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Collagen arrangements in ureter

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Summary. The collagen fibres of rabbit and human ureter were exposed by digestion with trypsin and hyaluronidase. The fibre structure was examined using an SEM and examples of the inner and outer fibre structures are shown together with the effects of different types of mechanical strain. An interesting difference between the arrangements of the inner fibres of human and rabbit was seen where the human ureter had a cross-ply structure while in the rabbit it was helical.

Key words: Collagen – Ureter – Enzyme treatment – Rabbit – Human

Collagen accounts for approximately 33% of total body protein and, in its various forms, constitutes the major structural component of the many and varied connective tissues of the body [14–16]. The orientation and ultrastructure of the collagen fibres within the connective tissues are directly related to their appropriate physiological functions.

The arrangement of collagen fibres in tissues such as skin [7, 15, 20, 25], tendon [1, 21, 24], liver [15, 22], bone [15], cartilage [15, 18], blood vessels [2, 17], cornea [6, 13, 15] and intestine [5, 11, 12] has already been well described in the literature.

The urinary tract has received less attention. The kidney in general has been studied [8, 15, 26] with some authors paying particular attention to the basement membrane of the glomerulus [3, 4, 10, 15]. The collagen framework of the kidney, renal cell carcinoma and tumour capsule has also been studied [9]. Very little work has been performed on the ureter distal to the renal pelvis.

Midel and Quesada [19] reported a scanning electron microscopic study of the normal ureter, primary obstructive megaureter and the megaureter occurring as a result of reflux. The main interest was the interrelation of collagen and muscle. The specific features of the collagen arrangement significant for mechanical characteristics were not well described.

The work reported in this paper describes the collagen arrangements in both rabbit and human ureter using enzymes to remove the non-collagenous material prior to scanning electron microscopic (SEM) studies. Differences between sub-mucosal and serosal structure will be described, as well as an interesting difference between human and rabbit ureter.

Materials and methods

Trypsin (bovine pancreas, 10000–13000 BAEE units/mg protein) and hyaluronidase (bovine testicle, 300 units/mg) were purchased from Sigma (Poole, Dorset, UK). Human and rabbit ureters were obtained at autopsy and either processed immediately for SEM studies or stored deep frozen until processed.

Specimens, not deep frozen, were collected in phosphate-buffered saline (pH 7.2, 0.01 M sodium phosphate, 0.15 M NaCl) and transported to the Bioengineering Unit. Adipose tissue was dissected away and the ureters having been cut into appropriate lengths, the relevant surfaces were exposed. Any tensile strain or dilation was applied to the tissue at this stage in the preparation. The specimens were incubated in trypsin at 37 °C (2.5 mg/ml), rinsed in saline, then incubated in hyaluronidase at 37 °C (3 mg/ml solution in 0.1 M sodium acetate buffer containing 0.15 M NaCl at pH 5.4). In both cases sodium azide was added as a bacteriocide. The samples were subsequently rinsed in phosphate-buffered saline and fixed for 24 h in a 2.5% solution of glutaraldehyde in cacodylate buffer (pH 7.3). After rinsing in saline, the specimens were dehydrated in methanol and dried in a critical point dryer using CO_2 before mounting onto a stub using double-sided adhesive tape and coating with gold in a sputter coater. All specimens were examined in a JEOL 840A scanning electron microscope.

Various regimes of enzyme treatment were used. In a third of the specimens a pre-treatment of continuous infusion of 10% saline into the ureter via a peristaltic pump was applied for 24 h before enzyme treatment in order to release the epithelium and submucosa, as used by Orberg et al. [23]. Details of the treatments are given in the legend of each micrograph.

In order to assess the response of the collagen network to mechanical strain the tissue was stretched in various ways before treatment. A longitudinal strain was maintained by glueing the tissue to a glass slide using cyanoacrylate adhesive. Dilation was simulated by introducing a

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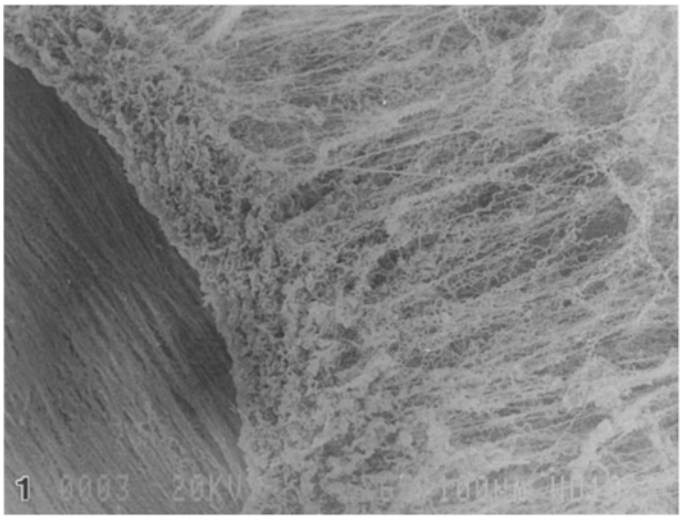


Fig. 1. Rabbit ureter, open end showing the inner (circumferential) and outer (longitudinal) fibres (48 h trypsin, 8 h hyaluronidase)

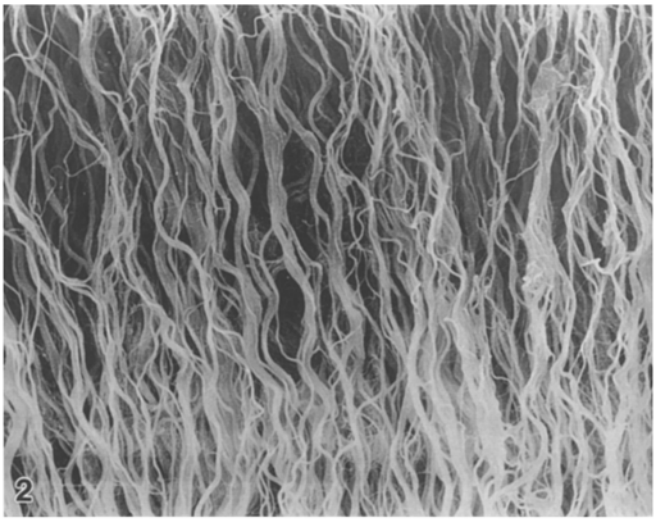


Fig. 2. Unstretched rabbit ureter, outer surface (48h trypsin, 8 h hyaluronidase)

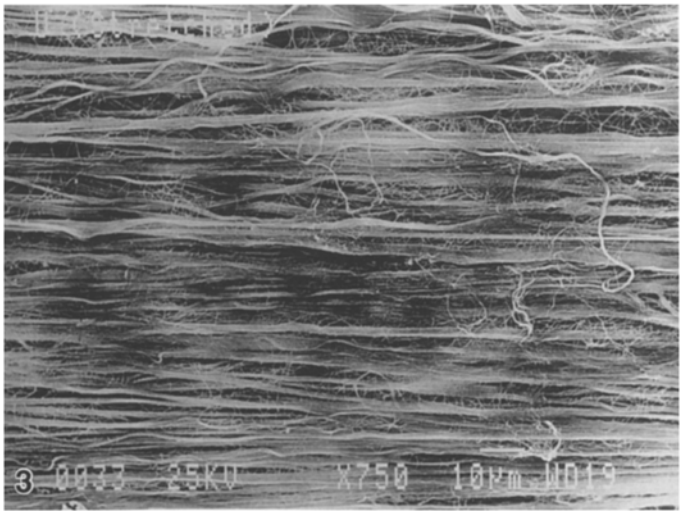


Fig. 3. Stretched rabbit ureter, outer surface (48 h trypsin, 16 h hyaluronidase)

Fig. 4. Unstretched human ureter, outer surface (48 h trypsin, 24 h hyaluronidase)

tube into the lumen, the protective sheath of a hypodermic needle having been found to be most suitable for human ureter. Short pieces of human ureter were everted before treatment or dilation. In all cases where mechanical strain was being investigated, fixation and dehydration were performed with the tissue held under the appropriate strain.

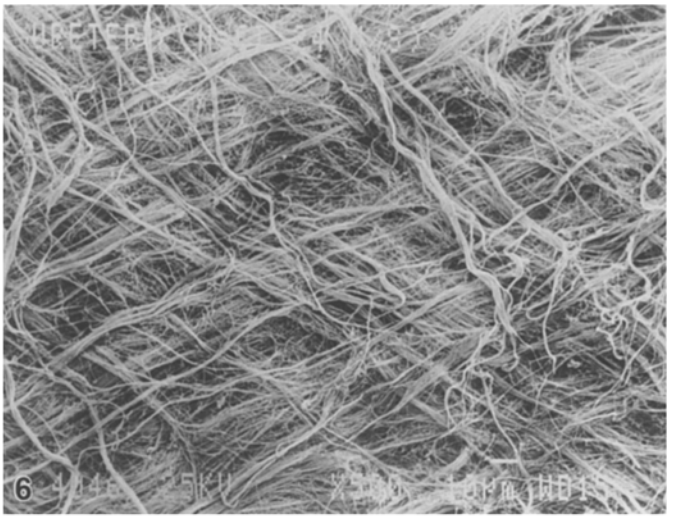
Results

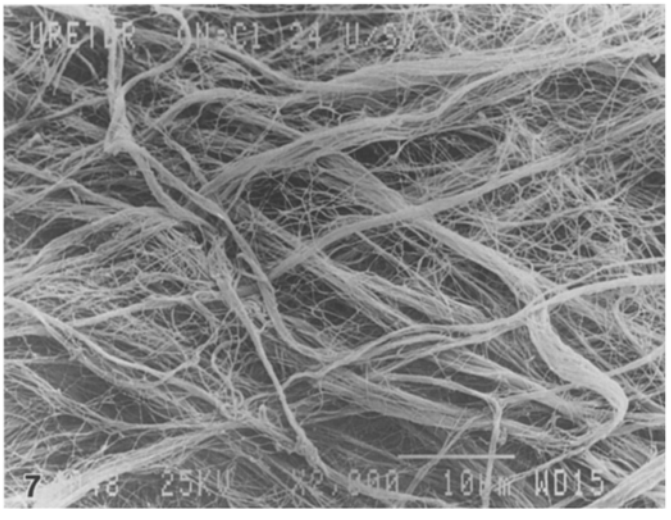
Two rabbit ureters were investigated; the central portion of each ureter was cut into eight specimens. Figure 1 shows an oblique section of rabbit ureter in which the outer serosal collagen is aligned parallel to the axis of the ureter, the inner sub-mucosal collagen being aligned in a helix of fine pitch. The two sets of fibres are almost perpendicular and show crimps, especially in the outer fibres. A higher magnification of the outer fibres in the unstretched state is shown in Fig. 2 after a longitudinal stretch in Fig. 3. It is clear that following stretching the crimp has been straightened and the axis of the fibres aligned even more accurately with the axis of the ureter.

Twelve human ureters were available; again the central portion of each ureter was cut into eight or more pieces. The outer serosal surface of human ureter is shown in Fig. 4, where it can be seen that the thicker fibre bundles are interspersed with a very fine filamentous network of fibres. The crimp is less obvious in human ureter than rabbit. When the tissue is stretched, the fibres once again are aligned with the axis of the ureter, but there is much less effect on the filamentous network (Fig. 5).

Examination of the sub-mucosal surface was less easy and often the mucosa had to be removed mechanically even after prolonged digestion. However, the cross-ply arrangement of the fibres can be seen through the mucosa (Fig. 6). Higher magnification views with the mucosa removed can be seen in Fig. 7.

If the ureter is distended as described above then the angle of the cross-ply with respect to the axis of the ureter is changed (Fig. 8).





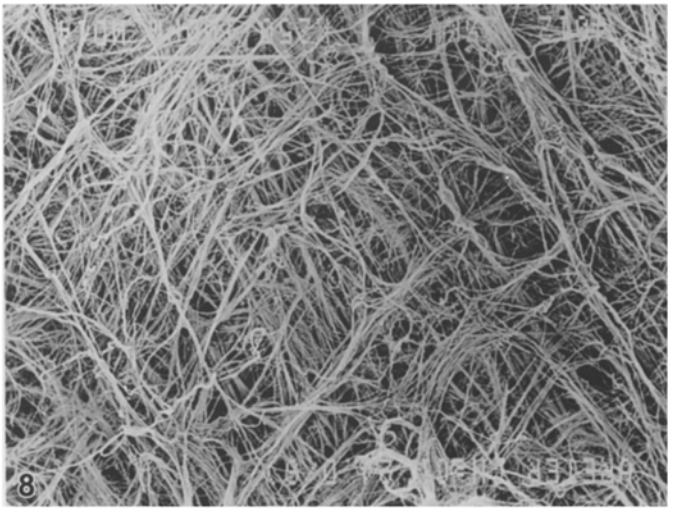


Fig. 5. Stretched human ureter, outer surface (48 h trypsin, 24 h hyaluronidase)

Fig. 6. Human ureter, internal surface after mechanical removal of endothelium (48 h NaCl, 24 h trypsin, 24 h hyaluronidase)

Fig. 7. Human ureter, internal surface after mechanical removal of endothelium (48 h NaCl, 24 h trypsin, 24 h hyaluronidase)

Fig. 8. Distended human ureter, internal surface after mechanical removal of endothelium (48 h NaCl, 24 h trypsin, 24 h hyaluronidase)

Discussion

Although some indication of fibre arrangement can be obtained even from tissue which has been fixed and dried with very little enzyme preparation, prolonged enzyme digestion is necessary to reveal structural details. The longitudinal arrangement of crimped fibres seen in the serous collagen on the outer ureter of both rabbit and human is consistent with a tissue in which large changes in length are not required and in which small changes may be accommodated by a change in the crimp configuration. Similar patterns are observed in tendon [1, 21, 24].

However, the difference in the sub-mucosal collagen arrangement between the two species is harder to explain. Both structures may accomodate dilation as the peristaltic action of the ureter forces a bolus of urine from kidney to bladder.

In the rabbit the angle of the inner helical fibres may alter, varying the pitch and diameter of the helix. The crimp may also change. In the human, stretching and dilation produce a change in angle of the cross-ply which would be consistent with an organ adapted for peristalsis. This arrangement of fibres has been seen in intestine, another peristaltic organ [5, 12]. The reasons for the species difference between human and rabbit are not clear but may be related either to the quantity of urine produced per day relative to the body weight, or to the typical posture of the animal.

Enzyme digestion using trypsin to remove non-collagenous proteins and hyaluronidase to remove proteoglycans permits detailed examination of the collagen fibres. This allows visualization of the filamentous network of fibres which is believed to play a role in recovery after removal of stress.

Further work is required to examine the arrangements of collagen fibres in a number of species. This may help to explain the reasons why two different fibre arrangements are seen in an organ which seems to have similar

physiological and mechanical functions in the two species studied. In addition, a more quantitative relationship between mechanical strain and fibre re-alignment is required, including the use of balloons to simulate the passage of a bolus.

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