PYELITIS, AN IMPORTANT FACTOR IN THE PATHOGENESIS OF RETROGRADE PYELONEPHRITIS*

By BURTON R. ANDERSEN,‡ M.D., and GEORGE GEE JACKSON,§ M.D.

(From the Research and Educational Hospitals, Department of Medicine, University of Illinois College of Medicine, Chicago)

Plates 36 to 39

(Received for publication, April 10, 1961)

In the now classic report of Weiss and Parker (1) on pyelonephritis, one of the important observations was that pyelitis and cystitis were almost invariably accompanied by pyelonephritis. Their observations have been confirmed and have won support to the extent that pyelitis as an entity has been overshadowed and disregarded. Few attempts have been made to determine the role of pyelitis in the natural history of pyelonephritis. In the experiments to be reported, histologic observations on rats after retrograde infection of the urinary tract show that pyelitis was a consistent finding and of considerable significance.

Pyelonephritis in animals has been difficult to produce by blood stream infection unless the kidneys were injured either by urinary obstruction (2), postinfectious scarring (3), massage (4), or cauterization (5). In addition, the type of pyelonephritis that has generally been studied in animals is an acute process with abscesses and granulocytic infiltration. The most common type of pyelonephritis in human beings, for which we seek an experimental model, is a chronic disease with mononuclear infiltration, fibrosis, and infrequent abscess formation. This report describes the production of pyelonephritis in rats by a single retrograde infusion of bacteria without renal trauma or urinary tract obstruction. By this method a persistent urinary infection was established in which pyelitis was regularly related to the development of pyelonephritis. Physiologic observations on the occurrence of vesicoureteral reflux in rats and anatomic factors important in the production of pyelonephritis are discussed.

Materials and Methods

Female albino rats of the Sprague-Dawley strain were used in the experiments. Bacterial cultures were prepared by inoculation in nutrient broth and incubation at 37°C for 24 hours.

* These studies were supported by a research grant from the United States Public Health Service, contract E 1949 (C3).

‡ United States Public Health Service Research Fellow in infectious diseases, Department of Medicine, University of Illinois College of Medicine, Chicago.

§ Professor of Medicine, Department of Medicine, University of Illinois College of Medicine Chicago.

Under thiopental anesthesia 0.6 ml of the bacterial culture was infused with minimal pressure into the urethra, bladder, and ureters through the hub of a 20 gauge needle from which the shaft had been broken. The smooth, blunt end of the hub was introduced a short distance up the urethra, and the slack urethral tissue was grasped with one hand and drawn up to prevent leaking of the solution upon injection. This method produced essentially no urethral injury and consequently no urethral obstruction.

The data reported below includes two separate groups of animals which were infected with bacterial strains of different virulence. In the first group of 30 animals all of the bacterial species used were from patients with active urinary tract infections and were subcultured in the laboratory only once or twice. Five strains of *Escherichia coli*, three of *Klebsiella-Aerobacter sp.*, and two of *Proteus mirabilis* were used. Single strains of *Herrellea* and *Pseudomonas sp.* also were tested. The animals were observed for periods varying from 2 days to 3 months at which time they were sacrificed for examination.

In the second experimental group, 16 animals were inoculated with a single strain of *Klebsiella* sp. which had been obtained from a quadriplegic patient with chronic pyelonephritis and cystitis. This strain was chosen because of its low virulence as shown by infusion into the urinary tract of a rat where it failed to produce cortical or medullary lesions but was successfully maintained in the urinary tract and identified after a period of 7 weeks. The strain which was isolated at 7 weeks was then grown in broth and inoculated into 16 rats using the same retrograde method as in the first experiment. These animals were sacrificed at intervals over a 13 week period. The tissues of the urinary tract were studied for gross and microscopic lesions and for the presence or absence of bacteria. 6 uninjected animals and 10 animals injected with sterile broth were simultaneously studied.

RESULTS

A. The Initiation of Infection:—In the initial group of 30 animals given a single retrograde urinary tract injection of one of five different bacterial species and sacrificed at different times during the subsequent 3 months, 18 (60 per cent) showed gross and microscopic evidence of pyelonephritis. Of these, 14 (47 per cent) had developed moderate or severe, acute, and chronic pyelonephritis manifested by cortical abscesses, deep or numerous cortical scars, or contracted kidneys, whereas 4 (13 per cent) had only a few or shallow cortical scars but were clearly abnormal. Among the remaining 12 animals 1 infected with a *Klebsiella sp.* had only pyelitis which had persisted 7 weeks and 11 (37 per cent) had no evidence of an established infection. *Escherichia coli*, *Aerobacter aerogenes*, and *Proteus mirabilis* each caused severe pathologic lesions in several animals while *Pseudomonas* and *Herrellea* species produced abnormalities of less severity. Uninfected culture medium caused no pathologic lesions.

The animals in the second series inoculated with a strain of klebsiella species and sacrificed between 2 and 13 weeks showed gross cortical scarring and depressions in only 3 (19 per cent) of the animals, but pyelitis was a prominent lesion in 11 (69 per cent).

B. Vesicoureteral Reflux:—In evaluating the retrograde infusion of bacteria into the bladder as a means of producing pyelonephritis, the bladder capacity and vesicoureteral reflux were studied. The usual volume of urine in the rat bladder was determined in normal animals by clamping the urethra and aspi-

rating the urine in the bladder after the animal was sacrificed. The volume generally was less than 0.5 ml, but occasionally it was as great as 0.8 to 1.0 ml. When methylene blue was injected through the urethra of anesthetized female rats, vesicoureteral reflux usually occurred in the range of 0.25 to 0.3 ml, and a volume of 0.6 ml or more caused reflux in all of the animals. The rate of infusion did not seem to affect the critical volume with which reflux occurred. Reflux was observed in some animals with a volume of 0.25 ml given over a 5 minute period and in other animals reflux did not occur when the same volume was infused as rapidly as possible.

Under direct observation using a volume of 0.6 ml, which approximated the normal bladder capacity, there was no evidence of distention of the ureters or renal pelvis when reflux occurred. It was observed, however, that methylene blue entered even the distal ramifications of the pelvis. The intrarenal pressure

TABLE I

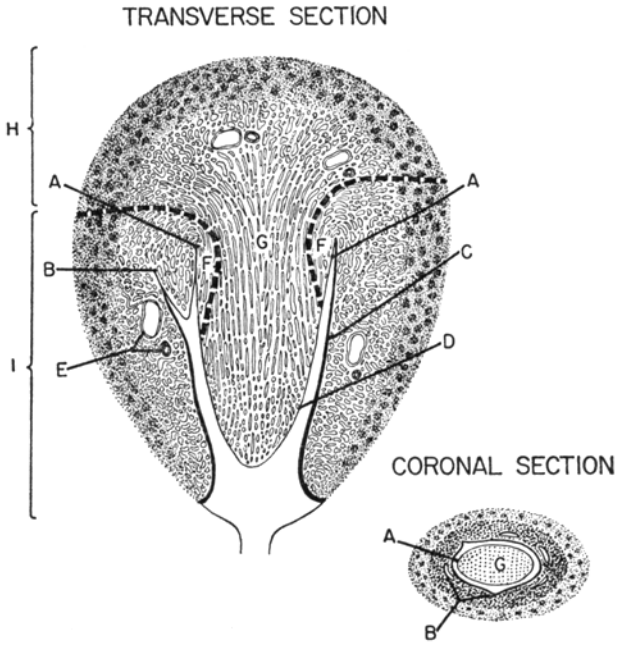
Tissue cultured	Positive cultures for *Klebsiella sp.* per total cultures			
	Uninoculated controls		Bacterial inoculation 2 to 13 weeks previously	
		per cent		*per cent*
Bladder	0/6	0	7/16	44
Ureter	0/12	0	11/32	34
Medulla	1/12	8	20/32	63
Cortex	0/12	0	7/32	22

from the inoculum was not great enough to give positive blood cultures 5 minutes after retrograde bacterial infusion except in 2 of 24 animals.

C. Bacteriology:—Urine and whole tissue from the kidneys, ureters, and bladder were obtained at the time of sacrifice for separate bacteriologic culture. Cultures of the bladder commonly showed bacterial species other than the one inoculated; these were believed to be contaminants introduced from the urethra by manipulation at the time of sacrifice. Contaminants were only rarely isolated from the other tissues. The frequency of positive cultures from different parts of the urinary tract following inoculation with a strain of *Klebsiella sp.* is shown in Table I.

In animals observed in the second experiment, in addition to the other cultures a separate culture of the renal cortex was obtained by slicing a section from each kidney, avoiding medullary and pelvic tissue. The medulla, pelvis, and the remainder of the cortex were incubated together in a separate vessel. As shown in Table I the cortex was often sterile and was never involved unless bacteria were also in the medullary portion of the kidney. Although some of the animals were not infected or had eliminated the klebsiella infection from

the genitourinary tract, the specific infection persisted in many animals throughout the entire period of 13 weeks that was available for observation after the inoculation. Also as shown in Table I cultures of urinary tract tissues from control animals and those previously infused with sterile broth yielded no bacterial growth with one exception.



TEXT-FIG. 1. Sketches of transverse and coronal cross-sections of a rat kidney showing the spatial relationships. *A*, pelvic apex; *B*, pelvic fornix; *C*, parietal epithelium (area of greatest pyelitis); *D*, papillary epithelium (area of least pyelitis); *E*, artery and vein; *F*, medullary area most severely involved; *G*, medullary area least severely involved; *H*, cortical area least often involved, *I*, cortical area most often involved. The heavy hatched lines separate the centrally located areas of cortex (*H*) and medulla (*G*), which are less frequently involved with inflammation and fibrosis, from the more frequently involved, lateral areas (*F* and *I*).

In other experiments a rise in the titer of specific serum hemagglutinin was observed, verifying the initiation of infection by the strain of bacteria given in the retrograde infusion (6).

D. Anatomy of the Renal Pelvis:—Certain factors in the anatomy of the normal rat kidney influenced the experimental infection (Text-fig. 1 and Fig. 1). In rat kidneys the collecting tubules flow into a single papilla. The lumen of the pelvis into which the papilla projects, has fornices that penetrate the renal parenchyma, usually associated with branches of the renal artery and vein. The

fornices are finger-like extensions of the pelvis which are generally collapsed and appear essentially functionless since no collecting tubules are seen entering into them. They are lined with a basophilic epithelium which is continuous with the epithelium of the renal pelvis and is not readily mistaken for lymph or blood vessels. These distal portions of the renal pelvis may represent developmental structures which form where the blood vessels penetrate the renal parenchyma.

E. Pathology:—In the first series of animals in which several species of bacteria were used a wide spectrum of lesions was observed. Some animals showed acute pyelonephritis and renal abscesses, others had chronic pyelonephritis with scarring, and 1 had only pyelitis. Cortical abscesses were common during the 1st week after infection. As shown in Fig. 2, they were contiguous with medullary abscesses. Large ischemic wedges radiating from the medulla were also common in the more fulminant infections which on microscopic examination showed acute polymorphonuclear leukocytic infiltration and tubular white blood cell casts. The acute lesions usually healed within 2 weeks and eventually resulted in scars and cortical depressions (Fig. 3) which in a few cases caused almost complete destruction of the involved kidney.

Chronic lesions of two types related to whether or not the kidney culture was positive were identified among animals in the first experiment. In both types, focal areas of damage were found with fibrosis and loss of tubules. In those cases with positive cultures mononuclear infiltrates were more prominent. Tubular dilatation suggesting intrinsic renal obstruction was seen in some areas but it did not approach the thyroid-like appearance sometimes seen in chronic pyelonephritis in human kidneys. Periglomerular fibrosis, although seen in some kidneys, was not a prominent feature. Pyelitis was always present with acute or chronic interstitial nephritis regardless of the degree of cortical or medullary involvement.

In the second series of animals, all of which were inoculated with a strain of *Klebsiella sp.*, the pathologic changes in those animals with cortical involvement were comparable to the chronic active infections previously described. Pyelitis was the primary histologic lesion, accompanied by bacteriuria and positive kidney cultures but with no gross or microscopic lesions beyond the pelvis or the immediate subepithelial tissue. This process can be seen in Fig. 4 where a mononuclear infiltration is noticeable beneath the parietal (non-papillary) pelvic epithelium. The severity of pelvic inflammation was less than that seen in the first experiment in which more fulminant infections were produced.

In animals with pyelitis the pelvic epithelium was thickened and infiltrated with inflammatory cells. In early lesions granulocytes were present in the subepithelial tissues. More common were chronic lesions with mononuclear infiltration of the epithelium and subepithelium (Fig. 5). The distribution of pelvic inflammation was striking in that the papillary epithelium especially

near the tip was remarkably free of inflammation and thickening which occurred in most other areas (Figs. 4 and 5). The most marked involvement was in the pelvic apex and fornices where epithelial thickening, inflammatory cell infiltrates, and fibrotic distortion were common. Granulocytic cells were frequently present within the pelvic lumen. Loculation of cells secondary to inflammatory adhesions and fibrosis were found in the pelvic apex and fornix where few or no collecting tubules entered (Figs. 5 and 6). Blunting of the pelvic apex was found occasionally, owing to these adhesions and to fibrotic distortion.

Where cortical and medullary inflammation and destruction occurred it was spatially related to an underlying pyelitis (Figs. 2, 3, and 7). The portion of medulla and its overlying cortex which was in closest proximity to the inflamed fornix of the pelvis was the first and most severely involved of the kidney tissue. The nephrons adjacent to the fornix originated in the cortex around the hilum (Text-fig. 1), resulting in a preponderance of parenchymal lesions in the half of the kidney nearest the hilum.

DISCUSSION

The simplest and most logical explanation for the common occurrence and pathogenesis of pyelonephritis in humans is retrograde renal infection. In these experiments this route of infection induced pyelonephritis in 60 per cent of rats when virulent strains were used. The results are in contrast to the difficulties encountered in establishing infection in the normal kidney by the hematogenous route (2, 4). Vesicoureteral reflux, which has been shown to occur under certain conditions in humans (7) and experimental animals (8), permits the introduction of bacteria into the renal pelvis with minimal, if any, trauma. Vivaldi, Zangwill, Cotran, and Kass (9) who produced retrograde infection of the rat kidney following the initiation of infection in the bladder showed that unilateral ureteral ligation protected one kidney, thus documenting the role of the ureter as an important route of infection.

The frequency and pathologic character of the renal lesions in our animals were influenced by the virulence of the bacterial species. With the more virulent strains, pyelitis was observed as a part of acute and chronic interestitial nephritis, whereas the use of an attenuated strain of *Klebsiella sp.* resulted in pyelitis as the outstanding pathologic feature. The persistence of positive medullary cultures was independent of the amount of histologic pyelonephritis. Thus, pyelitis was observed to be the primary chronic infectious process. It might be anticipated that with the passage of time active focal pyelitis would progressively involve the peripheral renal parenchyma causing varying degrees of chronic pyelonephritis as reported by Weiss and Parker (1).

The importance of pyelitis in the pathogenesis of retrograde infection also

was noted in the spatial distribution of parenchymal lesions. It was the perihilar region surrounding the pelvis in which the cortex and medulla were most severely involved. The anatomy of the renal pelvis has considerable importance in the localization of the pathologic process. Although the apex of the pelvis was often markedly involved, the pelvic fornix, because of its depth and greater opportunity to close off and loculate infection, was of even greater importance. The nephrons of the perihilar cortex and medulla, whose tubules pass near the pelvic fornix, were the site of severest and most frequent parenchymal involvement. The renal fornix *per se* and its importance as a site of initial renal infection has not been described before, to the authors' knowledge.

Regarding the spread of infection beyond the pelvis, the data suggest that contiguous spread rather than ascending infection up the collecting tubules was the route. The pelvic epithelium of the fornix and apex in an area where few or no collecting tubules entered the pelvic lumen was most severely involved. The epithelium covering the papilla, which is the outlet for many collecting ducts, was remarkably resistant to infection. If ascent in the collecting tubules was the path of spread, the distribution of cortical lesions would have radiated peripherally in all areas of the kidney rather than the predominantly perihilar distribution which was observed (Text-fig. 1). The distribution of cortical and medullary lesions can best be explained by interstitial invasion by contiguity from the infection in the pelvic epithelium. The reason for the sparing of the papillary epithelium is unknown, but such factors as urine flow, pH, and osmolarity may have an adverse effect on bacterial growth. In 1922, Helmholz (10) also observed a characteristic distribution of pelvic involvement in retrograde infections that was remarkably similar to that observed in this experiment.

While it is apparent that variation in bacterial virulence affected the extent of parenchymal lesions there seemed to be other factors that encouraged bacterial persistence even of less invasive organisms. Information obtained in this experiment would suggest that anatomic factors played some role in this process. This raises speculation as to whether chronic pyelitis owes its chronicity solely to these anatomic features or to unknown biochemical or immunologic characteristics of the host tissue.

The experimental model recapitulates an experience commonly found in man, that is chronic, often asymptomatic, urinary tract infection in an otherwise normal host. If a similar process is initiated in humans by reflux of infected urine producing a persistent low-grade pyelitis with surrounding inflammation and fibrosis, and eventual progression into the parenchyma, answers to many of the questions associated with pyelonephritis could be suggested. Negative bacterial cultures of renal biopsies in the face of persistent bacteriuria could be explained; the difficulty in curing the disease would be expected owing to the

sequestration of bacteria in the inaccessible areas of the pelvic fornices; and adhesions, fibrosis, and distortions in the pelvic apices could cause the calyceal blunting commonly seen in chronic pyelonephritis. The factor of pyelitis in the pathogenesis of pyelonephritis in man is worthy of additional study.

SUMMARY

Pyelitis with acute and chronic pyelonephritis and also primary chronic pyelitis were easily initiated in female rats by a single non-traumatic retrograde infusion of bacteria into the urinary tract. The virulence of the bacterial species and the time of observation were related to the type and extent of lesions, but pyelitis regardless of the virulence appeared to be an important factor. The apex and fornix of the renal pelvic lumen were anatomic sites of persistent pyelitis which permitted local proliferation of bacteria for long periods. Cortical and medullary lesions were anatomically related to these areas of underlying pyelitis. Spread of infection followed a pattern of contiguity rather than ascending tubular infection. The experimental model has many of the features of chronic urinary infections in man and suggests that pyelitis is an important factor in the pathogenesis of chronic pyelonephritis initiated by retrograde infection.

BIBLIOGRAPHY

1. Weiss, S., and Parker, F., Pyelonephritis: its relation to vascular lesions and to arterial hypertension, *Medicine*, 1939, **18**, 221.
2. Lepper, E., The production of coliform infection in the urinary tract of rabbits, *J. Path. and Bact.* 1921, **24**, 192.
3. DeNavasquey, S., Further studies in experimental pyelonephritis produced by various bacteria with special reference to renal scarring as a factor in pathogenesis, *J. Path. and Bact.* 1956, **71**, 27.
4. Braude, A., Shapiro, A., and Siemienski, J., Hematogenous pyelonephritis in rats. I. Its pathogenesis when produced by a simple new method, *J. Clin. Inv.* 1955, **34**, 1489.
5. Beeson, P., Rocha, H., and Guze, L., Experimental pyelonephritis: influence of localized injury in different parts of the kidney on susceptibility to hematogenous infection, *Tr. Assn. Am. Physicians*, 1957, **70**, 120.
6. Andersen, B., and Jackson, G., data to be published.
7. Forsythe, W., and Whelan, R., The occurrence and significance of vesico-ureteral reflux in children, *Brit. J. Urol.* 1958, **30**, 189.
8. Graves, R., and Davidoff, L., Studies on the ureter and bladder with especial reference to regurgitation of the vesical contents, *J. Urol.*, 1923, **10**, 185.
9. Vivaldi, E., Zangwill, D., Cotran, R., and Kass, E., Experimental pyelo-

nephritis consequent to induction of bacteriuria, *in* Biology of Pyelonephritis, (E. L. Quinn and E. H. Kass, editors), Boston, Little, Brown and Company, 1960, 27.

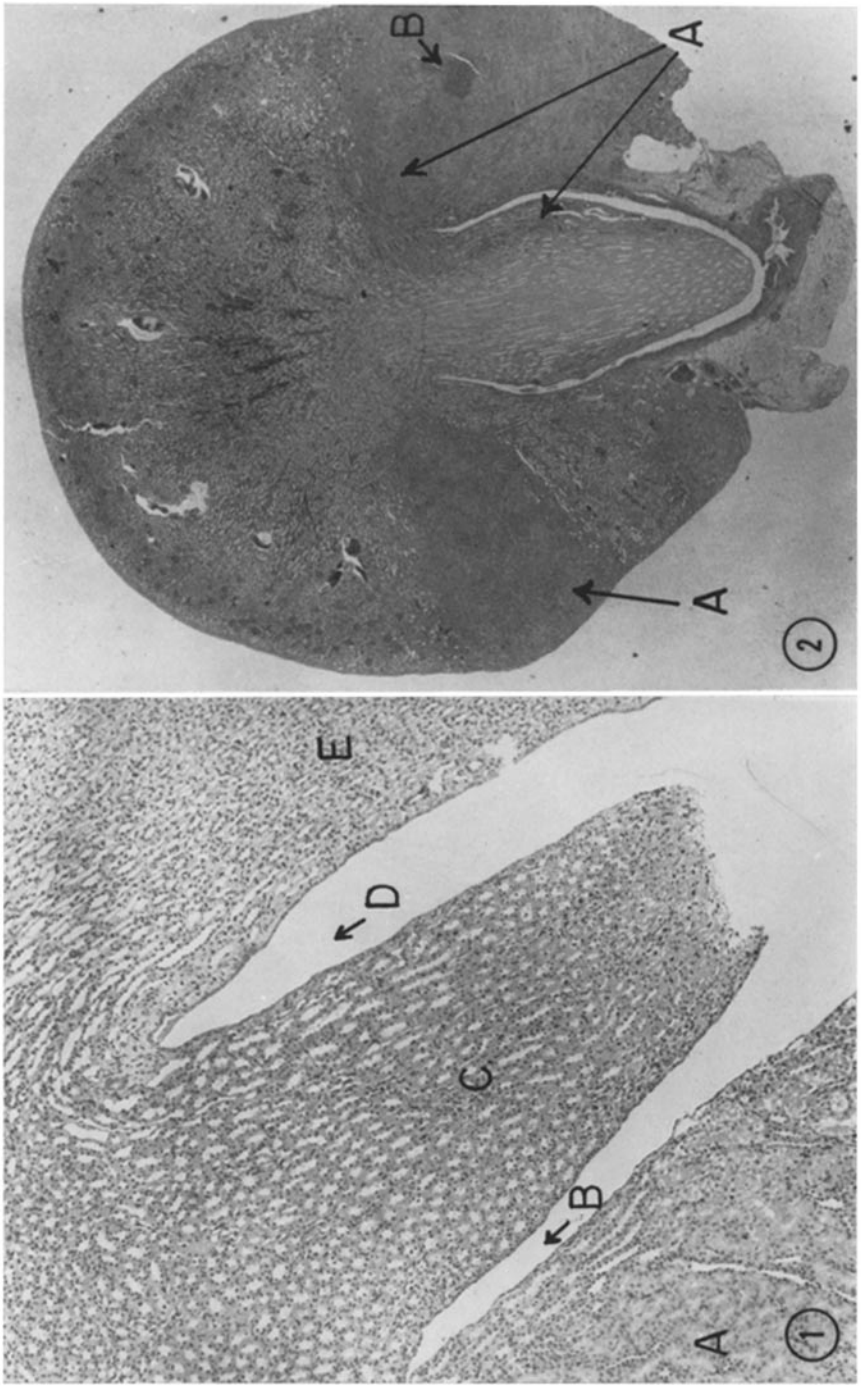
10. Helmholz, H., The pathologic changes in experimental ascending and hematogenous pyelitis, *J. Urol.*, 1922, **8,** 301.

EXPLANATION OF PLATES

PLATE 36

FIG. 1. Photomicrograph of a normal rat kidney. *A*, cortex; *B*, pelvic fornix; *C*, medulla; *D*, pelvic apex; *E*, papilla (hematoxylin and eosin). × 40.

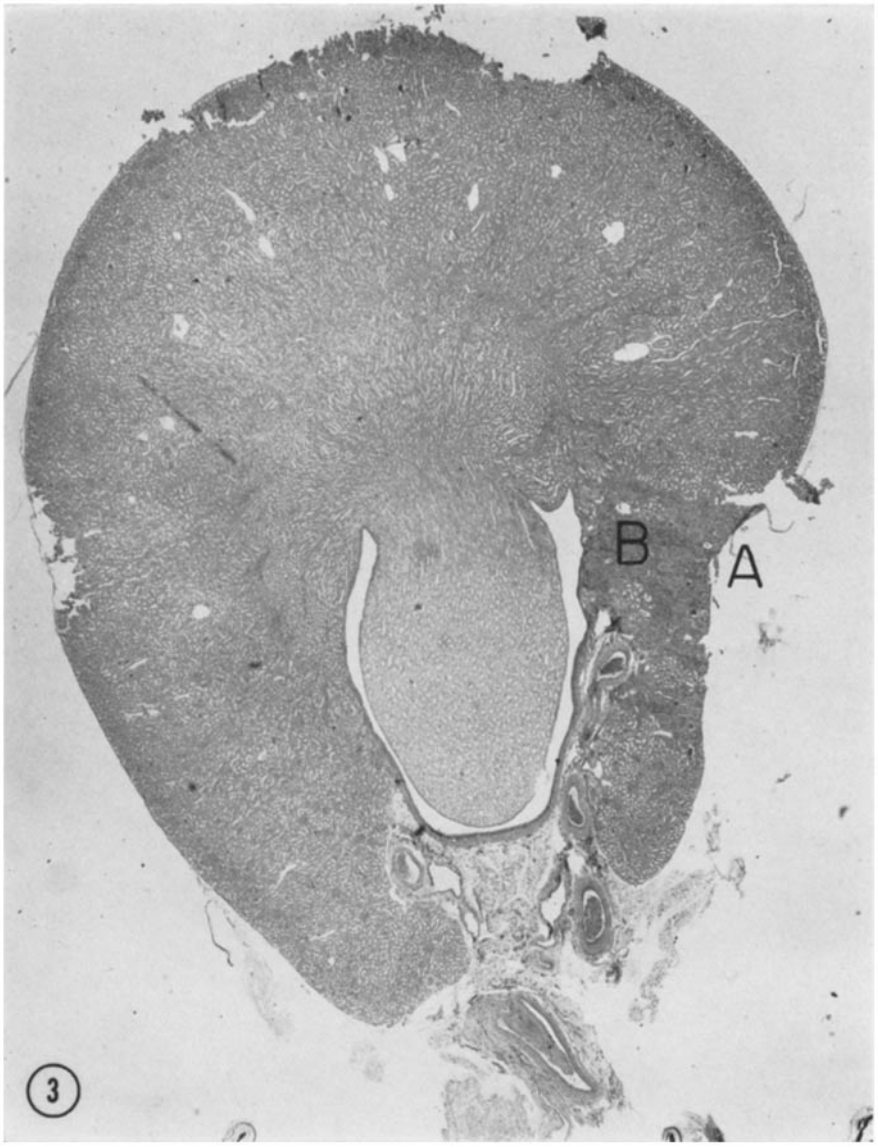
FIG. 2. Photomicrograph of a section through a rat kidney with an acute suppurative infection. *A*, area of mononuclear and polymorphonuclear cell infiltration; *B*, abscess (hematoxylin and eosin). × 10.



(Andersen and Jackson: Pyelitis)

PLATE 37

FIG. 3. Photomicrograph of a transverse section through a kidney with chronic scarring. *A*, cortical depression; *B*, fibrosis and mononuclear cell infiltrate (hematoxylin and eosin). × 10.

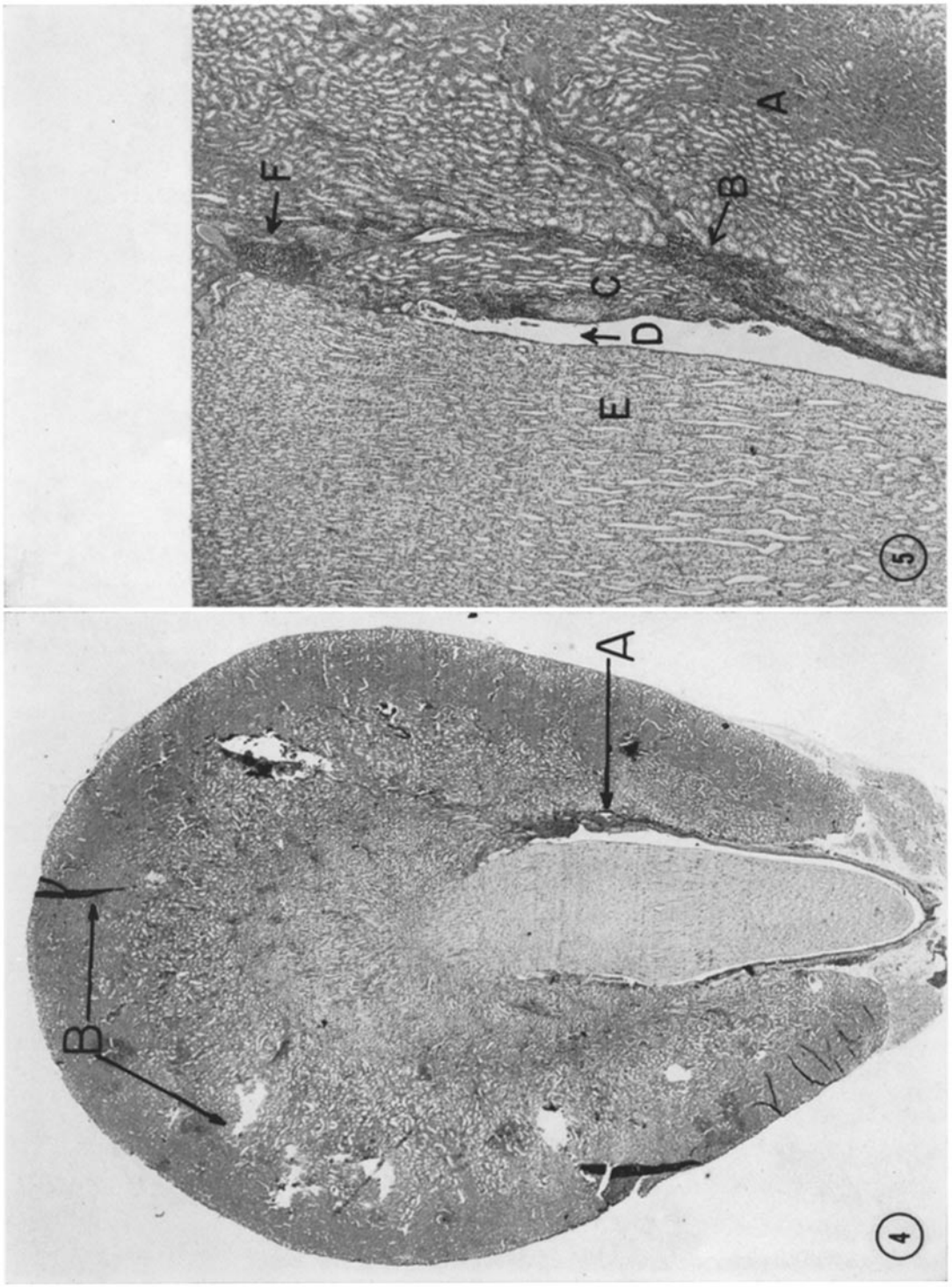


(Andersen and Jackson: Pyelitis)

PLATE 38

FIG. 4. Photomicrograph of a transverse section through a kidney with pyelitis alone. *A*, thickening and mononuclear cell infiltration of the parietal pelvic epithelium; *B*, artifact (hematoxylin and eosin). × 10.

FIG. 5. Photomicrograph of a transverse section through a kidney with pyelitis. *A*, cortex; *B*, obliterated pelvic fornix with a subepithelial mononuclear cell infiltrate; *C*, medulla; *D*, pelvic apex; *E*, papilla; *F*, localized area of mononuclear cells at the tip of the pelvic fornix (hematoxylin and eosin). × 35.

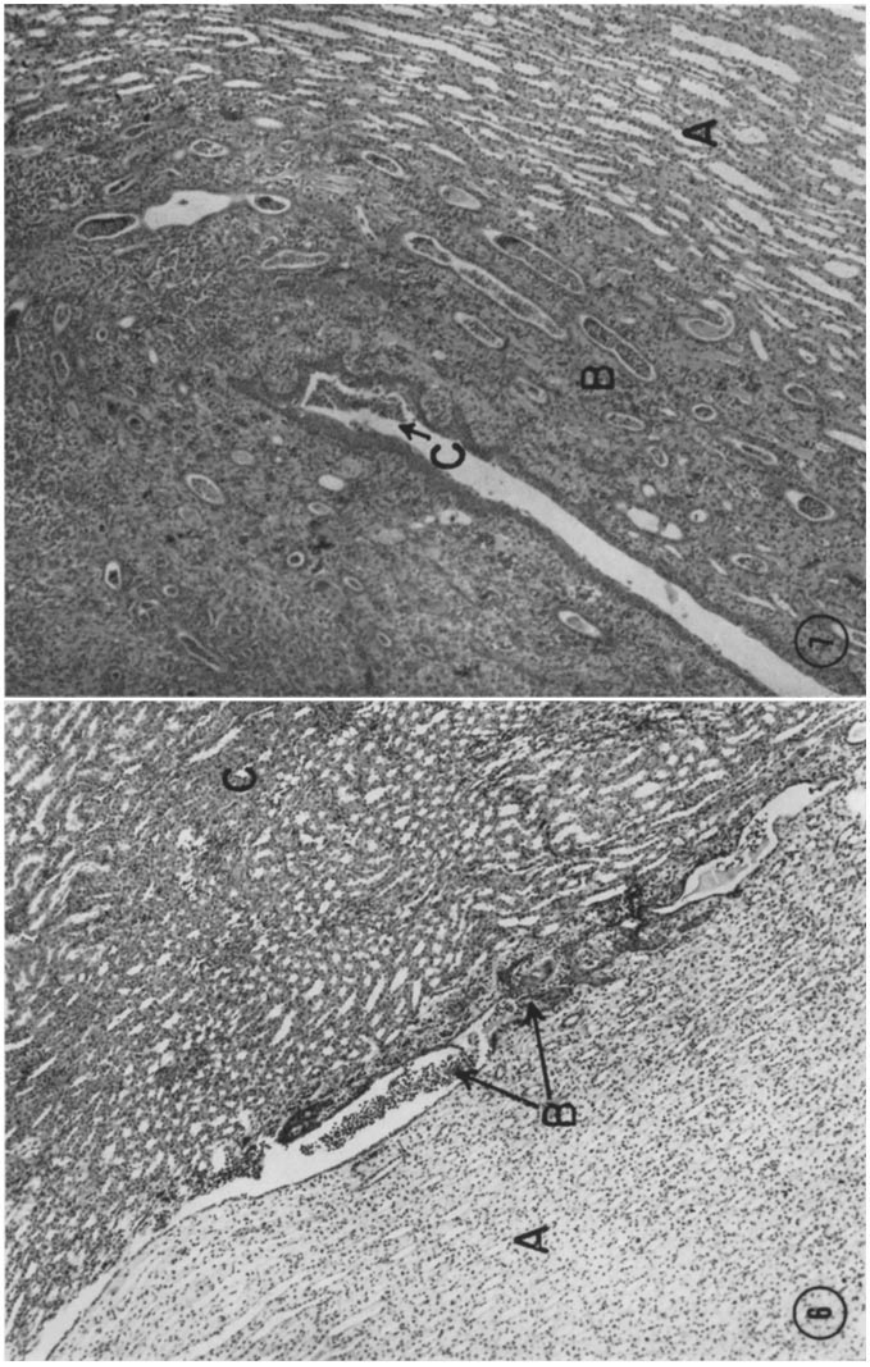


(Andersen and Jackson: Pyelitis)

PLATE 39

FIG. 6. Photomicrograph of a rat kidney showing pyelitis. *A*, papilla; *B*, loculations in the pelvic apex with polymorphonuclear cells in the lumen; *C*, cortex (hematoxylin and eosin). × 180.

FIG. 7. Photomicrograph of a pelvic apex showing pyelitis and surrounding medullary parenchymal involvement with mononuclear cell infiltration and cellular casts within the collecting tubules. *A*, papillary tissue, uninvolved; *B*, papillary tissue showing chronic inflammation; *C*, pelvic apex (hematoxylin and eosin). × 180.



(Andersen and Jackson: Pyelitis)