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Ureteral growth in animal models with increased renal excretion of urine

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Abstract The influence of increased functional load on the macroscopical and histological appearance of the ureter was investigated. Sixty rats were divided into five groups: (1) sucrose-fed rats with non-osmotic polyuria; (2) diabetic rats with osmotic polyuria; (3) uninephrectomized rats; (4) sham-operated control rats; and (5) control rats. The 24-hour urinary volume was measured on days 7, 14 and 21. Growth of the kidney, ureter and bladder was investigated and the histological appearance of the ureter was further evaluated. Diabetic and sucrose-fed rats had comparable polyuria with a seven-fold increase in urinary output. The urinary volume for the remaining kidney was doubled in uninephrectomized rats. After 3 weeks, diabetic rats had increased weight of the kidney, ureter and bladder, sucrose-fed rats had increased weight of the bladder, whereas uninephrectomized rats had increased weight of the kidney and ureter. The cross-sectional area (CSA) of the ureter wall from control rats increased from the proximal to the distal portion. The size of the whole ureter from diabetic rats was dramatically increased, the CSA of the wall of the distal ureter portion being four times that of the controls. The CSA of the ureter wall from sucrose-fed rats was increased only in the distal portion, whereas the ureter from uninephrectomized rats was increased only in the proximal portion. The results demonstrate the importance of differentiating between different portions of the rat ureter when examining histological sections of this organ. Moreover, polyuria per se is shown to induce growth of the bladder and of the adjacent distal part of the ureter, whereas uninephrectomy and diabetes caused growth of the kidney and the upper parts of the ureter, in addition to the growth induced by polyuria.

Key words Ureter · Histology · Polyuria · Diabetes mellitus · Nephrectomy · Rat

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Introduction

The ureter normally conducts urine by ureteral peristalsis from the kidney to the bladder. If urine production is increased, the frequency of ureteral peristalsis increases to accommodate the increase in volume load. When maximum peristaltic frequency is reached, further increases in flow occur by way of increase in bolus volume [15]. However, under conditions where volume load exceeds the ureteral transport capacity, e.g. ureteral obstruction, the ureter undergoes dilation and increases its wall thickness, and the muscular layer hypertrophies [3, 7]. Animal models with experimentally increased urine production represent a condition with altered functional load on the ureter. However, these models have mostly been associated with investigation of macroscopic growth of the kidneys and the bladder [1, 2, 4, 7], whereas no investigation has been performed to assess the effect of an increased functional load per se on the physical and histological changes in the ureter.

The present investigation was performed to assess the effect of increased renal excretion of urine on the ureter. Three groups of rats were employed: (1) rats with non-osmotic polyuria; (2) diabetic rats with osmotic polyuria; and (3) uninephrectomized rats, which have double functional load of the remaining kidney and ureter. More specifically, our goal was to distinguish between changes that occurred in the ureter as a result of increased renal

excretion of urine and those that might be due to pathogenic factors in diabetes and uninephrectomy.

Materials and methods

Animal study groups

All the animal experimental protocols were approved by the Animal Research Committee of Denmark. Ten-week-old female Wistar rats weighing 200–220 g (Panum Institute, Copenhagen, Denmark) were randomly allocated to the following groups:

(1) Streptozotocin-induced experimental diabetes, $n = 12$. A single intraperitoneal injection of 60 mg/kg streptozotocin (Sigma, St Loius, Mo.) dissolved immediately before administration in freshly prepared 50 mM citrate buffer (pH 4.0) was given to the rats 3 days before the first 24-hour urine collection. Blood glucose concentration was measured 24 hours after streptozotocin injection by a One Touch II instrument (Lifescan, Milpitas, Calif.) using the glucose oxidase method, and only rats with a blood glucose concentration exceeding 20 mmol/l were included in the study. Blood glucose concentration was subsequently measured twice weekly in the morning. The values were between 22.3 mmol/l and 33.1 mmol/l.

(2) Sucrose-fed rats on food-restriction, $n = 12.5\%$ sucrose was added to the drinking water and the availability of rat chow was restricted to 8 g/day from the first day of the experiment. This resulted in non-osmotic polyuria caused by a high water intake [2]. Urine samples were tested for glucose (BM-Test-5L, Boehringer Mannheim, Mannheim, Germany) to exclude glucosuria.

(3) Unilateral nephrectomy, $n = 12$. Nephrectomy was performed under barbiturate anaesthesia (Brietal, methohexital, 50 mg/kg i.p., Eli Lilly, Ind.). A small flank incision was made, the pedicle of the right kidney was then tied, and the kidney was removed. Care was taken not to damage the adrenal gland. The muscle and skin were then sutured. Analgesics (Temgesic, 0.1 mg/kg s.c., buprenorfin, Reckitt & Colman Pharmaceuticals, Hull, U.K.) were injected before the operative procedure and then postoperatively twice daily for 2 days.

(4) Sham-operated rats, $n = 12$. The pre- and post-operative procedures were as described for the uninephrectomized group. The right kidney was lightly manipulated manually, and the muscle and skin were then sutured.

(5) Control group, $n = 12$.

Experimental procedure

The rats were placed in the metabolic cages (Techniplast Gazzada, model 3700 M, Buguggiate, Italy) in the following periods: days 3–7, days 11–14 and days 18–21. The 24-hour urinary volume was measured on days 7, 14 and 21 after initial habituation of the rats to the metabolic cages. The room was temperature- (21°C) and moisture- (55%) controlled with a 12-hour light–dark cycle. The rats were allowed free access to rat chow (Altromin no. 1314, Altromin International, Lage, Germany) and drinking water, unless otherwise stated.

On day 21, rats were killed with barbiturate (Brietal, Methohexital), and the kidney, the ureter and the bladder were removed and weighed. The proximal end of the ureter was marked with a ligature approximately 4 mm distal to the renal pelvis. The ureters were then fixed by immersion in ice-cold, freshly prepared, buffered 4% paraformaldehyde.

Histological sections and area measurements

The ureters were cut at the ligature to exclude the part of the ureter adjacent to the renal pelvis. The remaining part of the ureter was then divided into thirds – proximal, middle and distal portions – and each of these was embedded in paraffin with the proximal end downwards. The paraffin-embedded tissue samples were cut perpendicular to the axis of their length into 10-μm sections using a microtome. The sections were then placed on gelatine-coated glass and stained with hematoxylin and eosin. The sections were examined by a Zeiss Axiophot microscope (Carl Zeiss, Oberkocken, Germany) connected with a high-resolution camera (Hamamatsu C2400, Hamamatsu Photonics, Hamamatsucity, Japan). The cross-sectional area of the urothelium, the submucosal and muscular layers were measured by means of an image-analysis program (NIH Image 1.59) run on a Power Macintosh (8500/120) computer. The ureteral lumen and the boundaries between the ureteral wall layers were outlined with the computer cursor, allowing the calculation of the cross-sectional area of the lumen and each layer in the wall. The examination and the computer analysis of the histological sections were performed without knowledge of the origin of tissue samples.

Statistical analyses

Results are shown as the mean ∓ SD. Comparison between groups was performed by two-way analysis of variance (ANOVA) followed by Fisher's protected least-significant difference post hoc analysis. Probability values of $P < 0.05$ were considered significant.

Results

Control group

In the control group, the relative weight of the kidney (Fig. 1a), of the ureter (Fig. 1b), and of the bladder (Fig. 1c) at the end of the study period was 4.56‰ (∓0.42‰), 0.10‰ (∓0.02‰) and 0.32‰ (∓0.14‰), respectively, of total body weight (Table 1). The cross-sectional area (CSA) of the ureter wall (Fig. 2a) which included the urothelium, the submucosal and the muscular layers, increased by approximately 30% from the proximal and middle portions to the distal portion of the ureter. Histological examination of the three different portions of the ureter (Fig. 3A, B) revealed a stellate configuration of the ureteral lumen, a urothelium consisting of four to five cell layers, a submucosal layer and well-developed smooth muscle layers.

Diabetic group

In the diabetic rats with osmotic polyuria, the body weight (Table 1) was decreased after 3 weeks by approximately 25% when compared to controls. The organ and relative weight of the kidney (Table 1, Fig. 1a) were increased by approximately 25% and 65%, respectively, the organ and relative weight of the ureter (Table 1, Fig. 1b) by 130% and 200%, respectively, and the organ and relative weight of the bladder (Table 1, Fig. 1c) were increased by 210% and 290%, respectively, when compared with controls. All three portions of the ureter from diabetic rats had increased CSA of the wall (Fig. 2a) when compared with controls, and like the ureter in the control animals, the CSA of the wall in the diabetic rats was largest in the distal portion of the ureter when compared with the middle and proximal parts. The increment in the area of the muscular layer (Fig. 2d)

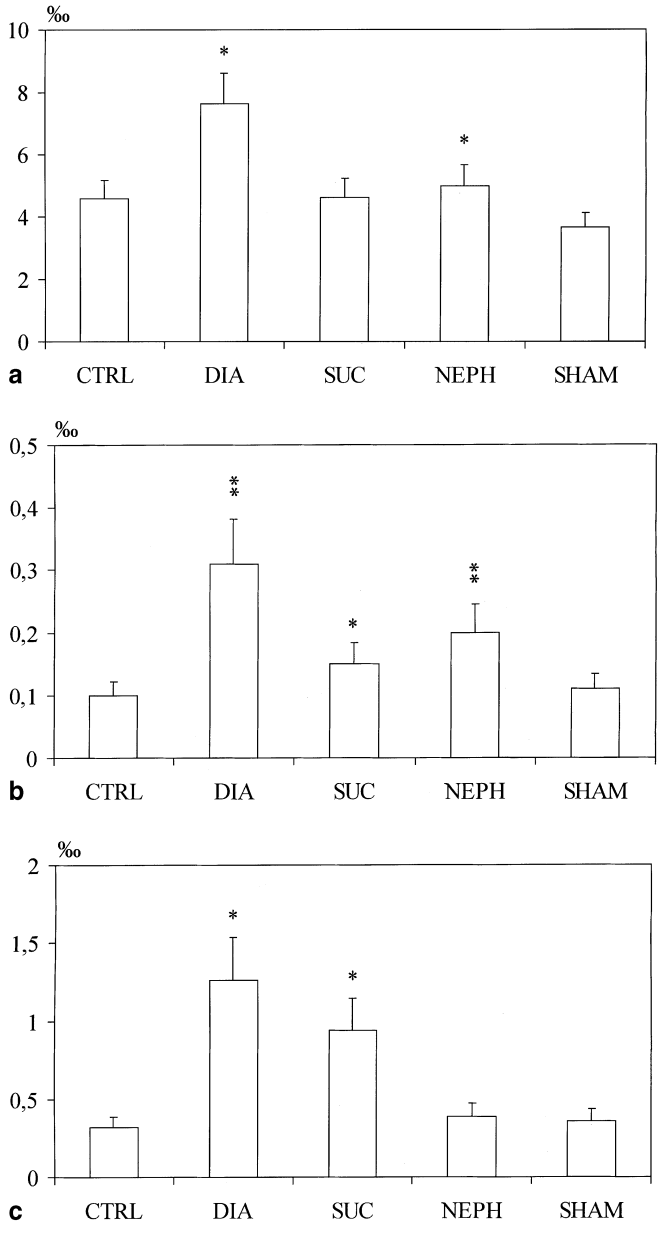

Fig. 1 The relative weight of the urinary organs in rats with increased renal excretion of urine for three weeks. **a** Kidney : body weight ratio, **b** ureter : body weight ratio and **c** bladder : body weight ratio. *CTRL* control, *DIA* diabetic, *SUC* sucrose-fed, *NEPH* uninephrectomized, *SHAM* sham-operated control rats. Each bar represents the mean, and the standard deviation of the mean of 12 observations is indicated. $*P < 0.05$ and $**P < 0.01$ vs appropriate controls

seemed most pronounced when the different wall layers were compared with each other, constituting 47% and 46% of the CSA in the proximal and middle ureter portions, respectively, compared with approximately 35% in the three portions of the ureter from controls. Histological examination of the proximal and distal portions of the ureter from diabetic rats (Fig. 3C, D) revealed pronounced luminal dilation with circular lumen, increased diameter and flattening of all wall layers. Also in the middle portion of the ureter from diabetic rats, a remarkable tendency to luminal dilation was present.

Sucrose-fed group

In sucrose-fed rats with non-osmotic polyuria, the body weight at the end of the study period (Table 1) was decreased when compared with controls. The organ weight of the kidney (Table 1) tended to be lower than the kidney weight in control rats, whereas the organ weight of the ureter tended to be higher than that of controls. The relative weight of the ureter (Fig. 1b) was increased by 50% and the weight of the bladder, expressed both as organ (Table 1) and relative weight compared with body weight (Fig. 1c), was increased by 165% and 190%, respectively, when compared with controls. Measurements of the CSA of the ureter wall of the sucrose-fed rats (Fig. 2a–d) revealed enlargement of all wall layers in the distal portion when compared with controls, whereas the proximal and middle portions were comparable to controls. All layers in the wall of the distal ureter portion (Fig. 2b–d) from sucrose-fed rats had increased area when compared with the same layers in the proximal portions. Histologically, the proximal portion of the ureter from sucrose-fed rats (Fig. 3E) appeared comparable to controls, whereas the lumen of the distal portion of the ureter from sucrose-fed rats (Fig. 3F) appeared to be more dilated than observed in controls.

Uninephrectomized group

In uninephrectomized rats, the body weight (Table 1) was reduced, but they had increased organ and relative weight of the kidney and the ureter (Table 1, Fig. 1a, b) when compared with sham-operated controls. The relative weight of these organs was lower than those measured in diabetic rats. Only the proximal portion of the ureter from uninephrectomized rats had increased CSA of the ureter wall (Fig. 2a) compared with sham-operated controls, and this was due to increased area of the urothelium and the submucosal layer (Fig. 2b, c), whereas the area of the muscle layer (Fig. 2d) was unchanged. Histologically, the proximal portion of the ureter from uninephrectomized rats (Fig. 3G) appeared enlarged with the normal stellate configuration of the lumen. The distal portion of the ureter from uninephrectomized rats (Fig. 3H) appeared comparable to controls.

Discussion

The present study demonstrates that the wall of the normal rat ureter increases in cross-sectional area when moving from the kidney towards the bladder, and that polyuria per se induces growth of the distal portion of the ureter and of the bladder.

A pronounced difference in the cross-sectional area was observed between the three different portions of the ureter from the control animals. The smallest cross-sectional area of the ureter wall was measured in the proximal portion, and it then increased by approxi-

Table 1 Body weight, weight of organs in the urinary tract and diuresis in rat models with increased urinary loads

	Control	Diabetic	Sucrose-fed	Uninephrectomy	Sham-operated
Final body weight (g)	244 ± 2	184 ± 3*†	223 ± 4*	222 ± 4*	239 ± 4
Kidney weight (g)	1.12 ± 0.03	1.41 ± 0.16**	1.03 ± 0.09	1.45 ± 0.18**	1.10 ± 0.04
Ureter weight (mg)	24.9 ± 5.1	56.9 ± 16.7**	33.5 ± 8.5	43.3 ± 12.2*	22.6 ± 3.9
Bladder weight (mg)	78.8 ± 33.9	243.9 ± 58.2*	210.0 ± 61.5*	85.1 ± 23.3	76.1 ± 29.6
Volume excreted (ml/24hr)					
Day 7	21 ± 3	129 ± 10 ‡	130 ± 18 ‡	23 ± 1	19 ± 1
Day 14	19 ± 3	155 ± 25 ‡	132 ± 12 ‡	29 ± 2	25 ± 5
Day 21	20 ± 3	153 ± 24 ‡	175 ± 12 ‡	22 ± 4	17 ± 2

Values represented are mean value ± SD, $n = 12$ in each group
*$P < 0.01$ vs appropiate controls; **$P < 0.01$ vs appropiate controls and sucrose-fed group; ‡$P < 0.01$ vs appropiate controls and uninephrectomized group; †$P < 0.01$ vs sucrose-fed and uninephrectomized groups

mately 30% towards the distal portion of the ureter. These results clearly demonstrate the importance of differentiating between different portions of the ureter when examining histological sections of this organ from the rat. The human ureter also differs in size along its length. The thickness of the wall gradually increases along the ureter [5], whereas the widest point is located just above the iliac vessel crossing [18].

It has been generally believed that the ureters, in the case of polyuria, become distended over their full length and convey urine in the form of a column [15], and that it is the pressure gradient created by the renal blood flow and not ureteral peristalsis that pushes the urine to the bladder [9]. However, the relationship between the physical and histological changes that occur in the ureter with increased functional load has not been studied in detail. In the present study, the ureters in the groups of rats with polyuria were affected differently depending on a diabetic-induced osmotic diuresis or a water-induced non-osmotic diuresis, although the functional load on the ureter was similar. Histological examination of the macroscopic enlarged ureters from the diabetic rats revealed tubes with open circular lumen, whereas the ureters from the sucrose-fed rats appeared luminally dilated only in the distal portion. These results indicate that polyuria per se, in addition to growth of the bladder, induces growth of the distal part of the ureter, whereas the growth of the kidney and the proximal and middle portions of the ureter in diabetic rats must depend on other growth-stimulating mechanisms.

Dilation of the ureteral lumen has previously been observed in rats and dogs with partially obstructed ureters [3, 7], and in patients suffering from diabetes insipidus, as part of hydroureteronephrosis [14]. Thus, the dimensions of the upper urinary tract are under the influence of the intraluminal pressure [6], which might be one physiological factor that explains the luminal dilation of the ureters in the present groups of rats with polyuria, at least in the distal and most distended parts.

In the present study, the uninephrectomized rats had increased cross-sectional area of the proximal portion of the ureter wall, due to increased area of the urothelium and the submucosal layer. The lumen was not dilated and remained in its normal stellate configuration. Nevertheless, the ureteral enlargement seems remarkable, in spite of only a doubled functional load of the ureter in these rats. However, it has recently been shown that the ureteric wall layers in rats are very sensitive to a growth-stimulating effect of epidermal growth factor (EGF) [20], a peptide normally synthesized in the kidneys and excreted in its biologically active form into urine. In a recent study, [19] we found that the 24-hour urinary excretion of EGF was increased in uninephrectomized rats by up to 140% when expressed as excretion per kidney. Thus, the remaining ureter in the uninephrectomized rat is exposed to more EGF in urine than in other groups. This finding supports the assumption that EGF in urine might be a potential candidate for a mitogen in urine with a growth-stimulatory effect on the ureter. Moreover, the ureter growth in uninephrectomized rats caused increased areas of the mucosal and submucosal layers that also support a role for a luminal "factor" like EGF.

The two groups with polyuria in the present study had, in addition to the increased size of the ureter, increased weight of the bladder. This finding corresponds to the results of previous studies and supports the hypothesis that increased diuresis results in an enlarged bladder [1, 4, 10, 12]. The bladders of the diabetic rats macroscopically appeared more atonic and distended than the bladders in rats with water-induced polyuria, even though the organ weight in the two groups was alike. It is not clear, however, whether the enlargement of the bladder in diabetes mellitus is the result of an autonomic neuropathy or a reflection of increased diuresis [1, 2, 11, 12]. But the present results indicate that the increased tissue mass of the bladder is caused by polyuria per se, whereas other mechanisms cause the atonic appearence of the bladder in diabetic rats.

Enlarged kidneys were observed in the present study in the groups of diabetic and uninephrectomized rats after three weeks. This finding is in agreement with the results of previous studies in which the weight of the kidney was found to be increased by 30% and 38%, respectively, 20 days after induction of diabetes or unilateral nephrectomy [16, 17]. Recent evidence has revealed that a number of polypeptide growth factors,

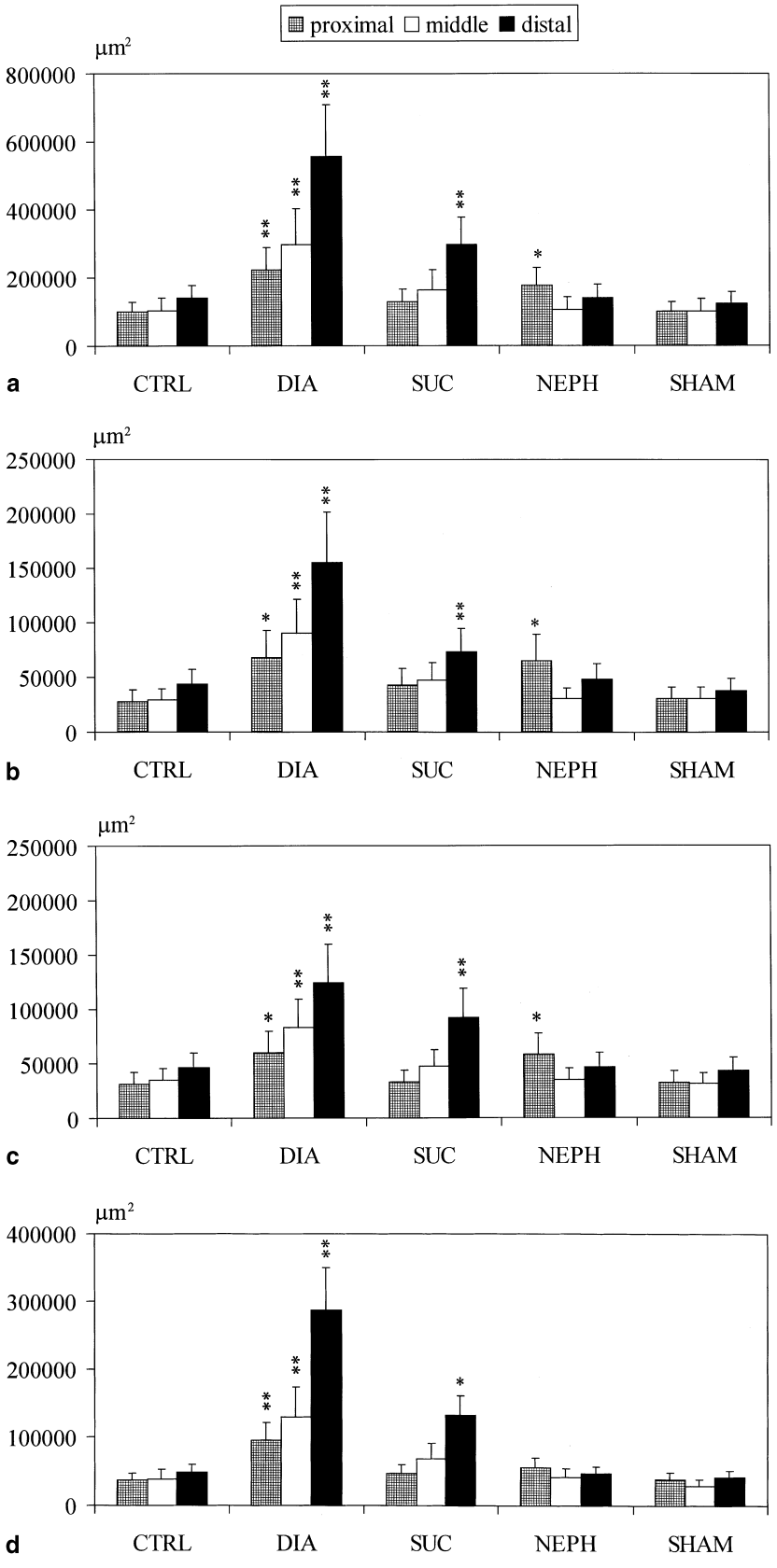

Fig. 2 The physical changes of the ureters in rats after 3 weeks with increased renal excretion of urine. **a** Cross-sectional area of the ureter wall, **b** urothelium, **c** lamina propria, **d** muscular layer of the ureter. *CTRL* control, *DIA* diabetic, *SUC* sucrose-fed, *NEPH* unine-phrectomized, *SHAM* sham-operated control rats. Each bar represents the mean, and the standard deviation of the mean of 12 observations is indicated. $*P < 0.05$, $**P < 0.01$ vs appropriate controls

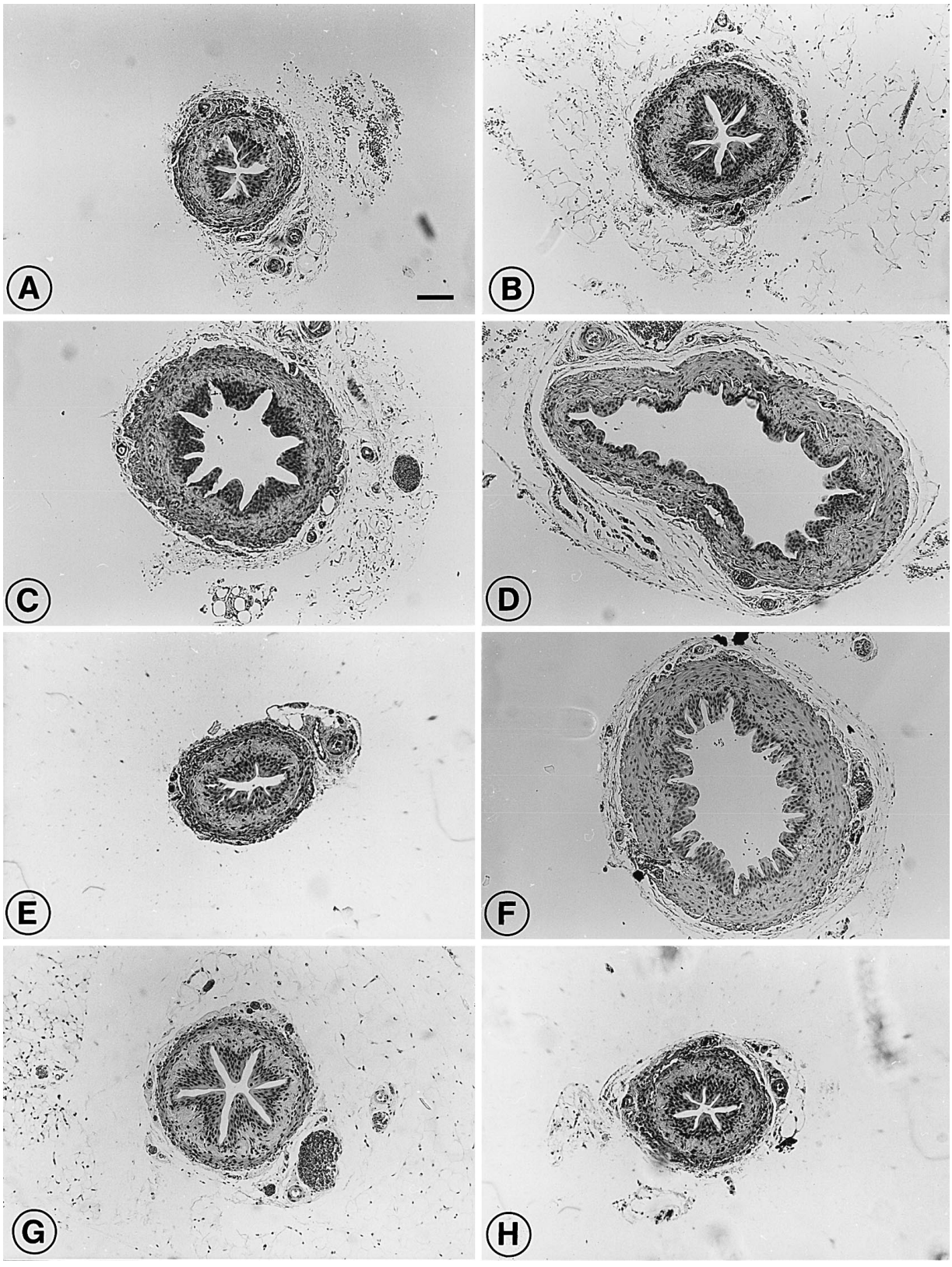

Fig. 3 The histological changes of the ureters in rats after 3 weeks with increased renal excretion of urine. The left panel shows the proximal part of the ureter and the right panel, the distal part of the ureter. **A** and **B** normal control rat, **C** and **D** diabetic rat, **E** and **F** sucrose-fed rat, **G** and **H** uninephrectomized rat. These photographs are representative of all sections examined from each group of rats ($n = 12$). Magnification, ×60, bar = 90 μm

including EGF, have potential functions in renal hypertrophy as paracrine agents within the nephron [8, 13].

The results have clearly demonstrated the importance of differentiating between different portions of the rat ureter when examining histological sections of this organ. Polyuria per se was shown to induce growth of the bladder and the adjacent distal part of the ureter. In contrast, uninephrectomy and diabetes caused growth of the kidney and the upper parts of the ureter. This growth is thus likely to be caused by other mechanisms than polyuria.

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