A STUDY OF CORYNEBACTERIUM RENALE-LIKE ORGANISMS ISOLATED FROM COWS

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THESIS

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A STUDY OF CORYNEBACTERIUM RENALE-LIKE ORGANISMS ISOLATED FROM COWS

by

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TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	
INTRODUCTION	1
REVIEW OF LITERATURE	2
MATERIALS AND METHODS	19
RESULTS	24
DISCUSSION	49
CONCLUSION	52
SUMMARY	52
BIBLIOGRAPHY	53
ILLUSTRATIONS	58

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INTRODUCTION

A study of ten strains of Corynebacterium renale-like organisms isolated from the urine of ten cows suffering from pyelonephritis is reported in this thesis. The organisms did not wholly fulfill the requirements as outlined by Bergey (1948) to be classified as C. renale. However, some of the characteristics such as morphology, growth in culture media, biochemical reactions and pathogenicity to laboratory animals were sufficiently similar to those of C. renale that they have been named C. renale-like organisms. The Corynebacteria of animal origin, however, have not been studied or classified adequately, although they were known to cause both acute and chronic diseases of animals, which were of great economic importance to animal health. Previous to 1946 there was a general opinion that laboratory animals were refractory to infection by C. renale. The primary objective of this investigation was to determine whether or not these C. renale-like bacteria would produce lesions in laboratory mice and rabbits.

REVIEW OF LITERATURE

The etiology of pyelonephritis in cattle has been described as poly-bacterial in nature. With few exceptions, however, the bulk of the evidence supports the view that C. renale is the specific etiological agent in infectious pyelonephritis and cystitis.

Bruckmuller (1869) described enlargement and abscessation of the kidney of an ox accompanied with dilatation of the renal pelvis.

Siedamgrotzky (1875) described the lesions in the renal parenchyma of a cow. Necrosis of the papillae associated with dilated pelvis was the main characteristic feature. Bacteria were isolated from the pus in the renal pelvis.

Pflug (1876) observed a case of nephritis in a cow. The author suspected that micro-organisms were responsible for the onset of pyelitis and pyelo-nephritis.

Dammann (1877) described the gross and microscopic lesions in a case of bacillary nephritis. Bacteria were demonstrated in the uriniferous tubules in addition to the leukocytic infiltration in the renal interstitial tissues. The affected kidneys increased in weight.

Guillebeau and Hess (1888 to 1892) described the symptomatology, post-mortem pathology and pathogenicity of the organisms isolated from the urine and the renal pelvis of affected animals. The authors could not produce the disease in cows, oxen, goats and pigs either by intravenous or intravesical inoculation with urine or cultures.

Guillebeau (1888) found non-motile, slender bacilli in the renal abscesses of cows affected with pyelonephritis.

Bang (1889) and Schmidt (1890) observed bacilli in the lesions of the kidney of affected cattle.

Friedberger (1890) in a study of pyelonephritis found clumped gram-positive bacilli occurring in the kidneys of affected cattle.

Hoflich (1891) described the gross and microscopic lesions of the bacillary pyelonephritis in the ox. Dilatation and leukocytic infiltration in uriniferous tubules associated with increase in amount of interstitial connective tissues of the kidney were noticed. Organisms were isolated from the renal abscesses, the pelvic pus and the urine. The organisms were small gram positive rods, measuring 1 to 3 x 0.7 u. He named the organism the "bacillus pyelonephritidis boum".

Enderlen (1891) isolated a small gram positive bacillus measuring 2.1 micra to 2.8 micra in length by 0.7 micra thick from cases of pyelonephritis in cattle. He was able to cultivate the organism in a pure state and also produced typical lesions of pyelonephritis in two ureter-ligatured rabbits following intravenous inoculation. This disease could not be produced by other methods. It was stressed that the infection was of hematogenous type.

Bollinger (1891) supported the work of Enderlen in that the infection was of hematogenous type. There was no generalization. The lesions were located for the most part in the renal pelvis and parenchyma.

Lucet(1892) found the bacillus pyogenes bovis as the cause of pyelonephritis in cattle. These were isolated from renal abscesses and were thought to be the same organism as previously identified by Hoflich in 1891.

Kitt (1893) described bacillary pyelonephritis in cattle. The author could not demonstrate the bacillus pyelonephritidis boum as the specific etiological agent in the causation of the disease. Diseased processes of varying degrees were observed in kidneys, ureters and bladder.

Mollereau and Porcher (1895) described the same type of bacteria as previously discovered by Hoflich and Enderlen in 1891, in the renal tissue of cattle affected with pyelonephritis.

Bartels (1897) described pyelonephrinephritis in cattle due to bacillus pyelonephritidis.

Albrecht (1900) described the preponderence of the bacillus of pyelonephritis in the affected kidneys of cattle.

Ernst (1905) studied the histopathology of bovine pyelonephritis. The organism could not be isolated in pure state. In 1907, he placed this organism in the group of corynebacteria and called it the "corynebacterium renalis bovis". It measured 2 to 4 micra x 0.5 to 0.6 micra. The bacteria occurred in clumps. Bacteria were present in the necrosed collecting tubes of the papillae. There was marked cellular infiltration into these tubes. With the degree of advancement of the disease a marked increase in the interstitial connective tissue was noticed. The

predilection of the organism was for urine. This was substantiated by observing the increased growth in agar medium containing urine.

Ritzenthaler (1910) described the microscopic findings in 28 out of a total of 88 cases encountered. The essential lesions in the renal parenchyma consisted of necrosis of lobules, dilatation of the pelvis, leucocytic infiltration and thickening of the mucous membrane of the pelvis. The author believed that the type of infection was an ascending one.

Gair (1918) described a bacterial pyelonephritis in a cow. Organisms isolated from the urine were aerobic, gram positive, non-motile rods, 2 to 4 micra long by 0.5 micran thick. They were pleomorphic and showed deeper staining at the ends and middle. Bacteria occurred in clumps in smears made directly from the urine. This clumping was a special characteristic in diagnosis of the organism which he called "corynobacillus".

Boyd (1918) described pyelonephritis in two cows - one a Guernsey, the other a Holstein. The kidneys were enlarged and the cortex was studded with numerous abscesses. The pelvis was dilated with yellow brown fluid which contained blood clots, some calcareous materials and tissue threads. The ureters were enlarged to the size of a man's middle finger. The bladder wall was thickened. Histopathologic examination of the affected kidney revealed cellular infiltration and club shaped bacteria in the cortex. Bacteria could not be isolated.

Boyd et al (1918) observed a case of pyelonephritis in a sheep.

Eberson (1918) in a review of the bacteriologic study of the diptheroid organisms stated:

> "Migula (1900) described the B. renalis bovis. Rods with thickened ends non-motile, gram-positive. In broth, a granular precipitate is formed, the medium remaining clear. Obligate aerobe do not grow at room temperature. The organism grows poorly as compared with the diphtheria bacterium. Babes-Ernst granules appear much later. It is club-shaped, lacet-like or cylindrical. No acid is produced in dextrose and glycerine, no spores. Pathogenicity appears doubtful. Ernst was unable to demonstrate virulence for guinea pigs or rabbits by means of intraperitoneal or intrapulmonary inoculations. Concludes that the organism is not etiologic for pyelonephritis, since he could not recover the organism from the lesions nor reproduce the disease."

Jones and Little (1925) stated: "Joest (1924) considers pyelonephritis a specific infectious disease of cattle and called attention to the fact that there is no disease having similar etiology and pathology."

From the protocols of 13 cattle autopsied and studied, it was evident that there were lesions in one or both kidneys and ureters. The bladder wall was thickened up to one to two cm. Bacteria were present in the urinary sediment in large numbers. When preparations from the pelvis of the infected animals were made and stained, bacteria were found in large number. An attempt was made to diagnose the infected animals by the agglutination test. However the titers of the healthy and the infected animals were about the same. No recognizable allergic reaction in the infected animals was observed when the skin and cornea were injected with the filtered culture.

Jones and Little (1926), on the basis of previous findings, made a bacteriologic and animal inoculation study. The organisms were non-motile, gram positive slender rods, two to three micra in length.

Plemorphism in artificial culture media was pronounced. The bacteria grew in ordinary laboratory media. There was bacterial sediment in broth cultures. On serum medium there were raised, grayish white, dry colonies which did not digest the serum. Blood was not hemolyzed nor was gelatin liquefied. There was reduction of litmus in litmus milk, the casein coagulated at the bottom, leaving the upper stratum blue. Only dextrose was fermented with a final pH of 4.9 to 5.0. The 26 strains studied were immunologically the same. These cultures were obtained from five herds. In a study of pathogenicity for laboratory animals it was found that white mice, rabbits and guinea pigs were resistant to infection. No toxin was demonstrated.

Renal lesions failed to develop in cattle following intravenous inoculation of 5 ml and 10 ml amounts of broth cultures of the organism. Cows failed to develop lesions in the bladder, when swab cultures were introduced into the vagina. On two instances when cows were fed large quantities of freshly isolated cultures, one developed cystitis and the other remained normal. When this part of the experiment was repeated the results were negative. Fecal examination of cows artificially fed with pure cultures, revealed no organisms. The introduction of broth cultures into the bladder produced the disease which pointed to an ascending type of infection.

Udall (1926) observed pyelonephritis in a 9 year old Jersey cow and a bacteriological examination revealed "B. pyelonephritis bovis - W. A. Hagan" from the left kidney and B. pyelonephritis and many cocci from the right kidney.

Boyd (1927) described five clinical cases of pyelonephritis and

stated that the disease was usually urogenous in origin, but might at certain occasions be hematogenous, especially when occurring after parturition. In one case the affected left kidney, ureter and bladder weighted 64 oz. The ureter was 4 cm in diameter and one cm thick. The mucous membrane of the bladder was deeply furrowed and the wall was one cm thick. The lesions were confined mainly to the ureters. There were also abscesses in the kidneys.

Cellular infiltration in the cortical zone and necrotic areas in the renal papillae were observed. Large amounts of erythrocytes, white blood cells, fibrin, diphtheroid bacteria and unrecognizable material were found in the calyces. Degeneration and desquamation of ureteral epithelium was accompanied by fibrosis. Fibrosis also was seen in the bladder wall. The urethra was apparently normal.

Jones and Little (1928) described the symptomatology of 20 head of cattle from three herds. Urine analysis of these cattle and many herds in New Jersey revealed white blood cells, erythrocytes, round cells, epithelial cells and masses of gram positive diphtheroids in the sediment.

The gross lesions found in the bladder were a hemorrhagic, swollen mucosa thrown into folds and accompanied by necrosis. The pelvis of the kidney was filled with yellow, purulent exudate. Most often the lesions were confined to the pelvis. When the involvement was diffuse in nature the enlargement of the kidneys was noticed. The capsule was adherent to the cortex. The microscopic findings seen in the renal parenchyma were fibrosis and leucocytic and round cell infiltration.

Cystitis and pyelonephritis were found to be related processes of the same disease.

The disease was an ascending type attacking first the bladder and then the kidneys through the ureters. This was substantiated by producing cystitis after the introduction of small quantities of a broth culture via the urethra but no lesions were found in the kidneys when five to ten cc of a broth culture was given intravenously. Furthermore, in the earlier cases bladders were alone involved. In the chronicity of the disease lesions were seen in the bladder, ureters and kidneys.

The diptheroids normally present in the urine of cows and bulls were not identical with the pyelonephritis bacillus. Vaginal swabbing with the cultures of the organism failed to transmit the disease. However, infection was produced in two cows which were groomed and brushed after infected animals.

The disease seemed to have no relation to the diseases of the genital tract. It occurred independently without genital infection. The only locus of the organism was the urinary tract of an infected animal.

McFadyean (1929) in a discussion of nephritis in animals described a pyelonephritis in which the pelvis and medulla of the kidney were the main sites of the disease. One or both kidneys might be involved. Even the extent of invasion in the latter case was not to the same degree in the two organs. It was stated that the hematogenous theory of infection was inadequate as the main sites of lesions were in the pelvis and medulla.

The macroscopic lesions observed were enlargement of the kidney and increase in weight - "up to 8-9 lbs". The lesions located in the pelvis varied from slight congestion to ulceration. In some cases the inflammatory process extended to the papillae of the medulla.

The microscopic alterations of the tissues were degeneration, destruction and desquamation of the epithelial lining of the tubules accompanied by polymorphonuclear leucocytic infiltration from the neighboring capillaries in the medulla. As the invasion of the bacteria was through the uriniferous tubules, recent exudation and cellular infiltration were in the cortex. He stated "Bollinger thought the pyelonephritis was the third most frequent disease of cattle in Germany."

Jones and Little (1930) isolated 128 strains of diphtheroids from the genito-urinary tract (sheath, urethra, bladder, urine and vagina) of 34 calves from a herd in which infections cystitis and pyelonephritis were present. They placed these strains into five cultural groups on the basis of morphology, biochemical and immunological reactions.

Olafson (1930) recorded a case of pyelonephritis and cystitis in a two-year-old English Shepherd female and was able to demonstrate "C. renalis" as the etiological agent. The organism isolated was identical with the bovine strains.

Palmer (1931) observed three cases of specific infectious pyelonephritis. Gram positive diptheroids were isolated from two cases. The lesions were alike in all cases.

Merchant (1935) in a study of the corynebacteria associated with the diseases of domestic animals stated that C. renalis isolated from the renal exudates were bacillary-shaped in all media and had a striated appearance in stained smears. Metachromatic granules were observed in the organisms when grown on Loeffler's serum medium. They occurredin clumps, were gram positive and non-acid fast. In solid media, the culture became drier with age. Nine of the 20 strains caused an alkalinity of

litmus milk with digestion of milk casein but no coagulation. There was no digestion of curd. There was no action on gelatin nor on solidified serum. The majority of the cultures fermented glucose, a few levulose and mannose and only one strain attacked dextrin. All the strains were not agglutinated by sera prepared against three strains.

Boyd and Bishop (1937) recorded eight cases of pyelonephritis in horses (seven mares and one stallion). C. renale was isolated from the urine of all animals. Pathologic findings were practically the same as observed in cattle.

McIntosh (1938) reported four clinical cases of pyelonephritis in cattle at the Ontario Veterinary College Clinic. The clinical symptoms were described, cornyebacteria were isolated and identified from two cases.

Palmer (1938) reported on a purebred Holstein-Friesian cow. At autopsy, in addition to the presence of pathologic changes in the urinary organs, gram positive diphtheroids were isolated from the kidney pus. These were thought to be the specific etiological agent of the disease.

Doll (1942) in an unpublished study found typical pyelonephritic lesions in the kidneys of 13 rabbits intravenously inoculated with C. renale.

Morgan (1942) mentioned an atypical C. renalis culture which did not ferment dextrose.

Thorp et al (1943) reported six cases of pyelonephritis among five cows and one male calf. C. renale was isolated from the cows and a diphtheroid from the calf. The organisms were non-motile, non-spore-forming, gram positive rods, occurring in palisade groups showing the beaded staining properties.

The bacteria fermented glucose, hydrogen sulfide and indole were not formed and nitrate was not reduced. Urine analysis indicated the presence of albumin, sediment, bacteria, erythrocytes, alkaline pH and decrease in creatinine and non-protein-nitrogen.

The affected kidneys were enlarged. In chronic cases the capsule was thickened and adhered to the cortical substance. They showed mottling, and grayish yellow foci on the lobes. On the cut surface necrotic exudates containing blood and pus were observed. Fibrosis developed in chronic cases. Necrosis, suppuration, sloughing and disappearance of some portions of the medulla were seen. The major and minor calyces were invariably distended with inflammatory and degenerative exudates. Numerous inflammatory, edematous, hyperplastic and focal degenerative changes were observed in the bladder and ureters.

Histopathologic changes were characterized by cellular infiltration and connective tissue proliferation in the interstitial tissues. Atrophy and cellular casts in the tubules were observed. The renal corpuscles showed various inflammatory and regressive changes leading to loss of architectural design. The medulla showed necrosis, suppuration and leucocytic infiltration.

The renal arteries displayed arteritis, thickening of the intima, necrosis and fibrosis in the media and numerous thrombi. The ureters showed inflammatory and degenerative changes. The ureteral epithelium showed metaplasia simulating stratified squamous epithelium. Varying degrees of congestion, edema, necrosis and desquamation of the bladder epithelium were noticed.

Coffin (1943) described C. renale as a gram positive pleomorphic rod occurring in palisade groupings. The banded appearance of the organism was seen with methylene blue staining.

Olney (1943) reported a case of pyelonephritis in a Holstein-Frisian cow. The hemoglobin content was 7.5 mg, the erythrocyte and leucocyte counts were 4.15 millions and 6.6 thousands mm^3 respectively. The urine was positive for albumin and blood. Grossly the kidneys were enlarged and weighted 6.0 to 7.5 lbs. In addition to ureteritis and cystitis fibronecrotic membranes were present.

Foss (1944) isolated C. renalis from the kidneys and bladder mucosa of a horse. The organism was a dextrose fermenter, indole non-producer, occurred as a rod in groups, showing typical bars and metachromatic granules and was gram positive.

Beck et al (1945) reported on the penicillin treatment of six cows affected with pyelonephritis and cystitis with encouraging results.

Feenstra et al (1945) artificially produced pyelonephritis and cystitis in a Jersey steer by intra-urethral inoculation. At autopsy nine months later gross and microscopic changes were identical with the lesions seen in naturally affected animals. C. renale was isolated from the kidneys.

Feenstra et al (1945A) studied morphological, cultural, biochemical and serological properties of 19 diphtheroids isolated from cases of pyelonephritis. These findings suggested that there were two distinct species of corynebacteria - fine growers and rapid growers - involved in the etiology of pyelonephritis. Of the 19 strains studied 6 were slow growers, xylose fermenters, hemolysis and H_2S producers.

Feenstra et al (1946) described the invasiveness of the diptheroids of pyelonephritis. In the acute fulminating type the lesions were more severe and were scattered throughout the kidney. In the mild and chronic type the lesions involved mostly the calyces, ureters and bladder.

Lovell (1946) in a morphological, cultural, biochemical and serological study of 36 strains of diphtheroids found 26 to be C. renale. All the 26 strains were gram positive, occurred in groups and showed a beaded appearance.

These grew well in plain broth and on agar. Bacterial deposit occurred in the broth leaving the supernatant fluid clear. No marked improvement in growth was seen in blood agar or serum agar. Neither hemolysis of blood nor liquefaction of gelatin was produced by any of the strains. Catalase was produced by all. Chromogenesis was marked on blood agar, solid serum and egg medium. A distinct "halo" around the colonies was produced when grown on 10% sterilized milk agar. High alkalinity in litmus milk, splitting of urea in urea broth were produced by most of these 26 strains. Glucose was attacked by all the 26 strains. Some of the strains were V. P. and M. R. positive. Most of the strains were serologically alike when treated with anti serum of three different strains of C. renale.

Mice when were infected intravenously with C. renale at a dosage rate of 20 millions living organisms, in most of the cases the organisms were isolated from the kidneys and urinary tract. In a few cases organisms were isolated from the blood and spleen. This suggested that C. renale was pathogenic to mice when given in adequate dosage.

Lovell and Cotchin (1946A) gave 20 millions of C. renale to 50 mice -

25 male, 25 female - intravenously. They were able to isolate the organisms from the blood, spleen, kidneys, urine of infected animals when they were killed after an interval of 6 hours to 14 days. More or less the same picture was noticed in those animals which died from the 3rd to 12th day after infection. In all these cases lesions were marked in the kidneys. There was papillitis, pyelitis and pyonephrosis with the invading degenerative processes extending downward in the ureter and outward to the peri-renal tissue.

In a second study they gave 20 million C. renale organisms intravenously, intraperitoneally, subcutaneously and vaginally to four groups of 10 mice. Kidney lesions occurred in three mice, infected intravenously and one mouse vaginally infected. The infection was not spread by contact as 12 healthy mice failed to show lesions in the urinary tract when placed with 12 infected mice for 56 days.

The renal damage in guinea pigs and rabbits to an intravenous inoculation of 60 million of C. renale was mild as compared to the lesions in mice.

Thickened edematous condition of the chorioallantoic membrane was noticed when an inoculum of C. renale was injected into a nine-day-old chick embryo.

Morgan et al (1946) identified 18 strains of C. renale isolated from the sheaths of 54 bulls.

Runnells (1946) mentioned that infections pyelonephritis has been recorded in swine.

Weitz (1947) isolated 44 strains of C. renale from the vagina of 100 normal cows in three herds. C. renale was not found in the abnormal discharge of 180 cows suffering from endometritis. C. renale was recovered from the normal urine. This led to the opinion that C. renale possibly might be a normal inhabitant of the posterior part of the vagina around the urinary meatus of dairy cows. Under certain favorable conditions they became pathogenic. Transmission of infection from animal to animal was not likely to occur because of the fact that many or most of the animals were potentially infected.

Morse (1948) in a study of 43 strains of C. renale found that a granular deposit occurred when they were grown in serum infusion broth. The cultural growths had a tendency to become mucoid when stored in the refrigerator for one month. No hemolysis was observed in blood agar plates. Appreciable luxuriant growth was more pronounced in blood or serum agar media. Chromogenesis appeared to be present.

Neither serum digestion nor gelatin liquefaction occurred. Dextrose was attacked by all strains. Urease was produced in urea medium. Inhibition of growth was noticed in the medium containing 12.5% to 15% sodium chloride. They were non-motile, H_2S, indole, M.R., V.P. negative. Except for two all the strains did not reduce nitrate to nitrite.

Morse (1948) made a bacteriological study of eight cases of pyelonephritis treated with penicillin and found the results encouraging.

Feenstra et al (1949) reported that C. renale was pathogenic for 22 rabbits artificially infected by the intravenous route. Very significantly altered clinical blood picture was noticed. The hemoglobin dropped and the heterophil percentage was increased in the infected rabbits.

C. renale could be recovered from the lungs, liver, bone marrow, spleen, kidneys and urinary bladder.

In early cases congestion of the capillaries, and with the chronicity of the infection necrosis of the pelvis, leucocytic infiltration and proliferation of fibroblasts were observed in the kidneys. Urethritis and cystitis were marked.

Hatch et al (1949) isolated eight cultures of C. renale from the cervix and vagina of infertile dairy cows.

Morse (1949) in a study of 51 strains of C. renale described the organisms as gram positive pleomorphic rods occurring in clumps with palisade arrangements. They fermented dextrose. All the strains produced alkalinity in litmus milk medium. Indole and hydrogen sulfide were not produced. Urea was hydrolyzed.

Morse (1950) studied six strains of C. renale in plain-extract-broth in which the pH became 8.2 after 28 days against the initial pH 6.8.

48 strains of C. renale produced acid in dextrose medium with the pH 6.1 against the initial 7.6.

A high alkalinity of pH 9.0 was observed in urea broth against the initial pH 7.5 by 51 strains. Increase in pH was noticed in litmus milk by 43 strains. Of the 50 strains studied in 10% milk agar media 7 did not produce a "halo". C. renale showed very poor growth in chocolate blood agar medium containing 5% of 0.9% of sodium tellurite solution.

Morse (1950A) made a survey of the incidence of C. renale in 523

animals. Of these, 215 animals were in herds where clinical cases were absent. The author was able to recover C. renale from the vaginal and penile swabs of clinically affected and non-affected cases, but failed to isolate the organisms from the urinary bladder of 108 animals. In an infected herd periodic examinations brought out latent infections. It appeared that C. renale was disseminated from the apparently healthy carriers to the neighboring healthy animals.

Morse (1950B) reviewed the various medicaments employed in the treatment of bovine pyelonephritis and found that penicillin was the effective therapy.

MATERIALS AND METHODS

1. Bacteriological Procedure:

For studying the morphology of these cultures Gram's stain (Hucker modification, 192/) and Wright's stain were used. Motility in broth culture was checked after 18-24 hours incubation at 37 C.

The media used in the study of the cultural characteristics are shown under results.

2. Pathological Procedure:

A. Mouse inoculation

I. Selection and Care of Mice

Apparently healthy white mice of two to three months of age, weighing an average of 20 to 25 gms each, were selected. They were housed in galvanized wire mesh cages having raised bottoms. Before use cages, trays, pans and all receptacles for food and water were thoroughly washed and were placed in flowing steam for an hour. The diet was a commercial rabbit-pellet* supplemented with tankage. During a preliminary period of at least a week animals not showing signs of illness were selected for use.

II. Preparation of Bacterial Suspension

The micro organisms were inoculated onto cryptose agar slants and incubated at 37 C for three to four days. The

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*Acrady Sunkist Rabbit Pellets Prepared by Acrady Farms Milling Co., Chicago 6, Illinois.

cultures were washed and suspended in sterile distilled water. A uniform, well mixed bacterial suspension was made by shaking the suspension in bottles containing glass beads in a Shaking Machine* for half an hour. The suspension was then kept at room temp rature for five to ten minutes allowing the foam to decrease.

The bacterial suspension according to the required dosage of organisms (15 million, 20 million, 40 million) was then standardized by the photelometer** using the standard opacity tubes of a McFarland Nephelometer (Kolmer and Boerner, 1945).

III. Technic for Injection

The device used for restraining the mice is shown in Fig. 1, page 21. After the mouse was placed into the filter mantle, its tail was wiped off with a pledget of cotton and immersed in warm water (approximate 45-50 C) just for a moment to make the vein more prominent. Holding the tail of the mouse with one hand the needle was inserted into the coccygeal vein.

B. Rabbit Inoculation

I. Selection and Care of Rabbits

Young healthy rabbits weighing an average of 2000 to 3000 gms were selected. They were housed in strong galvanized cages having wire-mesh bottoms.

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*Miller Paint Mixer Mfd by Miller Mfg. Co. 3238 BrynMawr Ave., Chicago, Illinois.

**Cenco-Sheard-Sanford-Photelometer Central Scientific Company, Chicago.

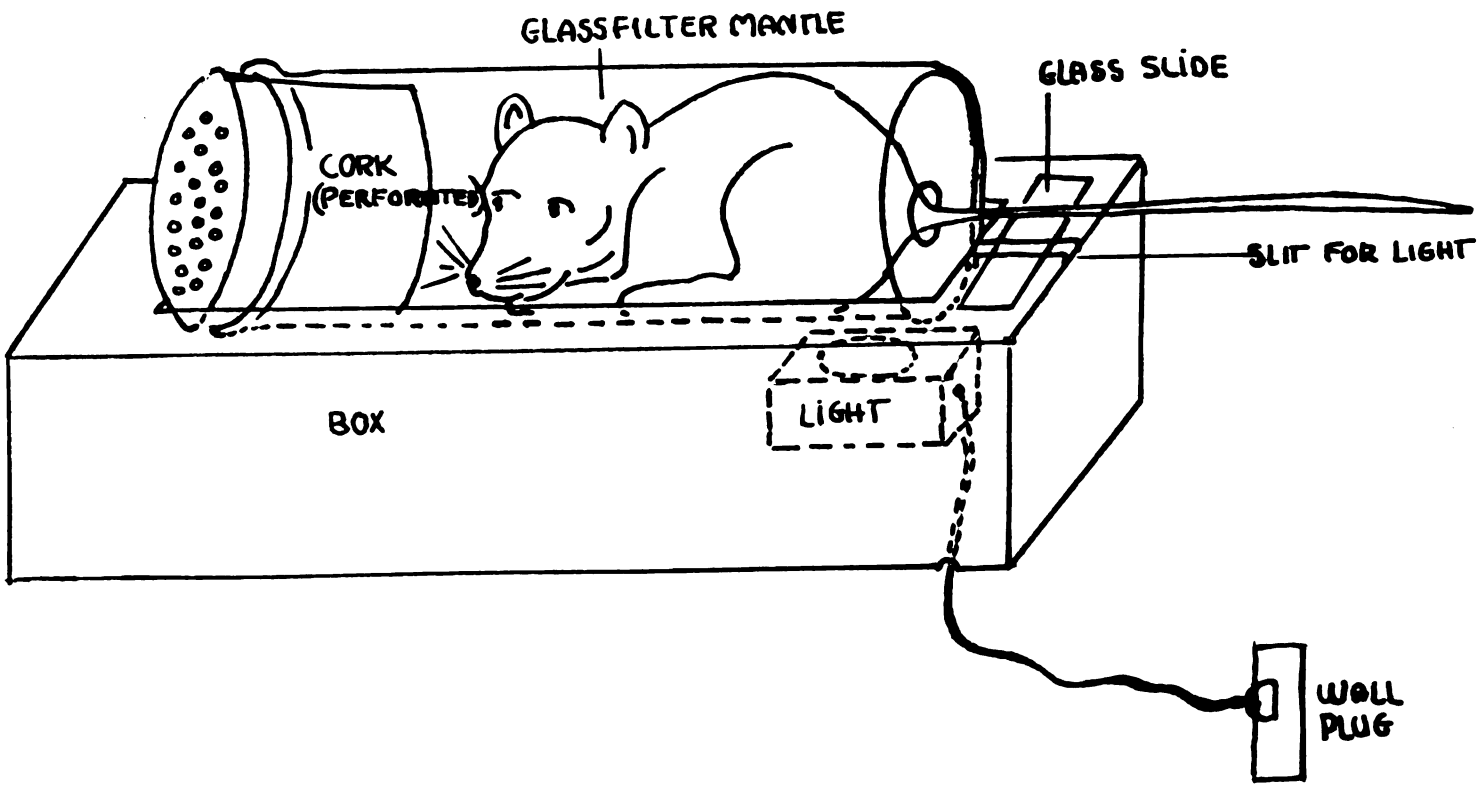

Fig. 1

Method of restraining mice

The diet was the same as given to the mice but supplemented with cabbage leaves and hay. Fresh water was given daily. Animals were observed for a preliminary period of five to seven days before inoculation.

II. Preparation of Bacterial Suspension

The procedure was the same as followed for the mice. The dose was 4 billion organisms for each rabbit.

III. Technic for Injection

The rabbit was restrained in a rabbit box. Bacterial suspension of specific quantity was given in the marginal ear vein after the site was clipped and 70% alcohol applied.

C. Hematological study

Blood smears for differential leucocyte counts (100 cells) stained with Wright's stain were prepared from both mice and rabbits. Hemoglobin determinations (Cenco-Sheard-Sanford photelometer, Hoffman method, 1941) were made on the rabbits only. For white cell and red cell counts, a Bright-line-improved-Neubauer-Counting Camber was used. The diluting fluid for erythrocyte count was that of Guy and Leake (Todd and Sanford, 1948) and for leucocyte count, 0.1% HCL (Coffin, 1945) plus 1% aqueous solution of 1% gentian violet. Thoma blood cell pipettes were used. Hematocrit reading and sedimentation rate were determined by the Wintrobe method (Kolmer and Boerner, 1945).

3. Technic of Isolation and Tissue Preservation.

Unless otherwise stated heart blood, lungs, liver, spleen, kidneys and urine of necropsied animals were cultured on litmus milk agar and blood agar plates. Tissue smears from heart, lungs, liver, spleen, kidneys and urine sediment were stained with Gram's stain.

For histopathologic study, tissues of heart, lungs, liver, spleen, kidneys, ureter and urinary bladder were fixed in Zenker's fluid (Mallory, 1938).

With mice tissue the usual procedure of decreasing the time for fixation, processing and embedding was followed.

Sections were stained with Hematoxylin and Eosin (Mallory, 1938). A few of the sections were stained by the Goodpasture's Method (MacCallum, 1919) for identifying bacteria in the tissues.

Some of the tissues (liver and kidney) of four rabbits were fixed in 10% neutral-formol-saline and the sections were cut on a Spencer Freezing Microtome and stained for fat with Sudan IV (Mallory, 1938).

RESULTS

I. Morphologic

All the strains studied were non-motile, non-spore forming gram positive rods usually arranged in a palisade, especially when isolated from animal tissue. Pleomorphism was the chief characteristic. Often deeper staining granules appeared to be present in the body of the organism which gave a banded or beaded appearance. Smears made from old cultures showed coccoid forms with a striking tendency toward gram negative appearance. This was noticed in all the strains.

II. Cultural Characteristics

Nutrient broth. After 48 to 72 hr. at 37 C the growth of these organisms in nutrient broth (M.M.P.C.S.B., 1948)* was discernible with the formation of granular sediments at the bottom of the inoculated tubes. The supernatant fluid was clear with not much clouding of the broth. No surface pellicle was produced by any of the cultures. In one there was growth on the side of the tube. The following table indicates the nature of growth produced by each culture.

*Manual of Methods for Pure Culture Study of Bacteria.

TABLE 1

Cultural characteristics in nutrient broth.

No. of Days	Culture number										Control
	1	2	3	4	5	6	7	8	9	10	
1	-	-	-	-	g.s.	-	-	-	-	-	-
2	g.s.	g.s.	g.s.	g.s.	g.s. (slight c)	g.s.	g.s.	g.s.	g.s.	f.g.s.	-
3	g.s.	g.s.	g.s.	g.s.	"	g.s.	g.s.	g.s.	g.s.	f.g.s.	-
4	g.s.	g.s.	g.s.	g.s.	"t	g.s.	g.s.	g.s.	g.s.	f.g.s.	-
7	g.s.	g.s.	g.s.	g.s.	"	g.s.	g.s.	g.s.	g.s.	f.g.s.	-
12	g.s.	g.s.	g.s.	g.s.	"	g.s.	g.s.	g.s.	g.s.	f.g.s.	-
14	g.s.	g.s.	g.s.	g.s.	"	g.s.	g.s.	g.s.	g.s.	f.g.s.	-

g.s. - granular sediment with supernatant fluid clear
f.g.s. - fine granular sediment with supernatant fluid clear
g.s.c. - granular sediment with clouding of the liquid
g.s.t. - granular sediment and growth on the side of the tube.

Tryptose agar plates. On tryptose agar plates (Difco, pH 7.4) incubated at 37 C for 48 to 72 hr. there developed small, raised greyish to creamy-white colored colonies, which microscopically appeared granular with slightly erose edges. The colonies were not brittle. On ageing they became more dry, appeared larger in size and showed a tendency tobecome chromogenic, i. e. from white-grey to pale-yellowish. The plates were kept 30 days for observation.

TABLE 2

Cultural characteristics on tryptose agar plates.

No. of Days	Cultural characteristics	Control
1	Very minute greyish white	-
3	Very minute greyish white but larger	-
6	Tendency to become dry, lacy, larger	-
11	Dry white yellowish colonies	-
13	More tendency towards yellow	-
26	Dry yellow	-
29	Dry yellow	-

Blood agar plates. Difco tryptose agar plates to which 10% defibrinated sheep blood was added were incubated at 37 C for 48-72 hr. The colonies initially were small dew-drop-like. Zones of alpha hemolysis were not large. The zones were better discernible after 72 to 96 hr. when viewed under the microscope. As these organisms were fine, slow growing and fastidious in nature, the cultural characteristics always were noted on the 3rd or 4th day.

TABLE 3

Cultural characteristics on blood agar plates.

No. of Days	Culture number										
	1	2	3	4	5	6	7	8	9	10	Control
1	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	-	∓	∓	+	+	∓	-
3	+	+	+	+	+	+	+	+	+	+	-
4	+	+	+	+	+	+	+	+	+	+	-
7	+	+	+	+	+	+	+	+	+	+	-

- = no hemolysis

∓ = slight hemolysis

\+ = complete hemolysis

Potassium tellurite agar. On potassium tellurite agar (M.M.P.C.S.B, 1948) black small raised colonies appeared in and along the streaks of inoculum made for seven days at 37 C.

TABLE 4

Cultural characteristics on potassium tellurite agar.

No. of Days	Culture number										
	1	2	3	4	5	6	7	8	9	10	Control
1	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	-	+	+	+	-	-
3	+	+	+	∓	+	∓	+	+	+	∓	-
4	+	+	+	∓	+	∓	+	+	+	+	-

- = no growth

∓ = poor growth

\+ = growth

Litmus milk agar. This medium was prepared by adding 10% of sterile litmus milk to tryptose agar (Difco) pH 7.4. Streaked cultures were made evenly on the plates and incubated at 37 C for 96 hr. The "halo" or zone of clearing (Lovell, 1946) around the colonies were observed as indicated in table 5.

TABLE 5

Occurrence of "halo" on litmus milk agar plates.

No. of Days	Culture number										Control
	1	2	3	4	5	6	7	8	9	10	
1	++	-	-	∓	++	∓	+	++	+	+++	-
2	+++	++++	++	∓	++++	++	+++	++	++	++++	-
3	++++	++++	++	++	++++	++++	++++	++++	++	++++	-
4	++++	++++	++	++	++++	++++	++++	++++	++	++++	-

\- = no "halo"

∓ = slight "halo"

\+ to ++++ = indicates size of "halo"

Starch agar. Cultures were streaked onto the freshly made starch agar plates (M.P.C.S.B., 1948) preferably on the day prepared. The inoculated plates were incubated 7 days at 37 C. A few drops of Lugol's solution was poured over each plate. There was a bluish appearance in all the plates except strain No. 10. This showed that starch was not hydrolysed by the remaining nine strains.

TABLE 6

Test for starch hydrolysis with Lugol's iodine.

Date	Culture number										Control
	1	2	3	4	5	6	7	8	9	10	
8/6/49	-	-	-	-	-	-	-	-	-	+	-

\- = no starch hydrolysis

\+ = starch hydrolysis

When seven day old cultures were treated with a saturated solution of iodine in 50% alcohol, hydrolysis of starch by cultures 6 and 10 was noticed.

For this test a saturated solution of iodine in 50% alcohol appeared to be more sensitive.

TABLE 7

Test for starch hydrolysis with 50% alcoholic solution of iodine.

Date	Culture number										Control
	1	2	3	4	5	6	7	8	9	10	
8/6/49	-	-	-	-	-	+	-	-	-	+	-

- = no starch hydrolysis

+ = starch hydrolysis

Liquefaction of gelatin. The gelatin was prepared as outlined in M.M.P.C.S.B., 1948. The inoculated tubes were incubated at 37 C. After intervals of 1,2,3,4,7,12, and 14 days they were placed in the refrigerator for evidence of liquefication. In no instance did the gelatin remain fluid except for culture 10.

TABLE 8

Liquefaction of gelatin.

No. of Days	Culture number										Control
	1	2	3	4	5	6	7	8	9	10	
1	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	+	-
12	-	-	-	-	-	-	-	-	-	+	-
14	-	-	-	-	-	-	-	-	-	+	-

- = no liquefaction of gelatin

+ = liquefaction of gelatin

Litmus milk. Litmus milk (Difco) was sterilized before use by heating to 100 C for 15 min. in an Arnold sterilizer for three consecutive days. The final pH was 6.4-6.5. In all cases the medium showed a tendency towards alkalinity, characterized by the bluish coloration of the supernatant fluid, with total or partial digestion of casein.

TABLE 9

Reaction of litmus milk.

Culture number	Number of days					
	1	2	4	7	12	14
1	-	b	b	b pd	b md	b md
2	-	b	b c	b c	b md	b md
3	-	b pd	b pd	b c md	b md	b md
4	-	b pd	b pd	b c md	b md	b md
5	b	b pd	b md	b c md	b md	b md
6	b c pd	b pd	b md	b c md	b md	b md
7	b c pd	b pd	b md	b c md	b md	b md
8	b c pd	b pd	b md	b c md	b md	b md
9	b c pd	b pd	b md	b c md	b md	b md
10	b c pd	b md	b md	b c md	b md	b md
Control	-	-	-	-	-	-

b = alkalinity

c = coagulation

pd = partial digestion

md = marked digestion

Two sets of litmus milk tubes from a recently prepared batch were inoculated at different times. The pH of the medium after 14 days incubation was determined by the Beckman pH meter as noted in table 10.

TABLE 10

Change of pH in litmus milk after 14 days incubation.

	Culture number										Control		
	1	2	3	4	5	6	7	8	9	10	1	2	3
1st set	6.9	7.1	7.2	7.0	7.2	7.0	7.6	7.2	7.4	7.1	6.4	6.2	6.3
2nd set	7.2	7.0	7.2	7.2	7.2	6.8	7.7	7.6	7.3	7.0	6.4	6.4	6.2

pH of litmus milk before inoculation, 6.5.

Hydrolysis of urea. Difco urea broth tubes were heavily inoculated with a 3 mm. loopful from a 72 hr. blood agar culture. Good urease production

was noticed after 24 hr. incubation at 37 C in some of the tubes. Tubes not showing urease activity in 24 hr. were incubated for an additional 48 hr. to 72 hr. Urease was produced by all the cultures except 6 and 10. Table 11 indicates the pH before and after 120 hr. incubation and the degree of change in the color of the medium.

TABLE 11

Hydrolysis of urea.

No. of Days	Culture number										
	1	2	3	4	5	6	7	8	9	10	Control
0	-	-	-	-	-	-	-	-	-	-	-
1	-	++	++	+	+++	-	++	-	++++	-	-
2	-	++	+++	+	++++	-	+++	+	++++	-	-
3	++	+++	++++	+	++++	-	++++	++++	++++	-	-
4	+++	+++	++++	+	++++	-	++++	++++	++++	-	-
5	+++	+++	++++	+	++++	-	++++	++++	++++	-	-
5 pH*	7.9	7.3	7.8	6.9	8.2	6.5	7.9	8.5	8.2	6.7	6.7

\- = yellow (normal color of medium)

\+ to ++++ = varying degrees of color change from yellow to violet.

* original pH 6.7

Digestion of serum. Loeffler's serum medium was made in accordance with M.M.P.C.S.B., 1948. A slight modification was made in that the tubes contained 3-4 ml of medium and were laid in a tray containing water. The temperature was slowly raised to 75 C and maintained for 6 hr., during which time the serum coagulated to a yellowish white solid. These tubes were then sterilized at 90 C (in the top of the Arnold steam cabinet) for 20 minutes on 3 consecutive days. After each day's heating the tubes were placed in the incubator. The medium was inoculated and incubated at 37 C for 14 days. The colonies appeared as small discrete yellow-cream discs. The serum was not liquefied by any of the cultures except 6 and 10 as noted in table 12.

TABLE 12

Liquefaction of Loeffler's serum.

No. of Days	Culture number 1	2	3	4	5	6	7	8	9	10	Control
1	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	∓	-	-	-	∓	-
7	-	-	-	-	-	∓	-	-	-	+	-
12	-	-	-	-	-	∓	-	-	-	+	-
14	-	-	-	-	-	∓	-	-	-	+	-

- = no digestion

∓ = slight digestion

\+ = complete digestion

Proteolysis of alkaline egg medium. The alkaline egg medium was made as outlined in the M.M.P.C.S.B., 1943. Mixing was done in a Waring Blendor.* The medium was sterilized in the same manner as for the Loeffler's serum medium. After each heating the medium was kept in the incubator at 37 C. Proteolysis was indicated by progressive clearing of the medium. The pH change after 14 days incubation was as noted in table 13.

TABLE 13

Proteolysis of the alkaline egg medium.

No. of Days	Culture number 1	2	3	4	5	6	7	8	9	10	Control
1	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	+	-	-	-	+	-
12	-	-	-	-	-	++	-	-	-	++	-
14	-	-	-	-	-	++	-	-	-	++	-
pH*	8.3	8.0	8.1	8.1	8.2	7.4	8.3	8.0	8.0	7.3	7.7

- = no proteolysis

\+ = proteolysis

* original pH 7.6

*Waring Corporation, 1697 Broadway, New York City, N. Y.

Utilization of citrate. The citrate medium was prepared as cited by Mackie and McCartney (1946). The pH was adjusted to 6.8, by the addition of NaOH. The final appearance of the medium was clear. The organisms were inoculated into the medium and incubated for 14 days at 37 C. Citrate utilization was determined by adding an equal volume of a saturated aqueous solution of lead acetate to the culture tubes. A flocculent white precipitate was noted in the uninoculated control tube and in those culture tubes exhibiting inhibition of growth. There was a slight variation in the pH of the medium after 14 days incubation.

TABLE 14

Reaction and pH change in citrate broth.

No. of Days	Culture number										
	1	2	3	4	5	6	7	8	9	10	Control
1	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-
pH*	7.4	7.3	7.4	7.3	7.3	7.2	7.3	7.3	7.3	7.1	7.0
Treatment with lead acetate	p	p	p	p	p	p	p	p	p	p	p

- = no growth

p = precipitate

* original pH 6.8

Fermentable media. The basic medium was prepared as described by Thorp (1936), modified by the addition of 2% Bacto-tryptose, 0.7% beef extract and the indicator was brom cresol purple. The fermentable media attacked are shown in table 15. There was no action on arabinose, dulcitol glycerol, inositol, inulin, mannitol, raffinose, salicin, sorbitol nor trehalose.

TABLE 15

The summary of the fermentation reactions of the C. renale-like organisms.

Strains	1					2					3					4					5					6					7					8					9					10				
Days on observation	1	2	4	7	12	14	1	2	4	7	12	14	1	2	4	7	12	14	1	2	4	7	12	14	1	2	4	7	12	14	1	2	4	7	12	14	1	2	4	7	12	14	1	2	4	7	12	14
Dextrin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	-	-	-	∓	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+
Glucose	-	-	+	+	+	+	-	-	-	∓	∓	-	-	-	-	+	+	+	-	-	-	∓	∓	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∓	∓	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	∓	+	+	-	-	-	∓	∓	-	-	-	-	-	-	-	-	-	±	±	±	-	-	-	-	-	-	∓	-	-	-	-	-	-	-	-	-	-	-	-	∓	∓	∓

- = negative + = positive ∓ = slight ± = marked ∓ = trace No gas was produced in any of the media.

Hydrogen sulfide production. Hydrogen sulfide determination was made as suggested by the M.M.P.C.S.B., 1948. Readings were made after the cultures had been incubated for 7 days at 37 C. None of the cultures produced hydrogen sulfide.

Nitrate reduction. The medium and manner of testing were those described in M.M.P.C.S.B., 1948. All cultures failed to reduce nitrate to nitrite.

Indole production. Indole production was determined according to the M.M.P.C.S.B., 1948 with the modification of Thorp (1936) of using narrow strips of dry filter paper. Cultures were incubated 4 days at 37 C. None of the cultures produced indole.

Methyl red test. The medium was prepared as described by Topley and Wilson (1946). The indicator was phenol red. The pH was adjusted to 7.5. Sterilization was at 100 C for 15 minutes for 3 consecutive days. After four days incubation at 37 C the cultures were treated with methyl red and were found to be M.R. negative.

Voges-Proskauer test. The glucose phosphate medium as used for the methyl red test was inoculated and incubated for four days at 37 C. One ml of 10% KOH was added to the medium as suggested by Topley and Wilson (1946). All cultures were V.P. negative when observed after 24 hr.

Ammonia test. A peptone water culture (Mackie and McCartney, 1946) was grown at 37 C for four days. Addition of a few drops of Nessler's reagent showed that ammonia was not produced by any of the cultures.

Methylene blue reduction. One drop of 1% aqueous solution of methylene blue was added to a broth culture incubated at 37 C for 72 hr. as recommended by Topley and Wilson (1946). All cultures reduced methylene blue.

Catalase test. This test was done on a 72 to 96 hr. tryptose agar slant culture incubated at 37 C by pouring 1 ml of H_2O_2 over the growth (Topley and Wilson, 1946). Gas bubble production was noticed in each of the ten cultures.

TABLE 16

Culture number

Culture number											
1	2	3	4	5	6	7	8	9	10	Control
++++	++++	++++	++++	+	+	++++	++++	++++	+	-

++++ = gas bubble production marked

\+ = gas bubble production slight

\- = no gas bubbles

TABLE 17

The summary of the cultural characteristics manifested by the ten cultures of C. renale-like organisms.

Cultures	Alk'ty in litmus milk	NH_4	Catalase	Citrate utilization	Digestion of serum	Gelatin liquefaction	H_2S	Blood Hemolysis	Indole	M.B.* reduction	M.R.** test	Nitrate reduction	Protein hydrolysis	Starch hydrolysis	Urea hydrolysis	V.P. test
1	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
2	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
3	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
4	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
5	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
6	+	-	+	-	+	-	-	+	-	+	-	-	+	+	-	-
7	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
8	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
9	+	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-
10	+	-	+	-	+	+	-	+	-	+	-	-	+	+	-	-

\+ = positive reaction

\- = negative reaction

*M.B. = methylene blue

**M.R. = methyl red

RESULTS

In vivo study using mice.

Symptoms and clinical findings. None of the 58 infected mice used in this study showed clinical signs suggestive of illness. The blood picture of 15 infected mice (table 19) showed marked variation in the total white cell and differential counts. In some, the total white cell count was 12 to 20 thousand as compared to the normal 6 to 8 thousand per mm^3. Little importance was attached to these results since they fell within the normal range (Kolmer and Boerner, 1945). In some of the mice a decrease in lymphocytes and an increase in neutrophils was noticed 10 to 14 days after infection. This was not a constant feature in all animals.

Gross pathology. No gross lesions were observed in any of the internal organs. The weight and size of the kidneys, ureters and bladders of 10 infected mice (table 18) did not show a significant difference when compared to the same organs of four healthy controls.

Micropathology.

Kidney. The pelvis of infected kidneys invariably showed distinct alterations varying in intensity from slight congestion to exfoliation of the epithelial lining extending to the papillae of the medullae. In the progress of pelvic necrosis the epithelia were destroyed and where this process was intensive the normal architectural design was partially or completely obliterated (Fig. 2). Frequently blood cells, plasma cells, polymorphornuclear leucocytes, desquamated epithelial cells and granular materials were present in the pelvis (Fig. 3). The peripelvic tissue

contained a serofibrinous exudate, neutrophils and plasma cells (Fig. 3). This appeared to be due to the presence of bacteria or their products of metabolism in the area. The immediate effect was degeneration and death of the epithelial cells of the collecting tubules (Fig. 2). The amount of necrosis was so intense and the material stained so deeply that it was not possible to identify bacterial forms. These changes were not uniformly observed in all papillae. The presence of glomerular material in the lumina of the collecting tubules would indicate obstruction and subsequent dilatation leading to pressure atrophy (Fig. 4). In the lumina of the collecting tubules and ascending limbs of Henele vacuolar material was often present, which suggested fatty changes.

Leucocytic infiltration occurred around the glomeruli, blood vessels and between the tubules. Along with the cellular infiltration connective tissue was proliferating in these areas. The new formed connective tissue together with cellular infiltration led to pressure atrophy of the convoluted tubules. Examination of other sections of the cortex showed that the distribution of the newly formed connective tissue and cellular infiltration was not uniform. The distribution of these lesions can be followed in the photomicrographs (Fig. 5).

The predominant alterations of focal necrosis and intertubular hemorrhage occurred between and around the convoluted tubules and Malpigian bodies of the cortex (Fig. 6). The convoluted tubules were more or less dilated and lined by an epithelium of altered shape (Fig. 7). The individual cells were abnormally distinct and the cytoplasm stained faintly with eosin (Fig. 7). Connective tissue was seen proliferating around these dilated tubules leading to compression of the adjacent tubules. Alterations were ballooning and loosening of the cells of the

convoluted tubules.

The glomeruli infrequently showed an increased number of polymorphonuclear cells in the capillary tufts giving the appearance of over distension and indicated an acute inflammatory process (Fig. 6).

The lesions occurring in the capsule were not uniform nor consistent. In a number of instances focal leucocytic infiltration in the subcapsular space was noticed. The capsule appeared to be thickened owing to the proliferation of connective tissue (Fig. 8).

Spleen. The alterations in the spleen were interesting in that the infiltration of lymphocytes had gone beyond the splenic corpuscles. Most of these lymphocytes appeared to be immature. The increased number of giant cells in some cases was more pronounced in the red pulp and this probably was the result of the chronicity of the diseased process in that area (Fig. 9).

Liver. Among the most striking changes found in the liver were those relating to the disturbances of the hepatic cords. The cords had undergone varying degenerative processes. The basic changes remained the same, but in one instance (Fig. 10), there appeared to be the presence of faintly eosin-staining hyaline droplets or fused masses within the liver cords and also around the hepatic vessels. The hyaline-like materials had a tendency to encroach upon the medial layer of hepatic vessels and actually replaced this layer. This was probably the beginning of amyloid degeneration. Within the same area round cells were observed in between the hepatic cords.

Lungs. The striking feature in the lung was the marked interstitial and peribronchiolar type of lesion. The inflammatory reaction

was often severe and was accompanied by extensive desquamation of the lining epithelium of the bronchioles. Some of the valveoli were filled with an exudate consisting almost entirely of degenerated polymorphonuclears and red blood cells. In other alveoli, large mononuclears, fibrin and blood cells were found. At the same time the alveoli walls were infiltrated with inflammatory cells. The peribronchiolar inflammation extended out into the interstitial lung parenchyma for a variable distance giving rise to interstitial pneumonitis (Fig. 11). The cellular activity of the peribronchiolar lymphoid tissue together with the hyperemic condition of the vessels (Fig. 11). The bronchioles showed catarrhal inflammation. Desquamated epithelial cells along with invading neutrophils were present in the lumina. Some of the polymorphonuclear leucocytes had undergone necrosis (Fig. 11).

Adrenal. A marked focal lymphocytic infiltration into the subcapsular space was noticed in one case.

TABLE 18

Weight of kidneys, ureters and bladders of infected and healthy mice in relation to the live weight.

	Infected										Normal			
Mice killed	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Live wt. in gms.	23.0	26.5	23.5	20.5	28.9	23.7	27.5	25.1	25.2	29.7	27.6	26.1	25.7	23.6
Millions of organisms injected	15	20	25	25	40	15	20	25	40	40				
Interval of days before killing	46	46	46	23	46	46	46	46	23	23				
Wt. of kidneys and ureters, mg.	436.8	469.8	355.1	326.4	532.4	386.2	526.6	432.2	431.5	601.8	451.3	439.8	492.4	537.7
Wt. of bladder, mg	23.5	25.5	19.1	17.9	47.0	27.1	27.5	32.1	29.9		25.0	23.4	27.4	43.0

TABLE 19

Hematological characteristic of infected and normal mice.

Animal		W.B.C. count	Differential count L	N	M	B	E
Normal Controls		6100	73	25	2	0	0
		6500	69	26	5	0	0
		8000	69	26	5	0	0
		7200	69	26	5	0	0
Infected - 6/23/49							
Dose in millions	Interval in days						
15	10	11,320	17	77	6	0	0
15	10	12,000	22	76	2	0	0
25	10	10,600	72	23	4	1	0
20	10	4,860	55	44	1	0	0
25	11	3,600	70	27	3	0	0
25	11	3,650	72	24	4	0	0
25	11	9,500	72	24	4	0	0
20	14	14,250	72	24	4	0	0
20	14	20,750	72	22	5	1	0
15	14	16,000	72	22	5	1	0
25	16	8,500	39	60	1	0	0
25	16	17,750	77	17	6	0	0
25	16	7,250	70	25	5	0	0
20	16	7,000	68	26	4	2	0
15	16	8,000	71	22	7	0	0

Owing to difficulty in securing blood, the white cell pipette was filled only to the 0.1 mark and the diluting fluid (o.1 HCL) was drawn to the 11 mark and thus making the dilution 1:100.

In vivo study using rabbits.

Symptoms and clinical findings. The first six infected rabbits did not show symptoms suggestive of illness. They gained in weight. The ten rabbits in the second and third groups lost weight and went off-feed.

The blood picture of all the 16 rabbits, on the other hand, showed definite change. There was an increase in the percentage of heterophils and decrease in the percentage of lymphocytes (table 20). As the percentage of polymorph increased immature forms appeared. The progressive drop in hemoglobin values after inoculation was striking (table 20). This drop in hemoglobin probably could be attributed to the fact that the bone marrow was concerned primarily with the production of heterophils in order to combat the infection and thereby interfering with the production of red blood cells.

Gross pathology. Mottling appearance of the livers and kidneys suggestive of fatty metamorphosis was noticed. Some of the livers of infected rabbits showed lesions of coccidiosis which were not referred to in the following description. Other organs appeared normal.

Micropathology.

Kidney. The lesions were similar to those observed in the mice. The pelvic epithelium showed focal necrosis which was extensive enough to cause exfoliation of epithelial lining. (Fig. 12)

Infiltrated into the pelvis were pseudoeosinphils, a few mononuclears, faintly stained acidophilic granules and desquamated epithelial cells. In the tunica propria of the pelvis edema was evident along with the presence of acidophilic material (Fig. 13).

A general vacuolization and granular degeneration of the epithelium of the collecting tubules were evident. In the sections stained with Sudan IV, fat droplets in the collecting tubules were discernible. The darker shade in the photomicrograph indicated the degree of fatty degenera-

tion in the affected tubules (Fig. 14). In some of the collecting tubules the granular and cellular casts had caused no damage at the point at which they were observed (Fig. 15). On the other hand in locations where the tubules were damaged and necrotic, the adjacent capillaries were congested and some a polymorphonuclear leucocytes had migrated into the interstitial spaces (Fig. 15).

Changes in the thick limbs of Henele loop were present in nearly all the kidneys. The epithelium was irregularly swollen and granular and showed much variation in staining. Considerable vacuolization of the cytoplasm was seen, some of which probably was due to hydropic degeneration or fatty metamorphosis. The tubules contained varying amount of cellular and granular materials with necrotic changes in the epithelium. With fat stain these tubules showed a considerable amount of intracellular fat (Fig. 14).

In general the thin limbs presented no marked nor consistent pathological changes. Granular acidophilic materials were frequently present in the tubules. The epithelium appeared to be in a state of ballooning degeneration.

In general, the glomeruli presented a mild cellular infiltration. The glomerular tufts showed an increased number of leucocytes. In some instances, there were partial or complete areas of granular degeneration in the glomeruli. The granular materials coalesced diffusely to form hyalinized mass and took a deep stain (Fig. 16). This hyalinized mass was also seen in some of the perivascular areas. Around the affected glomeruli newly formed connective tissue led to the thickening of the

capsules. Following this glomerulonephritis increase of the interstitial connective tissue in the adjoining intertubular spaces was seen.

Albuminous degeneration was present in the proximal convoluted tubules of all kidneys. This was more pronounced in the tubules adjacent to the glomeruli. The epithelial cells were not only swollen but completely filled the lumina of the tubules and also showed increased granularity of the cytoplasm. (Fig. 17) In some of the cells there were vacuoles near the nuclei. Cellular damage was severe in some of the tubules.

The varying process of necrosis in the cellular structure of the convoluted tubules was evident (Fig. 17). Many of the cells had ruptured as a result of severe ballooning degeneration. The nuclei were extruded into the lumina. Pyknotic or caryoclasic changes were seen in some areas. Faintly eosin stained granules were present in the tubules. The unusual appearance of intracellular fat in some of the tubules was also noticed.

The distal convoluted tubules presented the same pathological picture.

Bladder. The changes in the bladder were not consistent. Focal areas of necrosis in the epithelium were evident. The mucosa in the affected areas had undergone partial to complete exfoliation. Characteristic pyknotic changes were well marked (Fig. 18).

Ureter. Mild focal necrosis of the epithelium was noticed in the ureter of only one rabbit (Fig. 19).

Liver. The hepatic lesions were widely diverse ranging from fulminating lesions to a chronic form of hepatitis. In some areas there

was albuminous degeneration of the hepatic cells. This was most marked in the area around the central veins. The liver cells were markedly swollen, the cytoplasm was excessively granular and stained lightly with eosin. In other locations the liver cells showed varying degrees of necrosis. The architecture of the hepatic cords was still present. There was no line of demarcation between the healthy and necrotic liver cells (Fig. 20).

In some areas there appeared to be a reaction with giant cells forming a narrow zone of demarcation between the lesion and the living cells (Fig. 20). Within the same field fibroblasts were proliferating around the necrotic zone in an attempt to wall off degenerative process (Fig. 20).

Fat stain showed the degree of fatty degeneration present in the hepatic cells, especially around the central vein. This became less prominent towards the periphery of the lobule (Fig. 21).

No lesions were observed in the bile ducts.

Lungs. The primary lesion in the lungs was a general congestion. In some it was well marked in others it was very mild. The alveoli contained a number of pseudoeosinophils. The interalveolar walls were heavily infiltrated with these cells. The infiltration of inflammatory cells both in and out of the alveoli was so great and diffuse in some sections that the lung tissue was hardly recognizable (Fig. 22).

Exudate in the bronchioles was evident. Necrotic bronchiolar epithelium and polymorphonuclears formed the main constituents of the exudate. The polymorphonuclears in the exudate had undergone necrosis (Fig. 23).

Mononuclears, a few pseudoeosinophils and fine faintly stained granules were present in the subpleural space (Fig. 24).

Organisms identical with those inoculated were isolated from the kidney, urine, liver, lungs and heart blood of one rabbit and from the urine of two others.

TABLE 20

Hematological characteristics of the rabbits exposed to C. renale-like organisms.

Rabbit No.	Days of weighing	Wt. in gms	Culture No.	Dosage	Days of hematological examination	Hemoglobin mg %	W.B.C. per mm_3	Differential count L	H	M	B	E	Remarks
10 infected on 12/18/49	0	3798	10	4 billion	0	13.3	7500	60	34	4	2	0	
					1	11.4	7700	54	38	2	6	0	
					3	10.8	8500	44	54	1	1	0	
					5	10.1	9500	48	50	0	2	0	
					7	8.6	13500	38	56	1	5	0	
	9	3110			9	9.5	11350	35	65	0	0	0	Necropsy No. 208X 12/28/49.
2 infected on 12/18/49	0	4760	6	4 billion	0	14.1	11700	60	36	0	4	0	
					1	13.5	10800	66	30	2	2	0	
					3	13.3	8250	44	54	1	1	0	
					5	15.2	9700	27	70	1	2	0	
					7	11.4	12200	26	71	1	2	0	
	9	4510			9	10.4	10500	38	61	0	1	0	Necropsy No. 203X 12/28/49
1 control						13.3	7100	57	34	5	4	0	
2 control						13.7	8100	55	39	3	3	0	

L = lymphocytes H = heterophils M = monocytes B = basophils E = eosinophils

DISCUSSION

Ernst (1905) in a study of the etiology of pyelonephritis in cattle, found gram positive rod shaped bacteria in the urine of affected animals. This author classed these organisms with the Corynebacteria and designated them "Corynebacterium renalis bovis". Later Jones and Little (1926), Merchant (1935), Thorp, et al (1943), in extensive studies of the morphology and cultural characteristics of these organisms referred to them as Corynebacterium renale. Brooks and Hucker (1944) described the morphological and chemical properties of two cultures isolated from the urine of cattle affected with pyelonephritis. These cultures were designated Corynebacterium pseudodiphthericum. The properties of these cultures compared very closely to those of C. renale. Feenstra, et al (1945) on the basis of morphological, biochemical and serological properties described two distinct species of Corynebacteria involved in the etiology of pyelonephritis in cattle.

The ten cultures reported in this thesis were similar to C. renale in ecology, morphology and in that they fermented glucose and fructose (culture No. 9 did not attack fructose). Similarly there was no indole, nor H_2S production, and no liquefaction of gelatin (culture No. 10 liquefied gelatin). Alkaline reaction in litmus milk, formation of halos in milk agar and catalase production were shown by all the strains.

They differed from C. renale in that all 10 cultures produced alpha hemolysis. This hemolytic property was found in six cultures reported by Feenstra, et al (1945). All the cultures grew slowly, produced small colonies on artificial media, reduced methylene blue, failed to utilize citrate and to reduce nitrate, were ammonia, methyl red and Voges-Proskauer

negative. Out of 26 strains of C. renale studied by Lovell (1946) 23 were methyl red positive and 24 were Voges-Proskauer positive. Two of 51 strains of C. renale studied by Morse (1949) reduced nitrate. All these cultures except Nos. 6 and 10 split urea and failed to hydrolyze starch and protein, but digested serum.

The possibility that cultures no. 6 and 10 might be similar to C. pyogenes-like could be easily suggested, but not substantiated, in that although they fermented glucose, xylose, lactose, sucrose, hemolysed blood, digested serum they did not produce acid in litmus milk, and there was no beta hemolysis. Moreover, culture no. 6 fermented maltose but did not liquefy gelatin, while no. 10 produced an opposite effect on these two media.

The ten cultures studied in this investigation fell broadly into two main groups on the basis of cultural and biochemical properties. Cultures no. 6 and 10 appeared to comprise one group although minor dissimilarity occurred. The remaining eight cultures in spite of minor dissimilarity between one another were classed as a second group.

In view of these differences it was not possible to classify these cultures as C. renale. These groups of Corynebacteria may be a possible etiological factor in bovine pyelonephritis. This was further substantiated by an animal inoculation study using mice and rabbits.

Enderlen (1891) produced typical renal lesions in two ureter ligated rabbits. Jones and Little (1926) could not produce the disease in white mice, guinea pigs nor rabbits. Doll (1942) confirmed the findings of Enderlen and stated that lesions produced in rabbits were similar to those found in the cow. Lovell and Cotchin (1946a) described experimental

pyelonephritis in mice. Feenstra, et al (1949) produced typical renal lesions in 22 young rabbits which were identical with the lesions found in naturally affected cattle. These workers, in addition to reporting changes in the urinary tract, described lesions in the liver, lungs and spleen.

In this study variation in the lesions was noticed. Hematological study of the mice did not reveal any significant variation in the infected animals when compared to the normal. The white cell and differential counts remained within the normal limits (Kolmer and Boerner, 1945). Boyd (1913) observed an increase in size of the kidneys and ureters of cattle suffering from pyelonephritis. No significant difference in weight between the kidneys, ureters and bladders of the infected mice was noticed. With the higher infective dose of 40 millions, the severity of the lesions were not uniformly met with, bacteria could not be isolated from the viscera. However, it might be stated that these organisms were patnogenic to mice. They produced the same type of lesions as was described by Lovell and Cotchin (1948a). From the protocols it may be presumed that these organisms were not as invasive as typical C. renale.

In addition to the above, lesions in the spleen, liver and lungs were observed.

The lesions and hematological picture in the rabbits were similar to those described by Feenstra, et al (1949) with the exception that lesions were not seen in the spleen. Recording of giant cells in the liver was noteworthy in this study. Doll (1942) mentioned that giant cells were invariably found in the livers of rabbits infected with C. renale. The reason for the formation of these giant cells in the liver could not be established.

CONCLUSION

From the protocols of the results of both *in vitro* and *in vivo* studies it may be stated that these organisms isolated from cows suffering from pyelonephritis were in many features similar to, but not identical with typical *Corynebacterium renale* as described by various workers. They may be possible etiological factors in bovine pyelonephritis.

SUMMARY

The diphtheroids studied were non-motile, non-sporing pleomorphic gram positive beaded rods occurring in palisade groupings.

The bacteria were slow growers. All produced alkalinity in litmus milk and "halo" in milk agar, hemolyzed blood, reduced methylene blue, fermented glucose, and were catalase positive. With the exception of culture No. 9 all attacked fructose.

These cultures fell into two groups. Nos. 6 and 10 formed one group and the remaining 8 comprised the second group..

In mice, hematological study and weight of kidneys, ureters and bladders of infected animals did not show significant differences when compared to the normal.

In rabbits, hematological study revealed a drop in hemoglobin and an increase in the percentage of heterophils in the infected animals.

When these organisms were given to mice in adequate doses they produced pyelonephritis.

The patnogenesis of experimental pyelonephritis in and pathogenicity to rabbits were similar to those observed in mice.

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Fig. 2.

1. Pelvic necrosis
2. Necrosis of collecting tubules
3. Hyaline-like materials on the pelvic surface of the papilla.

The mouse was inoculated with 40 million organisms of culture no. 1. Necropsy after 7 days.

H. & E. stain 100X

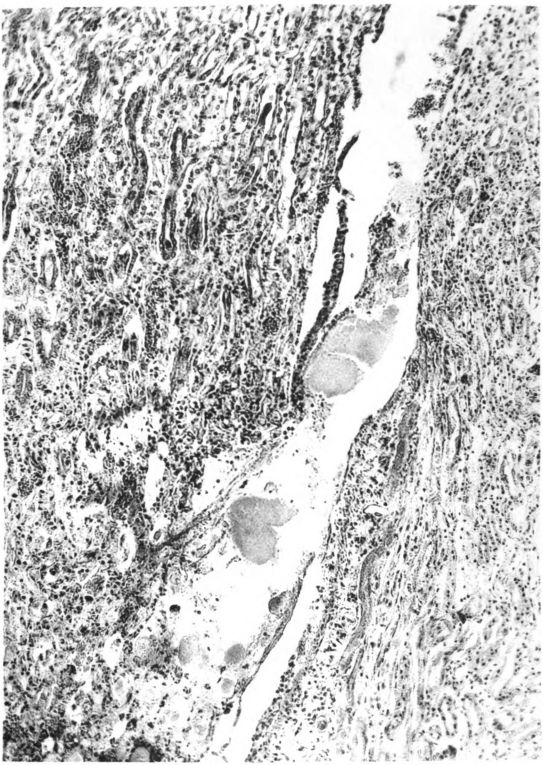

Fig. 2

Fig. 3.

1. Necrosis and exfoliation of the pelvic epithelium
2. Cellular deposits in the pelvis.
3. Leucocytic infiltration and fibrin in the peripelvic tissue.
4. Necrosis of the collecting tubules.

The mouse was inoculated with 40 million organisms of culture no. 1. Necropsy after 7 days.

H. & E. stain 100X

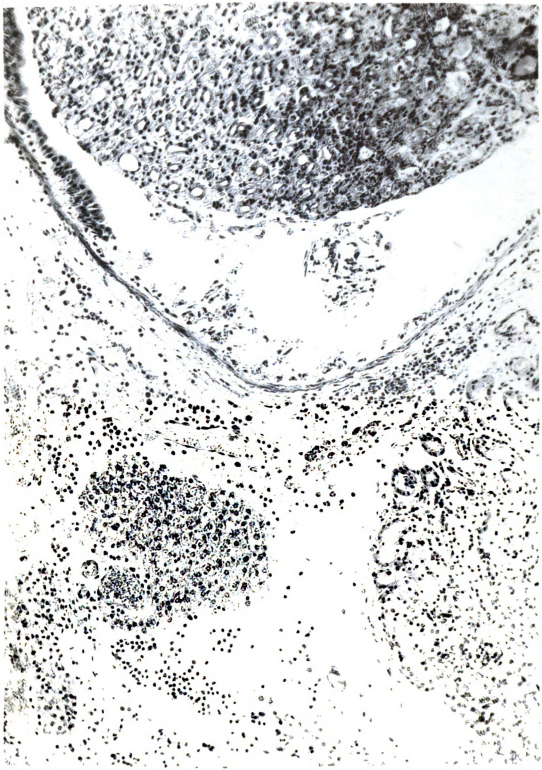

Fig. 3

Fig. 4. Dilatation of the collecting tubules.

The mouse was inoculated with 15 million organisms of culture no. 1. Necropsy after 55 days.

H. & E. stain 100X

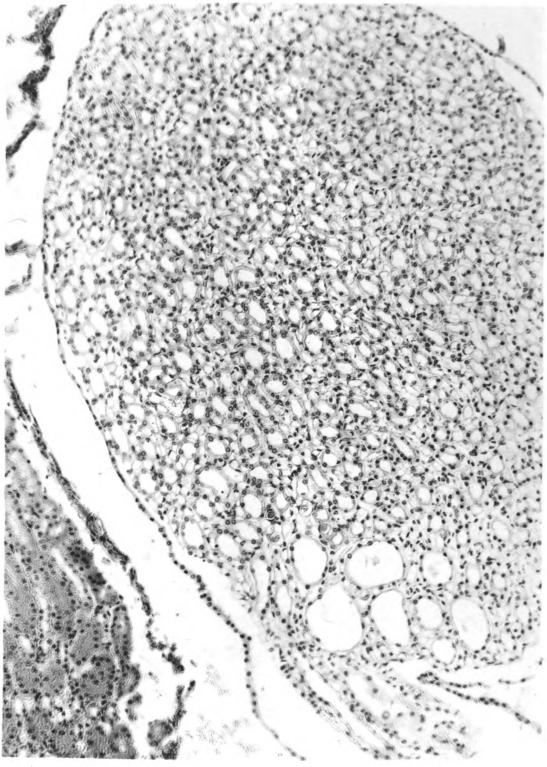

Fig. 4

Fig. 5. Periglomerular, perivascular and intertubular leucocytic infiltration with the appearance of new formed connective tissue.

The mouse was inoculated with 40 million organisms of culture no. 6. Necropsy after 30 days.

H. & E. stain 100X

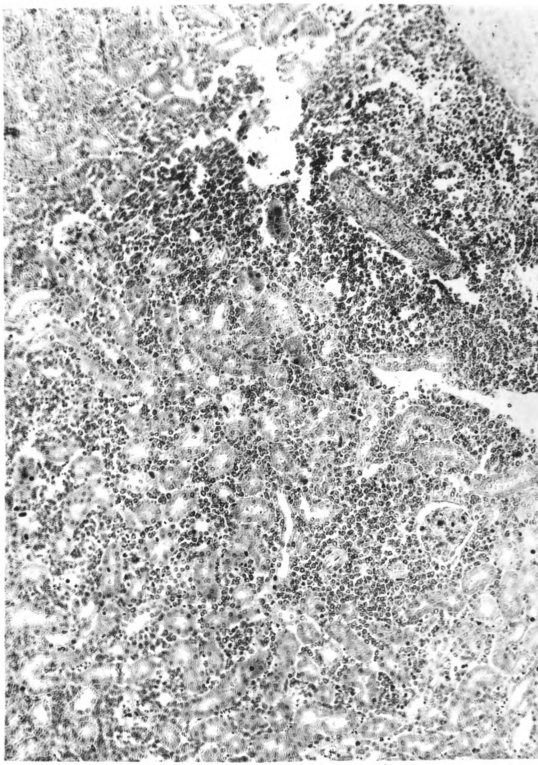

Fig. 5

Fig. 6.

1. Focal necrosis
2. Intertubular hemorrhage
3. Glomerulitis

The mouse was inoculated with 40 million organisms of culture no. 5. Necropsy after 7 days.

H. & E. stain 100X

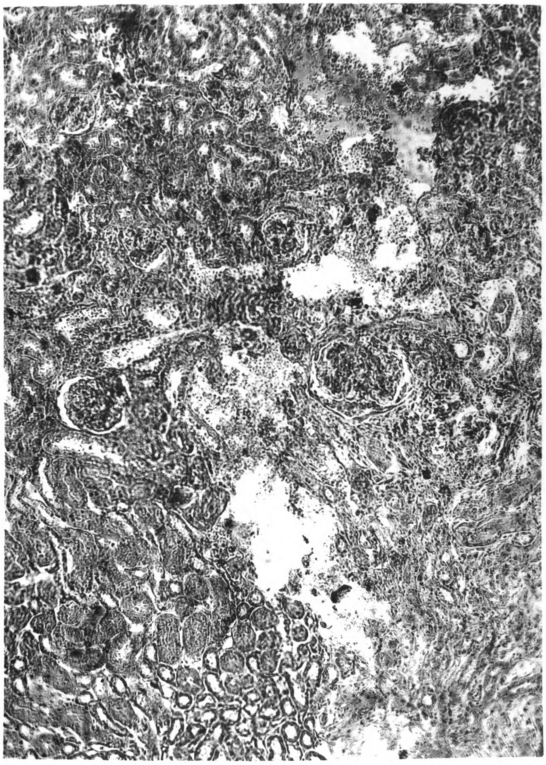

Fig. 6

Fig. 7.

1. Granular degeneration of the proximal convoluted tubules.
2. Dilatation of proximal convoluted tubules.
3. Newly formed connective tissue around the dilated tubules.

The mouse was inoculated with 15 million organisms of culture no. 7. Necropsy after 47 days.

H. & E. stain 100X

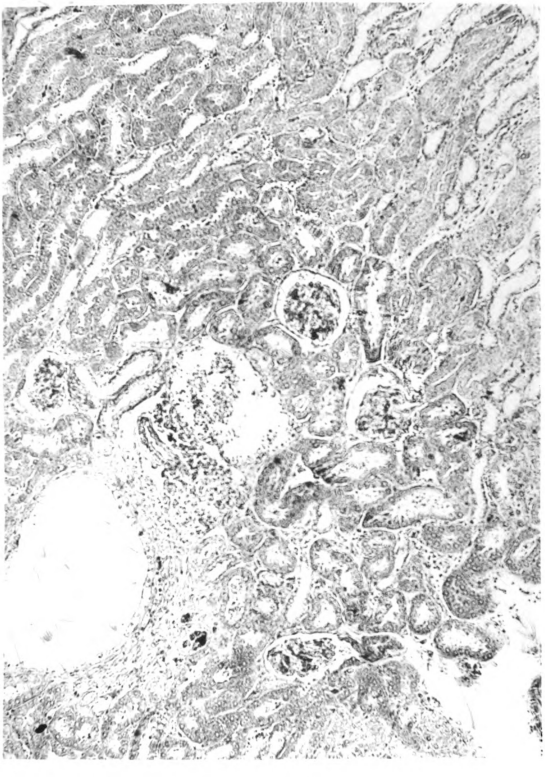

Fig. 7

Fig. 8. 1. Leucocytic infiltration in the subcapsular space.
2. Thickening of the renal capsule.

The mouse was inoculated with 20 million organisms of culture no. 1. Necropsy after 55 days.

H. & E. stain 100X

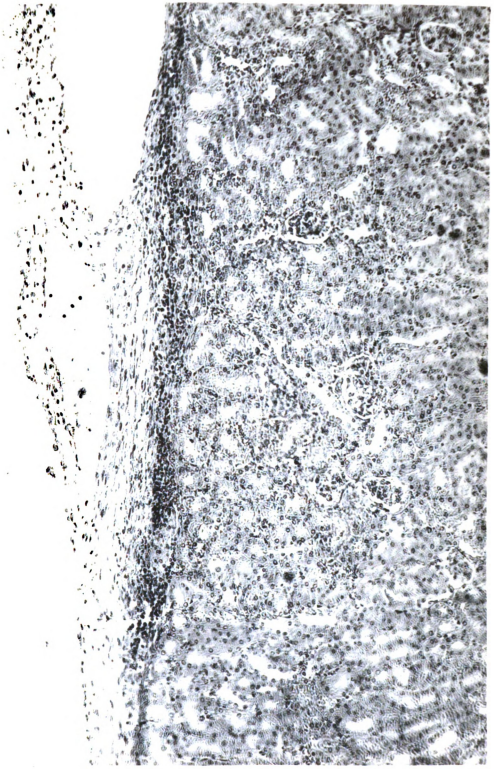

Fig. 8

Fig. 9. 1. Lymphocytes beyond the Malpighian corpuscles of the spleen.
2. Increased number of giant cells.

The mouse was inoculated with 40 million organisms of culture no. 3. Necropsy after 14 days.

H. & E. stain 65X

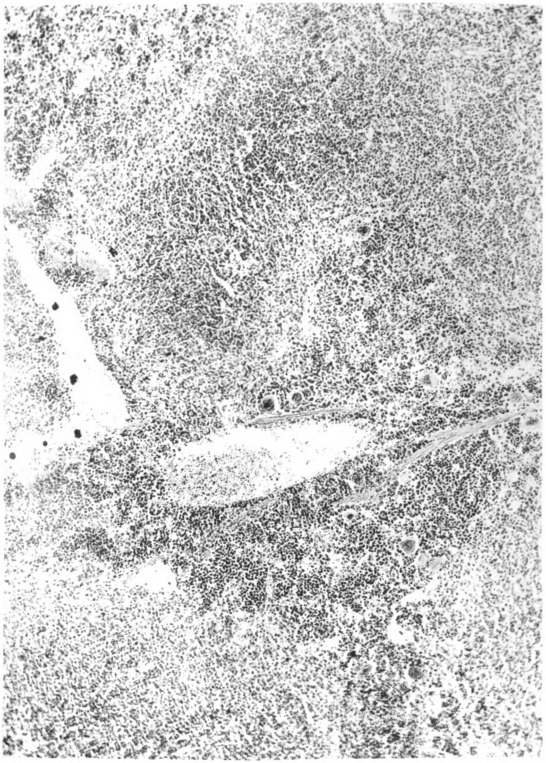

Fig. 9

Fig. 10. 1. Fatty metamorphosis in the hepatic cells.
2. Necrosis of the hepatic cells.
3. Amyloid degeneration of the hepatic cells.

The mouse was inoculated with 40 million organisms of culture no. 10. Killed 8 days after inoculation.

H. & E. stain 65X

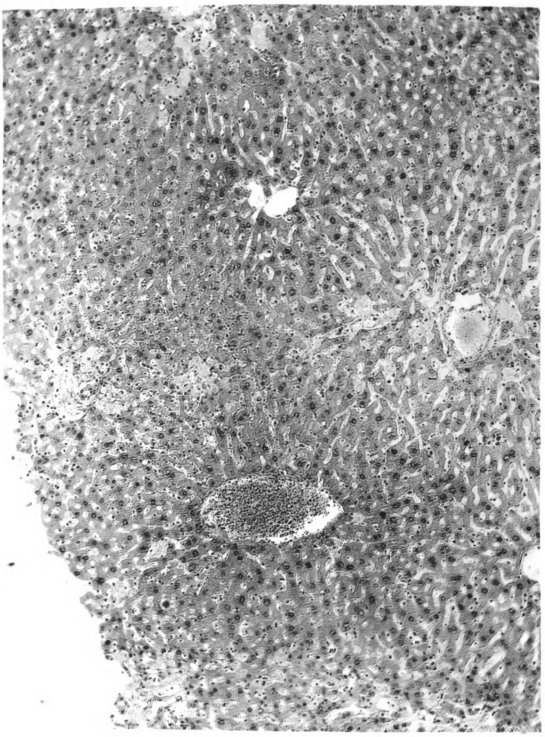

Fig. 10

Fig. 11. 1. Interstitial pneumonitis.
2. Peribronchiolitis.
3. Inflammatory exudate in the bronchioles.

The mouse was inoculated with 20 million organisms of culture no. 7. Necropsy after 7 days.

H. & E. stain 65X

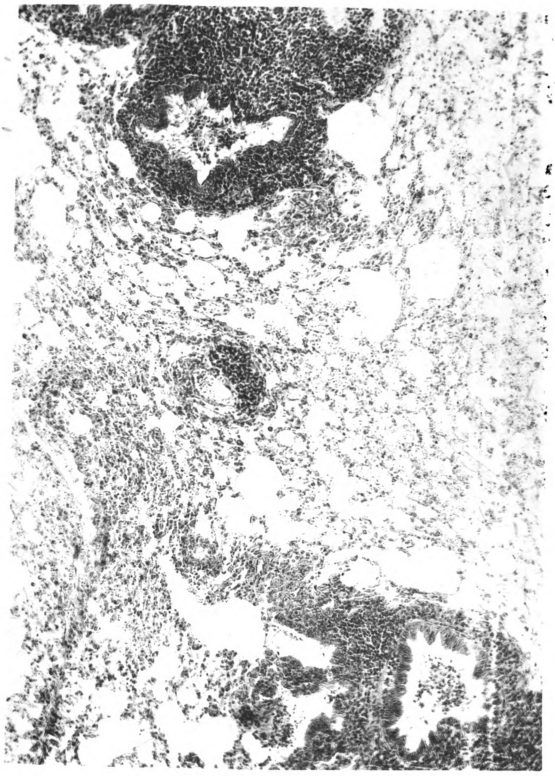

Fig. 11

Fig. 12. 1. Exfoliation of the pelvic epithelium.
2. Granular and cellular materials with pelvis.

The rabbit was inoculated with culture no. 10.
Necropsy after 10 days.

H. & E. stain 65X

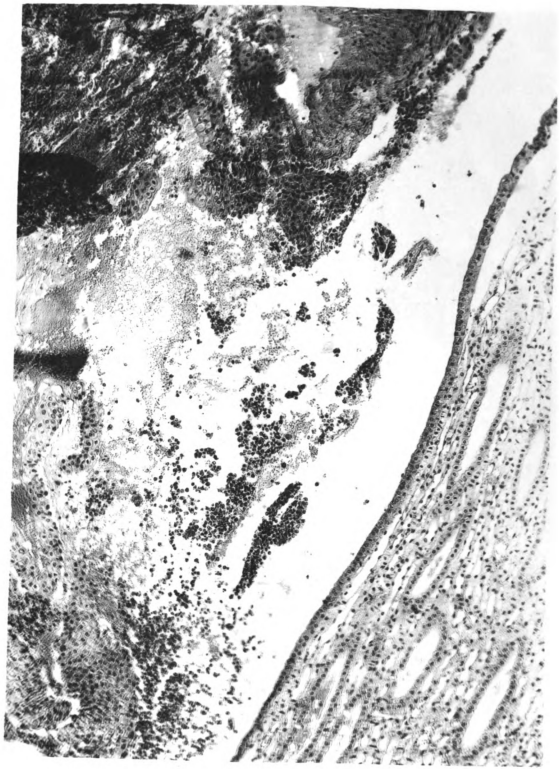

Fig. 12

Fig. 13. 1. Cellular materials in the pelvis.
2. Edema with acidophilic granules in the tunica propria.

The rabbit was inoculated with culture no. 10.
Necropsy after 10 days.

H. & E. stain 65X

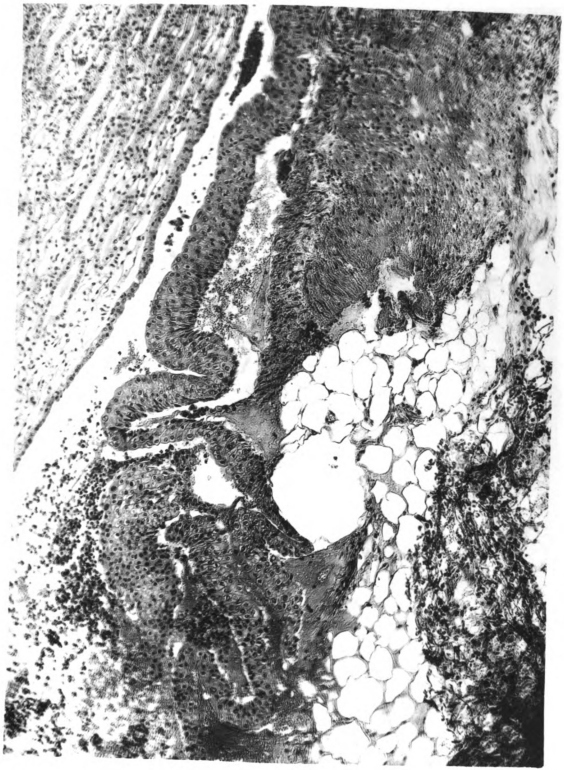

Fig. 13

Fig. 14. Fatty metamorphosis in the collecting tubules and ascending limbs of Henele.

The rabbit was inoculated with culture no. 6. Necropsy after 10 days.

Sudan IV 100X

Fig. 14

Fig. 15. 1. Granular and cellular materials in the collecting tubules.
2. Necrosis of the epithelium of the collecting tubules.
3. Hyperemia of the intertubular vessels.

The rabbit was inoculated with culture no. 10. Necropsy after 10 days.

H. & E. stain 100X

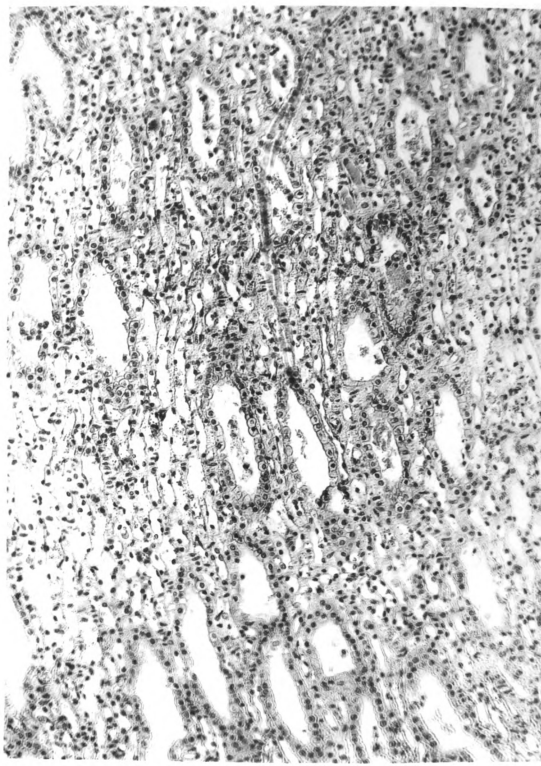

Fig. 15

Fig. 16. 1. Excessive number of leucocytes in the glomerular tufts.
2. Areas of granular degeneration.
3. Appearance of hyalinized mass.

Rabbit was inoculated with culture no. 10.
Necropsy after 9 days.

H. & E. stain 100X

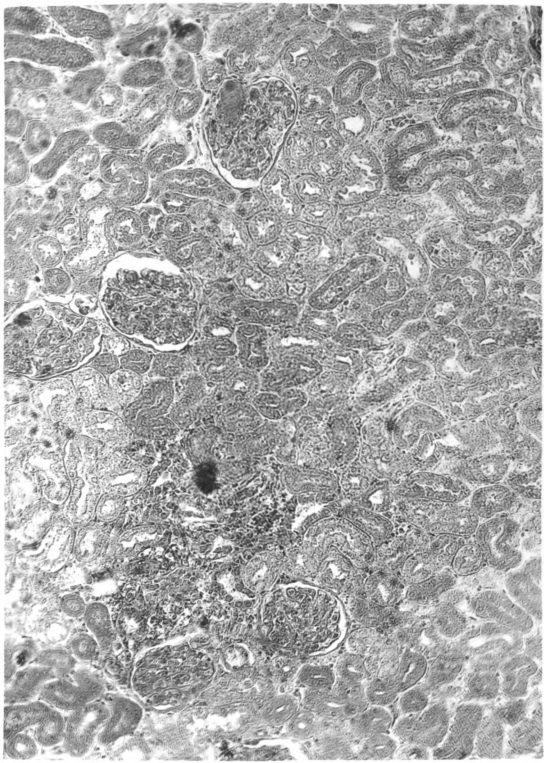

Fig. 16

Fig. 17.

1. Hyalinized mass in the glomerulus.
2. Connective tissue proliferation around the glomerulus.
3. Granular degeneration of the proximal convoluted tubules.

Rabbit was inoculated with culture no. 10.
Necropsy after 9 days.

H. & E. stain 100X

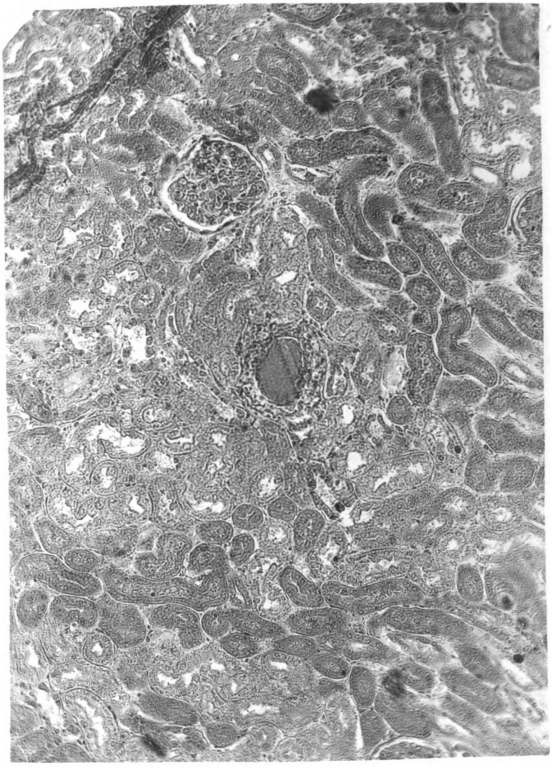

Fig. 17

Fig. 18. 1. Focal areas of necrosis of the bladder ep epithelium.
2. Desquamated epithelium in the lumen.

Rabbit was inoculated with culture no. 6.
Necropsy after 6 days.

H. & E. stain 100X

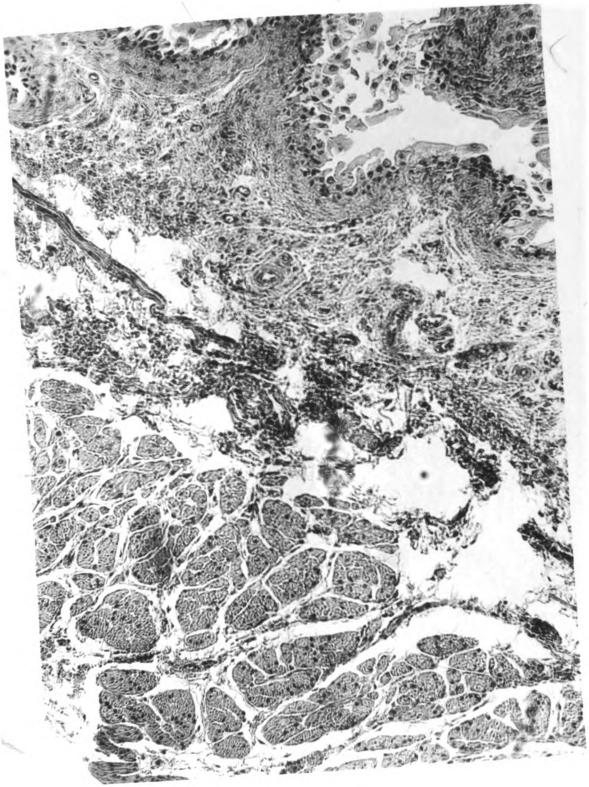

Fig. 18

Fig. 19. Mild focal necrosis of the ureteral epithelium.

The rabbit was inoculated with culture no. 6.
Necropsy after 6 days.

H. & E. stain 100X

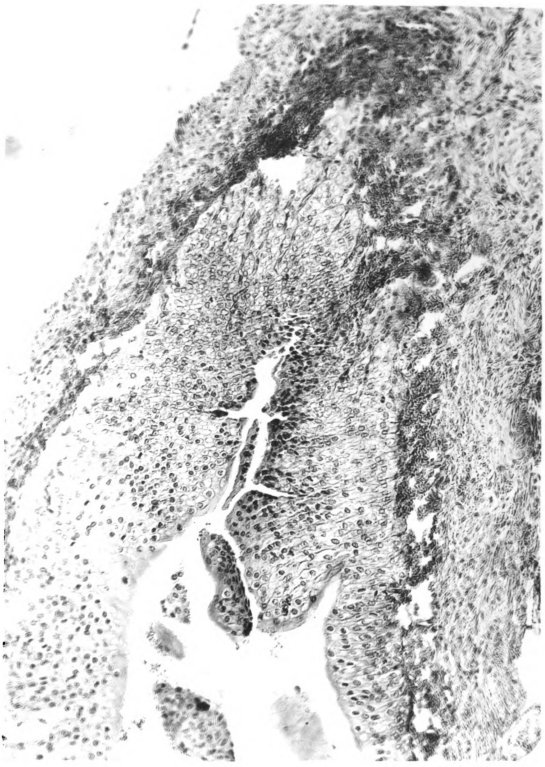

Fig. 19

Fig. 20. 1. Hepatic necrosis.
2. Giant cell formation.
3. Proliferation of fibroblasts.

The rabbit was inoculated with culture no. 10.
Necropsy after 10 days.

H. & E. stain. 100X

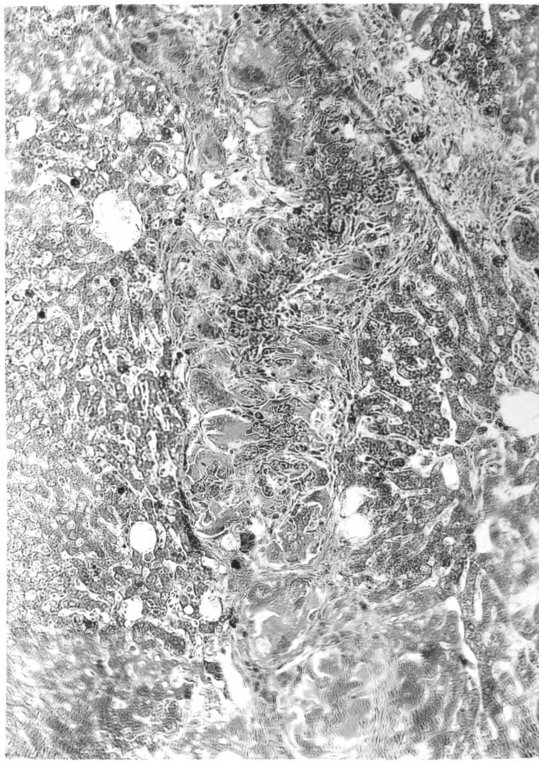

FIG. 20

Fig. 21. Fatty metamorphosis of the hepatic cells.

The rabbit was inoculated with culture no. 10.
Necropsy after 10 days.

[redacted] sudan IV 100X

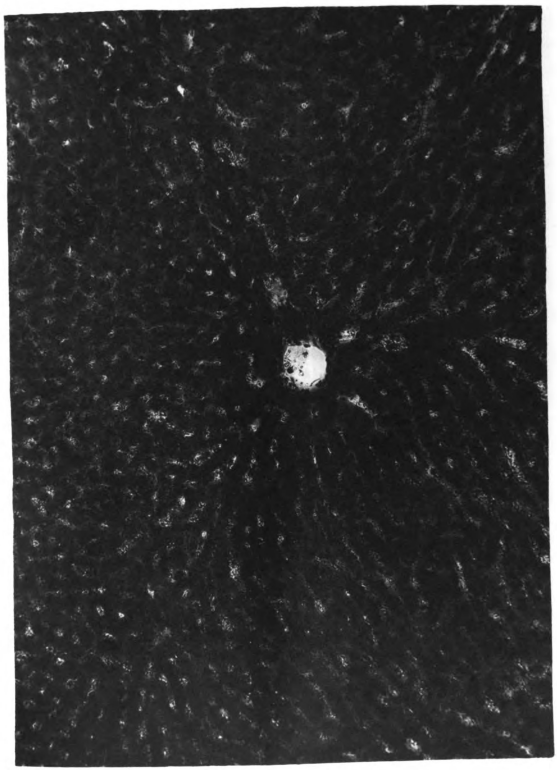

Fig. 21

Fig. 22

1. Cellular infiltration both inside and outside of the alveoli.
2. Inflammatory exudates in the bronchi.

The rabbit was inoculated with culture no. 6.
Necropsy after 8 days.

H. & E. stain 100X

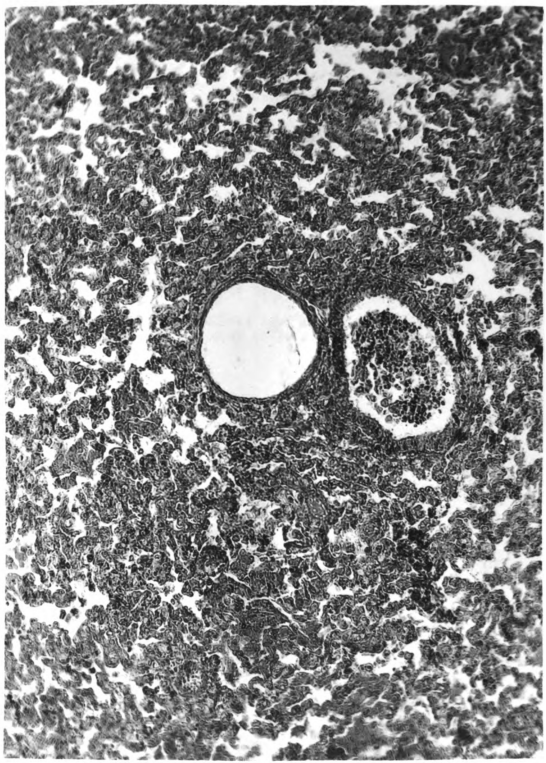

Fig. 22

Fig. 23. Inflammatory exudate in the bronchi.
Partial exfoliation of the bronchiolar epithelium.

The rabbit was inoculated with culture no. 6.
Necropsy after 6 days.

H. & E. stain 100X

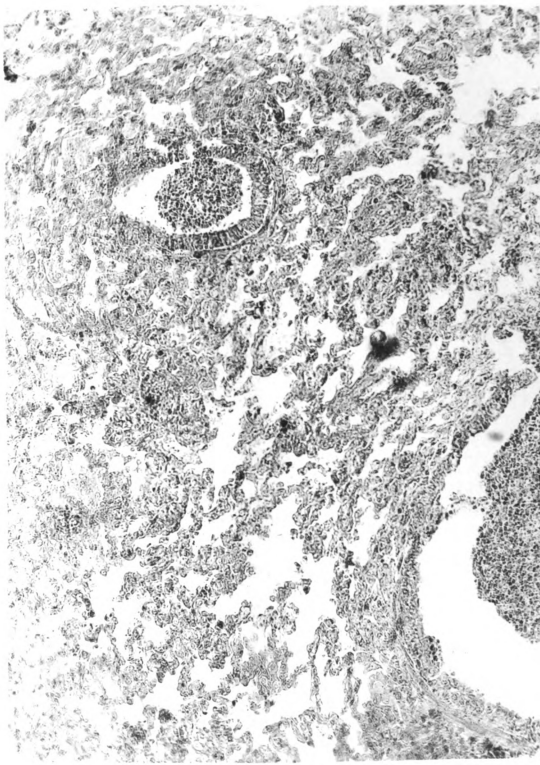

Fig. 23

Fig. 24. Mononuclears and heterophils in the subpleural space.

The rabbit was inoculated with culture no. 6. Necropsy after 8 days.

H. & E. stain 100X

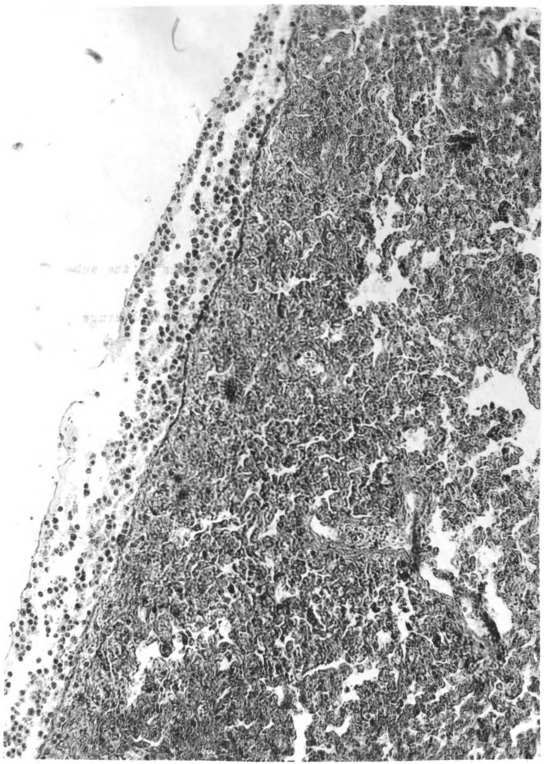

Fig. 24