Urol Res (1988) 16:441–447

Urological Research

The Effects of Urine on Mast Cells and Smooth Muscle of the Human Ureter*

L. Ugaily-Thulesius[1] and O. Thulesius[2]

Departments of [1]Anatomy and [2]Pharmacology-Toxicology, Faculty of Medicine, Kuwait University, Safat, Kuwait

Accepted: July 10, 1988

Summary. Electron microscopy was performed on normal human ureteral rings before and after incubation in human urine for 30 minutes. A large number of mast cells was detected subepithelially and in close proximity to smooth muscle fibres. Treatment with urine (346 mOsm/l) induced various degrees of degranulation in the majority of mast cells. Some membrane bound granules were found free in the surrounding connective tissue and near smooth muscle cells indicating rupture of the cell membrane. In the functional study frequency and amplitude of peristaltic contractions were studied in-vitro. Addition of urine increased frequency and amplitude of peristaltic contractions and addition of the histamine-1-blocker mepyramine (10^{-6} M) partially reversed these changes. It can be concluded that in a situation with urothelial damage such as ureteral calculus, urine can penetrate subepithelially and induce degranulation of mast cells with release of mediators. This is followed by forceful peristaltic contractions which are induced by histamine and other newly formed mediators such as prostaglandins. The process is likely to occur in renal colic with impacted kidney stones.

Key words: Mast cells – Ureter – Histamine – Renal colic

Introduction

In a previous study we demonstrated the presence of a large number of mast cells in the human and in the sheep ureter [12]. The functional role of mast cells has not been clarified except in allergic and anaphylactic reactions and no known physiological function can as yet be ascribed to the newly described mast cells in the ureter although their close association to the specialised LA-cells may hint at a cellular release mechanism controlling ureteral motility. Ultrastructural studies on experimental pyelonephritis [6] and bilharziosis [13] presented evidence that under these condition the urothelial barrier is damaged and therefore hyperosmolar and acidic urine may penetrate into the subepithelial space. Other instances in which a damage to the urothelial barrier can be expected are impacted renal calculi. Incubation of cut ureteral rings in Krebs solution with urine exposed the subepithelial structures to urine and induced profound disturbancies of peristaltic contractions [11]. The present study tests the hypothesis that ureteral rings incubated in freshly voided urine may initiate mast cell degranulation and therefore the release of histamine, a substance known to stimulate ureteral smooth muscle contractility [4, 12].

Material and Methods

Six normal ureters were obtained from kidney donors (mean age: 36 years). After operation the ureters were put into chilled Krebs-Henseleit solution and immediately transferred from the hospital to the laboratory were they were dissected free of fat and connective tissue and cut into rings for electron microscopy and pharmacological testing.

Electron Microscopy

Samples were cut into two 1 mm thick rings. One served as control and the other was incubated for 30 min in freshly voided urine from a healthy individual. The control and treated specimens were immediately fixed by immersion in 3% glutaraldehyde buffer of pH 7.3 at 4 °C for 3–4 h. The tissues were than post-fixed in 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Araldite. Orientation of the block was achieved by examining 1 μm sections stained with toluidine blue. Ultrathin sections were cut using an LKB Nova Ultratome, mounted on G200 grids, stained with uranyl acetate and lead citrate and viewed in Joel 1200 CX electron microscope.

* Supported by Grant No. MA 015, Kuwait University

Table 1. The effect of urine on the frequency and amplitude of ureteral contractions and treatment with mepyramine (10^{-6} M) in 17 experiments on human ureteral rings (mean ± SE, P = probability)

	Frequency c/min	P	Amplitude	P
Control	0.91 ± 0.26	0.005	2.88 ± 0.43	0.15
Urine	1.98 ± 0.25	0.2	3.54 ± 0.34	0.1
Mepyramine	2.47 ± 0.47		2.85 ± 0.38	

Functional Studies

Ureteral rings of 4 mm length were used for recording of rhythmic motility in an organ bath as described previously [10]. The 10 ml organ bath contained Kreb's-Henseleit solution at 37 °C and was areated with a mixture of 95% oxygen and 5% carbon dioxide. The lower end of the preparation was tied to a tissue holder and the upper end connected to a strain gauge force transducer (Dynometer, UF1) and tension continuously recorded on a Lectromed MX216 recorder. A pretension of 2 g was applied to the preparation which was allowed to equilibrate for 1 h, after which time urine and subsequently mepyramine were administered.

Osmolality of the urine samples added to the organ bath and the resultant final osmolality of the bathfluid was determined with a microosmometer Model 3MD of Advanced Instruments Inc., Needham Heights, Mass, USA, after calibration with a 290 mOsm/kg reference solution. The Krebs-Henseleit solution contained (mM): NaCl, 115.3; KCl 4.6; $CaCl_2$, 2.3; $MgSO_4$, 1.2; $NaHCO_3$, 22.1; KH_2PO_4; 1.1 and glucose 7.8 at pH 7.4.

Results

Electron Microscopy

Ureters of control experiments showed large numbers of mast cells in close proximity to smooth muscle cells, La-cells (Figs. 1 and 2), as well as small unmyelinated nerve fibres (Fig. 3). This is in keeping with our previously reported results on the normal human ureter [12].

Upon examination of ureteral specimens which were exposed to urine, a small number of mast cells was found intact (Fig. 4). A large population, however, was detected in various states of degranulation. Some cells appeared completely devoid of granules and instead, contained empty membrane-bound vesicles (Fig. 5). In others, the granules varied in their content and electron density (Fig. 6). Degranulation as a result of cell membrane disruption was also evident. In these cases, free membrane-bound granules were seen in the surrounding connective tissue and close to smooth muscle cells, with some cellular remains in the vicinity (Figs. 7, 8, 9). Some cells contained vesicles with electron-dense material some of which were lamellar in appearance, typical of human mast cell granules. Associated with these vesicles was a marked accumulation formed mediators such as prostaglandins and leukotrines and we have previously presented evidence that prostaglandins both in human and sheep ureters are essential for the motor control of peristalsis [1, 2, 10]. Moreover it is known that histamine-1 blockers like mepyramine in addition to their antagonism at histamine receptors have a direct, biphasic action on mast cells. At low concentrations they inhibit the histamine release produced by immunological and other stimuli whereas at higher concentrations they themselves induce release [8].

In ureters which were incubated with urine, a range of mast cells was observed. Very few were totally uneffected and remained intact while others showed various states of degranulation. This could be related either to the func- of filaments within the cytoplasm (Figs. 10, 11). Ocassionally, the whole cytoplasm appeared as a mass of filaments with few granular remains (Fig. 12).

Functional Studies

The ureteral rings suspended in organ baths displayed a pattern of continuous and regular rhythmic peristaltic activity. Addition of urine increased the osmolality of the bathing medium from 296 mOsm/l to 346 ± 10 mOsm/l. This was associated with a rapid increase in frequency (+117%) and amplitude (+23%) of peristaltic contractions. Moreover, basal tone also increased transiently (see Table 1). This pattern of hypermotility was partially blocked by the histamine –1 receptor blocker mepyramine (cf. Fig. 13).

Discussion

Mediator release from mast cells may take place by a degranulation mechanism that leaves the cell intact or by destruction of the cell membrane [7]. In our study using hypertonic urine which contains ionised molecules there is evidence that degranulation was associated with structural changes of the mast cell membrane. Previous studies have shown that hypertonic solutions can trigger release of histamine from human basophils [5]. Ureteral motility is relatively resistant to stimulation with autonomic transmittors but readily responds with increased motility to prostaglandins [10] and histamine [4, 12]. Histamine not only increases frequency of contractions but also base-line tone which may constitute the spastic component of ureteral colic [12]. Moreover histamine stimulates pain fibres [9] and is therefore, a strong candidate as a promotor of ureteral colic and other painful upper urinary tract disorders that involve damage of the urothelium such as in pyelonephritis and bilharziosis [13].

In the present study addition of the H-1 blocker mepyramine partially blocked the increased motility observed with urine. This is not surprising since degranulation of mast cells is also associated with the release of other newly tional characteristics of these cells or the variation in

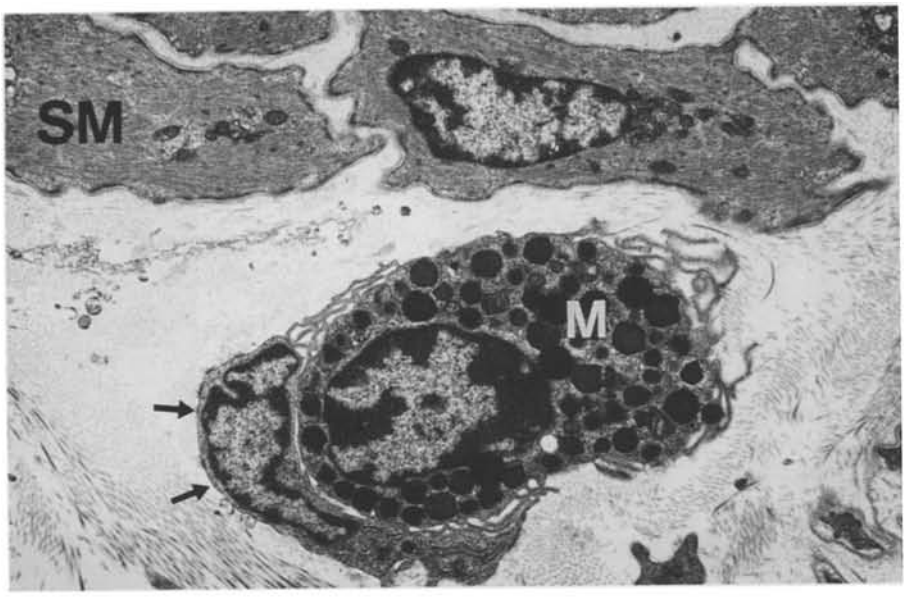

Fig. 1. Mast cell (*M*) in close proximity to smooth muscle cells (*SM*) and LA cell (*arrows*). Control human ureter. x6,400

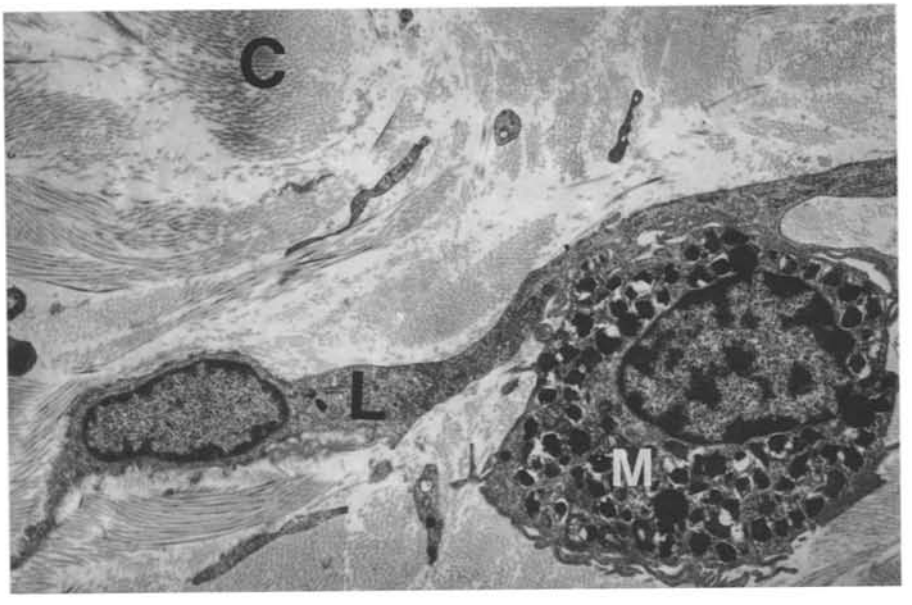

Fig. 2. Mast cell (*M*) in close contact with LA cell (*L*) in the control human ureter. *C* = Collagen fibres. x5,200

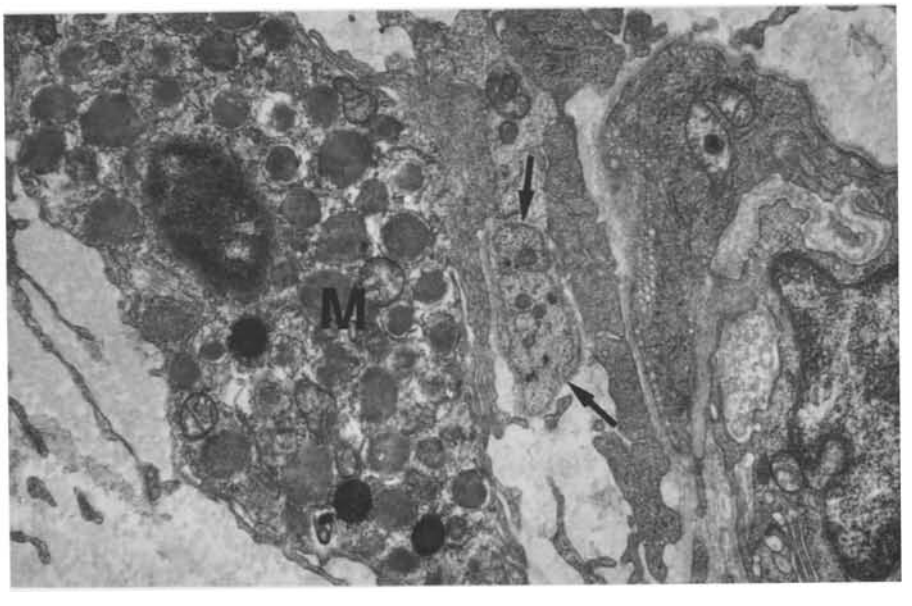

Fig. 3. Small unmyelinated nerve fibres (*arrows*) very close to a mast cell (*M*). Control human ureter. x13,000

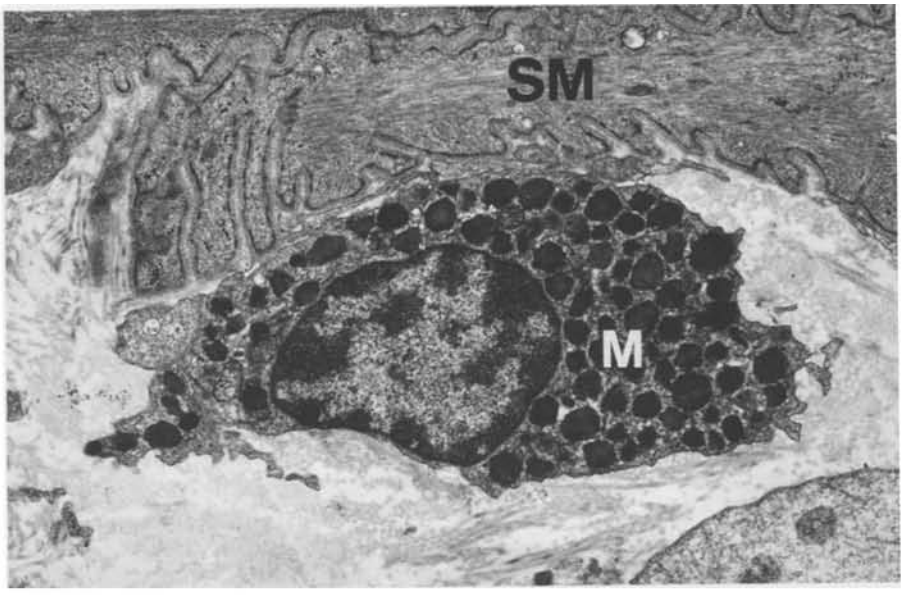

Fig. 4. Intact mast cell (*M*) in close association with contracted smooth muscle cells (*SM*). Experimental human ureter. ×7,800

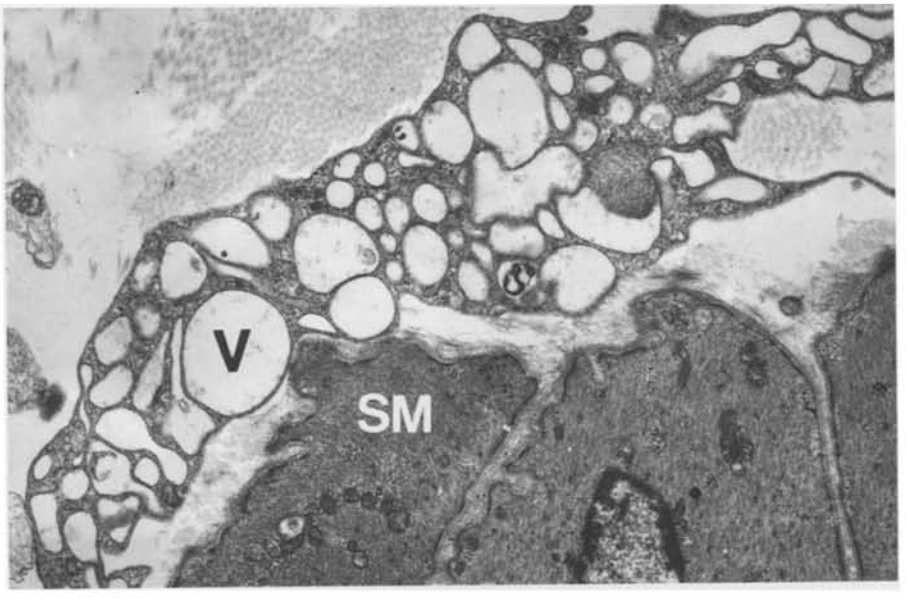

Fig. 5. Degranulated mast cell the cytoplasm of which is full of empty membrane-bound vesicles (*V*). Experimental human ureter. *SM* = smooth muscle. ×7,800

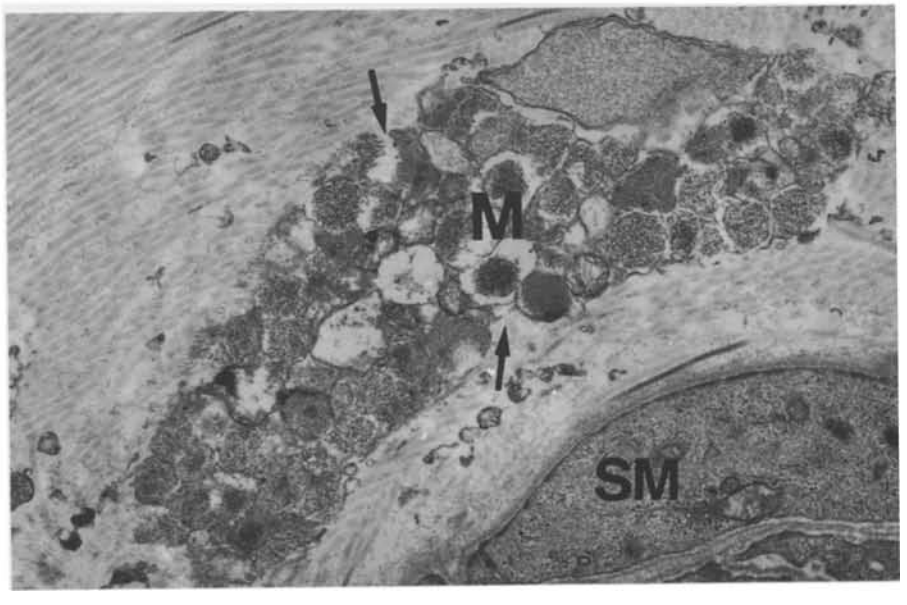

Fig. 6. Different stage of degranulation of mast cell (*M*). The granules are of various electron densities and contents. Membrane disruption is evident in places (*arrows*). Experimental human ureter. *SM* = smooth muscle. ×13,000

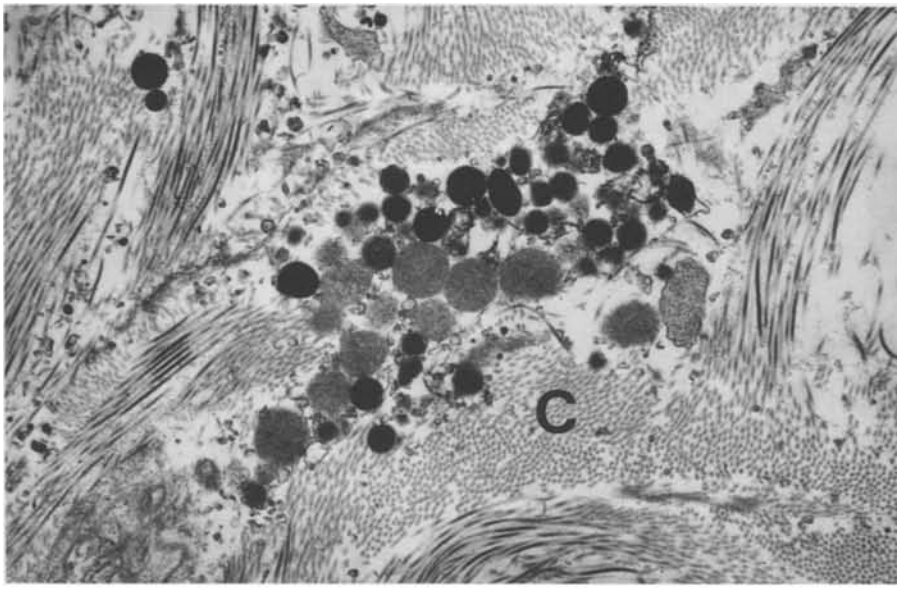

Fig. 7. Mast cell granules free in the connective tissue (*C*). Experimental human ureter. x7,800

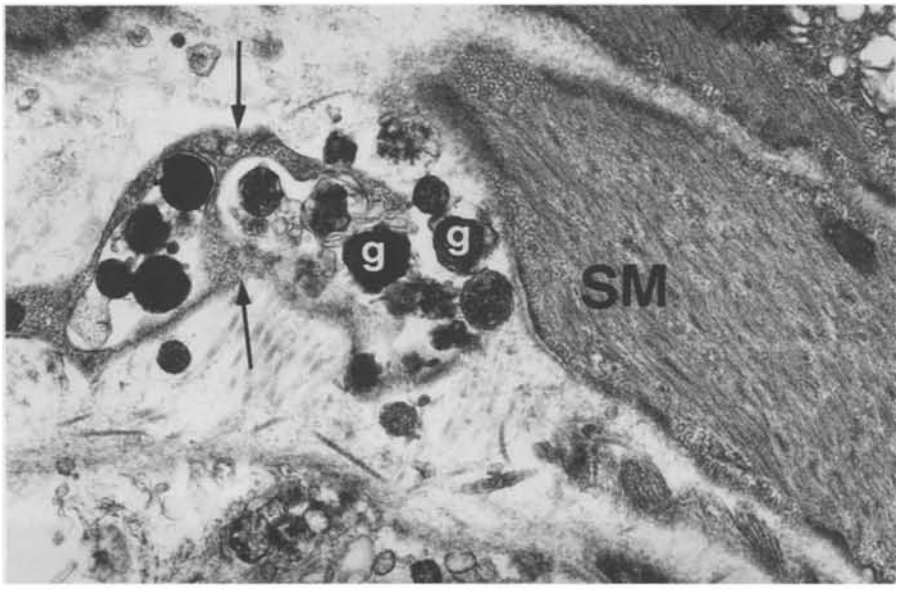

Fig. 8. Extracellular membrane-bound mast cell granules (*g*) close to smooth muscle cell (*SM*). Notice – cellular remains (*arrows*). Experimental human ureter. x16,000

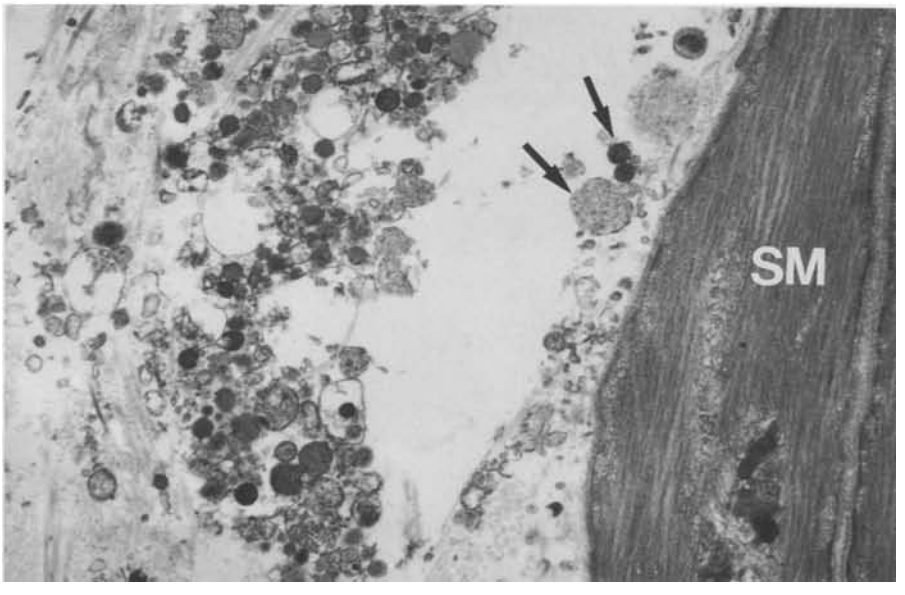

Fig. 9. Remains of degranulated mast cell. Notice – membrane bound granules some of which are seen close to smooth muscle (*arrows*). Experimental human ureter. *SM* = smooth muscle. x10,000

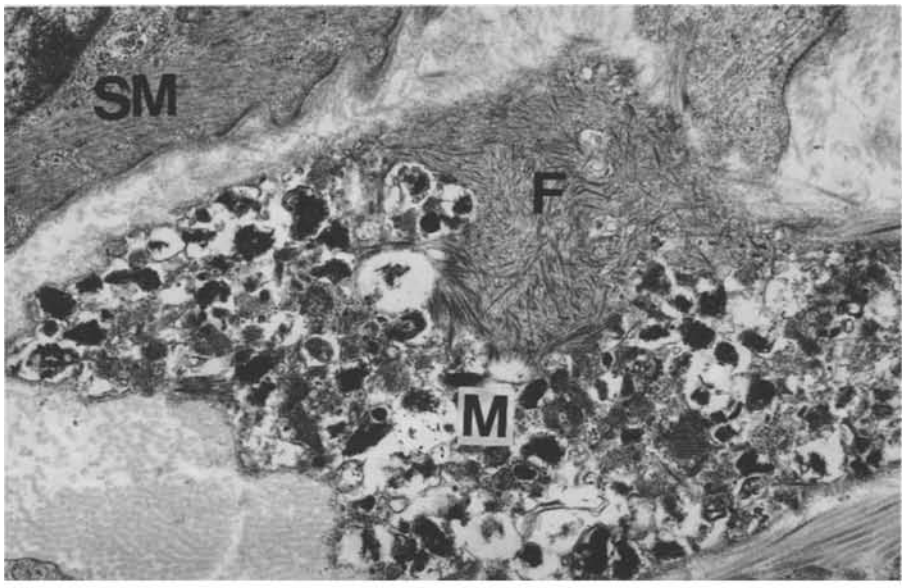

Fig. 10. Marked accumulation of filaments (*F*) in a degranulated mast cell (*M*). Granules vary in shape and electron density. Experimental human ureter. *SM* = smooth muscle. x10,400

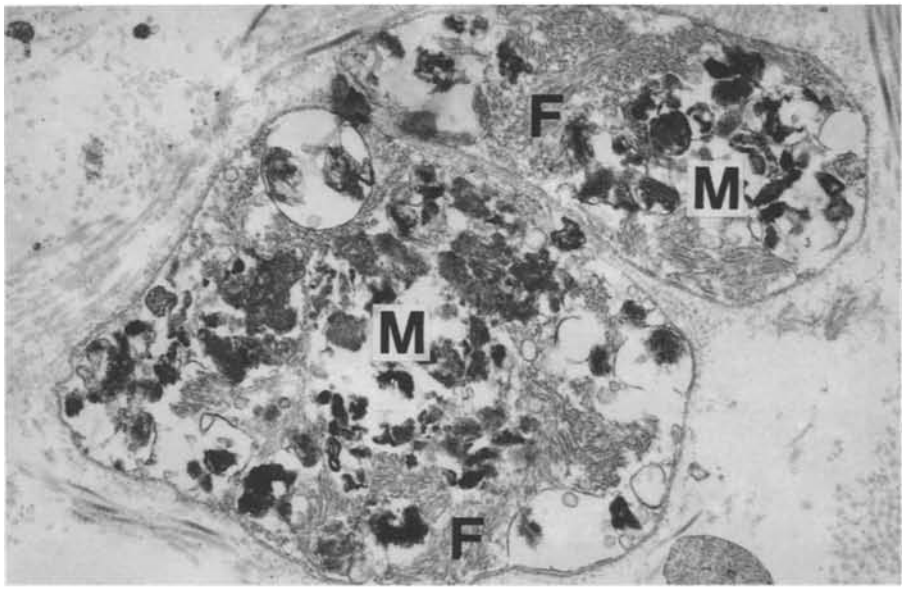

Fig. 11. Two degranulated mast cells (*M*) with bundles of intracellular filaments (*F*). Granules are of various shapes and electron densities. Experimental human ureter. *SM* = smooth muscle. x10,400

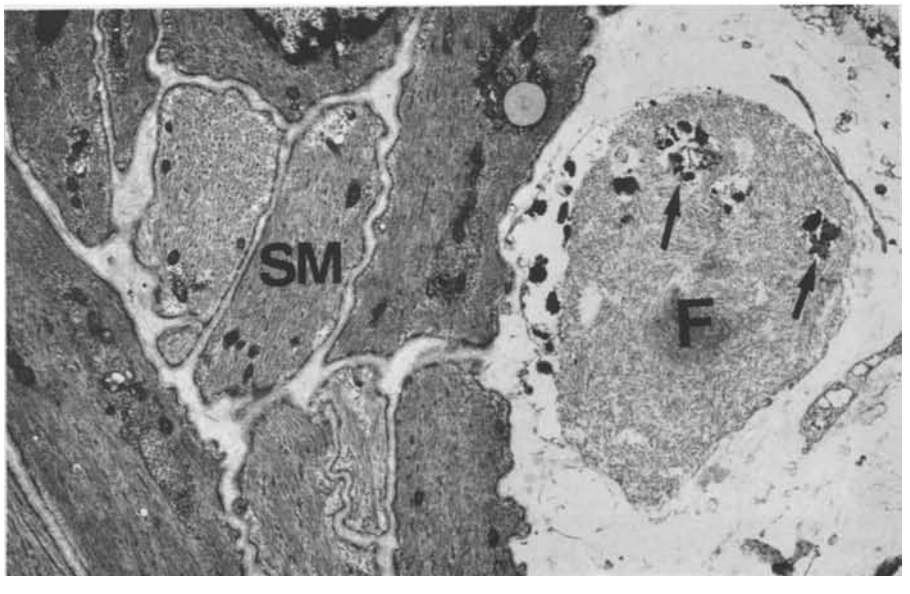

Fig. 12. Abundance of filaments (*F*) in the cytoplasm of degranulated mast cell. Remains of few granules can be seen (*arrows*). Experimental human ureter. *SM* = smooth muscle. x5,200

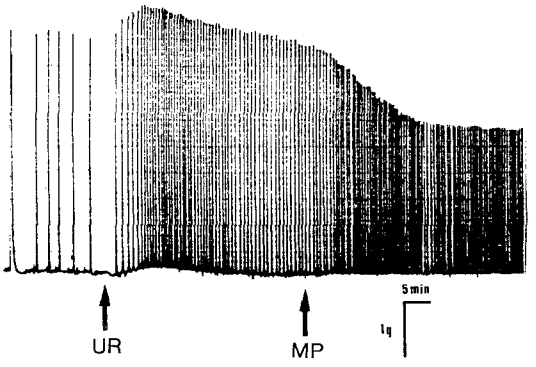

Fig. 13. Peristaltic contraction of isolated human ureter. At UR, 1 ml of human urine added to organ bath. Note increase in frequency and amplitude of contractions and increase in basal tone. Addition of 10 mepyramine reduces amplitude but not frequency of contractions

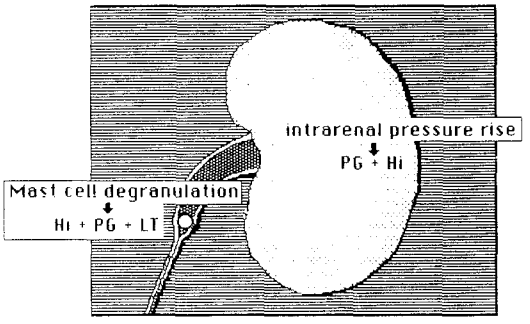

Fig. 14. Schematic illustration of the putative mediator release in renal colic with impacted calculus. Intrarenal pressure rise induces release of prostaglandins and histamine [3]. Local damage to the urothelium at the site of the impacted stone leads to penetration of hyperosmolar urine into the subepithelial space. This induces mast cell degranulation and release of preformed (histamine) and newly synthetised mediators (prostaglandins and leukotriens) which induce spastic contration and pain [11, 12, 13 and this paper]

osmolality of the urine they were incubated with. It is reasonable to assume that mast cell degranulation is promoted starting at a threshold concentration of the stimulating agent. Beyond that and at higher concentrations of urine other direct effects of urine on smooth muscle function are likely to appear, such as the tonic contraction due to a reduction in pH as shown previously [11].

Within the kidney and following ureteral occlusion, increases in histamine concentration and prostanoids have been observed [3]. This means that histaminergic mechanisms are not a novelty in obstructive nephropathy but our report seems to be the first observation linking histamine-release to mast cell degranulation in the ureter (cf. Fig. 14).

Acknowledgements. We are grateful to Prof. G. Abouna and the Organ Transplant Unit at the Faculty of Medicine, Kuwait University for providing samples of human ureters. The excellent technical assistance of Miss H. Khalaf and Mr. J. Thomas is greatly appreciated.

References

1. Al-Ugaily L, Thulesius O, Angelo-Khattar M (1986) New evidence for prostaglandin-induced motility of the urether. Scand J Urol Nephrol 20:225–229
2. Angelo-Khattar M, Thulesius O, Nilsson T, Cherian T, Joseph L (1985) Motility of the human ureter, with special reference to the effect of indomethacin. Scand J Urol Nephrol 19:261–265
3. Banks RO, Jacobson E (1985) Renal vasodilatation with ureteral occlusion and prostaglandins: Attenuation by histamine H-1 antagonists. Am J Physiol 249:F851–857
4. Bertaccini G, Zappia L, Bezzi E, Potnexoni D (1983) Histamine receptors in the human ureter. Pharmacol Res Commun 15:157–166
5. Findlay SR, Dvorak AM, Kagey-Sobotta A, Lichtenstein LM (1981) Hyperosmolar triggering of histamine release from human basophils. J Clin Invest 67:1604–1609
6. Fussel FN, Roberts JA (1978) Chronic pyelonephritis. Electron microscopic study III. The ureter. Invest Urol 17:108–119
7. Lagunoff D, Martin TW (1983) Agents that releae histamine from cells. Ann Rev Pharmacol Toxicol 23:331–351
8. Lau HYA, Pearce FL (1986) Effects of antihistamines on isolated mast cells from the rat, guinea pig and man. Agents Actions 18:107–109
9. Keele CA, Armstrong D (1964) Substances producing pain and itch. Arnold, London
10. Thulesius O, Ugaily-Thulesius L, Angelo-Khattar M (1986) Generation and transmission of ovine ureteral contractions with special reference to prostaglandins. Acta Physiol Scand 127:485–490
11. Thulesius O, El-Shirbiny A (1987) The effect of urine on ureteral motility. Urol Res 15:267–271
12. Ugaily-Thulesius L, Thulesius O, Angelo-Khattar M, Sivanandasigham P, Sabha M (1988) Mast cells and histamine responses of the ureter, ultrastructural features of cell-to-cell association and functional implications. Urol Res 16:287–293
13. Ugaily-Thulesius L, Thulesius O, Sabha M (1988) The effect of urothelial damage on ureteral motility, an ultrastructural and functional study. Br J Urol 62:19–25

Dr. L. Ugaily-Thulesius
Department of Anatomy
Faculty of Medicine
Kuwait University
P.O. Box 24923
Safat 13110
Kuwait