection of the lymph nodes from their respective locations to prevent internal contamination.

The right hindleg was dissected from the carcass and the right prefemoral, popliteal, ischiatic and left and right superficial inguinal lymph nodes were removed.

The attendant then made a ventral midline incision through the skin and, after dissecting it free from the abdominal and thoracic cavity reflected it laterally. Then, the abdominal cavity was opened by cutting from the dorsal costal arch to the xiphoid cartilage, finally posterior along the linea alba to the pubis. The abdominal wall was reflected dorsally.

During this time a ventral midline incision was made from the symphysis mandible to the cariniform cartilage of the sternum. The tongue was dissected from the mandible and the submaxillary lymph nodes removed. The soft palate was cut transversely and the greater cornua of the hyoid bones dissected from the larynx. The tongue, esophagus, larynx and trachea were then dissected from their attachment posteriorly to the thoracic inlet. The right and left medial and lateral retropharyngeal lymph nodes were identified and dissected free. The right and left parotid lymph nodes were similarly removed.

With the help of the attendant, the costal attachments of the diaphragm were dissected free and, with the use of rib shears, the dorsal ends of the ribs were cut and, similarly, the distal ends. The right chest wall was removed. Being careful not to contaminate the organs, a segment of the right diaphragmatic lobe of the lung was removed for culture.

Generous pieces of liver and spleen were also removed for culture. The attendant removed the intestine and dissected the mesenteric and colic lymph nodes free from the mesentery, as described by Jones and Gleiser (1954). The neck organs and lungs were removed *in toto*. The right and left bronchial lymph nodes, right apical bronchus, anterior and posterior mediastinal lymph nodes were removed and placed in individual plastic bags. The esophagus and trachea were opened; the lung palpated and cut tranversely at intervals for lesion location and identification.

The forestomachs were removed and their respective lymph nodes removed and pooled. While the attendant disarticulated the head and removed the skin; the right and left deep inguinal, left and right internal iliac and lumbar (including renal) nodes were removed and pooled separately. The liver was removed by cutting the diaphragmatic attachment and the hepatic lymph nodes removed. The liver was incised transversely approximately every cm. for lesion identification.

The uterus, cervix and ovaries were removed *in toto* by ligation of the vagina at the posterior external os of the cervix. The organ was placed in a plastic bag for bacteriological examination. The kidneys were removed and cut transversely at intervals.

Representative tissue was taken from the heart, kidney intestine, liver and spleen and fixed in 10% buffered formalin and Zenker's fluid. A sample of lung was fixed in

formalin. Approximately six inches of ileum were tied off and removed for bacteriological examination. The brain was removed and representative tissue samples, as described by McGavin et al. (1962), were fixed in 10% buffered formalin for histopathologic examination.

The carcass was turned onto the right side. The left foreleg and hindleg were removed and the following lymph nodes removed and placed in individual plastic bags: left prescapular, axillary, prefemoral, popliteal and ischiatic.

The mammary gland was cut transversely at intervals of about 1.5 cm. and, if lesions were detected, samples were saved for histopathologic examination.

7. Bacteriologic Technique

All lymph nodes and organs removed from the carcass were taken to the bacteriological laboratory to be examined under an isolation hood. The lymph nodes and organs for bacteriologic examination were trimmed of excess adventitial tissue and washed 5 times for 5 minutes each in 5% sodium hypochlorite solution. The lymph nodes were sliced every 2 to 3 mm. and examined for lesions. The organs were incised and a representative sample taken for bacteriologic examination. Sterile instruments were used for culturing each pool.

Depending on the size of each pool, they were either blended in a Waring blendor with nutrient broth or ground with nutrient broth in a mortar with pestle. An equal amount of NaOH (4%) was added to the tissue homogenate.

The sample was shaken and allowed to stand for 15 minutes and the pH adjusted to 7 by adding sufficient HCL (2%). The sample was then centrifugalized and the supernatant removed and seeded on Lowenstein-Jensen medium, Dubos Oleic agar and Middlebrook 7H10 agar.

The tissue pools examined bacteriologically included the following:

A. Right and left submaxillary, right and left medial and lateral retropharyngeal, and right and left parotid lymph nodes.

B. Right and left bronchial (including the right apical bronchial lymph node), and anterior and posterior mediastinal lymph nodes.

C. Mesenteric, colic, hepatic, and forestomach lymph nodes.

E. Lung.

G. Right and left superficial inguinal (supramammary) lymph nodes.

H. Intestine.

I. Liver and spleen.

J. Right and left internal iliac and right and left deep inguinal lymph nodes.

K. Uterus.

8. Histopathologic Technique

Representative tissue was fixed in 10% buffered formalin from each lymph node and organ mentioned above except those

in which tissues had been saved at the time of necropsy. In addition to the above, the following lymph nodes were incised and representative tissue saved for histopathologic examination: right and left prescapular, right and left axillary, right and left prefemoral, right and left ischiatic and right and left popliteal lymph nodes and omentum.

The tissues were embedded in a tissue embedding medium* and cut at 6 microns thickness. The sections were stained with new fuchsin-hematoxylin and eosin (Willigan, Garric and Trosko, 1961).

The following criteria were used to evaluate the tissues: 1) presence and description of gross or microscopic lesions; 2) lesions at the site of inoculation and regional lymph nodes; 3) generalized lesions either by lymphogenous or hematogenous route; 4) extent and distribution of the lesions; 5) progressive or nonprogressive (Feldman, 1943).

B. Aerosol Experiment

1. Source of Animals

Nine purebred Jersey male calves were obtained by Dr. R. M. Scott, ADED, USDA, Lansing, Michigan, from a Michigan herd. The herd had a history of being negative to the caudal fold test using 0.1 ml. of mammalian tuberculin. The calves were negative to comparative cervical tests using

---

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

0.1 ml. of avian tuberculin, 0.1 ml. of mammalian tuberculin and 0.2 ml. johnin. The calves varied in age from 3 to 6 months at the time of inoculation. Approximately two weeks after arrival, the calves were castrated by clamping the spermatic cord with Burdizzo forceps.

2. Isolation Procedures

The calves were divided into 3 groups, 3 animals to an isolation room. Strict isolation procedures as outlined in the intrauterine experiment were followed.

3. Aerosol Apparatus

A rectangular-shaped box large enough to house the animal was used for the experiment (Fig. 1). The box had previously been used for experimental euthanasia and thus contained some of the safety requirements needed for the experiment and required little modification. An inlet 2 inches in outside diameter (O.D.) was placed in the center of the door. This was connected to a tee 1 inch in inside diameter (I.D.). Two extension pipes, 16 inches long (1 inch I.D.) and each drilled with 5 5/8 inch holes, were attached to the tee running vertical to the box. This insured uniform dispersion of the aerosol cloud within the box. An exhaust outlet (1 inch O.D.) was placed in the center of the end of of the box. The inside measurements of the box were 29.5 inches wide by 46.5 inches high by 58 inches long, with a total of 46.04 cubic feet or 1303.67 liters of air displacement.

Fig. 1. Aerosol Apparatus
A. Aerosol chamber
B. Filter
C. Compressor
D. Exhaust tube
E. Inlet tube

Fig. 2. Atomizer-Venturi Apparatus
A. Primary air flow
B. Secondary air flow
C. Atomizer
D. Venturi


TOGRAPHIC LABORATO
Information Services
Michigan State University
East Lansing, Michigan

The external outlet was connected to a filter (Waxler, 1961) (using FG 50 glass-wool mat*) by tygon tubing (1 inch I.D.) and from the filter to the inlet of a two-cylinder compressor. A 25-ft. hose (3/4-inch I.D.) was connected to the outlet of the compressor and to a 25-gal. can of ortho-phenylphenol disinfectant-detergent** (0.5%) to destroy any organisms that might pass through the filter. The hose outlet was placed at the bottom of the can and covered with screening. This caused a breakup of large bubbles and insured better contact of any organism with the disinfectant.

An atomizer-venturi apparatus (Wells et al., 1948) was constructed from glass and mounted on the top of the aerosol box (Fig. 2). This was connected to the inlet on the aerosol box by 6 feet of tygon tubing (2-inch I.D.). The atomizer was calibrated using culture media and 20 lbs. of air pressure. It was found that an average of 15 ml. of culture medium could be atomized per hour (.25 ml./min.). This air will be referred to as the primary air flow.

The secondary air movement which consisted of air passed through the venturi apparatus with the compressor in operation was calibrated. The volume averaged approximately 21.8 L./min. If a culture with a cell count of 10^{8-9} per ml. were used, and loss of viability and change in primary air flow were negligible, there would be approximately 1.14

---

*American Air Filter Company, Louisville, Kentucky.
**Torsite, The Dow Chemical Company, Midland, Michigan.

x $10^7 \pm 10^2$ organisms/L. air/min. flow through the aerosol chamber.

The organisms were suspended in 0.1% bovine albumin and 0.1% Tween 80. This suspension maintains stability of the organisms in the aerosol cloud (Middlebrook, 1952).

Tests were made to determine the effectiveness of the aerosol cloud, and its potential ability to infect lung parenchyma of guinea pigs. The aerosol cloud contained particles less than 5 microns in diameter as demonstrated by an Anderson Air Sampler (Anderson, 1958).

Two tests were made with guinea pigs. The guinea pigs (5 each) were caged in a small wire cage and placed in approximately the same position. The animals' external nares were held for the experiments (17 inches from the floor of the box). The animals were exposed to an aerosol with 1.5 x $10^9 \pm 10^2$ organisms during a time interval of one hour, (.25 x $10^9 \pm 10^2$ per minute) using a culture of M. phlei. After sufficient decay of the aerosol cloud (approximately one-half hour) the animals were removed from the aerosol chamber and immediately euthanatized.

The lung was aseptically removed from the animals and divided into two parts, the anterior part consisted of the anterior half of the left lobe and the right superior and middle lobes, the posterior portion consisted of the posterior half of the left lobe and the right inferior and inferior medial lobes. The tissue was ground in a mortar with pestle and appropriate samples streaked on Middlebrook agar

plates, actidione, chloromycetin and erythromycin in Middlebrook plates and Dubos agar plates both prior to and after NaOH treatment of the ground tissues.

A control group of 5 guinea pigs was euthanatized and the lungs removed and cultured in a similar manner.

Due to the size of the aerosol chamber no attempt was made to maintain constant humidity. Therefore, any variation from day to day in the relative atmospheric humidity could cause a change in the percent viability of the organisms in the aerosol and also possibly the particle size. However, during the course of the inoculations, the relative humidity remained above 70% due to the moisture present in the expired air from the animal.

4. Inoculation of Animals

The calves in each isolation room were inoculated with a different Group-III isolant of bovine origin. The concentration of the organisms in the culture medium was 1 x 10^8 per ml. The total exposure for each animal was approximately 1.5 x $10^9 \pm 10^2$ organisms per hour since approximately 15 ml. of culture medium were used per hour. This amount was more or less arbitrarily selected based principally on previous reports and limited data collected from the pilot studies.

Three calves from an isolation room were inoculated each day with one culture. This allowed the operator time to

disinfect the aerosol chamber, and the contaminated working area, sterilize the atomizer-venturi apparatus and change the filter before inoculation of the next group of animals.

Two persons composed the team necessary to inoculate the calves. The operators wore white coveralls, disposable cap and mask, rubber apron, boots and surgical gloves while operating the aerosol chamber. After the completion of inoculating the three calves, each operator removed his contaminated attire and showered thoroughly.

Four corner tie ropes were attached at the entrance of the box in order to fix the position of the external nares of the calf in proximity to the aerosol cloud. The aerosol chamber was placed outside the isolation room door. The three animals within the isolation room were haltered and each injected with 400 mg. promazine* intravenously approximately ten minutes before placing them in the aerosol chamber. Each calf was backed into the chamber and the four tie ropes attached to the halter. The secondary air flow was allowed to run constantly to maintain a negative pressure away from the aerosol chamber door, thus reducing the danger of aerosol contamination to the operator.

After the calf was secured in position and the door was closed and sealed, the primary air flow was turned on and the timer set for one hour. Following this interval, the primary air flow was stopped and the aerosol cloud was

---

*Sparine, Wyeth Laboratories, Inc., Philadelphia, Pennsylvania.

allowed to decay for ½ hour before opening the aerosol chamber. With minimum contact between the operator and the aerosol chamber and calf, the tie ropes were unfastened and a lead rope tied to the halter. The calf was led from the aerosol chamber into the isolation room and tied to a wall ring. A second calf was backed into the aerosol chamber and the process repeated. The skin of the infected animal, with the exception of the head, was disinfected, using a high pressure (600 pounds per square inch) sprayer* containing orthophenylphenol disinfectant-detergent (0.5%). The head of the animal was scrubbed with a brush and the same disinfectant. After approximately 15 minutes the animal was flushed with warm running water.

5. Clinical Examinations

The same observations as described under the intrauterine experiment were followed, except that rectal examinations were not completed. In addition, lung auscultation was completed in order to detect and follow the clinical course of any pulmonary disturbance.

6. Tuberculin-Test Technique

The tuberculin tests were completed using identical procedures as outlined under the intrauterine experiment.

---

*Liquid Brush, Kleen King, Britt Teck Corporation, Britt, Iowa.

7. Necropsy Technique

The technique described under the intrauterine experiment was followed.

8. Bacteriologic Technique

The same bacteriologic techniques were followed for examination of the tissues as was described in the intrauterine experiment.

However, the following lymph nodes and organs were submitted for bacteriologic examination:

A. Right and left submaxillary, right and left medial and lateral retropharyngeal and right and left parotid lymph nodes.

B. Right and left bronchial (including the right apical bronchial)and anterior and posterior mediastinal lymph nodes.

C. Mesenteric, colic and forestomach lymph nodes.

E. Lung.

H. Intestine (ileum).

I. Liver and spleen.

9. Histopathologic Technique

The techniques were similar to the methods described in the intrauterine experiment.

In addition to the tissues mentioned above, the following were fixed in 10% buffered formalin for histopathologic examination: right and left prescapular, right and left axillary, hepatic, right and left internal iliac, right and left popliteal, right and left superficial inguinal, right

and left deep inguinal, right and left ischiatic, and right and left prefemoral lymph nodes; heart; kidneys and brain.

TABLE 1. PAGINATION OF RESULTS ACCORDING TO ANIMALS AND CULTURES USED

Intrauterine Experiment			Aerosol Experiment		
Animal No.	Culture No.	Page No.	Animal No.	Culture No.	Page No.
74	51C-0	3	91	51C-0	79
75	51C-0	46	84	51C-0	84
77	51C-0	49	90	51C-0	87
73	68C-0	54	82	68C-0	90
70	68C-0	58	86	68C-0	94
71	68C-0	65	83	68C-0	101
78	50B-0	73	89	50B-0	112
79	50B-0	75	87	50B-0	113
80	50B-0	76	88	50B-0	114

RESULTS

A. Intrauterine Experiment

1. Heifers Inoculated with Culture 51C-O

Heifer 74 - T. B. Lab. No. 90 - 63

Clinical History

On the 21st day following inoculation a yellow purulent vaginal discharge was noted. The highest temperature after inoculation was 102.4 F. On the 42nd day after inoculation a rectal examination revealed the following: 1) no indications of pregnancy, but both horns of the uterus were enlarged and the walls thickened, 2) the left ovary was larger than the right and also contained a developing follicle, 3) the cervix was slightly enlarged. The animal continued to lose condition from the time of inoculation until necropsy.

Necropsy Findings (62 days after inoculation)
Guernsey heifer, approximately 24 months old, fair condition, weight 450 lbs.

There were several circumscribed, raised plaques of reddish fibrinoid material scattered over the surface of the omentum. These lesions varied from .5 to 1.5 cm. in diameter.

Three of the mesenteric lymph nodes contained a few small caseous lesions (from 2 to 3 mm. in diameter) within the cortex.

The left supramammary lymph node was markedly enlarged (10 x 7 x 4.5 cm.) and contained a large encapsulated cavity (4 x 3 x 3 cm.) filled with purulent material.

The wall of the uterus was thickened and the mucosal surface was covered with numerous raised nodules. There was yellowish gritty material (from 1 to 3 mm.) in a few of the lesions.

The left ischiatic lymph node was markedly enlarged (3 x 3 x 3 cm. in diameter) and contained a caseous focus approximately 1 cm. in diameter.

Histopathologic Findings

The lesions of the left ischiatic lymph node consisted of several irregularly shaped to circular foci of macrophages and/or epithelioid cells (hereafter often referred to as a granulomatous reaction) with giant cell formation. In many of the inflammatory areas, central caseation necrosis was present and there was a mild polymorphonuclear infiltration.

The lesion of the left supramammary lymph node consisted of a large area of caseation necrosis surrounded by a granulomatous reaction with giant cell formation and early calcification. Peripherally, there was some fibroblastic proliferation.

The colic lymph node had an area of diffuse leukocytic infiltration predominantly in the cortex.

The lumbar lymph node had several microscopic foci of granulomatous reaction some of which had early central necrosis.

The submucosa of the intestine had several foci of necrosis surrounded by a mixed granulomatous reaction.

The lamina propria of the uterus had several developing tuberculous lesions characterized by granulomatous reaction with giant cells. The mucosal epithelium over some of these lesions had undergone necrosis.

Acid-fast bacilli were demonstrated in the lesions of all the lymph nodes and organs studied except the colic and lumbar lymph nodes and the intestine.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (J) the pool of the right and left internal iliac, right and left deep inguinal and lumbar lymph nodes; (G) the right and left supramammary lymph nodes; (I) the pool of the liver and spleen and (K) the uterus.

Group-III mycobacteria were isolated from 4 out of 10 (4/10) tubes from (A), from 2/10 tubes from (B), 2/10 tubes from (C), 6/10 tubes from (J), 3/10 tubes from (G), 10/10 tubes from (K) and 2/10 tubes from (I). Numerous colony forms were isolated similar to the original culture. One appeared to be a Group II which may be an orange-smooth variant of the original culture.

Heifer 75 - T. B. Lab. No. 116 - 63

Clinical History

A purulent vulvar discharge was first observed approximately 2 weeks after inoculation. On rectal examination 43 days after inoculation, there was no evidence of pregnancy and the cervix, uterus and ovaries were apparently normal. The rectal temperature remained normal. There was a progressive loss of weight starting approximately 2 months after inoculation.

Necropsy Findings (124 days after inoculation)
Guernsey heifer, approximately 24 months of age, fair condition, body weight approximately 800 lbs.

The posterior mediastinal lymph nodes had four circumscribed foci of yellowish caseous material 2 mm. in diameter.

The anterior mediastinal lymph node contained three circumscribed foci of yellowish caseous material from 1 to 3 mm. in diameter.

The right and left bronchial lymph nodes had several small caseous foci (1 to 3 mm. in diameter).

There were several reddish-colored plaques of solid tissue from 5 to 10 mm. in diameter on the serosal surface of the spleen.

The wall of the uterus was slightly thicker than normal and the mucosal surface had several yellowish-gray nodules (varying in diameter from 2 to 6 mm.) scattered throughout the mucosa. Many of the lesions had a central core of caseous material.

The left and right supramammary lymph nodes were markedly enlarged and had four circumscribed foci of caseous material from 2 to 8 mm. in diameter.

One of the colic lymph nodes had a circumscribed area of yellowish discoloration revealed on incision.

There were several raised, reddish plaques surrounding the ovaries and fimbriae.

The surface of the omentum was extensively covered with reddish-gray plaques from 2 to 15 mm. in diameter.

Histopathologic Findings

The cortical regions of the right and left bronchial lymph nodes had large areas of caseation necrosis and early calcification surrounded by granulomatous reactions with giant cells. There was an incomplete fibrous connective tissue encapsulation of the lesions.

The lesions of the anterior mediastinal lymph node consisted of several circumscribed foci of granulomatous reaction with giant cells, central caseation and early

calcification. Most of the advanced lesions were partially encapsulated. Confluent with the more advanced lesions were areas of non-caseating granulomas.

The lesions of the posterior mediastinal lymph node consisted of two circumscribed foci of caseation necrosis surrounded by granulomatous reaction. Also in the inflammatory area were a moderate neutrophilic infiltration and some giant cell formation.

A microscopic focus of macrophages was noted in the vicinity of a portal triad of the liver.

The lamina propria of the uterus had extensive areas of lymphocytic and monocytic infiltration. In some areas there was necrosis involving the surface epithelium and some of the adjacent glands. There were a few circumscribed foci of granulomatous reaction with central caseation necrosis beneath the epithelial surface. Occasionally, a similar reaction involved uterine mucosal glands accompanied by marked neutrophilic infiltration and loss of glandular epithelium. There was some increase in fibrous tissue in the lamina propria.

The lesions of the fimbriae consisted of extensive areas of non-caseating granulomas with giant cell formation.

The cortical regions of the right and left supramammary lymph nodes had few circumscribed foci of caseation necrosis, each surrounded by a zone of granulomatous reaction with giant cells. There was some fibroblastic proliferation around the lesions.

The right ischiatic lymph node had a circumscribed focus of granulomatous reaction with early central caseation necrosis. There was also some leukocytic infiltration, giant cell formation and some peripheral fibroblastic proliferation.

Acid-fast bacilli were demonstrated in all the lesions of the lymph nodes and organs studied except the liver.

Bacteriologic Findings

Acid-fast bacteria were recovered from (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (E) the lung; (G) the pool of the right and left supramammary lymph nodes; (J) the pool of the right and left internal iliac, right and left deep inguinal and lumbar lymph nodes; (I) the pool of the liver and spleen and (K) the uterus.

Group-III mycobacteria were isolated from 10 out of 10 (10/10) tubes from (B), 2/10 tubes from (C), 2/10 tubes from (E), 9/10 tubes from (G), 1/10 tubes from (J), 1/10 tubes from (I) and 5/10 tubes from (K).

Heifer 77 - T. B. Lab. No. 151 - 63

Clinical History

Forty-three days after inoculation, a rectal examination revealed the following: 1) adhesions of the left ovary, fimbria and Fallopian tube, 2) an apparently normal uterus,

and 3) no signs of pregnancy. There was some loss of body condition starting approximately 59 days after inoculation. A purulent vulvar discharge was first noted 146 days after inoculation. Rectal temperature was normal throughout the experiment.

A second rectal examination was completed 167 days after inoculation and the following were noted: 1) adhesions between the right fimbria and ovary, 2) a follicular cyst on the left ovary and adhesions between the latter and fimbria, 3) some fluid in both horns of the uterus.

Necropsy Findings (176 days after inoculation)
Guernsey heifer, approximately 29 months old, fair condition, body weight 700 lbs.

The cortical region of the right ischiatic lymph node contained a few circumscribed foci of caseous material from .5 to 2 mm. in diameter.

The left ischiatic lymph node had a few circumscribed caseous lesions in the cortical region from 1 to 5 mm. in diameter.

The right and left medial retropharyngeal lymph nodes were enlarged, one was 7 x 4 x 4.5 cm. and the other 5 x 4 x 4.5 cm. The lymph node contained multiple circumscribed foci of caseous material from 2 to 5 mm. in diameter.

The posterior mediastinal lymph node had several circumscribed caseous foci from 2 to 4 mm. in diameter.

The cortical region of two lymph nodes of the lumbar chain had several circumscribed foci of caseous material from 2 to 4 mm. in diameter.

The internal iliac lymph nodes were markedly enlarged and contained multiple circumscribed foci of caseous material from 1 to 4 mm. in diameter.

The serosal surface of the spleen and particularly the omentum had several raised, reddish circumscribed plaques. When incised, the lesions were solid throughout.

Multiple fibrous adhesions were noted between the serosal surface of the intestine and body wall, and between the liver and diaphragm.

One of the supramammary lymph nodes was markedly enlarged (6 x 2 x 3 cm.). Incision revealed several soft, yellow, caseous foci from 4 to 15 mm. in diameter.

One of the mesenteric lymph nodes had a discrete non-caseous focus of yellowish discoloration (3 mm. in diameter) in the cortex.

The mammary gland was enlarged, firm and had multiple circumscribed foci of caseous material throughout from 2 to 7 mm. in diameter.

The mucosal surface of the intestine had a few raised circumscribed nodules which contained greenish caseous material.

The right and left horns of the uterus were slightly enlarged and the mucosal surface was reddened and contained

numerous yellowish, circumscribed foci of caseous material approximately 2 mm. in diameter.

Histopathologic Findings

The lesions of the uterus were characterized by circumscribed foci of epithelioid cells, with an occasional giant cell, within the lamina propria. In some cases, the lesions were surrounded by lymphocytic infiltration.

The lesions of the supramammary lymph node consisted of several circumscribed and confluent foci of caseation necrosis surrounded by a narrow zone of granulomatous reaction with giant cells. The more advanced lesions were partially encapsulated. In some of the foci of inflammation the caseous material was moderately infiltrated with neutrophils and calcium.

The right and left internal iliac lymph nodes had several circumscribed foci of granulomatous reaction, some of which had early central caseation necrosis. The inflammatory process contained numerous giant cells and early calcification. The more advanced lesions were partially encapsulated.

The right ischiatic lymph node had a few small circumscribed foci of caseation necrosis with early calcification. These were surrounded by narrow zones of granulomatous reaction with giant cell formation. The granulomas were encapsulated.

The left ischiatic lymph node had a few circumscribed foci of granulomatous reaction with giant cells and central caseation necrosis. Some of the lesions were encapsulated.

The right and left medial retropharyngeal lymph nodes had several large circumscribed foci of caseation necrosis surrounded by narrow zones of epithelioid and giant cells. Around the periphery of the lesions were incomplete fibrous connective tissue capsules.

The cortical region of the mediastinal lymph node had a few small circumscribed foci of granulomatous reaction with some giant cells, central caseation necrosis and early calcification.

The lumbar lymph nodes had a few small circumscribed foci of granulomatous reaction with central caseation necrosis and early calcification. The inflammatory reaction contained giant cells and was in the cortical region adjacent to the capsule.

The cortical region of one colic lymph node had a small circumscribed focus of granulomatous reaction with giant cells, central caseation necrosis and calcification. Peripherally, there was an incomplete fibrous connective tissue capsule.

The left prescapular lymph node had a few circumscribed foci of granulomatous reaction with central caseation necrosis and early calcification. Giant cell formation was present in the inflammatory reaction and there was some peripheral fibrous connective tissue proliferation.

The lamina propria of the intestine had a marked diffuse granulomatous reaction and giant cell formation. There were also a marked neutrophilic infiltration and necrosis of much of the intestinal epithelium. There was fibrous connective tissue proliferation in some parts of the lamina propria.

Acid-fast bacilli were present in all lesions of the lymph nodes and organs examined except the uterus.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (C) the pool of the mesenteric, colic, and hepatic lymph nodes; (E) the lung; (G) the pool of the right and left supramammary lymph nodes; (J) the pool of the internal iliac, the right and left deep inguinal and lumbar lymph nodes; (I) the pool of the liver and spleen and (K) the uterus.

Group-III mycobacteria were isolated from ten out of 10 (10/10) tubes from (A), 6/10 tubes from (C), 1/10 tubes from (E), 9/10 tubes from (G), 8/10 tubes from (J), 3/10 tubes from (I) and 5/10 tubes from (K).

2. Heifers Inoculated with Culture 68C-0

Heifer 73 - T. B. Lab. No. 89 - 63

Clinical History

On the 42nd day after inoculation, rectal examination revealed the following: 1) no indication of pregnancy;

2) follicular development on the left ovary; 3) a thickened and enlarged uterus.

The animal had progressively lost weight following inoculation and the rectal temperature had not been observed above 103 F. Approximately 10 days after inoculation, the animal had a purulent vulvar discharge which persisted until the time of necropsy.

Necropsy Findings (62 days after inoculation)
Holstein heifer, approximately 22 months old, poor condition, body weight 400 lbs.

Two of the mesenteric lymph nodes contained several circumscribed foci, from 1 to 4 mm. in diameter, which contained a greenish, caseous material. The cortical region of one lymph node had a solid yellow focus 3 mm. in diameter and another an encapsulated abscess (8 mm. in diameter).

The mucosal surface of the uterus had multiple, raised, grayish-white, nodular foci (Fig. 3) from 2 to 6 mm. in diameter which were solid throughout. A gritty material was demonstrated in some lesions. The mucosal lining was hyperemic and covered with purulent exudate.

Scattered lesions were found over the serosal surface of the omentum and were characterized by multiple, circumscribed, raised, reddish fibrinous plaques from .5 to 1.5 cm. in diameter.

The right supramammary lymph node contained a circumscribed focus of caseous material .5 x 1 cm. in diameter.

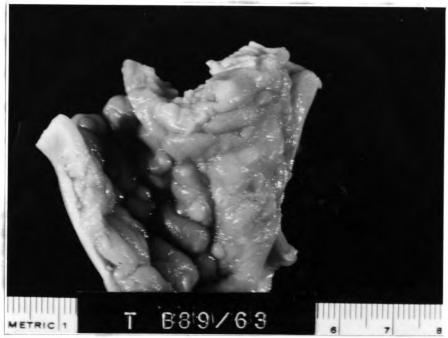

Fig. 3. Segment of uterus from heifer 73, 62 days after inoculation with culture 68C-0. Note raised nodules on mucosal surface.

The left supramammary lymph node contained several small circumscribed foci of caseous material from 2 to 3 mm. in diameter.

Histopathologic Findings

The mesenteric lymph nodes had large, irregular-shaped and circular areas of caseation necrosis and early calcification surrounded by a granulomatous reaction with giant cell formation. There was a marked leukocytic infiltration around some reactive areas. Several noncaseating granulomas (daughter tubercles) extended from the more advanced lesions.

The right medial retropharyngeal lymph node had several foci of granulomatous reaction with few giant cells and some early central caseation necrosis.

The left medial retropharyngeal lymph node had several microscopic foci of granulomas, some of which had early central caseation necrosis.

The right and left supramammary lymph nodes had several caseating and noncaseating granulomas. The larger lesions were encapsulated.

The intestinal submucosa had several foci of a mixed granulomatous reaction. The typical lesion consisted of a central area of necrosis surrounded by a granulomatous reaction, infiltrated with numerous eosinophils. Cross sections of nematodes were observed in some of the lesions.

The omental lesions consisted of granulation tissue within which were microscopic foci of tuberculous granulomas with leukocytic infiltration.

The uterine lesions consisted of foci of developing tuberculous inflammation in the lamina propria beneath the surface epithelium. There was a granulomatous reaction with some evidence of focal necrosis and leukocytic infiltration.

Acid-fast bacilli were demonstrated only in the mesenteric lymph nodes and the uterus.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes, a lesion from (C); (E) the lung; (G) the pool of the right and left supramammary lymph nodes; (J) the pool of the internal iliac, right and left deep inguinal and lumbar lymph nodes; (H) the intestine and (K) the uterus.

Group-III mycobacteria were isolated from nine out of 10 (9/10) tubes from (A), 4/10 tubes from (C), 8/10 tubes from a (C) lesion, 2/10 tubes from (E), 6/10 tubes from (G), 2/10 tubes from (J), 1/10 tubes from (H) and 7/10 tubes from (K).

Heifer 70 - T. B. Lab. No. 117 - 63

Clinical History

A loss of body condition was apparent approximately 42 days after inoculation and persisted until necropsy. On the 43rd day after inoculation a rectal examination revealed

the following: 1) no signs of pregnancy; 2) no developing ovarian follicles or corpora lutea; 3) enlargement of both horns of the uterus, the right horn appearing larger than the left.

A purulent vulvar discharge started approximately 83 days after inoculation and persisted until necropsy. The highest rectal temperature recorded after inoculation was 103 F.

Necropsy Findings

Guernsey heifer, approximately 24 months, fair condition, body weight 700 lbs.

One of the enlarged medial retropharyngeal lymph nodes had several caseous foci (up to 1 mm. in diameter). The other lymph node had similar foci from 2 to 5 mm. in diameter.

One of the lateral retropharyngeal lymph nodes was enlarged and, on incision, had a circumscribed focus of yellowish-white discoloration (approximately 5 mm. in diameter) with streaks of caseous material.

The liver had 2 circumscribed foci of caseous material which were 2 mm. in diameter. In another area of the liver, there was a focus of yellowish discoloration approximately 3 mm. in diameter.

The spleen contained several raised, reddish plaques of solid-appearing tissue over the border and surface. In some instances, the lesions extended into the parenchyma of the organ.

Two lymph nodes of the mesenteric group contained lesions. One had a focus of greenish-yellow material 5 mm. in diameter and the other contained a focus of yellowish caseous material 3 mm. in diameter.

The right and left internal iliac lymph nodes were enlarged and contained several foci of caseocalcareous material 2 to .5 mm. in diameter.

One of the two supramammary lymph nodes was markedly enlarged and contained multiple circumscribed foci of yellowish caseocalcareous material from 2 to 15 mm. in diameter. The other was slightly enlarged and contained two foci of caseocalcareous material (2 and 8 mm. in diameter respectively).

The wall of the uterus appeared to be thickened. There were raised nodules on the mucosal surface some of which had caseous centers and others a yellowish discoloration. There was a purulent exudate over the surface of the mucosa. The cervix was thickened and contained a yellowish circumscribed nodule on the mucosal surface.

The omentum was markedly thickened (up to 2 cm. in some areas) and was extensively covered with raised, reddish nodules (Fig. 4) of tissue which were solid throughout. Similar lesions occurred on the serosal surface of the pelvic cavity.

Histopathologic Findings

The lesions of the submaxillary lymph nodes consisted of circumscribed foci and confluent areas of granulomatous

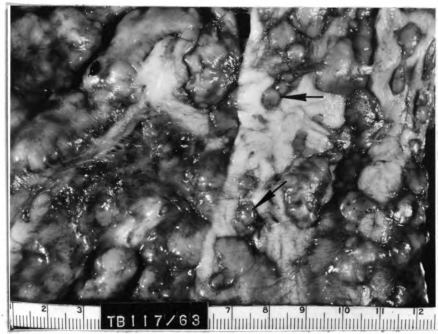

Fig. 4. Omentum from heifer 70, 124 days after inoculation with culture 68C-0. Note the multiple, raised, irregularly shaped nodules in the serosal surface (arrows).

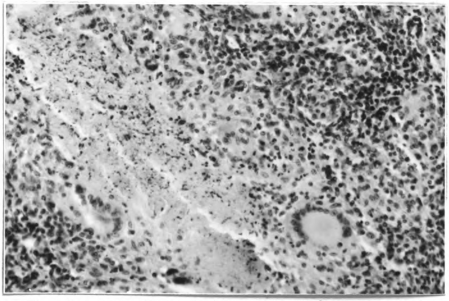

Fig. 5. Omentum from heifer 70, 124 days after inoculation with culture 68C-0. Note the area of necrosis, giant cells, surrounded by lymphoid and epithelioid cells. New Fuchsin--H & E. x187.

reaction with giant cells and some central caseation necrosis. Extending from many of the larger lesions were solid areas of granulomatous reaction typical of "daughter tubercle" formation. In some areas, the advanced lesions were partially encapsulated.

The medial retropharyngeal lymph node had irregular-shaped and circular foci of granulomatous reaction with giant cells scattered throughout the parenchyma. Many of the lesions were coalescing and areas of central caseation necrosis were evident.

The posterior mediastinal lymph node contained extensive areas of coalescing granulomas with giant cell formation. In some areas there was evidence of early caseation necrosis.

The cortical region of the lateral retropharyngeal lymph node had circumscribed foci of granulomatous reaction. There were numerous giant cells and early central caseation was evident.

The mesenteric lymph nodes had several partially encapsulated foci of caseation necrosis and early calcification surrounded by a narrow zone of epithelioid cells and a few giant cells. In another part of the node there was a leukocytic infiltration (predominantly eosinophils).

The omental lesions consisted of solid coalescing areas of granulomatous reaction with giant cells and some areas of early central caseation necrosis. There was a narrow incomplete fibrous connective tissue capsule partially surrounding the lesion.

The internal iliac lymph nodes had extensive areas of granulomatous reaction with giant cells throughout most of the parenchyma. In some areas there was confluence of the inflammatory reaction with caseation necrosis and calcification.

The right deep inguinal lymph node had a few circumscribed microscopic foci of granulomatous reaction and neutrophilic infiltration.

The left deep inguinal lymph node contained a small circumscribed focus of granulomatous reaction in the cortex and adjacent to the capsule. There was central caseation necrosis and giant cell formation.

The lumbar chain of lymph nodes had numerous circumscribed foci of non-caseating granulomas with giant cells. In some of the lesions early caseation necrosis and calcification were evident.

The submucosa of the intestine had an area of caseation necrosis infiltrated with neutrophils and surrounded by a mixed granulomatous reaction (macrophages and neutrophils).

The uterus had a marked granulomatous reaction with some giant cells and central caseation extending from the epithelial surface into the lamina propria. Much of the columnar epithelium over these lesions had undergone necrosis and was absent in some areas. There was considerable lymphocytic and neutrophilic infiltration in the lamina propria particularly in the immediate vicinity of some of the uterine mucosal glands.

The left and right supramammary lymph nodes had circumscribed foci and confluent areas of extensive granulomatous reaction and central caseation necrosis. There were numerous giant cells and early calcification throughout the reaction. Some of the lesions were partially encapsulated.

Acid-fast bacilli were demonstrated in the lesions of all the lymph nodes and organs examined except the mesenteric and left deep inguinal lymph nodes and the intestine.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (E) the lung; (G) the pool of the right and left supramammary lymph nodes; (H) the intestine; (J) the pool of the internal iliac, the right and left internal deep inguinal and lumbar lymph nodes; and (K) the uterus.

Group-III mycobacteria were isolated from seven out of 10 (7/10) tubes from (A), 7/10 tubes from (B), 3/10 tubes from (C), 6/10 tubes from (E), 5/10 tubes from (G), 2/10 tubes from (H), 7/10 tubes from (J) and 3/10 tubes from (K).

Heifer 71 - T. B. Lab. No. 150 - 63

Clinical History

The animal had some loss of condition and a purulent vulvar discharge starting approximately 30 days after inoculation. The rectal temperature remained normal during the experiment. Approximately 43 days after inoculation a rectal examination revealed the following: 1) no detectable signs of pregnancy, 2) no developing ovarian follicles or corpus luteum, and 3) enlargement of the uterine horns with fluid in the left horn.

At 72 and 146 days after inoculation there was a noticeable swelling of the vulva and the left anterior and posterior quarter of the mammary gland respectively. The animal continued to lose weight and condition until necropsy.

A second rectal examination was completed 169 days after inoculation and the following abnormalities were noted: 1) enlargement of both uterine horns with palpable nodules, and 2) the left ovary was enlarged and firm.

Necropsy Findings (176 days after inoculation)
Holstein heifer, approximately 30 months of age, extremely poor condition, body weight 800 lbs.

The left ischiatic lymph node was 4 x 3 x 4 cm. and contained multiple confluent foci of caseous material.

The cortical region of the right ischiatic lymph node contained a circumscribed focus of caseous material 3 to 4 mm. in diameter.

There were several noncaseous, raised, reddish fibrinous plaques on the surface of the omentum from 5 to 15 mm. in diameter.

The right anterior and posterior quarters of the mammary gland was markedly enlarged, cut with difficulty and had extensive circumscribed foci of caseous and purulent material throughout the parenchyma. The lesions were from 3 to 20 mm. in diameter.

The supramammary lymph nodes were markedly enlarged, one was 8.5 x 7 x 5 cm. and the other 6 x 2.5 x 5 cm. There were circumscribed foci and confluent areas of caseous material from 2 mm. in diameter to 2 x 4 x 4 cm.

The uterus was enlarged to approximately 7 cm. in diameter in some areas. The muccsa was congested and the uterine walls were markedly thickened. There were extensive areas of irregularly-shaped nodules some of which had caseous centers (Fig. 6). There were similar lesions on the serosal surface of the uterus. The lumen of the uterus contained approximately 2 L. of purulent material.

The vulva was markedly swollen, firm and had several focal areas of yellow caseous material and purulent exudate from 5 to 15 mm. in diameter. The ovaries were normal except for a few fibrinous adhesions on the surface.

The posterior mediastinal lymph nodes had several circumscribed foci of caseous material from 2 to 10 mm. in diameter.

Fig. 6. Uterus from heifer 71, 176 days after inoculation with culture 68C-0. Note the extensive tuberculous process with caseation (arrows) in the thickened walls.

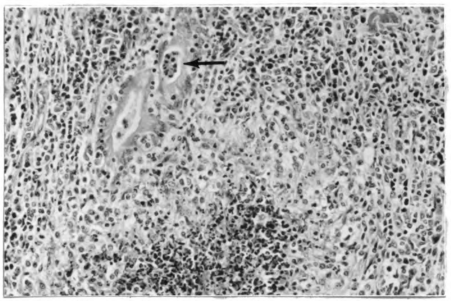

Fig. 7. Uterus from heifer 71, 176 days after inoculation with culture 68C-0. Note the mucosal glands (arrow) filled with neutrophils, epithelioid cells in the lamina propria, and suppuration (l) in the area of the destroyed epithelium of the endometrium. New Fuchsin--H & E. x187.

The right deep inguinal lymph node was slightly enlarged and, in the cortical region, there were several irregularly shaped to circular foci of caseous material from 2 to 4 mm. in diameter.

The left deep inguinal lymph node was markedly enlarged (8 x 4.5 x 5 cm.) and had several circumscribed caseous foci from 3 to 35 mm. in diameter.

Two lumbar lymph nodes were enlarged (4.5 x 3.5 x 3.5 cm.) The lesions consisted of circumscribed foci of caseous material which extended from the cortex into the medulla by linear streaks.

The right and left internal iliac lymph nodes were enlarged to 6.5 x 4 x 6 cm., 3 x 5.5 x 3.5 and 3.5 x 1.75 x 1.5 cm. in diameter. There were multiple irregularly-shaped and circular foci of caseous material in the cortex from 2 to 15 mm. in diameter.

The left and right parotid lymph nodes were slightly enlarged. Circumscribed foci of caseous material were present.

The medial retropharyngeal lymph nodes were markedly enlarged; one was 10 x 7 x 6 cm. and the other 6 x 9 x 4 cm. Multiple irregularly-shaped to circular foci of caseous material were found throughout the lymph nodes.

One of the anterior mediastinal lymph nodes had a circumscribed focus of caseous material which was 6 mm. in diameter.

The left bronchial lymph node had a few circumscribed foci (2 to 4 mm. in diameter) of caseous material.

The lung parenchyma had a few scattered circumscribed foci of caseous material from 3 to 5 mm. in diameter.

Histopathologic Findings

The lesions of the left bronchial lymph node consisted of extensive areas of caseation necrosis surrounded by a narrow zone of granulomatous reaction with some giant cells. The caseous centers had undergone early calcification and there was some neutrophilic infiltration.

The lumbar lymph nodes had circumscribed and confluent areas of caseation necrosis surrounded by a granulomatous reaction with pronounced giant cell formation. The caseous centers were undergoing early calcification.

The lesion of the left ischiatic lymph node consisted of large circumscribed foci and confluent areas of caseation necrosis surrounded by a granulomatous reaction with giant cells.

The right ischiatic lymph node had a circumscribed focus of caseation necrosis with early calcification. The lesion was surrounded by a zone of epithelioid cells with a few giant cells and was partially encapsulated.

Most of the posterior mediastinal lymph nodes had extensive confluent areas of caseation necrosis surrounded by a granulomatous reaction with abundant giant cells and peripheral connective tissue proliferation. The inflammatory reaction also contained some neutrophils.

The lesions in the mammary gland consisted of multiple circumscribed foci and confluent areas of granulomatous reaction. Some of the lesions had central caseation necrosis and neutrophilic infiltration.

The uterine lesions were characterized by a marked infiltration of the lamina propria with epithelioid cells and neutrophils (Fig. 7) and formation of giant cells (Fig. 8). In some areas there was early caseation necrosis and early calcification. The surface epithelium and epithelium of the mucosal glands were absent in many areas.

The pulmonary lesions were partially encapsulated and characterized by coalescing circumscribed foci of caseation necrosis surrounded by a granulomatous reaction with giant cells. In many areas there was extension of the lesions by noncaseating granulomas.

The left deep inguinal lymph node contained multiple circular and irregularly-shaped foci of granulomatous reaction with giant cells, central caseation necrosis and early calcification.

The parotid lymph nodes contained multiple circumscribed foci of granulomatous reaction and neutrophilic infiltration. The larger foci had central caseation necrosis and calcification. The lesions were partially encapsulated and primarily located in the cortical region adjacent to the capsule.

The internal iliac lymph node had circumscribed areas of caseation necrosis surrounded by a granulomatous reaction

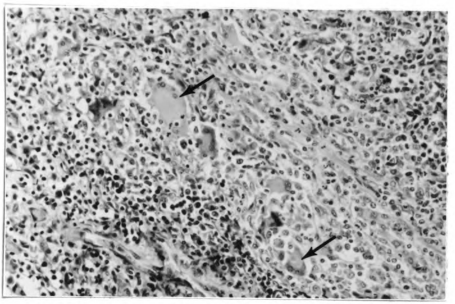

Fig. 8. Another area of the uterus shown in Fig. 7. Note the epithelioid cells, several giant cells (arrows) mixed with leukocytes. New Fuchsin--H & E. x187.

with some giant cells and neutrophils. In some parts of the caseous centers early calcification was noted.

The lesions in the right deep inguinal lymph node consisted of circumscribed foci of caseation necrosis surrounded by epithelioid cells with giant cell formation. In some parts of the caseous centers early calcification was present.

The posterior mediastinal lymph node had circumscribed and coalescing foci of caseation necrosis and early calcification surrounded by granulomatous reactions with giant cells. Extending from these lesions were solid nests of epithelioid cells typical of daughter tubercle formation.

The medial retropharyngeal lymph nodes had circumscribed and coalescing foci of caseation necrosis surrounded by a granulomatous reaction with giant cell formation.

The lesions in the left and right supramammary lymph nodes consisted of several circumscribed foci of granulomas with some giant cells and central caseation necrosis. The lesions were primarily located in the cortical region adjacent to the capsule.

Acid-fast bacilli were demonstrated in lesions in all the lymph nodes and organs examined.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes; (C) the pool

of the mesenteric, colic and hepatic lymph nodes; (E) the lung; (G) the pool of the right and left supramammary lymph nodes; (J) the pool of the right and left internal iliac, right and left deep inguinal and lumbar lymph nodes; (I) the pool of the liver and spleen and (K) the uterus.

Group-III mycobacteria were isolated from nine out of ten (9/10) tubes from (A), 9/10 tubes from (B), 1/10 tubes from (C), 2/10 tubes from (E), 10/10 tubes from (G), 10/10 tubes from (J), 1/10 tubes from (I) and 9/10 tubes from (K).

3. Heifers Inoculated with Culture 50B-0

Heifer 78 - T. B. Lab. No. 91 - 63

Clinical History

The rectal temperature remained normal during the experiment. Some purulent discharge was observed once around the tailhead. Rectal examination was completed 42 days after inoculation and the following were noted: 1) enlargement of the posterior portion of the cervix; 2) enlargement of the left ovary with apparent adhesions between it and the fimbria; 3) enlargement of the left horn of the uterus; 4) no sign of pregnancy.

Necropsy Findings (64 days after inoculation)
Guernsey heifer, approximately 22 months old, good condition, weight 550 lbs.

There were several raised, reddish, fibrinous plaques scattered over the surface of the omentum. The lesions were

solid and had a uniform color throughout. No other gross lesions were detected in this animal.

Histopathologic Findings

The intestine had a focus of mixed granulomatous reaction in the submucosa. The center of this lesion had undergone necrosis and there was moderate leukocytic infiltration around and within the lesion (many eosinophils).

A mesenteric lymph node had a focus of mixed granulomatous reaction (some neutrophils). In another lymph node there was a diffuse neutrophilic infiltration.

The cortical region of the left deep inguinal lymph node had several foci of macrophages and an occasional giant cell.

Acid-fast bacilli were not demonstrated in lesions of any of the lymph nodes and organs examined.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, the right and left parotid, the right and left medial and lateral retropharyngeal lymph nodes; (G) the pool of the right and left supramammary lymph nodes; (J) the internal iliac, the right and left deep inguinal and lumbar lymph nodes; and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from seven out of 10 (7/10) tubes from (A), 6/10 tubes from (G), 9/10 tubes from (J) and 1/10 tubes from (I).

Heifer 79 - T. B. Lab. No. 118 - 63

Clinical History

The animal's temperature remained normal and no vulvar discharge was noted throughout the experiment. On the 42nd day after inoculation the following were noted on rectal examination: 1) no evidence of pregnancy; 2) both ovaries appeared smaller than normal; 3) the cervix was enlarged, particularly the posterior half; 4) the uterus appeared to be normal.

The heifer continued to gain weight and was in excellent physical condition at the time of necropsy.

Necropsy Findings (125 days after inoculation)
Holstein heifer, approximately 26 months old, excellent condition, body weight 800 lbs.

The uterine mucosa was congested and there was considerable mucus in the cervical area. There were small raised yellowish nodules (2 to 3 mm. in diameter) which were uniformly scattered on the surface of the uterine mucosa. No caseous centers were noted in these areas. These findings probably represented normal caruncles and signs of impending estrus.

Histopathologic Findings

The tissue sections of the mesenteric lymph node had one microscopic focus of macrophages and one giant cell. Acid-fast bacilli were not demonstrated.

No microscopic lesions were found in the uterus.

Bacteriologic Findings

Although attempted, no isolations of mycobacteria were made.

Heifer 80 - T. B. Lab. No. 155 - 63

Clinical History

The heifer was in excellent condition and the rectal temperature remained normal throughout the experiment. A rectal examination was completed 43 days after inoculation and the following were noted: 1) the right uterine horn was enlarged; 2) no evidence of pregnancy; 3) the ovaries were normal.

A second rectal examination was completed 169 days after inoculation and the following were noted: 1) the uterus was normal; 2) developing follicle and corpus luteum on the left ovary; 3) the right ovary had questionable adhesions.

Necropsy Findings (181 days after inoculation)
Ayrshire heifer, approximately 30 months of age, excellent condition, body weight approximately 900 lbs.

The uterus was of normal size for a nonpregnant heifer. The mucosal surface appeared to be slightly congested and contained numerous slightly elevated yellowish nodules (2 to 3 mm. in diameter) within the mucosa. These areas revealed no caseous centers. These may have represented uterine caruncles.

No detectable gross lesions were found in the remaining lymph nodes or organs examined.

Histopathologic Findings

No microscopic lesions were detected in any of the organs or lymph nodes, including the uterus. Acid-fast bacilli were not demonstrated.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, the right and left parotid, the right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (E) the lung; and (K) the uterus.

Group-III mycobacteria were isolated from 1 out of 10 (1/10) tubes from (A), 1/10 tubes from (B), 1/10 tubes from (C), 2/10 tubes from (E), and 1/10 tubes from (K). One isolant when injected intradermally into a guinea pig produced a 2 x 5 mm. ulcer in 3 weeks.

B. Aerosol Experiment

1. Potential Infectivity of the Aerosol

Table 2 summarizes the results of the guinea pigs exposed to aerosol using a culture of M. phlei. Acid-fast isolations indistinguishable from the inoculum were recovered before sodium hydroxide treatment. No acid-fast isolations were made following sodium hydroxide treatment.

Trials I and II were conducted with essentially identical techniques.

TABLE 2. SUMMARY OF RESULTS OF BACTERIOLOGICAL EXAMINATION (ACID-FAST ISOLATIONS) OF LUNG TISSUE FROM GUINEA PIGS EXPOSED TO AEROSOL WITH A CULTURE OF M. PHLEI

Lobes of Lung	Infected Animals										Controls				
	Trial I					Trial II									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Anterior	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
Posterior	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-

-No acid-fast isolations.
+Acid-fast recovered on one or more culture media.

2. Calves Exposed to Aerosol with Culture 51C-O

Calf 91 - T. B. Lab. No. 131 - 63

Clinical History

Approximately 21 days after inoculation the animal had a persistent soft cough, seropurulent nasal discharge, a temperature of 103 F, and inappetance (feed consumption was approximately a third of normal). There was also some depression and loss of body weight. A diarrhea first occurred 34 days after inoculation and continued until the time of necropsy. The highest temperature after inoculation was 104 F. The calf continued to lose condition and developed a marked depression, purulent nasal discharge, complete anorexia, persistent soft cough, moist rales and some abdominal breathing. In moribund condition, the animal was euthanatized and necropsy was performed.

Necropsy Findings (44 days after inoculation)
Jersey steer, 7 1/2 months old, poor condition, weight 250 lbs.

The posterior mediastinal lymph node was markedly enlarged (17 x 7 x 4 cm.) and was cut with some difficulty. It had extensive confluent yellowish-gray areas with streaks and circular foci of caseous material. The capsule was thickened.

The left and right bronchial lymph nodes were respectively 9 x 4 x 7 and 8 x 6 x 5 cm. in diameter. The gross lesions were similar to the above.

The anterior mediastinal lymph nodes were markedly enlarged, one to 6 x 3 x 4 cm. and another to 9 x 4 x 4 cm. The gross lesions in the lymph nodes were similar to the above.

Approximately 10% of the liver tissue had a yellowish mottled appearance and the lobular markings were very prominent.

The intestine was markedly congested and there was excessive mucus on the mucosal surface.

The lungs had solid areas of red hepatization in the posterior portions of the apical and cardiac lobes. There were patchy areas of whitish-gray and red hepatization scattered over the surface of the remaining lung tissue. There were numerous circumscribed foci of solid grayish discoloration and caseous material uniformly scattered throughout all lobes of the lungs (Fig. 9). They measured from 1 to 6 mm. in diameter. There was marked interstitial edema of the lungs and mediastinum.

The heart was slightly enlarged and rounded at the apex. There was considerable loss of adipose tissue with serous atrophy, particularly around the base of the heart.

Histopathologic Findings

The cortical regions of the right and left submaxillary lymph nodes had several irregularly shaped to circular foci of caseation necrosis surrounded by granulomatous reaction with giant cells. Extending from these lesions into the

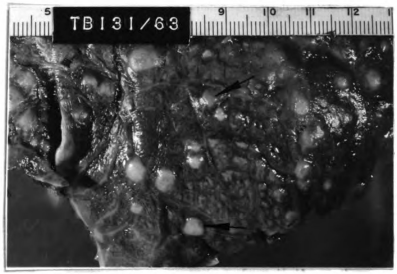

Fig. 9. Lung from calf 91, 44 days after aerosol exposure with culture 51C-0. There are several subpleural tuberculous lesions (arrows).

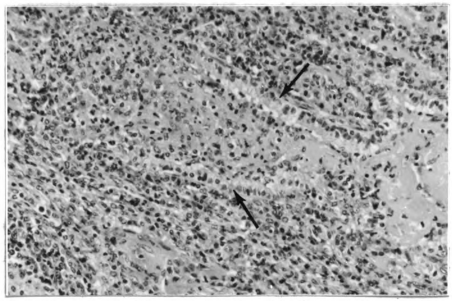

Fig. 10. Lung from calf 91, 44 days after aerosol exposure with culture 51C-0. Note bronchiole (arrows) filled with inflammatory exudate and surrounded by granulomatous process. New Fuchsin--H & E. x187.

deeper cortical and medullary areas were solid foci of granulomatous reaction (early tubercle formation).

The right and left medial retropharyngeal lymph nodes had a few circumscribed foci of granulomatous reaction with giant cells and caseation necrosis in the cortical region adjacent to the capsule.

The left ischiatic lymph node had areas of leukocytic infiltration and numerous multinucleated giant cells. Some of the blood and lymph vessels in the capsule and the subcapsular sinus were filled with inflammatory cells characterized by macrophages and leukocytic cells (predominantly neutrophils).

The anterior and posterior mediastinal and right and left bronchial lymph nodes were almost obliterated by extensive areas of necrosis surrounded by areas of granulomatous response with giant cell formation. The capsules of the lymph nodes were thickened by proliferation of fibrous connective tissue.

The pulmonary lesions consisted of numerous circumscribed foci of granulomatous reaction with central caseation necrosis. The lesions involved both alveoli and bronchioles (Figs. 10 and 11) of the lungs. The alveoli surrounding the lesions were filled with serous exudate and a few alveolar macrophages. In some of the caseous centers hemorrhage was also noted.

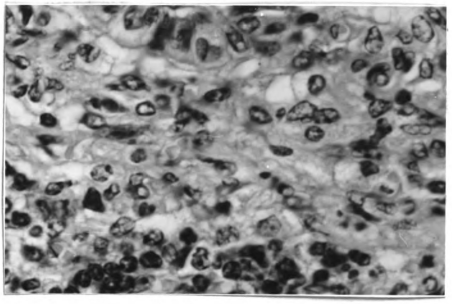

Fig. 11. Higher magnification of the respiratory bronchiole shown in Fig. 10. Note the epithelioid cells, lymphocytes and a few neutrophils. New Fuchsin--H & E. x400.

Acid-fast bacilli were demonstrated in the lesions of all the lymph nodes and organs examined except the left ischiatic lymph node.

Bacteriologic Findings

Acid-fast bacteria were isolated from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial, anterior and posterior mediastinal lymph nodes and (E) the lung.

Group-III mycobacteria were isolated from nine out of ten tubes (9/10) inoculated from (A), 9/10 tubes from (B) and 8/10 tubes from (E).

Calf 84 - T. B. Lab. No. 132 - 63

Clinical History

Approximately 12 days after inoculation the animal had signs of some loss of condition, a soft moist cough and slight seropurulent nasal discharge. The animal had an intermittent fever throughout the course of the experiment which reached a high of 104.6 F. The feed consumption had decreased approximately one-third due to partial anorexia and the animal was depressed. These signs persisted until necropsy. Approximately 40 days after inoculation the animal had a watery, fetid diarrhea and marked depression. Since death was anticipated the calf was euthanatized and necropsy performed.

Necropsy Findings (45 days after inoculation)
Jersey steer, 6 1/2 months old, poor condition, weight about 250 lbs.

The lungs were enlarged and had patchy areas of gray and red hepatization visible over the surface and throughout the incised parenchyma. The pulmonary parenchyma also had extensive irregularly shaped to circular solid grayish areas which in many cases had caseous centers from 2 to 8 mm. in diameter. The interstitial tissue of the lungs was markedly edematous. There was considerable frothy and some purulent exudate in the lumen of the bronchial tree. The interstitial tissue of the mediastinum was markedly edematous.

The heart was slightly enlarged and dilated and there was considerable interstitial edema of the mediastinum.

The posterior mediastinal lymph node was markedly enlarged (50 x 3 x 5 cm.). The architecture of the cortical and medullary regions was obliterated by a yellowish-gray discoloration with streaks and circumscribed foci of caseous material. The capsule was thickened.

The bronchial lymph nodes were markedly enlarged, one being 3.5 x 7 x 5 cm. and the other 4.3 x 1 x 3.5 cm. The gross lesions were similar to the above.

The anterior mediastinal lymph nodes were markedly enlarged, one being 5 x 3 x 3 cm. and another 3 x 2 x 1 cm. The gross lesions were similar to the above.

Histopathologic Findings

The lung had multiple circumscribed granulomas with some central caseation necrosis. Many of the lesions were located in the vicinity of the bronchioles as evidenced by remnants of smooth muscle and epithelium within the inflammatory area. The surrounding alveoli were filled with serous exudate and some alveolar macrophages. The interstitial tissue was markedly edematous and infiltrated in some areas with inflammatory cells (predominantly neutrophils, lymphocytes and monocytes).

The left ischiatic lymph node had two focal areas of necrosis adjacent to the capsule which were infiltrated with some neutrophils.

The lesions in the left and right bronchial and anterior and posterior mediastinal lymph nodes consisted of multiple circumscribed foci and confluent areas of caseation necrosis in the vicinity of the subcapsular region which extended by linear streaks into the medullary region. The areas of necrosis were surrounded by a marked granulomatous reaction with giant cells. The capsule was thickened by proliferation of fibrous connective tissue.

The liver contained a few scattered foci of epithelioid cells, occasional giant cells and some leukocytic infiltration. There was a moderate fatty metamorphosis of the hepatic cells.

Acid-fast bacilli were demonstrated in the lesions of all lymph nodes and organs examined except the left ischiatic lymph node and the liver.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial and anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (E) the lung; (H) intestines and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from one out of ten (1/10) tubes inoculated from (A), 6/10 tubes from (B), 4/10 tubes from (C), 9/10 tubes from (E), 1/10 tubes from (H) and 4/10 tubes from (I).

Calf 90 - T. B. Lab. No. 129 - 63

Clinical History

At approximately 21 days after inoculation, clinical signs such as persistent moist cough, partial anorexia and a general loss of body condition were noted. The feed consumption decreased to approximately one-third the consumption before inoculation. The highest temperature was 106.6 F which was observed 34 days after inoculation. The animal continued to lose weight, and developed dyspnea (characterized by abdominal breathing), marked anorexia, seropurulent

nasal discharge, moist rales and a terminal diarrhea. At 47 days after inoculation, the animal was found dead.

Necropsy Findings (47 days after inoculation)
Jersey steer, 7 1/2 months of age, poor condition, weight 250 lbs.

The left and right bronchial lymph nodes were markedly enlarged and were 7 x 3.5 x 8 cm. and 6 x 3 x 3.5 cm. One of the bronchial lymph nodes was cut with difficulty and the incised surface had an extensive yellowish-gray discoloration with streaks of caseous material extending from the capsular region into the medulla. The other bronchial lymph node had similar changes.

The anterior mediastinal lymph node was markedly enlarged (10.5 x 2 x 4 cm.) and had gross lesions similar to the above.

The posterior mediastinal lymph node was markedly enlarged and was 16 x 3.5 x 7 cm. The lymph node had gross lesions similar to the above.

The lungs were markedly enlarged and had patchy areas of red hepatization throughout the parenchyma. The pleura and pulmonary parenchyma had extensive circumscribed foci of solid yellowish-grayish tuberculous tissue some of which contained caseous foci from 1 to 3 mm. in diameter. There were more lesions in the apical and cardiac lobes of the lungs than in the diaphragmatic lobes. There was a marked interstitial edema in the cardiac and apical lobes. There were

reddish fibrinous tags on the visceral and parietal pleura. There was a hydrothorax consisting of approximately 200 ml. of amber colored fluid.

The heart appeared to be slightly enlarged and rounded at the apex and there was a marked interstitial edema of the mediastinum.

Histopathologic Findings

The lesions in the right and left bronchial and the anterior and posterior mediastinal lymph nodes consisted of large numbers of circumscribed foci and confluent areas of caseation necrosis surrounded by an area of granulomatous reaction with few giant cells. In other sections, the entire lymph node architecture was obliterated by confluent areas of caseation necrosis and granulomas.

The hepatic lobules had centrolobular atrophy and fatty metamorphosis throughout the parenchyma.

The pulmonary lesions were characterized by multiple circumscribed foci of granulomatous reaction with central caseation necrosis. The alveoli surrounding the above lesions contained serous exudate and numerous septal cells and some leukocytes. The lung parenchyma had marked interstitial edema. In some areas of the lung the lesions involved bronchioles as evidenced by strands of smooth muscle and epithelium mixed in the inflammatory reaction.

Acid-fast bacilli were demonstrated in lesions of all lymph nodes and organs examined.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial, and anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (E) the lung; (H) the intestines; and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from 5 out of 10 (5/10) tubes from (A), 8/10 tubes from (B), 1/10 tubes from (C), 9/10 tubes from (E), 1/10 tubes from (H), and 4/10 tubes from (I).

2. Calves Exposed to Aerosol with Culture 68C-0

Calf 82 - T. B. Lab. No. 140 - 63

Clinical History

On the 21st day after inoculation the animal had a soft moist cough, partial anorexia and a slight purulent nasal discharge. At approximately 34 days after inoculation the temperature was 104.2 F and it eventually reached 105 F. The persistent, soft moist cough with moist rales, continual loss of weight and partial anorexia resulted in rapid deterioration of the calf's condition. Due to anticipated death, the animal was euthanatized and a necropsy was performed.

Necropsy Findings (51 days after inoculation)
Jersey steer, very poor condition, weight 150 lbs.

The lungs were markedly enlarged and weighed 4707 gm. Thin slices of lung tissue measuring approximately 1 mm. in thickness were removed from the apical, cardiac and diaphragmatic lobes of the left and right lung and weighed to the nearest milligram. After fixation, the tubercles present in each slice were counted and an average number of tubercles per gram of lung parenchyma was determined. This was found to be .957 tubercles per gram of lung tissue. On the assumption that the distribution was the same throughout the lung there were approximately 4500 tubercles present. The lungs had patchy areas of red and gray hepatization over the surface and throughout the incised parenchyma. There were large numbers of circumscribed grayish-red and yellowish foci over the incised surface of the lung which were either solid or had caseous centers. These lesions were .1 to 1 cm. in diameter.

The posterior mediastinal lymph node was markedly enlarged and was 43 x 12 x 15.5 cm. The node was cut with difficulty and had extensive circumscribed and confluent areas of caseous material obliterating most of the normal architecture of the cortical and medullary regions. The second node was 10 x 3 x 4 cm. and, on incision, similar changes were noted. The capsules were markedly thickened.

The bronchial lymph nodes were markedly enlarged (one was 5.5 x 3 x 4 cm. and the other was 5 x 3 x 3 cm.), with gross lesions similar to the above.

The anterior mediastinal lymph nodes were markedly enlarged (one was 7 x 6 x 7 cm. and the other was 5 x 3 x 3 cm.), with gross lesions similar to the above.

The liver was friable and had extensive lobular markings and a yellowish discoloration.

Histopathologic Findings

One of the lateral retropharyngeal lymph nodes contained a microscopic focus of epithelioid cells adjacent to the capsule.

The right and left parotid lymph nodes had several microscopic foci of epithelioid cells in the peripheral cortical region. There was some infiltration of neutrophils in and around the focal lesions.

The cortical region of the medial retropharyngeal lymph nodes had few microscopic foci of epithelioid cells and neutrophils.

The anterior and posterior mediastinal and right and left bronchial lymph nodes had extensive circumscribed and confluent areas of necrosis obliterating most of the parenchyma and surrounded by a moderate to extensive granulomatous reaction. There was evidence of some early calcification and leukocytic infiltration. Many of the polymorphonuclear cells (predominantly neutrophils) were degenerating. The capsules were thickened by fibrous connective tissue.

The right prescapular lymph node had one microscopic focus of epithelioid cells in the cortical region.

Pulmonary lesions were characterized by circumscribed foci and confluent areas of caseation necrosis surrounded by a marked granulomatous reaction. Many of the lesions appeared to be within the alveolar parenchyma of the lung while others were located in the bronchioles as evidenced by remnants of epithelial or smooth muscle cells. The alveoli surrounding the lesions were filled with a serous exudate, some septal cells and inflammatory cells (neutrophils and lymphocytes). In other areas the visceral pleura of the lung was raised by a marked fibrous connective tissue proliferation in which foci of granulomatous reaction were found. There were very few giant cells in the inflammatory area.

There was fatty metamorphosis of the distal convoluted tubules of the kidneys.

The liver had a marked fatty metamorphosis and early centrolobular coagulation necrosis.

One tissue section of the internal iliac lymph node contained a microscopic focus of epithelioid cells.

The right and left deep inguinal lymph nodes contained microscopic foci of epithelioid cells in the cortical region.

Acid-fast bacilli were demonstrated in lesions of all the lymph nodes and organs examined except the right and left deep inguinal and medial retropharyngeal lymph nodes.

Bacteriologic Findings

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (E) the lung and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from three out of 10 tubes (3/10) from (A), 9/10 tubes from (E) and 8/10 tubes from (I).

Calf 86 - T. B. Lab. No. 173 - 63

Clinical History

The calf had a soft, moist, persistent cough, rales and some loss of body condition starting at approximately 21 days after inoculation. Thirty-five days after inoculation, there was in addition to the above a seropurulent nasal discharge. The rectal temperature of the animal varied throughout the experiment, reaching a high of 103.8 F on the 35th day after inoculation. The animal continued to lose condition and a fetid diarrhea was noticed starting approximately 65 days after inoculation. The animal steadily deteriorated and when death became imminent, euthanasia and necropsy were performed.

Necropsy Findings (76 days after inoculation)
Jersey steer, approximately 9 months of age, poor condition, body weight approximately 250 lbs.

The bronchial lymph nodes were markedly enlarged. One was 7 x 4 x 4.5 cm. and the other 8 x 7 x 7 cm. The lymph

nodes were cut with difficulty and the incised surface had widespread circumscribed foci and confluent areas of yellowish-gray discoloration and caseous material obliterating most of the normal architecture. The capsules of the lymph nodes were thickened.

The anterior mediastinal lymph nodes were markedly enlarged. The first was 5.5 x 4 x 7 cm., the second 6 x 4 x 4 cm., and the third 5.5 x 4 x 4.5 cm. The lymph nodes had gross lesions similar to the above.

The posterior mediastinal lymph nodes were markedly enlarged (Fig. 12). The first was 20 x 6.5 x 8 cm., the second 5 x 3.5 x 4 cm., the third 7 x 4 x 5 cm. and the fourth 5 x 3.5 x 2.5 cm. The lymph nodes had gross lesions similar to the above.

Several mesenteric lymph nodes had irregularly shaped and circular areas of yellowish caseous material 2 to 5 mm. in diameter.

The lungs were enlarged and had patchy areas of red to gray hepatization in all lobes. Many 2-to-3-cm. raised and irregularly shaped to circular caseous areas were found in the pleura. The lung parenchyma was cut with some difficulty and had large numbers of circumscribed foci of caseous material 2 to 30 mm. in diameter. The bronchi contained seropurulent exudate. There was marked interstitial edema involving all lobes of the lungs.

Approximately 50 ml. of serosanguineous fluid were present in the thoracic cavity. There was marked interstitial

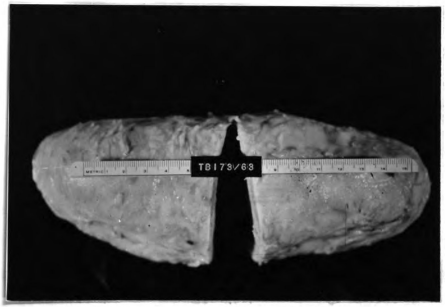

Fig. 12. Posterior mediastinal lymph node from calf 86, 76 days after exposure to culture 68C-0. Note the size of the lymph node.

edema of mediastinal tissue particularly in the vicinity of the heart. The heart was slightly enlarged and rounded at the apex. There was a circumscribed focus of yellowish discoloration approximately 4 cm. in diameter in the left coronary groove, approximately 2.5 cm. from the apex of the heart, in the left ventricle.

Scattered throughout the splenic parenchyma were several circumscribed yellowish foci from 2 to 4 mm. in diameter.

The liver had several yellowish, circumscribed foci (1 to 5 mm. in diameter), some of which had caseous material, scattered throughout the organ.

One of the hepatic lymph nodes had a few circumscribed yellowish foci of caseous material.

The right and left kidneys had several scattered caseous foci, from 2 to 3 mm. in diameter. There were a few large, yellowish, triangular-shaped lesions in the cortex, the apex of which extended to the cortico-medullary junction.

Histopathologic Findings

The pulmonary lesions consisted of several circumscribed foci of caseation necrosis and calcification surrounded by a marked granulomatous reaction. In some of the lesions there was also a marked lymphocytic infiltration. The alveoli surrounding the lesions and some of the bronchioles were filled with neutrophils. Extending from and confluent with the more advanced caseous lesions were non-caseating focal granulomas.

The hepatic lesions consisted of several circumscribed foci of granulomas, some of which had central caseation necrosis. There was diffuse fatty metamorphosis of the liver parenchyma.

The subcapsular sinus of the left ischiatic lymph node in some areas was partially filled with inflammatory cells consisting of macrophages and neutrophils. The lesions were suggestive of embolic origin.

There was a circumscribed microscopic focus of granulomatous reaction and leukocytic infiltration in the left popliteal lymph node.

The left and right medial retropharyngeal lymph nodes had multiple circumscribed foci and confluent areas of noncaseating and caseating granulomatous tissue, in the cortex adjacent to the capsule. The lesions also contained a moderate leukocytic infiltration.

The submaxillary lymph nodes had few circumscribed microscopic foci of granulomatous reaction and leukocytic infiltration.

The lesions in the right and left bronchial and the anterior and posterior mediastinal lymph nodes consisted of diffuse coagulation and caseation necrosis obliterating most of the lymph node parenchyma and surrounded by a narrow zone of granulomatous reaction. There were streaks of calcification, areas of edema and leukocytic infiltration within the areas of necrosis. No evidence of giant cell formation was

present. The capsule was thickened by fibrous connective tissue.

The spleen had several large foci of caseation necrosis surrounded by epithelioid cells and neutrophils. The latter cells also extended into the necrotic areas. The lesions appeared to have their origin within the white pulp.

The gastric lymph nodes were characterized by multiple circumscribed and confluent areas of caseation necrosis surrounded by a granulomatous reaction.

The mesenteric lymph nodes had multiple circumscribed and confluent foci of caseating and non-caseating granulomas.

The cortical region of the hepatic lymph node had many circumscribed foci of granulomatous reaction, some with caseation necrosis. There was also a moderate leukocytic infiltration.

The left prescapular lymph node had a few microscopic foci of epithelioid cells scattered throughout the cortex of the node.

The right and left prefemoral lymph nodes had few microscopic foci of granulomas in the cortical region.

The liver had several microscopic caseating and non-caseating granulomas. A few giant cells were seen in these areas and there was some lymphocytic infiltration into and around the granulomas. There was moderate fatty metamorphosis of the liver parenchyma.

The heart lesion was characterized by a focal area of degenerative changes in the muscle which included increase

of eosinophilic staining, loss of striations, cloudy swelling and in some areas early calcification. The nuclei of many of the muscle cells were pyknotic. In some areas, there was an increase of fibrous connective tissue. Numerous heart myocytes (Anitschkow's cells) were observed in the degenerated area of the heart muscle.

The cortical region of the superficial inguinal lymph nodes had few circumscribed foci of granulomatous reaction. In one of the foci, giant cells were present.

The right ischiatic lymph node lesions had few circumscribed granulomas with central caseation necrosis and some leukocytic infiltration.

The renal lesions consisted of circumscribed granulomas with giant cells and central caseation. There were also large areas of tubular necrosis and early calcification.

Acid-fast bacilli were demonstrated in lesions of all lymph nodes and organs examined except the right and left superficial inguinal and left ischiatic lymph nodes.

Bacteriologic Findings

Acid-fast organisms were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes; (E) the lung; (H) the intestines; and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from nine out of 10 tubes (9/10) from (A), 3/10 tubes from (B), 1/10 tubes from (C), 4/10 tubes from (E), 2/10 tubes from (H) and 1/10 tubes from (I).

Calf 83 - T. B. Lab. No. 172 - 63

Clinical History

At approximately 22 days after inoculation the animal had signs of partial anorexia, a persistent, soft moist cough, and a serous nasal discharge. A rectal temperature of 105 F was noted 35 days after inoculation, but it returned to normal in the terminal stages of the disease. There was considerable loss of body weight starting approximately 21 days after inoculation. A moderate depression and diarrhea started approximately 64 days after inoculation. Due to impending death the animal was euthanatized and necropsy performed.

Necropsy Findings (78 days after inoculation)
Jersey steer, 8 months old, poor condition, weight approximately 150 lbs.

The adipose tissue of much of the carcass, particularly around the base of the heart, was gelatinous in appearance.

Three lymph nodes of the hepatic group were slightly enlarged and had multiple circumscribed foci of caseous material from 3 to 5 mm. in diameter.

The liver was slightly enlarged, rounded at the free edges and contained a number of circumscribed foci of caseous

material 2 to 8 mm. in diameter. There were also circumscribed yellowish foci (approximately 1 x 1.5 x 1 cm.) in the liver which extended from the capsule into the parenchyma and resembled infarction. The liver had prominent lobular markings and a yellow discoloration.

Both kidneys had scattered, circumscribed, yellowish, caseous foci from 2 to 4 mm. in diameter in the cortical and medullary regions (Fig. 18). There were also multiple, well demarcated lesions, resembling infarction, in the cortex consisting of yellowish to greenish cone-shaped foci. The apex of the lesions started at the cortico-medullary junction and at the surface were 1.5 to 2 cm. in diameter.

The parietal and visceral pleura supported numerous, scattered, small, reddish colored, fibrinous plaques over the surface.

The heart was moderately enlarged and rounded at the apex. The epicardial adipose tissue was gelatinous in appearance.

The lungs were markedly enlarged and had scattered, patchy areas of red to gray hepatization over the surface of all lobes. There were numerous circular and irregularly shaped foci of yellow caseous material varying from .2 to 2.5 cm. in diameter (Fig. 13). The lesions were uniformly distributed throughout all lobes. Some of the larger lesions were encapsulated.

One lymph node of the mesenteric group had two circumscribed foci of caseous material from 3 to 5 mm. in diameter.

Fig. 13. Lung from calf 83, 78 days after aerosol exposure with culture 68C-O. Note extensive circumscribed foci and confluence of tuberculous lesions.

One lymph node of the colic group had a few circumscribed foci of caseous material 1 to 2 mm. in diameter.

Lymph nodes of the anterior mediastinal group were markedly enlarged; the first lymph node was 8 x 4.5 x 5 cm., the second, 4.5 x 3.5 x 4 cm. and the third, 7 x 2.5 x 4.5 cm. The lymph nodes were cut with difficulty and the cut surface had extensive circumscribed foci and confluent areas of caseous material obliterating most of the parenchyma.

The posterior mediastinal lymph nodes were also markedly enlarged; the first lymph node was 15.5 x 5 x 7.5 cm., the second lymph node 5 x 3 x 2.5 cm., and the third lymph node 4 x 3 x 2 cm. The lymph nodes had gross lesions similar to the above (Fig. 14).

The right and left bronchial lymph nodes were markedly enlarged, one being 8 x 3 x 5.5 cm., and the other 10 x 5 x 7 cm. The lymph nodes had gross lesions similar to the above.

Histopathologic Findings

The cortical region of the right and left medial retropharyngeal lymph nodes had three microscopic foci of granulomatous reaction and neutrophilic infiltration.

One of the right and left parotid lymph nodes had a small circumscribed focus of granulomatous reaction with early central caseation necrosis. The lesion was infiltrated with neutrophils. Adjacent to this lesion, there were foci composed of solid clusters of epithelioid cells.

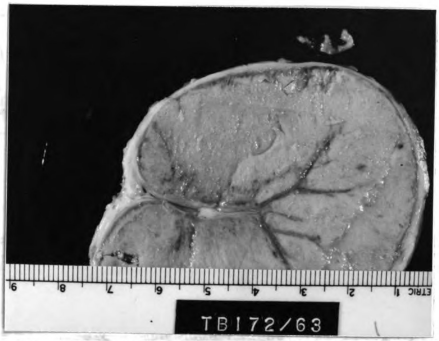

Fig. 14. Posterior mediastinal lymph node from calf 83, 78 days after exposure to culture 68C-0. The normal architecture was obliterated by necrotic tissue.

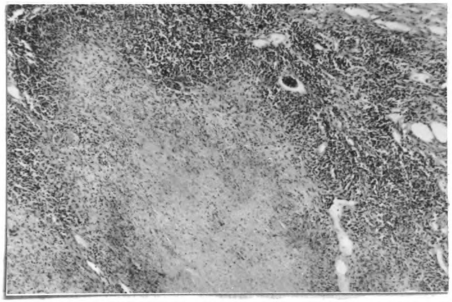

Fig. 15. Posterior mediastinal lymph node from calf 83, 78 days after aerosol exposure with culture 68C-0. Note area of caseous granuloma. New Fuchsin--H & E. x75.

The colic lymph node had several circumscribed foci of caseation necrosis surrounded by zones of granulomatous reaction with some neutrophilic infiltration. Extending from these lesions were several solid clusters of epithelioid cells.

The gastric lymph node had a few circumscribed foci of caseation necrosis surrounded by a granulomatous reaction. In other parts of the node noncaseating granulomas were found.

The mesenteric lymph node had several irregularly shaped and rounded foci of caseation necrosis surrounded by a granulomatous reaction and neutrophilic infiltration.

The pulmonary lesions were characterized by multiple circumscribed foci and confluent areas in which there was granulomatous tissue with central caseation necrosis. The lesions were located in bronchioles, alveoli, and, in some cases, the interstitial tissue. Many of the lesions were surrounded by infiltrates of lymphoid cells. In other areas the alveoli around the lesions were filled with macrophages, some neutrophils and serous fluid. The lung parenchyma had areas of atelectasis and emphysema. There was a marked interstitial edema in which lymphocytic infiltration and fibroblastic proliferation occurred.

The liver had a large circumscribed focus of caseation necrosis surrounded by a narrow zone of epithelioid cells and lymphocytes. The parenchymal cells of the liver had undergone cloudy swelling and early fatty metamorphosis.

Throughout the parenchyma were microscopic foci of monocytic and lymphocytic infiltration.

The spleen had a microscopic focus of coagulation necrosis in which there was an infiltration of neutrophilic cells and a few epithelioid cells.

The right popliteal lymph node had a small circumscribed focus of caseation necrosis surrounded by a narrow zone of epithelioid cells. Only an occasional giant cell could be found in the inflammatory reaction.

The left ischiatic lymph node had a circumscribed focus of caseation necrosis surrounded by a narrow zone of epithelioid and some neutrophilic cells. An occasional giant cell was noted.

The right prefemoral lymph node had two cortical foci of granulomatous reaction. Both of these lesions had early central caseation necrosis.

The left axillary lymph node had a circumscribed focus of caseation necrosis surrounded by a narrow zone of epithelioid cells, neutrophils and occasional giant cells.

The architecture of the anterior and posterior mediastinal and right and left bronchial lymph nodes was almost obliterated by circumscribed and confluent areas of caseation necrosis surrounded by narrow zones of epithelioid cells which themselves had undergone considerable necrosis (Figs. 15, 16 & 17). The inflammatory area also contained some neutrophils and lymphocytes. There was evidence of scattered areas of early calcification throughout the caseous centers.

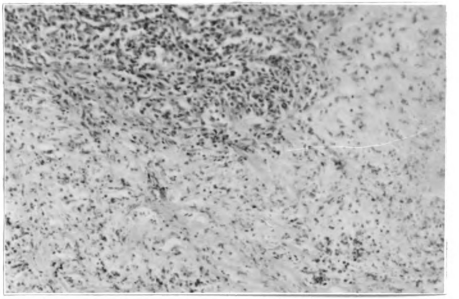

Fig. 16. A different area of the same lymph node as in Fig. 15. Note the extensive cellular necrosis of the zone bordering the area of caseation necrosis. New Fuchsin--H & E. x187.

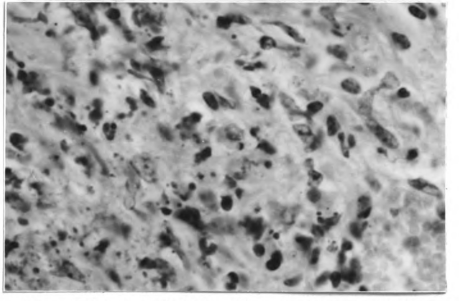

Fig. 17. Higher magnification of zone of cellular necrosis shown in Fig. 16. There is extensive pyknosis and karyorrhexis. New Fuchsin--H & E. x400.

Fig. 18. Kidney from calf 83, 78 days after aerosol exposure to culture 68C-0. Note infarcted area (1) and several subcapsular tuberculous lesions (arrows).

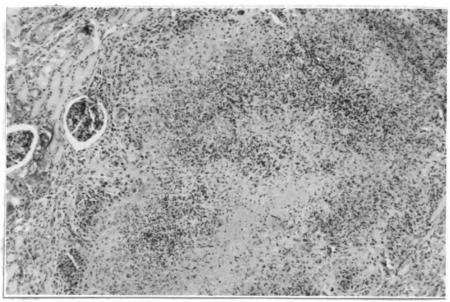

Fig. 19. Kidney from calf 83, 78 days after aerosol exposure to culture 68C-0. There is a large area of caseation necrosis surrounded by a zone of granulomatous reaction. New Fuchsin--H & E. x75.

The capsules of the lymph nodes were thickened by fibrous connective tissue. Occasionally a giant cell was noted in the inflammatory areas.

The hepatic lymph node had several irregularly shaped to circular foci of caseation necrosis surrounded by a zone of epithelioid cells with some neutrophils and early calcification in the caseous centers.

The subcapsular renal lesions consisted of circumscribed foci of caseation necrosis surrounded by narrow zones of granulomatous reaction (Fig. 19). In other parts of the cortex, there were wide areas of tubular necrosis and calcification (Figs. 20 & 21).

Acid-fast bacilli were demonstrated in the lesions of all lymph nodes and organs except the right popliteal and prefemoral lymph nodes and spleen.

Bacteriologic Findings

Acid-fast organisms were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial, and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes, (E) the lung; (H) the intestine; and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from ten out of 10 tubes (10/10) from (A), 5/10 tubes from (B), 2/10 tubes from (C), 10/10 tubes from (E), 1/10 tubes from (H) and 2/10 tubes from (I).

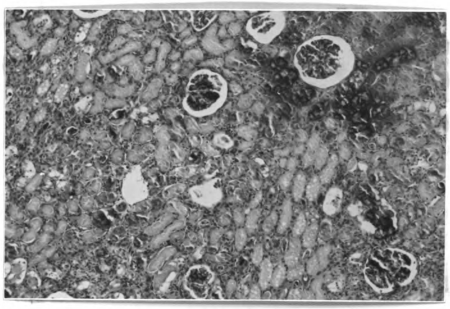

Fig. 20. Kidney from calf 83, 78 days after aerosol exposure with culture 68C-0. Note the extensive tubular degeneration and calcification. New Fuchsin--H & E. x75.

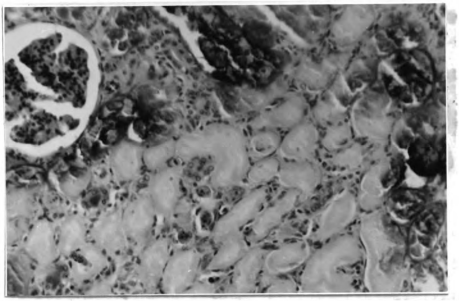

Fig. 21. Higher magnification of tubules in Fig. 20. There is a marked coagulative necrosis and karyolytic loss of nuclei of the renal tubules. New Fuchsin--H & E. x187.

3. Calves Exposed to Aerosol with Culture 50B-0

Calf 89 - T. B. Lab. No. 146 - 63

Clinical History

The animal had no clinical signs of disease throughout the course of the experiment. The rectal temperature remained normal.

Necropsy Findings (64 days after inoculation)
Jersey steer, 8 months old, excellent condition, weight 450 lbs.

There were no detectable gross lesions in any of the lymph nodes or organs examined.

Histopathologic Findings

The liver had a few circumscribed foci of necrosis surrounded by an eosinophilic infiltration. Acid-fast bacilli were not demonstrated.

There were no detectable microscopic lesions in the remaining organs and lymph nodes. Acid-fast bacilli were not demonstrated.

Acid-fast bacteria were recovered from (A) the pool of the right and left submaxillary, right and left parotid, right and left medial and lateral retropharyngeal lymph nodes; (B) the pool of the right and left bronchial, and the anterior and posterior mediastinal lymph nodes; (C) the pool of the mesenteric, colic and hepatic lymph nodes, (E) the lung; and (I) the pool of the liver and spleen.

Group-III mycobacteria were isolated from one out of 10 (1/10) tubes from (A), 3/10 tubes from (B), 1/10 tubes from (C), 4/10 tubes from (E) and 1/10 tubes from (I).

Calf 87 - T. B. Lab. No. 228 - 63

Clinical History

There were no signs of disease throughout the course of the experiment.

Necropsy Findings (128 days after inoculation)
Jersey steer, weight approximately 600 lbs, excellent physical condition.

There were no detectable gross lesions in any of the organs or lymph nodes examined.

Histopathologic Findings

There were no detectable microscopic lesions in any of the organs or lymph nodes examined. Acid-fast bacilli were not demonstrated.

Bacteriologic Findings

Acid-fast bacteria were recovered from (B) the pool of the right and left bronchial and anterior and posterior mediastinal lymph nodes. Group-III mycobacteria were isolated from all ten tubes of culture media used (10/10).

Calf 88 - T. B. Lab. No. 294 - 63

Clinical History

The calf had no signs of overt disease during the course of the experiment.

Necropsy Findings (188 days after inoculation)

Jersey steer, excellent condition, weight approximately 450 lbs, approximately 12 months of age.

The cut surface of some of the mesenteric lymph nodes exuded a white fluid resembling chyme. No other gross lesions were noted in the animal.

Histopathologic Findings

No microscopic lesions were found in any of the organs or lymph nodes examined. Acid-fast bacilli were not demonstrated.

Bacteriologic Findings

Acid-fast bacteria were recovered from (B) the pool of the right and left bronchial and the anterior and posterior mediastinal lymph nodes. Group-III mycobacteria were recovered from seven of the ten tubes of culture media used (7/10).

C. Tuberculin Sensitivity Studies

Tables 3, 4 and 5 contain the tuberculin test results for the intrauterine experiment. All test measurements were recorded in millimeters and corrected by subtraction of the zero hour skin thickness from skin thickness at 24, 48 and 72 hours after injection of the tuberculin.

Tables 6, 7 and 8 contain the tuberculin test results of the aerosol experiment.

TABLE 3. Tuberculin Test Results of Inoculating Heifers by Intrauterine Route with Culture 51C-0

Animal No.	Days after Inoc.	Anterior 0.1 ml. Avian O.T.				Cervical Middle 0.1 ml. Mam. O.T.				Posterior 0.2 ml. Johnin				Comparative Mammalian-Avian			Caudal Fold 0.1 ml. Mam. O.T.			
		0	24	48	72	0	24	48	72	0	24	48	72	24	48	72	0	24	48	72
74	47	3*	12	19	22.5	3.5	10	19	21	3.5	5.5	10	9				3	9	13	12
			9	16	19.5		6.5	15.5	17.5		2	6.5	5.5	-2	0	-1.5		6	10	9
75	47	5	9	10	12	4	18	29	32	4.5	7	7	6				4.5	11	6	14
			4	5	7		14	25	28		2.5	2.5	1.5	10	20	21		6.5	1.5	9.5
	110	5.5	13	12.5	11	6.5	18	15.5	15	5.5	7.5	7	7				4	6	8	9
			7.5	7	5.5		11.5	9.0	9.5		2.0	1.5	1.5	4	2	4		2	4	5
77	47	5.5	9.5	12	15	4	13	21	28	4	6.5	6	7				4	8	12	13
			4	6.5	9.5		9	17	24		2.5	2	3	5	10.5	14.5		4	8	9
	110	6.5	7	8.5	9	5.5	10	16	15	4	5	5	5				5.5	6.5	9.5	10
			0.5	2	2.5		4.5	10.5	9.5		1	1	1	4	8.5	7		1	4	4.5
	167	7	9	8.5	11	6	9	9.5	10	4	6	5	6				6	8	10.5	5x41
			2	1.5	4		3	3.5	4		2	1	2	1	2	0		2	4.5	---**

*Measurements were recorded in millimeters of skin thickening using a dermal thickness gauge.
**Not determined due to dimensions of tuberculin reaction.

TABLE 4. Tuberculin Test Results of Inoculating Heifers by Intrauterine Route with Culture 68C-0

Animal No.	Days after Inoc.	Anterior 0.1 ml. Avian O.T.				Cervical Middle 0.1 ml. Mam. O.T.				Posterior 0.2 ml. Johnin				Comparative Mammalian-Avian			Caudal Fold 0.1 ml. Mam. O.T.			
		0	24	48	72	0	24	48	72	0	24	48	72	24	48	72	0	24	48	72
73	47	4.5*	7	9.5	11	4	15	24	28	4.0	5.5	5.0	6.5				4	7	14	12
			2.5	5	6.5		11	20	24		1.5	1	2.5	8.5	15	17.5		3	10	8
70	47	5.5	10	11.5	11.5	5	18	21	19.5	3.5	7.5	7	7				4	9	11	13
			4.5	6	6		13	16	14.5		4	3.5	3.5	8.5	10	7.5		5	7	9
	110	6	9	8.5	9	4	13	16	17	3.5	7	7.5	6				4.5	9	12	15
			3	2.5	3		9	12	13		3.5	1.5	2.5	6	9.5	10		4.5	7.5	7.5
71	47	7	10	16	18	6	16	32	29	4.5	7	8	8.5				4.5	7	14	17
			3	9	11		10	26	23		2.5	3.5	4	7	17	12		2.5	9.5	12.5
	110	6	18.5	31	30	4	24	61	60	4	7	12	14				4	25	42x	47x
																			72x	71x
																			47	44
			12.5	25	24		20	57	56		3	8	10	7.5	32	32		21	---	---
	167	10	20	22	25	6	65x	110x	110x	6	11	8	14				9	23x	38x**	36x**
							120	175	185									102x	82x	84x
																		25	32	41
			10	12	15		---***				5	2	8		---				---	

*Measurements were recorded in millimeters of skin thickening using a dermal thickness gauge.
**Ulceration on the surface of the skin.
***Not determined due to dimensions of tuberculin reaction.

TABLE 5. Tuberculin Test Results of Inoculating Heifers by Intrauterine Route with Culture 508-O

Animal No.	Days after Inoc.	Anterior 0.1 ml. Avian O.T.				Cervical Middle 0.1 ml. Mam. O.T.				Posterior 0.2 ml. Johnin				Comparative Mammalian-Avian			Caudal Fold 0.1 ml. Mam. O.T.			
		0	24	48	72	0	24	48	72	0	24	48	72	24	48	72	0	24	48	72
78	47	9*	11	10	9	8	9	8	7	6	7	7	5.5				5	5	5	5
			2	1	0		1	0	-1		1	1	-.5	-1	-1	-1				
79	47	6	7.5	6	6	5	6	5	5	4.5	6.5	5	4.5				3	4	4	4.5
			1.5	0	0		1	0	0		2	0.5	0	-.5	0	0		1	1	1.5
	110	6.5	9.5	9	8	5	7	5.5	5.5	4	6	6	5.5				4	5	4	4.5
			3	2.5	1.5		2	0.5	0.5		2	2	1.5	-1	-2	-1		1	0	0.5
80	47	6	7	7	7	5	5.5	5	5	5.5	6.5	6	5				4	4	4.5	4.5
			1	1	1		0.5	0	0		1	0.5	-0.5	-.5	-1	-1		0	0.5	0.5
	110	6	10	10	11.5	5	7	6	7	4	5	5	5.5				4.5	4	4	5.5
			4	4	5.5		2	1	2		1	1	1.5	-2	-3	-3.5		-.5	-.5	1
	167	7	9.5	8.5	12	6.5	7	6.5	7.5	5	6	6	6				4	4	4	3.5
			2.5	1.5	5		0.5	0	1		1	1	1	-2	1.5	-4		0	0	-.5

*Measurements were recorded in millimeters of skin thickening using a dermal thickness gauge

TABLE 6. Tuberculin Test Results of Inoculating Calves by Aerosol With Culture 51C-O

Animal No.	Days after Inoc.	Cervical Anterior 0.1 cc. Avian				Cervical Middle 0.1 cc. Mam.				Posterior 0.2 cc. Johnin				Comparative Mammalian-Avian			Caudal Fold 0.1 cc. Mam.			
		0	24	48	72	0	24	48	72	0	24	48	72	24	48	72	0	24	48	72
91	41	5.5*	9	8.5	10	3.5	34.0	44	81x	3	7	---**	---						11x	11x
									77								25	14	51x	53x
																			14	16
			3.5	3	4.5		30.5	40.5	---***		4	---	---	27	37.5	---		11.5	---	---
84	41	4	6	7	7.5	3.5	59	70x	74x	3	3	---	---				25	14	13x	26x
								124	130										15x	20x
																			50	58
			2	3	3.5		55.5	---	---		0	---	---	53.5	---	---		11.5	---	---
90+																				

*Measurements were recorded in millimeters of skin thickening using a dermal thickness gauge.
**Not determined due to size of mammalian tuberculin reactions.
***Unable to determine due to size of mammalian tuberculin reactions.
+No tuberculin tests due to death of animal.

TABLE 7. Tuberculin Test Results of Inoculating Calves by Aerosol with Culture 68C-O

Animal No.	Days after Inoc.	Cervical Anterior 0.1 ml. Avian				Cervical Middle 0.1 ml. Mam.				Cervical Posterior 0.2 ml. Johnin				Comparative Mammalian-Avian			Caudal Fold 0.1 mm. Mam.			
		0	24	48	72	0	24	48	72	0	24	48	72	24	48	72	0	24	48	72
83	41	6*	7	7	7	4	64	120x 180	125x 180	3	3	---**	---				3	15.5	22	17
			1	1	1		60	---***	---		0	---	---	59	---	---		12.5	17	14
86	41	7	23	25	18	5	45	95x 115	95x 120	4	11	17	---				3	20x 47x 47	42x 25x 50	41x 21x 41
			16	18	11		40	---	---		7	13	---	24	---	---		---	---	---
82	41	5	6	6	5.5	4	16	16	10	3.5	3.5	3.5	3.5				3	6.5	6	5
			1	1	0.5		12	12	6	0	0	0	0	11	11	5.5		3.5	3	2

*Measurements were recorded in millimeters of skin thickening using a thermal thickness gauge.
**Not determined due to overlapping by the mammalian tuberculin reactions.
***Unable to determine due to size of mammalian tuberculin reactions.

TABLE 8. Tuberculin Test Results of Inoculating Calves by Aerosol with Culture 50B-O

Animal No.	Days after Inoc.	Anterior 0.1 cc. Avian				Cervical Middle 0.1 cc. Mam.				Posterior 0.2 cc. Johnin				Comparative Mammalian-Avian			Caudal Fold 0.1 cc. Mam.			
		0	24	48	72	0	24	48	72	0	24	48	72	24	48	72	0	24	48	72
89	41	6.5*	9	9	10	6	6	6.5	8	4.5	4.5	6	4.5				3.5	3	3	3
			2.5	2.5	3.5		0	0.5	2		0	1.5	0	-2.5	-2.0	-1.5		-.5	-.5	-.5
87	41	7	7	7	9	5	6	6	6	4	6	6	4				3	5	5	5
			0	0	2		1	1	1		2	2	0	1	1	-1		2	2	2
	90	6	8	7	8	5.5	7	6.5	6.5	4.5	5	6	5				4	5	4	4
			2	1	2		1.5	1	1		0.5	1.5	0.5	-.5	0	1		1	0	0
88	41	5	9	10	10	4	5	4	4	3.5	4	3.5	3.5				3.5	3.5	3.5	3.5
			4	5	5		1	0	0		0.5	0	0	-3	-5	-5		0	0	0
	90	4.5	6	9	10	4	6	5.5	4.5	3.5	5	4.5	4				3.5	3	3.5	3.5
			1.5	4.5	5.5		2	1.5	0.5		1.5	1.0	0.5	0.5	-3	-5		-.5	0	0
	174	5	6.5	7	9	4.5	6	5	5	4	5	5	4.5				3	4	4	3.5
			1.5	2	4		1.5	0.5	0.5		1	1	0.5	0	-1.5	-3.5		1	1	0.5

*Measurements were recorded in millimeters of skin thickening using a dermal thickness gauge.

TABLE 9. Location of Macroscopic and Microscopic Lesions from Intrauterine Experiment

Culture	Animal No.	Days after Inoc.	Macroscopic Lesions	Microscopic Lesions
51C-0	74	62	Mesenteric, L supramammary, L ischiatic lymph nodes; peritoneum and uterus.	L ischiatic, L supramammary, colic and lumbar lymph nodes; uterus
51C-0	75	124	Ant. and post. mediastinal, R & L bronchial, R & L supramammary, colic lymph nodes; uterus, peritoneum and fibria.	R & L bronchial, ant. and post. mediastinal, R & L supramammary, R ischiatic lymph nodes; liver and uterus.
51C-0	77	176	R & L ischiatic, R & L med. retropharyngeal, post. mediastinal, lumbar, R & L int. iliac, R & L supramammary, mesenteric lymph nodes; peritoneum, mammary gland and uterus.	R & L supramammary, R & L int. iliac, R & L ischiatic, R & L med. retropharyngeal, post. mediastinal, lumbar, colic, L prescap. lymph nodes; uterus and intestine.
68C-0	73	62	Mesenteric, R & L supramammary lymph nodes; uterus and peritoneum.	Mesenteric, R & L med. retropharyngeal, R & L supramammary lymph nodes; peritoneum and uterus.
68C-0	70	124	R & L med. retropharyngeal, R & L lateral retropharyngeal, mesenteric, R & L int. iliac, R & L supramammary lymph nodes; liver, peritoneum, uterus and cervix.	R & L submaxillary, R & L medial retropharyngeal, post. mediastinal, R & L lat. retropharyngeal, mesenteric, R & L int. iliac, R & L deep inguinal, lumbar lymph nodes; liver, uterus and cervix.
68C-0	71	176	R & L ischiatic, R & L supramammary, ant. and post. mediastinal, R & L deep inguinal, lumbar, R & L int. iliac, R & L parotid,	L bronchial, lumbar, R & L ischiatic, ant. and post. mediastinal, R & L deep inguinal, R & L parotid, R & L int. iliac, R & L med. retro-

TABLE 9 (continued)

Culture	Animal No.	Days after Inoc.	Macroscopic Lesions	Microscopic Lesions
			R & L med. retropharyngeal, L bronchial lymph nodes; mammary gland, uterus, vulva, peritoneum and lung.	pharyngeal, R & L supramammary lymph nodes; mammary gland, uterus and lung.
50B-0	78	64	Peritoneum.	L deep inguinal lymph node.
50B-0	79	125	NGL.	Mesenteric lymph node.
50B-0	80	181	NGL.	NML.

NGL - No detectable gross lesions.
NML - No detectable microscopic lesions.

TABLE 10. Summary of Bacteriological Results (Acid-Fast Isolations) From Heifers with Intrauterine Inoculation

Culture	Animal No.	Days after Inoc.	Lymph Nodes and Organ Pools*									Contamination
			A	B	C	E	G	H	I	J	K	%
51C-0	74	62	4**/10***	2/10	2/10		3/10	---	2/10	6/10	10/10	40
51C-0	75	124	---	10/10	2/10	2/10	9/10	---	1/10	1/10	5/10	30
51C-0	77	176	10/10	---	6/10	1/10	9/10	---	3/10	8/10	5/10	40
68C-0	73	62	9/10	---	4/10 8/10#	2/10	6/10	1/10	---	2/10	7/10	27
68C-0	70	124	7/10	7/10	3/10	6/10	5/10	2/10	---	7/10	3/10	35
68C-0	71	176	9/10	9/10	1/10	2/10	10/10	---	1/10	10/10	9/10	23
50B-0	78	64	7/10	---	---	---	6/10	---	1/10	9/10	---	26
50B-0	79	125	---	---	---	---	---	---	---	---	---	49
50B-0	80	181	1/10	1/10	1/10	2/10	---	---	---	---	1/10	32

*A - R & L submaxillary, R & L parotid, R & L medial and lateral retropharyngeal lymph nodes.
B - R & L bronchial, ant. and post. mediastinal lymph nodes.
C - Mesenteric, colic, hepatic lymph nodes.
E - Lung.
G - R & L supramammary lymph nodes.
H - Intestine.
I - Liver and spleen.
J - R & L internal iliac, R & L deep inguinal, lumbar lymph nodes.
K - Uterus.
**Numerator - no. of tubes positive for acid-fast isolations.
***Denominator - total no. of tubes used for acid-fast isolations.
#C lesion.

TABLE 11. Location of Macroscopic and Microscopic Lesions of Calves Inoculated by Aerosol

Culture	Animal No.	Days after Inoc.	Macroscopic Lesions	Microscopic Lesions
51C-0	91	44	R & L bronchial, ant. and post. mediastinal lymph nodes; lung.	R & L submaxillary, R & L mediastinal retropharyngeal, L ischiatic, R & L bronchial, ant. and post. mediastinal lymph nodes; lung.
51C-0	84	45	R & L bronchial, ant. and post. mediastinal lymph nodes; lung.	L ischiatic, R & L bronchial, ant. and post. mediastinal lymph nodes; liver and lung.
51C-0	90	47	R & L bronchial, ant. and post. mediastinal lymph nodes; lung.	R & L bronchial, ant. and post. mediastinal lymph nodes; lung.
68C-0	82	51	R & L bronchial, ant. and post. mediastinal lymph nodes; lung.	R & L lat. retropharyngeal, R & L parotid, R & L mediastinal, retropharyngeal, ant. and post. mediastinal, R & L bronchial, R prescapular, R & L internal iliac, R & L deep inguinal lymph nodes; lung.
68C-0	86	76	R & L bronchial, mesenteric, ant. and post. mediastinal, and hepatic lymph nodes; lung, liver, spleen and kidney.	R & L ischiatic, L popliteal, R & L mediastinal retropharyngeal, R & L submaxillary, R & L bronchial, ant. and post. mediastinal, stomach, mesenteric, hepatic, R & L superficial inguinal, L prescapular, R & L prefemoral, colic lymph nodes; kidney, liver and lung.

TABLE 11 (continued)

Culture	Animal No.	Days after Inoc.	Macroscopic Lesions	Microscopic Lesions
68C-0	83	78	Hepatic, mesenteric, colic, ant. and post. mediastinal, R & L bronchial lymph nodes; liver, kidney and lung.	R & L mediastinal retropharyngeal, R & L parotid, ant. and post. mediastinal, colic, stomach, mesenteric, R popliteal, L ischiatic, R prefemoral, L axillary, R & L bronchial, hepatic lymph nodes; lung, liver and spleen.
50B-0	89	64	NGL	NML
50B-0	87	128	NGL	NML
50B-0	88	188	NGL	NML

NGL - No detectable gross lesions.
NML - No detectable microscopic lesions.

TABLE 12. Summary of Bacteriologic Results (Acid-Fast Isolations) of Inoculating Calves by Aerosol

Culture	Animal No.	Days after Inoc.	Lymph Nodes and Organ Pools*						Contamination
			A	B	C	E	H	I	%
51C-0	91	44	9**/10***	9/10	---	8/10	---	---	20
51C-0	84	45	1/10	6/10	4/10	9/10	1/10	4/10	15
51C-0	90	47	5/10	8/10	1/10	9/10	1/10	4/10	20
68C-0	82	51	3/10	---	---	9/10	---	8/10	45
68C-0	86	76	9/10	3/10	1/10	4/10	2/10	1/10	20
68C-0	83	78	10/10	5/10	2/10	10/10	1/10	2/10	21
50B-0	89	64	1/10	3/10	1/10	4/10	---	1/10	43
50B-0	87	128	---	10/10	---	---	---	---	25
50B-0	88	188	---	7/10	---	---	---	---	10

*A - R & L submaxillary, R & L parotid, R & L medial and lateral retropharyngeal lymph nodes.
B - R & L bronchial, ant. and post. mediastinal lymph nodes.
C - Mesenteric, colic, and forestomach lymph nodes.
E - Lung.
H - Intestine.
I - Liver and spleen.
**Numerator - no. of tubes positive for acid-fast isolations.
***Denominator - total no. of tubes used for acid-fast isolations.

DISCUSSION

A. Intrauterine Experiment

1. Analysis of Clinicopathologic Findings

The results indicated that two of the three cultures (51C-O and 68C-O) were highly virulent for heifers as manifested by generalized disease in all six animals infected.

The heifers inoculated with cultures 68C-O and 51C-O developed the following signs: 1) loss of body weight and condition beginning approximately 1 1/2 months after inoculation, and 2) a purulent vulvar discharge starting approximately 1.5 to 10 weeks after inoculation.

Although all six heifers had signs compatible with the disease, detectable abnormalities of the uterus were found in only 4 heifers (Nos. 74, 73, 70 and 71) on rectal examination. Yet all six heifers had rather obvious gross lesions of the uterus at necropsy.

The gross and microscopic lesions progressively increased with time in heifers inoculated with cultures 51C-O and 68C-O. This increase in scope and distribution of the lesions is well pointed out in the summary of the findings at necropsy (Table 9).

The lesions involving the peritoneum, right and left supramammary and left ischiatic lymph nodes were quite

advanced and extensive in heifer 74, culture 51C-0 and heifer 73, culture 68C-0. This suggested that the infection rapidly spread from the uterus to the peritoneal cavity through the opening in the fimbria between the Fallopian tube and the abdominal cavity (Sisson and Grossman, 1953). Afferent lymphatic vessels from the visceral peritoneum to the above mentioned lymph nodes would explain the disease process observed in these lymph nodes. There was additional evidence confirming the supposition in the similar distribution of the disease process (involving the peritoneal cavity and the lymph nodes with afferent lymphatic vessels from this tissue) in the remaining 4 heifers, 75, 77, 70 and 71, inoculated with cultures 51C-0 and 68C-0.

The presence of lesions in the mesenteric and right and left medial retropharyngeal lymph nodes suggested oral infection. However, some of the mesenteric lymph nodes may have become infected via lymphatics from the lesions of the peritoneum (Sisson and Grossman, 1953).

Heifers 75 and 70, examined post-mortem 124 days after inoculation, had some differences in the distribution and progression of the disease process (Table 9). Heifer 75, inoculated with culture 51C-0, had (in addition to the gross lesions like those noted in heifers 74 and 73) gross lesions in the thoracic lymph nodes. Heifer 70 had gross lesions in the lymph nodes of the head region (Table 9). Both animals had gross lesions of the lymph nodes (colic and mesenteric) with afferent lymphatic vessels from the intestine. Microscopic lesions were found in the right and left deep inguinal,

right and left internal iliac, and lumbar lymph nodes of heifer 70. Infection of these lymph nodes can be explained on the basis of afferent vessels from the genital organs (Sisson and Grossman, 1953). Microscopic lesions were found in the liver of heifers 75 (culture 51C-0) and 70 (culture 68C-0).

The lesions of the thoracic lymph nodes and liver were indicative of hematogenous spread of the infection. However, lung infection in heifer 70, culture 68C-0, may also have occurred through the oral route since there were lesions in the lymph nodes of the head region.

The heifers (77, culture 51C-0 and 71, culture 68C-0) examined post-mortem at 176 days after inoculation had lesions, in addition to the lesion distribution found in the previous four heifers (74, 75, 73 and 70), in the mammary gland (heifers 77 and 71), intestine, left prescapular lymph node (heifer 77), lungs and vulva (heifer 71). These findings suggested extension of the disease by either the lymphatic system or the vascular system.

Culture 50B-0 caused little evidence of disease in the three heifers (Nos. 78, 79 and 90) inoculated. None of the heifers had any overt clinical signs which could be attributed to the occurrence of a tuberculous process.

Heifer 78, examined post-mortem 64 days after inoculation, had slight gross lesions in the peritoneum, particularly the omentum (Table 9). This finding was not present in the heifers examined post-mortem subsequently.

Microscopic lesions were found in the left deep inguinal lymph node of heifer 78 and in the mesenteric lymph node of heifer 79, examined post-mortem 125 days after inoculation. Both of these lesions appeared to be nonprogressive when measured by the criteria devised by Feldman (1943) for guinea pigs. In contrast, the histopathologic features of the lesions found in the heifers inoculated with cultures 51C-O and 68C-O were considered progressive.

The gross and microscopic lesions may have resulted from the mere presence of these organisms and their metabolic by-products, which, having reached the peritoneal cavity soon after inoculation, induced a mild foreign-body reaction. If multiplication of the organisms occurred, there was an apparent lack of virulence.

Clinical and pathologic findings in heifers 77, 79 and 80, inoculated with culture 50B-O, indicated that the reproductive organs remained normal throughout the experiment and yet conception did not occur. These findings suggested that: 1) ovulation did not occur, or did not occur soon enough for fertilization of the ovum to take place (this may have been related to the artificially induced estrus); 2) the inoculum of culture introduced concurrently with the semen was unfavorable for sperm survival; or 3) heifers inoculated with cultures 51C-O and 68C-O had sufficient lesions of the uterus to prevent conception or cause abortion, had conception occurred. Since the reproductive organs of heifers 78, 79 and 80

were normal, the last reason probably was not a contributing factor.

Group-III mycobacteria have been isolated from bovine semen (Mallmann, 1962). Thus the intrauterine route of transmission can not be ignored as a possible source of infection to cattle. However, the unusual distribution of lesions resulting from the inoculation of heifers 74, 75, 77, 73, 70 and 71 with highly virulent Group-III mycobacteria would indicate that this is not an important route of transmission under natural conditions, even in the case of virulent Group-III mycobacteria.

Francis (1947) reported that about 5% of tuberculous cows have tuberculous metritis. Although infection of the uterus occurred from tuberculous peritonitis or from the external genitalia, the commonest route was hematogenous spread of the disease from primary lesions elsewhere in the body, particularly the thoracic organs. The lesions were associated with pregnancy in nearly all cases.

2. Analysis of Bacteriologic Findings

In general, there was agreement between tissue pools with gross and microscopic lesions and acid-fast isolations (Table 10). However, acid-fast isolations were made from tissue pools with no detectable gross and microscopic lesions. Although the possibility that lesions were overlooked in these tissues can not be ignored, the most probable factor in explaining this finding is the presence of infection without

overt disease due to lack of local tissue hypersensitivity to the organisms (Rich, 1951). Other workers (Willis, 1925; Soltys and Jennings, 1950) have demonstrated a bacteremia in laboratory animals with generalized tuberculosis. This also could account for the spread of acid-fast organisms to non-tuberculous tissues.

3. Evaluation of Tuberculin Sensitivity

Heifers 75 and 77, inoculated with culture 51C-O, following an initial hypersensitivity had a progressive decrease in tuberculin sensitivity (Table 3). There was approximately a three-fold decrease in the cervical reaction to mammalian tuberculin within 63 days in heifer 75 and a six-fold decrease within 120 days. These findings were also reflected in the comparative cervical test readings which varied from -1.5 to 21 mm. increase. Although a decrease in sensitivity may occur as a result of repeated frequent tuberculin testing, the author believes that,in view of the time intervals involved herein and the extent of the lesions encountered, the decrease in sensitivity observed is more likely to have been related to the sensitivity pattern not infrequently observed in advanced tuberculosis.

Heifers 73, 70 and 71, inoculated with culture 68C-O, in general had a progressive increase in tuberculin sensitivity (Table 4). The sensitivity to mammalian tuberculin in the cervical test varied in its manifestation from circumscribed thickening of 13 mm. to more extensive flattened

swellings up to 110 x 185 mm. in surface area and at least 32 mm. thickness; the comparative test results varied from 7.5 to 32 mm.

Heifers 78, 79 and 80, inoculated with culture 50B-0, had no significant increase in mammalian tuberculin sensitivity (Table 5). One animal (No. 80) had a slight increase in avian tuberculin sensitivity (5.5 mm. and 5 mm. respectively) 110 and 167 days after inoculation. The comparative cervical test readings were -3.5 and -4 mm. respectively. However acid-fast bacilli, indistinguishable from the organisms inoculated, were recovered from 5 of the 9 tissue pools cultured. The presence of the organisms may explain the slight avian tuberculin sensitivity even in the absence of lesions.

In comparison to the above results, calves inoculated intradermally with Group-III mycobacteria (McGavin, 1964) had lesions considered as a primary complex (skin inoculation site and lymph node draining the site of inoculation), and had variable tuberculin sensitivity to avian and mammalian tuberculin. In most cases, the animals with generalized disease had a larger mammalian tuberculin response than avian tuberculin response.

Tuberculin sensitivity induced in cattle inoculated by intradermal (McGavin, 1964) and intrauterine routes were essentially similar to that reported by Mallmann et al., (1964) in which 12 of 40 isolants were classified as Group-III by growth characteristics. Two of the strains induced

Battey sensitivity; 4, avian sensitivity; 3, mammalian; 1, nondefinitive; 2, no sensitivity when inoculated intradermally into guinea pigs. In general, the more virulent the strain, the greater the tendency to produce mammalian sensitivity.

Swine-origin Group-III mycobacteria (Scammon et al., 1963) produced more intense dermal hypersensitivity to avian tuberculin than to human tuberculin. Cross reactions also occurred in human infections but, in general, most investigators (Runyon, 1959; Merckx et al., 1963; Lewis et al., 1959) found the majority of the patients had a positive reaction to human PPD's and particularly to homologous PPD's. The reactions were generally considered weak.

From these results, it is obvious that there is great heterogenicity in tuberculin sensitivity from different sources of Group-III mycobacteria. The virulent Group III of bovine origin appeared to be more closely related antigenically to mammalian tubercle bacilli than to swine or human Group-III mycobacteria.

B. Aerosol Experiment

1. Evaluation of Potential Infectivity of the Aerosol

Limited tests with the Anderson Air Sampler indicated that there were sufficient infective particles to cause infection of the lung parenchyma of the calves. Other workers (Wells, 1948; Middlebrook, 1952) have found that particle size must be less than 5 microns to insure lung penetration.

In seven of the ten guinea pigs exposed to aerosol (Nos. 1-10), acid-fast organisms were recovered from lung tissue (Table 2). There was no explanation for the differences observed in the two trials. Since no isolations were made from the control group, the isolations from the infected group indicated lung penetration.

2. Analysis of Clinicopathologic Findings

Little difficulty was encountered in inoculating the calves. The prevailing temperature was approximately 85 to 90 F and the humidity was above 70%. This caused some discomfort and increased respiration during inoculation.

The results of the six calves inoculated with cultures 51C-0 and 68C-0 compared favorably with the same cultures used in the intrauterine experiment, in that the strains were readily demonstrated to be highly virulent for cattle.

The six calves (Nos. 91, 84, 90, 82, 86 and 83) inoculated with culture 51C-0 and 68C-0 had, in general, the following signs: 1) a soft moist cough starting at approximately 3 weeks (auscultation revealed moist rales),

2) pyrexia (varying from 103 to 106.6 F), 3) anorexia, 4) depression, 5) seropurulent nasal discharge beginning approximately 3 to 4 weeks after inoculation, 6) fetid diarrhea starting approximately 1 to 2 weeks before extremis. One (No. 90-culture 51C-0) of the six calves died (47 days after inoculation) and the other five were euthanatized ahead of schedule due to their moribund condition.

The disease process was essentially similar in both groups and the extent of lesions increased with time following inoculation. Calves 83 (culture 68C-0) and 86 (culture 68C-0), which survived the longest, had the most advanced and extensive gross and microscopic lesions.

Analysis of the pathologic findings of calves 91, 84, 90 (culture 51C-0) and 82 (culture 68C-0) examined postmortem from 44 to 51 days revealed comparable distribution and extent of gross lesions (Table 11). The gross lesions were confined to the thoracic organs. There appeared to be a rather uniform distribution of gross lesions throughout all lobes of the lungs.

The markedly enlarged lungs with extensive lesions of tuberculosis and markedly enlarged thoracic lymph nodes apparently caused a circulatory impairment. This was evident by the marked interstitial edema of the mediastinum and by the enlarged heart. Histopathologic examination of the liver also revealed a distension of the centrolobular veins.

The anorexia and terminal diarrhea, which undoubtedly resulted in protein and carbohydrate imbalance with marked

dehydration (in addition to the above) doubtless were contributing factors in the rapid debilitation of these calves (Nos. 91, 84, 90 and 82).

The microscopic lesions found in the lymph nodes of the head region in calves 91 and 82 may have resulted from upper respiratory infection at the time of infection or from expiration of infective exudate from the lungs.

The microscopic lesions of the liver of calf 84, left ischiatic lymph node of calf 91, and the right prescapular, right and left internal iliac, right and left deep inguinal lymph nodes of calf 82 probably resulted from hematogenous spread of the infection.

The gross lesions found in calves 83 and 86 were much more extensive and advanced than calves 91, 84, 90 and 92. Gross lesions were also found in the kidneys, spleen (calf 86), liver, hepatic and mesenteric lymph nodes in addition to the thoracic lymph nodes and lungs of both calves. Microscopic lesions were found in many lymph nodes and organs in addition to the above (Table 11).

The disease was rapidly fulminating in these calves. With the possible exception of the lymph nodes of the head and digestive system, there was a marked generalized hematogenous spread of the infection. This could be expected when the anatomical considerations of the lung are considered. There is no lymph node barrier between the blood vascular system and the pulmonary lesions.

The cause of the renal and hepatic infarcts was not determined but septic emboli are the most likely explanation.

The disease process was more extensive, advanced, and consistent in the calves inoculated with aerosol and the heifers inoculated by the intrauterine route than calves inoculated intradermally by McGavin (1964). This may be due primarily to the route of inoculation (Feldman, 1938).

One of the three calves inoculated intradermally by McGavin (1964) with a single dosage (1 mg. wet weight) of culture 51C-O had generalized disease. A second calf had lesions at the site of inoculation and the lymph node draining the site of inoculation. A third calf had lesions at the site of inoculation only. One calf inoculated with culture 68C-O had generalized lesions.

Calves 87, 88 and 83, inoculated with culture 50B-O, had no clinical signs of disease, and no macroscopic or microscopic lesions were detected (Table 11). These results were essentially the same as in the intrauterine experiment.

The distribution of the lesions in calves 91, 84, 90, 82, 86 and 83, inoculated by the aerosol route with cultures 51C-O and 68C-O, were remarkedly similar to lesions naturally acquired following pulmonary infection. Francis (1958) emphasized the importance of the pulmonary route of transmission of pathogenic mycobacteria by summarizing the findings of other investigators from 1898 until 1946. Except for one report, the incidence of lesions involving the respiratory system varied from 60.1% to 94%.

3. Analysis of Bacteriologic Findings

Table 12 summarizes the bacteriologic findings of the calves inoculated with cultures 51C-O and 68C-O. Tissue pools which included gross lesions had the highest incidence of acid-fast isolations. The organisms were indistinguishable from the Group-III mycobacteria inoculated. As in the results of the intrauterine experiment, isolations were made from tissue pools which had no detectable gross or microscopic lesions.

Calves 89, 87, and 88, inoculated with culture 50B-O, are worthy of comment. Five of the six tissue pools cultured from calf 89 (examined post-mortem at 64 days after inoculation) were positive for acid-fast isolations. Only the thoracic lymph node pools of the remaining two calves (No. 87, 128 days after inoculation, and No. 88, 188 days after inoculation) were positive. The organisms again were indistinguishable from the strain inoculated. Since the isolations were made from the lymph nodes with afferent lymphatic vessels from the lungs (target organ in this experiment), the isolations were considered significant. Since no gross or microscopic changes were detected, these findings suggested that mycobacteria of little or no pathogenic ability may reside in normal tissues for a considerable period of time before being destroyed.

4. Evaluation of Tuberculin Sensitivity

The tuberculin sensitivity results (Tables 6, 7 and 8) were not as complete as in the intrauterine experiment, due

to the rapidly fulminating disease in the calves inoculated with cultures 51C-O and 68C-O. Five of the six calves (Nos. 91 and 84, receiving culture 51C-O, and Nos. 83, 86 and 82, receiving culture 68C-O) had one tuberculin test before necropsy. Four of the five calves (91, 84, 83 and 86) had very marked mammalian tuberculin reactions in comparison to the avian tuberculin reactions. The cervical responses varied from 40 mm. (24-hr. reading) to 125 by 180 mm. and larger. In some cases the responses made it impossible to record the johnin reactions. The caudal fold test varied from 14 to 41 mm. high by 21 to 41 mm. long. The avian tuberculin test response varied from 1 to 11 mm. in diameter.

Calf 82 had only a 6 mm. increase to mammalian tuberculin in the cervical area and a .5 mm. increase to avian tuberculin. The caudal fold test site had a 2 mm. increase at 72 hours.

Calves 89, 87, and 88, inoculated with culture 50B-O, had a slight increase in avian tuberculin sensitivity. The size of the response varied from 2 to 5.5 mm. (comparative test results varied from 1 to -5 mm.). These results are comparable to the findings in the intrauterine experiment.

5. Comparison of Histopathologic Lesions Produced by Group-III Mycobacteria and M. bovis

Except for minor differences, the histopathologic changes in the calves and heifers inoculated with cultures 51C-O and 68C-O were similar. There was little evidence of giant cell

formation, but considerable cellular necrosis of the tissue of calves in the aerosol experiment. This can be explained on the basis of the fulminating character of the tuberculous process in these animals.

When the lesions were compared with lesions produced by M. bovis, there were no salient differentiating feature. Other investigators (Corpe, 1963; Merckx et al., 1964) have reported similar findings.

SUMMARY

Two experiments were conducted to determine the relative pathogenicity of Group-III mycobacteria of bovine origin in cattle when inoculated by the intrauterine route and by aerosol.

The results were determined by tuberculin testing, serologic procedures, clinical observations and detailed macroscopic, microscopic and bacteriologic examination of tissues.

Intrauterine Experiment

Nine heifers between 21 and 23 months of age were divided into three groups of three heifers each. Each group was inseminated with semen containing a different strain (1 mg. wet weight) of bovine-origin Group III mycobacteria after artificial production of estrus.

One week prior to each scheduled necropsy, all heifers were tuberculin tested in the caudal fold with mammalian tuberculin and in the cervical region with mammalian and avian tuberculins and johnin. Following the tuberculin tests, one heifer from each group was examined post-mortem at 2, 4 and 6 months after inoculation.

The six heifers, three inoculated with culture 51C-O and three with culture 68C-O, had evidence of generalized

disease. Three heifers inoculated with culture 50B-0 had little evidence of disease.

The distribution of the lesions indicated that the infection spread from the uterus to lymph nodes via the lymphatics and to the peritoneal cavity through the opening of the fimbria of the Fallopian tube. Further spread of the infection occurred from the peritoneum to lymph nodes via the lymphatics.

The heifers examined post-mortem at 4 months or at 6 months after inoculation (except those inoculated with culture 50B-0) had evidence of both lymphatic and hematogenous spread of the infection to other organs and lymph nodes. The lesions found in the lymph nodes of the head region suggested oral reinfection.

There was a marked dissimilarity between the distribution of lesions observed in naturally acquired bovine tuberculosis and the lesions found in the heifers inoculated by the intrauterine route with cultures 51C-0 and 68C-0.

Group-III mycobacteria indistinguishable from the strains inoculated were recovered from most of the lymph node pools which had lesions. Frequently isolations were made from lymph nodes with no detectable gross or microscopic lesions.

Heifers inoculated with culture 51C-0 had a progressive decrease in tuberculin sensitivity after an initial hypersensitivity. There was a negative correlation between the results of the tuberculin sensitivity and the presence of

gross and microscopic lesions. In contrast, heifers inoculated with culture 68C-O had a progressive increase in tuberculin sensitivity. The mammalian tuberculin sensitivity was greater than the avian tuberculin sensitivity.

The heifers inoculated with culture 50B-O had no significant increase in sensitivity to mammalian tuberculin. One heifer had a slight increase in sensitivity to avian tuberculin.

Aerosol Experiment

Nine calves, between 3 and 6 months of age, were divided into three groups of three calves each. Each group was inoculated with a different strain of bovine-origin Group-III mycobacteria by aerosol containing approximately $1.5 \times 10^9 \pm 10^2$ organisms. The calves were tuberculin tested as described previously. The calves were euthanatized and a necropsy performed ahead of the scheduled 2, 4 and 6 months after inoculation due to anticipated death.

The six calves, three inoculated with culture 51C-O and three with culture 68C-O, had extensive lesions of pulmonary disease. The thoracic lymph nodes with afferent lymph vessels from the lungs also had extensive lesions. The microscopic lesions could not be distinguished from lesions found in cattle infected with Mycobacterium bovis. This was also true of lesions studied from the intrauterine experiment. The disease rapidly spread to most of the lymph nodes, the

abdominal organs (liver, spleen, kidney), and throughout the body, principally by the hematogenous route.

The distribution of lesions in the six calves exposed by aerosol to cultures 51C-O and 68C-O was markedly similar to lesions found in naturally acquired bovine tuberculosis. Calves inoculated with culture 50B-O had no detectable gross or microscopic lesions.

Group-III mycobacteria, indistinguishable from the strains inoculated, were recovered from most of the lymph node pools in which lesions were found. Acid-fast organisms were recovered from the thoracic lymph nodes of the group of calves inoculated with culture 50B-O.

Five of the six calves, inoculated with culture 51C-O and 68C-O, had a marked mammalian tuberculin sensitivity.

Calves inoculated with 50B-O had a slight increase in sensitivity to avian tuberculin.

REFERENCES

Anderson, A. A. 1958. New sampler for the collection, sizing and enumeration of viable airborne particles. J. Bact., 76:471.

Anonymous. 1960. Why tuberculosis in livestock is increasing. Agric. Res. Service, U. S. Dept. Agric., 91-21.

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.

Atwell, R. J. and Pratt, P. C. 1960. Unclassified mycobacteria in the gastric contents of healthy personnel and of patients of a tuberculosis hospital. Am. Rev. Resp. Dis., 81:888.

Baldwin, E. R. 1942. Non-pathogenic acid-fast bacilli: a case of chronic pneumonitis associated with a non-pathogenic acid-fast bacillus. Am. Rev. Tuberc., 45:756.

Beach, B. A. and Hastings, E. G. 1924. Proc. 28th Ann. Mtg. U. S. Livestock San. Assoc., 17.

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

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.

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

Carpenter, C. M. and Goldberg, S. A. 1925. A study of skin tuberculosis. Cornell Vet., 15:148.

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. A. V. M. A., 80:414.

Day, L. E. 1928. Skin lesions in tuberculin-reacting cattle. J. A. V. M. A., 72:782.

Dixon, W. J. and Massey, F. J. 1957. Introduction to statistical analysis. McGraw-Hill Book Co., Inc., New York.

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. Am. Rev. Resp. Dis., 80:876.

Edwards, L. B. and Palmer, C. E. 1959. Isolation of "atypical" mycobacteria from healthy persons. Am. Rev. Resp. Dis., 80:747.

Edwards, L. B., Palmer, C. E., Affronti, L. F., Hopwood, L. and Edwards, P. Q. 1960. Epidemiologic studies of tuberculin sensitivity. II. Response to experimental infection with mycobacteria isolated from human sources. Am. J. Hyg., 71:218.

Ekdahl, M. O. and MacFarlane, D. J. W. 1963. Diagnosis of bovine tuberculosis. 3. Some observations on isolation and types of Mycobacterium tuberculosis from tuberculin-sensitive cattle in New Zealand. New Zealand Vet. J., 11:15.

Feldman, W. H. 1938. Avian tuberculosis infections. Williams & Wilkins, Baltimore, Md.

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. 1963. Pathogenicity studies of group III (battey) mycobacteria from pulmonary lesions of man. Dis. of Chest, 43:26.

Floodgate, G. D. 1962. Some remarks on the theoretical aspects of bacterial taxonomy. Bact. Rev., 26:277.

Francis, J. 1947. Bovine tuberculosis. Staples Press Ltd., London.

Francis, J. 1958. Tuberculosis in animals and man: A study in comparative pathology. Cassell & Co., London.

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

Goyings, L. S. 1962. Final report on research contract #12-14-100-4511 (45) between the U. S. Dept. of Agr. and Mich. State Univ.

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

Hall, W. H., Stief, M. and Song, H. S. 1957. Comparative studies of pathogenic chromogenic mycobacteria. Tr. 16th VA-Armed Forces Conference on the Chemotherapy of Tuberculosis, 286.

Hastings, E. G., Beach, B. A. and Weber, C. W. 1924. No lesion and skin-lesion tuberculin-reacting cattle. J.A.V.M.A., 66:36.

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.

Hauduroy, P. 1955. Several aspects of special problems of mycobacteria. Paris, Masson et cie. Cited by Xalabarder, C. 1961. Am. Rev. Resp. Dis., 83:1.

Hedstrom, H. 1949. Studies on so-called skin tuberculosis in cattle, concerning its prevalence in Sweden, its diagnosis, etiology and allergy to tuberculin. Gernandts Boktryckeri, Stockholm. Cited by Karlson, A. G. 1962. Advances in Vet. Sci., 7:147.

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

Hull, T. G. 1963. Disease transmitted from animals to man, 5th Ed. Charles C. Thomas, Publisher, Springfield, Ill.

Johnson, H. W., Baisden, L. A. and Frank, A. H. 1961. Recent research findings on bovine tuberculosis. J.A.V.M.A., 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. and Feldman, W. H. 1940. Studies on an acid-fast bacterium frequently present in tonsillar tissue of swine. J. Bact., 39:461.

Karlson, A. G., and Feldman, W. H. 1953. Mycobacteria of human origin resembling Mycobacterium avium. Proc. 15th Intern. Vet. Congr., Stockholm, Part I, 159.

Karlson, A. G., Davis, C. L. and Cohn, M. L. 1962. Skotochromogenic Mycobacterium avium from a trumpeter swan. Am. J. Vet. Res., 23:575.

Karlson, A. G. 1964. Personal communication.

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.

Krantz, G. T. 1938. Om sa kallt hudtuberkulose hos notkreatur. Skant. Vet. Tidskr., 28:1-26.

Kubica, G. P. 1964. Personal communication.

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. Am. J. Med. Tech., 26:241.

Lewis, A. G., Dunbar, F. P., Lasche, E. M., Bond, J. O., Lerner, E. N., Wharton, D. J., Hardy, A. V. and Davies, R. 1959. Chronic pulmonary disease due to atypical mycobacterial infections. Am. Rev. Resp. Dis., 80: 188.

Mallmann, V. H. 1962. Final report on research contract #12-14-100-4511 (45) between the U. S. Dept. of Agr. and Mich. State Univ.

Mallmann, V. H. 1963. Unpublished data.

Mallmann, V. H., Mallmann, W. L. and Robinson, P. 1961. Relationship of atypical bovine and porcine mycobacteria to those of human origin. 89th Annual Meeting of the A.P.H.A., Detroit, Mich. Published in 1964, Health Lab. Sci., 1:11.

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.

Mallmann, W. L. 1964. Significance of the Runyon group III mycobacteria in animals. Conference of Tuberculosis Epidemiologists and Symposium on Relationship of M. avium and Runyon type III mycobacteria (Unpublished data).

Mallmann, W. L., Mallmann, V. H. and Ray, J. A. 1962. Mycobacteriosis in swine caused by atypical mycobacteria. Proc. U. S. Livestock San. Assoc. Mtg., 180.

McCusker, J. J. and Green, R. A. 1962. Generalized non-tuberculous mycobacteriosis. Am. Rev. Resp. Dis., 86:405.

McGavin, M. D. 1964. The lesions resulting from inoculation of calves with atypical mycobacteria. Ph. D. Thesis, Michigan State University.

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

McGavin, M. D., Mallmann, V. H., Mallmann, W. L., Ray, J. A. and Morrill, C. C. 1964. Pathological changes and alterations in the tuberculin sensitivity of calves injected with atypical mycobacteria. Medical abstracts of National Tuberculosis Association.

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.

Merckx, J. J., Soule, E. H. and Karlson, A. G. 1964. The histopathology of lesions caused by infection with unclassified acid-fast bacteria in man. Am. J. Clin. Path., 41:244.

Middlebrook, G. 1952. An apparatus for airborne infections of mice. Proc. Soc. Exp. Biol. Med., 80:105.

Mitchell, C. A. 1928. Bovine subcutaneous tuberculosis. J.A.V.M.A., 73:493.

Nocard and Roux. 1888. Sur la cultur du bacille de la tuberculose. Ann. Inst. Pasteur (Paris), 2:285. Cited by Xalabarder, C., 1961. Am. Rev. Resp. Dis., 83:1.

Paterson, A. B. 1956. The incidence and causes of tuberculin reactions in non-tuberculous cattle. Advances in Tuberc. Res., 7:101.

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. 1951. The pathogenesis of tuberculosis. Charles C. Thomas, Springfield, Ill.

Runnells, R. A. 1932. The histopathology of cutaneous and subcutaneous nodules of cattle. J.A.V.M.A., 81:173.

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

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 ileocecal 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.

Stuart, P. and Harvey, P. 1951. Bovine mastitis resembling tuberculosis caused by Mycobacterium lacticola and other rapidly growing acid-fast bacteria. Vet. Rec., 63:881.

Thomann, H. 1949. Untersuchungen uber das vorkommen der "skin-lesion" beim schweizerischen braunvieh. Schweiz. Arch. Tierheilk., 91:237.

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.A.V.M.A., 49:254.

Tucker, E. W. 1953. Infection of the bovine udder with Mycobacterium species. Cornell Vet., 43:576.

Waxler, G. L. 1961. Studies on gnotobiotic pigs. Ph. D. Thesis, Michigan State University.

Wayne, L. G. 1964. The mycobacterial mystique: Deterrent to taxonomy. Am. Rev. Resp. Dis., 90:255.

Weed, L. A., McDonald, J. R. and Needham, G. M. 1956. The isolation of "saprophytic" acid-fast bacilli from lesions of caseous granulomas. Proc. Staff Meet. Mayo Clin., 31:246.

Wells, W. F., Ralcliffe, H. L. and Crumb, C. 1948. On the mechanics of droplet nuclei infection: II quantitative experimental airborne tuberculosis in rabbits. Am. J. Hyg., 47:11.

Wilder, C. W. 1962. No gross lesions and the tuberculosis eradication program. J.A.V.M.A., 140:41.

Willigan, D. A. 1961. Fourth semi-annual progress report of co-operative agreement #12-14-100-2372 (91) between Mich. State Univ. and the U. S. Dept. of Agr.

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.

Willis, H. S. 1925. Studies on tuberculosis infection: X. The early dissemination of tubercle bacilli after intracutaneous inoculation of guinea pigs of first infection. Am. Rev. Tuberc., 11:427.

Wood, L. E., Buhler, V. B. and Pollak, A. 1956. Human infection with the "yellow" acid-fast bacillus, a report of fifteen additional cases. Am. Rev. Tuberc., 73:917.

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

Yoder, W. D. 1964. Isolation and typing of mycobacteria at N.A.D.L. Conference of Tuberculosis Epidemiologists and Symposium on Relationship of M. avium and Runyon type III mycobacteria (Unpublished data).