RETROGRADE PROTEUS PYELONEPHRITIS IN RATS

Localization of Antigen and Antibody in Treated Sterile Pyelonephritic Kidneys

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Plates 51 to 54

(Received for publication, October 31, 1962)

The role played by bacteria or bacterial products in the pathogenesis of the renal lesion in chronic pyelonephritis in man is unclear. While there is little doubt that bacterial invasion is the casue of the acute inflammatory changes in acute or recurrent acute pyelonephritis, there is no clear evidence that progression of the disease and the development of the classical lesions of chronic pyelonephritis (1, 2) are always related to continued bacterial proliferation. Clinicopathological studies have emphasized the discrepancy between pathological and bacteriological findings as well as the frequent absence of a history of repeated attacks of the acute disease in human chronic pyelonephritis (1, 3–6).

A study of one aspect of the problem was stimulated by the finding that amorphous bacterial antigen without recognizable bacterial bodies persisted for long periods of time in the areas of renal parenchymal inflammation in retrograde chronic pyelonephritis produced by *Proteus mirabilis* in the rat (7). However, in the reported experimental model, bacteria could be cultured from the kidneys in all animals with chronic pyelonephritis, so that it was impossible to determine the degree of persistence of bacterial antigen and the associated renal pathologic changes that might occur in the absence of bacterial proliferation. We therefore have studied the course of retrograde proteus pyelonephritis in infected animals that were treated until their kidneys became sterile. It will be shown that *P. mirabilis* antigen, identified by fluorescent antibody (FA) techniques, persists in the kidneys for at least 20 weeks after sterilization of renal tissue. The persistent antigen is accompanied by renal inflammatory reactions associated primarily with antibody-producing cells.

* Aided by grants from the National Institutes of Health, United States Public Health Service (Dr. Edward H. Kass).

Materials and Methods

These have been described previously (7) except as noted below. In brief, female rats weighing 150 to 200 gm were inoculated with 10^8 *P. mirabilis* into the bladder by the transurethral procedure, and after the infection had become established antibacterial therapy was begun. 9 days to 20 weeks after cessation of treatment with antibiotics (see below) animals were sacrificed and their kidneys removed under sterile conditions. Half of each kidney was cultured and the other half used for histologic and immunohistologic studies. When gross lesions were present, a portion of the gross lesion was always cultured. Bladder wall and/or bladder urine was also cultured.

Treatment of infected animals.—4 days following infection, the animals were given 100 mg of kanamycin per kilogram of body weight, injected subcutaneously once daily for 7 to 12 days. Urinalysis and postmortem examination of a random group of animals showed that the infection was not eradicated in most animals. They were then given 50 mg/kg of kanamycin and 50 mg/kg of chloramphenicol twice daily subcutaneously for 14 days. This regimen eradicated the infection in the majority of animals.

In addition, six animals received the combination of kanamycin and chloramphenicol without prior administration of kanamycin alone.

Fluorescent Antibody Studies.—

1. Localization of proteus antigen: Direct staining of sections by fluorescein isothiocyanate (FITC)—labeled rabbit anti proteus serum was used. The antisera and conjugates against *P. mirabilis* were prepared as in the previous report (7).

2. Localization of rat gamma globulin: The direct staining method was used. Antisera against rat gamma globulin were prepared as follows: Rat serum was fractionated by one-step chromatography on columns of diethylaminoethyl cellulose (DEAE) using pH 6.6, 0.015 M phosphate as the buffer. The eluate was concentrated by dialysis against 25 per cent polyvinylpyrolidone and dialyzed against pH 7.2 phosphate-buffered saline (PBS). 25 mg were mixed with Freund's adjuvant and injected into rabbits in four intramuscular sites. After 3 to 4 weeks a second similar injection was given; the rabbits were bled 1 week after the last injection and the sera were pooled. The pooled antiserum was studied by immunoelectrophoresis against whole rat serum and gave a single line of precipitation against gamma globulin and 3 to 4 faint lines against β-globulin components. The antiserum was then conjugated with fluorescein isothiocyanate and fractionated using column chromatography according to Riggs *et al.* (8).

3. Localization of proteus antibody: The "sandwich" technique (9), used successfully by White to localize *Proteus vulgaris* antibody in tissues (10), was employed. The method consisted of layering the tissue sections with unlabeled *P. mirabilis* antigen for 30 to 45 minutes, followed by washing with PBS, followed by staining with the specific antiproteus conjugate. This procedure results in staining of both proteus antibody and proteus antigen; therefore the staining was always compared with a contiguous section stained for antigen alone. The proteus antigen used for the first layer was prepared according to White (10).

Since it was desirable to study localization of antigen, antibody, and rat globulin in the same tissues, and since formalin fixation abolished staining of the latter by FA, tissues for FA studies were processed by cold alcohol fixation and paraffin embedding according to Sainte-Marie (11). This procedure resulted in adequate preservation of both the proteus antigen and rat globulin. After fixation in 95 per cent alcohol at 4°C for 16 to 24 hours, the tissues were dehydrated in three changes of absolute alcohol and passed through three changes of xylene (1 hour for each change), all at 4°C. They were then infiltrated with and embedded in a mixture of Waterman's paraffin (75 per cent) and Harleco's synthetic resin (25 per cent) at 56°C. Sections were cut at 2 to 4 μ, placed on non-albuminized slides and dried at 37°C

for 2 hours. They were then deparaffinized in three changes of xylene, followed by three of alcohol and one of PBS, all at room temperature. The conjugate was then layered on the section for 45 minutes; sections were washed three times in PBS, mounted, and sealed.

Controls for specificity of staining were similar to those previously employed (7).

RESULTS

Table I is a summary of the bacteriological and pathological findings in the kidneys of a group of animals sacrificed 1½ to 20 weeks after cessation of treatment.

Two animals did not respond to treatment in that *P. mirabilis* was cultured from the infected kidneys at the time of sacrifice. Fourteen animals had bacteriologically sterile kidneys and showed neither gross nor microscopic evidence of pyelonephritis; these animals were considered not to have had initial renal infection or to have had only mild pyelitis which responded to treatment without residuum. Twenty-two animals had bacteriologically sterile kidneys bilaterally but showed morphologic evidence of pyelonephritis. The description of the gross, microscopic, and immunohistologic findings which follows is based on the study of these twenty-two animals. Untreated animals with retrograde proteus pyelonephritis all have culturable organisms in the infected kidneys at comparable times of sacrifice (7).

TABLE I

Bacteriologic and Histologic Findings 1½ to 20 Weeks after Cessation of Treatment

Time after cessation of treatment	No. of animals	No. of animals with:		
		Histologic evidence of pyelonephritis		No evidence of pyelonephritis
		Sterile	Infected	
wks.				
1½ to 4	12	7	1	4
5 to 10	10	5	0	5
11 to 15	9	5	1	3
16 to 20	7	5	0	2
Total	38	22	2	14

Gross Pathology.—The gross lesions consisted of irregular shallow scars on the cortical surfaces of the pyelonephritic kidneys. The scars were often confluent and some kidneys showed considerable contraction. The number and size of the gross scars were generally less than in the untreated animals. As in the untreated animals, the infection was usually bilateral and unequal in severity. Several animals had gross lesions in one kidney and only microscopic pyelitis or pyelonephritis in the other.

None of the twenty-two sterile treated pyelonephritic animals had either vesical or renal calculi. Gross hydronephrosis was absent. Comparable untreated animals have calculi in 25 to 83 per cent of cases (7). The elimination of formation of calculi is undoubtedly due to elimination of multiplication of *P. mirabilis* with consequent absence of both urease production and consequent precipitation of magnesium ammonium phosphate stones. Vermuelen (12) found a decrease in average size of bladder stones in experimental proteus infections in rats after treatment with nitrofurantoin.

Histologic Lesions.—Treated and untreated animals differed in the extent of the acute inflammatory infiltrate throughout the course of infection. This was appreciated even in animals sacrificed 4 weeks after infection. In the untreated animals considerable renal acute inflammation and pelvic ulceration and necrosis was usually present, but in treated animals acute inflammation was slight and focal. Extensive areas of renal inflammation were found, (Fig. 1) but the cells were mostly plasma cells, monocytes, lymphocytes, and histiocytes (see below). Large pleomorphic macrophages, similar to those described in untreated animals, were seen frequently. Fibrosis and hyalinization (Fig. 2) were common and appeared promptly after treatment. However, foci of neutrophils in the interstitium, including occasional tubular pus casts, were found in treated sterile animals up to 14 weeks after presumed renal sterilization (Figs. 3 and 4). In some such kidneys, which were bacteriologically sterile at autopsy, fluorescent antibody studies (see below) failed to show antigen either within or in the vicinity of the pus casts. The pelvic epithelium was smooth in all cases, whereas pelvic ulceration and acute inflammation was common in untreated animals. In seven kidneys, the changes were confined to inflammation of the pelvis and lower medulla.

In spite of the absence of gross pelvic hydronephrosis, there were several microscopic foci of tubular dilatation suggesting areas of intrarenal hydronephrosis due to scarring.

Fluorescent Antibody Studies.—

Localization of antigen: (Table II). Sections from most kidneys positive in the gross and random kidneys negative in the gross were stained with anti-proteus conjugate. Identifiable specifically staining rods were uniformly absent; this is in contrast to untreated animals in which organisms were present in the pelvis, pelvic mucosa, within concretions and in renal abscesses.

The specific fluorescent staining in the pyelonephritic sterile kidneys was in the form of irregular granular and amorphous material. The material was mostly in macrophages but in some areas it was in the interstitium between atrophic tubules and apparently not within cells. The macrophages containing the antigen had round, oval, and sometimes elongated nuclei (Figs. 8–10). In some areas the cells were large and contained two or more nuclei (Fig. 8).

With the hematoxylin and eosin stain their cytoplasm was slightly eosinophilic and often granular, and their nuclei were faint-staining, finely stippled, and had no prominent nucleoli (Figs. 5 and 6). Sometimes an orange-brown granular pigment was also present in such cells. The pigment was iron-positive.

The amount of fluorescent proteus antigen was correlated roughly with the size of the lesion; the kidneys that had large scars contained the most antigen, while small scars were devoid of antigen even within 9 days of treatment. The antigen was found equally in the cortex and the medulla. The pelvic mucosa was usually free, but the submucosal layers sometimes contained abundant antigen (Fig. 7). There was no correlation between the presence of antigen and either interstitial neutrophils or white blood cell tubular casts. Some kidneys

TABLE II

Localization of P. mirabilis Antigen by FA in Kidneys with Treated P. mirabilis pyelonephritis

Time after cessation of treatment	No. of animals	No. of kidneys with antigen per No. of kidneys studied
wks.		
1½ to 4	7	5/8
5 to 10	5	3/5
11 to 15	5	2/4
16 to 20	5	4/6

with obvious tubular pus casts (Fig. 4) had no identifiable antigen in several sections, either in or around the tubules or in the cortical areas drained by these tubules. The cell type most often associated with antigen was the plasma cell, and to a lesser degree the lymphocyte. In some areas sheets of plasma cells, including young large forms and forms containing Russell bodies infiltrated the interstitium (Figs. 11 and 12).

In addition to plasma cells and lymphocytes, infiltrates contained cells with large nuclei, prominent nucleoli and basophilic cytoplasm resembling reticulum cells. These were sometimes present in the pelvic infiltrates (Fig. 13) and within the renal tissue proper. In a few sections from kidneys containing abundant antigen, the infiltrate in the cortex took the form of a lymphatic follicle, with large reticulum cells in the center and a mantle of lymphocytes around (Figs. 14 and 15).

In those scars showing little or no antigen by FA, the interstitial tissue showed fibrosis and a cellular infiltrate composed predominantly of lymphocytes.

Localization of rat gamma globulin: When representative sections were stained with FITC-labeled rabbit anti-rat globulin, the cells showing specific staining were the plasma cell forms in the interstitial infiltrates (Figs. 16 and 17). The

round forms stained adequately, but the eccentric and Russell body forms were brightest.

Comparison of the FA-stained sections with hematoxylin and eosin–stained sections showed that many more plasma cells stained with hematoxylin and eosin were seen than those stained by FA. The antigen-containing macrophages that stained brightly with anti-proteus antigen were non-fluorescent or only autofluorescent when stained with rabbit anti-rat globulin. In several areas, gamma globulin–containing plasma cells were seen to surround foci of macrophages that were unstained with the anti-rat globulin.

In addition to plasma cells, the rabbit anti-rat globulin stained some "colloid" casts within tubular lumina (presumable filtered protein), granules within tubular cells (absorbed protein) as well as some tubular basement membranes and intertubular tissue. These structures did not stain with rabbit anti-proteus fluor; their staining was specific, as confirmed by inhibition tests.

Although the anti-rat globulin conjugate did contain anti-rat beta globulin antibodies in addition to anti-rat gamma globulin, it can be assumed from other studies that the fluorescent substance stained in the plasma cells was gamma globulin (13).

Localization of proteus antibody: No attempt was made to study the details of the times and sites of antibody production in pyelonephritis. Selected sections from sterile pyelonephritic kidneys were studied in order to determine whether some of the gamma globulin–containing cells described above contained antibody to the proteus antigen. It was found that many cells of the plasma-cell series in the renal interstitial infiltrates showed the characteristic staining of the cytoplasm and of Russell bodies, when the sections were stained for specific proteus antibody. The method also stained the antigen containing macrophages. Figs. 18 and 19 are from contiguous sections stained for proteus antibody and proteus antigen respectively.

Comparison of sections stained for proteus antibody with sections stained for rabbit anti-rat globulin showed approximately the same number of plasma cells stained by both techniques, although it was not possible to determine whether the same cells stained both for rat gamma globulin and for proteus antibody. The sections stained for proteus antibody showed no staining of the tubular protein casts, basement membranes and interstitial material as did those stained for rat-globulin.

DISCUSSION

The reported experiments provide supplementary information to that derived from previously described studies on the course of experimental retrograde *P. mirabilis* pyelonephritis in the rat. The tentative conclusion that *P. mirabilis* antigen can persist in renal tissues for a considerable time after infection is here confirmed, since it was present at least 20 weeks after the kidneys had

presumably become sterile. The large amount of antigen released from the initial proliferation of organisms in the kidneys is apparently more than can be handled immediately, and persistence in the interstitium and in macrophages follows. The antigen so released is eventually cleared. Areas of scarring without antigen are found as early as 9 days after sterilization of the infection. It is presumed that eventually all antigenic material is broken down, the duration of persistence of antigen depending upon the degree of initial or continued bacterial proliferation and thus upon the severity of the lesion. The antigen was seen in the form of aggregates of amorphous and granular material, resembling the microscopic appearance of disrupted bacteria or crude preparations of cell wall antigen.

The distribution and the persistence of Gram-negative rod antigens has been the subject of a few fluorescent antibody studies (14–17). In particular, Wood and White (14) found that *P. mirabilis* antigen persisted locally and in the liver at least 3 weeks after a single subcutaneous injection of a heat-killed vaccine in mice. They also described localization and persistence of *P. mirabilis* antigen in the kidney glomeruli with the development of glomerulonephritis, after repeated subcutaneous injections of the vaccine in mice. In similar experiments in rats, we have confirmed the persistence of *P. mirabilis* endotoxin locally and in the liver for at least 8 weeks after intracardiac and subcutaneous injections, but have failed to detect any preferential persistence in the glomerular basement membranes or to find any glomerular lesions after repeated injections of vaccine.

In the pathogenesis of chronic pyelonephritis, the possible role of antigenic bacterial products, including endotoxin, in the progression of the renal lesion has been the subject of speculation. Sanford *et al.* (18) found that *E. coli* somatic antigen persisted in renal scars at least 4 weeks following hematogenous *E. coli* pyelonephritis in rats, at a time when the kidneys were sterile. At that time the scars showed "moderate to minimal mononuclear" infiltration. The authors concluded that the antigen did not contribute to continuing inflammatory changes or progressive scarring.

From the studies reported here, it seems likely that the persistence of antigen contributes to the continuance of a chronic inflammatory reaction associated with production of antibody. The FA studies indicate (*a*) that the presence of gamma globulin–containing cells in the interstitial infiltrates is related to the presence of proteus antigen and (*b*) that at least some of those cells contain antibody to proteus antigen. Whether the persistent inflammatory reaction is also directed to renal tissue immunologically altered by bacterial endotoxin cannot be answered from these studies.

In spite of persistent antigen and chronic inflammatory cells in the chronic scars, there was no evidence in our experiments that the renal lesions had progressed. The size and numbers of gross lesions in the infected kidneys were

essentially similar throughout the course of study, and individual scars were completely healed even at early intervals of sacrifice. In addition, although some neutrophils and white blood cell casts were present in the bacteriologically healed lesions, these were uncommon and did not correlate with the presence of antigen. It is therefore possible to assume that persistent antigen did not result in continued acute inflammation or in progressive scarring.

Comparison of the findings with untreated proteus pyelonephritis (7) indicates that the development of calculi as well as the persistence of substantial acute pelvic and renal inflammation in the untreated animals is related to continued bacterial proliferation.

SUMMARY

Rats with retrograde proteus pyelonephritis were treated with antibiotics until their kidneys became sterile. Using fluorescent antibody techniques, specific *P. mirabilis* antigen was found in some sterile pyelonephritic kidneys 20 weeks after cessation of treatment and presumed renal sterilization. Persistent antigen was associated with interstitial chronic inflammation but not with acute inflammation or progressive scarring. Rat gamma globulin and proteus antibody were localized in plasma cells of the renal inflammatory infiltrates. It is suggested that persistent antigen in chronic pyelonephritis may lead to the continued local appearance of antibody-producing cells.

The advice and help of Dr. Edward H. Kass and the technical assistance of Miss Marilyn Miller and Miss Mary Kendrick are gratefully acknowledged.

BIBLIOGRAPHY

1. Weiss, S., and Parker, F., Pyelonephritis: its relation to vascular lesions and to arterial hypertension, *Medicine*, 1939, **18**, 221.
2. Kimmelsteil, P., Significance of chronic pyelonephritis, *in* Biology of Pyelonephritis, (E. Quinn, and E. H. Kass, editors), Boston, Little, Brown and Co., 1960, chapter 14, 215.
3. Relman, A. S., Some clinical aspects of chronic pyelonephritis, *in* Biology of Pyelonephritis (E. Quinn, and E. H. Kass, editors), Boston, Little, Brown and Co., 1960, 355.
4. Jacobson, M. H., and Newman, W., Study of pyelonephritis using renal biopsy material, *A.M.A. Arch. Int. Med.*, 1962, **110**, 211.
5. Biology of Pyelonephritis (E. Quinn, and E. H. Kass, editors), Boston, Little, Brown and Co., 1960, discussions by Beeson, P., p.109; Kark, R., p. 274; Relman, A., p. 413; Jackson, G., p. 480; Kass, E. H., p. 695.
6. Kleeman, S., and Freedman, L., The finding of chronic pyelonephritis in males and females at autopsy, *New England J. Med.*, 1960, **263**, 988.
7. Cotran, R., Vivaldi, E., Zangwill, D., and Kass, E. H., Retrograde proteus pyelonephritis in rats: Bacteriologic, pathologic and fluorescent-antibody studies, *Am. J. Path*, in press.

8. RIGGS, J. L., LOH, P., AND EVELAND, W. C., A simple fractionation method for preparation of fluorescein-labelled gamma-globulin, *Proc. Soc. Exp. Biol. and Med.*, 1960, **105,** 655.
9. Coons, A. H., Leduc, E. H., and Connolly, J. M., Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit, *J. Exp. Med.*, 1955, **102,** 49.
10. White, R. C., Observations on the formation and nature of Russell bodies, *Brit. J. Exp. Path.*, 1954, **35,** 365.
11. Sainte-Marie, G., A paraffin embedding technique for studies employing immuno-fluorescence, *J. Histochem. and Cytochem.*, 1962, **10,** 250.
12. Vermeulen, C. W., and Goetz, R., Experimental urolithiasis. VIII. Furadantin in treatment of experimental proteus infection with stone formation, *J. Urol.*, 1954, **72,** 99.
13. Ortega, L. G., and Mellors, R. C., Cellular sites of formation of gamma globulin, *J. Exp. Med.*, 1957, **106,** 627.
14. Wood, C., and White, R. G., Experimental glomerulonephritis produced in mice by subcutaneous injections of heat-killed *Proteus mirabilis*, *Brit. J. Exp. Path.*, 1956, **37,** 49.
15. Cremer, N., and Watson, D., Influence of stress on the distribution of endotoxin in the reticuloendothelial system by fluorescent-antibody technique, *Proc. Soc. Exp. Biol. and Med.* 1957, **95,** 510.
16. Tanaka, N., Nishimara, T., and Yoshiyaki, T., Histochemical studies on the cellular distribution of endotoxin of *Salmonella enteritidis* in mouse tissues, *Japan J. Microbiol.*, 1959, **3,** 191.
17. Rubenstein, H. S., Fine, J., and Coons, A. H., The distribution of endotoxin in the dog in lethal endotoxemia as determined by fluorescent antibody, *Fed. Proc.*, 1962, **21,** 275.
18. Sanford, J., Hunter, B., and Donaldson, P., Localization and fate of *Escherichia coli* in hematogenous pyelonephritis, *J. Exp. Med.*, 1962, **116,** 205.

EXPLANATION OF PLATES

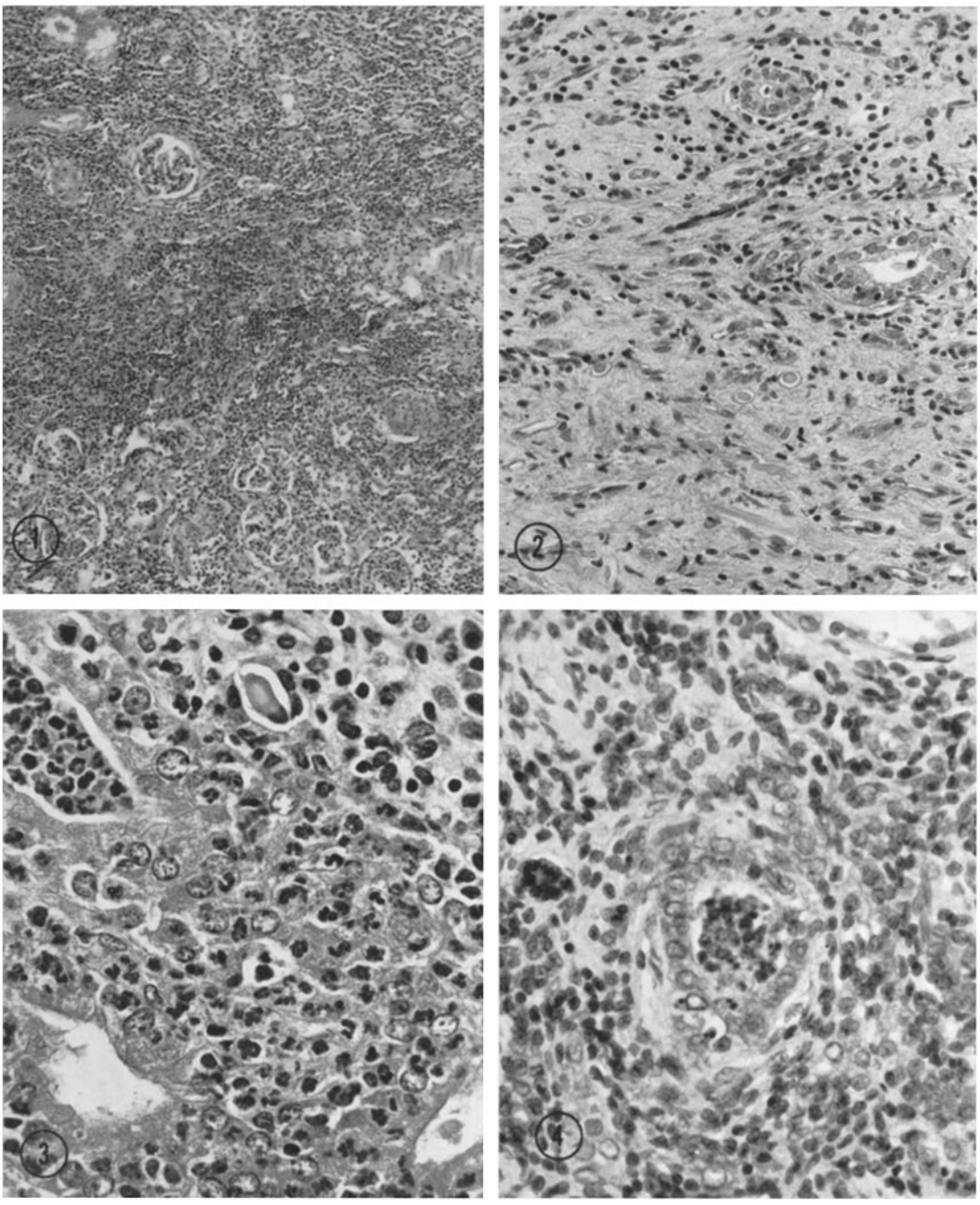
PLATE 51

FIG. 1. Cortical focus of chronic inflammation, periglomerular fibrosis, and tubular atrophy. 16 weeks after cessation of treatment. Hematoxylin and eosin. × 90.

FIG. 2. Fibrosis and hyalinization, with only little inflammatory reaction. 7 weeks after treatment. × 210.

FIG. 3. Neutrophils in a tubule (top left) and in the interstitium 6½ weeks after treatment. The kidney was sterile, but contained abundant antigen. Hematoxylin and eosin. × 400.

FIG. 4. Neutrophils in a tubule 14 weeks after sterilization. The kidney was sterile and no antigen was found in 8 sections studied. Hematoxylin and eosin. × 400.



(Cotran: Retrograde proteus pyelonephritis)

PLATE 52

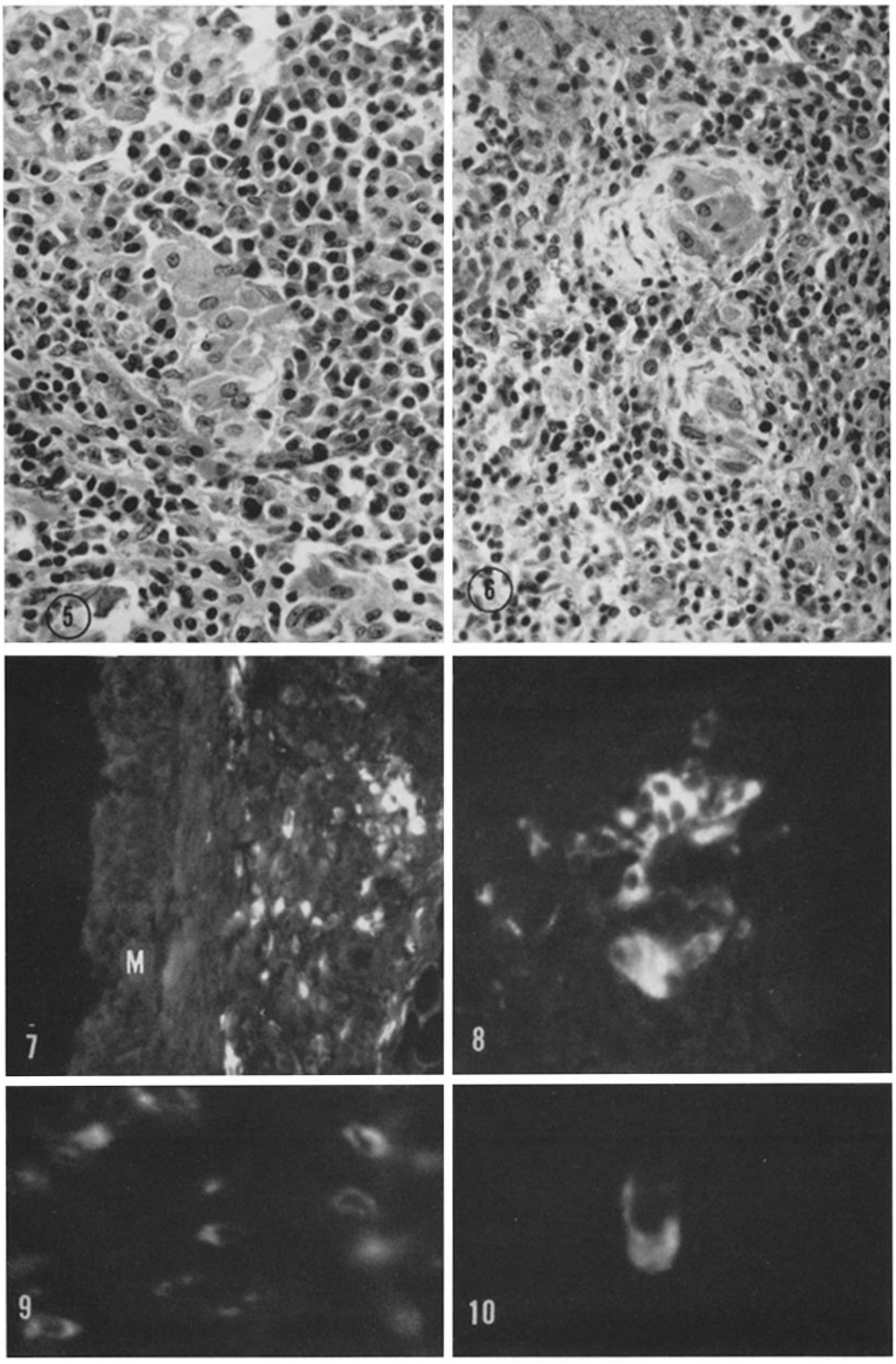
FIGS. 5 and 6. Collections of macrophages surrounded by lymphocytes and many plasma cells Antigen was present in such collections when contiguous sections were stained with anti-proteus fluor. × 400.

FIG. 7. Section from sterile pyelonephritic kidney stained with rabbit anti-proteus fluor. Antigen in cells in connective tissue beneath pelvic mucosa. (*M*) and into peri-pelvic fat (left lower corner). × approximately 300.

FIG. 8. Antigen in mononucleate and a binucleate cell around a glomerulus, Note granular appearance of antigen. × approximately 500.

FIG. 9. Higher magnification shows amorphous granular antigen in macrophages with elongated nuclei. × approximately 750.

FIG. 10. Same as Fig. 9. Cell with a more rounded nucleus. × approximately 1000.



(Cotran: Retrograde proteus pyelonephritis)

PLATE 53

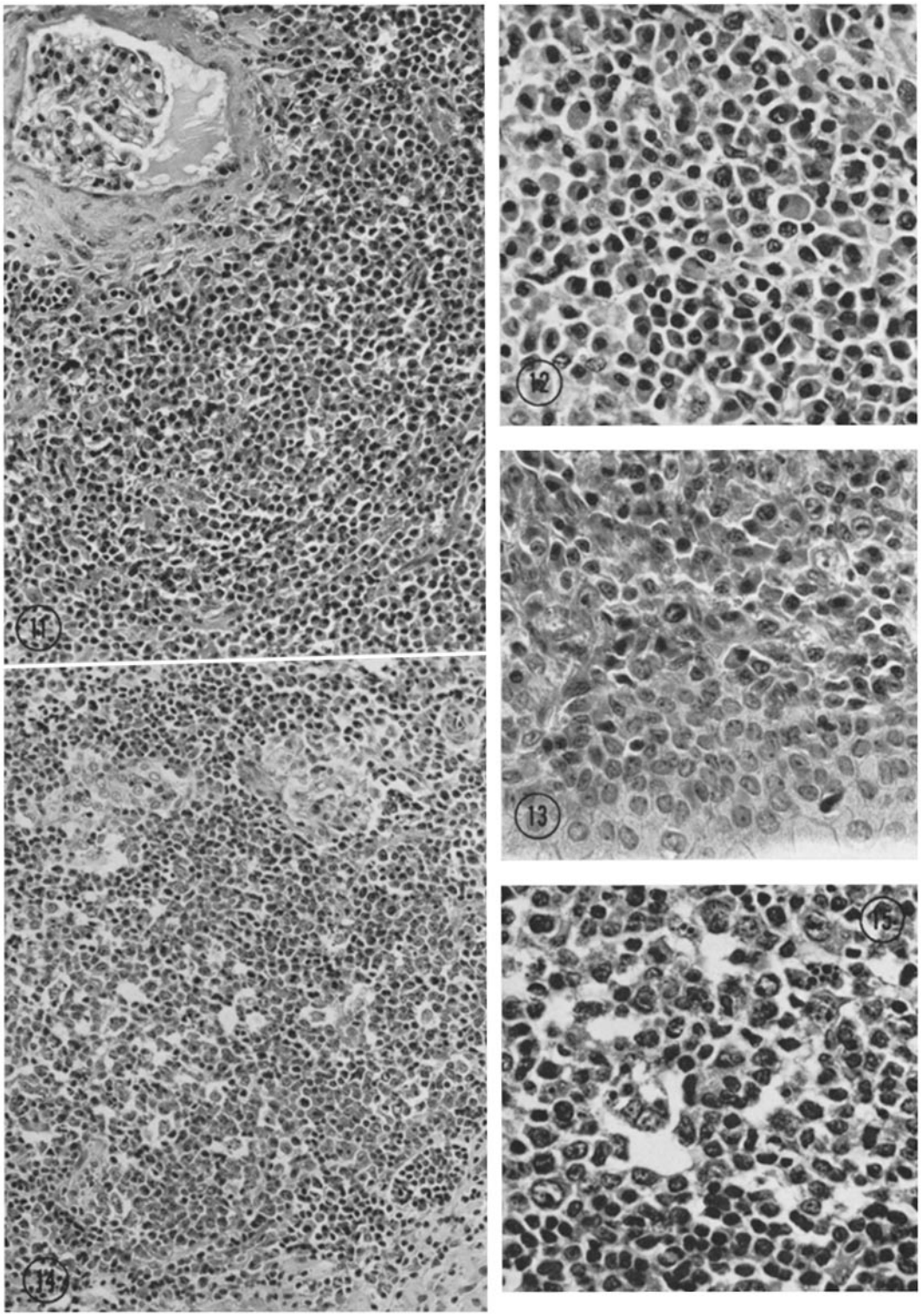
FIG. 11. A sheet of plasma cells surrounding glomerulus with periglomerular fibrosis. 7 weeks after cessation of treatment. Hematoxylin and eosin. × 210.

FIG. 12. One area from same field as Fig. 11, containing mostly mature plasma cells. Hematoxylin and eosin.

FIG. 13. Chronic inflammatory cells, including lymphocytes, plasma cells, and reticulum cells beneath mucosa (bottom of picture). 8 weeks after treatment. Hematoxylin and eosin. × 400.

FIG. 14. Focus of severe cortical interstitial inflammation. The infiltrate is in a form suggesting a follicle, with large reticulum cells (Fig. 15) in center and smaller cells around. Hematoxylin and eosin.

FIG. 15. Higher magnification of same field as in Fig. 14. Note the large reticulum cells and lymphocytes. Hematoxylin and eosin. × 400.



(Cotran: Retrograde proteus pyelonephritis)

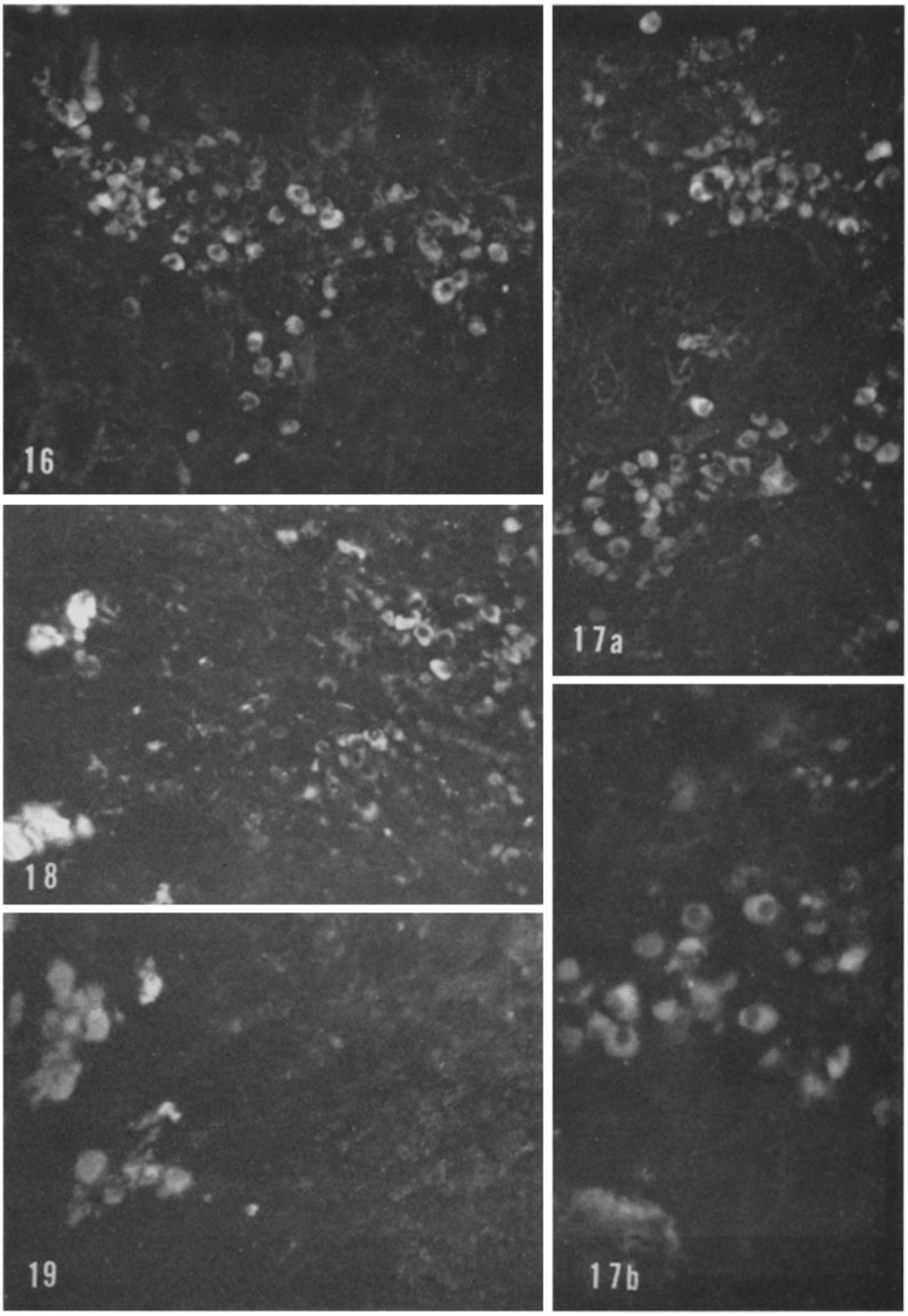
PLATE 54

FIG. 16. Plasma cells in the interstitial intertubular infiltrate stained with rabbit anti-rat globulin. Note characteristic cytoplasmic staining of eccentric and round plasma cell forms. × approximately 300.

FIG. 17. (*a*) Staining of the cytoplasm of plasma cells around a glomerulus (center) with rabbit anti-rat globulin. × approximately 300. (*b*) Same as *a*. × approximately 500.

FIG. 18. Section from pyelonephritic kidney stained for proteus antibody by the sandwich technique (see text). The fluorescent structures on the left are collections of antigen-containing macrophages. On the right are plasma cells with the characteristic staining of the cytoplasm for antibody. × approximately 300.

FIG. 19. Same area in contiguous section to that photographed in Fig. 18, stained with rabbit anti-proteus fluor. The staining of the antigen on the left is present, but the plasma cells in the field to the right do not stain. × 300.



(Cotran: Retrograde proteus pyelonephritis)