THE PATHOGENICITY OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS FOR LABORATORY WHITE MICE

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INTRODUCTION

Corynebacterium pseudotuberculosis was first recognized as the causative agent of caseous lymphadenitis in sheep by Preisz and Guinard in 1891 (Carne 1939). Norgaard and Mohler (1899) showed the disease was widespread in the United States and other countries but was of minor economic importance to the sheep industry. Since then, Maddy (1953) has shown that this disease has increased in importance in the United States, New Zealand, Australia, Argentina, South Africa and parts of Europe. At the present time it ranks third in importance as a cause for the condemnation of sheep carcasses in the United States.

At the present time little evidence has been found in the literature concerning the pathogenicity of any number of strains of C. pseudotuberculosis for laboratory animals. For this reason research was undertaken to determine the relative pathogenicity of sixteen strains of C. pseudotuberculosis for laboratory white mice and to characterize the course of the infection in this experimental host. An attempt has been made to determine the relationship between hemolysin and urease production and pathogenicity.

LITERATURE REVIEW

Infections produced by C. pseudotuberculosis in domestic and wild animals have been characterized in textbooks of veterinary bacteriology by Merchant (1950), Hagan (1947) and by Kelser and Schoening (1943). In sheep, this organism produces a condition known as caseous lymphadenitis. An inflamatory enlargement of the lymph glands occurs followed by caseous degeneration. Marsh (1947) also recognized C. pseudotuberculosis to be one of the causes of "stiff lambs" disease. It has also been incriminated as an infectious agent for other animals. In the horse, it produces an ulcerative lymphangitis (pseudo-farcy) and in cattle it causes a suppurative lymphangitis. It also has been found in the bovine reproduction tract. The diseased condition, which sometimes is found in deer, resembles the lesions observed in sheep.

According to Norgaard and Mohler (1899) caseous lymphadenitis of sheep and C. pseudotuberculosis were first described by Preisz and Guinard in France in 1891. Nothing more was published until 1894 when Preisz undertook further research concerning the cellular and colonial morphology of this organism and it s role in pathological processes. Preisz noted the resemblance between this organism and the diptheria bacillus and gave it the name Bacillus

pseudotuberculosis ovis. Other investigators, during the 1890's named the organism The Bacillus of Preisz and Nocard or Bacillus pseudotuberculosis. Eberson (1918) classified it as a diptheroid and placed it in the genus Corynebacterium (C. pseudotuberculosis).

Nocard (1896) described a case of lymphatic ulceration in a horse which resembled glanders. Laboratory examination of the causative organism showed it to be the same as that previously described by Preisz in 1984. Nocard found that this diptheroid was capable of producing orchitis when inoculated intraperitoneally in male guinea pigs. Nocard's observations led to the term "pseudo-farcy" for the infection in horses.

Norgaard and Mohler (1899) reported on the incidence of caseous lymphadenitis in sheep in the United States. They also described the cultural characteristics and pathogenicity of the microorganism for various species of domestic and laboratory animals. Microscopic examination of smears from typical purulent lesions showed Gram positive, pleomorphic bacilli, these cells upon culture often appeared as coccobacilli. The organism was aerobic or facultative anaerobic, did not form spores and was non-motile. Growth occurred on nutrient agar but was increased by the addition of blood or serum. Dextrose was fermented with the production of acid. Gas was not evolved. Lactose was not attacked. Growth occurred at 16 to 43 C. The optimal

temperature was 37 C. The organism was able to survive at 6 to 8 C. for a period of several weeks. Animal inoculations indicated that guinea pigs, rabbits, mice and sheep were susceptible to infection whereas pigeons and chickens were not. Intravenous inoculation of rabbits and guinea pigs produced a generalized infection. Intraperitoneal inoculation of rabbits, guinea pigs and mice produced infection primarily in the liver, kidneys and spleen with abscess formation on the peritoneum. Pulmonary lesions were observed occasionally in rabbits and guinea pigs. Subcutaneous inoculations resulted in localized abscesses at the site of inoculation followed by extension of the infection to the viscera. Sheep inoculated *via* the intravenous route developed abscesses in the liver and lungs. Renal and splenic degenerations were reported. Food containing the organisms, when fed to guinea pigs and rabbits, produced generalized infections. Sheep were not infected by feeding. This report also indicated that the disease was of minor economic importance to the American sheep industry. In contrast, Maddy (1953) showed that caseous lymphadenitis in sheep had increased to the extent of being the third most important cause of condemnation at the abattoirs in the United States.

Nicolle, Loiseau and Forgeot (1912) were the first to recognize the ubiquity of this organism. They attempted to divide strains into two groups, virulent and avirulent.

Their virulent type was characterized by it's ability to produce a soluble poison or exotoxin. Hall and Stone (1916) obtained cultures of C. pseudotuberculosis from infected horses and calves. Intraperitoneal inoculations of guinea pigs with these cultures resulted in orchitis, generalized peritonitis, and toxic degeneration of abdominal organs similar to that observed in animals injected with diphtheria toxin.

Bull and Dickinson (1933 and 1934) and Seddon and Belschner (1927) studied the transmission of caseous lymphadenitis among sheep. Their reports indicated that the organism was quite resistant to drying and exposure to sunlight. The primary route of entrance in lambs was through open wounds due to docking, castration or through the umbilicus shortly after birth.

Further characterization of the infection in sheep was accomplished by Rottgardt (1928) as well as by DeKock and Fourie (1931). Morcas (1932) produced experimental ulcerative lymphangitis in a horse by subcutaneous inoculation of C. pseudotuberculosis.

Petri and McClean (1934) observed the relationship between Corynebacterium diphtheriae and C. pseudotuberculosis and concluded that although virulent strains of both species produce an exotoxin, the type of lesion produced in a susceptible experimental animal by the individual toxins is not similar and therefore the toxins are not similar.

However, they presented evidence that an unclassified group of diphtheroids isolated from the human nasopharynx was in certain respects related to both species on the basis of colonial morphology.

Merchant (1935), in a study of the corynebacteria associated with diseases of domestic animals, reported that there was a serological relationship between C. pseudotuberculosis and Corynebacterium renale. There was also a limited relationship between C. pseudotuberculosis and Corynebacterium equi.

An extensive study on the morphological, biochemical and hemolytic characteristics of 134 strains of C. pseudotuberculosis was made by Carne (1939). He defined the organism as a Gram positive, coccobacillus when cultivated on solid media. Pleomorphic forms were found only in smears obtained directly from lesions. The organisms grew poorly on plain nutrient media but the addition of serum or blood as enrichment resulted in increased growth. The strains were capable of fermenting dextrose, galactose and fructose with the formation of acid. No gas was produced. Sucrose fermentation was variable and lactose was not attacked. Indole and acetyl methyl carbinol were not produced. Nitrate reduction was variable but usually negative. The hemolytic activity varied according to the type of blood used. The order of susceptibility to hemolysis for erythrocytes of various animals tested was: guinea pig > rabbit >

horse> sheep. The hemolysin produced by the various strains appeared to be oxygen labile. Hemolysis was observed under anaerobic conditions whereas it was not observed under aerobic conditions with the same strains.

Lovell (1937) observed that the toxin production of Corynebacterium pyogenes was correlated with hemolytic activity. Those strains which were actively hemolytic and produced a toxin, whereas non-hemolytic strains were atoxogenic. It is possible that these data would prove useful in pathogenicity studies for other species of this genus.

Howard and Jann (1955) found that strains of C. pseudotuberculosis were lysed by a bacteriophage. Further research might permit the use of phage in typing various closely related diphtheroids.

MATERIALS AND METHODS

PART I

Blood for media enrichment was collected aseptically in flasks containing glass beads or broken glass and rotated to accomplish defibrination. Sterile citrated (0.5 percent sodium citrate) human blood was obtained from a local hospital.

Serum was obtained by allowing horse or cow blood to clot and sterilizing the decanted serum by Seitz filtration.

Media for biochemical tests were prepared for indole production, the Methyl Red test, the Voges Proskauer test, nitrate reduction, urease production and gelatin liquefaction from the dehydrated form according to the manufacture's directions.

Stock cultures of the sixteen strains of C. pseudotuberculosis were maintained on Blood Agar Base slants (Difco) containing a final concentration of 5 percent defibrinated blood in screw cap culture tubes. The cultures were incubated for 48 hours at 37 C and were then kept in the refrigerator ($\pm$ 4 C). The cultures were transferred to fresh media every four weeks.

Cultures of C. pseudotuberculosis were obtained from various veterinary colleges or veterinary science departments in the United States. With four exceptions these

cultures had been stored in the lyophilized state for three to eight years. The date and history of isolation for the sixteen strains are shown in Table I.

The cellular morphology of each strain was determined from Gram stained smears made from 48 hour agar cultures. The fermentation reactions were determined using dextrose, galactose, lactose and sucrose. Ten percent solutions of the respective sterilized carbohydrates were added to Phenol Red Broth Base (Difco) to give a final concentration of 1.0 percent in the medium. Sterile serum was added to give a final concentration of 1.0 percent in the broth. Sterility of the medium was determined prior to inoculation with strains of C. pseudotuberculosis. Following inoculation the tubes were returned to the incubator and observations were made at weekly intervals for a period of four weeks at which time a final determination was made. Indole formation was determined according to the method of Kovacs as found in the Manual of Methods for Pure Culture Study (1946). Acetyl methyl carbinol was determined according to the Standard Methods for the Examination of Water and Sewage of the American Public Health Association (1946). Urease production was ascertained on Christensen's (Difco) agar slants. Gelatin liquefaction was observed using the method of Smith (1946). The action of each strain on litmus milk (Difco) was also ascertained.

The hemolytic effect of the sixteen strains on sheep,

cow, horse, human, rabbit, guinea pig and dog erythrocytes was determined for aerobic cultures which were prepared by streaking the cultures on the surface of Blood Agar Base plates (Difco) containing a final concentration of 5 percent defibrinated or citrated blood. Semianaerobic cultures were prepared using Blood Agar Base (Difco) pour plates containing a final concentration of 5 percent defibrinated or citrated blood. Anaerobiosis was obtained by overlaying the pour plates with 10 ml of 1.5 percent plain sterile agar.

RESULTS

PART I

Examination of the Gram stained smears of the sixteen strains prepared from 48 hour agar cultures exhibited minor morphological differences. Two strains, 2335 and 1965, were predominatly rod shaped while the rest of the strains were coccoid in shape. All of the strains were Gram positive. The Gram stain reaction and the morphology of each strain is shown in Table II.

Two of the strains, CCP-1 and 1741, were able to reduce nitrate to nitrite. The other strains did not. Three of the strains, OV-10, OV-7 and 2335, did not ferment sucrose and two strains, 2335 and 1965, did not produce detectable urease. All strains complied, with minor exceptions, with the characteristics for C. pseudotuberculosis as found in Bergey's Manual 6th edition (1946). The biochemical results are presented in Table III.

On aerobic culture 14 strains produced beta hemolysis on sheep blood, 11 on cow blood, 10 on rabbit blood, 8 on horse blood and 5 on human blood. Guinea pig and dog erythrocytes were not hemolyzed by any of the strains.

Several strains exhibited either alpha or beta hemolysis when cultured under semianaerobic conditions. The following results were obtained from semianaerobic culture. On cow blood 14 strains produced beta hemolysis and 1 strain

gave alpha hemolysis. On rabbit blood, 11 strains were beta hemolytic and 4 strains produced alpha hemolysis. On guinea pig blood, 10 strains produced beta hemolysis and 2 strains gave alpha hemolysis. Nine strains were beta hemolytic on horse blood, while no alpha hemolysis was observed. On human blood, 8 strains exhibited beta hemolysis, while 6 strains were alpha hemolytic. Six strains produced beta hemolysis on sheep blood, while dog erythrocytes were hemolyzed by only 1 strain. Alpha hemolysis was not observed on either of the latter two bloods. The results of the aerobic and semianaerobic culture of the 16 strains on the blood from 7 different species of animals are presented in Table IV.

MATERIALS AND METHODS

PART II

Tryptose Broth (Difco) was used for culturing the organisms. Sterile serum was added to give a final concentration of 1.0 percent to provide necessary growth factors. Tween 80, to give a final concentration of 0.1 percent, was also added to reduce clumping of the organisms and maintain dispersion of the cells.

Gelatin Saline Diluting Fluid developed by Read and Read (1946) was used to wash the organisms, as a suspending medium for the inocula and as a diluent.

Cultures were inoculated in Tryptose Broth (Difco) in either 15 ml centrifuge tubes containing 8 ml of broth or in 250 ml Erlenmeyer flasks containing 50 ml of broth. The centrifuge tubes or flasks were incubated at 37 C for 48 hours. The cultures were then centrifuged at 3,000 revolutions per minute for 20 minutes to sediment the cells. The supernate was discarded and the organisms resuspended in gelatin saline diluting fluid. The cells were washed three times by this method to remove any possible traces of toxin. The cells were again suspended in gelatin saline diluting fluid. The suspension was standardized to a density of 50 percent light transmission at a wave length of 575 mu in a Bausch and Lomb, model 120 spectrophotometer. This material was used for the inocula, direct microscopic counts and

plate counts.

Direct microscopic counts were made from an aliquot of each inoculum. A Helber counting chamber and a phase microscope were employed. Forty squares were counted and the average number of bacteria per square determined. The number of organisms per ml was ascertained by multiplying the number of cells per square by 2×10^7.

Plate counts were determined using 1×10^{-3}, 1×10^{-4}, 1×10^{-5} and 1×10^{-6} dilutions of the inocula. Four plates were used for each dilution. The plates were prepared by first pouring a layer of Blood Agar Base (Difco) containing 1.0 percent sterile serum on to a sterile plate and allowing it to solidify. The dilutions were made in gelatin saline diluting fluid. One ml of this was transferred to 9 ml of 0.7 percent plain sterile agar maintained at 48 C in a water bath. Two ml of the agar was then overlayed onto the layer of Blood Agar Base (Difco) and allowed to solidify. The plates were incubated at 37 C for 48 to 72 hours. The number of colonies on each plate was determined for plates containing 30 to 300 colonies. The number of organisms per ml was then ascertained.

Mice of Rockland Farms, strain C-57, weighing between 18 and 22 gm were used for the pathogenicity studies. A preliminary titration, using strains 409H and 409NH, was conducted to obtain the dilution which would effectively produce infection. Dilutions from 1×10^0 to 1×10^{-8}

were prepared from the standardized suspensions and 0.5 ml was inoculated intraperitoneally into groups of 7 mice per dilution. The results from this preliminary titration indicated that only the undiluted suspension was capable of producing infection in all of the mice.

For the pathogenicity experiments groups of 10 mice each were inoculated by the intravenous, intraperitoneal or subcutaneous route with each of the test strains. The inocula consisted of the undiluted suspension of the organisms prepared in the manner previously described. Each mouse received 0.5 ml of the inoculum.

Intravenous inoculations were made into the tail vein. The tail was first cleansed with xylene to dilate the vein. Intraperitoneal inoculations were made into the posterior portion of the abdominal wall with the mice held in dorsal recumbency. Subcutaneous inoculations were made above the scapula on the back. A 1 ml tuberculin type syringe with a 27 gauge needle was employed.

Each group of mice was housed in a separate cage containing food and water. They were checked for evidence of infection at least twice daily. Mice which showed signs of infection (ruffled coat, discharge around the eyes, inactivity or lack of appetite) were killed and autopsied immediately. Seven days from the date of inoculation one-half of the surviving mice in each group were sacrificed, autopsied and cultured. At the end of 14 days the remaining mice

were killed, autopsied and cultured. At autopsy each mouse was examined for the presence of abscesses and other gross pathological changes in the spleen, kidneys, reproductive tract (testes in the male and ovaries and uterus in the female), lungs, liver and heart. Bacteriological cultures of the organs listed above were made from all mice. The organs were removed from the animals aseptically, transferred to a blood agar plate and minced with sterile scissors. The minced material was spread over a sector of the plate with a sterile inoculating loop. The plates were incubated at 37 C for 48 to 72 hours and examined for the presence of typical colonies of C. pseudotuberculosis.

RESULTS

PART II

The number of organisms per ml of inoculum as found by the plate count method, ranged from 7.7×10^5 to 124.0×10^5 with a mean of 44.8×10^5. The direct counting method gave values which ranged from 20.2×10^7 to 41.8×10^7 with a mean of 34.0×10^7 organisms per ml of inoculum. The number of bacteria per ml of inoculum for each strain as determined by the two methods is presented in Table V.

Following intravenous inoculation, 15 out of 16 strains produced 90.7 percent infection. One strain did not produce infection, 1 strain caused 40 percent infection, 1 strain produced 50 percent infection, 2 strains produced 90 percent infection and the remaining strains caused 100 percent infection. By the intraperitoneal route of inoculation, 13 out of 16 strains produced 70.8 percent infection. Three strains did not cause infection, 1 strain produced 10 percent infection, 1 strain resulted in 30 percent infection, 2 strains caused 40 percent infection, 1 strain produced 60 percent infection, 1 strain produced 70 percent infection, 3 strains caused 90 percent infection and 4 strains produced 100 percent infection. Sixteen strains produced 83.3 percent infection following subcutaneous inoculation when abscess formation at the site of inoculation was included as evidence of infection. One strain produced 10 percent

infection, 1 strain caused 40 percent infection, 2 strains produced 70 percent infection, 2 strains elicited 80 percent infection, 2 strains produced 90 percent infection and the remaining strains caused 100 percent infection. When abscess formation at the site of subcutaneous inoculation was not included as evidence of infection, 10 out of 16 strains produced 21.2 percent infection. Six strains did not produce infection, 3 strains elicited 10 percent infection, 2 strains produced 20 percent infection, 1 strain produced 30 percent infection, 2 strains caused 40 percent infection, 1 strain produced 50 percent infection and 1 strain caused 80 percent infection. When the percentage of infection did not include abscess formation at the site of subcutaneous inoculation, the differences in the percentage of infection for the 3 routes of inoculation was significant at the 1 percent level. This was determined by the Chi square method of analysis. The difference in the percentage infection between the intravenous and subcutaneous routes was not significant at the 5 percent level when abscess formation at the site of subcutaneous inoculation was used in determining the percentage of infection. A summary of the results with each strain, by the various routes of inoculation is presented in Tables VI and VII.

Intravenous inoculation produced infection in 76.8 percent of the total number of organs cultured, intraperitoneal inoculation caused infection in 36.2 percent of the

organs and subcutaneous inoculation produced 13.0 percent infection in the organs cultured. The differences in the percentage of the total number of organs infected for each route of inoculation was significant at the 1 percent level as determined by the Chi square method.

The total percentage of individual organs infected following intravenous inoculation were: 75.4 percent of the kidneys, 63.5 percent of the reproductive tracts, 62.9 percent of the spleens, 62.2 percent of the livers, 60.3 percent of the lungs and 58.3 percent of the hearts. The difference in infection rate between the various organs was not significant, at the 5 percent level, for this route of inoculation.

The total percentage of individual organs infected following intraperitoneal inoculation were: 50.3 percent of the kidneys, 38.2 percent of the reproductive tracts, 36.9 percent of the spleens, 36.9 percent of the livers, 25.5 percent of the lungs and 20.1 percent of the hearts. The difference in the infection rate between the various organs was significant at the 1 percent level except for the infection rate between the livers and the spleens which was not significant at the 5 percent level.

The total percentage of individual organs infected following subcutaneous inoculation were: 20.6 percent of the kidneys, 15.3 percent of the reproductive tracts, 13.3 percent of the livers, 12.6 percent of the spleens, 12.6

percent of the lungs and 9.3 percent of the hearts. The difference in the infection rate between the various organs was not significant at the 5 percent level for this route of inoculation.

The number of organs infected by each strain per route of inoculation are presented in Table I of the appendix. The number of organs infected per mouse by each strain for the 3 routes of inoculation are shown in Table III of the appendix.

Of the mice exposed by the intravenous route, females showed 79.1 percent of infection and males 61.4 percent, while intraperitoneal inoculation caused 26.2 percent infection in the females and 46.6 percent infection in the males and subcutaneous inoculation resulted in 12.3 percent infection in the females and 17.6 percent infection in the males. A significant difference, at the 1 percent level, existed in the reproductive tract infection between male and female mice by the intravenous and intraperitoneal routes of inoculation. There was no significant difference in the reproductive tract infection between male and female mice by the subcutaneous route. The number of male and female mice which developed infection with each strain by various routes of inoculation are presented in Table VIII.

On autopsy the animals were observed for signs of gross pathological change. The kidneys showed congestion or necrosis usually accompanied by one or more encapsulated

abscesses. The liver was enlarged or congested. Widespread, pin point or large multiple encapsulated abscesses were also present in many cases. The spleen was congested and/or enlarged. Changes in the lungs were characterized by hemorrhagic pneumonic areas. Reproductive tract alterations involved abscess formation only. Multiple abdominal abscesses of the mesentery and peritoneum were observed. Abscesses were also found at the site of subcutaneous or intraperitoneal inoculation. Abscesses were occasionally found on the surface of the heart. It is realized that the pathological changes, other than abscess formation, as seen in the kidneys, liver and spleen may not be due to the presence of the infective agent. The changes, however, observed in organs from which the infective agent was recovered were similar to those which were bacteriologically negative.

Intravenous inoculation produced a total of 59.0 percent pathological changes, intraperitoneal inoculation caused a total of 56.7 percent and subcutaneous inoculation produced a total of 70.3 percent pathological changes. The differences between the total percentage of pathological changes for the 3 routes of inoculation was not significant at the 5 percent level.

The total percentage of gross pathological changes in mice inoculated intravenously was as follows: 83.5 percent of the kidneys, 56.2 percent of the livers, 37.0 percent of the spleens, 3.9 percent of the lungs, 1.3 percent of the

reproductive tract and 1.3 percent of the hearts. Intraperitoneal inoculation produced the following total percentage of gross pathological changes: 82.5 percent of the kidneys, 55.0 percent of the livers, 44.2 percent of the spleens, 14.0 percent abdominal abscesses, 5.3 percent of the reproductive tracts, 0.6 percent of the lungs and 0.0 percent of the hearts. The total percentage of gross pathological changes following subcutaneous inoculation was: 83.3 percent abscesses at the site of inoculation, 72.0 percent of the kidneys, 44.6 percent of the livers, 37.3 percent of the spleens, 3.3 percent of the lungs and 0.0 percent of the reproductive tracts and hearts. The total number of organs or sites showing gross pathological alterations with each strain for the 3 routes of inoculation are presented in Table II of the appendix.

DISCUSSION

The cultural characteristics of the 16 strains of C. pseudotuberculosis used in the experiments were similar to those observed by Carne (1939), Norgaard and Mohler (1899) and other investigators. Dextrose and galactose were fermented with the formation of acid only, lactose was not attacked and sucrose fermentation was variable. Carne (1939) found that lactose and sucrose fermentation was variable but that only acid was produced and that dextrose and galactose were attacked with the formation of acid. Norgaard and Mohler (1899) observed that dextrose and sucrose were fermented with the formation of acid, and that lactose was not attacked. Carne (1939) found that only a few strains would reduce nitrates to nitrites. Two of the strains used in the experiments reported in this thesis were able to reduce nitrate to nitrite. Gelatin was not liquified and indole was not formed which conformed with the findings of Norgaard and Mohler (1899) and Carne (1939). Litmus milk was not changed, the Methyl Red test was negative and acetyl methyl carbinol was not produced. These results were similar to those observed by Carne (1939). Fourteen of the strains used in the experiments were able to hydrolyze urea. These results were similar to those found by Morse (1949 and 1950). The cultural characteristics of the strains

used in the experiments and those reported by other investigators indicated that the strains used were typical of C. pseudotuberculosis.

The hemolytic characteristics of the 16 strains indicated differences depending upon the type of blood, the method of culture and the particular strain.

Hemolytic activity of aerobic culture showed the following order of erythrocyte susceptibility: sheep> cow> horse> rabbit> human> guinea pig> dog. These results are in contradiction to those reported by Carne (1939). He found the following order of susceptibility: guinea pig> rabbit> horse> sheep. The differences between the two can be accounted for because of possible differences in the individual strains, the method of incubation or the type of base media employed.

Fourteen of the strains were beta hemolytic on sheep blood when cultured aerobically. It follows from this that a hemolysin is produced by these 14 strains, even if it is not demonstrated with the blood from other species of animals. This indicates that the hemolysin is specific or that the erythrocytes of the different species may differ in their susceptibility to the hemolysin produced by the various strains of C. pseudotuberculosis. This postulation is in agreement with that of Carne (1939).

Bernheimer and Cantoni (1945) showed that the hemolysin from Group A streptococci was enzymatic in nature. Other

work with Group A streptococci, (Merchant, 1950 and Smythe and Harris, 1940), indicated that more than one type of hemolysin may be produced by the same organism. The possibility then exists that C. pseudotuberculosis is also capable of producing more than one hemolysin and that the variation in hemolysis is not due to the source of the erythrocytes but to the chemical nature and number of distinct or specific hemolysins produced by the particular strain.

Semianaerobic culture of the 16 strains on the blood from the 7 different animals gave results different from those found on aerobic culture. Alpha or beta hemolysis was observed with the same strain on different bloods and with different strains on the same blood. Production of alpha or beta hemolysis on semianaerobic culture was observed, while on aerobic culture the same strain did not produce hemolysis. Carne (1939) indicated that this change was probably due to the oxygen labile nature of the hemolysin. However, the experimental results reported in this thesis show that some strains capable of producing beta hemolysis on aerobic culture did not produce any type of hemolysis on semianaerobic culture.

The results from aerobic and semianaerobic culture indicate that the difference in susceptibility of erythrocytes, from various animals, to the hemolysin of different strains of C. pseudotuberculosis may be due to a number

of different factors such as: the nature of the erythrocytes, the chemical and biological nature of the hemolysin produced, the presence or absence of oxygen and the number of specific hemolysins produced. In reasoning from the research concerning the hemolysins of Group A streptococci it is evident that further elucidation on the chemical nature and the possible enzymatic properties of the hemolysin(s) of C. pseudotuberculosis is necessary before the results obtained in this experiment are fully understood.

Determination of the number of bacteria per ml of inoculum by the plate count method and the direct count method showed relatively large differences between each strain and each technique, although all of the inocula were standardized to the same degree of light transmission. The difference in the number of bacteria by the two methods was attributed to: 1) the variable clumping of the cells which occurred even with the addition of Tween 80 to the culture medium, 2) errors in performing the dilutions, 3) the presence of nonviable organisms, and 4) errors in counting the number of colonies or cells.

The 16 strains of C. pseudotuberculosis gave divergent results depending both upon the strain inoculated and the route of inoculation. The 3 routes of inoculation produced infection in the mice in the following order: intravenous> intraperitoneal> subcutaneous. The difference in the total percentage of infection between the 3 routes of inoculation

was significant at the 1 percent level. These results are similar to those obtained by Morse, Robertstad, Wipf and Glattli (1952), using mice inoculated with C. pyogenes and Morse and Wipf (1951), working with mice inoculated with C. renale. The results also seem to follow the general pattern for any pathogenic bacterium when inoculated by different routes into an experimental host. The intravenous route will result in the greatest incidence of generalized infection because of the immediate dissemination of the organisms to all parts of the body. Infection following subcutaneous inoculation requires the penetration of the organisms into the tissues of the host in order to produce other than a localized infection. The defensive mechanisms of the body in this case are capable of preventing a generalized infection more often than when intravenous inoculation is employed.

A comparison of the total percentage of bacteriologically positive organs for each route of inoculation showed a significant difference at the 1 percent level. However, an identical trend in the total percentage of individual organs infected existed for the 3 routes of inoculation. This trend gave the following order: kidneys > reproductive tract > spleen > liver > lungs > heart. In all cases the kidneys showed the highest total percentage of infection as compared to the other organs. The total percentage of kidney infection as compared to the total percentage of

infection in organs, by each route of inoculation, indicated a significant difference at the 1 percent level. This significant difference in the rate of kidney involvement would seem to indicate that this diphtheroid had an affinity for kidney tissue. This predilection may be the result of two factors: 1) the ability of many of the strains to hydrolyze urea which would be found in a high concentration in kidney tissue and 2) the inability of clumps of these cells to pass through the capillaries of the glomerular tuft. However, other unrecognized factors may also be responsible for this apparent predilection. Other diphtheroids have also been shown to produce a high percentage of kidney infection similar to that found with C. pseudotuberculosis. Morse, Robertstad, Wipf and Glattli (1952) working with C. pyogenes and Kuzdas, Morse and Ellis (1951) and Morse and Wipf (1951) using C. renale have shown that these diphtheroids consistently produce infection of the kidneys to a greater extent than any other organ when using mice as the experimental host. However, C. pyogenes does not produce urease therefore it is evident that some other property must be the cause of the high percentage of kidney infections. On the other hand C. renale does produce urease and this seems to be one of the factors responsible for the high percentage of kidney or urinary tract infections.

Hall and Stone (1916), Norgaard and Mohler (1899) and Nocard (1896) observed that intraperitoneal inoculation of

male guinea pigs with C. pseudotuberculosis produced orchitis which occasionally terminated in a suppurative lesion if the animal was allowed to live for a sufficient period of time. Neither orchitis nor suppurative lesions around the scrotal sac were observed in any of the male mice following inoculation of C. pseudotuberculosis via the intraperitoneal route. However, on the basis of bacteriological results male mice showed a total of 46.6 percent reproductive tract infection as compared with 26.2 percent for female mice. This difference proved to be significant at the 1 percent level. These results indicate that male mice are more susceptible to reproductive tract infections, following intraperitoneal inoculation of C. pseudotuberculosis, than are female mice and that orchitis and/or suppurative lesions around the scrotal sac might have developed if the mice had been allowed to survive for longer than 14 days.

Ten strains of C. pseudotuberculosis, when inoculated subcutaneously, produced metastatic lesions in internal organs as well as localized abscesses. The kidneys again showed the greatest incidence of infection. If this property is considered to be a characteristic of virulence, then those strains that are able to metastasize to internal organs are more virulent than those strains capable only of producing a localized abscess following subcutaneous inoculation. These results indicate that characterization of various strains of this diphtheroid into pathogenic and

nonpathogenic groups on the basis of invasiveness in mice may further aid in the delineation of C. pseudotuberculosis.

Gross pathological alterations in the experimentally infected mice most commonly involved the kidneys, liver and spleen. A consistent descending incidence of pathological change trend of kidneys-liver-spleen was exhibited for all 3 routes of inoculation. With the exception of the reproductive tract alterations, this trend parallels that observed in the bacteriological results.

Nicolle, Loiseau and Forgeot (1912), Hall and Stone (1916) and Petri and McClean (1934) have shown in vitro and in vivo toxin production by C. pseudotuberculosis. The type of pathological lesions observed in the experimentally infected mice were similar to those reported by these investigators as being due to the action of a toxin. This toxic degeneration may account for the presence of pathological lesions in the absence of bacteriological evidence of infection in certain organs.

Attempts to divide the 16 strains of C. pseudotuberculosis into groups on the basis of cultural or hemolytic characteristics was not conclusive. Results of the pathogenicity experiments indicated that certain strains, on subcutaneous inoculation in mice, had the ability to produce as abscess at the site of inoculation and to metastasize to internal organs whereas other strains could only produce an abscess at the site of inoculation. A comparison

of the hemolytic characteristics and urea hydrolyzing ability of the 16 strains with their ability to produce a generalized infection in mice was undertaken to ascertain if a combination of characteristics might indicate a means of grouping the various strains. The results indicated that two strains (2335 and 1965) which were non-hemolytic on all of the bloods tested and were not able to hydrolyze urea were also relatively nonpathogenic in the experimental host. This indicates in a limited way that hemolysis and urease activity may be involved in the pathogenicity of these two strains. Further elaboration of this point is required. In addition, further research involving serological relationships, toxin production and possibly the nature of the hemolysin should be undertaken before definite classification of the strains into groups or types can be accomplished.

SUMMARY AND CONCLUSION

The cultural characteristics of the 16 strains of C. pseudotuberculosis indicated that they were relatively homogeneous with the exception of their hemolytic activity. The hemolytic activity of the strains depended upon the individual strain, the type of blood used and the amount of oxygen present during incubation. The results indicated the possible presence of more than one type of hemolysin for a single strain.

The virulence of the cultures was dependent upon the route of inoculation. The differences in the rate of infection by the various routes of inoculation proved to be significant. It appeared that renal predilection was demonstrable since kidney tissue showed the greatest percentage of infection as compared to the other organs. In general, correlations could be made regarding the frequency of infections in other organs. Intraperitoneal inoculation resulted in a greater percentage of reproductive tract infections in male mice than in female mice. This difference was significant. Intraperitoneal inoculations were characterized by the formation of abscesses on the peritoneum and on the mesentery. Subcutaneous inoculations indicated that certain strains had considerable invasive ability. A few strains produced localized abscesses but

did not cause a generalized infection. Pathological changes in the kidneys, liver and spleen were observed which resembled those seen in organs undergoing toxic degeneration.

All strains proved to have some degree of pathogenicity for laboratory white mice. Some strains proved to be highly virulent by all routes of inoculation, while others were only capable of inducing localized abscesses at the site of subcutaneous inoculation. Some degree of infectivity was therefore demonstrated for all cultures examined.

A combination of hemolytic characteristics, urease producing ability and pathogenicity indicated a method which might possibly be used to group the various strains of this species.

TABLE I

History of Sixteen Strains of
Corynebacterium pseudotuberculosis

Strain	History
2335	Isolated August 1943 from a synovial culture from a lamb. On autopsy infarcts in both kidneys were noted. The synovial fluid was turbid, and erosion of the joints was evident. Obtained from H. Marsh, University of Montana, Bozeman.
1742	Isolated November 1940 from the thoracic wall of a deer. The pleura showed a large number of nodules which contained pus. Obtained from H. Marsh, University of Montana, Bozeman.
5364	Isolated January 1953 from a sheep. On autopsy abscesses were found in the lungs, mediastinal lymph nodes, liver and kidneys. Obtained from H. Marsh, University of Montana, Bozeman.
5362	Isolated January 1953 from a sheep with multiple submaxillary abscesses and miliary abscesses of both lungs. Obtained from H. Marsh, University of Montana, Bozeman.
1648	Isolated April 1940 from a caseous lymph node abscess in a sheep. Obtained from H. Marsh, University of Montana, Bozeman.
434	Isolated August 1955 from a lung abscess in an experimental ewe at the University of Wisconsin, Madison. Obtained from E.V. Morse, Michigan State University, East Lansing.
409H	Isolated August 1955 from a pulmonary lymph node of a sheep. Obtained from E.V. Morse, Michigan State University, East Lansing.
409NH	Isolated October 1955, a nonhemolytic variant of culture 409H. Michigan State University, East Lansing.
1965	Isolated October 1941 from the brain of a lamb. Obtained from H. Marsh, University of Montana, Bozeman.

TABLE I CONTINUED

Strain	History
CCP-1	A recently isolated culture. The exact date and history of isolation was unknown. Obtained from D. Bruner, Cornell University, Ithaca.
1741	Isolated November 1940 from a horse. Obtained from H. Marsh, University of Montana, Bozeman.
OV-1	Isolated 1950 from a case of caseous lymphadenitis in a sheep. Obtained from G.J. Jann, University of California Medical Center, Los Angeles.
OV-7	Isolated 1930 from a caseous lymph node of a sheep. Obtained from C.J. Jann, University of California Medical Center, Los Angeles.
OV-9	Isolated 1952 from a caseous lymph node of a sheep. Obtained from C.J. Jann, University of California Medical Center, Los Angeles.
OV-10	Isolated 1952 from a caseous lymph node of a sheep. Obtained from C.J. Jann, University of California Medical Center, Los Angeles.
0-2-56	Isolated February 1956 from the periscapular lymph node of a lamb. Obtained from J.P. Newman, Michigan State University, East Lansing.

TABLE II

Morphological Characteristics of
Corynebacterium pseudotuberculosis

Strain	Characteristics
2335	Gram positive rod shaped
1742	Gram positive coccoid shaped
5364	Gram positive coccoid shaped
5362	Gram positive coccoid shaped
1648	Gram positive coccoid shaped
434	Gram positive coccoid shaped
409H	Gram positive coccoid shaped
409NH	Gram positive coccoid shaped
1965	Gram positive rod shaped
CCP-1	Gram positive coccoid shaped
1741	Gram positive coccoid shaped
OV-1	Gram positive coccoid shaped
OV-7	Gram positive coccoid shaped
OV-9	Gram positive coccoid shaped
OV-10	Gram positive coccoid shaped
O-2-56	Gram positive coccoid shaped

TABLE III

Cultural Characteristics of
Corynebacterium pseudotuberculosis

Strain	Dextrose	Galactose	Lactose	Sucrose	Nitrate Reduction
2335	A	A	-	-	-
1742	A	A	-	A	-
5364	A	A	-	A	-
5362	A	A	-	A	-
1648	A	A	-	A	-
434	A	A	-	A	-
409H	A	A	-	A	-
409NH	A	A	-	A	-
1965	A	A	-	A	-
CCP-1	A	A	-	A	+
1741	A	A	-	A	+
OV-1	A	A	-	A	-
OV-7	A	A	-	-	-
OV-9	A	A	-	A	-
OV-10	A	A	-	-	-
O-2-56	A	A	-	A	-

A = Acid production, no gas

\- = No reaction

\+ = Positive reaction

TABLE III CONTINUED

Methyl Red	Voges Proskauer	Gelatin Liquefaction	Urea Hydrolysis	Litmus Milk	Indole
-	-	-	-	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	-	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-

TABLE IV

Hemolytic Characteristics of Sixteen Strains of *Corynebacterium pseudotuberculosis* on Aerobic and Semianaerobic Incubation

Strain	Blood					
	Sheep		Cow		Horse	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
2335	-	-	-	x	-	-
1742	/	-	-	/	-	-
5364	/	/	/	/	/	-
5362	/	/	/	/	/	-
1648	/	-	-	/	-	-
434	/	/	/	/	-	/
409H	/	/	/	/	/	/
409NH	/	-	-*	/	-	-
1965	-	-	-	-	-	-
CCP-1	/	-	/	/	-	/
1741	/	-	/	/	/	/
OV-1	/	-	/	/	/	/
OV-7	/	-	/	/	-	/
OV-9	/	-	/	/	/	/
OV-10	/	/	/	/	/	/
0-2-56	/	/	/	/	/	/

/ = Beta hemolysis - = No hemolysis

x = Alpha hemolysis

* Showed cold hemolysis following 24 hours incubation at 4 C after incubation at 37 C for 48 hours.

TABLE IV CONTINUED

Blood							
Rabbit		Human		Guinea pig		Dog	
Aer-obic	Anaer-obic	Aer-obic	Anaer-obic	Aer-obic	Anaer-obic	Aer-obic	Anaer-obic
-	≠	-	-	-	x	-	-
-	≠	-	x	-	-	-	-
≠	≠	-	x	-	-	-	-
≠	x	-	x	-	x	-	-
≠	x	-	x	-	-	-	-
-	≠	-	≠	-	≠	-	-
≠	≠	≠	≠	-	≠	-	-
-	≠	-	x	-	-	-	-
-	-	-	-	-	-	-	-
-	x	-	x	-	-	-	-
≠	x	≠	≠	-	≠	-	-
≠	≠	≠	≠	-	≠	-	-
≠	≠	-	≠	-	≠	-	-
≠	≠	≠	≠	-	≠	-	-
≠	≠	-	≠	-	≠	-	-
≠	≠	≠	≠	-	≠	-	≠

TABLE V

Organisms per ml of Inoculum by Direct Microscopic Count and by Plate Count

Strain	Direct Count Method	Plate Count Method
2335	20.2×10^7	63.6×10^5
1742	39.1×10^7	33.6×10^5
5364	40.7×10^7	101.0×10^5
5362	29.4×10^7	7.7×10^5
1648	45.5×10^7	33.5×10^5
434	43.5×10^7	124.0×10^5
409H	27.2×10^7	56.0×10^5
409NH	26.8×10^7	58.0×10^5
1965	22.9×10^7	15.5×10^5
CCP-1	48.1×10^7	15.4×10^5
1741	43.5×10^7	84.0×10^5
OV-1	24.3×10^7	38.3×10^5
OV-7	25.8×10^7	40.1×10^5
OV-9	41.8×10^7	17.7×10^5
OV-10	28.7×10^7	11.3×10^5
O-2-56	36.8×10^7	17.7×10^5
Range:	$20.2 - 48.1 \times 10^7$	$7.7 - 124.0 \times 10^5$
Mean:	34.0×10^7	44.8×10^5

TABLE VI

Total Number of Mice Showing Infection by Each Route of Inoculation**

Strain	Route of Inoculation		
	Intravenous	Intraperitoneal	Subcutaneous*
2335	4/10	0/10	1/10
1742	5/10	1/10	4/10
5364	10/10	9/10	8/10
5362	9/10	6/10	10/10
1648	9/10	0/10	7/10
434	10/10	3/10	10/10
409H	10/10	10/10	10/10
409NH	10/10	10/10	10/10
1965	0/10	0/10	9/10
CCP-1	10/10	10/10	10/10
1741	10/10	10/10	10/10
OV-1	10/10	9/10	8/10
OV-7	10/10	4/10	9/10
OV-9	10/10	7/10	10/10
OV-10	10/10	9/10	7/10
0-2-56	10/10	4/10	10/10
Total	136/160	92/160	133/160
Percent	85.0	57.0	83.3

* Includes those mice with generalized infections and/or abscesses at the site of inoculation.

** Includes those mice with generalized infections and those dead and not autopsied.

TABLE VII

Generalized Infection and Abscess Formation by the Subcutaneous Route of Inoculation *

Strain	Organ+ Site+	Organ + Site -	Organ - Site +	Organ - Site -
2335	0/10	1/10	1/10	8/10
1742	0/10	0/10	4/10	6/10
5364	0/9	0/9	7/9	2/9
5362	1/7	0/7	6/7	0/7
1648	0/10	0/10	7/10	3/10
434	1/10	0/10	9/10	0/10
409H	8/8	0/8	0/8	0/8
409NH	4/8	0/8	4/8	0/8
1965	0/10	0/10	9/10	1/10
CCP-1	0/10	0/10	10/10	0/10
1741	3/10	0/10	7/10	0/10
OV-1	4/7	0/7	3/7	0/7
OV-7	1/10	0/10	8/10	1/10
OV-9	2/10	0/10	8/10	0/10
OV-10	5/7	0/10	2/7	0/7
O-2-56	2/10	0/10	7/10	0/10
Total	31/146	1/146	93/146	21/146
Percent	21.2	0.7	63.7	14.4

* Includes only those mice with generalized infections and/or those with abscesses at the site of inoculation.

TABLE VIII

Reproductive Tract Infection
in Male and Female Mice*

Strain	Intravenous		Intraperitoneal		Subcutaneous	
	Female	Male	Female	Male	Female	Male
2335	0/0	1/10	0/0	0/10	0/0	0/10
1742	0/4	0/6	0/5	0/5	0/7	0/3
5364	3/3	5/5	2/3	4/6	0/4	0/6
5362	3/4	0/4	2/6	2/3	0/6	0/1
1648	3/5	1/3	0/6	0/4	0/4	0/6
434	4/5	4/4	1/5	0/5	0/3	0/7
409H	0/0	8/9	0/0	8/8	0/0	8/8
409NH	0/0	8/8	0/0	5/10	0/0	2/8
1965	0/0	0/10	0/7	0/3	0/4	0/6
CCP-1	5/5	4/4	6/6	2/2	0/5	0/5
1741	2/2	5/5	3/3	4/4	0/0	1/8
OV-1	0/0	9/9	0/0	7/10	0/0	3/8
OV-7	0/1	4/9	0/5	3/5	0/3	0/7
OV-9	5/5	3/3	0/2	4/8	0/5	1/5
OV-10	1/1	5/5	2/5	2/3	7/10	0/0
0-2-56	8/8	2/2	0/8	0/2	0/6	0/4
Total	34/43	59/96	16/61	41/88	7/57	16/91
Percent	79.1	61.4	26.2	46.6	12.3	17.6

* Includes only those mice showing positive bacteriological culture. Does not include those mice which died and were not autopsied.

REFERENCES

American Public Health Association 1946 Standard Methods for the Examination of Water and Sewage. 9th ed. Published by the Association, New York p 231.

Bernheimer, A.W. and Cantoni, G.L. 1945 The Cardiotoxic Action of Preparations Containing Oxygen Labile Hemolysin of Streptococcus pyogenes. J. Exp. Med., 81, pp 295-306.

Brooks, R.F. and Hucker, G.J. 1944 Cultural Characteristics of Seventy-nine Strains of Corynebacteria. J. Bact., 48, pp 295-312.

Bull, L.B. and Dickinson, C.G. 1933 Studies on Infections by and Resistance to the Preisz-Nocard Bacillus. Australian Vet. Res. Jour., 9, pp 82-93. Original article not seen. Obtained from Biol. Abs., 9, p 361 1935.

Bull, L.B. and Dickinson, C.G. 1934 Caseous Lymphadenitis-Factors Associated with Spread Under Natural Conditions. J. Counc. Sci. and Indust. Res. Australia, 7, pp 78-86. Original article not seen. Obtained from Biol. Abs., 9, p 1061 1935.

Carne, H.R. 1939 A Bacteriological Study of 134 Strains of Corynebacterium ovis. J. Pat. and Bact., 49, pp 313-328.

DeKock, G. and Fourie, P.J.J. 1931 Necrosis in the Musculature and Myocard of Sheep. Union S. Africa Rept. Dir. Vet. Educ. and Res., 13, pp 595-599. Original article not seen. Obtained from Biol. Abs., 5, p 146 1931.

Eberson, F.A. 1918 A Bacteriologic Study of the Diphtheroid Organism with Special Reference to Hodgkin's Disease. J. Infect. Dis., 23, pp 1-42.

Hagan, W.A. 1947 The Infectious Diseases of Domestic Animals. Comstock Publishing Co. Inc., Ithica, New York, pp 109-111.

Hall, I.C. and Stone, R.V. 1916 The Diphtheroid Bacillus of Preisz-Nocard from Equine, Bovine and Ovine Abscesses. J. Infect. Dis., 18, pp 195-208.

Howard, D.H. and Jann, G.J. 1955 The Isolation of a Bacteriophage Active Against Corynebacterium pseudotuberculosis. J. Bact., 69, p 108.

Kelser, R.A. and Schoening, H.W. 1943 Manual of Veterinary Bacteriology. 4th ed, The Williams and Wilkins Co., Baltimore, Md., pp 353-356.

Kuzdas, C.D., Morse, E.V. and Ellis, R.H. 1951 A Systematic Study of the Pathogenicity of Sixty-two Strains of Corynebacterium renale for Laboratory White Mice. J. Bact., 62, pp 763-766.

Lovell, R. 1937 Studies on Corynebacterium pyogenes with Special Reference to Toxin Production. J. Path. and Bact., 45, pp 339-355.

Maddy, K.T, 1953 Caseous Lymphadenitis of Sheep. J. Am. Vet. Med. Assoc., 122, pp 257-259.

Manual of Methods for Pure Culture Study. 1946 Biotech Publications. Geneva, N.Y., Leaflet V, p 10.

Marsh, H. 1947 Corynebacterium ovis Associated with an Arthritis in Lambs. Am J. Vet. Res., 8, pp 294-298.

Merchant, I.A. 1950 Veterinary Bacteriology and Virology. 4th.ed., The Iowa State College Press, Ames, Iowa, pp 168 and 549-552.

Morcas, Z. 1932 The Preisz-Nocard Bacillus and Ulcerative Lymphangitis. J. Egypt. Med. Assoc., 15, pp193-197. Original Article not seen. Obtained from Biol. Abs., 7, p 894 1933.

Morse, E.V. 1949 The Urease Activity of Certain Pathogenic Bacteria Isolated from Animal Infections. The Cornell Vet., 39, pp 77-78.

Morse, E. V. 1950 Further Studies on the Cultural and Biochemical Characteristics of some Diphtheroid Bacilli Isolated from Animals. The Cornell Vet., 40, pp 49-55.

Morse, E.V. and Wipf,L. 1951 Experimental Pyelonephritis in Mice, Rabbits and Cattle. Proceedings Book, Am. Vet. Med. Assoc. Eighty-eighth Annual Meeting, pp 89-98.

Morse, E.V., Robertstad, G.W., Wipf, L. and Glattli, H.R. 1952 A Study of the Pathogenicity of Corynebacterium pyogenes for Laboratory White Mice, Hamsters and Rabbits. The Cornell Vet., 42,

Nicolle, M., Loiseau, G. and Forgeot, P. 1912 Les Facteurs De Toxicite Des Bacteries-Etude Des Bacilles Preisz-Nocard. Ann. Inst. Pasteur, 26, pp 83-86.

Nocard, E. 1896 Sur Une Lymphangite Ulcereuse. Ann. Inst. Pasteur, 10, pp 609-617.

Norgaard, V.A. and Mohler, J.R. 1899 The Nature, Cause and Economic Importance of Ovine Caseous Lymphadenitis. Sixteenth Annual Report of the Bureau of Animal Industries, U. S. Dept. of Agriculture, Washington, pp 638-662.

Petrie, G.F. and McClean, D. 1934 The Inter-relationships of Corynebacterium ovis and Corynebacterium diphtheriae and Certain Diphtheroid Strains Derived from the Human Nasopharynx. J. Path. and Bact., 39, pp 635-663.

Read, R. and Read, J. 1948 A Drop Plate Method for the Determinations of Coliforms. Can. J, Res., 26, pp 317-326.

Rottgardt, A. 1928 Le Supuracion Caseousa de los Ovinos. Rev. Sud-Americana Endrocrinol. and Immunol., 11, pp 777-785. Original article not seen. Obtained from Biol. Abs., 4, p 1448 1930.

Seddon, H.R. and Belschner, H.G. 1927 Bacterial Infection Associated with Grass Seed Infestation in Sheep. Dept. of Agriculture, New So. Wales Sci. Bull., 29, pp 26-30. Original article not seen. Obtained from Biol. Abs., 3, p 947 1929.

Smith, N.R. 1946 Aerobic Mesophilic Spore Forming Bacteria. U.S. Dept. of Agriculture, Washington, Misc. Pub. #559, p 4.

Smythe, C.V. and Harris, T.N. 1940 Some Properties of a Hemolysin Produced by Group A Beta Hemolytic Streptococci. J. Immunol., 38, pp 283-300.

APPENDIX

TABLE I

Total Number of Organs Showing Infection
by Each Route of Inoculation *

Strain	Intravenous Route					
	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
2335	0/10	3/10	1/10	1/10	1/10	0/10
1742	1/10	4/10	0/10	0/10	1/10	1/10
5364	8/8	8/8	8/8	8/8	8/8	8/8
5362	5/8	6/8	3/8	6/8	3/8	3/8
1648	0/8	6/8	5/8	0/8	0/8	0/8
434	9/9	9/9	8/9	6/9	6/9	4/9
409H	10/10	10/10	10/10	10/10	10/10	10/10
409NH	8/9	9/9	8/9	9/9	8/9	7/9
1965	0/10	0/10	0/10	0/10	0/10	0/10
CCP-1	9/9	9/9	9/9	9/9	9/9	9/9
1741	7/7	7/7	7/7	7/7	7/7	7/7
OV-1	9/9	9/9	9/9	9/9	9/9	9/9
OV-7	5/10	10/10	4/10	5/10	5/10	6/10
OV-9	8/8	8/8	8/8	8/8	8/8	8/8
OV-10	6/6	6/6	6/6	6/6	6/6	6/6
0-2-56	10/10	10/10	10/10	10/10	10/10	10/10
Total	95/151	114/151	96/151	94/151	91/151	88/151
Percent	62.9	75.4	63.5	62.2	60.3	58.3

* Does not include those mice which died and were not autopsied.

TABLE I CONTINUED

Strain	Intraperitoneal Route					
	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
2335	0/10	0/10	0/10	0/10	0/10	0/10
1742	1/10	1/10	0/10	1/10	0/10	0/10
5364	5/10	8/10	6/10	6/10	3/10	2/10
5362	4/9	5/9	4/9	3/9	2/9	2/9
1648	0/10	0/10	0/10	0/10	0/10	0/10
434	2/10	3/10	1/10	1/10	0/10	0/10
409H	8/8	8/8	8/8	8/8	8/8	8/8
409NH	4/10	9/10	5/10	3/10	0/10	0/10
1965	0/10	0/10	0/10	0/10	0/10	0/10
CCP-1	8/8	8/8	8/8	8/8	7/8	7/8
1741	7/7	7/7	7/7	7/7	7/7	7/7
OV-1	6/10	8/10	7/10	7/10	4/10	4/10
OV-7	4/10	3/10	3/10	2/10	0/10	0/10
OV-9	1/10	5/10	4/10	3/10	1/10	0/10
OV-10	5/8	7/8	4/8	5/8	4/8	0/8
0-2-56	0/10	3/10	0/10	1/10	0/10	0/10
Total	55/149	75/149	57/149	55/149	38/149	30/149
Percent	36.9	50/3	38.2	36.9	25.5	20.1

TABLE I CONTINUED

Strain	Subcutaneous Route					
	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
2335	0/10	0/10	0/10	0/10	0/10	0/10
1742	0/10	0/10	0/10	0/10	0/10	0/10
5364	0/9	0/9	0/9	0/9	0/9	0/9
5362	1/7	0/7	0/7	0/7	0/7	0/7
1648	0/10	0/10	0/10	0/10	0/10	0/10
434	0/10	0/10	0/10	1/10	0/10	0/10
409H	5/8	8/8	8/8	5/8	5/8	5/8
409NH	2/8	4/8	2/8	2/8	2/8	2/8
1965	0/10	0/10	0/10	0/10	0/10	0/10
CCP-1	0/10	0/10	0/10	0/10	0/10	0/10
1741	1/10	3/10	1/10	3/10	2/10	1/10
OV-1	3/8	5/8	3/8	2/8	2/8	1/8
OV-7	0/10	1/10	0/10	0/10	0/10	0/10
OV-9	0/10	1/10	1/10	0/10	0/10	0/10
OV-10	7/10	7/10	7/10	7/10	8/10	5/10
0-2-56	0/10	2/10	0/10	0/10	0/10	0/10
Total	19/150	31/150	23/150	20/150	19/150	14/150
Percent	12.6	20.6	15.3	13.3	12.6	9.35

TABLE II

Total Number of Mice Exhibiting Pathological Alterations and Site or Abdominal Abscesses by Each Route of Inoculation

Strain	Intravenous Route					
	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
2335	6/10	10/10	1/10	6/10	0/10	0/10
1742	0/10	2/10	0/10	1/10	0/10	0/10
5364	0/8	8/8	0/8	6/8	0/8	0/8
5362	5/8	7/8	0/8	4/8	0/8	0/8
1648	2/8	8/8	0/8	1/8	0/8	0/8
434	1/9	9/9	0/9	5/9	0/9	0/9
409H	4/8	8/8	0/8	6/8	0/8	0/8
409NH	8/8	3/8	0/8	7/8	1/8	0/8
1965	3/10	9/10	0/10	9/10	0/10	0/10
CCP-1	0/8	8/8	0/8	1/8	0/8	0/8
1741	0/7	7/7	0/7	6/7	0/7	0/7
OV-1	6/9	8/9	0/9	8/9	4/9	0/9
OV-7	5/10	10/10	0/10	8/10	0/10	1/10
OV-9	6/8	8/8	0/8	2/8	1/8	1/8
OV-10	6/6	6/6	1/6	6/6	0/6	0/6
0-2-56	4/10	10/10	0/10	9/10	0/10	0/10
Total	56/151	126/151	2/151	85/151	6/151	2/151
Percent	37.0	83.5	1.3	56.2	3.9	1.3

* Does not include those mice which died and were not autopsied.

TABLE II CONTINUED

Strain	Intraperitoneal Route						
	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Abdominal Abscesses
2335	3/10	10/10	0/10	8/10	0/10	0/10	0/10
1742	0/10	2/10	0/10	0/10	0/10	0/10	0/10
5364	5/9	8/9	0/9	1/9	0/9	0/9	4/9
5362	7/9	7/9	0/9	2/9	0/9	0/9	0/9
1648	3/10	5/10	0/10	4/10	0/10	0/10	4/10
434	6/10	8/10	0/10	7/10	0/10	0/10	2/10
409H	3/8	6/8	0/8	7/8	1/8	0/8	0/8
409NH	8/10	9/10	1/10	6/10	0/10	0/10	0/10
1965	0/10	10/10	0/10	8/10	0/10	0/10	0/10
CCP-1	4/8	8/8	0/8	7/8	0/8	0/8	4/8
1741	1/7	7/7	0/7	6/7	0/7	0/7	0/7
OV-1	7/10	9/10	3/10	9/10	0/10	0/10	0/10
OV-7	4/10	9/10	4/10	5/10	0/10	0/10	0/10
OV-9	4/10	10/10	0/10	4/10	0/10	0/10	4/10
OV-10	6/8	8/8	0/8	6/8	0/8	0/8	0/8
0-2-56	5/10	7/10	0/10	2/10	0/10	0/10	3/10
Total	66/149	123/149	8/149	82/149	1/149	0/149	21/149
Percent	44.2	82.5	5.3	55.0	0.6	0.0	14.0

TABLE II CONTINUED

Strain	Subcutaneous Route						
	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Site Abscesses
2335	6/10	6/10	0/10	6/10	0/10	0/10	1/10
1742	0/10	0/10	0/10	0/10	0/10	0/10	4/10
5364	3/9	9/9	0/9	5/9	0/9	0/9	7/9
5362	0/10	0/10	0/10	0/10	0/10	0/10	7/7
1648	2/10	5/10	0/10	1/10	0/10	0/10	7/10
434	1/10	8/10	0/10	1/10	0/10	0/10	10/10
409H	6/8	8/8	0/8	8/8	5/8	0/8	8/8
409NH	1/8	6/8	0/8	4/8	0/8	0/8	8/8
1965	0/10	8/10	0/10	2/10	0/10	0/10	9/10
CCP-1	10/10	10/10	0/10	10/10	0/10	0/10	10/10
1741	10/10	8/10	0/10	4/10	0/10	0/10	10/10
OV-1	4/8	8/8	0/8	6/8	0/8	0/8	8/8
OV-7	0/10	5/10	0/10	6/10	0/10	0/10	9/10
OV-9	2/10	9/10	0/10	3/10	0/10	0/10	10/10
OV-10	9/10	10/10	0/10	9/10	0/10	0/10	7/10
0-2-56	2/10	8/10	0/10	2/10	0/10	0/10	9/10
Total	56/150	108/150	0/150	67/150	5/150	0/150	124/150
Percent	37.3	72.0	0.0	44.6	3.3	0.0	82.6

TABLE III

Organ Infection in Individual Mice
by Each Route of Inoculation

Strain	Mouse No.	Route of Inoculation																			
		Intravenous						Intraperitoneal						Subcutaneous							
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart		
2335	1	-	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	2	-	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	3	-	/	-	/	/	-	-	-	-	-	-	-	-	-	/	-	-	-		
	4	-	-	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

/ = Infection - = No Infection

TABLE III CONTINUED

Strain	Route of Inoculation																					
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous					
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
1742	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE III CONTINUED

Strain	Route of Inoculation																							
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous										
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart					
5364	1	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	Died - Not Autopsied										
	2	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-	-	-					
	3	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-	-	-					
	4	Died - Not Autopsied						Died - Not Autopsied						-	-	-	-	-	-					
	5	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-	-	-					
	6	Died - Not Autopsied						-	≠	≠	≠	≠	-	-	-	-	-	-	-					
	7	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-					
	8	≠	≠	≠	≠	≠	≠	-	≠	-	-	-	-	-	-	-	-	-	-					
	9	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-	-	-	-	-	-	-					
	10	≠	≠	≠	≠	≠	≠	-	≠	-	-	-	-	-	-	-	-	-	-					

TABLE III CONTINUED

Strain	Route of Inoculation																					
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous								
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart			
5362	1	+	+	+	+	+	+	+	+	+	+	-	-	Died - Not Autopsied								
	2	-	+	-	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-			
	3	+	-	-	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-			
	4	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-			
	6	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+			
	7	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+			
	8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	10	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-			

TABLE III CONTINUED

Strain	Route of Inoculation																							
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous										
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart					
1648	1	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	3	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	4	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	5	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	6	Died - Not Autopsied						-	-	-	-	-	-	-	-	-	-	-	-					
	7	Died - Not autopsied						-	-	-	-	-	-	-	-	-	-	-	-					
	8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	10	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					

TABLE III CONTINUED

Strain	Route of Inoculation																							
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous										
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart					
434	1	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-					
	2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					
	3	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-					
	4	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					
	5	Died - Not Autopsied						-	-	-	-	-	-	-	-	-	-	-	-					
	6	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-					
	7	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	8	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	9	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					

TABLE III CONTINUED

Strain	Route of Inoculation																							
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous										
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart					
409H	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
	6	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-					
	7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-					
	8	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-					
	9	+	+	+	+	+	+	Died - Not Autopsied						Died - Not Autopsied										
	10	+	+	+	+	+	+	Died - Not Autopsied						Died - Not Autopsied										

TABLE III CONTINUED

Strain	Route of Inoculation																			
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous						
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	
409NH	1	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	
	3	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
	4	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	
	5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
	6	Died - Not Autopsied						-	+	-	-	-	-	-	-	-	-	-	-	
	7	+	+	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-	-	
	8	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	
	9	+	+	+	+	+	-	-	+	-	-	-	-	Died - Not Autopsied						
	10	+	+	+	+	+	+	-	+	-	-	-	-	Died - Not Autopsied						

TABLE III CONTINUED

Strain	Route of Inoculation																							
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous										
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart					
1965	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					

TABLE III CONTINUED

Strain	Mouse No.	Route of Inoculation																					
		Intravenous						Intraperitoneal						Subcutaneous									
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart				
CCP-1	1	≠	≠	≠	≠	≠	≠	Died - Not Autopsied						-	-	-	-	-	-				
	2	≠	≠	≠	≠	≠	≠	Died - Not Autopsied						-	-	-	-	-	-				
	3	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	4	Died - Not Autopsied						≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	5	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	6	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	7	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	8	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	9	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-				
	10	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠	-	-	-	-	-	-	-	-				

TABLE III CONTINUED

Strain	Route of Inoculation																		
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous					
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
1741	1	Died - Not Autopsied						Died - Not Autopsied						-	+	+	+	+	+
	2	Died - Not Autopsied						Died - Not Autopsied						+	+	-	+	+	-
	3	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-
	4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	6	+	+	+	+	+	+	Died - Not Autopsied						-	-	-	-	-	-
	7	Died - Not Autopsied						+	+	+	+	+	+	-	-	-	-	-	-
	8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	10	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

TABLE III CONTINUED

Strain	Route of Inoculation																			
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous						
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	
OV-1	1	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	
	2	+	+	+	+	+	+	+	+	+	+	-	-	Died - Not Autopsied						
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	
	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	
	5	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	
	6	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	
	7	+	+	+	+	+	+	-	+	+	-	-	+	Died - Not Autopsied						
	8	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	
	9	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	
	10	Died - Not Autopsied						+	+	+	+	+	+	-	-	-	-	-	-	

TABLE III CONTINUED

Strain	Route of Inoculation																			
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous						
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	
OV-7	1	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	
	2	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	
	3	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	
	4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
	5	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
	6	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	
	7	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	9	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	10	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

TABLE III CONTINUED

Strain	Route of Inoculation																	
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous					
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
OV-9	1	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-
	2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	3	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
	4	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-
	5	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-
	6	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	7	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
	8	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-
	9	Died - Not Autopsied						-	+	+	-	-	-	-	-	-	-	-	-
	10	Died - Not Autopsied						-	-	+	-	-	-	-	-	-	-	-	-

TABLE III CONTINUED

Strain	Route of Inoculation																		
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous					
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart
OV-10	1	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
	4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	6	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	8	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+
	9	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
	10	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-

TABLE III CONTINUED

Strain	Route of Inoculation																							
	Mouse No.	Intravenous						Intraperitoneal						Subcutaneous										
		Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart	Spleen	Kidneys	Reproductive Tract	Liver	Lungs	Heart					
0-2-56	1	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-					
	2	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-					
	3	+	+	+	+	+	+	-	-	-	+	-	-	-	+	-	-	-	-					
	4	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					
	5	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					
	6	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-					
	7	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					
	8	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					
	9	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-					
	10	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-					