

## Renal Lesions After Unilateral Ureterosigmoidostomy in the Rabbit: Preliminary Results

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**Summary.** Unilateral ureterosigmoidostomy (U.S.S.) was performed on 15 rabbits and then the animals were sacrificed after 3, 8 and 15 months. The gross appearance of the kidney and radiological investigations revealed enlargement of the pelvis and of the ureter on the operated side although the anastomosis was patent in all cases. In rabbits sacrificed 8 months after U.S.S. an adenomatous polyp was present at the site of anastomosis.

Histological examination of the affected kidney showed pyelonephritis secondary to obstruction and immunopathological studies revealed in some rabbits glomerular and tubular deposition of immunoglobulins and complement. The contralateral kidney was always normal. Biochemical investigations showed only a moderate increase in blood urea nitrogen and plasma creatinine values.

**Key words:** Ureterosigmoidostomy, Pyelonephritis, Adenomatous polyp, Urinary obstruction, Immunological changes.

the morphological and immunological features of the affected kidney with those observed in the contralateral kidney.

### MATERIAL AND METHODS

U.S.S. was performed in 15 male New Zealand rabbits (mean weight 2,800 kg, mean age 3 months).

The animals were given tap water for 12 h before and after surgery, and thereafter they were kept on a standard integrated diet. None of the rabbits received antibiotics or other drugs. Blood urea nitrogen, plasma creatinine and sodium and potassium concentrations were measured after cardiac venepuncture before surgery and later at monthly intervals. General anaesthesia was induced with Alfaxalone, injected into a peripheral ear vein in a dose of 0.3-0.4 ml/kg (maintenance dose: 3 ml/kg/h).

U.S.S. was performed using the Coffey I technique (5, 6) but incorporating a submucosal tunnel, as Mirot-Worzoff suggests (15). Chromic catgut 0000 was used for the anastomosis. Three to 12 months after surgery, intravenous pyelography was performed in all animals. 5 animals were sacrificed after 3 months (Group 1), 5 after 8 months (Group 2) and 5 after 15 months (Group 3).

Immediately after death, the following specimens were obtained:

- urine samples from the bladder and from the pelvis of the operated kidney for microscopic and cultural examination.
- tissue specimens from the operated and contralateral kidneys for light microscopy and immunofluorescence studies.
- bone specimens from a lumbar vertebra, iliac crest and sterno-costal junction for electron microscopic examination.

### INTRODUCTION

Renal damage is a well-known consequence of U.S.S., in spite of the various techniques which have been introduced to allow a free urinary flow into the gut and to avoid ureteric reflux (5, 6, 9, 18). These techniques have been only partially successful in reducing renal damage, which is mainly due, on both experimental and clinical grounds, to pyelonephritis developing even in the absence of reflux. This investigation was undertaken with the purpose of evaluating the onset and the evolution of renal lesions, the biochemical abnormalities and the morphology of bone after U.S.S. Furthermore, an attempt was made to compare

- the intestinal tract including U.S.S. and approximately 4 cm of the ureter, through which a catheter was placed.

For light microscopy studies, the kidney and intestinal specimens were fixed in Bouin's solutions, embedded in paraffin, cut in 3-4  $\mu$  thick sections and stained with hematoxylin-eosin, PAS and Masson trichrome. For immunofluorescence studies, the kidney specimens were deep-frozen, cut in 4  $\mu$  thick sections in a cryostat, and incubated with fluorescing antiserum (the cappel sheep antirabbit serum: anti-IgG; IgM; C<sub>3</sub>). The bone specimens were fixed in 2.5% paraformaldehyde, cut at 2  $\mu$  thick section, post-fixed in osmium tetroxide and embedded in Epon-Araldite.

## RESULTS

Post-mortem examination revealed the operated kidney to be either of normal size and pale in colour or increased in size and consistency.

Only in one animal, sacrificed after 3 months, was the kidney size reduced. An increased consistency of the kidney was regularly present in those animals examined 1 year after surgery.

In all animals the contralateral kidney was normal. The ureter on the operated side was somewhat enlarged in rabbits from Groups 2 and 3. The anastomosis was patent in all cases, although in rabbits from Groups 2 and 3 there were, at the intestinal lumen, some granulations whose size ranged from a maize grain to a cherry-stone.



Fig. 1. Intravenous pyelography 12 months after left U.S.S. Right kidney is normal while left kidney is enlarged and the appearance of contrast medium is delayed



Fig. 2. Late radiographs of intravenous pyelography 12 months after left U.S.S. The pelvis and the ureter are dilated down to the anastomosis. It is also possible to see the contrast medium in the sigmoid

Intravenous pyelography showed delayed appearance and low concentration of contrast medium on the operated side (Fig. 1). The pelvis and the ureter appeared enlarged in direct proportion to the time from surgery. In all cases, the late radiographs showed the passage of contrast medium into the colon (Fig. 2). Blood urea nitrogen and plasma creatinine were increased in all rabbits 1 month after surgery (Fig. 3). Eventually, creatinine values remained stable but blood urea nitrogen showed a continuous and slow increase.

No change in serum electrolyte values was observed. Urine cultures from the bladder and renal pelvis were sterile. Microscopic examination of the urinary sediment showed some white blood cells in the bladder urine and a significantly greater number in urine from the renal pelvis.

Histological examination gave the following results:

- the right, non-operated kidney was normal in all animals.
- the left, operated kidney had diffuse lesions involving all its components, namely:

a. numerous inflammatory cells (lymphocytes and monocytes) in the interstitium, sometimes grouped, sometimes diffuse, with consequent disruption of the renal structure (Fig. 4). This lesion was more evident in rabbits sacrificed 3 months after the surgery.

b. vacuolar degenerative changes of tubules, (which also had atrophy and PAS-positive widening of their walls) was observed in all rabbits from the 3 groups.

c. dilatation of tubular lumina was often found, which was sometimes the most obvious change and associated with disruption of the wall of the tubule (Fig. 5). This dilatation was more evident in rabbits from Groups 2 and 3.

d. patchy interstitial fibrosis, affecting both cortex and deep medulla. This histological alteration was seldom present in rabbits from Group 1, more frequent in rabbits from Group 2 and very marked in animals from Group 3.

e. widening of the walls and narrowing of the lumina of many small and middle sized arteries (Fig. 6).

f. segmental or diffuse sclerosis of some glomeruli, and widening of Bowman's capsule associated with periglomerular fibrosis in many others. Vascular and glomerular lesions were more frequently observed in rabbits from Group 2 and 3 than in those from Group 1.

In rabbits from Group 3 the glomerular tuft was often collapsed and in some glomeruli the filtration space was dilated (Fig. 7).

Immunofluorescence studies showed positive staining for C<sub>3</sub> along some tubular membranes in the operated kidney of one rabbit from Group 1 (Fig. 8) and focal segmental glomerular staining for C<sub>3</sub> and IgM in the operated kidney of one rabbit from Group 3 (Fig. 9). No immunological lesions were observed in the non-operated kidney. Bone morphology was normal in rabbits from Group 1 and showed early defective mineralisation, seldom associated with increased bone resorption, in 2 animals from Group 2 and in 4 animals from Group 3 (Fig. 10).

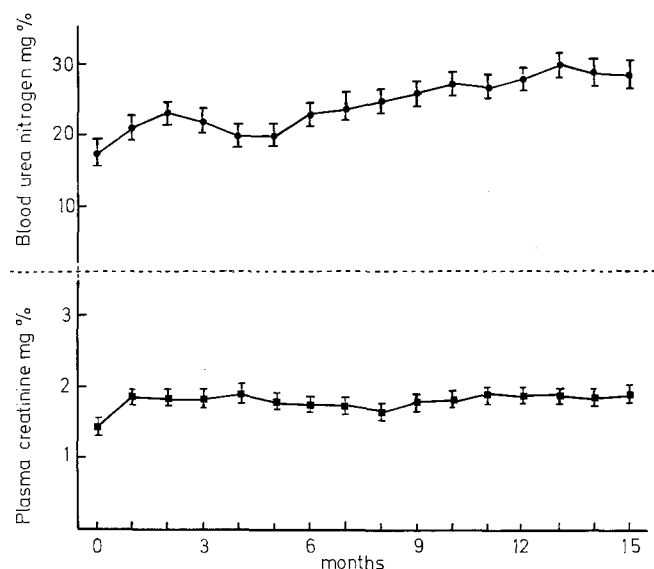


Fig. 3. Blood urea nitrogen and plasma creatinine in rabbits (mean values  $\pm$  S.D.) before surgery (time 0) and at monthly intervals. The results are given as mean values of biochemical determination in 15 animals after 3 months, in 10 animals after 9 months and in 5 animals after 15 months

## COMMENT AND CONCLUSIONS

In spite of the well-known sensitivity to general anaesthetics, alphaxalone administration was well tolerated in our rabbits. The surgical technique was chosen from those which fulfilled our investigational criteria. The results were good: in fact, at the end of the study, the anastomosis was still open and the affected kidney appeared to have residual function in spite of the hydronephrosis.

An adenoma near the anastomosis (Fig. 11, Fig. 12 and Fig. 13) has already been reported as a complication of U.S.S. in man (13). It is possible that it was the cause of ureteric dilatation and hydronephrosis. However, in all rabbits the anastomoses showed no stenosis and a catheter was easily placed through them. The late radiographs also showed the passage of contrast medium into the sigmoid.

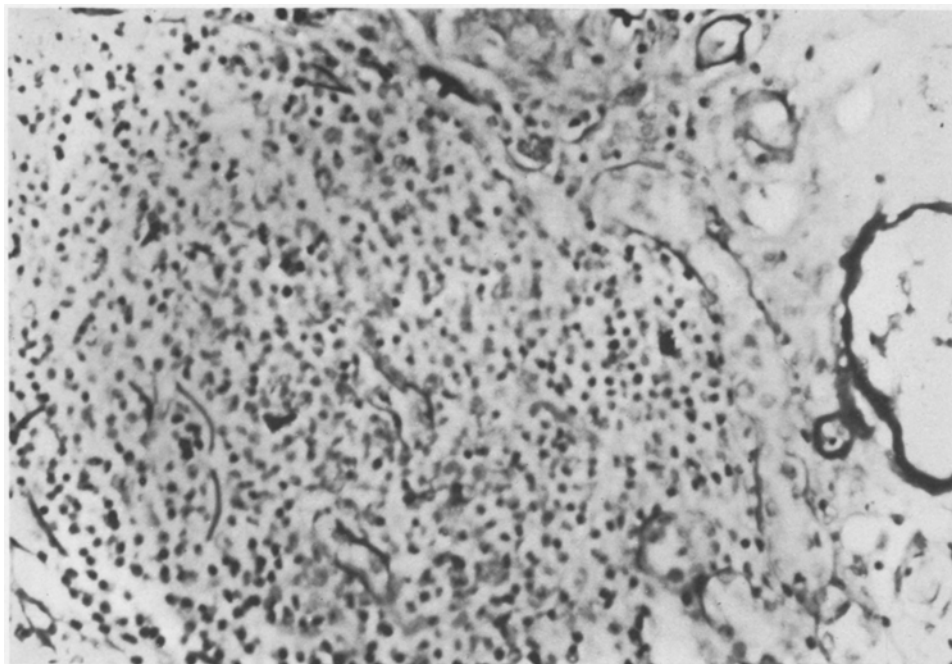


Fig. 4. Rabbit sacrificed 3 months after surgery. Numerous inflammatory cells in the interstitium with disruption of the renal structure. (PAS 40 X)

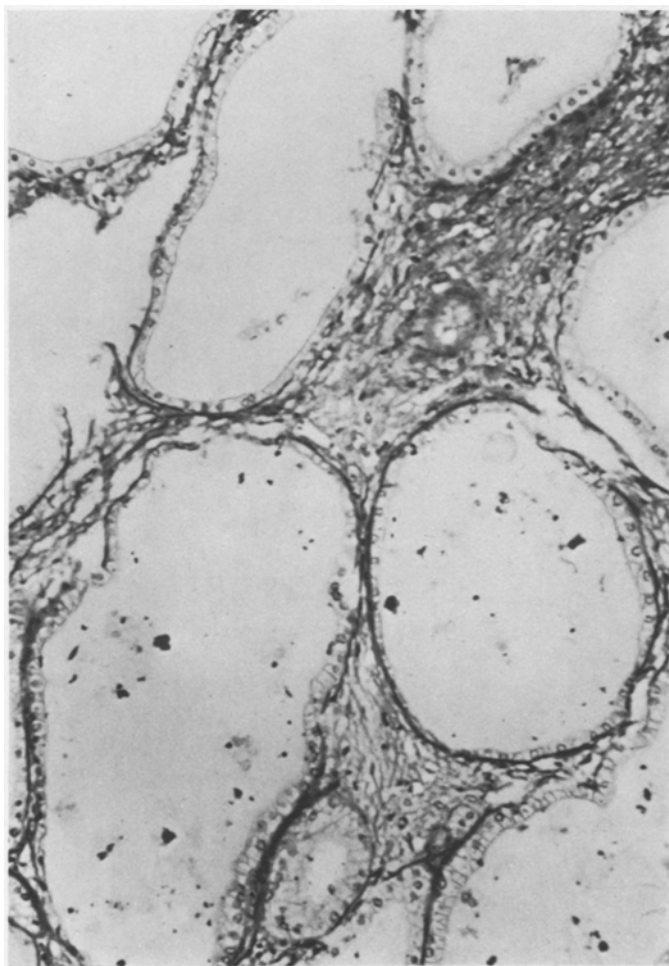


Fig. 5. Marked dilatation of tubular lumina with disruption of their walls. Interstitial fibrosis is also evident. No inflammatory cells were found; some glomeruli were sclerosed. This is a photograph taken of a rabbit in Group 3. (MASSON 40 X)

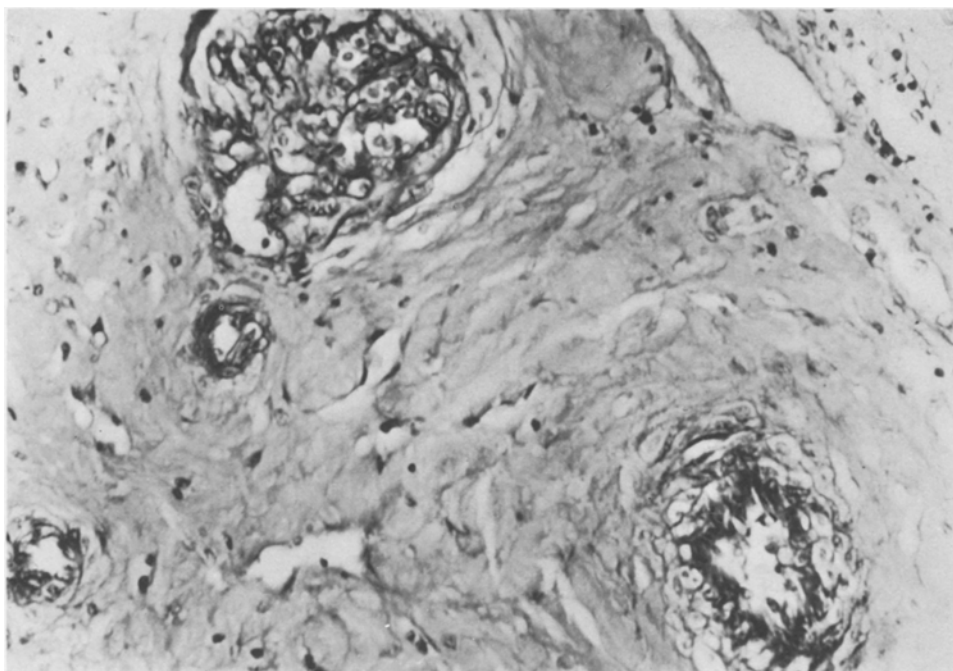


Fig. 6. Interstitial fibrosis and narrowing of the lumina of many small and medium-sized arteries. Thickening of Bowman's capsule and widening of mesangial spaces are also shown. Rabbit from Group 2. (PAS 40 X)

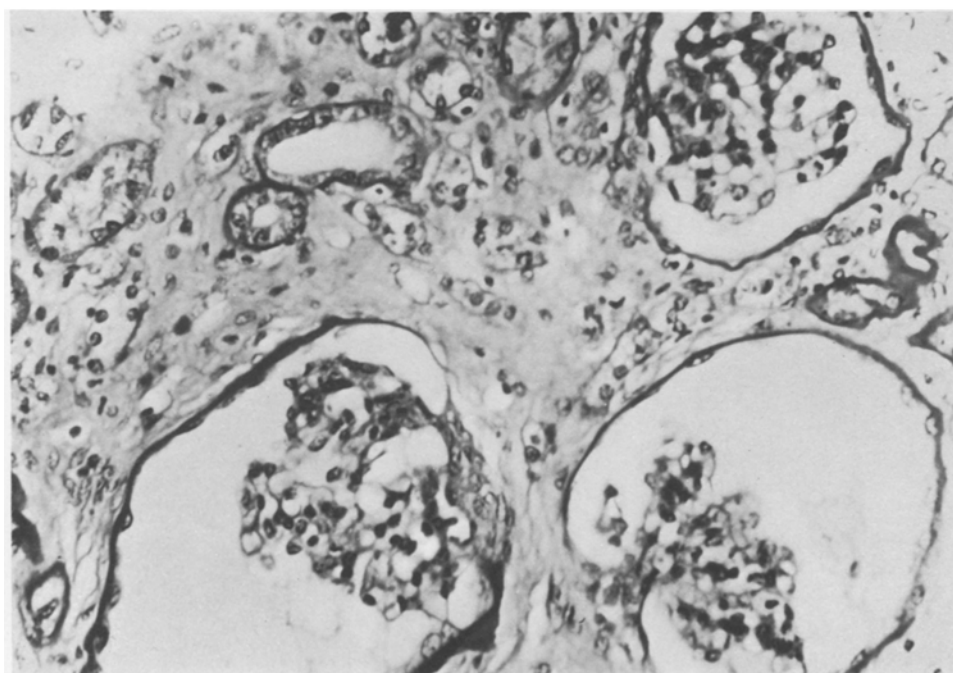


Fig. 7. Glomerular tufts collapsed and filtration space dilated. Interstitial fibrosis, tubular atrophy and PAS-positive thickening of tubular walls are shown. Rabbit from Group 3. (PAS 40 X)

The slight increase in blood urea nitrogen and plasma creatinine after surgery was, in our opinion, related to the early injury of the affected kidney: Tubulo-interstitial lesions were also evident in rabbits sacrificed 3 months after surgery.

The eventual steady state is probably explained by the normal function of the non-operated kidney still evident 15 months after surgery. The negative urine cultures from the pelvis and blad-

der, in spite of the microscopic evidence of pyuria, are not easily explained.

It is known, however, that a sterile pyuria may well mean bacterial infection even in the absence of bacteria, as bacteriuria may be an intermittent feature of chronic renal infections (7). Histological lesions typical of chronic pyelonephritis have also been observed in patients without evidence of bacterial infection at the time of examination (10, 1, 2). Begue, (3) in his experi-



Fig. 8. Immunofluorescence: Positive staining for  $C_3$  along some tubular membranes. No positive staining was found in glomeruli. Rabbit from the Group 1

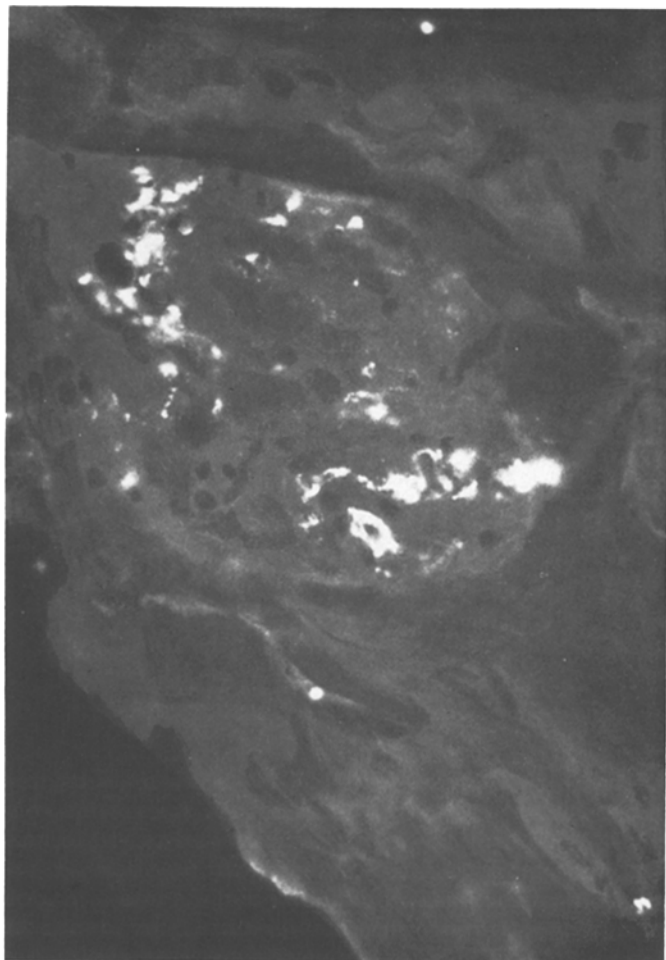


Fig. 9. Immunofluorescence: Positive glomerular staining for IgM in mesangial spaces and along some capillary walls. In this case glomerular  $C_3$  deposition had the same pattern. No  $C_3$  was found in the tubules. Rabbit from Group 3

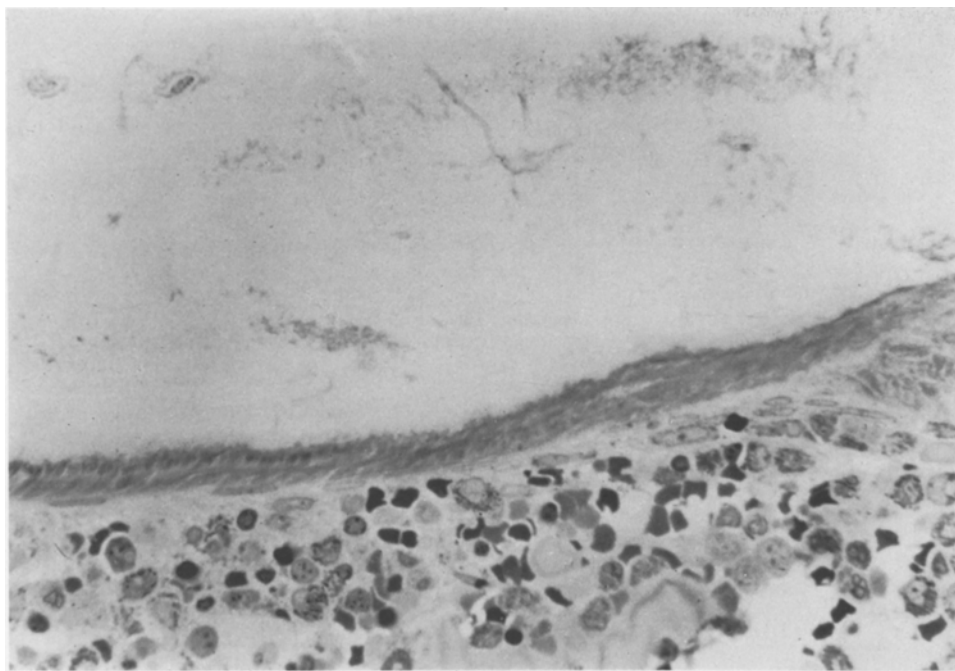


Fig. 10. Bone histology:early defective mineralization (little increase in osteoid) (Azur II, methylene blue, X 130)

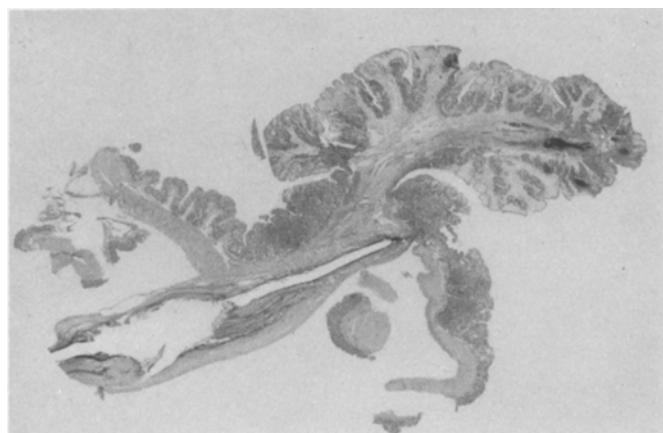


Fig. 11. H&E X 4. Low-power view of the polyp in the site of U.S.S.

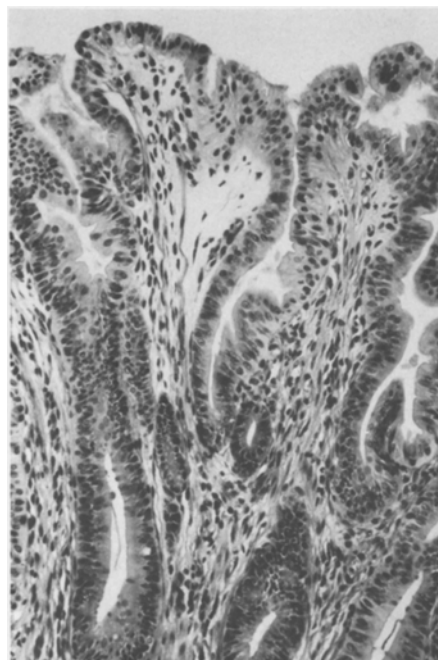


Fig. 12. H & E X 160

mental studies on rabbits has also observed abacterial pyelonephritis, which may suggest an immunological mechanism for the evolution of infectious renal disease.

The histological lesions observed in our rabbits suggest bacterial infection and hydronephrosis as the main causes of renal damage. Inter-

stitial inflammatory cells are the markers of renal parenchymal infection. Tubular atrophy and interstitial fibrosis may be regarded as the result of scarring after a primary inflammatory lesion. On the other hand, tubular dilatation and widening of filtration spaces are usually observed either in obstructive nephropathy (17)



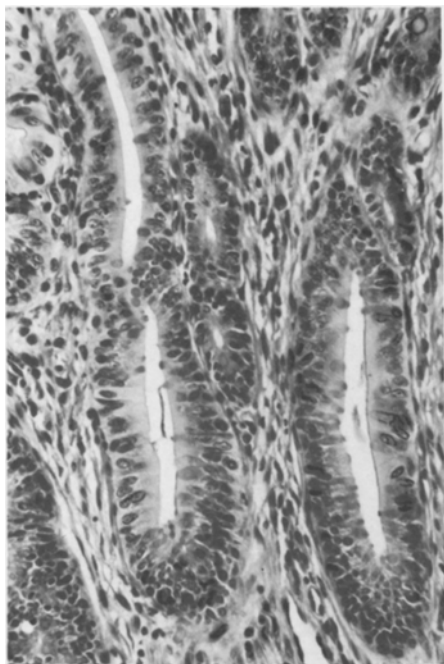


Fig. 13. H & E X 250. The polyp is composed of adenomatous tubules with mild to moderate dysplasia

or in reflux nephropathy, and suggest in our rabbits an associated hydronephrotic component of renal damage (which, in turn, may worsen the bacterial infection). The tubular immunofluorescence pattern may be regarded as a non-specific trapping of complement by the altered tubular basement membranes. It must be remembered, however, that bacterial antigens are known to activate the complement system by an alternative pathway and that small depositions of immunoglobulins and complement may be found inside the epithelial tubular cells together with bacterial antigens (12).

A glomerular immunofluorescence pattern similar to that observed in our animals has also been described in some patients with chronic pyelonephritis, and has been interpreted as being the result of deposition of immune complexes following a bacterial antigen stimulation (4).

The electron microscopic findings in bone tissue, namely slight and early defective mineralisation, are akin to those sometimes observed in incipient renal failure. Yet, although in our animals the contralateral kidney was intact, they appeared to have a moderate degree of renal insufficiency. It is known that bone lesions may be present also in this initial stage of renal failure (14).

In conclusion, those who attempted to improve the anastomosis in dogs and pigs (19, 21, 20, 16, 22, 11), succeeded, at best, to lower the incidence of pyelonephritis but not to eliminate it.

Since pyelonephritis is not apparently prevented nor improved by surgical techniques, we feel that the understanding of this renal pathology should take into account immunological factors.

Some interesting results have been reported by other authors (8, 3) who reproduced an experimental model of pyelonephritis by combining a short-lived obstruction and a direct injection of intestinal bacteria into the kidney. In human pathology, the main cause of pyelonephritis after U.S.S. is bacterial infection which reaches the kidney from the gut through the ureters: therefore, our experimental model seems to be valid for future studies.

We feel U.S.S. should be further investigated not only in the search for more sophisticated surgical techniques but also in an attempt to demonstrate (and possibly potentiate) in renal tissue a valid antibacterial immunity.

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