ACUTE INFLAMMATION IN THE RENAL CORTEX AND MEDULLA FOLLOWING THERMAL INJURY*

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PLATES 2 TO 4

The striking difference in resistance to infection of the cortex and medulla of the kidney has been documented by Beeson and his colleagues (1, 2). In rabbits 10 organisms are sufficient to initiate infection in the medulla following direct inoculation of *Escherichia coli*, while 100,000 organisms are required in the cortex. The reasons for this difference are not established. Localized intrarenal hydronephrosis probably occurs following medullary injections, and hydronephrosis is known to increase susceptibility to pyelonephritis. However, even in the presence of hydronephrosis the cortex is far more resistant than is the medulla (1). In addition, the same disparity is noted in the absence of hydronephrosis following intravenous challenge (3), and it is not explained by the selective localization of organisms or their access to stagnant urine (2). The anticomplementary effect of kidney tissue, as demonstrated by Beeson and Rowley (4), offers an explanation for some peculiar features of the susceptibility of the kidney to infection; but this effect was not shown to be localized to the medulla. Peculiarities of blood flow, lymphatic drainage, and the chemical environment of the medulla have accordingly been invoked to explain these findings, but these factors have not been precisely defined.

Chernew and Braude (5) demonstrated that solute concentrations identical with those found in the kidney and urine inhibited the phagocytosis of bacteria *in vitro*, but the significance within the kidney of these interesting observations is not certain. It is likely the most important events are occurring not in the urine, but in the interstitium of the kidney, since organisms capable of causing hematogenous pyelonephritis in animals do not appear in urine until after bacterial multiplication in the kidney (3). Furthermore, leukocytic infiltration must occur at the site of primary lodgement of bacteria before phagocytosis can

occur, and it is well established that the promptness of leukocyte mobilization
is an important determinant of the outcome of many infectious processes. Since
very little is known about the dynamics of the acute inflammatory response in
the kidney, it was decided to compare the development of acute inflammation
in the renal cortex and medulla, and to attempt to determine the effect of hy-
dronephrosis upon this process. In order to avoid the effects of progressive
bacterial infection upon leukocyte integrity, a thermal burn was selected for
initiating the inflammatory reaction. A marked difference between the speed
of leukocyte mobilization in the cortex and medulla was found, and is the
subject of this report.

Materials and Methods

Albino New Zealand male rabbits weighing 2 to 3 kg were used. Kidneys were exposed
using a midline abdominal incision and light pentobarbital anesthesia. In the experiments
concerning hydronephrosis, the midportion of the ureter was doubly ligated with silk sutures.
Burns were made either immediately thereafter or 24 hours later. Appropriate controls were
included.

Cortical burns were made on the lateral aspect of the kidney with the head of a heated
tack or with a fine electrocautery wire introduced 1 to 2 mm into the parenchyma. The re-
sults with both techniques were similar. Medullary burns were made with a fine cautery wire
insulated so that only 1 to 2 mm was exposed, and introduced 1.5 cm into the lower pole of
the kidney in the direction of the papilla. The current was applied for about 1 second, using a
Birtcher blendtome unit. After burns were made, the incisions were closed with silk sutures
and skin clips, and rabbits were allowed to recover from the anesthesia.

Animals were sacrificed 2, 4, 6, 8, 12, or 24 hours after burns were made, and their kidneys
were removed and fixed in 10 per cent formalin. Histologic sections of the lesions were pre-
pared and stained with hematoxylin and eosin. Multiple sections (18 to 24) from at least four
lesions were studied in each category. The evaluation of the inflammatory response included
an estimate of congestion, edema, necrosis, leukocyte margination, and the cellular infiltration
of tissues. Exudative responses were graded from 0 to 4+, but these attempts at quantitation
will not be presented, since the only differences noted in this study were gross ones.

Leukopenia was induced by the intravenous administration of 7.5 mg nitrogen mustard
(mechlorethamine hydrochloride). When the peripheral white blood cell count was less than
1000/mm^3 (usually 3 or 4 days following the injection), the animals were operated upon as
before, and 0.1 ml of a dilution (containing 10^2 organisms) of a 4 hour trypticase soy broth
culture of the *E. coli* strain used by Beeson (1, 4) was inoculated into the cortex of the kidney.
Animals were sacrificed 8 hours later, blood cultures were taken by cardiac puncture, the
kidneys were removed and ground under sterile conditions in a virtis homogenizer, and
serial pour plates were prepared for enumeration of bacterial populations.

RESULTS

*Comparison of the Acute Inflammatory Responses in the Cortex and Medulla
of Normal (Non-Hydronephrotic) Kidneys.*—During these acute experiments the
cellular exudate consisted predominantly of granulocytes (pseudoeosinophiles).
The responses in the peripheral areas of the burn provided the most interesting
information and were given the most attention. Observations on the relatively

avascular, necrotic center of the burn were of little note in these acute experiments. The results are illustrated by representative sections of cortex and medulla shown in Figs. 1 to 6. In the cortex, at 2 hours, focal areas of granulocyte margination in small blood vessels and clusters of cells in the interstitium were noted. This was more marked and diffuse at 4 hours and by 8 hours an intense and widespread peritubular granulocyte infiltrate was noted. Similar events were noted in the capsule. The inflammatory response in the medulla was markedly different and was characterized by its strikingly delayed and diminished intensity when compared with the cortex. Granulocyte margination and exudation were not readily detected until 12 hours after the burn, and even at 24 hours cellularity there was not as marked as in the 8 hour cortical lesions. At 12 and 24 hours, an infiltrate, visible in the gross, had encircled the cortical lesions (Fig. 7). Mononuclear cells were noted in the 24 hour lesions, but were not dominant. In so far as granulocyte mobilization is concerned, the cortical responses were grossly comparable to those obtained on serial biopsy of rabbit skin following mechanical injury (6). As illustrated in Fig. 8, medullary burns were usually located in the inner medulla or papilla, and marked leukocyte accumulation at the periphery of the lesion was never noted.

In some sections (Figs. 9 and 10), the inflammatory reaction along the cautery wire tract could be followed from the cortex to the papilla, and in these it was found that the intensity of the granulocyte response progressively decreased beginning in the outer medullary zone until it was virtually absent in the inner zone. Study of burns fortuitously located in the outer zone led to the same conclusion: delayed granulocyte mobilization was most marked in the inner or papillary zone made up of collecting ducts and the loops of Henle. It was of interest that marked exudation was noted in capsular connective and adipose tissue overlying the pelvic aspect of the papilla in many sections, even though the nearby intrarenal tissue revealed minimal cellular responses.

Glomerular congestion, edema, and necrosis were noted at all intervals studied, but granulocyte exudation in glomeruli was not striking (Fig. 3). While granulocyte migration across intact tubular epithelium was never observed, migration across disrupted walls of tubules was seen on rare occasions. Consistent with these observations, cellular casts and exudate were seldom seen within tubular lumina, which ordinarily contained only small numbers of erythrocytes or hyaline casts, usually in the region of the papilla. Despite the differences in the architecture of the cortex and medulla, congestion, edema, and necrosis seemed comparable in these areas. Hemorrhage was slightly more marked in the kidneys burned in the medulla, but this seemed in keeping with the likelihood that a greater amount of tissue was injured in order to produce medullary burns.

The Effect of Hydronephrosis upon the Inflammatory Response.—It was anticipated that hydronephrosis would alter the inflammatory response in the

medulla because of its effects upon intrarenal pressure and blood flow. However, exudation was so minimal in the medulla of the normal kidneys that it was not possible to detect any differences in the hydronephrotic medulla. The latter showed obvious generalized tubular dilatation that was not seen in animals with non-ligated ureters. The cortical responses in normal and hydronephrotic kidneys were also indistinguishable.

The Effect of Leukopenia Induced by Nitrogen-Mustard upon Cortical Resistance to Infection.—The preceding results suggested that a deficiency of leukocyte mobilization in the medulla of the kidney might be an important factor responsible for its increased susceptibility to infection; and conversely, that the

TABLE I

Effect of Leukopenia (Nitrogen-Mustard) upon Resistance Following Intracortical Inoculation of 10^2 E. coli

Group	No. E. coli recovered 8 hrs., postinoculation	
	Per kidney	Per ml cardiac blood
Leukopenic animals (9)...................	10^2, 10^4, 10^6, 10^4, 10^4, 10^5, *	0, 0, 10^1, 10^1, 10^2, 10^2, *
Controls (6)............................	0, 0, 0, 0, 10^1, 10^1	0, 0, 0, 0, 10^1, 10^1

* $>10^4$ Gram-positive organisms isolated from each kidney of 3 animals, blood cultures also positive.

capability of brisk granulocyte mobilization in the cortex was an important factor in determining its relative resistance. To test the latter hypothesis, the effect of leukopenia upon resistance to *E. coli* following its direct inoculation into the cortex was studied. The results are shown in Table I. It can be seen that proliferation of the organism occurred in the kidneys of leukopenic animals during the 8 hours following challenge, while the expected reduction in bacterial populations was noted in the normal controls. That containment of the infection was not accomplished in the leukopenic animals was further suggested by the finding that 4 of 6 leukopenic animals had *E. coli* bacteremia when blood cultures were performed at the time of sacrifice (3 leukopenic animals had bacteremia and renal infection with Gram-positive organisms). In leukopenic animals, the susceptibility of the cortex approached that of the medulla of normal animals. Since 10 or fewer organisms are adequate to induce medullary infection (1), it was not practical to test the alternate hypothesis that leukopenia would have relatively little influence upon medullary susceptibility. Notwithstanding the fact that nitrogen-mustard may have effects upon resistance in addition to those of granulocytopenia, the results of this experiment concerning the events that occur in the first few hours following the primary lodgement

of bacteria strongly suggest the importance of granulocytes in the early defense against bacterial multiplication in the cortex of the kidney, and lend support to the concept that the alterations in granulocyte mobilization seen in normal kidneys do relate to increased susceptibility of the medulla.

The Cellular Response Following the Injection of E. coli.—Since Hurley and Spector (7) have suggested that the inflammatory response following cutaneous burns has certain peculiar features, especially in the relatively avascular center of the lesion, these experiments were repeated in part using a bacterial challenge to induce inflammation. The cortex or medulla of normal rabbits was inoculated with 0.1 ml of a dilution of an overnight broth culture of *E. coli* which contained 10^5 organisms/ml, using a no. 27 needle introduced in a fashion similar to that used to induce burns, except that in the cortex the needle was introduced 1 cm into the kidney parallel to and 1 mm beneath the surface. Animals were sacrificed 8 hours later, and histologic sections of the inoculum site were prepared. These showed results comparable to those obtained following burns. The granulocyte exudative response was much more marked in the lesions in the cortex than in the medulla. Granulocyte mobilization in the papilla was particularly delayed and deficient. Organisms were not identified in the sections. Even though the deficiency of granulocytes in the medulla in this experiment may have reflected adverse effects of progressive infection there, these results suggest the burn experiments have application to the problem of experimental pyelonephritis.

DISCUSSION

The delayed leukocyte mobilization observed in the renal medulla affords an attractive and plausible explanation for the inordinate susceptibility of the medulla to bacterial infection. It is consistent with the hypothesis that leukocyte function and its disorders are major determinants of whether infection will occur following the primary lodgement of bacteria, especially in the nonimmune host. In this context, many observers have emphasized that the speed with which granulocytes are mobilized in the tissues is often of importance equal to, if not greater than, that of the magnitude of the ultimate response (8–10). The present studies indicate that the speed with which leukocytes are mobilized in the cortex of the kidney is comparable to the speed with which they are mobilized in normal skin (6), but that granulocyte mobilization is markedly delayed and diminished in the medulla of the kidney where it is akin to that observed in the skin of leukopenic, endotoxin-treated, or alloxan-diabetic acidotic rabbits (6, 8, 9). In these circumstances, bacteria may be given the opportunity to proliferate extensively, so that the issue of eventual infection is settled prior to maximal display of host defenses. Using immunofluorescent techniques, Sanford and his associates were able to demonstrate bacterial multiplication within the interstitium of the kidney of the rat prior to leukocyte

mobilization, but they did not specifically relate this to the medulla in their report (11). In preliminary experiments similar to those reported herein, we observed delayed leukocyte migration in the medulla of the kidney of the rat.

These observations serve to enhance the importance of alterations in other mechanisms of resistance that are operative in the medulla of the kidney. Because of deficiencies in leukocyte activity in the medulla, a factor such as the anticomplementary effect of renal tissue described by Beeson and Rowley (4) would assume greater significance in the medulla than it would in the cortex, where its ill effects upon the antibacterial action of serum might be inconsequential in the face of other powerful defense mechanisms.

These studies also suggest that bacteria within the lumen of kidney tubules are largely protected from cellular defense mechanisms. Granulocytes did not readily gain access to these extensive urinary spaces except where the disruption of tubular integrity had taken place. In addition, leukocytic function within the lumen may be further compromised by deficiencies in antibody, by less than optimal conditions for surface phagocytosis, and by the osmolar, electrolyte, and acid-base conditions implicated by Chernew and Braude (5).

Many explanations for the alterations in leukokinetics observed in these studies have been considered, but further study is required to document their importance. One possibility concerns the unusual features of the blood supply in the kidney, resulting in diminished blood flow and intravascular pressures in the medulla. In addition, there is evidence that the composition of blood bathing the inner medulla differs from that in the cortex in that it has a low erythrocyte content (12), and a similarly low leukocyte content would influence the delivery of granulocytes to inflammatory sites in the inner medulla. Edema, hydronephrosis, or disruption of blood vessels induced by the burn might also compromise blood flow there. However, the effects of hydronephrosis alone do not appear to explain the increased susceptibility of the medulla, since this is demonstrable in the absence of hydronephrosis (3). Furthermore, hydronephrosis produced by ureteral ligation did not appear to influence leukocyte migration in the cortex. The second possibility concerns the effects of the biochemical milieu of the medulla upon capillary permeability, leukocyte margination and mobility, and vascular reactivity were considered. In this context, the ill effects of acidosis (9) and hypertonicity (5) upon leukocyte function should be mentioned again, since these conditions are especially encountered in the inner zone of the medulla where leukocyte mobilization was especially slow. Leonhardt and Landes showed the oxygen tension (pO_2) of renal parenchyma progressively declined along the corticomedullary axis, and that marked hypoxia prevailed in the inner zone (13). Finally, biochemical mediators of the inflammatory response might diffuse away rapidly into urine, lymph, or blood in the inner medulla, or they might not function optimally there.

These studies concerned with experimental pyelonephritis have several clini-

cal implications. The difficulty of curing chronic pyelonephritis with presently available antibiotics is well known. The observations suggesting that leukocytes are relatively ineffective in containing infection in the medulla emphasize the special need for therapeutic measures which are capable of eradicating bacteria within the medulla without much assistance from host defenses. The problem thus would appear very similar in theory to bacterial endocarditis, where truly bactericidal antibiotics are practically essential if a cure is to be effected. Moreover, measures designed to restore leukocyte function in the medulla towards normal might significantly improve the efficacy of currently available therapeutic measures. For example, Leonhardt and Landes demonstrated the ease with which medullary pO_2 may be altered favorably in man by hydration or oxygen administration (13); osmolarity and pH are also subject to manipulation. Consequently, the important effects of the biochemical environment of the medullary interstitium upon leukocyte, antibiotic, and bacterial activity are worthy of further study.

SUMMARY

As determined by serial histologic study, the leukocyte component of the acute inflammatory response to thermal injury in the medulla of the kidney of rabbits was markedly delayed and diminished in intensity when compared with the response of the cortex. Similar differences in granulocyte mobilization between cortex and medulla were seen following the inoculation of bacteria. Hydronephrosis produced by ureteral ligation had no discernible effect upon the inflammatory response in either cortex or medulla following thermal burns. The susceptibility of the cortex to *E. coli* infection was markedly enhanced during leukopenia induced by nitrogen-mustard. These results suggest that deficiencies in granulocyte mobilization play an important role in the increased susceptibility of the renal medulla to bacterial infection.

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EXPLANATION OF PLATES

PLATE 2

Fig. 1. Cortical burn, 2 hours. Focal peritubular and subcapsular granulocyte infiltration. Hematoxylin and eosin. × 400.

Fig. 2. Cortical burn, 4 hours. Widespread interstitial granulocyte accumulation. Hematoxylin and eosin. × 400.

Fig. 3. Cortical burn, 8 hours. Marked peritubular exudation. Glomerular congestion and edema with minimal leukocyte margination. Hematoxylin and eosin. × 400.

Fig. 4. Margin of medullary burn, 2 hours. Minimal congestion, rare granulocytes. Hematoxylin and eosin. × 400.

Fig. 5. Margin of medullary burn, 4 hours. Leukocyte infiltration practically absent. Hematoxylin and eosin. × 400.

Fig. 6. Medullary burn, 8 hours. Focal granulocyte accumulation and congestion. Hematoxylin and eosin. × 400.
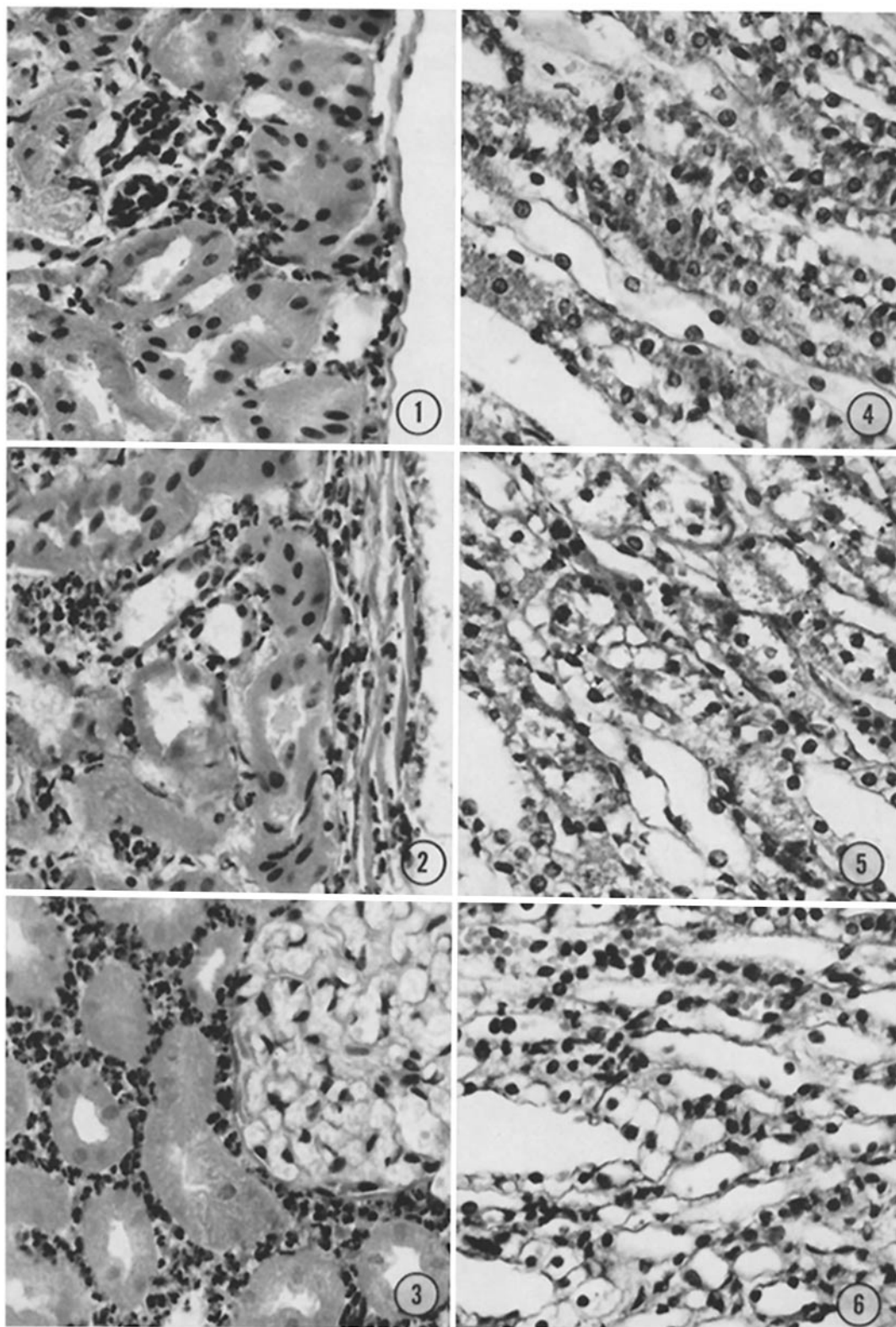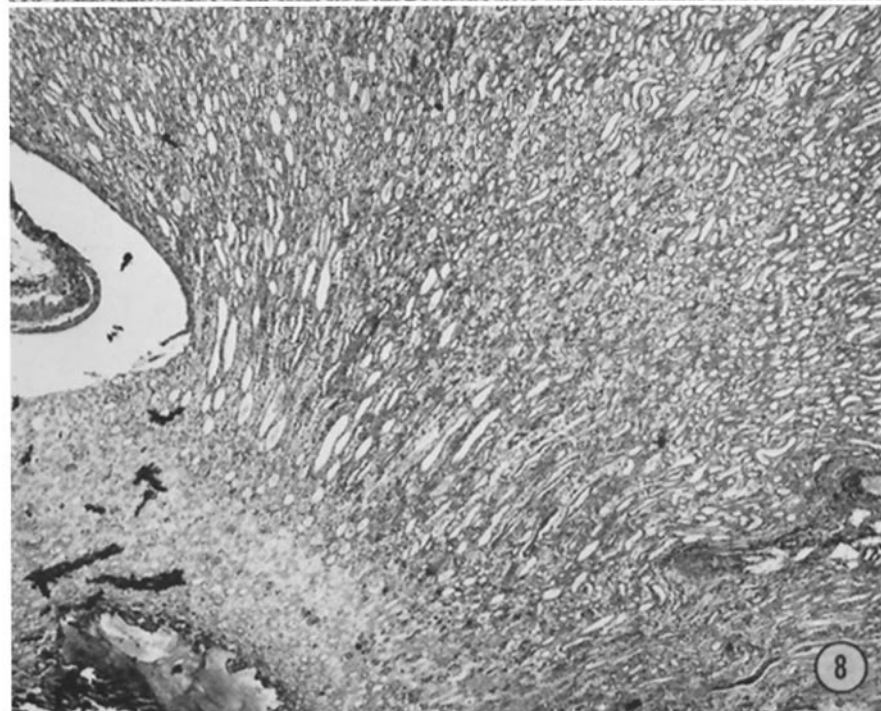
(Rocha and Fekety, Jr.: Renal inflammation)

PLATE 3

FIG. 7. Cortical burn, 24 hours. Leukocytes have accumulated at the periphery of the lesion and have encircled its necrotic center. Hematoxylin and eosin. × 35.

FIG. 8. Medullary burn at junction of inner and outer zones, 24 hours. Hydronephrosis is minimal, and leukocyte accumulations in the periphery are not seen. Hematoxylin and eosin. × 35.
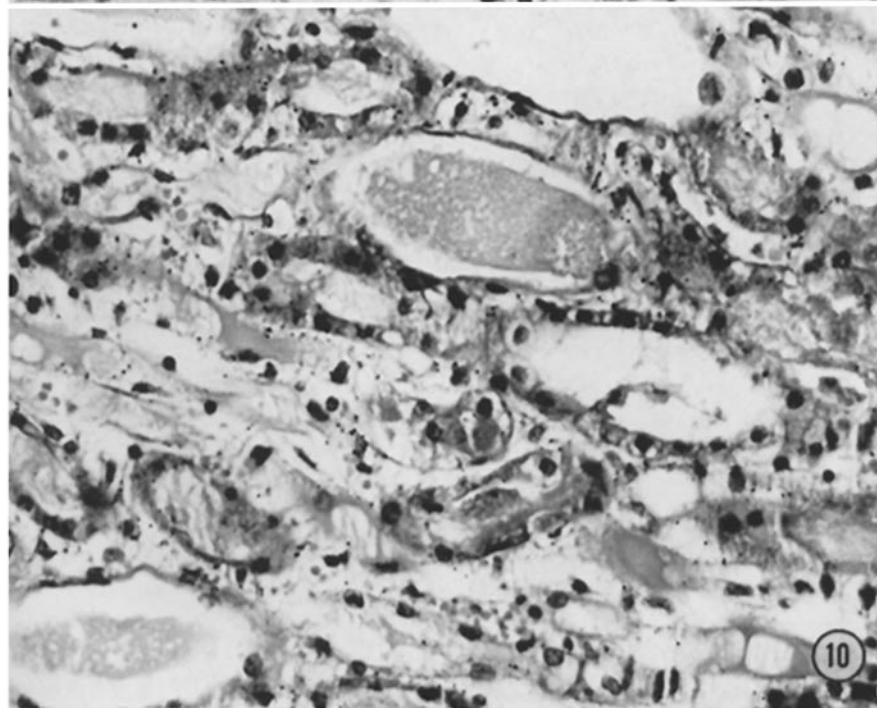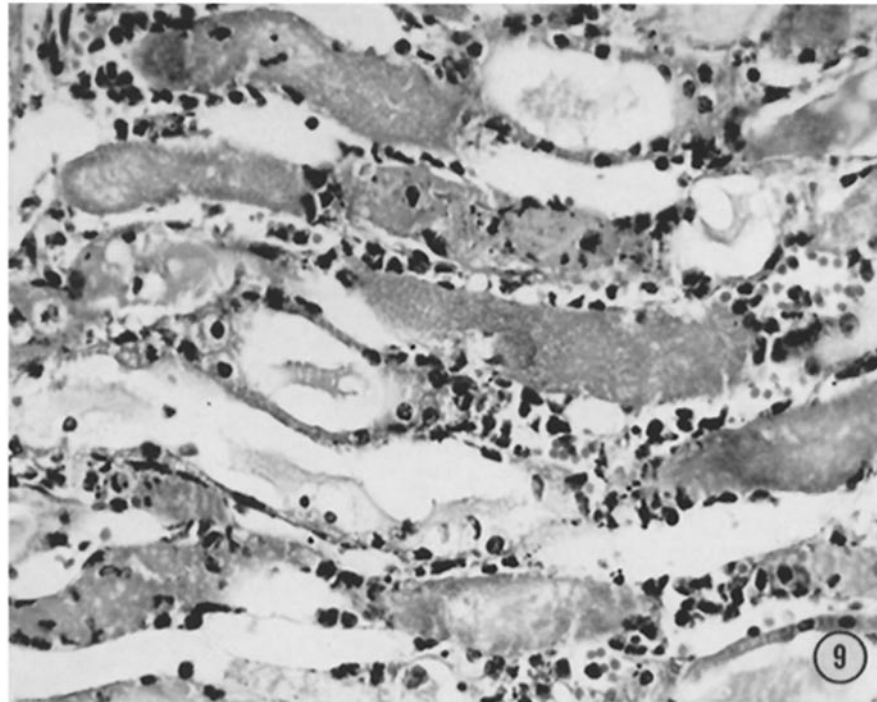
PLATE 3

(Rocha and Fekety, Jr.: Renal inflammation)

FIG. 9. Burn, outer zone of medulla, 24 hours. Peritubular leukocyte infiltration, few leukocytes within tubules. Hematoxylin and eosin. × 400.

FIG. 10. Burn, inner zone of medulla, 24 hours. Same specimen shown in Fig. 9. Minimal granulocyte accumulation in this area near the papilla. Hematoxylin and eosin. × 400.

(Rocha and Fekety, Jr.: Renal inflammation)