STUDIES ON THE ETIOLOGY OF SPECIFIC INFECTIOUS BOVINE PYELONEPHRITIS

Thesis for the Degree of Ph.D.

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An Abstract

Part I. Pathogenicity of Corynebacterium renale for Rabbits.

In order to determine the efficacy of the rabbit as an experimental animal for use in the study of bovine pyelonephritis, 22 white rabbits were inoculated intravenously with a suspension of Corynebacterium renale which had been grown on tryptose agar (Difco) at 37°C for 24 hours. These rabbits were necropsied at intervals of one-half hour, one, two, three, four, five, six, seven, nine, eleven, eighteen and nineteen days after inoculation. Hemoglobin determinations, total leukocyte counts and differential leukocyte counts were made on alternate days. The heart blood, lungs, liver, spleen, kidney, sternal bone marrow and urine were cultured on tryptose agar. For microscopic study, specimens of the lung, liver, spleen, kidney, ureters and urinary bladder were fixed in Zenker's fluid; paraffin sections prepared, and stained with hematoxylin and eosin and by the Gram-Weigert method for staining bacteria in sections.

The earliest change in the kidney was the accumulation of pseudo-eosinophils in the capillaries and collecting tubules with congestion in the papillary region of the medulla on the second day. Following this, at three and four days after inoculation, necrosis and erosion of the papilla were found. Masses of bacteria were found in the remains of capillaries and tubules and in the debris in the pelvis. The polymorphonuclear leukocyte (pseudoeosinophil) was the predominant inflammatory cell found in

the early lesion. On about the fourth day the renal pelvic mucosa appeared necrotic and the peripelvic tissues contained serous fluid, fibrin, erythrocytes and pseudoeosinophils. Lymphocytes appeared on the sixth day and mononuclear phagocytes and active fibroblasts appeared on the seventh day. These latter three cells increased in number as time passed while the pseudoeosinophils decreased in number but never disappeared entirely. At 17 and 18 days there were changes in the cortex such as an increase in interstitial connective tissue, dilation and constriction of tubules and accumulations of lymphocytes. The blood picture was one characteristic of acute infections and showed changes such as an increase in leukocytes on the fourth day and an increase in percent of pseudoeosinophils with a decrease in percent of lymphocytes in the differential leukocyte count.

Part II. Properties of Some Colonial Phase Variants of Corynebacterium renale.

Five cultures, representing at least three different colony types were chosen from stock cultures of Corynebacterium renale for study in order to determine their morphological, biochemical, serological and pathogenic relationships.

One culture which might be classified as "smooth" showed the weakest action in the fermentation of glucose, digestion of casein, showed the least resistance to the bactericidal action of bovine plasma, and was the least virulent for rabbits.

Two cultures which might be classified as "rough" showed the most resistance to the bactericidal action of bovine plasma and were the most virulent for rabbits.

The other two cultures were intermediate in morphology and pathogenicity.

The results of agglutination tests showed that there were antigenic

differences and similarities between the different cultures but there was little or no correlation with the morphological characteristics of the colonies.

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by

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A Thesis

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INTRODUCTION

For many years laboratory animals have been considered refractory to infection with Corynebacterium renale. The advantages of the use of the small laboratory animals in the study of disease is obvious. A fortuitous circumstance in this laboratory pointed to the fact that rabbits seemed to be susceptible to infection under certain conditions. The first part of this thesis is devoted to a study of the pathogenicity of C. renale in the rabbit.

The second part of this thesis is devoted to a study of the colony morphology of cultures of C. renale and the relation of these morphological characteristics to the pathogenic and antigenic properties.

Part I. PATHOGENICITY OF CORYNEBACTERIUM RENALE FOR RABBITS

The advantages of the use of the small laboratory animals in the study of the diseases of large animals are well known. This is particularly true if a similar disease process is evinced by the laboratory animals. Previous to 1946, laboratory animals were considered to be refractory to infection by Corynebacterium renale.

Enderlen (1891) reported typical renal lesions eight and eleven days after intravenous inoculation of an organism isolated from a case of bovine pyelonephritis into two rabbits with ligated ureters. This was repeated by Doll (1942). Jones and Little (1926) reported negative results after using white mice, guinea pigs and rabbits. Lovell (1946) and Lovell and Cotchin (1946) describe experimental pyelonephritis in mice which resulted from the intravenous inoculation of 20 million organisms. The results after intravenous inoculation of 60 million organisms into guinea pigs and rabbits they report as much less satisfactory. They also describe the results of inoculation of C. renale onto the chorio-allantoic membrane of nine day old chick embryos.

The pathogenicity of C. renale for the rabbit was discovered in this laboratory while attempting to produce immune sera in rabbits for several colonial phase variants of C. renale. A total of nine rabbits died of the 21 started on immune serum production. Experiments were then designed to study the pathogenesis of experimental pyelonephritis in rabbits.

Materials and Methods

Fifteen rabbits (about 2 Kg each) were inoculated intravenously with approximately four billion microorganisms each. The culture (Culture No. 2, Thesis, Part II) chosen for inoculation was from a cow

with a typical case of pyelonephritis; it satisfied all morphological, cultural and biochemical requirements for C. renale (Bergey, 1939) and had proved pathogenic for several rabbits. The first ten rabbits were necropsied one-half hour, one, two, three, four, five, six, seven, nine and eleven days after inoculation. The remaining five were allowed to live for 18-19 days in order to determine the mortality which prevailed under the conditions of the experiment (one died at eight days). Blood samples were taken on alternate days to allow determination of any changes in hemoglobin levels (Cenco-Sheard-Sanford photelometer, Hoffman method, 1941), numbers of leukocytes, and differential leucocyte counts (100 cells counted on each slide; Wright stain). In most cases the heart blood, lungs, liver, spleen, kidney, sternal bone marrow and urine were cultured on tryptose agar (Difco). Tissue smears of lung, liver, spleen, kidney, urine and bone marrow were made and stained with gram stain. Gross pathological changes were recorded. For microscopic study, specimens of the lung, liver, spleen, kidney, ureters and urinary bladder were fixed in Zenker's fluid; paraffin sections prepared, and stained with hematoxylin and eosin and by the Gram-Weigert method for staining bacteria in sections.

After the first experiment was completed another was designed to investigate more thoroughly the happenings of the first four days. Eight rabbits (series A) were used, one was killed as a normal control at the time the other seven were inoculated and these were necropsied at the rate of two at the end of one day, two days, and three days, and one at the end of four days. The technics and procedures were similar to those employed in the first experiment.

Almost without exception the livers exhibited lesions of coccidiosis. These lesions were recorded but will not be referred to in

the individual case reports. This condition may explain the high initial leukocyte counts obtained in some cases, especially in the series A rabbits.

Occasionally, the lesions reported in the gross examination were missed in sectioning the tissue. However, sufficient lesions were found to allow adequate descriptions of the microscopic changes.

Results

Case reports of rabbits used in the experiment follow. The results of the study of the tissue smears so nearly paralleled the results of the culture work that they will not be further reported.

Case 1

Rabbit 1 was necropsied one-half hour after inoculation. Organisms were cultured from the heart blood, liver, lung and kidney. No gross lesions were found.

Microscopic findings. Diphtheroids were found in the larger blood vessels of the liver and lung. The section of the lung showed congestion, slight serous exudation, and in the alveolar capillaries were many pseudoeosinophils, some of which appeared to contain diphtheroids.

Time	Hb	WBC	M	L	P	B	E
0	13.3	8,100	1	75	19	4	1
1/2 hr.	13.3	8,850	2	73	24	1	0

(See next page for key to headings)

Time - Interval after inoculation; 0 being just prior to inoculation.

Hb - Hemoglobin in grams per 100 ml blood.

WBC - Leucocytes per cubic mm.

M - Monocytes, percent.

L - Lymphocytes, percent.

P - Polymorphonuclear leukocytes (pseudoeosinophils) percent.

B - Basophils, percent.

E - Eosinophils, percent.

Case 2

Rabbit 2 was necropsied one day after inoculation. Organisms were cultured from the liver and kidney. No visible lesions were present except a possible enlargement of the spleen.

Microscopic findings. The spleen was congested.

Time	Hb	WBC	M	L	P	B	E
0	13.9	10,900	1	72	24	3	0
1 day	11.8	8,800	3	46	49	2	0

Case 3

Rabbit 3 was necropsied two days after inoculation. No organisms were cultured. The spleen appeared slightly swollen.

Microscopic findings. A slight congestion of the spleen, excess mucus in the pulmonary bronchi, and one focus (250 micra diam.) in the outer medulla of the kidney consisting of pseudoeosinophils in the thick limbs of Henle's loops were the only apparent lesions.

Time	Hb	WBC	M	L	P	B	E
0	10.1	8,900	1	79	18	2	0
2	9.0	4,650	5	63	30	2	0

Case 4

Rabbit 4 was necropsied three days after inoculation. Organisms were cultured from the kidney and urine. The spleen seemed slightly enlarged. The kidneys were congested and there were several long, slender, radiating (toward the cortex) abscesses in the medulla.

Microscopic findings. The liver was somewhat foamy in appearance. There were several foci (0.5-1 mm in diam.) in the renal medulla consisting mainly of pseudoeosinophils and necrotic tissue debris. There were also many small lesions in the medulla which appeared to be the beginnings of the above mentioned foci consisting of pseudoeosinophils in capillaries and tubules (mainly thick limbs and collecting tubules) and congestion of the capillaries in the area. There were erythrocytes, pseudoeosinophils and tissue debris in the pelvis. The parts of the tips of the papillae found in the stained sections showed slight necrosis.

Time	Hb	WBC	M	L	P	B	E
0	11.4	8,550	1	73	24	1	1
2 days	9.5	8,200	6	60	28	6	0
3 "	10.8	15,900	5	57	37	1	0

Case 5

Rabbit 5 was necropsied four days after inoculation. Organisms were cultured from the kidney, urine and heart blood. The kidneys were swollen and congested. There was papillary necrosis with radiating lesions extending through the medulla toward the cortex. The urine was blood tinged.

Microscopic findings. The spleen exhibited an increase in pseudoeosinophils and much phagocytosis of blood pigment by the phagocytes. The ureters were enlarged, the epithelium was necrotic and desquamated, hyaline

bodies were present in the lamina propria, and the lumens were filled with erythrocytes, pseudoeosinophils, fibrin, diphtheroids and tissue debris. All the structures in the renal papillae in an area about 1 mm thick were necrotic. Masses of bacteria were present in what had been tubules and capillaries. Pseudoeosinophils were scattered throughout the lesion and appeared to contain diphtheroids. Several collecting tubules and capillaries in the inner medulla were filled with pseudoeosinophils and cellular debris. There was a necrotic focus in the pelvic mucosa with the presence of serofibrinous exudate, congestion, hemorrhage and pseudoeosinophils. There was a slight amount of cloudy swelling of the proximal convoluted tubules.

Time	Hb	WBC	M	L	P	B	E
0	11.8	5,300	1	69	30	0	0
2 days	10.1	6,600	5	55	32	8	0
4 "	11.8	25,500	13	39	41	7	0

Case 6

Rabbit 6 was necropsied five days after inoculation. This animal was a pregnant female which died shortly before necropsy. Organisms were cultured from the liver, lung, spleen, heart blood, bone marrow, kidney, placenta, fetal fluid and fetus. The lung showed a few hemorrhages. The spleen was slightly enlarged. The kidneys were much swollen and congested. The kidney pelves were enlarged and filled with bloody fluid. The papillae were necrotic with radiating abscesses in the medulla. There were also several subcapsular abscesses. The ureters were enlarged.

Microscopic findings. There were vacuoles in the hepatic cells in central part of the lobules suggestive of fatty degeneration. The lungs were slightly congested. The spleen was congested and there was

phagocytosis of erythrocytes and blood pigment. The ureters were distended with blood, tissue debris, and bacteria. Much of the ureteral epithelium was necrotic and the lamina propria was infiltrated with pseudoeosinophils. The renal papillae were eroded. Large masses of bacteria and cellular debris were observed in the collecting tubules in the medulla around the necrotic area. The pelvic epithelium was necrotic and the peripelvic connective tissue contained erythrocytes, fluid, fibrin, and pseudoeosinophils. Some proximal convoluted tubules showed a slight amount of cloudy swelling.

Time	Hb	WBC	M	L	P	B	E
0	12.9	5,350	2	58	30	8	2
2 days	10.8	5,400	7	45	47	1	0
4 "	9.8	11,050	18	34	47	1	0

Case 7

Rabbit 7 was necropsied six days after inoculation. Organisms were cultured from the kidney and urine. The spleen appeared slightly enlarged. The left kidney showed erosion of papilla and cortical and subcapsular abscesses. The papilla of the right kidney was normal, although the cortex contained a large white lesion which was cone shaped with the apex in the medulla. The ureters were slightly congested, especially near the kidneys.

Microscopic findings. The liver was slightly congested, the central veins were enlarged and there was a small (2 mm diam.) abscess near the surface. The lumens of the ureters contained bacteria. The renal medullae contained scattered foci which consisted of groups of pseudoeosinophils and cellular debris and which were surrounded by an area of capillary congestion. The tip of the left renal papilla was lost from

the stained sections but the pelvic mucosa opposite the papilla exhibited a necrotic focus with infiltration of the area by serous and fibrinous exudates, pseudoeosinophils and a few lymphocytes.

Time	Hb	WBC	M	L	P	B	E
0	13.3	8,900	1	64	33	2	0
2 days	11.1	7,550	5	62	29	4	0
4 "	12.1	17,000	14	52	33	1	0
6 "	12.7	14,700	5	41	48	5	1

Case 8

Rabbit 8 was necropsied seven days after inoculation. This animal had appeared ill for several days and was moribund. Organisms were cultured from the kidney. The spleen appeared slightly enlarged. The kidneys were much enlarged, exhibiting papillary necrosis with purulent material in the pelves, and cortical and subcapsular abscesses. The ureters were enlarged and congested. The bladder was filled with bloody urine.

Microscopic findings. The liver cells showed cloudy swelling and there were vacuoles indicating central fatty degeneration. The pulmonary alveolar walls were thickened by an increase of pseudoeosinophils and erythrocytes. The spleen was congested and showed some phagocytosis of blood pigment. The urinary bladder epithelium appeared slightly degenerated and the lumen contained bacteria and tissue debris. The ureters were enlarged and the epithelium was necrotic; bacteria and tissue debris were found in the lumens. An abscess (1 x 1.5 mm) in the renal medulla near the papillary tip contained pseudoeosinophils and tissue debris with bacteria on the border. There was a zone of necrosis along the tip of the renal papilla about .25 mm to 1.5 mm in thickness surrounded by congestion

and an infiltration with inflammatory cells. Several cortical abscesses were present and some of the convoluted tubules exhibited the characteristics of cloudy swelling. Diphtheroids and pseudoeosinophils were scattered through the tissue debris in the pelvis. The pelvic mucosa was necrotic and the peripelvic tissue contained a serofibrinous exudate, pseudoeosinophils, lymphocytes, mononuclear phagocytes and active fibroblasts. Several areas (5-10 mm diam.) of tubular degeneration and hemorrhage in the cortices and medullae appeared to be hemorrhagic infarcts.

Time	Hb	WBC	M	L	P	B	E
0	12.9	6,850	0	87	12	1	0
2 days	11.8	7,400	0	36	36	18	0
4 "	11.8	10,450	7	50	28	14	1
6 "	11.8	15,600	21	32	45	2	0
7 "	10.0	14,450	25	23	49	3	0

Case 9

Rabbit 9 was necropsied nine days after inoculation. Organisms were cultured from the kidney and urine. The spleen appeared slightly enlarged. The kidneys were very slightly enlarged and exhibited papillary necrosis and pyelitis (yellowish lesion 0.5 cm in diameter). The right kidney also had a small white cortical lesion.

Microscopic findings. The pulmonary alveolar walls were slightly congested and contained an increased number of pseudoeosinophils. The spleen was congested and exhibited a moderate phagocytosis of blood pigment. The ureteral lumens contained some tissue debris and the epithelial cells contained a few hyaline bodies (cytoplasmic). The kidneys were congested and showed the characteristic papillary erosion (progressive necrosis, masses of bacteria, infiltration with pseudoeosinophils, and

congestion). The pelvis was filled with blood, tissue debris, bacteria and pseudoeosinophils; the pelvic mucosa was necrotic and the peripelvic tissues were congested and contained fibrin, lymphocytes, mononuclear phagocytes and proliferating fibroblasts. There were lymphocytic foci scattered throughout the cortex and peripelvic tissue. The convoluted tubules were slightly degenerated as evidenced by the presence of cloudy swelling.

Time	Hb	WBC	M	L	P	B	E
0	12.3	5,600	0	73	22	5	0
2 days	10.4	8,250	0	69	25	5	1
4 "	11.1	9,250	3	72	18	7	0
6 "	11.8	12,350	7	60	28	5	0
9 "	12.4	16,550	1	32	55	12	0

Case 10

Rabbit 10 was necropsied eleven days after inoculation. Organisms were cultured from the kidney and urine. The kidneys and ureters seemed to be of about average size and presented no external lesions. The left kidney showed papillary necrosis with purulent material in the pelvis.

Microscopic findings. The lung section showed a slight congestion of the alveolar walls. There was a slight increase in the number of pseudoeosinophils in the spleen. The urinary bladder epithelial cells contained a few hyaline bodies (cytoplasmic). A few scattered collecting tubules of both kidneys were filled with cellular debris, also both cortices exhibited some cloudy swelling and fatty degeneration. There was the characteristic papillary erosion of the left kidney with early fibroblast proliferation. Also present was marked necrosis of the pelvic mucosa and in the peripelvic tissue were found inflammatory cells such as

pseudoeosinophils, lymphocytes, mononuclear phagocytes and proliferating fibroblasts.

Time	Hb	WBC	M	L	P	B	E
0	11.8	7,750	0	69	29	2	0
2 days	11.1	8,950	4	67	26	3	0
4 "	10.1	10,900	8	51	32	9	0
6 "	11.6	12,550	4	53	34	9	0
9 "	11.3	13,400	4	50	46	0	0
11 "	10.8	9,450	3	33	56	8	0

Case 11

Rabbit 11 was necropsied seventeen days after inoculation. Organisms were cultured from the kidney and urine. The carcass was rather emaciated. The kidneys were enlarged and displayed large pale areas in the cortices, papillary necrosis and erosion, and necrotic debris in the pelves. The left ureter was slightly enlarged.

Microscopic findings. The spleen was congested and showed phagocytosis of blood pigment. One of the ureters exhibited necrosis and desquamation of the epithelium. The mucosa was congested and infiltrated with lymphocytes while the lumen contained bacteria, pseudoeosinophils and tissue debris. The renal cortices showed a slight increase in activity of interstitial connective tissue, constriction of masses of tubules and dilation of others, notably the collecting tubules, distal convoluted tubules and some few proximal convoluted tubules. The medullae were congested. The papillae were eroded and the lesions were surrounded by a zone of chronic inflammatory processes. The pelves were filled with pseudoeosinophils, bacteria and tissue debris. The pelvic epithelium was necrotic and the peripelvic tissues contained serous fluid, pseudoeosinophils,

lymphocytes, mononuclear phagocytes and proliferating fibroblasts. There were small foci of lymphocytes scattered throughout the kidneys.

Time	Hb	WBC	M	L	P	B	E
0	12.3	5,400	2	50	41	6	1
2 days	11.6	7,550	9	47	37	7	0
4 "	11.1	28,650	9	51	33	7	0
6 "	12.3	7,850	11	31	50	6	2
9 "	13.3	52,200	15	38	46	1	0
11 "	13.3	19,950	15	37	41	7	0
14 "	12.9	16,200	12	41	41	6	0
17 "	13.5	9,400	13	39	46	1	1

Case 12

Rabbit 12 was necropsied seventeen days after inoculation. No organisms were cultured from any of the organs. There appeared to be organisms in the stained smears of the kidney and urine. The carcass was in good physical condition. The kidneys were of average size and exhibited pyelitis, papillary necrosis and accumulation of necrotic debris in the pelves.

Microscopic findings. The pulmonary alveolar walls contained a slightly increased number of pseudoeosinophils. The ureteral epithelium was necrotic and the walls were thickened with edematous fluid, congestion, pseudoeosinophils, lymphocytes and mononuclear phagocytes. The kidneys exhibited a moderate amount of papillary necrosis (250 micra thick) and the cells of the collecting tubules in the areas contained hyaline bodies. The very tips of the papillae were not in the sections. The cortices exhibited a slight increase in interstitial connective tissue, dilation of some tubules and constriction of others. Some tubules showed

evidence of degeneration and atrophy. A few lymphocytic foci were scattered throughout the kidneys. The pelves contained pseudoeosinophils and tissue debris. The pelvic mucosa was necrotic in some areas and hyperplastic in others, and the peripelvic tissue contained increased numbers of connective tissue elements, lymphocytes and mononuclear phagocytes.

Time	Hb	WBC	M	L	P	B	E
0	12.9	10,150	0	79	16	5	0
2 days	13.1	11,000	4	65	24	7	0
4 "	11.1	24,550	4	61	22	12	1
6 "	12.3	14,050	3	62	24	10	1
9 "	13.8	15,650	2	70	25	3	0
11 "	12.5	25,400	6	57	32	5	0
14 "	14.6	10,000	9	45	32	14	0
17 "	15.0	17,750	5	35	52	7	1

Case 13

Rabbit 13 was necropsied eighteen days after inoculation. Organisms were cultured from the kidney and the urine. The carcass was in poor condition. The kidneys were slightly enlarged and exhibited white cortical lesions causing the surface to appear mottled, medullar abscesses, papillary erosion, pyelitis and accumulation of purulent fluid in the pelves.

Microscopic findings. The renal cortices exhibited an increase in interstitial connective tissue, areas of dilation and of constriction, and also atrophy and degeneration of some of the tubules. One medulla contained an abscess characterized by tissue debris, pseudoeosinophils and mononuclear phagocytes. Several small foci similar to the above

abscess were also present. There was dilation of the papillary ducts and slight papillary erosion; the dominant inflammatory cell being the mononuclear phagocyte. There was much necrosis of the pelvic mucosa. The pelvis contained tissue debris and bacteria, and in the peripelvic tissue pseudoeosinophils, lymphocytes, mononuclear phagocytes and proliferating fibroblasts were present.

Time	Hb	WBC	M	L	P	B	E
0	12.9	8,400	0	80	16	3	1
2 days	11.8	10,150	3	49	34	14	0
4 "	12.9	13,750	4	40	44	9	3
6 "	13.3	18,750	2	43	45	9	1
9 "	13.7	16,550	10	44	42	4	0
11 "	14.2	24,100	4	47	46	3	0
14 "		37,950	5	56	33	6	0
18 "	14.8	19,650	1	52	35	9	3

Case 14

Rabbit 14 was necropsied eighteen days after inoculation. Organisms were cultured from the kidney and urine. The kidneys were of average size and exhibited scattered white foci, especially in the medullae. A pyelitis was present in the left kidney.

Microscopic findings. The interstitial connective tissue was present in increased amounts in scattered foci in both kidneys, especially in the cortices. The left kidney showed a slight amount of desquamation of epithelium on the tip of the papilla and a severe pyelitis characterized by necrosis of the pelvic mucosa and a zone of chronic inflammatory changes such as the presence of lymphocytes, mononuclear phagocytes, active fibroblasts and a few pseudoeosinophils. The pelvis contained

blood, tissue debris and bacteria.

Time	Hb	WBC	M	L	P	B	E
0	12.5	11,200	0	80	14	6	0
2 days	11.4	13,550	8	70	13	9	0
4 "	10.8	14,150	14	49	29	8	0
6 "	11.1	14,950	7	55	29	8	1
9 "	12.1	13,850	6	59	31	3	1
11 "	12.6	14,150	3	64	31	2	0
14 "	11.4	14,500	7	41	41	10	1
18 "	11.6	14,550	9	45	33	12	1

Case 15

Rabbit 15 died and was necropsied eight days after inoculation. This rabbit had appeared sickly on the day previous to death. Blood tinged urine had stained the fur around the urinary opening. Organisms were cultured from the kidney and urine. The kidneys were enlarged and exhibited several subcapsular abscesses (1 mm diam.), papillary erosion and bloody mucous fluid in the pelves. The ureters were enlarged.

Microscopic findings. The liver was congested and there was a slight central fatty degeneration. The lung was mildly congested and many pseudoeosinophiles were present in the alveolar walls. The spleen was congested and there was phagocytosis of blood pigment. Bacteria were present in tissue debris in the lumen of the urinary bladder and the epithelium appeared slightly degenerated. The ureteral mucosa was necrotic and bacteria were present in tissue debris in the lumen. The kidneys exhibited the characteristic papillary erosion. Abscesses (1 x 1.5 mm) characterized by tissue debris, pseudoeosinophils and bacteria were present in the medullae. The pelves contained tissue debris, pseudoeosinophils

and bacteria. The pelvic mucosa was necrotic and the peripelvic tissue was infiltrated with pseudoeosinophils and fibrin. There were scattered foci of lymphocytes, especially in the cortex. Some of the proximal convoluted tubules exhibited cloudy swelling.

Time	Hb	WBC	M	L	P	B	E
0	13.9	7,050	0	78	18	4	0
2 days	11.4	3,800	3	45	39	13	0
4 "	11.8	6,200	7	40	34	18	1
6 "	12.9	23,050	10	26	59	4	1

Case 16

Rabbit 1A was necropsied as a normal control for series (A) of seven rabbits. All organs were normal except the kidneys which exhibited a few small white foci in medulla and cortex.

Microscopic findings. There were a few lymphocytic foci scattered throughout the kidneys and a few of the collecting tubules contained cellular debris.

Time	Hb	WBC	M	L	P	B	E
0	11.9	11,050	1	72	26	1	0

Case 17

Rabbit 2A was necropsied one day after inoculation. Organisms were cultured from the liver, spleen, kidney and urine. The spleen appeared swollen and dark; the other organs appeared normal.

Microscopic findings. There was in increase in pseudoeosinophils and congestion of the lung and spleen. The epithelium on the tip of the renal papillae appeared slightly degenerated.

Time	Hb	WBC	M	L	P	B	E
0	12.9	11,300	0	78	20	2	0
1 day	13.3	8,750	0	45	51	4	0

Case 18

Rabbit 3A was necropsied one day after inoculation. Organisms (very few) were cultured from the spleen and heart blood. All of the organs appeared normal except for a small white focus in one kidney.

Microscopic findings. The lung and spleen were mildly congested and contained an increased number of pseudoeosinophils. One renal medulla exhibited a few small foci of pseudoeosinophils.

Time	Hb	WBC	M	L	P	B	E
0	11.5	14,650	2	76	18	3	1
1 day	11.8	17,850	0	61	36	3	0

Case 19

Rabbit 4A was necropsied two days after inoculation. No organisms were cultured from any of the organs. No lesions were apparent.

Microscopic findings. There was a slight increase in the number of pseudoeosinophils in the lung and a slight congestion of the spleen. One section of the kidney contained a focus (100 x 125 micra) of pseudoeosinophils near the side of the medulla.

Time	Hb	WBC	M	L	P	B	E
0	13.1	12,900	1	75	22	2	0
1 day	12.9	13,450	1	40	53	6	0
2 days	12.1	12,500	2	70	22	5	1

Case 20

Rabbit 5A was necropsied two days after inoculation. Organisms

were cultured from the kidney. All organs appeared normal except one small white focus in cortico-medullary region of one kidney.

Microscopic findings. The lung was congested, many pseudoeosinophils were present and bacteria were present in one of the larger blood vessels. The spleen was congested. Both renal medullae contained small abscesses (200 micra diam.) characterized by pseudoeosinophils, tissue debris, and local congestion.

Time	Hb	WBC	M	L	P	B	E
0	12.1	12,300	2	71	23	2	2
1 day	11.4	13,050	1	62	35	2	0
2 days	10.3	12,100	1	49	46	4	0

Case 21

Rabbit 6A was necropsied three days after inoculation. Organisms were cultured from the spleen, kidney and urine. The kidneys were of normal size but exhibited white foci in the cortices and medullae, medullar congestion, and a necrotic focus on the tip of each renal papilla.

Microscopic findings. The lung contained an increased number of pseudoeosinophils. The spleen was congested and showed increased phagocytosis of blood pigment. There were foci in the urinary bladder mucosa which likely represented early lesions in the bladder. These were characterized by some desquamation and cavity formation of the epithelium, hyaline bodies in some epithelial cells, congestion of the mucosa and infiltration with serous fluid, fibrin and pseudoeosinophils. In the section of the right kidney there was a focus at the tip of the papilla characterized by necrosis, masses of bacteria, congestion, and infiltration by pseudoeosinophils. Also present in the medulla were several small foci characterized by pseudoeosinophils in the tubules and congestion.

Time	Hb	WBC	M	L	P	B	E
0	12.9	13,350	1	60	31	8	0
1 day	12.9	15,850	2	22	69	7	0
2 days	11.1	11,100	7	52	35	6	0
3 "	11.4	16,150	0	52	42	5	1

Case 22

Rabbit 7A was necropsied three days after inoculation. Organisms were cultured from the kidney and urine. The kidneys were of normal size and exhibited medullar congestion with a necrotic focus on the tip of each renal papilla.

Microscopic findings. The lung exhibited mild congestion and edema with an increase in pseudoeosinophils. The spleen was congested. The lesions at the tips of the renal papillae were very similar to those in rabbit 6A. The renal medullae were markedly congested and contained several foci of pseudoeosinophils. The pelvic epithelium showed slight degenerative changes where it contacted the papillar lesion.

Time	Hb	WBC	M	L	P	B	E
0	12.1	13,600	2	78	15	5	0
1 day	11.6	11,400	0	56	41	3	0
2 days	11.2	13,600	0	52	40	8	0
3 "	11.0	11,450	2	69	26	3	0

Case 23

Rabbit 8A was necropsied four days after inoculation. Organisms were cultured from the liver, heart blood, kidney and urine. The spleen appeared slightly enlarged. In the heart was a small red nodule on base of the aortic valve and a small (1 mm x 2 mm) thrombus attached to the base of the left atrioventricular valve. The kidneys were of average size

and exhibited a necrotic area on the tip of each renal papilla. The ureters appeared very slightly congested.

Microscopic findings. The lung showed a slight congestion and edema. The bladder epithelium appeared slightly degenerated. The ureteral epithelium appeared degenerated and the lumen contained blood and bacteria. The lesions in the renal papillae and medullae were similar to those in rabbit 6A only much more extensive. The pelvic epithelium showed degenerative changes where it contacted the papillar lesion. There was a slight infiltration of fibrin into the peripelvic tissue in these places.

Time	Hb	WBC	M	L	P	B	E
0	12.3	10,950	0	71	25	4	0
1 day	12.3	11,000	1	45	51	3	0
2 days	10.5	13,850	0	36	56	7	1
3 "	10.4	12,600	0	73	23	4	0
4 "	11.8	20,750	-	-	-	-	-

Table I

Summary of Kidney Lesions and Culture Results

Rabbit	Time	Kidney Lesions	Culture (1)
1	1/2 hr.	-	+ hbl, lv, l, kd
2	1 day	-	+ lv, kd
3	2 days	+	-
4	3 "	+	+ kd, ur
5	4 "	+	+ hbl, kd, ur
6 (2)	5 "	+	+ hbl, kd, spl, bm, l, fetus
7	6 "	+	+ kd, ur
8 (3)	7 "	+	+ kd
9	9 "	+	+ kd, ur
10	11 "	+	+ kd, ur
11	17 "	+	+ kd, ur
12	17 "	+	-
13	18 "	+	+ kd, ur
14	18 "	+	+ kd, ur
15 (2)	8 "	+	+ kd, ur
1A	0	-	-
2A	1 day	-	+ spl, lv, kd, ur
3A	1 "	-	+ hbl, spl
4A	2 days	-	-
5A	2 "	+	+ kd
6A	3 "	+	+ spl, kd, ur
7A	3 "	+	+ kd, ur
8A	4 "	+	+ hbl, lv, kd, ur

(1) cultures; l-lung, lv-liver, bm-bone marrow, spl-spleen, kd-kidney, ur-urinary bladder, hbl-heart blood.

(2) Died

(3) Moribund

Table II

Averages of Hematologic Results

Days (1)	No. An (2)	Hb (3)	WBC (4)	M (5)	L	P	B	E
0	15	12.6	7,893	0.8	72.	23.3	3.5	0.4
2	13	11.0	7,927	4.5	56.2	31.7	7.5	0.1
4	11	11.3	15,585	9.2	49.0	32.8	8.5	0.5
6	9	12.2	14,872	7.8	45.	40.	6.4	0.8
9	6	12.7	21,366	6.3	48.7	41.	3.8	0.2
11	5	12.7	18,610	6.2	47.6	41.2	5.	0
14	4	13.0	19,662	8.3	45.4	37.	9.	0.3
17	2	14.3	13,575	9.0	37.	49.	4.	1.0
18	2	13.2	17,100	5.0	49.	34.	10.	2.0
0	8	12.4	12,512	1.1	72.6	22.5	3.4	0.4
1	7	12.3	13,050	0.7	47.3	48.	4.	0
2	5	11.0	12,630	2.0	51.8	39.8	6.	0.4
3	3	10.8	13,016	0.7	65.	30.3	4.	0.3
4	1	11.8	20,750	-	-	-	-	-

(1) Days after inoculation.

(2) Number of animals in the group under consideration at that time.

(3) Hemoglobin in grams per 100 ml of blood.

(4) Leucocytes per cubic mm.

(5) Percent of monocytes, lymphocytes, pseudoeosinophils, basophils and eosinophils.

DISCUSSION

The lesions of experimental pyelonephritis in the rabbit are very similar to those found in spontaneous cases of pyelonephritis in cattle as described originally by Ernst (1905) and later by others (Jones and Little, 1925; Thorp et al, 1943). The lesions are also similar to those found in experimental pyelonephritis in mice by Lovell and Cotchin (1946).

The fact that the condition in the rabbit was produced by intravenous injection of C. renale should not be considered as evidence that the spontaneous disease in cattle is of hematogenic origin. The weight of evidence in the case of the cow would indicate that it is an ascending type of infection which may gain entrance by means of vulvar or urethral contamination (Jones and Little, 1926; Feenstra and Thorp, 1945). Eisendrath and Schultz (1917) have demonstrated, experimentally in rabbits and dogs, the presence of an anastomosing network of lymphatics in the wall of the bladder and of the ureter which communicates above with a similar lymphatic network in the renal pelvis and parenchyma and below with the lymphatics of the pelvic structures in both the male and female. They suggest that this is the route of an ascending infection of the urinary tract.

This report should not be taken as implying that only C. renale causes lesions of pyelonephritis in the rabbit. Dudgeon and Goadby (1930) reported that a suppurative pyelonephritis followed the intravenous inoculation of Staphylococcus aureus into rabbits.

The fate of the organisms from the time of injection until the beginning of lesion formation in the kidney is of interest. The data in Table I would indicate that, in general, the organisms were found initially in the lung, spleen and liver and later in the kidney and urine. The

interval between necropsies was too great to obtain a more accurate picture of the route of the organisms through the animal body. A study of the results of intravenous inoculation of other organisms by various workers may cast more light on the process. Bull (1914) showed that virulent streptococci and pneumococci tended to disappear from the circulating blood of rabbits in two to four hours and to reappear at five to six hours and eventually cause death. Less virulent organisms may not reappear or may cause a chronic infection. Bartlet and Ozaki (1917) reported that "Micrococcus aureus" injected into the left ventricle of dogs was almost immediately stored in the lung capillaries, being at first chiefly extracellular and then rapidly ingested by polymorphonuclear leukocytes. Shortly after injection the cocci increased in number in the liver and spleen as they decreased in number in the lung. The bacteria completely disappeared from these organs within 48 to 72 hours. The cocci did not primarily lodge in the kidney in any considerable numbers, but were secondarily deposited there after a period of adaptation elsewhere (cocci were found in the kidney at 16 hours but not at eight hours). Their location here was probably not embolic. Hopkins and Parker (1918) showed that injected hemolytic streptococci tended to disappear from the blood in about two hours and to reappear in increasing numbers after four and one-half hours. In cats and rabbits the organisms withdrawn from the circulation were found mainly in the lung, spleen and kidney. Nagao (1920) injected killed non-hemolytic streptococci into guinea pigs and observed that the organisms accumulated almost immediately in the lung capillaries inside polymorphonuclear leukocytes and later in the reticulo-endothelial cells of the liver and spleen. Dudgeon and Goadby (1931) reported that intravenously injected Staphylococcus aureus organisms were held in the lungs where they were actively phagocytosed by the polymorphonuclear

leukocytes within five minutes. Subsequently, the cocci were distributed to the other organs such as the liver and spleen and to the kidney. It may be concluded from study of the above mentioned results that, in general, the bacteria travel first to the lung and later are released to be picked up by the liver and spleen and later may be found in the kidney.

The mechanism of infection in the kidney is of special interest. Lovell and Cotchin (1946) have made observations on this process in the mouse kidney. They first saw the organisms in clumps in the capillaries between the collecting tubules near the apex of the papilla. This was accompanied by local dilation of capillaries with an accumulation of polymorphonuclear leukocytes which later passed through the normal or necrotic epithelium of the collecting tubules into their lumens. Small abscesses developed in the papilla often just under the visceral pelvic epithelium. Pus was found in the renal pelvis as early as thirty hours. Necrosis of the papilla extended toward the cortex. Large clumps of gram positive bacteria were seen in the necrotic material with a zone of pseudoeosinophils at the extending edge of the necrotic area. Pseudoeosinophils were also found in streaks up the medulla toward and into the cortex. The renal pelvis was dilated with purulent material. The pelvic epithelium showed acute inflammatory changes with abscess and ulcer formation. The pathogenesis of experimental pyelonephritis in the rabbit, as reported in this paper was essentially similar to that described in the case of the mouse. The earliest change in the kidney was the accumulation of pseudoeosinophils in the capillaries and collecting tubules with congestion in the papillary region. Bacteria may have been present in these pseudoeosinophils but they could not be clearly identified. Following this, at three and four days after inoculation, necrosis and erosion of the papilla were found. Masses of bacteria were seen in the remains

of capillaries and tubules, and in the debris in the pelvis as has been noted in the study of bovine pyelonephritis (Feenstra and Thorp, 1946). Pseudoeosinophils were numerous in and around the necrotic lesion. The pseudoeosinophils appeared, in many cases, to contain bacteria but the granularity of the cells and faint staining quality of any bacteria inside them interfered with precise observations. Streaks of pseudoeosinophils were found in the medulla extending toward the cortex. At this stage (about four days) the pelvic mucosa appeared necrotic and the peripelvic tissues contained serous fluid, fibrin, erythrocytes and pseudoeosinophils. Lymphocytes appeared on about the sixth day and mononuclear phagocytes and active fibroblasts appeared on the seventh day. These latter three cells increased in number as time passed while the pseudoeosinophils decreased in number but never disappeared entirely. Later (17 and 18 days), changes in the cortex such as an increase in interstitial connective tissue, dilation and constriction of tubules and accumulation of lymphocytes were found. In these more chronic cases the papillary erosion seemed to be progressing very slowly if at all but the pyelitis appeared to be increasing in severity. Necrosis and inflammatory changes resembling the pyelitis to a great extent were found in the ureters shortly after the appearance of the pyelitis.

As may be expected in animal experimentation, there were individual variations in reaction to inoculation and the reaction in series A was slightly different from the other group. For this reason mice could be used to advantage because there is less care and expense connected with the use of greater numbers.

As may be seen in Table II there was a slight initial drop in the hemoglobin values followed by an increase. This could be due to the fact that during the first week after inoculation the function of the

bone marrow was concerned mainly with production of granular leukocytes and thereby the production of erythrocytes was hampered.

In both series of rabbits it was on the fourth day that the blood leukocyte count showed a marked increase. The slight initial drop in the numbers of leukocytes is likely due to the fact that many of them are temporarily lodged in the pulmonary capillaries and in other organs.

As might be expected, there was an increase in the percentage of pseudoeosinophils in the blood and a decrease in the percentage of lymphocytes. In the case of the pseudoeosinophils, immature forms were often seen as the percentage increased but were not recorded as such because of the difficulty in precise identification of these forms due to the brilliantly red staining granules present. There was an increase in the percentage of monocytes. Less expected and not easily explainable was the increase in percentage of basophils. The main difficulty is that little or nothing is known of the function of the basophil. There was not a significant or meaningful change in the percentage of eosinophils.

Summary

The pathogenesis of experimental pyelonephritis in rabbits produced by intravenous inoculation of Corynebacterium renale has been described and discussed. Most inoculated rabbits showed kidney lesions and several died. The lesions in the kidney consisted of a papillitis and pyelitis characterized by necrosis and acute inflammatory changes early in the course and by chronic inflammatory changes later in the course. Bacteria were present mainly in the necrotic debris of the lesions and in the pelvis.

The lesions in the rabbit kidney were similar to those found in pyelonephritis in the cow. The fact that the experimental pyelonephritis

was of hematogenic origin in no way negates the generally accepted theory of an ascending infection in the cow.

The rabbit may be used, advantageously, in the study of pyelonephritis and also in the study of the pathogenic and antigenic properties of Corynebacterium renale.

REFERENCES

Bartlett, C. J. and Ozaki, Y. 1917 The fate of Micrococcus aureus introduced into the blood stream of dogs. J. Med. Res. 35, 465-486.

Bergey, D. H., et al. 1939 Manual of determinative bacteriology. 5th ed. The Williams and Wilkins Co., Baltimore.

Bull, C. G. 1914 A method for estimating the bacteria in the circulating blood in rabbits. J. Exp. Med., 20, 237-248.

Doll, E. R. 1942 Unpublished data. Dept. of Animal Pathology, Michigan State College.

Dudgeon, L. S. and Goadby, H. K. 1930 Further observations on the staphylococci with special reference to their hemolysis and variability. J. Hyg., 30, 180-195.

Dudgeon, L. S. and Goadby, H. K. 1931 The examination of the tissues and some observations on the blood platelets of rabbits at intervals of five minutes and later after intravenous inoculation of Staphylococcus aureus and India ink. J. Hyg., 31, 247-256.

Eisendrath, D. N. and Schultz, O. T. 1917 The path of involvement in ascending infection of the urinary tract. J. Med. Res., 35, 295-335.

Enderlen, D. 1891 Z. Tiermed., 17, 346. Cited by Ritzenthaler, M. 1910 The pathology of bacillary pyelonephritis in cattle. J. Comp. Path. Therap., 23, 33-50.

Ernst, W. 1905 Zentr. Bakt. Parasitenk., 39, 549-666; 40, 79. Cited by Ritzenthaler (see Enderlen).

Feenstra, E. S., Clark, C. F. and Thorp, Frank, Jr. 1945 Artificially induced case of pyelonephritis in a bovine. M.S.C. Vet., 5, 147-150.

Feenstra, E. S. and Thorp, Frank, Jr. 1946 Bacterio-pathology of infectious bovine pyelonephritis. Am. J. Vet. Res., 7, 432-436.

Hoffman, W. S. 1941 Photelometric clinical chemistry. William Morrow and Co., N.Y.

Hopkins, J. G. and Parker, J. T. 1918 The effect of injections of hemolytic streptococci on susceptible and insusceptible animals. J. Exp. Med., 27, 1-26.

Jones, F. S. and Little, R. B. 1925 Specific infectious cystitis and pyelonephritis of cows. J. Exp. Med., 42, 593-607.

Jones, F. S. and Little, R. B. 1926 The organism associated with specific infectious cystitis and pyelonephritis of cows. J. Exp. Med., 44, 11-20.

Lovell, R. 1946 Studies on Corynebacterium renale. I. A systematic study of a number of strains. J. Comp. Path., 56, 196-204.

Lovell, R. and Cotchin, E. 1946 Studies on Corynebacterium renale. II. The experimental pathogenicity for mice. J. Comp. Path., 56, 205-214.

Nagoa, K. 1920 The fate of killed non-hemolytic streptococci injected into the blood and the resulting cellular changes. J. Infect. Dis., 27, 327-362.

Thorp, Frank, Jr., Langham, R. F., Clark, C. F. and Doll, E. R. 1943 The pathology of bovine pyelonephritis. Am. J. Vet. Res., 4, 240-249.

Fig. 1. Rabbit pyelonephritis at necropsy. This animal died after a series of inoculations with culture No. 2 (Thesis, Part II). The digestive system has been removed. The kidneys are enlarged and exhibit white foci. The ureters are much enlarged. The urinary bladder is distended with bloody urine. Color print from Kodachrome negative.

Fig. 2. Rabbit kidney, showing papillary necrosis and erosion. This animal died after a series of inoculations with culture No. 5 (Thesis, Part II). Color print from Kodachrome negative.

Fig. 3. Same as Fig. 1, except color and size.

Fig. 4. Essentially normal rabbit kidneys from an uninoculated rabbit. Cross section, sagittal section and external view.


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Fig. 5. Rabbit kidneys, removed four days after inoculation, showing enlargement, white streaks in the medulla and early papillary necrosis.

Fig. 6. Rabbit kidneys, removed at death six days after inoculation, showing enlargement, papillary necrosis and white streaks in the medulla and cortex.

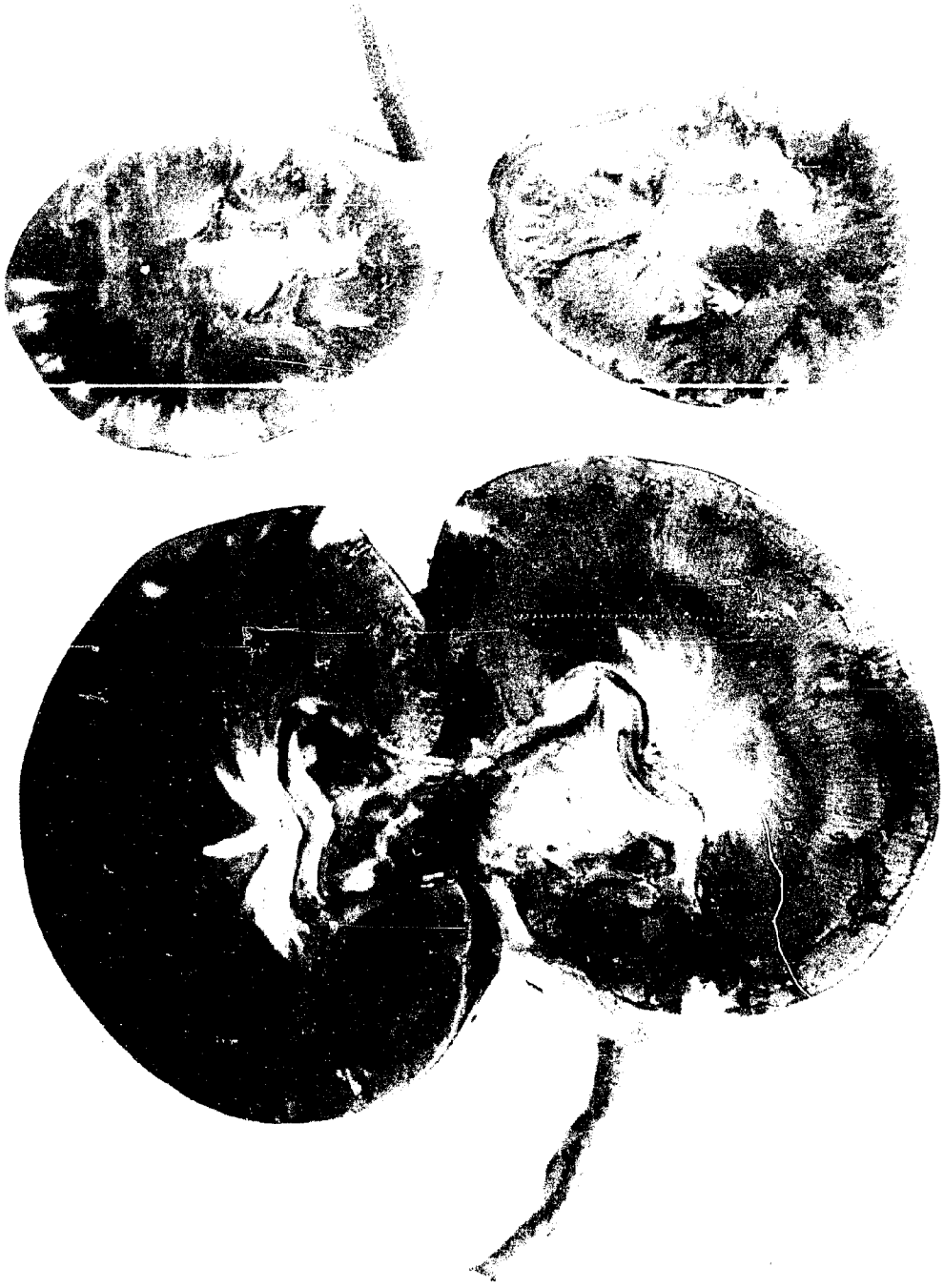


Fig. 7. Rabbit kidneys, removed at death seven days after inoculation, showing enlargement, papillary necrosis and white streaks in the medulla and cortex.

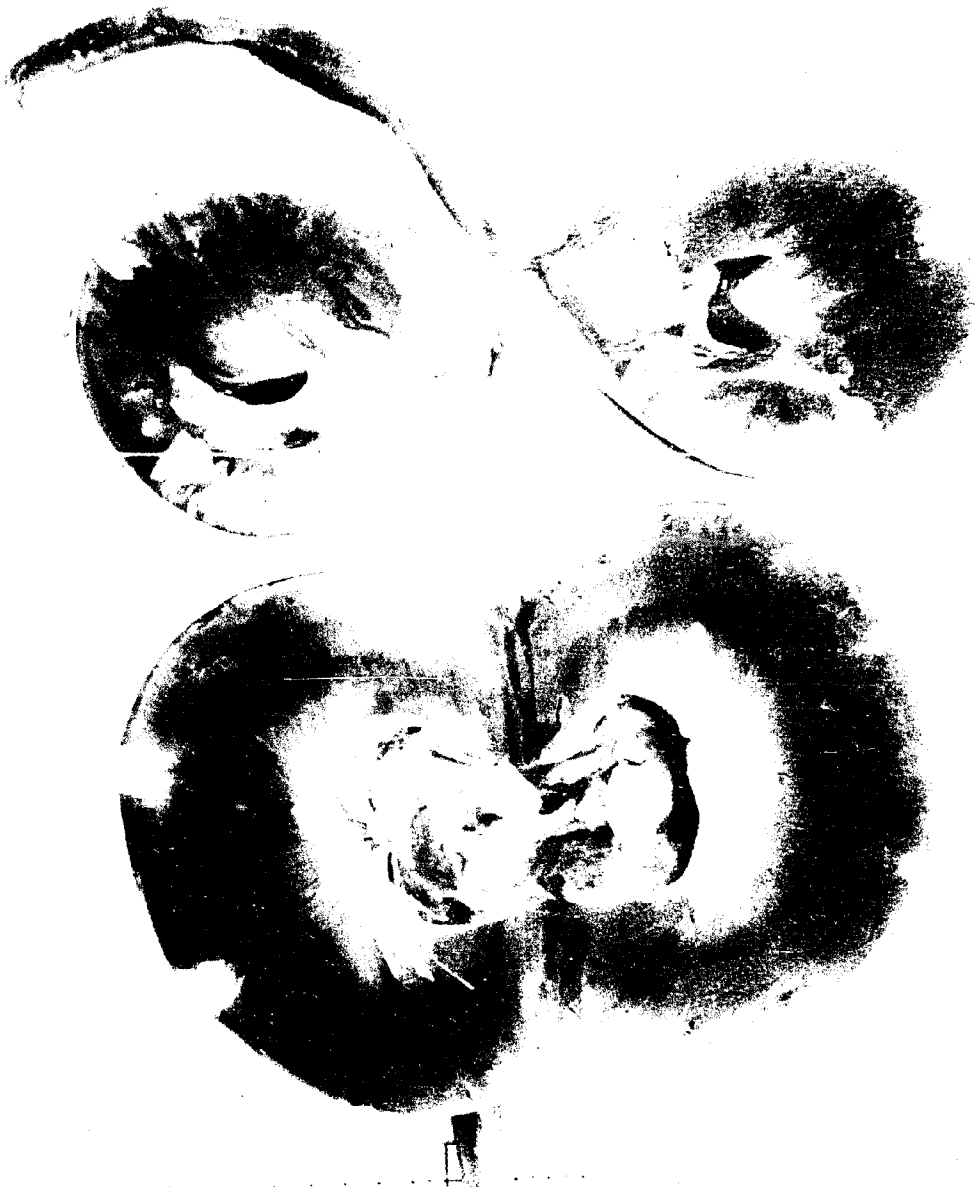


Fig. 8. Cross section of kidney from rabbit 4A which was necropsied on the second day after inoculation. No lesions are evident. The pelvic mucosa was lost from the specimen. Gram-Weigert stain. X9.

Fig. 9. Cross section of kidney from rabbit 6A which was necropsied three days after inoculation. A slight amount of papillary necrosis is evident. Figs. 16 and 23 show some of these changes in more detail. Hematoxylin and eosin stain. X9.

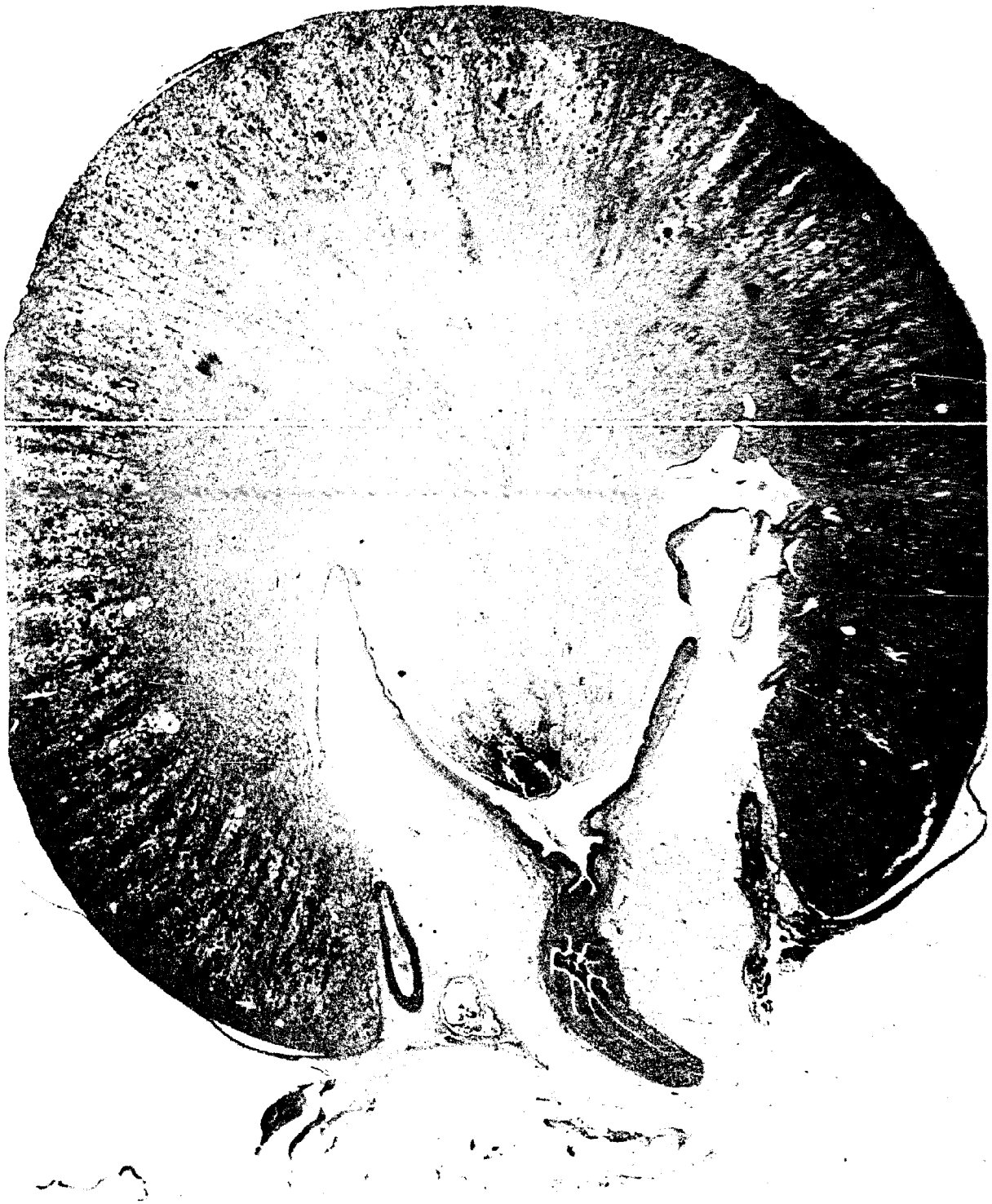


Fig. 10. Cross section of kidney from rabbit 8A which was necropsied four days after inoculation. Papillary necrosis and erosion, and the beginning of streaks (pseudoeosinophils) in the medulla are evident. Fig. 17 shows some of these changes in more detail. Gram-Weigert stain. X9.

Fig. 11. Sagittal section of kidney from rabbit 8A. Papillary necrosis and erosion, and a streak of pseudoeosinophils extending from the papilla to the cortex are evident. Fig. 18 shows some of these changes in more detail. Gram-Weigert stain. X9.

Fig. 12. Cross section of a kidney from a rabbit with a chronic form of pyelonephritis. This animal had been given a series of ten weekly inoculations with culture No. 5 (Thesis, Part II). Pyelitis and papillitis are evident. Figs. 15, 20 and 25 show some of these changes in greater detail. Hematoxylin and eosin stain. X9.

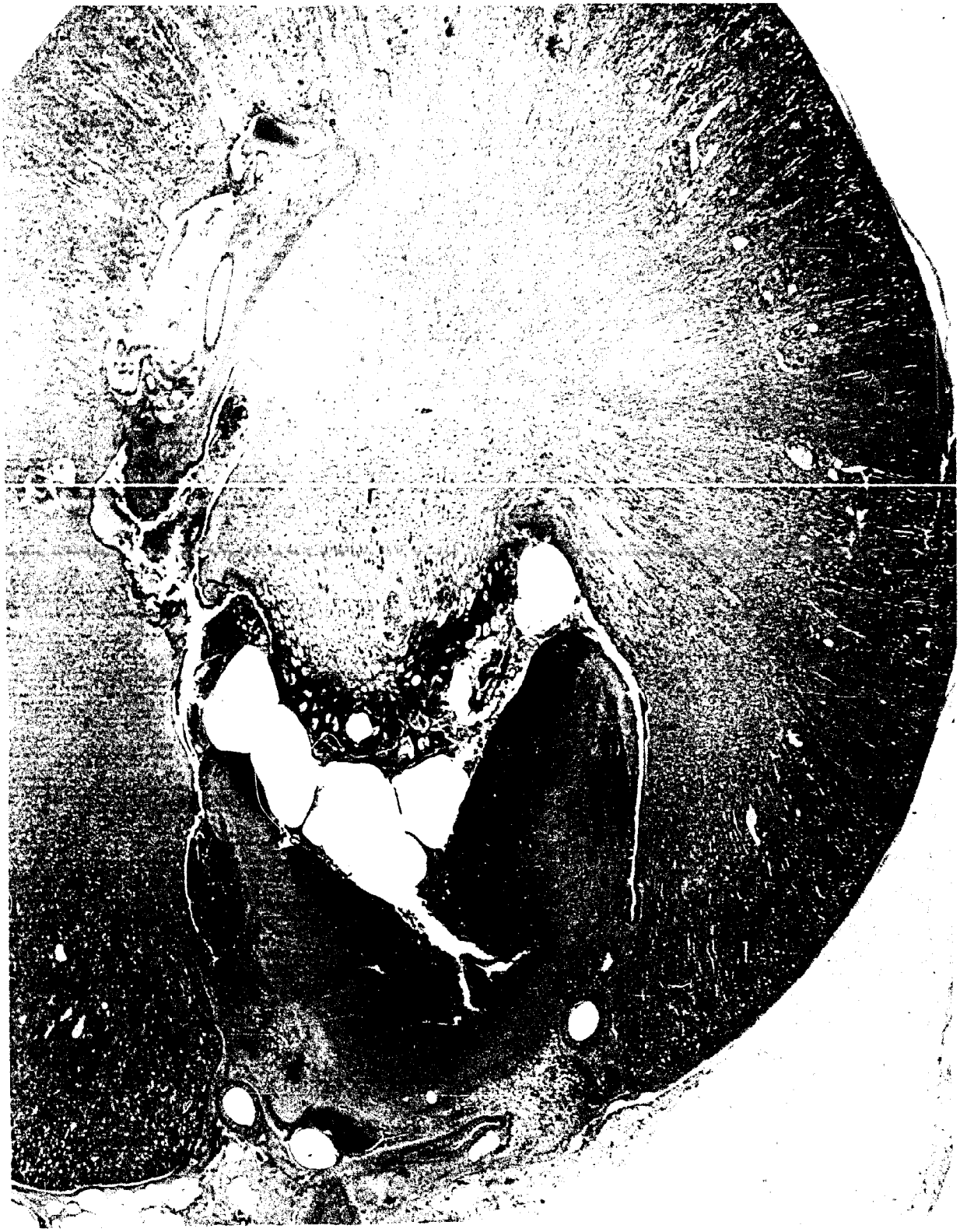


Fig. 13. Pyelitis and papillitis in the kidney of rabbit 9 which was necropsied nine days after inoculation. The papillitis seems to be regressing while the pyelitis is still very severe. Gram-Weigert stain. X45.

Fig. 14. Pyelitis and papillitis in the kidney of rabbit 10 which was necropsied eleven days after inoculation. The dark masses in the pelvis are made up of bacteria. Gram-Weigert stain. X45.

Fig. 15. Chronic form of pyelitis and papillitis. Fig. 12 includes this field and Figs. 20 and 25 show some of these changes in more detail. Hematoxylin and eosin stain. X35.

Fig. 16. Papillitis. The dark masses are made up of bacteria. Fig. 9 contains this field. Fig. 23 shows the bacteria and tubules in more detail. Gram-Weigert stain. X160.

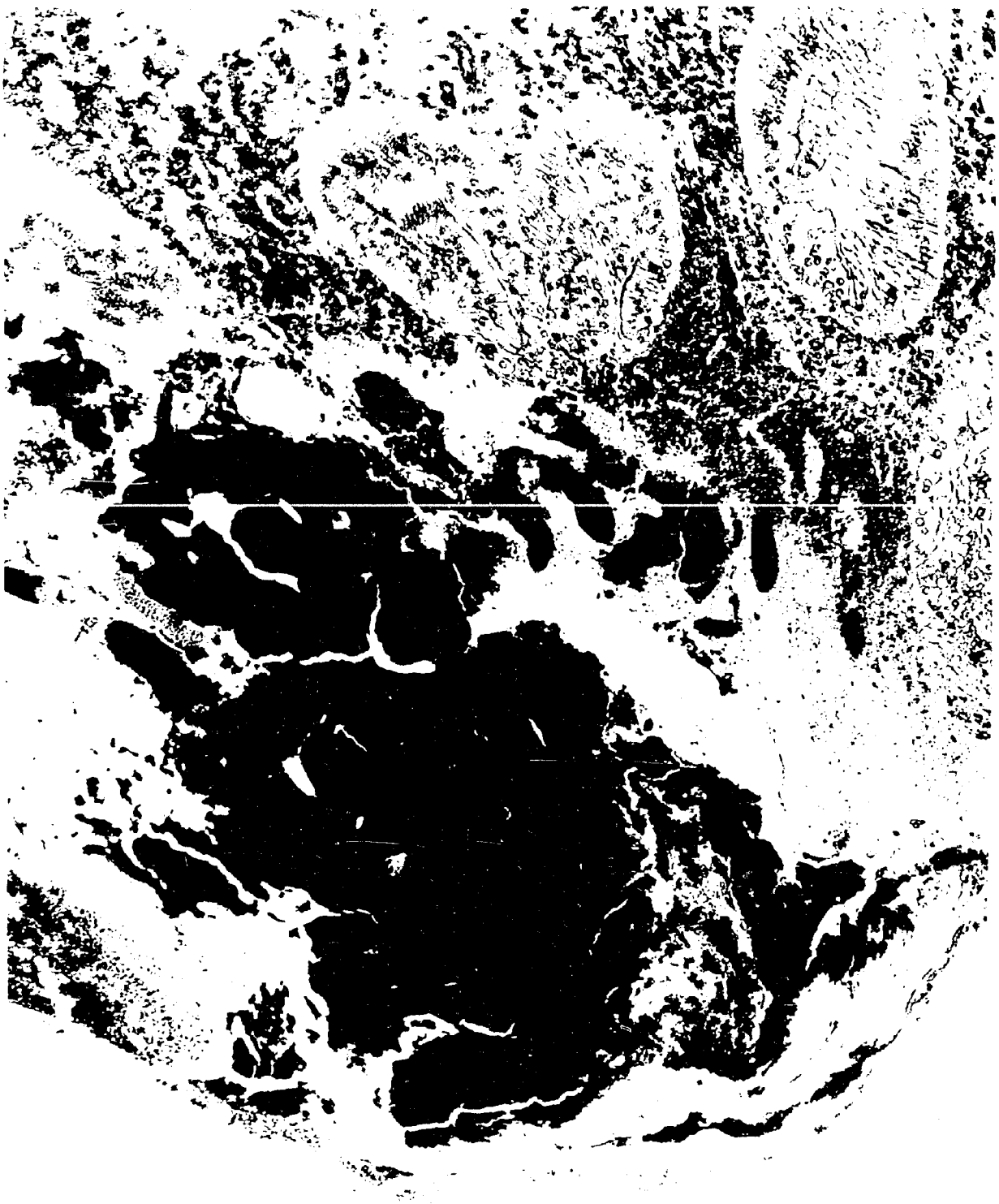


Fig. 16

Fig. 17. Papillitis. Bacteria may be seen in the lower half of this photomicrograph while many pseudo-eosinophils are present between the tubules and in the necrotic debris. Fig. 10 contains this field. Gram-Weigert stain. X160.

Fig. 18. Papillitis. Collecting tubules, necrotic tissue and bacteria may be seen. Fig. 11 contains this field. Gram-Weigert stain. X160.

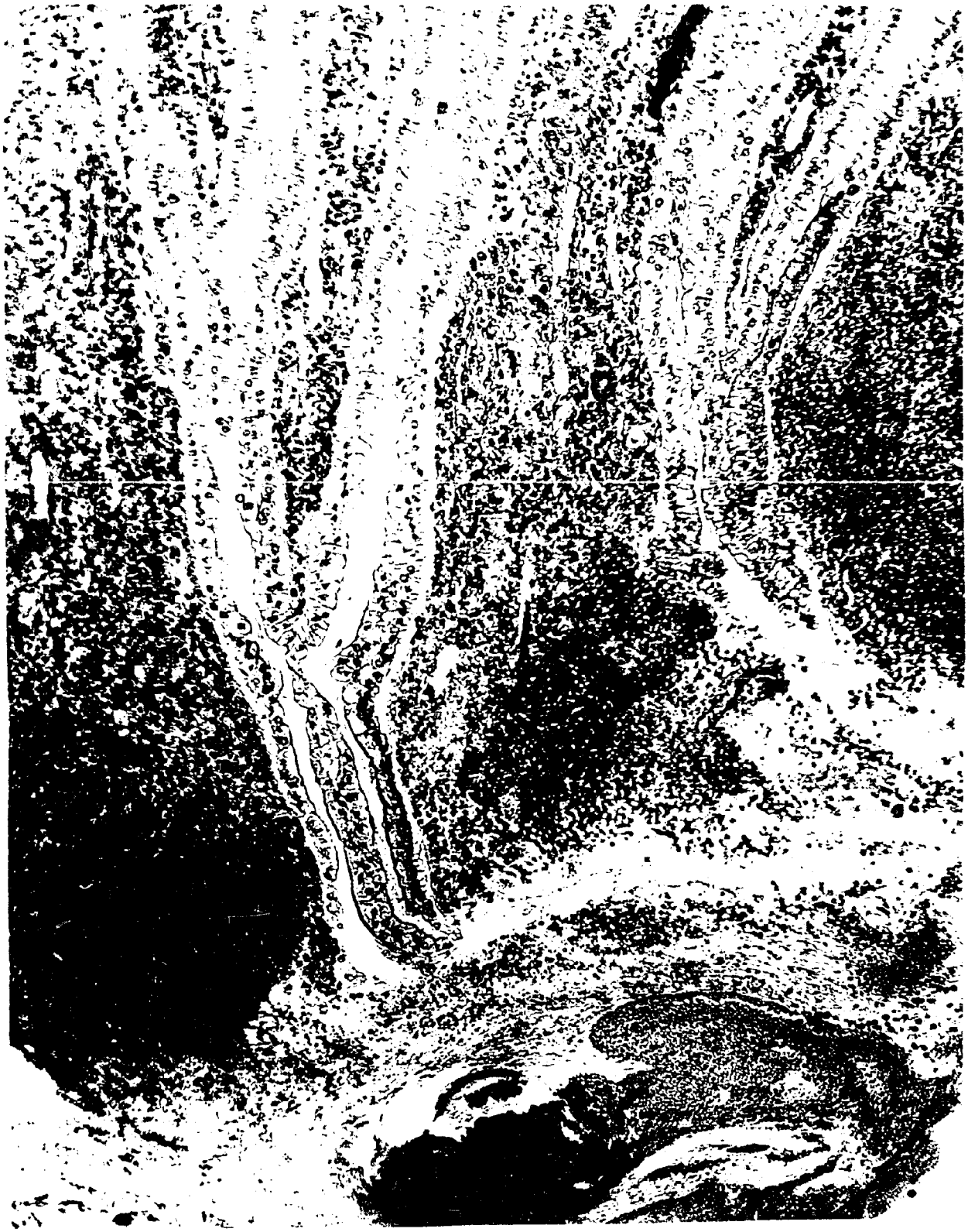


Fig. 19. Papillitis in kidney of rabbit 6 which was necropsied five days after inoculation. Masses of bacteria may be seen in the capillaries and collecting tubules. Gram-Weigert stain. X160.

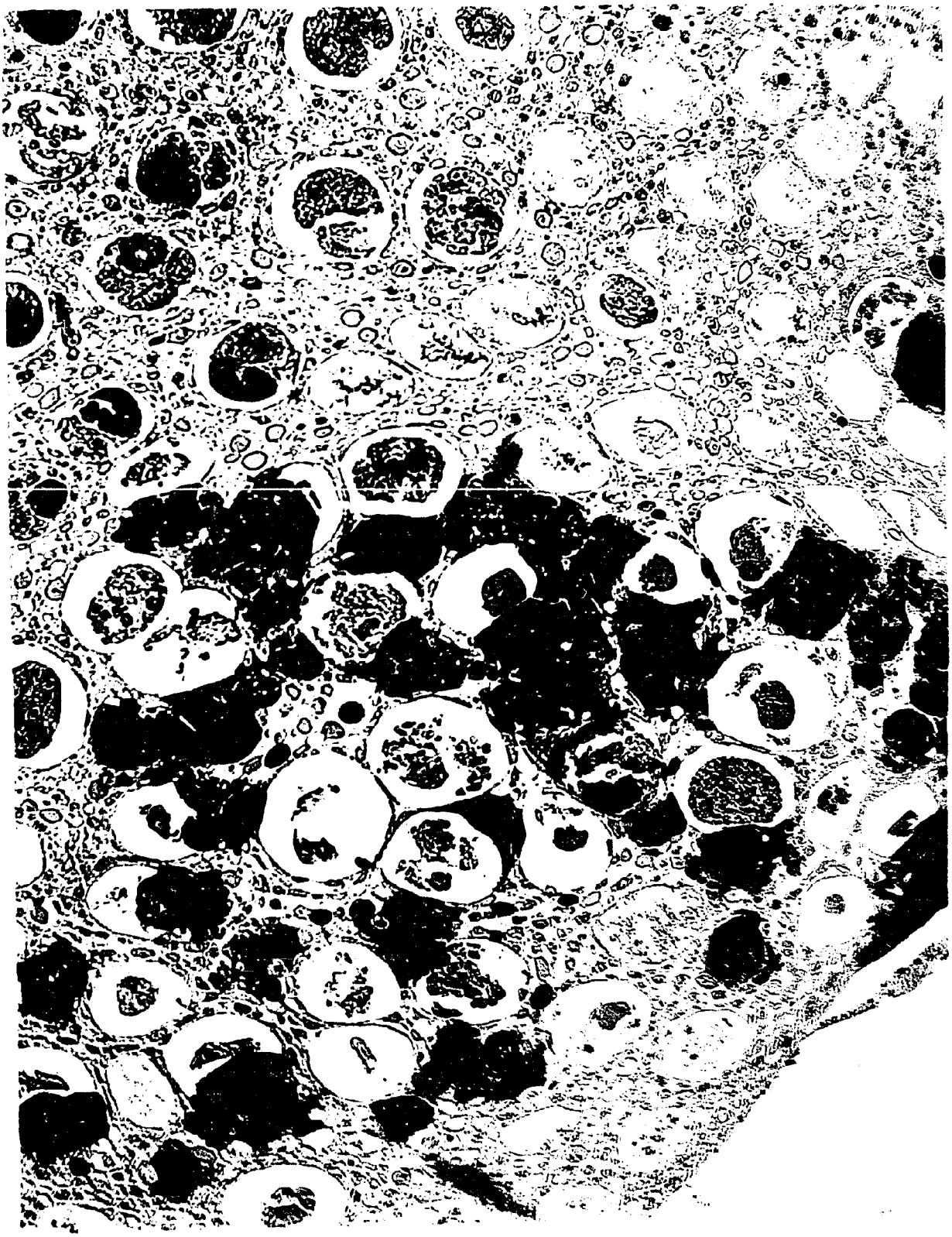


Fig. 19

Fig. 20. Papillitis, showing necrosis, accumulation of inflammatory cells and formation of crystals. Figs. 12 and 15 contain this field. Fig. 25 shows the crystals in greater detail. Hematoxylin and eosin stain. X160.

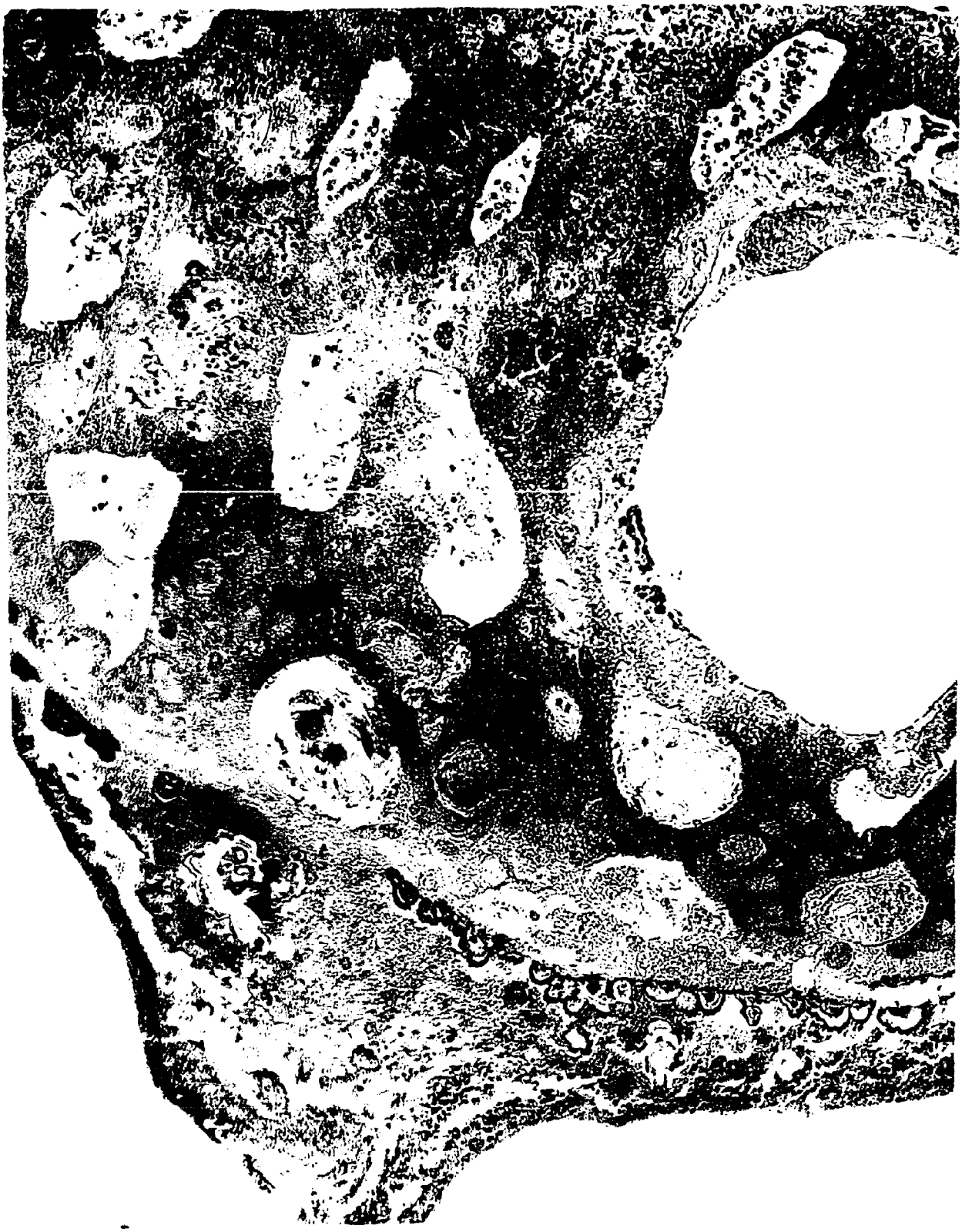


Fig. 21. Ureter from rabbit 6 which was necropsied five days after inoculation. Necrosis of the mucosa, infiltration by pseudoeosinophils, edema and fibrin are in evidence. The lumen contains bacteria, cellular debris, fibrin and pseudoeosinophils. Gram-Weigert stain. X160.

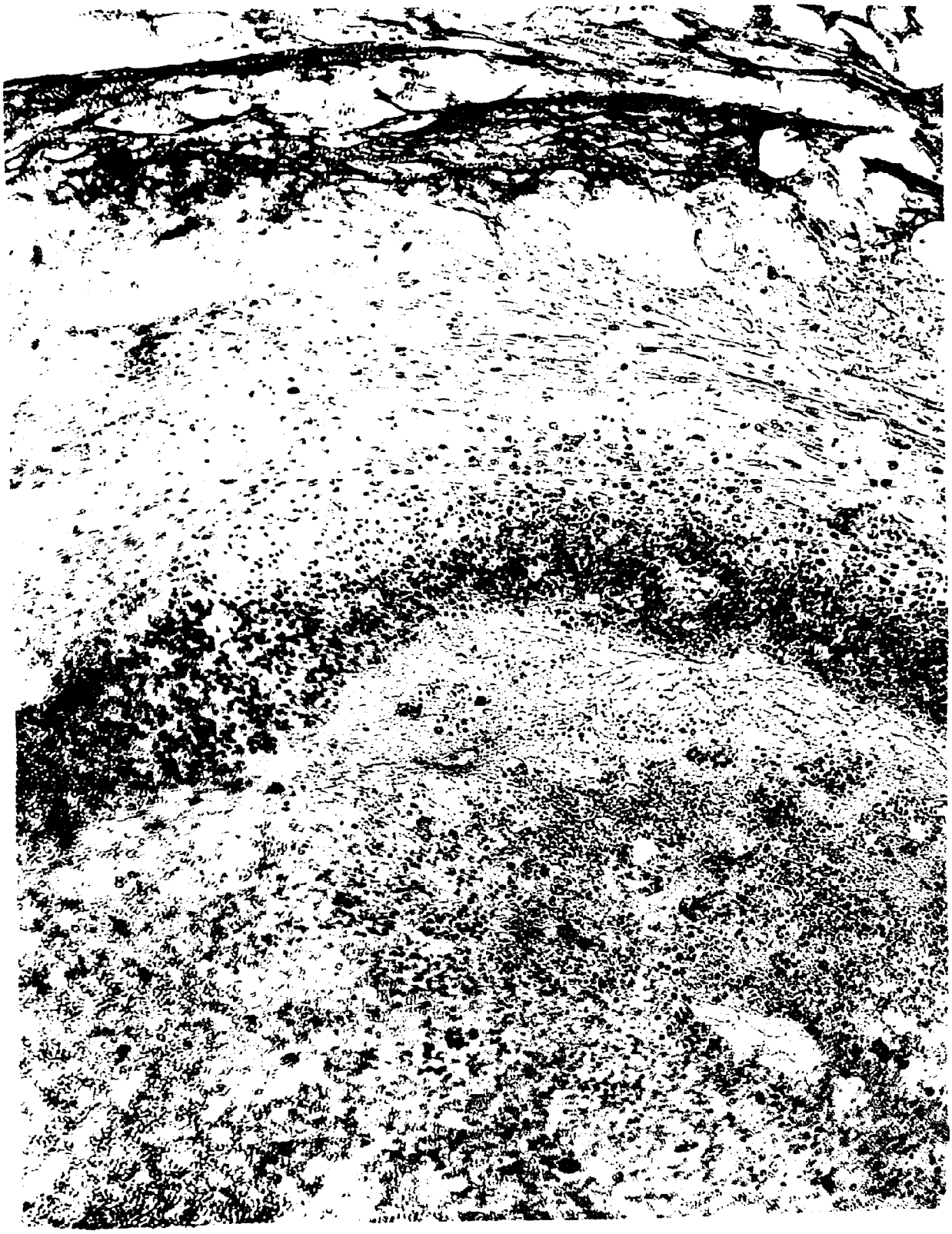


Fig. 22. Focus of pseudoeosinophils in and between the collecting tubules. Rabbit 5, which was necropsied four days after inoculation. Hematoxylin and eosin stain. X885.

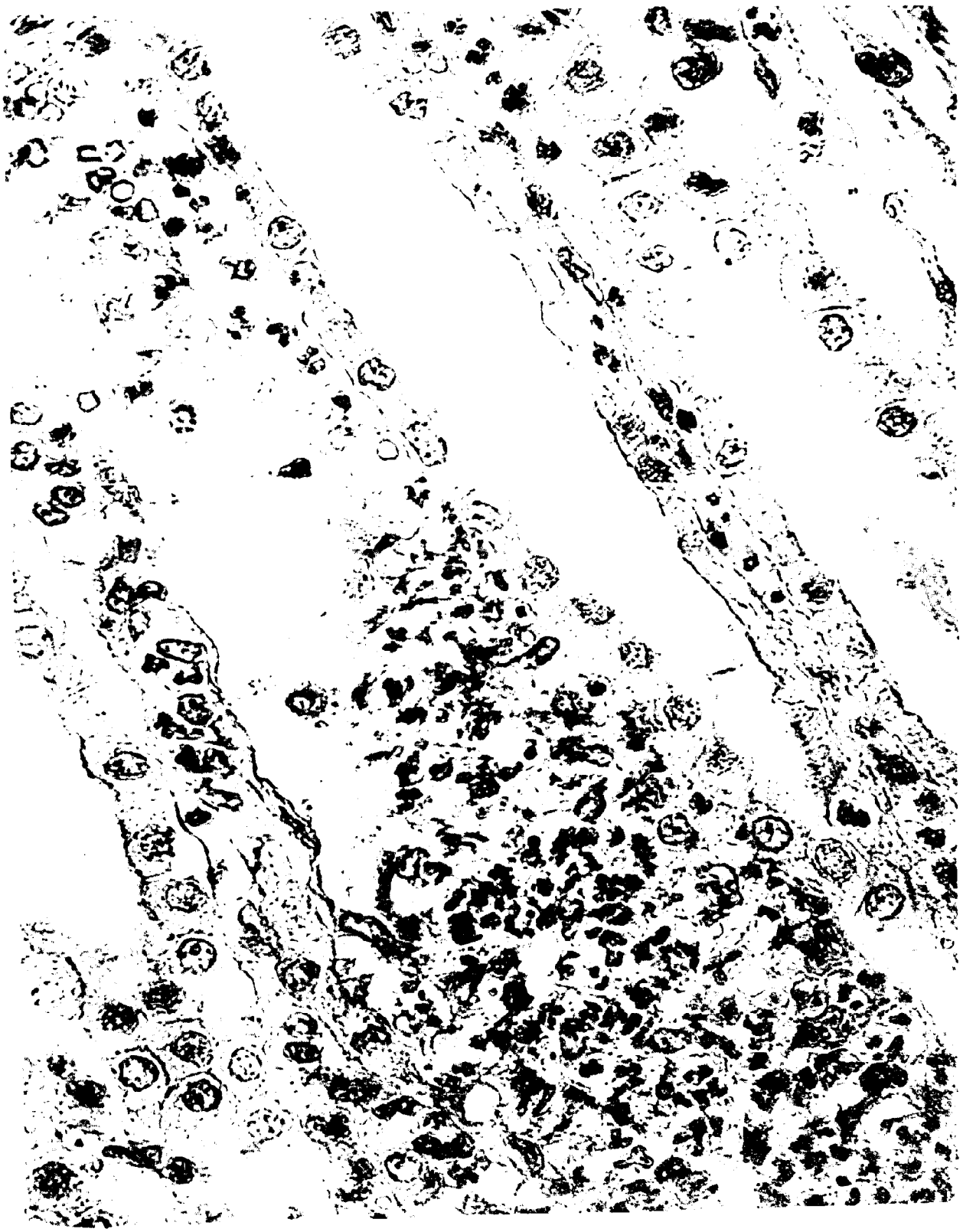


Fig. 23. Bacteria, necrotic debris, pseudoeosinophils, and collecting tubules. Figs. 9 and 16 contain this field. Gram-Weigert stain. X885.

Fig. 23

Fig. 24. Collecting tubule showing hyaline bodies in the cytoplasm of the cells. Rabbit 12 which was necropsied seventeen days after inoculation. Gram-Weigert stain. X885.

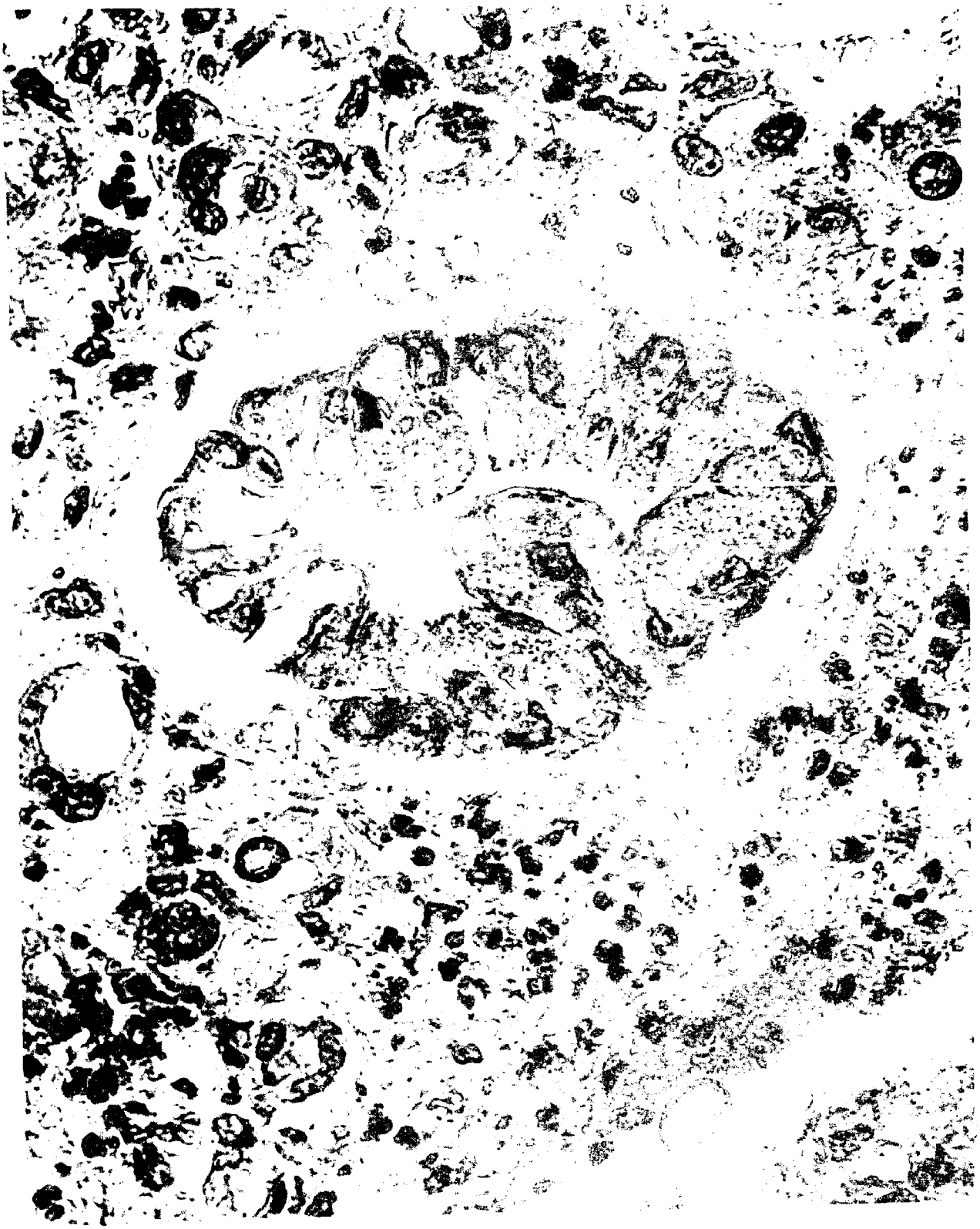
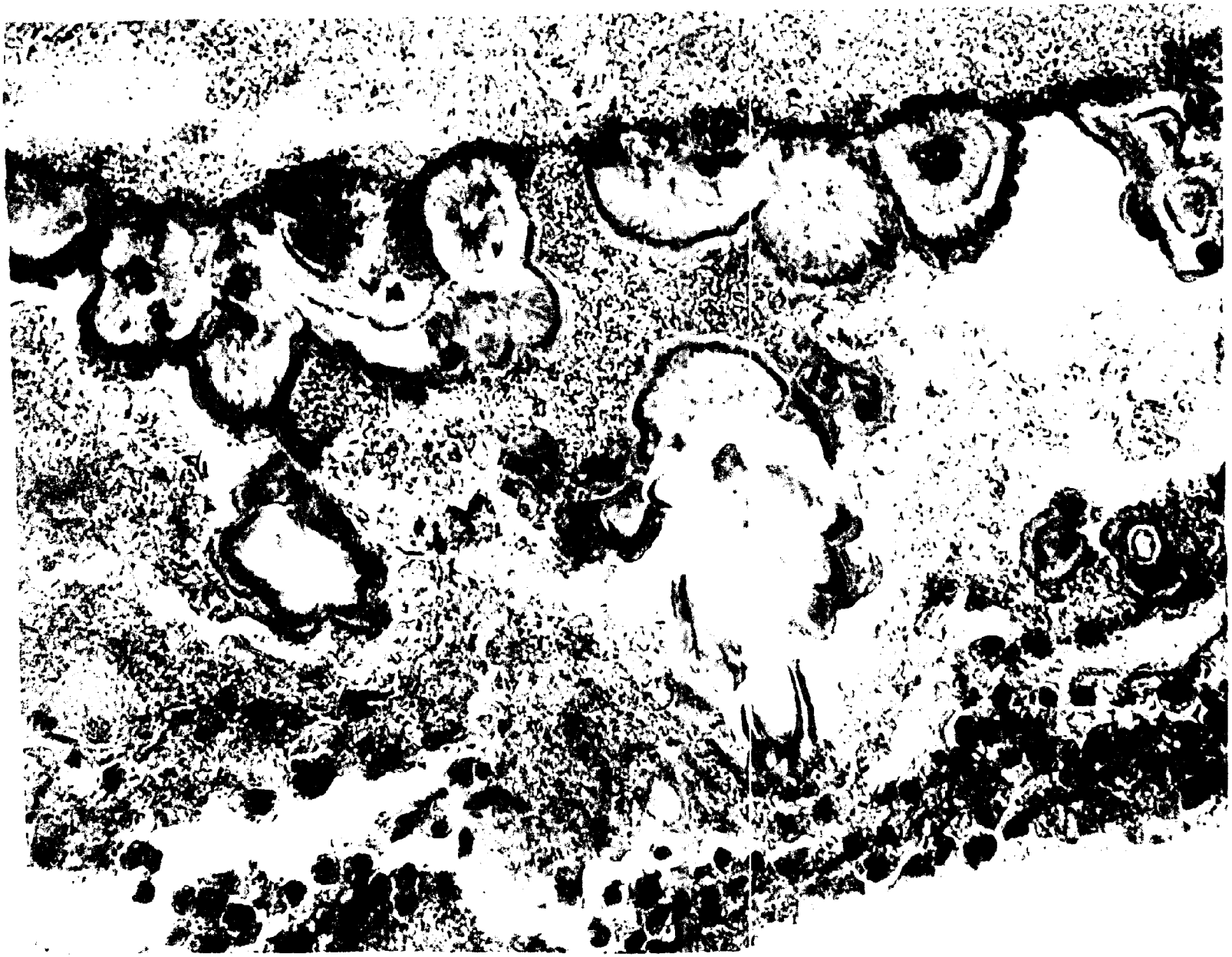


FIG.

Fig. 25. Formation of crystals in necrotic material at the edge of the renal papilla. Figs. 12, 15 and 20 contain this field. Hematoxylin and eosin stain. X885.



PART II. PROPERTIES OF SOME COLONIAL PHASE VARIANTS OF CORYNEBACTERIUM RENALE

In previous work (Feenstra, 1944) it had been noted that the characteristics of the Corynebacterium renale cultures isolated from cases of bovine pyelonephritis were, in general, identical with those described by Bergey (1939), but often produced colonies which exhibited marked dissemblance.

The tendency of diphtheroids to vary is well known (Merchant, 1935), but the colonial phase variation of C. renale has received scant attention or publicity.

In this preliminary work a survey of the colonial properties of the stock cultures of C. renale was made. Inoculum was streaked out on thick tryptose agar (Difco) plates so that separate colonies could develop to maturity (2-3 cm apart). Colonies were examined as to texture by probing and morphologically under a dissecting microscope by means of reflected light and also by oblique transmitted light which was directed toward the colonies from below at approximately a 45 degree angle by means of a mirror and a microscope lamp (Huddleson, 1946).

Five cultures were chosen as representing at least three different colony types for further study to determine the biochemical, serological and pathogenic properties and relationships. The colonies were roughly classified into groups by means of the following key:

1. Smooth, soft pasty and no marked tendency to hold shape when probed.

    a. Circular, entire, convex, creamy white with ground glass appearance.

    b. Irregular.

    c. Yellow to orange color.

    d. Tend to produce flat secondary growth upon aging.

2. Only slightly pasty, marked tendency to hold shape and fragment when probed.

a. Circular, entire, convex, creamy white with ground glass appearance.

b. Irregular.

c. Yellow to orange color.

d. Tend to produce flat secondary growth upon aging.

The five cultures chosen for preliminary study were classified as follows:

Culture No. 1	1.a.
" " 2	2.b.
" " 3	2.b.
" " 4	2.a.d.
" " 5	2.a.d.

Agglutination Relationship

Agglutination tests were carried out as suggested in the Manual of Methods for Pure Culture Study of Bacteria (1945) with the exception that distilled water rather than 0.85 percent NaCl solution was used as the diluent and tests were incubated at 37°C. for 24 hours. Antigens were prepared by growing the organisms in two percent tryptose (Difco), in distilled water, at a pH of 7.5 for 24 hours at 37°C.; centrifuging; washing once in 0.5 percent phenol solution and three times in distilled water; and allowing to remain in distilled water in the refrigerator until used. A stable suspension was obtained by suspending the organisms in distilled water, allowing one hour for sedimentation of clumps and removing a portion of the supernatant for use. These suspensions were standardized by means of a Cenco-Sheard-Sanford photelometer to approximately the density of tube 0.5 of McFarland's nephelometer.

Antisera were produced in rabbits by six to ten intravenous injections of a heavy distilled water suspension of living organisms at approximately weekly intervals. Results of two trials are given in Table I.

Table I

Antigens		Antisera				
		1	2	3	4	5
1	1st	1-1280*	640	640	80	5120
	2nd	1280	640	640	80	1280
2		160	320	1280	320	80
		320	640	320	40	320
3		160	640	1280	160	320
		320	640	1280	160	320
4		80	80	160	320	160
		40	160	40	80	40
5		320	320	320	160	320
		640	640	320	320	640

* Indicates highest dilution at which complete agglutination took place.

Susceptibility to the Bactericidal Action of Bovine Plasma

Varying numbers of organisms were exposed to a constant amount of bovine plasma in an attempt to measure any possible difference in antigenic properties or any difference in virulence. The tests were set up as indicated in the protocol using 13 x 125 mm tubes and were incubated in a water bath at 37°C. The plasma (.2 percent sodium citrate) used was from an infected cow which had been exposed intraurethrally 14 months previously with a culture suspension of C. renale. The antigens were standardized by means of a Cenco-Sheard-Sanford photelometer to approximate tube 1.5 of McFarland's nephelometer. Subcultures of each tube were made at intervals on tryptose agar plates by streaking a loopful of inoculum on an area approximately 2 x 3 cm.

The results were not conclusive. Repeated tests showed that

culture No. 1 was most susceptible and that culture No. 3 was most resistant to the bactericidal action of bovine plasma. The other three cultures gave somewhat variable results ranging between the two cultures first mentioned. The results of a typical trial are given in Table II.

Protocol of Bactericidal Test

Tube	1	2	3	4	5	6	7	8
Distilled H_2O	1 (1)	1	1	1	1	1	1	1
Culture Susp.	0.5	1(2)	0.5	0.5	0.5	0.5	0.5	0.5(2)
Plasma	0 (3)	1	1	1	1	1	1	1
Dilution of Culture	1-4	1-4	1-12	1-36	108	324	972	2916
Dilution of Plasma	0	1-2	1-2	1-2	1-2	1-2	1-2	1-2

(1) All quantities given are in ml.

(2) Discard 0.5 ml.

(3) 0.5 ml broth added to the control tube.

Table II

Results of a Typical Trial

Culture	Time[1]	1	2	3	4	5	6	7	8
1	0	++++[2]	++++	++++	++++	++++	+++	++	45[3]
	24	++++	++++	+++	+++	+	++	+	47
	48	++++	++++	5	2	5	1	0	0
	72	++++	+	11	3	1	0	0	2
2	0	++++	++++	++++	++++	++++	++++	++	++
	24	++++	++++	++++	++++	++++	++++	+++	+++
	48	++++	++++	++++	++++	++++	++++	++	++
	72	++++	13	15	12	13	11	2	+++
3	0	++++	++++	++++	++++	++++	+++	++	+
	24	++++	++++	++++	++++	++++	++++	+++	+++
	48	++++	++++	++++	++++	++++	+++	+++	++
	72	++++	+	+++	+++	++	4	18	2
4	0	++++	++++	++++	++++	++++	++++	+++	++
	24	++++	++++	++++	++++	++++	++++	+++	++
	48	++++	++	++	20	14	30	2	5
	72	++++	1	12	7	2	4	0	2
5	0	++++	++++	++++	++++	+++	++	++	+
	24	++++	++++	++++	++++	++++	+++	++	+
	48	++++	+++	+++	+++	+	++	4	4
	72	++++	++	+++	9	5	5	3	4

(1) Time in hours.

(2) Indicates amount of growth; (+) indicating approximately 50 colonies.

(3) Number of colonies present.

Biochemical Properties

Glucose was fermented with resulting acid production by all five cultures, but the action of culture No. 1 was somewhat weaker than that of the other four cultures.

A similar situation resulted in the study of casein digestion in ten percent skim milk agar (tryptose); culture No. 1 lagged behind the others in halo production.

Pathogenicity for Rabbits

In order to obtain data concerning the differential virulence of the five cultures under study, approximately four billion organisms (distilled water suspension) of each culture grown on tryptose agar for 24 hours at 37° C. were injected intravenously, each into two rabbits. Determination of the body weights, hemoglobin levels (Cenco-Sheard-Sanford photelometer, Hoffman method, 1941), numbers of leukocytes and differential leukocyte counts were made at intervals. The rabbits which did not die were sacrificed at nine and ten days after inoculation, necropsied and the lesions recorded. The lung, liver, spleen, heart blood, bone marrow, left kidney and urinary bladder were cultured on tryptose agar. Tissues for microscopic examination (lung, liver, spleen, kidney, urinary bladder and ureters) were fixed in Zenker's fluid and paraffin sections were prepared and stained with hematoxylin and eosin and by the Gram-Weigert method for staining bacteria in sections.

All the rabbits had gross or microscopic lesions of coccidiosis in the liver. This may also account for some of the higher than average initial leukocyte counts obtained. Other lesions will be recorded separately. Occasionally the lesions reported in the gross examination were missed in sectioning the tissue, but they were present in a sufficient number of cases to allow adequate description. Reports on individual rabbits follow.

Cases 1 and 2

Rabbits 1a and 1b were inoculated intravenously with culture No. 1. None of the organisms were recovered by culture technic and no gross or microscopic lesions were found. What appeared to be a clump of

diphtheroids was seen in the pelvis of the right kidney of rabbit 1b.

Rabbit 1a

Time(1)	Hb(2)	WBC(3)	M(4)	L	P	B	E	Wt(5)
0	12.4	13,600	3	60	35	1	1	2024
1	11.0	11,150	3	46	44	6	1	-
3	11.0	11,450	2	49	37	9	3	2010
5	11.5	12,350	2	52	38	6	2	2022
7	11.5	15,450	2	39	51	7	1	2002
9	11.2	11,850	3	62	29	6	0	2030

Rabbit 1b

0	11.6	12,750	2	62	31	4	1	1763
1	11.1	5,850	1	46	42	9	2	-
3	10.4	13,850	4	53	33	7	3	1914
5	10.8	10,900	2	52	38	6	2	1320
7	11.9	16,350	4	43	45	7	1	1816
9	11.3	12,050	3	55	35	6	1	1848

(1) Time in days after inoculation; 0 indicating the bleeding just prior to inoculation.

(2) Hemoglobin in grams per 100 ml of blood.

(3) Leukocytes per cubic mm.

(4) Percent of monocytes, lymphocytes, pseudoeosinophils, basophils and eosinophils.

(5) Body weight in grams.

Case 3

Rabbit 2a was inoculated with culture No. 2. Organisms were cultured from the pelvis of the kidney and from the urine. The lungs

were slightly congested. The kidneys appeared normal except for the presence of a yellow concretion in the pelvis of the left kidney and a slight papillary necrosis which was also noticed in the microscopic examination.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	12.1	9,700	0	63	34	1	2	2050
1	12.1	7,950	2	45	43	8	2	-
3	12.1	12,400	4	52	42	1	1	1865
5	12.9	26,800	3	46	30	17	4	1828
7	12.7	11,100	3	49	45	1	2	1824
9	12.3	18,000	0	53	39	7	1	1846

Case 4

Rabbit 2b was inoculated with culture No. 2 and died on the seventh day. Organisms were cultured from the kidney pelvis and cortex and from the urine. Both kidneys were enlarged and exhibited congestion, cortical abscesses, papillary erosion, and purulent bloody mucous material in the pelves, ureters and bladder. The ureters were enlarged and congested. Upon microscopic examination the liver and spleen appeared congested. The kidneys showed edema, congestion, hemorrhage, degeneration of the convoluted tubules, abscesses in the cortices and at the tips of the papillae, and bacterial masses in the collecting tubules at the tips of the papillae. The pelvis was filled with blood, tissue debris and diphtheroids. The pelvic epithelium was necrotic and the peripelvic tissues were infiltrated with fibrin, erythrocytes and serous fluid.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	14.5	8,550	2	75	19	3	1	2128
1	13.3	3,700	1	43	56	0	0	-
3	13.7	7,950	7	49	42	2	0	1983
5	14.8	28,550	16	39	33	11	1	1810

Case 5

Rabbit 3a was inoculated with culture No. 3. Organisms were cultured from the kidney pelvis and from the bladder. The kidneys were swollen and pale and exhibited whitish streaks in the medulla radiating toward the cortex, papillary necrosis, and yellow necrotic foci in the pelvic mucosa. The ureters were slightly enlarged. Microscopically, the kidneys showed degeneration of the convoluted tubules, papillary erosion characterized by necrosis, congestion, hemorrhage, pseudoeosinophils, and diphtheroids in the necrotic debris and in several collecting tubules. The pelvic mucosa was necrotic and the peripelvic tissue was infiltrated with serous fluid, fibrin and pseudoeosinophils.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	13.3	12,650	1	79	12	2	6	2320
1	12.1	5,350	0	30	60	10	0	-
3	10.4	9,350	9	64	21	5	1	2382
5	10.4	9,650	7	68	22	2	1	2288
7	13.2	9,000	3	32	62	1	2	2254
9	11.8	10,450	3	66	30	1	0	2282

Case 6

Rabbit 3b was inoculated with culture No. 3. Organisms were cultured from the kidney pelvis and from the bladder. No gross or microscopic alterations were apparent but diphtheroids were seen in the

bladder, ureter and kidney. These organisms were very likely different from the ones injected as judged from their cultural characteristics.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	11.7	8,200	3	70	24	1	2	2240
1	11.4	7,250	2	47	50	0	1	-
3	11.1	11,050	6	52	31	8	3	2384
5	11.8	8,300	3	63	32	1	1	2300
7	12.1	8,200	5	65	26	3	1	2410
9	12.3	9,300	6	59	31	3	1	2540

Case 7

Rabbit 4a was inoculated with culture No. 4. Organisms were cultured from the kidney pelvis. The kidneys were normal in size and color but the left kidney exhibited papillary necrosis and abscess formation and the right kidney exhibited very slight papillary necrosis. Sections did not show the tips of the renal papillae, but the pelvic epithelium showed slight degenerative changes and the peripelvic tissues contained some fibrin. There was necrotic tissue debris in the pelvis.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	14.1	12,100	1	83	10	3	3	2266
1	11.8	3,550	0	37	61	1	1	-
3	11.4	22,300	6	70	23	0	1	2111
5	12.5	11,650	5	74	15	5	1	2056
7	12.9	16,450	3	61	22	14	0	2030
9	13.2	10,700	2	60	30	8	0	2176

Case 8

Rabbit 4b was inoculated with culture No. 4. No organisms were

cultured from this rabbit. The right kidney exhibited a patchy congestion of the cortex, a small white focus in the medulla and congestion of the papilla. In stained sections the right kidney showed a small fibrotic nodule in the inner medulla and the epithelium of the collecting tubules in the medulla of the left kidney appeared slightly degenerated.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	12.1	9,850	0	60	36	2	2	2400
1	12.5	6,500	2	30	66	1	1	-
3	13.1	8,000	6	56	32	3	3	2396
5	13.5	4,750	1	51	41	6	1	2308
7	13.2	8,250	1	55	36	7	1	2312
9	13.7	10,100	2	45	48	4	1	2440

Case 9

Rabbit 5a was inoculated with culture No. 5. No organisms were cultured from this rabbit. The only lesions found were in the stained sections. There was a slight increase of pseudoeosinophils in the lung, and a slight increase of phagocytosis of blood pigment in the spleen.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	11.8	8,950	2	65	30	2	1	2152
1	11.8	7,800	0	59	40	1	0	-
3	11.8	10,700	5	53	39	2	1	2162
5	12.3	12,750	4	62	28	5	1	2120
7	14.6	16,800	4	59	34	1	2	2182
9	12.4	9,250	5	48	44	3	0	2238

Case 10

Rabbit 5b was inoculated with culture No. 5. No organisms were

cultured from this rabbit. No gross or microscopic lesions were apparent.

Time	Hb	WBC	M	L	P	B	E	Wt.
0	11.4	9,250	1	76	18	3	2	2300
1	11.1	5,400	0	44	50	5	1	-
3	11.6	13,750	2	48	45	5	0	2280
5	12.5	9,800	3	56	31	8	2	2282
7	12.9	11,050	3	49	42	5	1	2335
9	12.4	10,450	1	54	41	2	?	2444

Table III

Summary of Data

Culture	Rabbit	Kidney Lesions (1)	Culture (2)	Max. Wt. Loss (3)	Wt. at end Comp. to Begin (4)	Max.% inc. in WBC (5)
1	1a	-	-	22	+6	13
1	1b	-	-	None	+95	28
2	2a	+	+	225	-204	156
2	2b	+	+	400	-400	234
3	3a	+	+	66	-38	drop
3	3b	-	∓	None	+300	35
4	4a	+	+	233	-90	84
4	4b	∓	-	92	+40	3
5	5a	-	-	32	+86	65
5	5b	-	-	20	+144	49

(1) Microscopic or gross lesions in the kidneys.

(2) Recovery of the original organism by culture at necropsy.

(3) Lowest weight recorded during experiment compared to weight at the beginning (Grams).

(4) Weight at the end of the experiment compared to the weight at beginning (Grams).

(5) The maximum percent increase in the leukocyte count.

Discussion

The results of this preliminary work indicate that there were marked differences in the morphology and properties of the colonies chosen for study from stock cultures which were originally isolated from cattle having pyelonephritis. That all these variations were present in the cultures as originally isolated is very doubtful and could not be maintained.

As may be noted by examination of the photographs and the classification of the colonies, the greatest morphological differences were between culture No. 1 and the other four cultures. The morphology of cultures No. 2 and No. 3 is somewhat similar and also that of cultures No. 4 and No. 5.

The serological reactions of Corynebacterium renale are at best none too satisfactory. The results of the agglutination tests show that there are antigenic differences and similarities between the different cultures but there is little or no correlation with the morphological characteristics of the colonies.

The results of the bactericidal reactions show a fairly close correlation with the morphological characteristics. Culture No. 1 was the most susceptible, cultures No. 2 and No. 3 were the least susceptible and the reactions of cultures No. 4 and No. 5 were intermediate. The plasma used in this case very likely contained some specific antibodies because this animal had been exposed, intraurethrally, 14 months before and was still passing organisms in the urine. Other tests, not recorded here, showed that the plasma from a cow not passing organisms was less potent in action.

The pathogenicity test, using rabbits, was of course woefully inadequate because so few animals were used to test each culture. The

results, as summarized in Table III, would indicate marked differences in pathogenicity of the cultures. The bacteriology, pathology, and hematology of the infected rabbits will not be discussed here because these subjects were covered more thoroughly in the first part of this thesis (Part I). The weights of these rabbits were recorded at intervals after noticing in previous work that infected rabbits lost weight during the course of the infection. In general, the rabbits lost weight after inoculation and the infected ones continued to lose weight whereas the ones which did not become infected gained weight after the initial slight loss.

It is apparent that the cultures which would likely be classed as "rough", morphologically, were more pathogenic for rabbits and less susceptible to the bactericidal action of bovine plasma and the culture that would most likely be classed as "smooth" was markedly less pathogenic for rabbits and more susceptible to the bactericidal action of bovine plasma.

In the past, much work has been done on the relative virulence of bacterial variants. It is not considered desirable at this time to review the literature on this subject. Sample references to such work would include DeKruif (1921), Huddleson et al (1945), and Irwin and Beach (1946).

Summary

A brief preliminary study and discussion of the morphology, agglutination relationships, reaction to the bactericidal action of bovine plasma, and the pathogenicity of some of the colony types found in stock cultures of Corynebacterium renale have been presented.

At present, it may be concluded that the "rough" type of culture is more pathogenic for rabbits and more resistant to the bactericidal action of bovine plasma than the smooth type.

References

Bergey, D. H., et al. 1939 Manual of determinative bacteriology. 5th ed. Williams and Wilkins Co., Baltimore, Md.

DeKruif, P. H. 1921 Dissociation of microbic species. I. Coexistence of individuals of different degrees of virulence in cultures of the bacillus of rabbit septicemia. J. Exp. Med., 33, 773-789.

Feenstra, E. S. 1944 Studies on the etiology and pathology of infectious bovine pyelonephritis. Thesis. School of Graduate Studies. Michigan State College.

Hoffman, W. S. 1941 Photelometric clinical chemistry. William Morrow and Co., N.Y.

Huddleson, I. F., Wood, E. E., Cressman, A. R. and Bennett, G. R. 1945 The bactericidal action of bovine blood for Brucella and its possible significance. J. Bact., 50, 261-277.

Huddleson, I. F. 1946 The mucoid phases of the genus Brucella. Am. J. Vet. Res., 7, 5-10.

Irwin, M. R. and Beach, B. A. 1946 Differential bactericidal activity of bovine serum toward strains of Brucella abortus of high and low virulence. J. Agr. Res., 72, 89-91.

Manual of methods for pure culture study of bacteria. 1939 Biotech Publications, Geneva, N.Y. (Current revisions used).

Merchant, I. A. 1935 Corynebacteria associated with diseases of domestic animals. J. Bact., 30, 95-118.

Fig. 26. Colony of culture No. 1. Two weeks growth on tryptose agar at room temperature after the first day at 37°C. X10.
(The colonies depicted in Figs. 27, 28, 29 and 30 were grown under similar conditions).

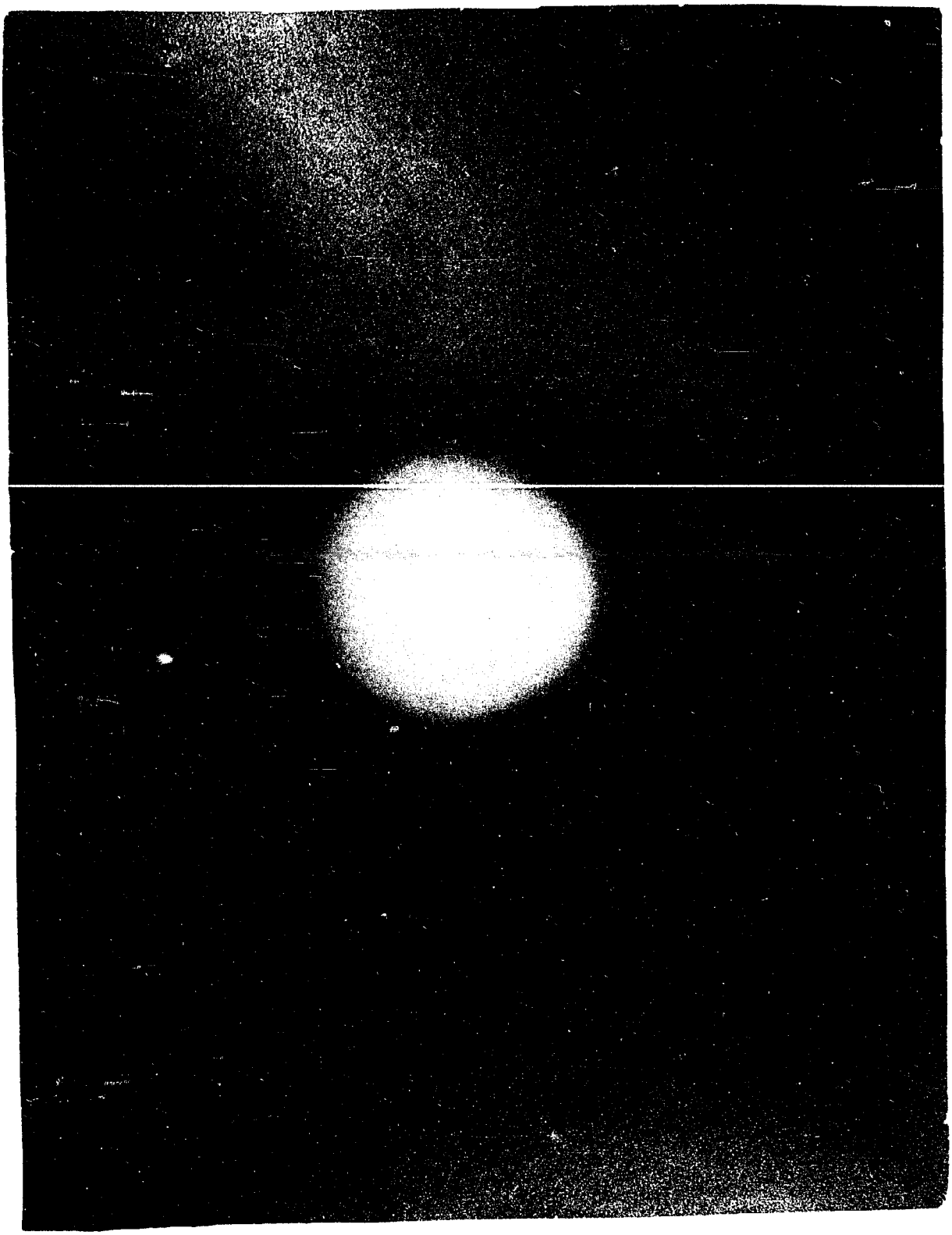


Fig. 27. Colony of culture No. 2. X10.

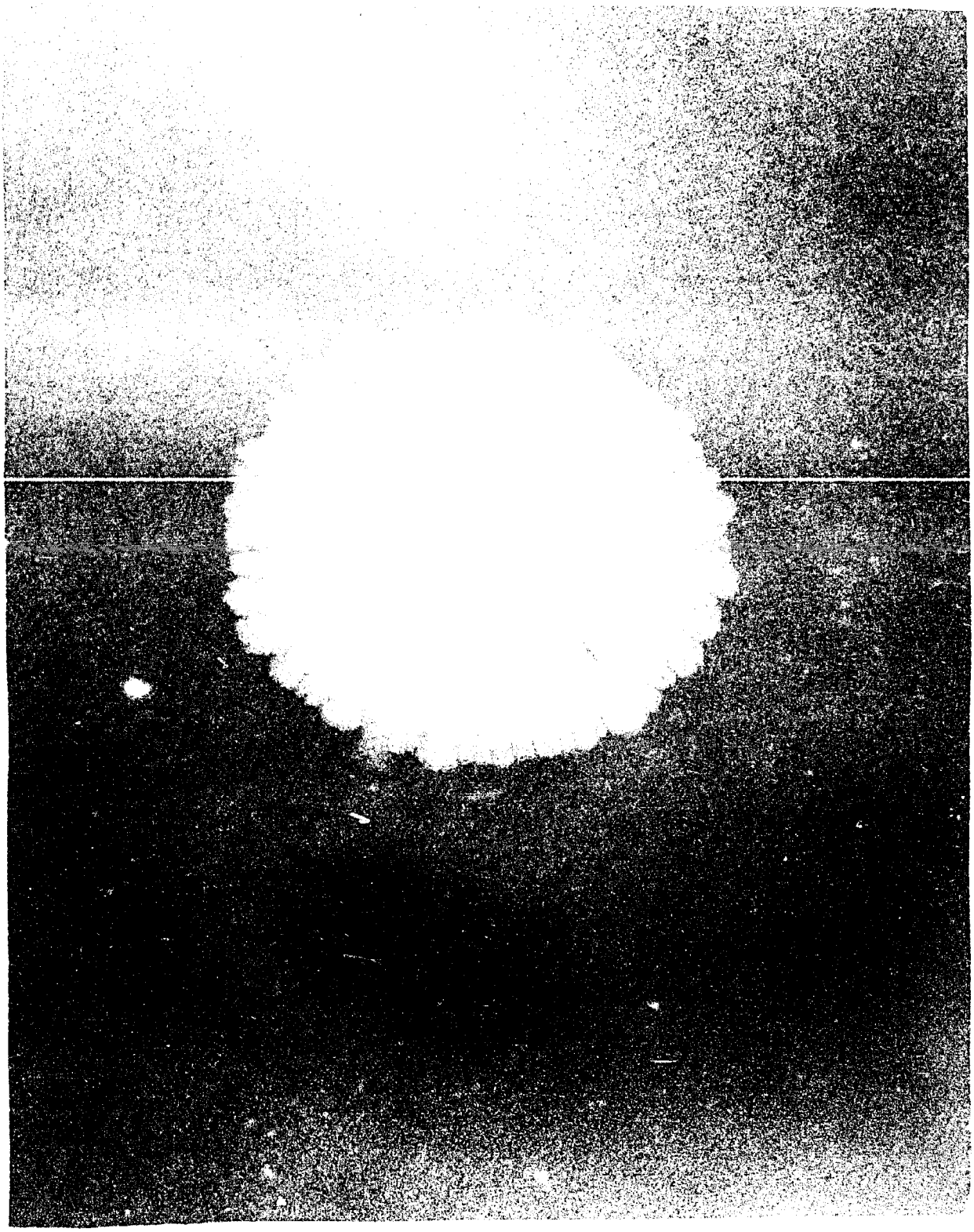


Fig. 28. Colony of culture No. 3. X10.

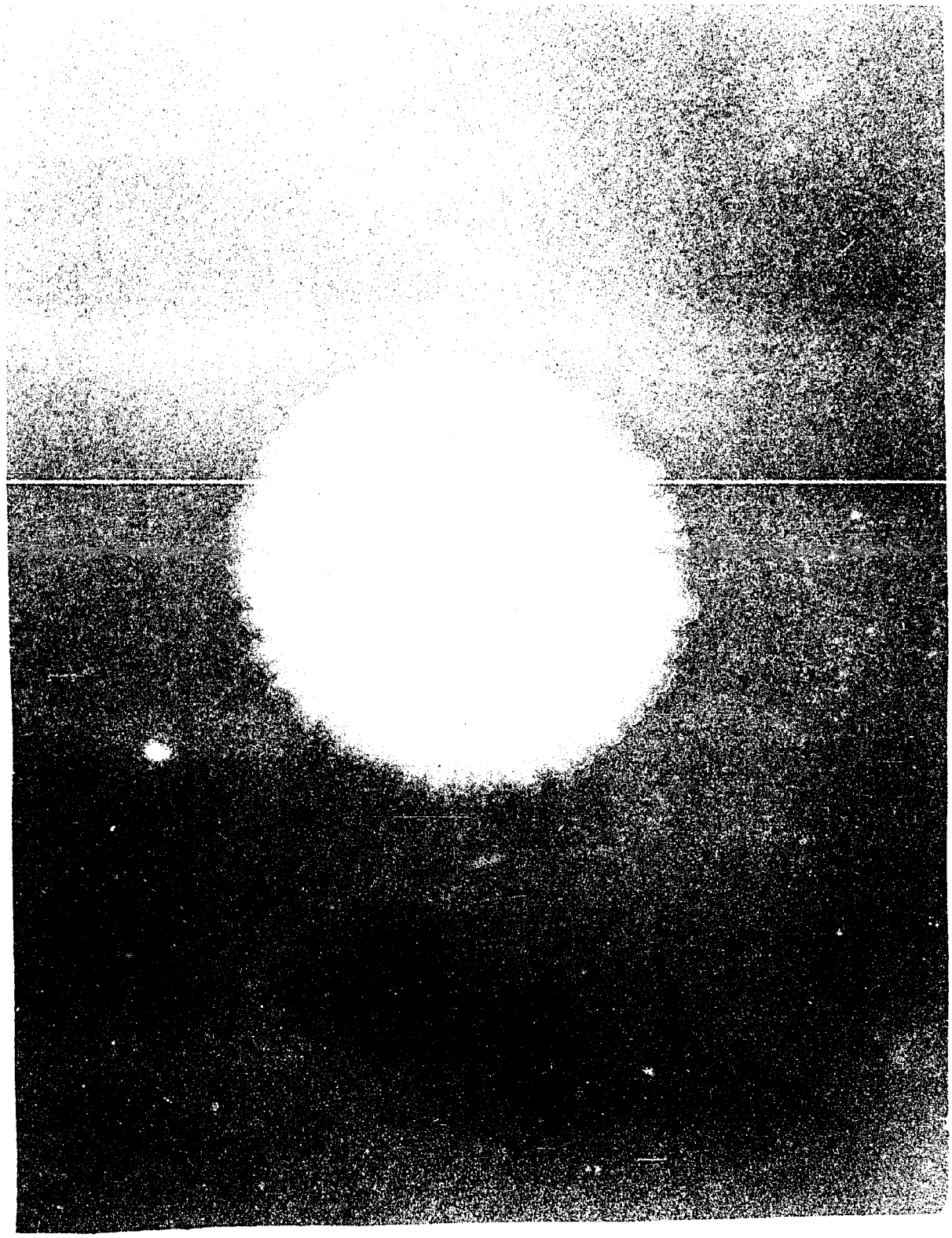


Fig. 22

Fig. 29. Colony of culture No. 4. X10.

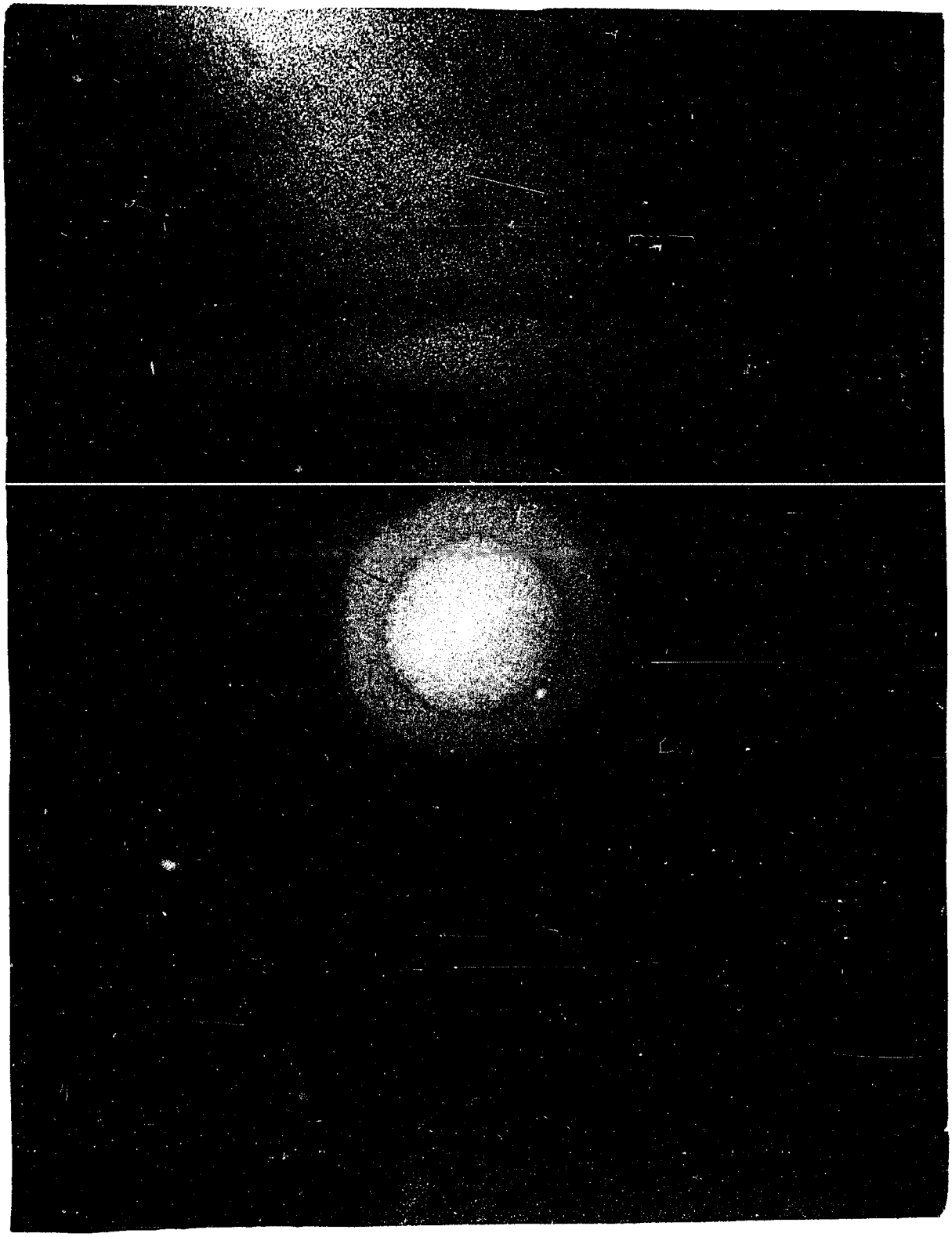


Fig. 30. Colony of culture No. 5. X10.

Fig. 10