PATHOGENESIS OF CHRONIC PYELONEPHRITIS

II. Effect of Repetitive Infection

Sheldon C. Sommers, M.D.; Harvey C. Gonick, M.D.;
George M. Kalmanson, M.D., and Lucien B. Guze, M.D.

From the Medical Service, Wadsworth Hospital, Veterans Administration Center, Los Angeles, Calif.; Department of Medicine, UCLA Center for the Health Sciences, Los Angeles, Calif.; and Francis Delafield Hospital, New York, N.Y.

Clinically, pyelonephritis may present as a progressive disease characterized by increasing tissue destruction and associated functional insufficiency. Hypertension has also been described as a consequence.[1] The course of the disease may be very variable; in some instances it progresses slowly over many years (pyelonephritis lenta), while in other instances an accelerated form may occur.[2] Factors which may influence the rate of progression include (a) the infecting bacterial species, (b) the frequency of re-infection or exacerbations of a preexistent infection, (c) the nature of predisposing anatomic deformities, and (d) the presence of certain associated disorders, such as hypertension, diabetes mellitus and gout. In a previous communication the pathologic sequence of events following a single injection of *Streptococcus faecalis* into the rat was described.[3] The present experiment was designed to determine whether or not repetitive infections accelerated the pathologic changes.

Material and Methods

Thirty male Wistar rats were used. Twelve animals were included in each of 2 experimental groups. Three uninfected animals of the same age and size constituted the controls for each experimental group. At the time of first bacterial injection these animals weighed 90 to 125 gm; when sacrificed they weighed 250 to 325 gm. Multiple bacterial injections were made to simulate repetitive episodes of infection. Intravenous injections of 4.0×10^8 *Str. faecalis* were made at intervals of 3 weeks for 12 weeks (4 injections) or for 21 weeks (7 injections).

The animals were sacrificed 3 weeks after the last injection. At necropsy the kidneys were removed and fixed in 10 per cent calcium formalin. Kidney tissues were stained with hematoxylin and eosin, Masson trichrome method for collagen, Korson technique for DNA and RNA, periodic acid-Schiff (PAS) method for connective tissues and basement membranes, colloidal iron-PAS method for acid and neutral mucopolysaccharides (colloidal iron delineating acid mucopolysaccharide and PAS defining neutral mucopolysaccharide), and Turnbull's stain for iron.

Supported by grants from the United States Public Health Service (AI-02257 and AI-03343).

Accepted for publication, June 9, 1964.

Results

Table I summarizes the observations and compares the pathologic findings after 12 and 21 weeks of repetitive injection with the changes seen 1, 12 and 21 weeks following a single injection as contrasted to uninfected normal controls.[3]

Table I

PATHOLOGIC CHANGES FOLLOWING REPETITIVE INFECTION CONTRASTED TO SINGLE INFECTION

	1 Week *Single*	12 Weeks *Single*	21 Weeks *Single*	12 Weeks *Repetitive*	21 Weeks *Repetitive*
Glomerular sclerosis	0*	0-1+	0-1+	1+	2+
Collecting duct					
Dilatation	2+	2+	2+	1+	2+
Destruction	1+	0	1+	0	1+
Hyperplasia	1+	2+	1+	2+	2+
Casts	1+	0	0	0	0
Hydropic	1+	1+	0	1+	0
Distal tubule					
Dilated	1+	2+	2+	1+	2+
Destruction	0	0	0	0	0
Hyperplasia	0	1+	1+	1+	1+
Casts	0	0	1+	0	1+
Hydropic	1+	0	0	0	0
Henle's loop					
Dilated	2+	2+	2+	1+	3+
Destruction	0	0	0	0	0
Hyperplasia	0	0	0	0	1+
Casts	0	0	0	0	0
Hydropic	0	0	0	0	0
Papilla, Interstitium					
Scars	0	1+	1+	1+	2+
Collagen	0	1+	1+	1+	2+
Mucopolysaccharide	1+	2+	1+	1+	2+
Inflammation	2+	0	0	0-1+	2+
Arteries thickened	0	0	0	1+	1+
Arterioles thickened	1+	0	0	0	1+

* Slides were examined without knowledge of the experimental group to which the animals belonged and abnormalities found were graded as absent (0), slight (1+), moderate (2+), or severe (3+). Following this the results were analyzed with reference to the experimental or control groups and compared to the animals with single injections previously reported.[3]

Twelve Weeks. Certain alterations at 12 weeks were comparable to those seen at the same interval after a single injection of streptococci. There was minimal edema of the renal papillae with small focal stellate collagenous scars. The interstitial medullary tissue was slightly increased in amount and in its affinity for PAS stain. The collecting ducts were focally hypertrophied and hyperplastic, with increased epithelial cytoplasmic staining for RNA. Some collecting tubular epithelial cells ap-

peared hydropic. Both the proximal and distal convoluted tubular epithelium were mildly hyperplastic, and the enlarged cells were richer in cytoplasmic RNA than were those in the uninfected controls. Proximal to the collecting ducts there were no casts, hydropic changes or destruction of tubular epithelium. Periglomerular collagen deposition was similar to that noted at this stage after a single injection.

Other lesions were more extensive and advanced after repeated injections for 12 weeks. Stellate scars at the cortico-medullary junction were larger and more numerous than after one injection of bacteria (Fig. 1). Calcospherites, of laminated rounded appearance, and other foci of calcification were present in the interstitial tissue in the deeper cortical layers, close to the cortico-medullary junction (Figs. 1 and 2). These were not noted in the animals with single injections. Some calcified bodies were also seen in the lumens of collecting tubules. Dilatation of collecting ducts, distal convolutions and Henle segments was less marked 12 weeks after repeated injections than at the same interval following one injection. This was possibly due to increased collagenous scarring and less interstitial edema found after repeated injections.

The glomeruli in general had slight sclerotic thickening of the capillary walls, with heavier than usual colloidal iron staining. Bowman's capsular basement membranes were thickened and strongly stained with PAS (Fig. 3). The walls of arteries, but not of arterioles, were focally thickened by intramural collagen deposition (Fig. 4). Both the glomerular and arterial sclerosis were comparable to the changes 36 weeks after one bacterial injection.

Twenty-one Weeks. At 21 weeks certain findings were similar in the animals receiving repetitive injections and in those with single injections. In both groups collecting ducts were often moderately dilated, with slight epithelial degeneration but without hydropic changes. The distal convoluted tubules likewise were somewhat dilated, without any apparent epithelial degeneration but with slight cellular hyperplasia, an associated increase in cytoplasmic RNA and with moderate numbers of colloid casts. No hydropic changes were evident in the distal convolutions or Henle's loops. Squamous metaplasia and epithelial hyperplasia of the renal pelvic epithelium, associated with pyelitis, was the same at 21 weeks after either single or repeated injections.

Other pathologic changes differed more between the repetitively and singly injected animals at 21 than at 12 weeks. Scarring of medullary interstitial tissue, particularly along the cortico-medullary junction, was comparatively advanced, and in some cases a collagenous network was formed by coalescence of the linear scars generally present in the group with re-injection (Fig. 5). At times, there were adjacent stellate collagenous scars in the deeper cortical tissue or collections of calcospher-

ites, as described in the 12 week repetitive group. The extent of scarring 21 weeks after repeated injections was comparable to that noted 60 weeks after one injection. The PAS staining reaction of the medullary interstitial tissue also was stronger after repeated injections and more collecting ducts showed epithelial hyperplasia. Acute exudative inflammation in the interstitial tissue of the papillae (with or without abscesses) was moderately active and corresponded to the peak activity observed one week after a single injection.

Collections of interstitial phagocytic cells were present in moderate numbers in the papillary region 21 weeks after repeated injections (Fig. 6). They were not seen to this extent in any other experimental or control animals. These macrophages were of moderate size and were filled with eosinophilic granules. Similarly stained granular and vacuolated material was found free in the surrounding medullary interstitium. This substance also stained with colloidal iron.

An unusual finding, not previously seen in experimental pyelonephritis, was extensive lymphangiectasis and lymph leakage, predominantly in the cortico-medullary zone (Fig. 7). No definite obstruction of the lymphatics due to scars could be identified. Further, a number of kidneys had a curiously loose, edematous appearance in glomerular capillary walls, without equivalent sclerosis, particularly in the cases with prominent lymph stasis (Fig. 8).

Henle segments in the group 21 weeks after repeated injections were markedly dilated and showed evidence of mild or moderate epithelial hyperplasia, comparable to that found at 36 to 54 weeks following a single injection.

Glomerular and arteriolar changes were comparatively more advanced in the 21 week group than any other lesions. Most of the glomeruli appeared moderately sclerotic with conventional staining. The basement membrane of the glomerular capillary walls showed increased colloidal iron staining and was split into laminae. The degree of glomerular capillary sclerosis was out of proportion to what might have been expected to accompany the periglomerular fibrosis present.

Slight, but definite, arteriolar hypertrophy was present. This was not associated with any evident juxtaglomerular apparatus alterations (as seen in Fig. 8). Both the degree of glomerular and arteriolar sclerosis 21 weeks after repeated injections corresponded to the findings 72 weeks after one injection of bacteria.

Discussion

The most notable effects of repetitive infection were the acceleration of interstitial scar formation, glomerular sclerosis and arteriolar sclero-

sis. Exudative inflammation, cast formation and tubular degeneration appeared relatively unaffected. These observations of accelerated pathologic changes were in contrast to the findings of Sanford, Hunter and Souda [4] and Shapiro, Braude and Siemiensky,[5] who indicated that re-infection failed to increase the severity of lesions produced by experimental pyelonephritis. In the study by Sanford and co-workers [4] a lesser degree of infectibility, as judged by quantitation of abscesses and organisms, was shown to follow healing in *E. coli* pyelonephritis. An acquired resistance to re-infection, possibly on an immune basis, was postulated. Chronic pyelonephritis did not ensue. Shapiro and co-workers,[5] while demonstrating that the severity of the lesion was dependent, in part, on the species of infecting bacteria, could not show any increase in the gross scarring produced by re-infection with *E. coli, Proteus morganii,* or *Streptococcus zymogenes.*

In the present investigation, coarse scar tissue formation, both nascent and mature, was invariably stimulated by repeated infections. After 21 weeks the medullary, cortico-medullary, and occasionally the cortical collagenous scars that had formed corresponded to changes observed only after an interval 3 times as long following a single infection. While abundant, the scars were still focal, and did not interfere with the renal circulation generally or produce noticeable ischemic degeneration or atrophy. It was of interest that coarse scar formation was not associated with the severe arterial thickening described by Kincaid-Smith.[6] These findings support the contention of Heptinstall, Michaels and Brumfitt [7] and Lagergren and Ljungqvist [8] that scarring may be a manifestation of inflammation rather than of ischemia produced by arterial narrowing.

The peritubular and periglomerular collagen deposition that was apparently responsible for a delayed diffuse renal atrophy did not appear until 24 weeks after a single bacterial injection,[3] and repeated injections did not accelerate its onset or development.

Glomerular and arteriolar sclerosis were also exaggerated after 21 weeks of repetitive infection. However, the arteriolar narrowing did not appear sufficiently severe to stimulate any increased granularity of the juxtaglomerular apparatus [9] and necrotizing arteriolitis of the type described as accompanying hypertension in the pyelonephritic rat [10] was not demonstrable.

The association between hypertension and chronic pyelonephritis has generated considerable interest since the classic studies of Weiss and Parker.[1] The high incidence of hypertension in chronic pyelonephritis has been confirmed recently in clinical studies by Brod [11] and Kincaid-Smith.[6] Yet development of hypertension in the experimental animal

with induced pyelonephritis has only been shown in the presence of advanced renal disease[12-14] or after removal of one kidney.[10] Shapiro and co-workers[5] failed to demonstrate hypertension after repetitive injections of several species of bacteria for periods up to one year. Similarly, Guze and Kalmanson[15] found normal blood pressures in rats up to 20 months after a single injection of *Streptococcus faecalis,* despite progressive atrophy and scarring. In the present study blood pressure determinations were not made, but the absence of advanced vascular lesions may be considered a presumptive indication that severe hypertension was not present. Nevertheless, since arteriolar sclerosis could be accelerated by repetitive infection, the possibility remains that a more prolonged series of injections might have led to the development of hypertensive vascular alterations.

The appearance of extensive lymphangiectasis and lymph stasis, not seen in animals with single injections, is worthy of comment. Several investigators[14,16-18] have suggested that infection spreads from the external surface of the kidney to medullary and cortical interstitial tissue via lymphatics. A late consequence may be intrarenal obstruction of the lymph channels. Although this sequence might in theory account for the lymphangiectasis in the present experiment, no definite obstruction could be identified. An alternative explanation, suggested by the experiments of Fujimoto,[16] is that the lymphatic changes might represent an antigen-antibody reaction. Fujimoto had sensitized rabbits to egg white or egg albumin by repeated intramuscular injections and then administered the antigen into the pelvis of one kidney following ligation of the ureter. A pyelonephritis was consistently produced in which distinctive perifocal thrombo- and peri-lymphangitic changes were seen around renal and arcuate blood vessels. Although antibody to kidney tissue *per se* has not been demonstrated in pyelonephritis,[19] the sensitizing antigen may be bacterial in origin. Cotran,[20] utilizing fluorescent techniques, has demonstrated anti-Proteus antibody in plasma cells within pyelonephritic scars.

A relationship between pyelonephritis and calculus formation has frequently been postulated.[21-23] A focal area of degeneration, particularly in the renal papilla, has been thought to act as a nidus for calcification. Stone formation has previously been demonstrated in experimental infections produced by *Proteus mirabilis*[4] and *Proteus morganii*[5] but not by *E. coli* or *Streptococcus zymogenes*.[5] It is of interest that the calcospherites described in the present experiment were found at the cortico-medullary junction, rather than in the papilla. From a conceptual standpoint, however, the site of origin of the calculi is of importance only in that communication with the renal pelvis must be

achieved by passage through collecting ducts rather than direct sloughing from papilla into pelvis.

Summary

Experimental pyelonephritis induced in rats by repeated injections of *Streptococcus faecalis* was studied pathologically and histochemically. Compared to pyelonephritis induced by one bacterial injection, the formation of interstitial scars, glomerular sclerosis and arteriolar sclerosis were greatly enhanced, while exudative inflammation, cast formation and tubular degeneration were relatively unaffected. Calcospherites were noted in areas of cortico-medullary scar formation. Extensive lymph stasis and extravasations of lymph were unique findings, not seen in the animals with single injections.

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Requests for reprints should be addressed: Lucien B. Guze, M.D., Veterans Administration Center, Wilshire and Sawtelle Boulevards, Los Angeles, California, 90073.

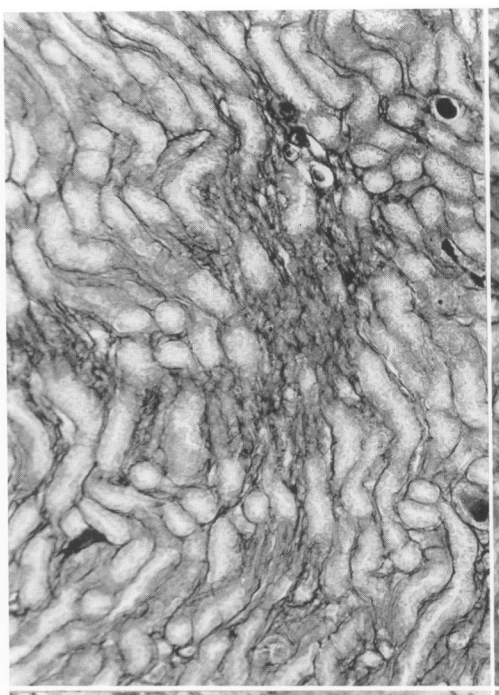
LEGENDS FOR FIGURES

FIG. 1. Stellate scars at the cortico-medullary junction are associated with localized calcific deposits and calcospherites. Twelve weeks of repeated bacterial injections. Periodic acid-Schiff (PAS) stain. × 60.

FIG. 2. Multiple calcospherites appear in the juxtamedullary cortex after 12 weeks of repeated bacterial injections. Colloidal-iron PAS stain. × 100.

FIG. 3. Thickened glomerular capillary walls exhibit increased colloidal iron staining and a thickened basement membrane in Bowman's capsule, stained with PAS. Twelve weeks of repeated injections. Colloidal iron-PAS stain. × 500.

FIG. 4. Irregular focal thickening appears in arterial walls after 12 weeks of repeated bacterial injections. Masson stain. × 500.



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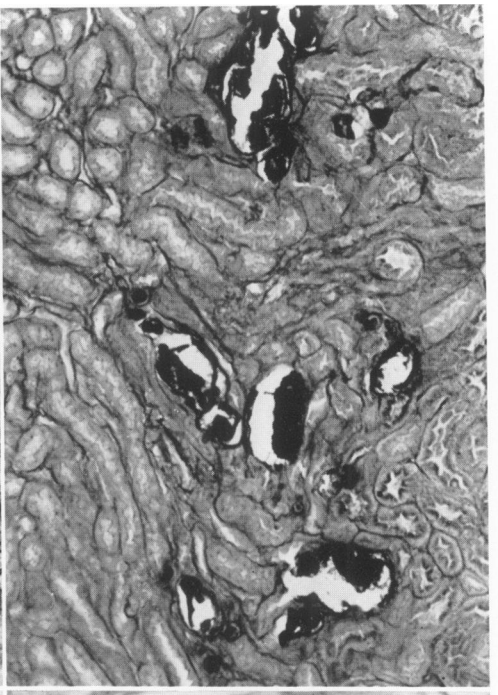

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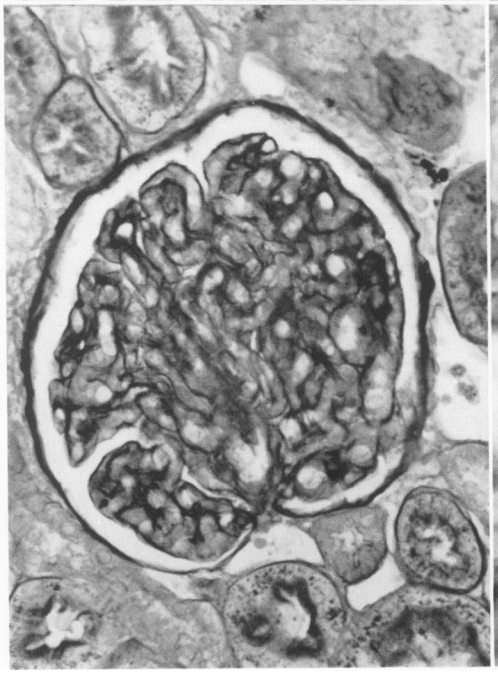

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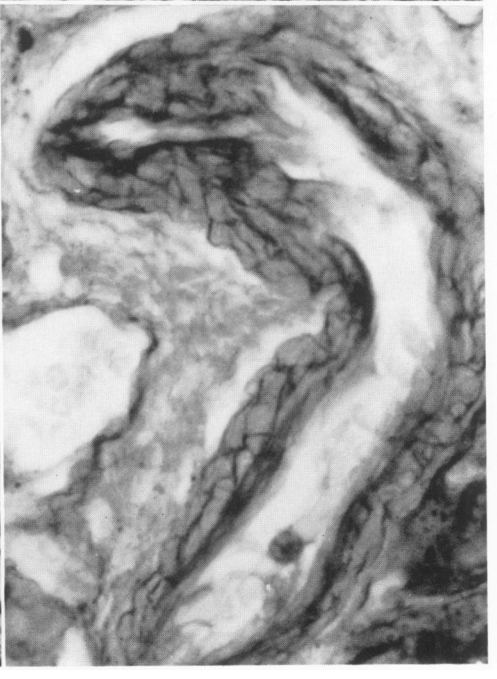

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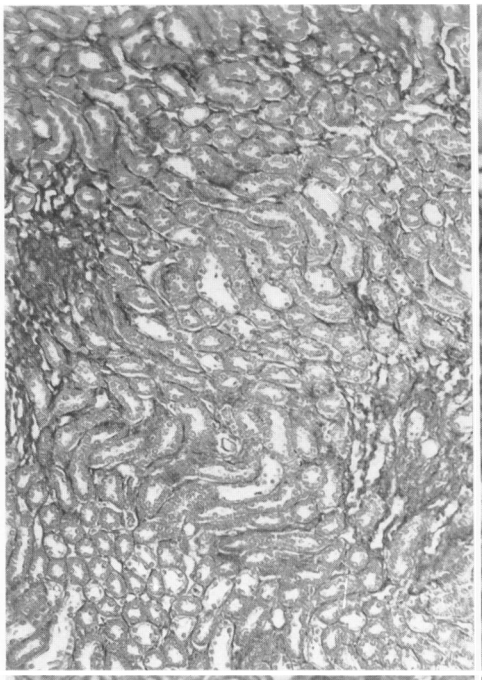
FIG. 5. Coalescence of stellate scars is evident after 21 weeks of repeated injections. Masson stain. × 60.

FIG. 6. Colloidal iron-stained granules appear in macrophages in the renal sub-pelvic interstitial tissue after 21 weeks of repeated injections. The granules may represent lysosomes.[24] Colloidal iron-PAS stain. × 500.

FIG. 7. The periarterial, perivenous and interstitial spaces are distended by excessive lymph after 21 weeks of repeated injections. The lymphatics are dilated but not apparently mechanically obstructed. Colloidal iron-PAS stain. × 100.

FIG. 8. Swollen glomerular capillary walls are seen in kidneys with other indications of lymphedema 21 weeks after repeated injections. Hematoxylin and eosin stain. × 500.



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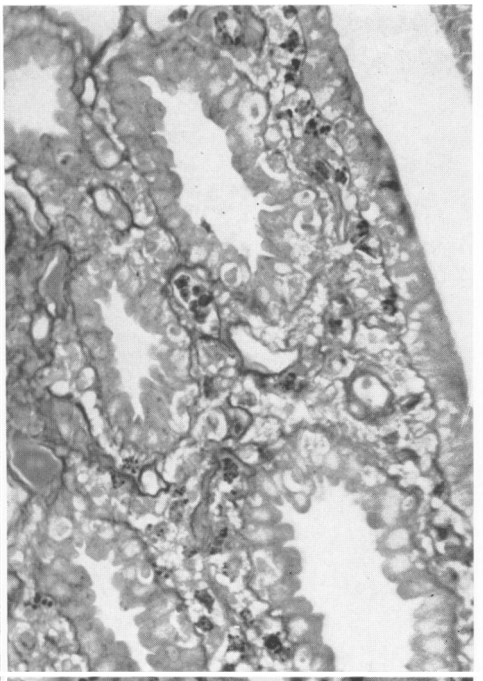

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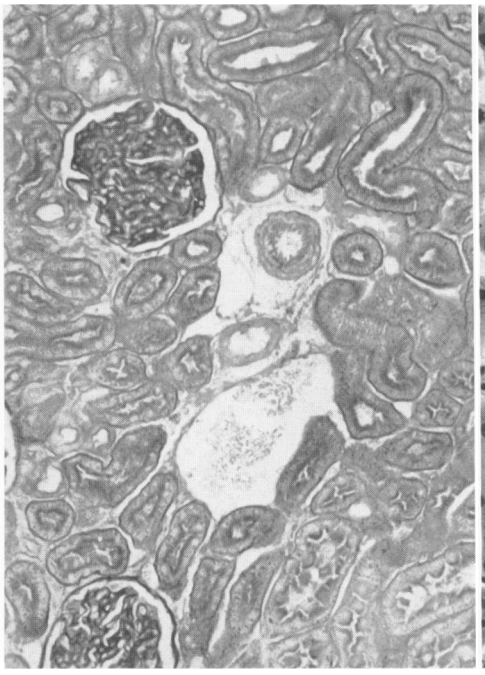

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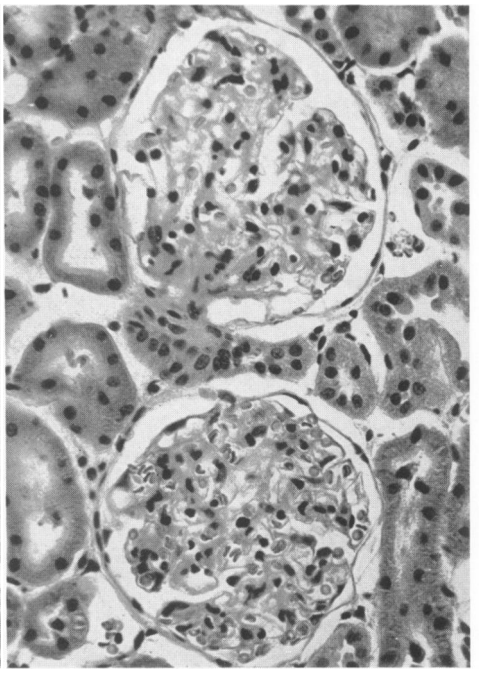

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