

Tubular Dimensions and Juxtaglomerular Granulation Index in Rat Kidneys after Unilateral Obstruction of the Ureter

A Study of the Morphogenesis of Hydronephrosis

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Summary. The effect of ligation of a ureter on the tissues in the kidneys in male Wistar rats was studied by morphometry. Dilation of the lumen in the obstructed kidney persisted after ligation only in distal convolutions and collecting ducts. Swelling of epithelial cells in the obstructed kidney was noted only in the distal convolutions and collecting ducts. The Juxtaglomerular Granulation Index (JGI) in the obstructed kidney increased to a maximum 7 days after ligation of the ureter. In the control kidney the lumen of Bowman's space was expanded, epithelial cells in both proximal and distal parts of the nephron were swollen, and the JGI was increased after ligation of the contralateral ureter. The morphological findings support the assumption that reduced cortical blood flow and decreased intratubular flow are the cause of proximal tubular atrophy rather than a persisting increase of proximal intratubular pressure.

Key words: Hydronephrosis, Tubular dimensions, Juxta-glomerular granulation index, Proximal convolution, Distal convolution, Collecting tubules.

Hydronephrosis occurs when urine collects in the pelvis of the kidney due to an obstruction of the ureter. The renal pelvis typically expands (1, 2, 3, 5, 18, 24, 26), but the tissue of the renal cortex seems to remain intact for several weeks after obstruction of the ureter provided no infection occurs. Since dilatation of the renal pelvis can subside after removal of even longlasting obstruction, some authors have attributed the kidney with a good regenerative capability (6, 12, 13, 21, 42, 43, 45). However irreversible disturbances of kidney function occur a few days after obstruction of a ureter (7, 8, 9, 10, 11, 19, 41).

Investigations of the structure of kidney tissue soon after obstruction of a ureter are rare (17, 31, 29) and, as far as we know, morphometric studies of kidneys with obstructed ureters have not been reported. In this study structural changes in different segments of the nephron in early hydronephrosis have been examined. The effect of obstruction on the juxtaglomerular granulation index (JGI) was al-

so examined because disturbances of kidney function in hydronephrotic kidneys have been related to changes in the Renin-Angiotensin system (7, 8, 9, 10, 11, 20).

Material and Methods

Thirty-eight male Wistar rats were used. They weighed 193-248 g at the start of the experiment and had free access to Altromin standard diet (R 10) and tap water. The left ureter was ligated through a lateral abdominal incision in 30 rats while the other 8 rats received sham operations and served as controls. Both kidneys were removed from groups of 6 rats under ether anesthesia at 6 hrs, 24 hrs, 3 days, 7 days and 14 days after ligating the ureter. Both kidneys were removed from control animals at 14 days. The kidneys were weighed after opening the renal pelvis and fixed for 24 hrs in Bouin's solution.

Paraffin sections (6-8 μ) were prepared from

the anteromedial border of each kidney using Goldner Trichrom staining. Zeiss equipment was used with a magnification of 500 times (objective 40x, ocular 12.5x) to draw the following structures in each kidney:

1. 50 glomeruli from all regions of the cortex of the medial convexity except near the upper and lower portions of the hilus.
2. 20 proximal convolutions, pars contorta, from the medial region of the cortex.
3. 20 distal convolutions at the point of contact with the glomerulus
4. 20 collecting tubules in the medial cortex. Only tubules of almost round outline, cut transversely and lying well apart from one another were drawn in order to prevent several measurements being carried out on one nephron

The lumen of the proximal convolution was drawn following the middle of the brush border. An Ott-planimeter (Type 31, 43/47), calibrated in such a way that 10 units of the planimeter (PE) corresponded to 1 cm^2 of the drawing or $400 \mu^2$ in the object, was used to measure the structures. We determined the free Bowman's space in the glomeruli, the total cross section area of tubules, and the area of the lumen. We deduced the epithelial cross sectional area from the difference between total cross sectional area of tubules and the area of the lumen. The juxta-glomerular granulation index (JGI) was determined according to Hartroft and Hartroft (1953) in Bowies stain.

We calculated the arithmetic mean of free Bowman's space in the glomeruli, the total cross sectional area of the tubules, the epithelial

elial cross sectional area and the JGI in each kidney.

By adding the mean values for the different animals of one test-group, the mean values for the left and right kidneys in each group were calculated. Statistical analysis was carried out by the student T-test. The average value for each group was compared with the corresponding average value of the control group.

Results

The effect of obstruction of the left ureter on the weight of the left and right kidneys is shown in Fig. 1. The weight of the obstructed (left) kidney increased significantly ($p < 0.001$) to a maximum on the 3rd day after ligation of the left ureter. The weight of the unobstructed (right) kidney also increased significantly above control values after 3 days ($p < 0.001$). Although the weight of the left and right kidneys tended to decrease after the 3rd day, they continued to weigh significantly more than control kidneys ($p < 0.05$). The difference between the weight of the left and right kidneys tended to diminish, but the left kidney continued to be much larger than the right kidney throughout the period of study (Fig. 2).

Fig. 3 shows the changes in the free Bowman's space and in the lumen area in the proximal convolution, distal convolution and collecting tubules after obstruction of the left ureter. A significant increase in the free

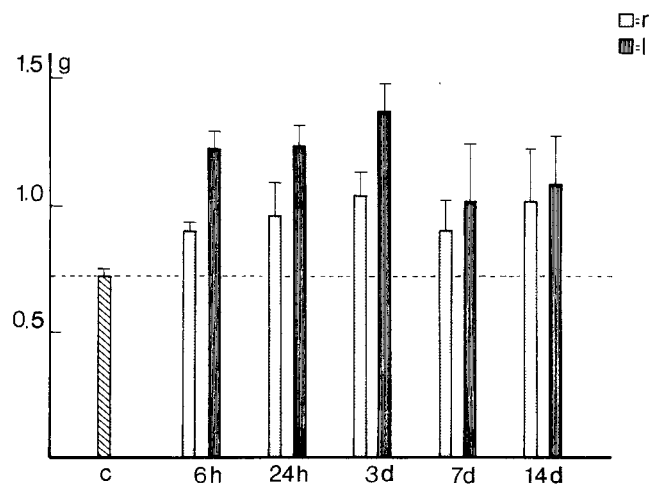


Fig. 1. Weight of kidneys after obstruction of the left ureter. The average weight of the kidneys in grams appears on the ordinate and the time after ligation of the ureter appears on the abscissa. c = control values; h = hours; d = days; r = right kidney; l = left kidney



Fig. 2. Photograph of rat kidneys 14 days after ligation of the left ureter. The left hydronephrotic kidney (shown on the right) has a pale cortex and flattened medullary cone. The right kidney with free urine flow (shown to left) has a normal cut surface

Bowman's space in the left kidney occurred 6 hrs after obstruction of the left ureter. Thereafter, the free Bowman's space in the left kidney decreased to a level significantly below the control values ($563.6 \pm 87.6 \mu^2$) at 24 hrs and 3 days after ligating the ureter. The free Bowman's space in the right kidney showed a significant decrease on the 1st day after obstruction of the left ureter, and then increased significantly throughout the experiment. The free Bowman's space in the left kidney was significantly less than that in the right kidney on the 3rd, 7th and 14th day after obstruction of the left ureter ($p < 0.05$).

The lumen areas of the proximal convolutions in the left kidney increased significantly 6 hrs after obstruction of the left ureter (Fig. 4), decreased significantly at 24 hrs, and thereafter (Fig. 3) failed to differ significantly from control values ($206.7 \pm 15.5 \mu^2$). The changes in the lumen areas in the proximal convolutions in the right kidney resembled those seen in the left kidney, except for a significant increase in the right kidney but not in the left kidney 3 days after obstruction of the left ureter.

The lumen areas of the distal convolutions in the left kidney increased significantly 6 hrs after obstruction of the left ureter (Fig. 3). Thereafter, the lumen areas of the distal convolutions in the left kidney showed a tendency to decline but, nevertheless, significantly exceeded the control values ($152.7 \pm 26 \mu^2$) throughout the test (Fig. 5 and 6). The lumen areas of the distal convolutions in the right kidney increased significantly above control values only on the 7th day. After ligation of the left ureter, the lumen areas of the distal convolutions in the left kidney were significantly greater than in the right kidney at all times (Fig. 4, 5, 6) except on the 7th day ($p < 0.05$).

The lumen areas of the collecting tubules in the left kidney increased significantly above control values ($258.2 \pm 34.4 \mu^2$) after ligation of the left ureter; the increase was apparent 6 hrs after ligating the ureter (Fig. 4) and persisted throughout the experiment (Figs. 5 and 6), except for a temporary decline on the 7th day. The lumen area of the collecting tubules in the right kidney tended to decrease ($0.5 > p > 0.1$) 6 hrs after ligation of the left ureter and then tended to increase above control levels on the 14th day ($0.5 > p > 0.1$).

Fig. 7 shows the changes in the cross sectional area of the epithelium in the proximal convolutions, distal convolutions, and collecting tubules after obstruction of the left ureter. The cross sectional area of the proximal tubular epithelium in the left kidney decreased significantly below control values ($925.7 \pm 74.1 \mu^2$) 6 hrs and 24 hrs after ligation of the left ureter and then increased to control levels. The cross sectional area of the proximal tubular epitheli-

um in the right kidney increased significantly above control values 7 days after ligation of the left ureter. Compared to the left kidney, the cross sectional area of the epithelium in the right kidney was significantly greater throughout the experiment ($p < 0.05$).

The cross sectional area of the epithelium in the distal convolution of the obstructed kidney decreased significantly below control levels ($478.7 \pm 28.1 \mu^2$) 6 hrs after ligation and then rose to a level significantly above control values on the 7th and 14th days. The cross sectional area of the distal tubular epithelium in

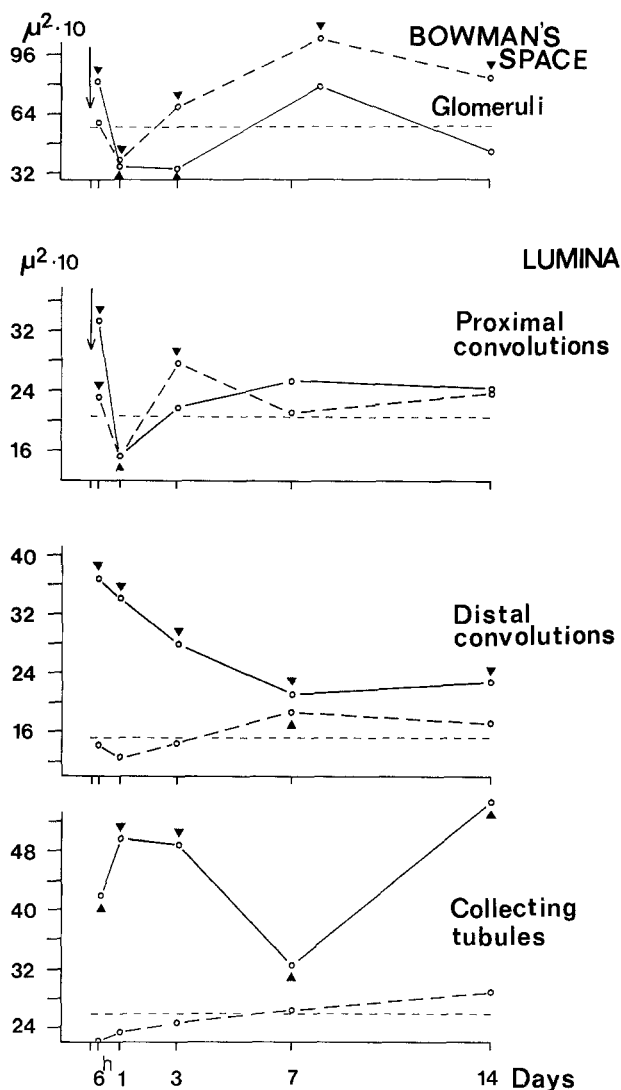


Fig. 3. Cross section areas obtained by planimetric measurements of free Bowman's space and of the lumen in different segments of the nephron in the left kidney (—) and in the right kidney (---) after ligation of the left ureter, and in control rats (----). Filled triangles indicate significant difference from control values

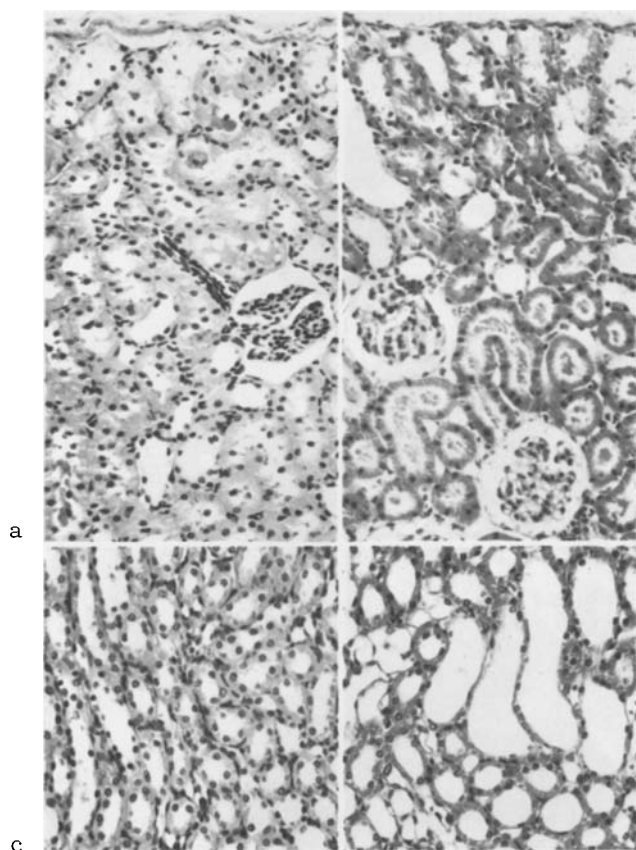


Fig. 4. Photomicrographs (Häematoxylin-Eosin staining, 153x) of the renal cortex and of the inner stripe of the outer medulla 6 hours after ligation of the left ureter. Renal cortex (a) and outer medulla (c) of the right kidney show a normal histological picture. Cortex (b) and outer medulla (d) of the left kidney show distinct dilatation of all nephron segments

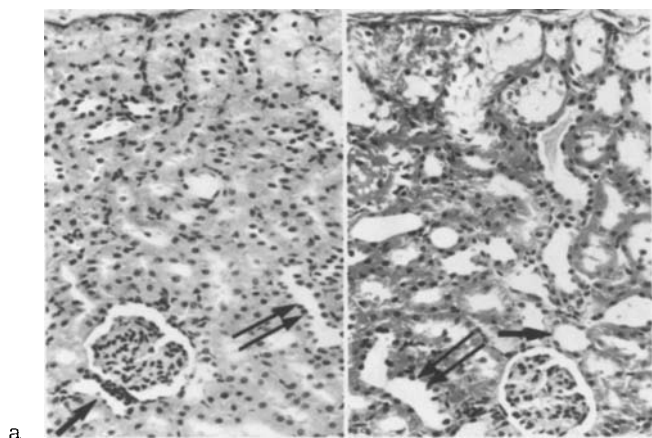


Fig. 5. Photomicrographs (Häematoxylin-Eosin staining, 153x) of the right kidney (a) and of the left kidney (b) 3 days after ligation of the left ureter. Note the distinct dilatation of distal convolutions. (→) and collecting tubules (⇒) in the left kidney while the proximal convolutions are not dilated

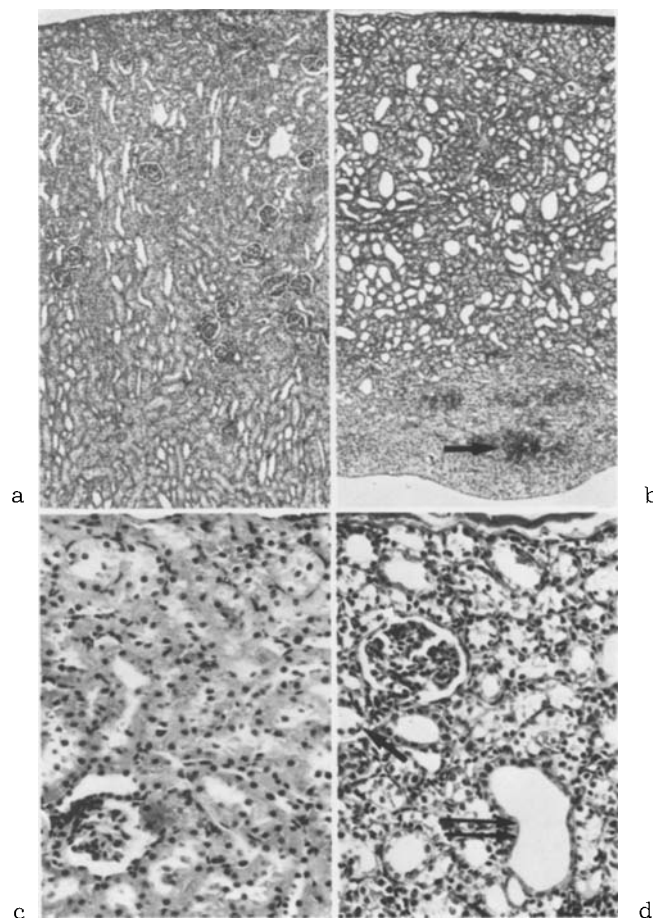


Fig. 6. Photomicrographs (Häematoxylin-Eosin staining) of the renal cortex and outer medulla of the right kidney (a 34x and c, 153x) and of the left kidney (b, 34x and d, 153x) 14 days after ligation of the left ureter. The diameter of the lumen in all segments of the tubules in the right kidney are normal with no discernible enlargement of the epithelial cross sectional areas. The distal convolutions (→) are slightly dilated and the collecting tubules (⇒) are considerably expanded in the left kidney. Considerable narrowing of the total parenchyma accompanied by extreme flattening of the medullary cone which contains a small hemorrhagic necrotic zone, is present in the left kidney (see Fig. b. →). Narrow lumina and hydropically changed epithelial cells are present in the proximal convolutions of the left kidney

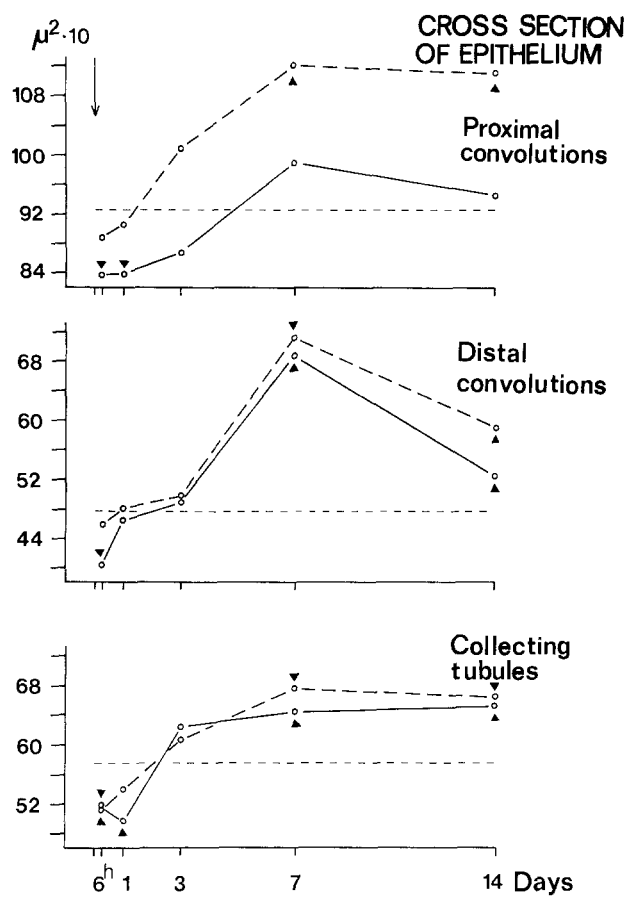


Fig. 7. Epithelial cross sectional areas in the different segments of the nephron after ligation of the left ureter. See the legend to Fig. 3 for the key to the symbols

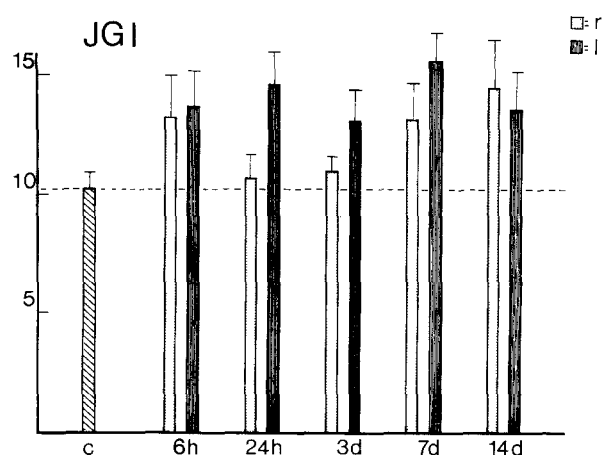


Fig. 8. The juxtaglomerular granulation index (JGI) in the left kidney (speckled bars) and in the right kidney (black bars) after ligation of the left ureter, and in intact rat kidneys (control value: striped bar and dotted line). Other symbols are described in the legend to Fig. 1

the unobstructed kidney failed to differ from control values until the 7th day on which a significant increase occurred and persisted until the end of the experiment.

The cross sectional area of the epithelium in the collecting tubules of the left and right kidneys showed similar changes after obstruction of the left ureter; a significant decrease occurred at 6 hrs followed by a rise to levels significantly above control values ($574.0 \pm 46.9 \mu^2$) on the 7th and 14th days.

Ligation of the left ureter for 6 hrs increased the JGI significantly above control levels (10.4 ± 0.7) (Fig. 8) in both kidneys. At 24 hr and at 3 days after ureteric ligation, the JGI was significantly elevated only in the left kidney. On the 7th day, the JGI in the left kidney reached its maximum and the JGI in the right kidney also exceeded control levels significantly. At the end of the experiment, the JGI in the left and right kidneys were similar and significantly greater than control levels.

Discussion

Although ligation of the ureter led to an initial rise in the cross sectional area of the lumen in both proximal and distal parts of the nephron, long-lasting expansion of tubules in kidneys with ligated ureters occurred only in the distal structures. Our direct measurements indicated that in the obstructed kidney the expansion of Bowman's space and of the lumen of the proximal convolutions had diminished within 24 hrs, while distension of the lumen of the distal convolutions and collecting tubules persisted for up to 14 days. Our findings in rats agree with observations made in rabbits (28, 34) and in dogs (31) in which either no change or slight transient distension was found in Bowman's space and in the proximal convolutions of kidneys with ligated ureters, while increasing ectasia was present in the distal convolutions and collecting tubules. Our findings do not agree with the view (29) that the distal convolutions are not dilated between the first and 7th day after unilateral ligation of the ureter.

On the first day following obstruction a decrease occurred in the cross sectional area of the epithelium in the proximal convolutions, distal convolutions and collecting tubules of the kidneys with ligated ureters but in collecting tubules only in the opposite kidneys. Following this, the area of the epithelial cells in these regions in both kidneys increased. In the proximal convolutions, the greatest rise occurred in the kidney with the non-obstructed ureter, while in the distal convolutions and collecting tubules comparable increases above control levels occurred in both kidneys.

An accumulation of fluid at different sites in the kidney (32, 33) and an increased synthesis of DNA, RNA and protein (16, 35, 36) are thought to be responsible for the increase in weight of hydronephrotic kidneys. In the present study increase in the cross sectional area of the lumen and of the epithelium was observed only in the distal convolutions and collecting tubules of kidneys with ligated ureters and suggests that the increase in weight of these hydronephrotic kidneys might be due mainly to an accumulation of fluid and hypertrophy in the distal portions of the nephron. On the other hand, the changes observed in the cross section area of the lumen and of the epithelium in the non-obstructed kidneys suggest that changes in both proximal and distal parts of the nephron may be involved in the increase in weight of these kidneys.

Our findings cannot be accounted for by the changes in the dimensions of the tubules in the renal cortex that occur after removal of the kidney from the animal (4, 2, 7, 30) since microscopic studies carried out on kidneys *in situ* also showed dilatation of the tubules in the distal convolutions and cortical collecting tubules after obstruction of the ureter (23, 39, 44). Evidently, expansion of tubules in the distal parts of the nephron in hydronephrotic kidneys is irreversible. In agreement with our results, a temporary dilatation of proximal convolutions has been seen by microscopic studies of kidneys *in situ* during the early phase after obstruction of the ureter (14, 25), but microscopic studies have not been carried out in kidneys *in situ* during the later phases of ureteric obstruction. Our histological observations show that dilatation does not persist in the proximal convolutions of kidneys with ligated ureters and permanent dilatation of tubules occurred only in the distal convolutions and collecting tubules of the obstructed kidney. Micropuncture studies also indicate that persistent dilatation of proximal convolutions will not occur in obstructed kidneys as long as the contralateral kidney remains intact (20, 44).

Pressure atrophy of the tubular epithelium in the kidney did not occur after ligation of the ureter. On the contrary, the epithelium was swollen. The failure of pressure atrophy to occur is consistent with micropuncture studies that have demonstrated the intratubular pressure to be at (44) or below (20) control levels in the obstructed kidney within one day of ligation of the ureter. In addition, after an initial rise, the renal blood flow and glomerular filtration rate in the obstructed kidney decreased (20, 40). An augmented sodium load in the distal convolution of the obstructed kidney is thought to be the most important cause of the reduction in renal blood flow (7-11, 20). Ac-

cording to these studies, reduction of renal cortical blood flow in acute hydronephrosis is produced by a feedback mechanism between the macula densa and the vascular pole of the glomerulus (37, 38). The rise in the juxtaglomerular granulation index observed in hydronephrotic kidneys in the present study may have been caused by a reduction of renal blood flow. The failure of Kelemen and Endes (22) to observe a rise in the juxtaglomerular granulation index in kidneys after ligating the ureter may have been due to their fixation (formalin instead of Helly or Bouin) and staining procedure (Goldner-Trichrom stain) that gave elevated values in the control kidneys compared to the levels obtained both in the present and in previous studies (15). Since we did not measure systemic blood pressure, total blood volume and renal blood flow, a conclusive interpretation of these findings in terms of the Renin-Angiotensin system is difficult and will be the subject of further investigations.

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