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RETROGRADE PROTEUS PYELONEPHRITIS IN RATS

BACTERIOLOGIC, PATHOLOGIC AND FLUORESCENT-ANTIBODY STUDIES

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Although many significant contributions have come from recent clinical and experimental studies, the natural history of human pyelonephritis is still imperfectly understood.[1-3] An opportunity to observe the natural history of experimental pyelonephritis arose from the demonstration that ascending infection could be produced readily in rats.[4-8] Increasing evidence has also suggested an important role for the ascending process in clinical disease in man.[1,9]

Since strains of Proteus were found to produce consistent, severe and chronic infections of the urinary tract in rats, detailed observations of the bacteriologic and morphologic sequence of events after intravenous and intravesical injection of Proteus were undertaken. The localization and fate of bacterial antigen throughout the course of such infections was determined by fluorescent-antibody methods. Comparative studies of infections due to less virulent organisms such as *Escherichia coli* and *Klebsiella pneumoniae* have also been performed and will be reported separately.

MATERIAL AND METHODS

White rats, of the Charles River strain, weighing 150 to 200 gm., were used in all experiments. They were fed laboratory chow and water *ad libitum*.

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Bacteria

Several strains of *Proteus mirabilis* and *Proteus vulgaris* were initially tested for their ability to produce renal infections in rats. These were isolated from various sources, including human urine and rat feces. All strains produced essentially similar disease. Subsequently, one strain of *P. mirabilis* was chosen for the majority of the experiments reported here. The organisms were incubated in a large volume of nutrient broth and frozen in 3 to 5 ml. aliquots. When needed, one aliquot was thawed, transferred to flasks containing 10 to 100 ml. of nutrient broth and incubated at 37° C. for 18 hours before injection.

Intravesical Inoculations

Two procedures were used to introduce the organisms into the urinary bladder: direct inoculation into the bladder lumen and transurethal inoculation. In the initial experiments, the rats were anesthetized with ether, a small suprapubic incision was made, the bladder exposed, and 0.01 to 0.05 ml. of nutrient broth containing approximately 10^6 organisms was injected into the bladder with a 25-gauge needle. The peritoneum, muscle, and skin were then closed with silk sutures and skin clips respectively. In the majority of the experiments reported here the bacteria were injected transurethrally. In this procedure female rats were used exclusively. The animals were anesthetized with ether or sodium pentobarbital, and a 26-gauge needle, 1½ inches long with a blunt polished end, was carefully introduced through the urethra into the bladder. With practice this was accomplished with a minimum of trauma. Approximately 10^7 organisms in 0.05 to 0.2 ml. of broth were injected. Experiments in our laboratory and elsewhere have shown that immediate reflux through the ureterovesical orifices occurs regularly with volumes of 0.2 ml. or more and occasionally with smaller volumes.[7,8,10]

Intracardiac Inoculation

The required number of organisms was suspended in 0.5 ml. of sterile 0.85 per cent sodium chloride and injected by percutaneous cardiac puncture, using a 25-gauge needle. The animals were under light ether anesthesia.

Examination of Animals

Animals were sacrificed at intervals from 1 day to 13 months after infection. They were anesthetized with ether and bled from the heart by percutaneous puncture for culture and determination of blood urea nitrogen. The animals were then killed by replacing them in the ether jar. The hair was shaved from the abdomen and flanks, and the skin thoroughly washed with benzalkonium hydrochloride. The abdomen was opened with instruments that had been sterilized by repeated immersion in alcohol and flaming. Bladder urine was aspirated with a 25-gauge needle. If no urine was present, a section of bladder was cultured. Each kidney was removed, placed in sterile Petri dishes and divided in half. One half was used for culture and the other for morphologic studies. In some instances the animals were given sodium pentobarbital parenterally 1 hour before sacrifice. The latter procedure led to delayed micturition so that the urinary bladder generally contained enough urine for quantitative cultures.

Bacteriologic Studies

One half of each kidney, the bladder wall, and other tissues were cultured quantitatively by homogenizing the tissues with sterile glass homogenizers in 5 ml. of nutrient broth followed by serial plate dilution of the homogenate in heart infusion nutrient agar. The urine was also cultured quantitatively.

Histologic Studies

Tissues fixed in 10 per cent neutral formalin or Zenker's solution were stained with hematoxylin and eosin and, when indicated, with the McCallum-Goodpasture or Brown-Brenn stain for bacteria, or with Schiff's periodic acid stain (PAS) or the ferrocyanide iron stain.

Fluorescent Antibody Studies

Preparation of Antiserums. Specific antiserums to the strain of *P. mirabilis* that was used were prepared in rabbits by the intravenous injection of acetone-killed organisms in increasing doses, 3 days each week for 3 weeks, followed by 3 consecutive daily injections of live suspensions during the fourth week. One week after the last injection, serum was tested for agglutination and the rabbits bled by cardiac puncture.

Preparation of Conjugates. Two procedures were used: cold-ethanol fractionation of antiserums followed by conjugation with fluorescein isothiocyanate [11] or conjugation of whole serum with fluorescein isothiocyanate followed by separation of the gamma globulin fraction by DEAE (diethylaminoethyl cellulose) chromatography, according to Riggs, Loh and Eveland.[12] In the latter procedure, the eluate was concentrated by dialyzing against 25 per cent polyvinyl pyrrolidone. The final protein concentration was 10 mg. per ml. in the alcohol-fractionated fluors and 5 mg. per ml. in the chromatographed fluors. The agglutination titers of unconjugated serums were usually between 1:1024 and 1:4096. The conjugates usually stained smears of bacteria brightly in dilutions corresponding to 25 to 100 per cent of the initial serum titer. The serums were diluted with pH 7.2 phosphate buffered saline (PBS) before use.

Preparation of Tissues. Initial studies showed that formalin fixation of tissues for 6 to 24 hours did not abolish the specific staining of Proteus by fluorescent antibody. This was further evaluated by the following procedure. Two contiguous sections were taken from acutely and chronically infected kidneys. One was frozen in isopentane-liquid nitrogen and the other fixed in neutral 10 per cent aqueous or alcoholic formalin for 18 to 24 hours. The frozen pieces were cut in the cryostat at 4 μ and fixed in 95 per cent alcohol, acetone, or 10 per cent formalin for 15 minutes to 18 hours. The pieces fixed in formalin were dehydrated in alcohol-xylene, embedded in paraffin, and cut at 4 μ. All sections were stained with the same fluor preparation for 45 minutes, washed and mounted. With undiluted and 10 times diluted fluors, there was no appreciable difference in the intensity and localization of specifically fluorescent material. In 2 instances, such sections were compared, after staining, with serial dilutions of fluors. Table I shows that differences were detected, but that formalin-fixed sections continued to stain adequately with higher concentrations of antiserum. It is obvious, however, that small amounts of antigen could escape detection after fixation in formalin. Because of the practical advantages of the formalin-paraffin method over the cryostat method, the majority of observations described in this paper have been made on tissues processed in the following manner: Small pieces were fixed in 10 per cent aqueous formalin for 6 to 24 hours, dehydrated in 3 changes of alcohol and in either 3 changes of dioxane or 2 changes of cedarwood oil followed by 2 changes of xylene. They were then embedded in a mixture of 25 per cent Harleco's synthetic resin and 75 per cent Waterman's paraffin, cut at 1 to 4 μ, deparaffinized in 3 changes of xylene followed by 3 changes of alcohol (100, 95 and 80 per cent) and 1 of PBS. The fluor was then layered over the tissues for 45 minutes, the sections were then washed in 3 changes of PBS, mounted in PBS glycerine, and sealed with dentists' stick wax.[13] Slides were stored at 4° C.

Autofluorescence and Nonspecific Staining. Formalin-fixed tissues were considerably more autofluorescent than alcohol or acetone-fixed sections that had been cut

in the cryostat. The autofluorescence could easily be differentiated from the green specific fluorescence with UG2 and UG5 (Zeiss) filters.

With chromatographed unabsorbed fluors, the major nonspecifically fluorescent elements were the eosinophils and the elastic tissue, which were easily recognizable. Absorption with mouse liver powder (100 mg. MLP per 1 ml. of fluor) abolished

TABLE I

EFFECT OF VARIOUS PROCEDURES OF FIXATION AND EMBEDDING ON THE SPECIFIC STAINABILITY OF *P. mirabilis* ANTIGEN IN TISSUE SECTIONS

Fixative	Time	Cutting	Dilution of fluor *	
			Whole bacteria †	Amorphous antigen †
95% alcohol	15 min.	Cryostat	1:512	1:256
95% acetone	15 min.	Cryostat	1:1024	1:512
10% formalin	15 min.	Cryostat	1:512	1:256
10% formalin	2 hr.	Cryostat	1:512	1:256
10% formalin	18 hr.	Cryostat	1:512	
10% formalin	18 hr.	Paraffin	1:512	1:128; 1:64
10% formalin	2 mo.	Paraffin	1:128	
95% alcohol at 4° C.‡	18 hr.	Paraffin		1:256; 1:128

* Same fluor layered on all sections. Fluor stained smears of heat-fixed bacteria in a dilution of 1:2048.

† "Whole bacteria" refers to fluorescence of rod-shaped bacteria in kidneys with acute pyelonephritis. Amorphous antigen refers to fluorescence of antigenic material within macrophages in chronic infections.

‡ According to G. Sainte-Marie.[27]

this nonspecific staining. In certain instances, the fluors were mixed with 2 to 4 per cent solution of Evans blue (3 parts of fluor to 1 part dye) and the sections layered with the fluor-Evans blue mixture. This procedure, developed by Teplitz,[10] resulted in staining of various tissue elements by the Evans blue, which under ultraviolet illumination fluoresced orange-red. The eosinophilic granules and the elastic tissue of vessels stained bright red. The green specific staining of bacteria and bacterial fragments was not affected. When Evans blue was used, an additional step in the staining procedure was followed: after staining was complete, the sections were differentiated in 20 per cent diethylene-glycol warmed to 40° to 50° C. until most of the blue color of the section had been extracted. Sections were then washed in PBS and mounted as usual.

Staining of Other Bacteria by Anti-Proteus Conjugate. Heat-fixed smears from strains of *E coli,* group A beta-hemolytic streptococcus, pneumococcus and *Pseudomonas aeruginosa* failed to stain with the anti-Proteus conjugate. Two *Staphylococcus aureus* strains stained with a 1:8 and 1:32 dilution. Of 14 *P. mirabilis* cultures from rat feces, 2 did not stain, 4 stained with undiluted fluor and 8 with fluor dilutions of 1:32 (3), 1:64 (2), 1:128 (2) and 1:256 (1). The fluor stained the infecting strain in a dilution of 1:2048.

Controls for Specificity of Staining. The specificity of the staining was confirmed by: (a) absence of staining of contiguous sections when the fluor was prepared from heterologous antiserums which contained similar protein and fluorescein concentrations; (b) inhibition of specific staining by previous absorption of the fluors with homologous antigens and retention of staining after absorption with heterologous bacterial antigen[14]; and (c) significant diminution of staining by previous treatment of sections with unconjugated homologous but not heterologous antiserum. Significant but not complete inhibition was accomplished when unconjugated antiserum was layered for 45 minutes and the fluor for 15 minutes. Complete inhibition was

possible, however, when the antiserum was applied for 2 hours and the fluor for only 5 to 10 minutes. Direct application of the fluor in contiguous sections for 5 to 10 minutes resulted in adequately bright staining. This experience is similar to that of Wood and White.[14] Treatment of sections with heterologous serums for equal periods of time followed by application of the fluor did not inhibit the specific staining.

Microscopy and Photography. A GFL Zeiss microscope or a Zeiss photomicroscope equipped with an Osram HBO 200 mercury lamp as the ultraviolet light source was used. The combinations of filters most frequently employed were: exciter filters UG2, UG5, BG12; barrier filters 410, 440, 470 with or without 650. For photography, high speed super Ektachrome type B film was used. Black and white prints were then made from the color transparencies.

RESULTS

General Data

A single intravesical inoculation of one of several strains of Proteus, using either of the two procedures described above, resulted regularly in infections of the kidneys and the development of pyelonephritis. The incidence and severity of infection in any one experiment depended on several factors, such as the volume of fluid introduced into the bladder, the number of organisms, and factors favoring urine retention, such as anesthesia with pentobarbital. These factors will be discussed in detail in a separate communication. The usual proportion of pyelonephritis in the experiments described here was 60 to 80 per cent, but 95 per cent of animals developed severe pyelonephritis if a small smooth glass bead was also inserted in the bladder.[4] Both sexes were susceptible to direct inoculation into the bladder; there was no significant sex difference when the same infecting dose was inoculated under identical experimental conditions (Table II). Since transurethral inoculation is more readily achieved in the female rat, the experiments involving transurethral inoculation were performed on female rats.

TABLE II

EFFECT OF SEX OF ANIMAL ON INCIDENCE OF PYELONEPHRITIS DUE TO DIRECT INOCULATION OF *P. mirabilis* IN RATS *

Sex	Duration of infection	No. of animals	No. with pyelonephritis
M	2 days to 1 mo.	10	7 (70%)
	3 to 5 mo.	27	15 (56%)
F	2 days to 1 mo.	15	10 (67%)
	3 to 5 mo.	23	8 (39%)

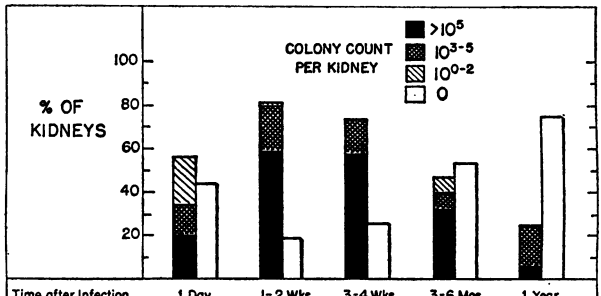
* The differences are not statistically significant ($p > 0.05$).

Bacteriologic Data

Retrograde Inoculation. Text-figure 1 presents the bacteriologic data from animals sacrificed 1 day to 13 months after introduction of *P.*

mirabilis into the urinary bladder. Since the animals that died spontaneously were not cultured, they are not included in the tabulation.

After 24 hours, organisms were cultured from 56 per cent of the kidneys, though the urine contained bacteria in 96 per cent of animals.



Time after Infection	1 Day	1-2 Wks.	3-4 Wks.	3-6 Mos.	1 Year
No. of Kidneys	134	94	36	126	16
% with Pos. Urine culture	96%	88%	74%	47%	25%
Infected animals with Positive Blood culture	2%	16%	18%	0	0
Mean ± S.D. of B.U.N. of Infected (pos.) animals*	23.2 ±6.8	49.3± 53.7	—	63.8± 31.2	33.3±9.2

*Normal value = 23.8 ± 5.4 ; n = 41

TEXT-FIG. 1. Bacteriologic content of kidneys, bladder and blood from 1 day to 1 year after the induction of retrograde pyelonephritis by *P. mirabilis* in the rat. (Both sexes; intravesical and transurethral inoculations of 10^6 to 10^7 organisms).

The infection was bilateral in all animals given injections directly into the bladder and in 86 per cent of animals given transurethral injections.

Eighty-one per cent of kidneys examined between the second and 14th days, and 74 per cent of kidneys examined at 3 to 4 weeks had positive cultures. More than 100,000 colonies were cultured from the majority of the kidneys, and all infected kidneys obtained after the second day showed either gross or microscopic evidence of pyelonephritis. Although the infection was generally bilateral, the severity was usually unequal.

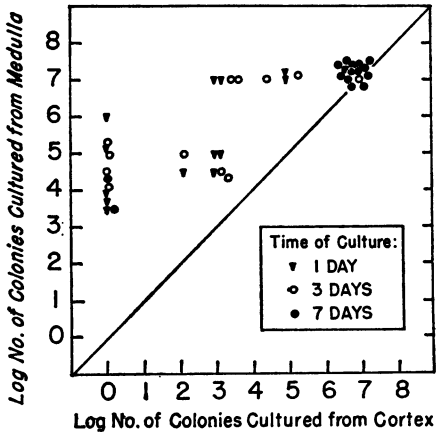
At 1 and 2 weeks, instances in which the urine culture was positive and the kidneys were sterile were occasionally encountered, but after the second week, all animals with Proteus in their urine or bladder walls had bacteria in their kidneys as well.

After 3 to 13 months of infection, bacteria were cultured from the kidneys in all animals with gross or microscopic evidence of pyelonephritis. A large proportion of the infected animals died with renal failure between the second and eighth month; a smaller percentage of surviving animals, sacrificed at 3 to 6 months and at 1 year, had persistent infection. The bacteriologically negative kidneys had no histologic evidence of previous pyelonephritis.

In one series of experiments, slices of cortex and medulla (including pelvis) were cultured separately at 1, 3 and 7 day intervals after trans-

urethral inoculation of 0.1 ml. of Proteus. As seen in Text-figure 2, 5 of 13 kidneys at 1 day, 4 of 12 at 3 days and 2 of 16 at 7 days had sterile renal cortices while their respective medullas and pelves contained 10^3 to 10^6 organisms. There was no instance in which the cortex contained bacteria and the medulla was sterile. Infected kidneys at 1 and 3 days after onset of infection had higher numbers of bacteria in the medulla than in the cortex, but when gross cortical infection occurred, the numbers of bacteria in both cortex and medulla were over 10^7.

Cultures of heart's blood just before sacrifice were negative in all but 1 of the blood cultures taken 1 day after inoculation into the bladder. However, the blood cultures were positive in 16 per cent of infected animals after 1 day to 2 weeks of infection and in 18 per cent after 2



TEXT-FIG. 2. Bacterial counts in the renal cortex and medulla 1, 3, and 7 days after transurethral injection of *P. mirabilis* in female rats.

to 4 weeks, during the period of acute renal inflammation with abscess formation. All blood cultures in animals sacrificed 3 to 13 months after infection were sterile. The livers were sterile in all of 11 animals after 1 day, but contained 10^3 to 10^5 Proteus in 2 of 10 animals after 7 days of infection.

In general, after retrograde infection, occasional animals succumbed to the infection during the first week, about ¼ during the first 6 weeks, and an additional fourth during the next 6 to 8 months. Virtually all of the animals dying after the first day of infection had severe pyelonephritis. The animals surviving longer than 6 months either escaped infection or had severe chronic pyelonephritis in 1 kidney and mild chronic pyelonephritis in the other.

Azotemia and Calculi. Azotemia, as measured by the blood urea nitro-

gen (BUN), developed as early as the first week after infection (Text-fig. 1). The mean BUN in 34 pyelonephritic animals in the first 2 weeks was 49.3 mg. per cent with 10 having a BUN above 40, and 5 above 80 mg. per cent. The elevated BUN values were found in animals with severe gross pyelonephritis or papillary necrosis, although many animals with considerable renal involvement had normal values. Animals sacrificed from 3 to 6 months after infection had a mean BUN of 63.8 mg. per cent and 42 per cent of these had a BUN above 40 mg. per cent. The higher values were found in animals with bilateral disease or in those with renal calculi.

Vesical calculi developed as early as the fourth day and were found initially as soft gravel adherent to the mucous membrane of the urinary

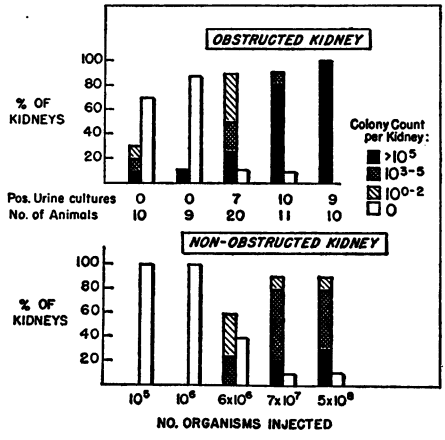
TABLE III

INCIDENCE OF URINARY TRACT CALCULI IN RATS WITH UNTREATED RETROGRADE PYELONEPHRITIS DUE TO *P. mirabilis* *

Time after infection	Kidneys with calculi	Bladders with calculi
1 to 2 wk.	5%	25%
3 to 4 wk.	25%	66%
3 to 5 mo.	32%	84%
6 to 13 mo.	83%	83%

* Both sexes. Intravesical and transurethral inoculations. All animals with kidney calculi had bladder calculi also.

bladder. Well-formed stones generally were not found until the second week. Table III shows the proportion of infected animals with renal and vesical calculi. Chemically, the calculi were magnesium-ammonium-

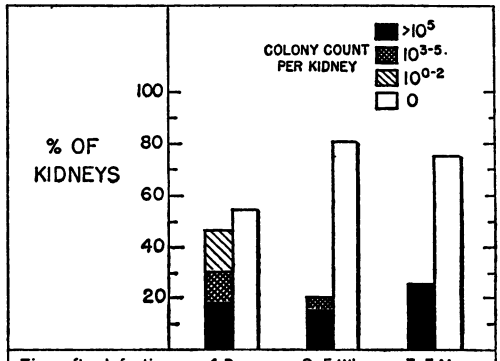


TEXT-FIG. 3. Bacterial counts in the kidneys and urine of animals 1 day after receiving varying doses of *P. mirabilis* intracardially. All animals had sham cystotomy and ligation of left ureter before injection.

phosphate complexes, and their formation was undoubtedly enhanced by the alkalinity of Proteus-infected urine. Hydroureter and hydronephrosis usually were found in the presence of well-formed stones.

Intracardiac Inoculation. The occurrence of renal infection after intracardiac inoculation of Proteus depended upon the number of bacteria injected. Text-figure 3 is a summary of the bacteriologic data from animals given varying doses of bacteria and sacrificed 1 day after the injection. One kidney was totally obstructed by ureteral ligation prior to the injection in order to determine whether the incidence and magnitude of infection increased after urinary obstruction as in other intravascular models.[15,16] In addition, cystotomy without injection of bacteria was performed. The data indicate that in the absence of obstruction, large numbers of bacteria were cultured only after the injection of 7×10^7 or more organisms. A smaller inoculum resulted in little or no infection. In the presence of obstruction, occasional animals were heavily infected with lower doses of bacteria, and with large infecting doses the number of heavily infected kidneys was greater.

Animals were given injections with 5×10^8 organisms intracardially and sacrificed at intervals up to 5 months thereafter (Text-fig. 4). In



Time after Infection	4 Days	2-5 Wks.	3-5 Mos.
No. of Kidneys	32	40	16
% with Pos. Urine culture	36%	20%	25%

TEXT-FIG. 4. Bacteriologic findings in kidneys and bladder after the intracardiac inoculation of 5×10^8 *P. mirabilis* into rats. Sham cystotomy was performed before infection, but the ureter was not ligated.

this series both ureters were left intact, but cystotomy was performed as an added control. Although half of the kidneys of these animals still contained organisms after 4 days, only those with heavy infection (25 per cent) had histologic lesions of pyelonephritis. At later stages, about 25 per cent of animals were found to have bacteriologic and pathologic evidence of pyelonephritis. In several series of animals that received

this inoculum of Proteus without sham infection of the bladder by cystotomy, the percentage of animals that developed pyelonephritis after intracardiac injection of 5×10^8 organisms was 0 to 18 per cent.

Morphologic Findings at Different Stages of Infection

Retrograde Infection, 1 to 2 Days. A few animals already had gross or microscopic renal abscesses 1 and 2 days after infection; kidneys from such animals were similar to those of the majority of the animals studied after the third day and will be described below. Most kidneys appeared grossly normal at 24 hours, but in ⅓ there was histologic evidence of pyelonephritis. At 2 days all bacteriologically positive kidneys had either pyelitis or frank pyelonephritis. The earliest microscopic lesion was in the pelvis. In the lumen of the pelvis, neutrophils, red cells, desquamated epithelial cells and occasional clumps of bacteria could be seen, while in the pelvic mucosa there was infiltration with neutrophils in several foci, sometimes localized only to the most superficial layers of the mucous membrane. The layer of epithelium at the tip of the papilla, the epithelium in contact with the poles of renal parenchyma and the so-called "fornices" of the pelvis were involved. The inflammatory infiltrate occasionally involved the interstitial tissues of the papilla and medulla beneath the mucosa (Fig. 1). In some instances, neutrophils were found in the lowermost collecting ducts.

The earliest renal localization of organisms by the fluorescent-antibody method was in the lumen of the pelvis and the inner aspect of the pelvic mucosa. Bright green rods singly and in small clumps were seen within masses of necrotic cells and neutrophil exudate (Fig. 2). In the more severe infections, the pelvic lumen was full of bacteria and of bright green amorphous noncellular material. Occasionally, the lowermost collecting ducts contained organisms without neutrophils (Fig. 3).

The urinary bladder also showed various degrees of inflammation. Early submucosal edema and vascular dilatation were followed by neutrophil infiltration and eventually by focal ulceration of mucosa. In sections stained with fluorescent antibody, bacteria were present in the lumen and within the mucous membrane but rarely beneath the mucosa.

Hematogenous Infection, 1 Day. At 1 day the microscopic lesions after intracardiac inoculation differed strikingly from the early changes in retrograde pyelonephritis. The morphologic findings after hematogenous infection were clumps of bacteria and bacterial fragments in the glomerular capillaries and in capillaries and the interstitium of the medulla and papilla. The bacteria were seen in hematoxylin and eosin (H and E) stained sections as basophilic masses (Figs. 4 and 6). The bacteria stained brilliantly with fluorescent antibody (FA) and were

relatively easy to locate (Figs. 5 and 7). Sometimes variable numbers of neutrophils were present around the bacterial clumps, both in the glomeruli and around the medullary clumps of bacteria (Fig. 9). Capillaries filled with bacteria were found close to the pelvic mucous membrane in the papilla (Fig. 8), but the pelvic mucosa was intact at this stage of the infection and contained no inflammatory cells. The presence of focal collections of bacteria in vessels, glomeruli and interstitium throughout the cortex and medulla of the kidneys was the most characteristic lesion of the hematogenously produced pyelonephritis at this early stage and was different from the lesion in early stages of retrograde pyelonephritis. Similar findings in hematogenous pyelonephritis have been reported by Mallory, Crane and Edwards in obstructive *E. coli* pyelonephritis in rabbits,[17] and by Gorrill in *B. pyocyaneus* and *S. aureus* renal infections in mice.[18,19] Kennedy[20] described similar differences in the earliest histologic lesions of *E. coli* pyelonephritis produced by intravenous and intraureteral injection of bacteria in rabbits.

Recently, Sanford, Hunter and Donaldson,[21] using the FA technique, described the earliest distribution of bacteria in hematogenous *E. coli* pyelonephritis produced in rats with renal massage. They observed initial localization of bacteria in glomerular and interstitial blood vessels; subsequently bacterial multiplication occurred in the interstitium with formation of abscesses and rupture into tubules.

Retrograde Infection, 4 to 30 Days. Animals sacrificed after the fourth day of retrograde infection showed classical gross and microscopic features of acute pyelonephritis. In the most severe infections, the kidneys were enlarged and the capsular surfaces were studded with abscesses. Abscesses were present in the cortex, medulla and pelvis (Figs. 11 and 12). In the less severe lesions, the kidneys were of normal size but contained scattered abscesses. In many animals, whitish gray necrotic tissue formed part of the lining of the pelvis, as well as the tip and sides of the papilla, and occasionally this was the only gross lesion seen (Fig. 10). The urinary bladder was hyperemic and occasionally hemorrhagic. Gravel was sometimes present by the end of the first week and stones by the end of the second week as described above.

Histologically (Figs. 13 to 18) all infected kidneys had inflammatory infiltration in the pelvis and renal parenchyma adjacent to the pelvic mucosa, and in some animals this was the only significant finding. The pelvic mucosa was usually ulcerated in one or several foci. The papilla, medulla and cortex were variably involved by an intense inflammatory infiltrate, in the interstitium as well as in the tubules. The tubules contained neutrophils, pus casts, necrotic debris and desquamated epithelial cells and, in some instances, bacteria (Fig. 13). Some tubules had lost

their cell outlines and were completely filled with amorphous basophilic material, and surrounded by masses of neutrophils (Fig. 15). Evidence of healing occurred early, and even after 4 days macrophages, regenerating blood vessels and fibroblasts could be seen, especially around the pelvis (Fig. 17). After the second week, a significant proportion of the inflammatory cells were mononuclears and plasma cells, although areas of necrosis and neutrophil infiltrates were still extensive. Occasional tubules now contained amorphous eosinophilic material confined by irregular basement membranes and surrounded by plasma cells and macrophages (Fig. 18).

Specific antigen and organisms were distributed in the following locations as determined by FA:

1. Pelvic lumen and mucosa: There were rods and much granular green material, not shaped like bacterial bodies, but staining specifically. Much of the material that stained as "cellular debris" in conventionally stained sections contained specifically staining fragments.

2. Tubules: Both rods and amorphous fragments were found in conjunction with neutrophils and cellular debris (Fig. 14). The tubules seen in H and E stained sections were packed with bright green, specifically staining material (Fig. 16).

3. Interstitium of the medulla and the cortex: Clumps of bacteria and amorphous fragments were found. In kidneys in which the cortex had no lesions the specifically staining component was present in the interstitium of the papilla and in the lowermost medulla.

The lesions produced after hematogenous inoculation of the organisms could be distinguished from those produced by retrograde infection, in most instances, for about 4 days after the initial infection. The abscesses formed after hematogenous infections were found at random throughout the cortex and medulla, whereas those following ascending infection tended to be more focalized. After the fourth day, however, it was not possible to differentiate hematogenous from retrograde lesions as ulceration of the pelvis occurred in the former. Pelvic inflammation became very conspicuous and the consequent lesions were similar to those described for retrograde pyelonephritis. This difficulty in differentiating hematogenous from retrograde lesions except very early in the course of infection has been stressed by Kennedy [20] and is the general experience in human pathologic material.

Retrograde Infections, 3 to 13 Months. Kidneys from infected animals that had been sacrificed at intervals of 3 to 13 months presented one of several gross appearances: (a) small contracted kidneys without hydronephrosis and without renal calculi (Fig. 20); (b) hypertrophied kidneys, with one or several cortical scars radiating from the pelvis; these

were present when the other kidney was atrophic (Fig. 19); (c) contracted kidneys with slightly dilated pelves containing stones or gravel (Figs. 22 and 24); and (d) kidneys of relatively normal size, without stones, but with considerable hydronephrotic atrophy due to obstruction by vesical calculi (Fig. 23). Deformity of the papilla was uniformly observed in infected kidneys; instead of the usual pointed tip, the distal end of the papilla was either flattened or grossly deformed (Figs. 20 to 22).

Microscopically, the pelvic lumen was filled with debris of cells, nuclei and calculi. The pelvic mucosa was often thickened and showed papillary projections (Fig. 23). Inflammatory cells (including mononuclear cells and many neutrophils) were abundant in the pelvic mucosa and submucosa. There were various degrees and combinations of interstitial inflammatory infiltrate, tubular atrophy, periglomerular and interstitial fibrosis and hyalinization in the renal parenchyma. The inflammatory infiltrate varied from one area to the other, but the predominant cells in the interstitium were mononuclears, including macrophages and plasma cells. Neutrophils and eosinophils were found in all instances, however. Some of the macrophages had abundant cytoplasm which contained orange-brown granular pigment as well as faintly eosinophilic granular or amorphous material. The pigment stained as iron. The cytoplasm of some of the macrophages contained granules and globules that stained with the PAS stain but not metachromatically with toluidine blue (Fig. 28). In many areas the cytoplasm of several macrophages fused, forming multinucleated cells resembling to some degree foreign-body giant cells. As will be seen below, a number of these macrophages contained specifically staining bacterial antigen when stained with FA.

The tubules were either small and without lumens or dilated and filled with casts. Casts formed predominantly of neutrophils were found easily in all infected kidneys (Fig. 19) and, in addition, many of the casts contained epithelial cells, macrophages and homogeneous eosinophilic material (colloid). The glomeruli were rarely completely hyalinized. The blood vessels in the uninvolved part of renal parenchyma were normal. However, in the areas of inflammation and scarring, the vessels were conspicuously close to one another, and their walls appeared hypercellular; there was no hyalinization or necrosis, and the walls of the arteries in other organs were not remarkable.

Specific antigen was found by the fluorescent-antibody technique to be localized in the following areas:

1. Pelvic lumen and mucosa: Twenty-four of 29 infected kidneys in which an adequate section of pelvis was present contained specific

Proteus antigen in the pelvic lumen and mucosa. The staining was in the form of recognizable bacterial bodies as well as small granules in the masses of gravel and necrotic debris (Fig. 25). In the pelvic mucosa, the bacteria were adherent to the surface of the interstices between the mucosal projections (Fig. 25).

2. Interstitium: *P. Mirabilis* antigen was found in the cortical and medullary areas of inflammation in 23 of 36 chronically infected kidneys, including 1 of 2 kidneys from an animal sacrificed in the 13th month after infection. The predominant pattern of staining consisted of bright green, amorphous, granular material in the cytoplasm of cells considered to be macrophages (see above) and in the interstitial tissue around the tubules (Fig. 27). The mononuclear as well as the multinucleated macrophages contained antigen (Fig. 29). Not all macrophages identifiable in contiguous H and E sections stained with FA. The cells not containing antigen had either a bluish gray cytoplasmic autofluorescence or contained an orange-brown autofluorescent pigment that corresponded to the iron-positive pigment present in contiguous sections stained for iron. Much of the PAS positive material did not stain with FA.

No systematic attempt to quantitate the amount of antigen present was made. Generally, however, the larger scars contained the largest quantities of specifically staining material. In addition, the amount of antigen was less in kidneys of animals sacrificed at later intervals; such kidneys often contained fairly extensive areas of scarring without any antigen.

3. Tubules: In 6 of 36 kidneys, antigen was present within the cellular casts in the tubular lumens. In 4 of these 6, the antigen consisted of well-formed bacteria. Examination of areas drained by these tubules revealed acute cortical abscesses in 3 and a peripelvic abscess in 1. These 4 kidneys were from animals sacrificed 80, 90, 105 and 126 days after infection. In 2 kidneys, the specific material in the tubules consisted of amorphous antigen in the cytoplasm of large mononuclear cells.

4. Concretions: Bacteria and granular antigen were present uniformly within the concretions in the pelvis.

Control Studies

Throughout the experiments the following studies helped to control the specificity of the bacteriologic, pathologic and FA correlations:

1. The kidneys from 80 rats not subjected to any experimental procedure were cultured and examined histologically. All had essentially sterile kidneys. Minor histologic lesions were present in 10 per cent. The commonest lesions consisted of focal small interstitial accumula-

tions of lymphocytes, plasma cells and histiocytes in the cortex, medulla or pelvis. These were not accompanied by tubular necrosis, atrophy or fibrosis. In one kidney neutrophils were present in a medullary tubule, and in 3 there were a few neutrophils in the pelvis. Some kidneys had variable numbers of eosinophils in the peripelvic fat. FA stained sections in representative lesions showed no specific staining of Proteus antigen.

2. One hundred twenty rats were subjected either to suprapubic cystotomy or to transurethral inoculation of sterile broth. Eight (6.7 per cent) had pyelonephritis and bacteria in their kidneys 4, 14 and 20 days after the procedure. The organisms were Proteus in 3, Proteus and *E. coli* in 2, *E. coli* in 1, *E. coli* and *S. aureus* in 1, and *S. aureus* in 1. Thus, the incidence of pyelonephritis induced by contamination during instrumentation was 6.7 per cent.

3. Twenty-seven rats were subjected to unilateral ureteral ligation without injection of bacteria. Ten were sacrificed after 1 week, 9 after 3 to 4 weeks and 8 after 5 months. All kidneys were sterile at the time of sacrifice. The histologic lesions after 1 week included tubular dilatation, flattening of the tip of the papilla, infarction of the layer of tubules beneath the visceral pelvis at the papilla and occasional focal interstitial infiltrates, mostly of round cells but with occasional neutrophils. Tubules rarely contained neutrophils. Three to 4 weeks after ureteral ligation there were atrophy of renal parenchyma, focal lymphocytic interstitial infiltration and an occasional pus cast. In all 8 animals with hydronephrosis, sacrificed after 6 months, the renal parenchyma was converted to a thin cystic lining containing a few tubules, an occasional glomerulus and round cells. FA studies were uniformly negative for Proteus antigen. Although focal lesions in obstructed kidneys did resemble pyelonephritic lesions, differentiation of the over-all picture presented no difficulty.

4. Pyelonephritic kidneys infected hematogenously and intravesically with *E. coli* were stained with rabbit anti-Proteus fluor. In spite of the presence of numerous lesions, no specific staining was present.

Discussion

Studies of experimental retrograde pyelonephritis have indicated that following introduction of Proteus into the urinary bladder the organisms reached the kidneys by way of the ureter,[4] and ascended into the lumen.[10] The organisms then produced inflammation of the pelvic mucosa, followed by ulceration, involvement of the collecting tubules and interstitium of the papilla, and finally inflammation in the cortex. The disease produced was generally severe, and bacterial proliferation during the active stages resulted in tubular necrosis and extensive renal

inflammation. Bacterial proliferation was accompanied by release into the interstitial tissue of antigenic (and nonantigenic) bacterial products. The particulate antigenic material was phagocytized first by neutrophils and later by macrophages. This was followed by repair which began early and was associated with the appearance of fibroblasts, macrophages and plasma cells followed by scarring and contracture. In the untreated animal, however, healing was not complete, even in animals surviving the acute attack. The development of calculi and of consequent obstruction might contribute to the persistence of organisms, but the mechanism by which obstruction altered renal susceptibility to infection is still unclear.[1] With healing, fewer organisms were present in renal tissue proper, and after 1 to 3 months of chronic infection the organisms were confined to the bladder, pelvis and occasional renal abscesses. The finding of renal abscesses with organisms in a few animals with chronic infection might indicate re-infection of previously uninvolved areas by bacteria from pelvic urine.[5] Recognizable bacteria could be found in the chronically infected kidney only in the pelvis and occasionally in abscesses. However, specific bacterial antigen was found in the interstitium and in the macrophages even in animals that had been infected for 13 months. The present study did not allow an assessment of the length of time that antigen persisted in renal tissue in the absence of bacterial proliferation, since cultures were positive and rods were seen in the pelvis in all animals having interstitial antigen. However, animals that were infected and then treated with antibiotic agents until the kidneys became sterile have shown specific antigen in the renal parenchyma at least 20 weeks after sterilization was achieved.[22] The finding, in many sections, of areas of scarring without antigen signified that bacterial antigen was eventually cleared. In hematogenous *E. coli* pyelonephritis in rats with renal massage, somatic *E. coli* antigen could be detected in the renal scars 4 weeks after infection, at a time when the kidneys were sterile by culture.[21] Wood and White[14] found that *P. mirabilis* antigen persisted in local macrophages, in the liver and in glomeruli at least 3 weeks after subcutaneous injection of *P. mirabilis* vaccine into mice. They reported glomerulonephritis in 50 per cent of mice that received repeated injections of vaccine prepared from 1 of 2 *P. mirabilis* strains. In our experience with repeated injections of live and dead *P. mirabilis* in rats, no glomerular lesions were produced.

The role of the persistent bacterial antigen in the progression of the lesion could not be determined from this study since bacteria elsewhere in the kidney might have contributed to the active inflammation. In a subsequent communication[22] it will be shown that persistent bacterial antigen in the absence of culturable bacteria is accompanied by an in-

flammatory reaction associated with antibody-producing cells. At present there is no adequate experimental or clinical basis for evaluation of the role of hypersensitivity in the pathogenesis of chronic pyelonephritis. The nature of the persistent antigen demonstrated in the infected kidneys is under study. Preliminary results so far suggest that it may be in part a polysaccharide. The material probably does not represent antigen-antibody complexes since sections stained with fluorescent rabbit anti-rat gamma globulin have shown the gamma globulin to be localized in plasma cells around the antigen-containing macrophages, but not within them.[22]

The present model for the production of retrograde pyelonephritis further emphasizes the various parameters of experimental pyelonephritis, such as the route of infection, the pathogenicity of the infecting organism, the species of animal and the premorbid condition of the urinary tract. The ease of ascension of organisms from the urinary bladder to the kidneys in rats may be related to the frequency with which vesico-ureteral reflux occurs in this animal. However, the relative difficulty of producing pyelonephritis after hematogenous injection of a large number of identical organisms must be explained by other factors, such as the smaller numbers of organisms trapped by the kidney after hematogenous infection, the bactericidal action of serum, and possibly the differences in initial localization described. Obstruction (Text-fig. 3), as well as renal massage,[23,24] increased the incidence of pyelonephritis due to hematogenous Proteus in rats, but the mechanisms of increased susceptibility in either case are not understood. On the other hand, in mice, Phillips[25] showed that several strains of Proteus could produce renal abscesses after hematogenous injection even when the kidneys were intact. He reported significant differences of renal pathogenicity among different strains of Proteus.

The importance of bacterial species demands consideration. Studies in this laboratory[26] have shown that injection of *E. coli* into the urinary bladder resulted in their ascension to the kidney and their persistence for a variable period of time, but the infection produced was significantly different from that due to Proteus; the inflammatory action was limited to the pelvis and lower medulla, and in most instances localization by fluorescent antibody showed the organism to be confined to the pelvis without invasion of renal parenchyma.

Chronic retrograde pyelonephritis produced by Proteus was associated with an unusually high incidence of urinary tract calculi and, as such, presented the problems associated with the complications of obstructive disease. However, the experimental model had many similarities to human chronic bacterial pyelonephritis, with its many ramifica-

tions. There are relatively few experimental models for the study of chronic infections; chronic pyelonephritis provides such a model.

Summary

Retrograde pyelonephritis has been produced in rats by the intravesical inoculation of *P. mirabilis*. The pathogenesis and course of pyelonephritis by this route has been studied in an attempt to correlate the morphologic and bacteriologic changes. It was found that organisms appeared in the kidneys by 24 to 48 hours after injection into the bladder, that they first invaded the pelvis and that the infection involved the medulla and the cortex by continuity through the interstitium and the tubules. The infection was usually bilateral but unequal in both kidneys. The surviving animals developed chronic active pyelonephritis with persistence of bacteria and of morphologic evidence of pyelonephritis for at least 13 months after initiation of infection. Chronic pyelonephritis was also bilateral and unequal and was associated with sufficiently widespread scarring to produce unilateral atrophy. In chronic infection there was often azotemia. Renal and vesical calculi were present in the majority of animals with chronic disease.

Fluorescent-antibody studies indicated that bacterial antigen persisted in the renal parenchyma following the initial bacterial proliferation. After the acute stage recognizable bacterial bodies were limited to the pelvis and occasional abscesses; however, variable amounts of amorphous bacterial antigen were present within some renal scars for periods up to 13 months.

After hematogenous infection the initial localization was in cortical and medullary blood vessels, followed by passage into the interstitium and production of scattered abscesses. The hematogenously induced lesions could not be differentiated from the retrograde lesions by the fourth day, presumably because rupture of bacteria into the pelvis, medulla and tubules was followed by intrarenal retrograde spread.

References

1. Beeson, P. B. Factors in the pathogenesis of pyelonephritis. *Yale J. Biol. & Med.*, 1955, **28**, 81–104.
2. Kleeman, C. R.; Hewitt, W. L., and Guze, L. B. Pyelonephritis. *Medicine*, 1960, **39**, 3–116.
3. Quinn, E. L., and Kass, E. H. (eds.). Biology of Pyelonephritis. Henry Ford Hospital International Symposium. Little, Brown & Co., Boston, 1960, 708 pp.
4. Vivaldi, E.; Cotran, R.; Zangwill, D. P., and Kass, E. H. Ascending infection as a mechanism in the pathogenesis of experimental non-obstructive pyelonephritis. *Proc. Soc. Exper. Biol. & Med.*, 1959, **102**, 242–244.

5. VIVALDI, E.; ZANGWILL, D. P.; COTRAN, R., and KASS, E. H. Experimental Pyelonephritis Consequent to the Induction of Bacteriuria. In: The Biology of Pyelonephritis. Henry Ford Hospital International Symposium. QUINN, E. L., and KASS, E. H. (eds.). Little, Brown & Co., Boston, 1960, Chapt. 3, pp. 27–37.
6. MCCABE, W. R., and JACKSON, G. G. The Natural Course of Retrograde Infections of the Urinary Tract of Rats with Different Serotypes of *Escherichia coli* or Enterococcus. In: The Biology of Pyelonephritis. Henry Ford Hospital International Symposium. QUINN, E. L., and KASS, E. H. (eds.). Little, Brown & Co., Boston, 1960, Chapt. 4, pp. 39–52.
7. ANDERSEN, B. R., and JACKSON, G. G. Pyelitis, an important factor in the pathogenesis of retrograde pyelonephritis. *J. Exper. Med.*, 1961, **114**, 375–384.
8. SOMMER, J. L. Experimental pyelonephritis in the rat, with observations on ureteral reflux. *J. Urol.*, 1961, **86**, 375–381.
9. KASS, E. H. Bacteriuria and pyelonephritis of pregnancy. *Arch. Int. Med.*, 1960, **105**, 194–198.
10. TEPLITZ, C., and ZANGWILL, D. Unpublished observations.
11. MELLORS, R. C. Analytical Cytology. Methods for Studying Cellular Form and Function. McGraw-Hill Book Co., Inc., New York, Toronto and London, 1959, ed. 2, Chapt. 1.
12. RIGGS, J. L.; LOH, P. C., and EVELAND, W. C. A simple fractionation method for preparation of fluorescein-labeled gamma globulin. *Proc. Soc. Exper. Biol. & Med.*, 1960, **105**, 655–658.
13. CONGER, A. D. Dentists' stick wax: a cover-sealing compound for temporary slides. *Stain Technol.*, 1960, **35**, 225.
14. WOOD, C., and WHITE, R. G. Experimental glomerulonephritis produced in mice by subcutaneous injections of heat killed *Proteus mirabilis*. *Brit. J. Exper. Path.*, 1956, **37**, 49–60.
15. GUZE, L. B., and BEESON, P. B. Experimental pyelonephritis. 1. Effect of ureteral ligation on the course of bacterial infection in the kidney of the rat. *J. Exper. Med.*, 1956, **104**, 803–815.
16. GORRILL, R. H. The effect of obstruction of the ureter on the renal localisation of bacteria. *J. Path. & Bact.*, 1956, **72**, 59–71.
17. MALLORY, G. K.; CRANE, A. R., and EDWARDS, J. E. Pathology of acute and of healed experimental pyelonephritis. *Arch. Path.*, 1940, **30**, 330–347.
18. GORRILL, R. H. Bacterial localisation in the kidney with particular reference to *Pseudomonas pyocyanea*. *J. Path. & Bact.*, 1952, **64**, 857–864.
19. GORRILL, R. H. Experimental staphylococcal infections in mice. *Brit. J. Exper. Path.*, 1951, **32**, 151–155.
20. KENNEDY, R. L. J. The pathologic changes in pyelitis of children interpreted on the basis of experimental lesions. *J. Urol.*, 1932, **27**, 371–398.
21. SANFORD, J. P.; HUNTER, B. W., and DONALDSON, P. Localization and fate of *Escherichia coli* in hematogenous pyelonephritis. *J. Exper. Med.*, 1962, **116**, 285–294.
22. COTRAN, R. S. Retrograde Proteus pyelonephritis in rats: localization of antigen and antibody in treated sterile pyelonephritic kidneys. *J. Exper. Med.*, 1963. (In press)
23. SHAPIRO, A. P.; BRAUDE, A. I., and SIEMIENSKY, J. Hematogenous pyelonephritis in rats. IV. Relationship of bacterial species to the pathogenesis and sequelae of chronic pyelonephritis. *J. Clin. Invest.*, 1959, **38**, 1228–1240.

24. SANFORD, J. P.; HUNTER, B. W., and SOUDA, L. L. The role of immunity in the pathogenesis of experimental hematogenous pyelonephritis. *J. Exper. Med.*, 1962, 115, 383–410.

25. PHILLIPS, J. E. The experimental pathogenicity in mice of strains of Proteus of animal origin. *J. Hyg.*, 1955, 53, 212–216.

26. COTRAN, R.; THRUPP, L.; HAJJ, S.; ZANGWILL, D.; VIVALDI, E., and KASS, E. H. Retrograde *E. coli* pyelonephritis in rats: bacteriologic, pathologic and fluorescent-antibody studies. *J. Lab. & Clin. Med.* (In press)

27. SAINTE-MARIE, G. A paraffin embedding technique for studies employing immunofluorescence. *J. Histochem.*, 1962, 10, 250–256.

LEGENDS FOR FIGURES

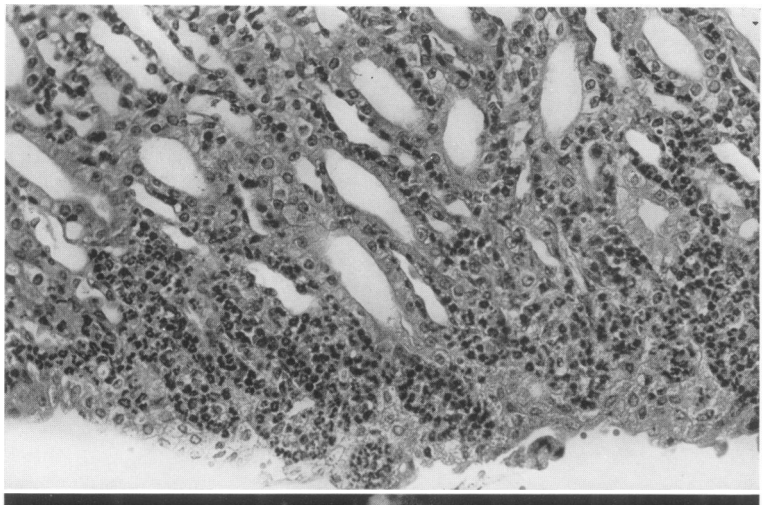
Unless otherwise stated, photomicrographs were prepared from sections stained with hematoxylin and eosin.

Figures 1 to 3: Retrograde Proteus pyelonephritis, 1 to 2 days.

FIG. 1. Visceral pelvis and papilla 2 days after infection. A neutrophil exudate is manifest. × 200.

FIG. 2. Pelvis from rat sacrificed 24 hours after retrograde infection. Bacteria and bacterial clumps underlie the pelvic mucosa. The fluorescent structures in the right upper corner of the photomicrograph are autofluorescent cells. L = pelvic lumen. M = pelvic mucosa. Stained with anti-Proteus fluor. Approximately × 800.

FIG. 3. A collecting tubule contains organisms. The large fluorescent structures (R) are red cells that stained orange-red with Evans blue. Anti-Proteus fluor. Approximately × 1,200.



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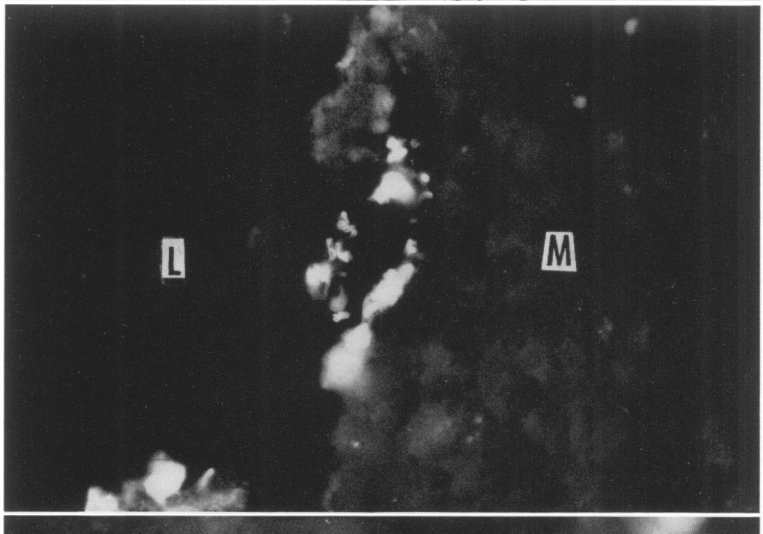

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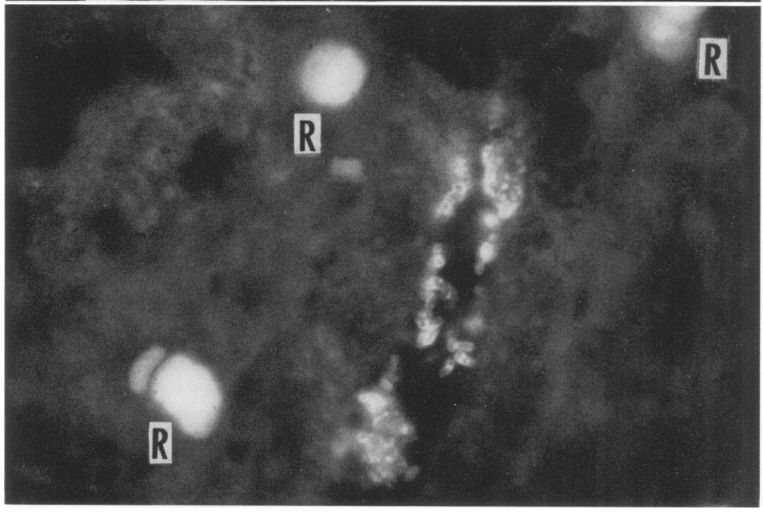

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Figures 4 to 9: Hematogenous Proteus pyelonephritis, 1 day. All figures are from animals given injections of 5×10^8 *P. mirabilis* intracardially and sacrificed 1 day later.

FIG. 4. A glomerulus contains clumps of organisms (arrow). × 400.

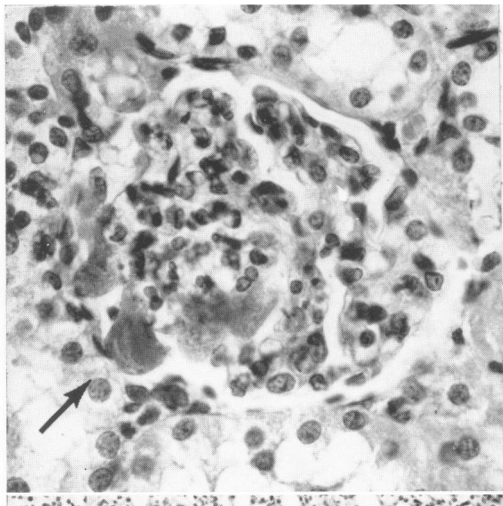
FIG. 5. A glomerulus similar to that shown in Figure 4, stained with anti-Proteus fluor. The bacterial antigen within the glomerulus is fluorescent. The tubules are autofluorescent.

FIG. 6. The medulla contains clumps of bacteria in the interstitium (arrow). × 95.

FIG. 7. Area similar to that shown in Figure 6, stained with anti-Proteus fluor. Approximately × 90.

FIG. 8. The renal pelvis shows a clump of bacteria in the interstitium just inside the visceral pelvic lining. Cells in the pelvic lumen are red cells. × 200.

FIG. 9. Neutrophils surround an interstitial clump of bacteria. × 205.



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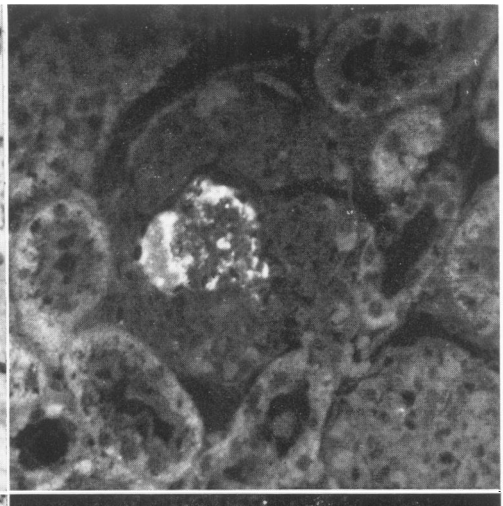

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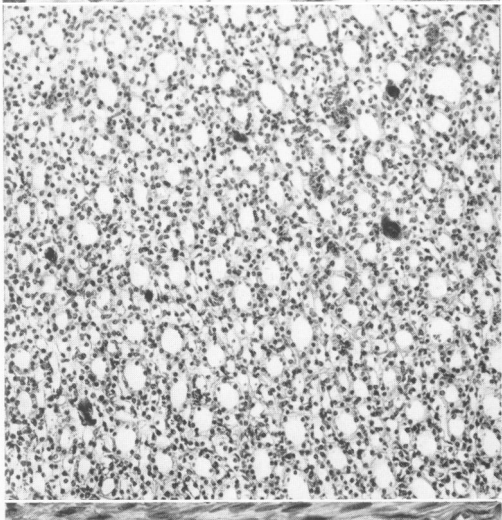

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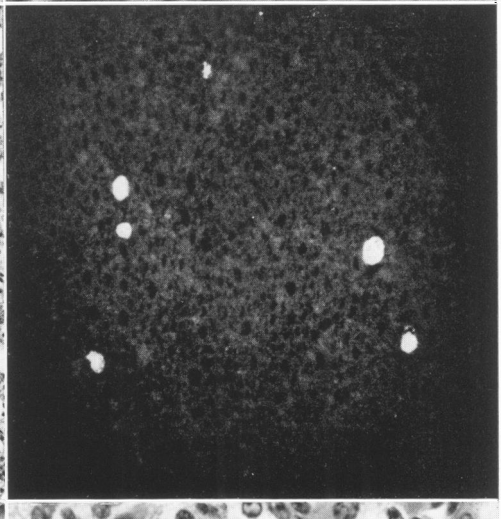

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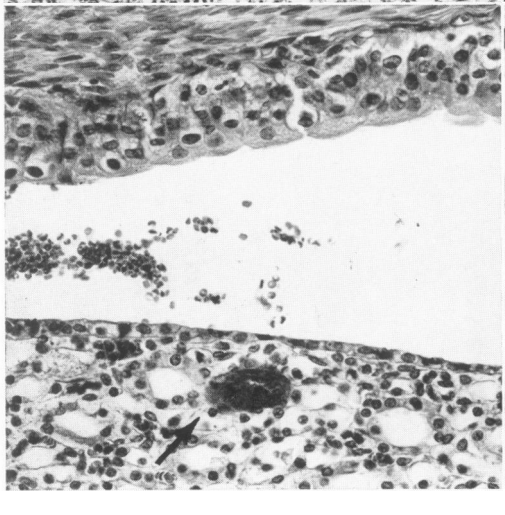

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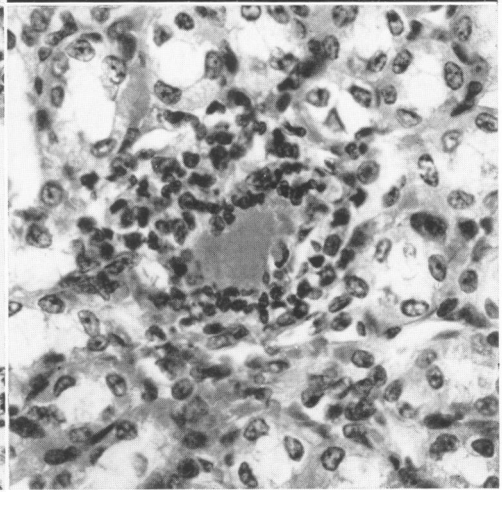

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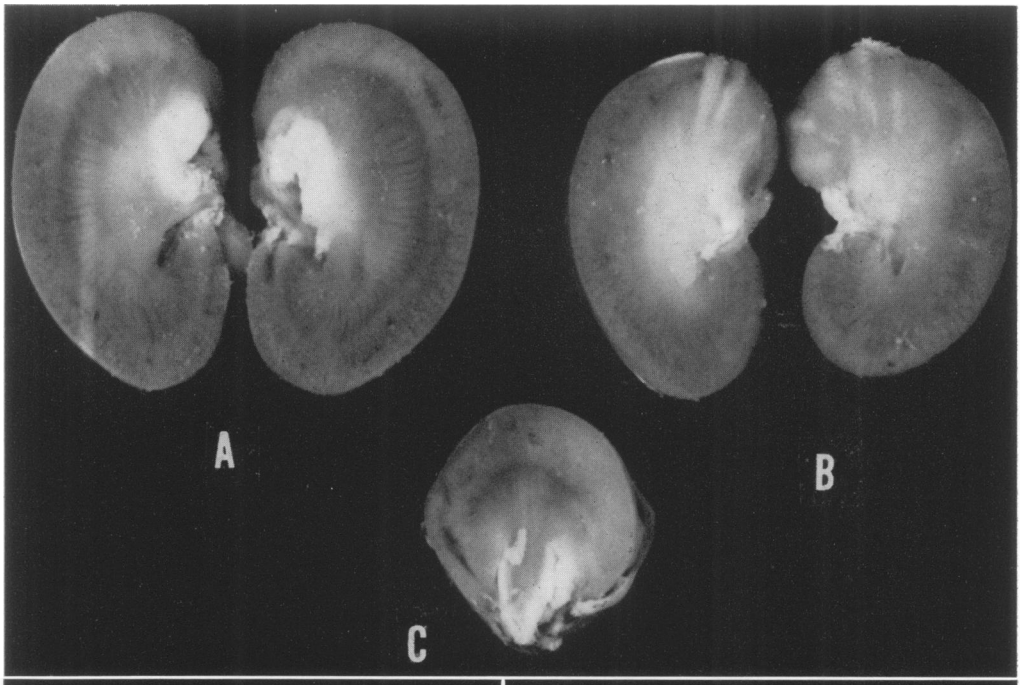
Figures 10A to 12: Acute retrograde pyelonephritis.

FIG. 10A. Kidney from an animal sacrificed 1 week after infection. Necrosis is limited to the lining of the pelvis in one pole of the kidney.

FIG. 10B. A wedge-shaped area of necrosis radiates to the cortex.

FIG. 10C. The necrosis involves the sides and tip of the papilla as well as the pelvis.

FIGS. 11 and 12. Kidneys from animals 1 week after retrograde infection. There is cortical involvement and abscess formation.



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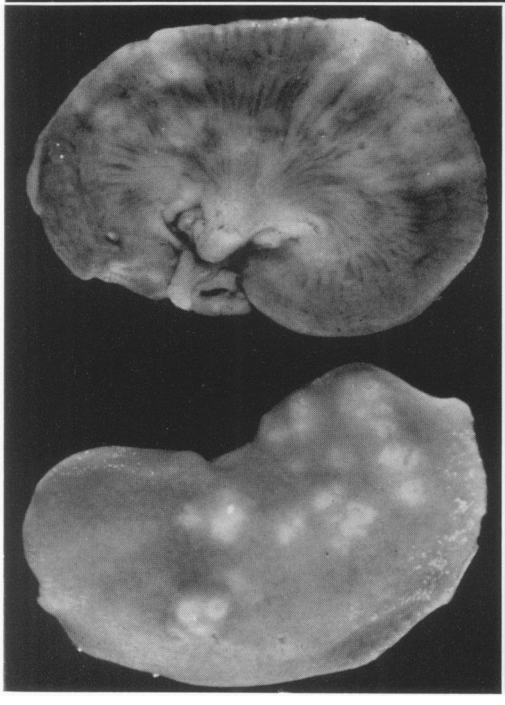

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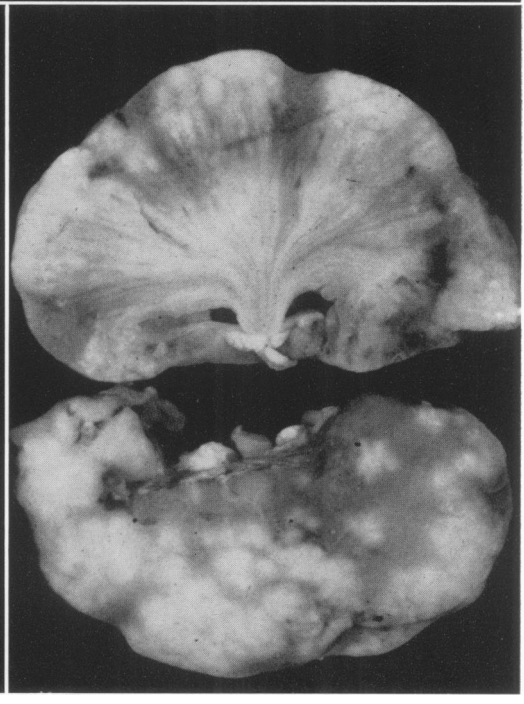

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Figures 13 to 18: Acute retrograde pyelonephritis.

FIG. 13. Four days. The medulla exhibits tubules containing neutrophils and necrotic debris. × 200.

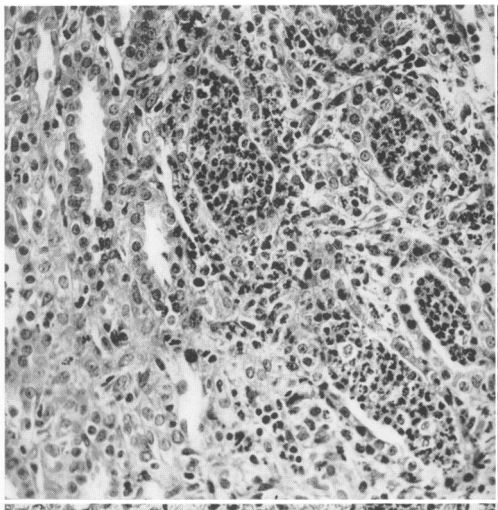
FIG. 14. Four days. The medulla is stained with anti-Proteus fluor. Bacteria and bacterial antigen are fluorescent within two tubules and (at arrow) in the interstitium. The round autofluorescent cells in the tubules are neutrophils. Approximately × 500.

FIG. 15. Four days. In the medulla totally necrotic tubules are filled with bacteria and bacterial fragments and surrounded by an intense neutrophil infiltrate. × 200.

FIG. 16. A field similar to that shown in Figure 15. Stained with anti-Proteus fluor. Approximately × 90.

FIG. 17. Renal pelvis in an animal sacrificed 4 days after infection. Fibroblastic and vascular proliferation are evident. × 200.

FIG. 18. Renal medulla, 4 weeks after infection. A mononuclear interstitial infiltrate now surrounds the tubules. × 200.



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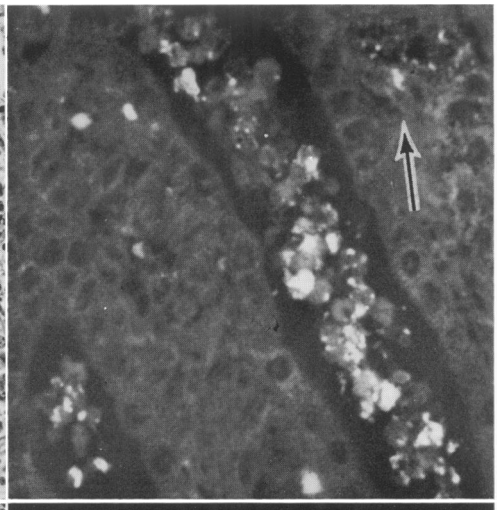

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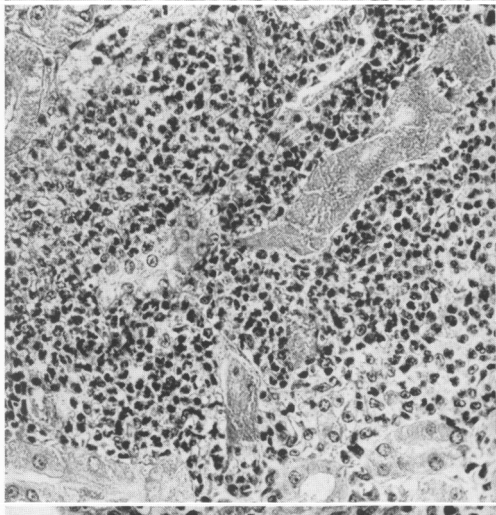

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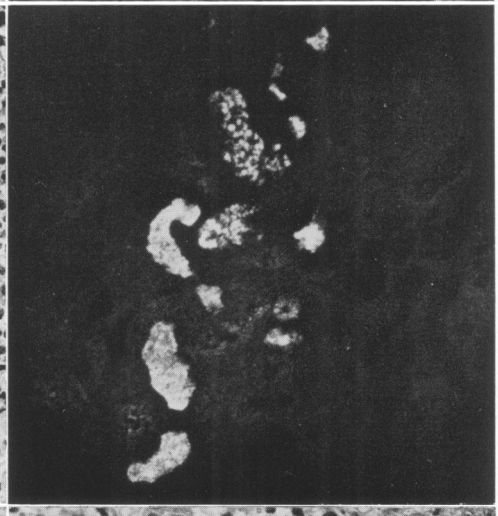

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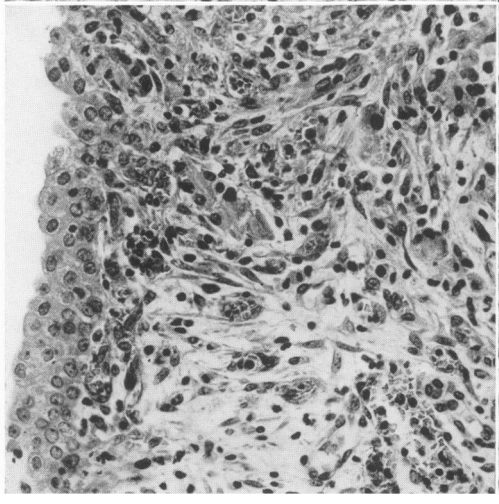

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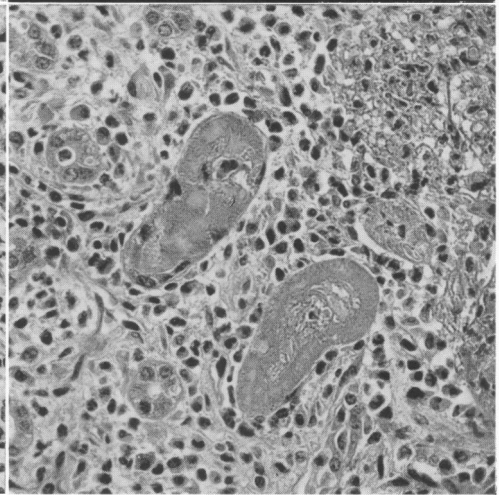

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Figures 19 to 24: Chronic retrograde pyelonephritis. All kidneys were bacteriologically positive for *P. mirabilis*.

FIG. 19. Right kidney from an animal sacrificed 4 months after infection. This kidney is larger than normal and has one deep scar. The tubules (arrow) are filled with "pus casts." × 5.

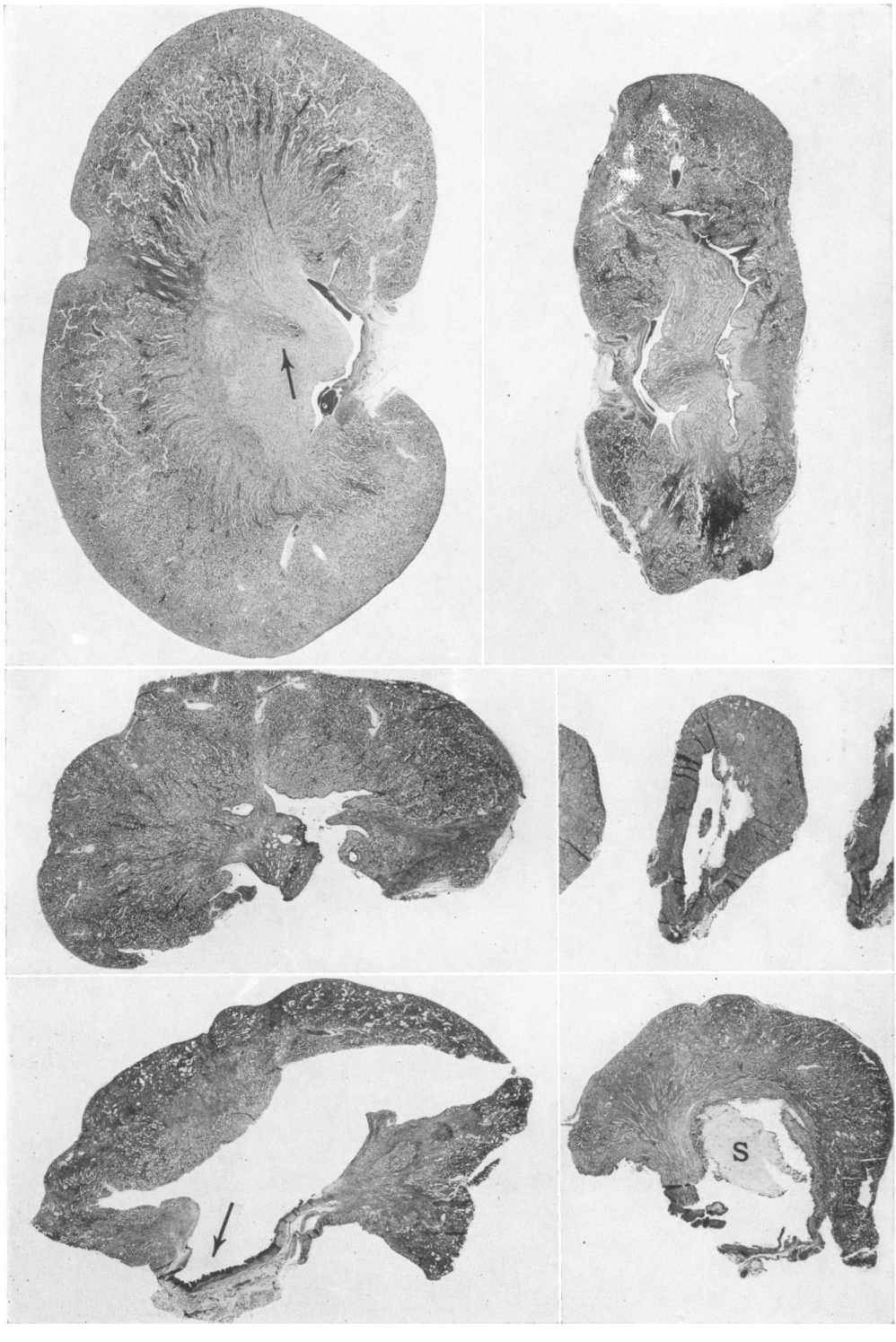
FIG. 20. Left kidney from the animal shown in Figure 19. Contraction is associated with extensive scarring and deformity of the papilla. × 5.

FIG. 21. A kidney, 5 months after infection, is normal in size and shows slight scarring. There is marked deformity of the papilla. × 5.

FIG. 22. A kidney, 12½ months after infection, is contracted and diffusely scarred. × 5.

FIG. 23. A kidney 6 months after infection. Hydronephrosis and atrophy are attributable to obstruction by vesical calculi. The pelvic mucosa exhibits papillary hyperplasia (arrow). × 5.

FIG. 24. A kidney, 5 months after infection, exhibits a stone (S) filling the pelvis. × 5.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Figures 25 to 29: Chronic retrograde pyelonephritis.

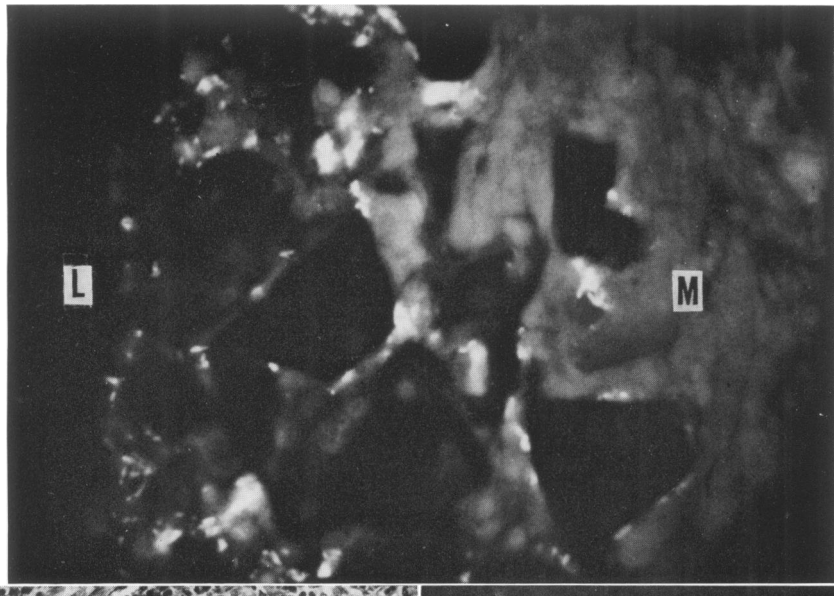
FIG. 25. Renal pelvis in an animal sacrificed 6 months after infection. Bacteria and antigenic material appear in the lumen (L) and are adherent to the pelvic mucosa (M).

FIG. 26. A mononuclear cell infiltrate, macrophages and atrophic tubules are evident 4 months after infection. × 200.

FIG. 27. Specific amorphous antigen is demonstrable in macrophages and in the interstitium about the tubules. Four months after infection. Stained with anti-Proteus fluor. Approximately × 200.

FIG. 28. PAS-positive granular and globular material is evident in cells (see text). Plasma cells and lymphocytes are shown. Periodic acid-Schiff stain. × 400.

FIG. 29. Two binucleate macrophages contain specific antigen and are surrounded by autofluorescent (gray background) plasma cells and lymphocytes. Approximately × 500.



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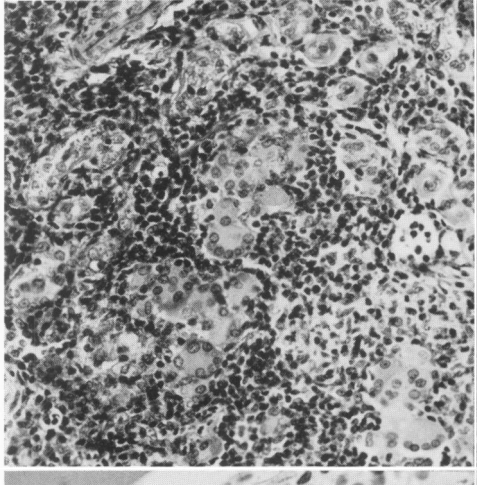

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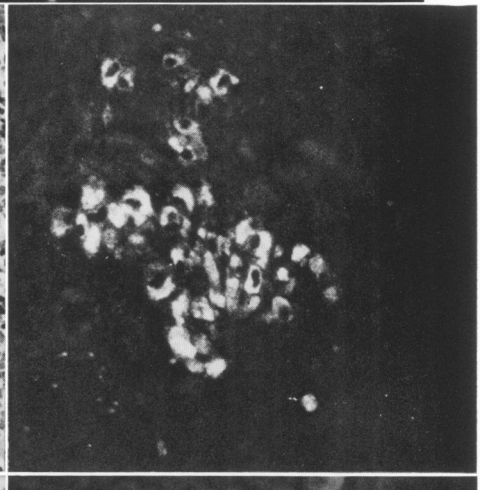

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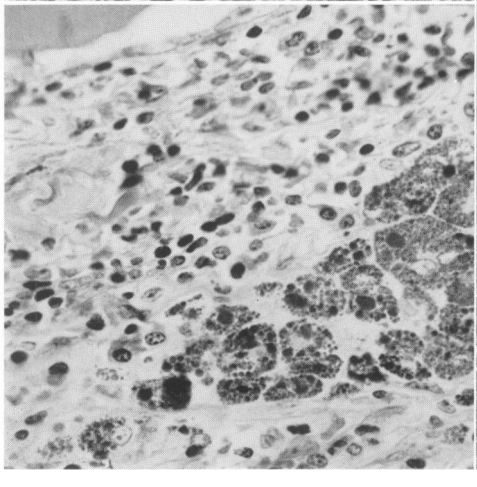

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