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THE MICROVASCULATURE OF THE GUINEA PIG URETER. A SCANNING ELECTRON MICROSCOPIC INVESTIGATION

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Abstract

In 24 albinotic guinea pigs (Cavia porcellus) the gross vasculature and the microvascular architecture of the ureter were studied by light microscopy of tissue blocks and by scanning electron microscopy of vascular casts. The guinea pig ureter is supplied by the renal artery proximally, by the aorta and the internal iliac artery in its mid-segment, and by the uterine and prostatic as well as by the vesical arteries distally. The main arterial trunks run alongside the ureter before they branch to send perforating arterioles to the muscular coat and the mucosal lining. The draining venules are found on both sides of the ureter and form transverse anastomoses. Communications between the arterioles are also located on both sides, but longitudinally arranged. The capillary network of the mucosal lining shows an undulating pattern with tortuous vessels and lies just below the epithelium. The muscular coat and the adventitia have no prominent capillaries of their own. Large arteries are embedded in the adventitia, large veins in the lamina propria.

In analogy to human anatomy the vascular arrangement found suggests that, if the ureters are excised in transplant surgery, a lateral incision should be used for the abdominal portion, while the pelvic portion is best approached by a medial incision.

KEY WORDS: Ureter; blood supply; microcirculation; guinea pig; scanning electron microscopy (SEM); corrosion casts.

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Introduction

The blood supply of the human ureters was first described by Haller in 1747. He distinguished 3 vascular territories and reported that the upper part of the ureter was supplied by the renal arteries, the middle part by the aorta, the common iliac (middle ureteric) or internal iliac arteries, and the lower part by the vesical or uterine arteries. His account was the first to mention the middle ureteric artery as the vessel supplying the mid-portion of the ureter. This vessel is a branch of the abdominal aorta or the common iliac artery. Haller's observations were later confirmed by Protopow (1897), Feitel (1901), Merkel (1915) and Frommolt (1927, 1928).

While there is general agreement about the blood supply of the proximal ureteral segment, that of the middle and distal segments is controversial. While Disse (1902), Rauber and Kopsch (1922), Poirier and Charpy (1923) and Waldeyer (1942) found the mid-ureteral segment to be supplied by no more than fine twigs from the testicular artery, a middle ureteric artery was mentioned by Merkel (1915) and Braus and Elze (1956). In the distal segment Disse (1902), Merkel (1915), Waldeyer (1942) and Braus and Elze (1956) thought the middle rectal artery to be the supplying vessel, whereas Protopow (1897) and Feitel (1901) followed Haller's description from 1747 and reported that the distal segment received its blood through the vesical and uterine arteries. A periureteral arterial plexus which is fed by the aorta, the testicular and the iliac arteries and communicates with the peritoneal vessels was described by Sampson (1904). In Gisel's description (1969) the largest ureteral vessel originates from the common iliac artery, but may also take its origin from the internal iliac, superior gluteal, vesical or uterine arteries.

This brief review shows that accounts of the arterial supply of the ureter vary considerably (Varverikos, 1952). The microvascular architecture of the ureter has so far not been investigated. Yet its understanding is clinically important for various reasons, e.g. preventing ischemia by ligating ureteral vessels, locating and sizing ureteral grafts, preventing fistulation following colorectal surgery, and identifying the optimal incision site (medial versus lateral to the ureter).In addition, ureteral vessels are also of particular interest during surgical approaches to the urinary bladder and ureter, which are performed to maintain the continence (Ghoneim et al.,1987; Hinman,1988; Schreiter and Noll,1989). The purpose of this investigation was to shed light on the microcirculation (Murakami, 1971; Hodde and Nowell, 1980; Lametschwandtner et al.,1984) of the ureter and the in situ topography of ureteral vessels in guinea pig with a view to contribute to a better understanding of clinical and morphological problems.

Material and Methods

Vascular corrosion casting: Twenty albinotic guinea pigs (Cavia porcellus) of both sexes weighing 200 to 250 grams were used. Casts of 14 ureters were obtained for scanning electron microscopy (SEM). Animals were anesthetized with ether and thoracotomized. A plastic catheter (Argyle, 0.8 mm x 1.9 mm; Sherwood Medical, St. Louis, Mo. USA) connected to a two-way connector (LS-2, B. Braun Melsungen AG, FRG) was introduced into the thoracic aorta just above the diaphragm and tightly ligated into place. The right ventricle was incised and the circulatory system was rinsed with 100 ml of warm (37°C), heparinized (5,000 IU/l) saline solution using manual pressure until ventricular efflux was clear. Then Mercox-CI-2B (Dainippon Ink & Chemicals, Tokyo, Japan) diluted with monomeric methyl methacrylate (v/v 4 : 1; Hodde, 1981) was injected manually along the same route. For polymerization of the injected resin, the bodies were kept at room temperature (20°C) for 2 hours before overnight tempering in a water bath at 60°C. Maceration was done in 15% potassium hydroxide at 40°C for 2 days or longer. Ureters with surrounding vessels were dissected free from the whole specimen under the binocular, cleaned in 5% formic acid for 15 minutes, rinsed in several passages of distilled water and frozen in it. Freeze-dried casts were mounted with colloidal silver onto copper foils fixed to specimen stubs by using the conductive bridge method of Lametschwandtner et al. (1980). Mounted casts were sputtered with gold for 600 seconds and examined under the scanning electron microscope (Cambridge 250) at an accelerating voltage of 5 to 15 kV (Aharinejad et al., 1989).

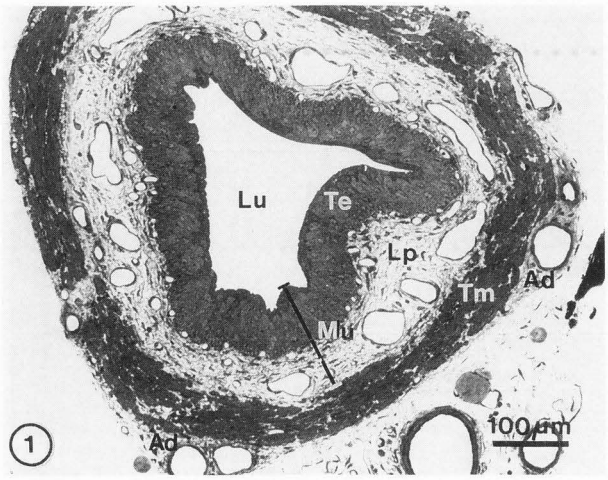
Light microscopy: Ureters of 4 guinea pigs (Cavia porcellus) of both sexes were studied. Guinea pigs were fixed by perfusion with 0.2 M phosphate buffered glutaraldehyde (2.5%; pH 7.2) through the thoracic aorta. Ureters were removed, cut into small blocks and fixed by immersion into the same fixative for another 2 hours. After an overnight rinse in buffer specimens were postfixed in buffered 1% osmium tetroxide (Michaelis buffer, pH 7.2; 2 hours), rinsed in the same buffer, dehydrated in a graded series of ethanol and propylene oxide and finally embedded in Epon 812 (Luft, 1961). 1µm semithin sections were stained with alkaline toluidine blue O (Trump et al., 1961) and examined by light microscopy.

Terminology: The denominations of anatomical regions are those of Cooper and Schiller (1975); the terms used for describing ureteral histology are those suggested by Kessel and Kardon (1979). According to Rhodin's (1980) proposal not to use vessels diameter for categorization of arterial and venous vessels, these vessels are classified by the analysis of the relief indented into the cast's surface by endothelial cell nuclei (Miodonski et al.,1976; Hodde et al., 1977); subclassification of feeding arterioles (=first order arterioles, foa) and draining venules (=first order venules, fov) is according to their branching order (Anderhuber et al., 1989).

Results

Anatomy of the guinea pig ureter: Guinea pig ureters consist of 3 layers. These are, from inside out, the mucosal lining (Mu), the tunica muscularis (Tm) and the adventitia (Ad), (Fig. 1). Guinea pigs do not have a submucosal layer in the ureter.

The mucosal lining (tunica mucosa) is composed of transitional epithelium (Te) and a lamina propria (Lp). The transitional epithelium is smooth without any prominent folding on the luminal side, while the lamina propria consists of loose fibrous tissue in which regularly spaced venous vessels are embedded along the entire circumference of the ureter (Fig. 1). Between the transitional epithelium and the lamina propria lies a subepithelial capillary network with evenly spaced capillaries that also span the entire circumference of the ureter (Fig. 1).



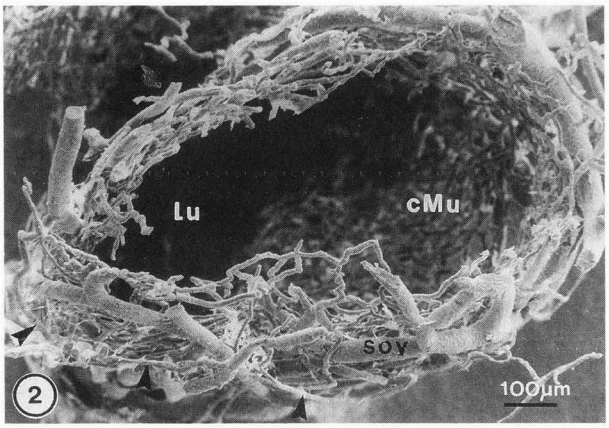


Fig. 1. Histology of the proximal segment of the guinea pig (Cavia porcellus) ureter. Transverse section, alkaline toluidine blue O staining. Ad = adventitia; Lp = lamina propria; Lu = lumen; Mu = mucosa; Te = transitional epithelium; Tm = tunica muscularis. Note the numerous large venules within the lamina propria (Lp) and the large arterioles in the adventitia (Ad).
Fig. 2. Vascular architecture of the proximal segment of the ureter. Corrosion cast; transverse section. cMu = (subepithelial) capillary bed of the mucosa; Lu = lumen; sov = second order venules. Arrowhead marks a vessel in the tunica muscularis.

The muscular coat (tunica muscularis), (Tm) (Fig. 1) consists mainly of circular smooth muscle fibers with some sporadic transverse or longitudinal fibers. Unlike in humans (Protopow, 1897) and rabbits (Engelmann, 1869), there is no clear distinction between an inner longitudinal and an outer circular muscle layer in guinea pigs. Only few vessels (capillaries) are present in the muscular coat.

The adventitia (Ad) of the ureter is very thin except around the large longitudinal vessels, most of which are arteries, where it becomes somewhat thicker (Fig. 1).

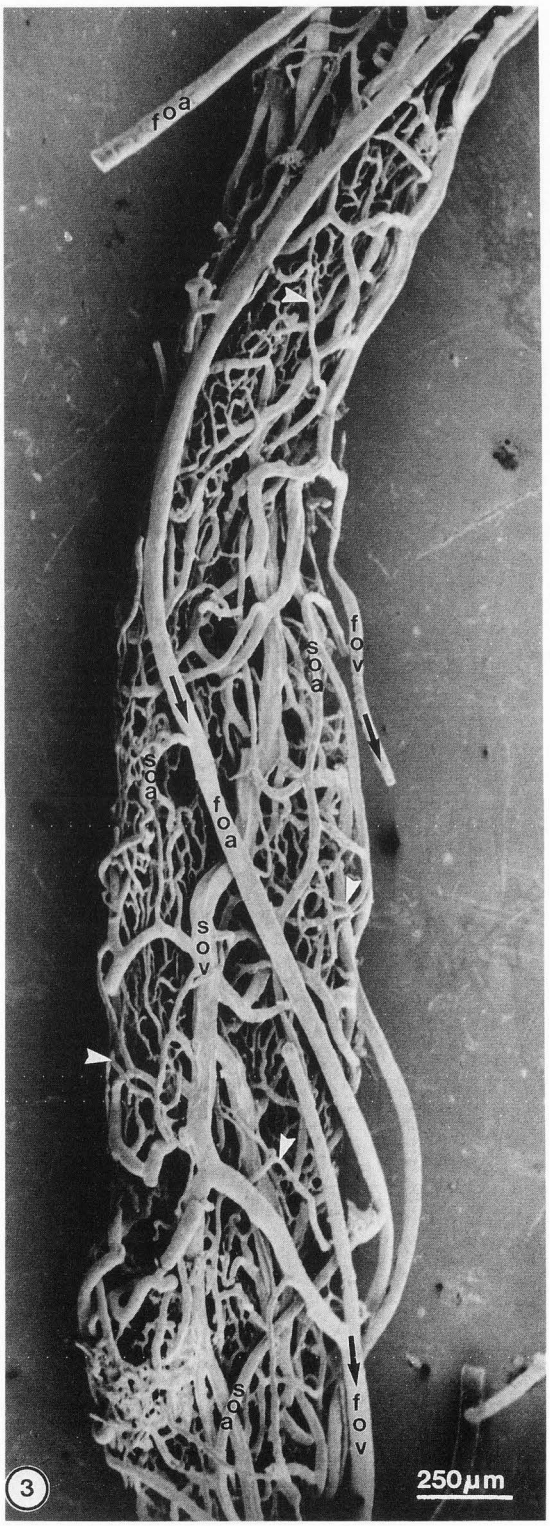
Main blood supply and drainage: Corrosion casts give an impressive three-dimensional view of the topography of ureteral vessels (Fig. 2) found in tissue sections (Fig. 1). Vessels can be allocated to the different coats of the ureteral wall. Intraindividually, corrosion casts of the ureters do not show any differences in their caliber between the 2 sides throughout their length. Interindividual differences are unrelated to the animals' sex. In our material ureters had a caliber of 500 to 1,000 µm (Figs. 2 and 3).

Proximal segment. The proximal segment is invariably supplied by branches of the renal artery with occasional contributions by branches of the testicular or ovarian arteries. Rarely (on both sides in 4 specimens) small twigs from the aorta ascend towards the kidneys to supply the uppermost part of the ureter. Venous drainage is into the inferior vena cava and the testicular or ovarian veins.

Middle segment: This segment is mainly supplied by branches from the abdominal aorta and the internal iliac artery (middle ureteric artery). Contributions from the external iliac artery are rarely seen (on both sides in 5 specimens). Branches of the right and left colic arteries are not involved in the blood supply of the mid-ureteral segment. Venous drainage is through the internal and external iliac veins and the inferior vena cava.

Distal segment: The distal segment of the ureter close to the urinary bladder is supplied by the internal iliac, prostatic or uterine and vesical arteries.
The internal pudendal artery was not involved in the blood supply of this segment in any one of the specimens examined. Venous drainage is by the uterine or prostatic and the vesical veins, exceptionally (on the left side in 2 specimens) by the internal iliac vein.

Intrinsic ureteral vasculature: Branches of the above arteries approach the ureter and accompany it along variable distances towards cranial and caudal before crossing it obliquely to reach the contralateral side (Figs. 3 and 4). We term these vessels, which have a caliber of 80 to 120 µm, "first order arterioles". They give off 30 to 50 µm thick branches cranially and caudally, which we term "second order arterioles" (Figs. 3 , 4 and 9). Both first and second order arterioles are embedded in



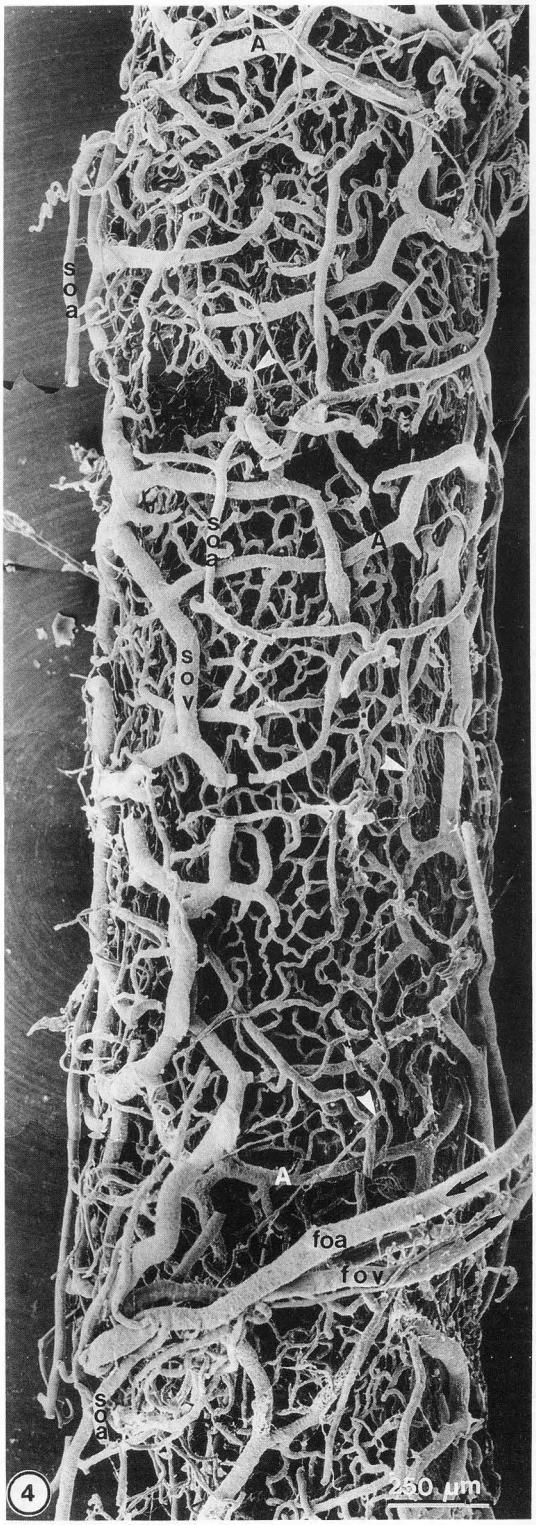
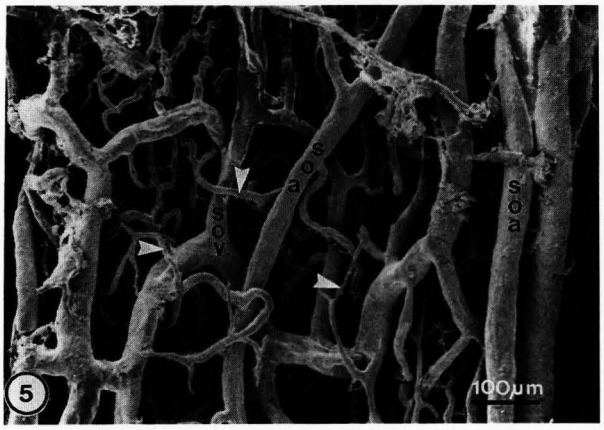
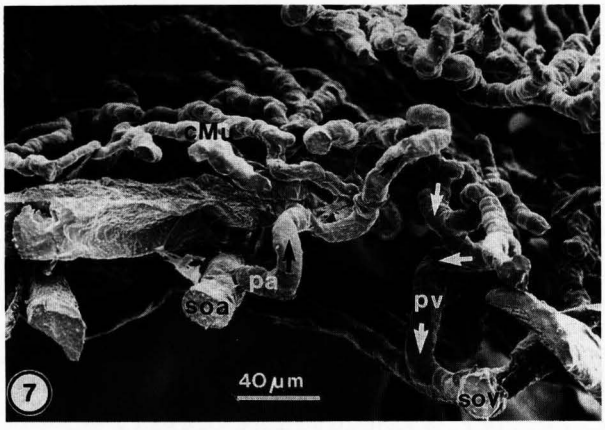
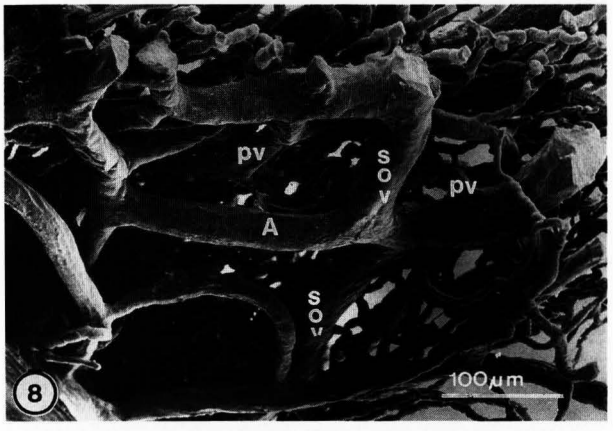


Fig. 3. Mid-ureteral segment with feeding first order (foa) and second order (soa) arterioles and draining second order (sov) and first order (fov) venules. Arrows indicate direction of blood flow; arrowheads point to vessels of the tunica muscularis.
Fig. 4. Proximal ureteral segment. Note transverse "segmental" anastomoses (A) between longitudinal second order venules (sov). For further abbreviations, see Fig. 3. Arrowheads indicate vessels in muscle coat.







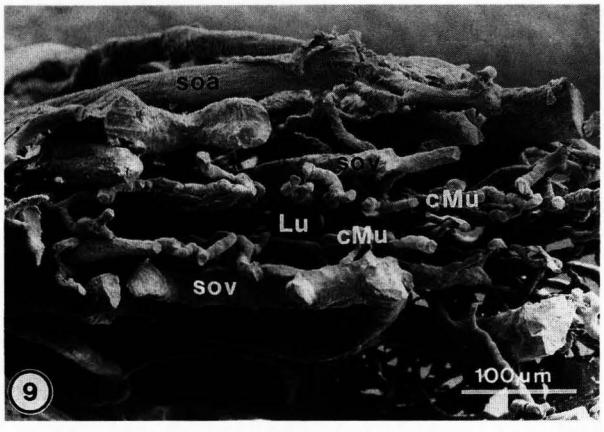


Fig. 5. Topography of second order arterioles (soa) and second order venules (sov) in the guinea pig ureter. Arrowheads point to vessels of the tunica muscularis.

Fig. 6. Second order arterioles (soa) giving off third order arterioles (=perforating arterioles; pa) to supply the subepithelial capillary bed of the mucosa (cMu).

Fig. 7. Supply and drainage of the subepithelial capillary bed of the ureteral mucosa (cMu) through second order (soa) -third order arterioles (=perforating arterioles (pa) - postcapillary venules (pv)(=third order venules) - second order venules (sov). Arrows indicate direction of blood flow.

Fig. 8. Transverse anastomosis (A) connecting longitudinal second order venules (sov). pv = postcapillary venules (=third order venules).

Fig. 9. Vascular architecture of a collapsed ureter. Transverse section. cMu = capillary bed of the mucosa; Lu = ureteral lumen; soa = second order arterioles; sov = second order venules.

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the adventitia, which contains few capillaries (Fig. 1).

Tunica muscularis (Tm): As can be seen from the semithin sections, there are very few vessels in the muscle coat (Fig. 1). Their identification and topographical allocation in corrosion casts is problematic (Figs. 3 to 5).

Lamina propria (Lp): Second order arterioles give off internal branches which pierce the muscle coat (Fig. 6). Following Engelmann's suggestion (1869), these vessels which feed the subepithelial capillary network at the base of the transitional epithelium were termed "perforating arterioles" (i.e. "third order arterioles") (Figs. 6 and 7; pa). Figure 1 impressively shows that the

Fig. 10. Subepithelial mucosal capillary bed. Luminal aspect. Note the Y- (arrow) and T-shaped (arrowhead) anastomoses between capillaries. Postcapillary venules (pv)(third order venules) drain into second order venules (sov) every 200 to 300 µm.

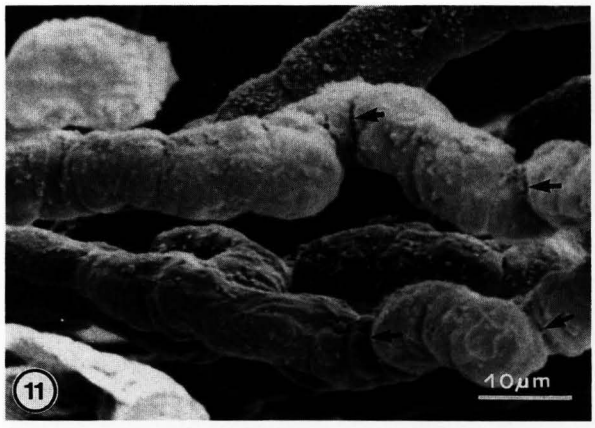

Fig. 11. Subepithelial capillaries of the ureteral mucosa. Note the "microkinks" and numerous infoldings (arrows) of the vessels.

most prominent structures in the lamina propria are large, mostly longitudinal venules. In analogy to the nomenclature used for arterial vessels, these were termed "second order venules" (Figs. 3, 4 and 9 ; sov). They have a wide lumen and communicate with one another through transverse anastomoses (Figs. 4 and 8; A). These anastomoses show a "segmental" arrangement recurring every 600 to 800 µm (Fig. 4) both on the anterior and posterior aspects of the ureter. Every 200 to 300 µm the second order venules (sov) of the lamina propria are joined by postcapillary venules (pv)(i.e. "third order venules") draining the subepithelial capillary network (Figs. 7, 8 and 10).

Mucosal capillaries: On longitudinal sections through a corrosion cast of the ureter the vasculature underlying the transitional epithelium (Fig. 1) can be seen to be almost entirely composed of capillaries arranged parallel to the longitudinal axis of the ureter (Figs.9 and 10). At higher magnification these present an undulating pattern with many shallow infoldings and "kinks" (Fig. 11). The capillaries communicate through Y- and T- shaped anastomoses (Fig. 10). Every 200 to 300 µm they empty through short postcapillary venules (pv) into the second order venules (Fig. 10; sov).

Discussion

Main blood supply and drainage: In our studies on guinea pigs the mid-ureteral segment was supplied by the middle ureteric artery, i.e. by the abdominal aorta. While this agrees with the observations of Haller (1747), Protopow (1897), Feitel (1901) and

Frommolt (1928) in humans, it is contradictory to what Disse (1902), Rauber and Kopsch (1922), Poirier and Charpy (1923) and Waldeyer (1942) reported. Like in humans (Daniel and Shackman; 1952), the internal iliac artery was consistently found to contribute to the supply of the mid-ureteral segment in guinea pigs.

An involvement of the middle rectal artery in the blood supply of the distal ureteral segment, which was postulated by Disse (1902), Merkel (1915), Harper (1942), Waldeyer (1942) and Braus and Elze (1956), was not seen in guinea pigs. This agrees well with what Frommolt (1928) found in man. Like most examiners who studied human ureters (Haller, 1747; Protopow, 1897; Feitel, 1901; Poirier and Charpy, 1923; Frommolt, 1928; Michaels, 1948; Racker, 1951; Daniel and Shackman, 1952; Poisel, 1979), we found the uterine and the prostatic arteries to be the main contributors to the blood supply of the distal ureter. By contrast, Harper (1942) described these arteries as "...rarely contributing to the supply of the ureter". As regards an involvement of the internal iliac artery in the supply of the distal ureter, our observations agree well with those of McCormack and Anson (1946), Daniel and Shackman (1952) and Poisel (1979). In all of the specimens examined branches of the internal iliac artery were seen to approach the distal ureter. We also found the testicular or ovarian arteries to contribute consistently to the supply of the distal ureter. In Michaels' (1948) view the contribution of the latter vessel is at best inconsistent. Like Racker (1951), we found no evidence confirming Gisel's (1969) observation that the superior gluteal, lateral sacral, inferior gluteal or middle rectal arteries supplied the distal ureteral segment. But unlike Racker (1951), we did not find the colic artery to be involved. This agrees with observations by other examiners.

In agreement with all of the authors quoted we found the proximal ureteral segment to be supplied by branches of the renal artery with contributions, in the guinea pig, by branches of the testicular or ovarian arteries and an occasional twig from the aorta.

Intrinsic blood supply: Like in humans (Engelmann, 1869; Sampson, 1904; Daniel and Shackman, 1952), the arterial trunks supplying the ureter (our first order arterioles) are surrounded by a thin layer of fibrous tissue so that they can easily be stripped away from the subjacent ureteral wall by blunt dissection. Multiple branching of first order arterioles before they give off perforating branches, as described by Protopow (1897) in humans, was not seen in guinea pigs. There was also no evidence of transverse anastomoses between first order arterioles such as those reported in humans by Frommolt (1928). The arterial anastomoses seen were longitudinal rather than transverse and recall the "longitudinal anastomoses" described by Daniel and Shackman (1952) and Poisel (1979). While Protopow (1897) reported the renal artery to communicate with the vesical artery, we found no evidence of such an anastomosis in guinea pigs. Veno-venous anastomoses, by contrast, were numerous; on account of their arrangement corrosion casts looked like ladders.

Engelmann (1869) reported perforating veins to accompany the perforating arteries. This was not confirmed by our studies. We rather found the venules draining the subepithelial capillaries to pierce the muscle coat at sites other than those used by their arteriolar counterparts.

The tortuosity and "kinking" of the subepithelial capillaries, in our view, reflects the capacity of the capillary bed to adapt to the filling volume of the ureter. The subepithelial location of the capillary network appears to explain why the ureter is so vulnerable and tends to bleed profusely even on blunt dissection.

Clinical implications: Nitch (1931) felt that, while ureteral grafts with their vascular supply high off the pelvinic rim were likely to be successful, the chances of successfully grafting the pelvinic portion of the ureter decreased with increasingly distal supply. Our approach to this problem is a more differentiated one, because we consider the relation between the incision and the main supplying arteries to be critical for the success of a ureteral graft. This view is shared by Daniel and Shackman (1952), who maintained that incisions made 2 cm below the entry of a main artery did not compromise the blood supply of the organ so that complications (ischemia) were unlikely to occur. Relating the length of the guinea pig ureter to that of human ureters, the view expressed by Daniel and Shackman (1952) would also appear to apply to guinea pigs. When excising any ureteral portion, the main vessels in the adventitia should be stripped away bluntly and preserved. This approach agrees with suggestions of Poisel (1979).If unavoidable, ligation of longitudinal arteries after skeletonizing them along the ureteral segment to be excised will still leave the blood supply proximal and distal to the incision intact. This is ensured by the lateral longitudinal anastomoses between the arterial trunks. Whether or not the distal ureteral segment is likely to become necrotic, if its supplying vessels are cut, is still controversial. Like Frommolt (1928) and Harper (1942), we find it to be unlikely

on account of the ureteral blood supply seen in guinea pigs, while Racker (1951) considers it to be a likely consequence of ligating the uterine and vesical arteries.

Clinically, the optimal site for the incision in operations involving the ureters is another point of interest. As the abdominal portion of the ureter is largely supplied from medial, while the pelvic portion receives most of the blood from lateral, the least traumatic approach with the lowest risk of causing vascular damage and consequent complications is from lateral for the abdominal portion and from medial for the pelvic portion.

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Discussion with Reviewers

A.J. Miodonski: What procedure was used to make the transverse sections of the ureters cast (as shown in Fig.9) without collapsing them.
Authors: Casts were embedded in ice and cut transversally under binocular control with a mini wheel-saw placed in the chamber of a cryomicrotome (see Lametschwandtner A. and U. Lametschwandtner (1990) Historical review and technical survey of vascular casting and scanning electron microscopy. In: Motta PM, Murakami T, Fujita H (eds.) Scanning electron microscopy of vascular casts: Methods and Applications. Kluwer Academic Publisher, The Hague, Boston, London (in press).

A.J. Miodonski: Would the authors discuss the distal ureter segment which is important in bladder cancer?
Authors: Angioarchitectonically the distal ureter resembles proximal and middle segments. But the closer it approaches the urinary bladder the more circumferential arteries are present.

D.E. Schraufnagel: Why do you think the surface of the casts at high magnification is often rough? Do you think the peristaltic expansion and contraction may be associated with a redundant endothelial surface?
Authors: We cannot answer this question directly. But if peristaltic movements account for cast surface roughness we wonder why in cast vessels of the rodent small and large intestine with much stronger peristaltic movements than in the ureter no such findings are reported.

D.E. Schraufnagel: Do you think there is a characteristic capillary pattern for different endothelial surfaces?
Authors: At the moment there is a lack of data which are in favor of your suggestion.

J.G. Walmsley: Recent reviews indicate that arterioles are usually categorized according to branching order, where the feeding vessel is the first-order arteriole and the subsequent branches are termed the next higher order until reaching the capillaries. Would this criterion be more in line with the one which you are using for the ureter? If this is consistent with the general classification which you are using, how do "perforating arterioles" fit into the scheme? Can you consider classification of the resistance arteries also?
Authors: Yes, we also consider the concept of branching order of arterioles (as well as of venules) in cast preparations superior to other means of vessel categorization. "Perforating arterioles"

represent "third-order arterioles". We do not consider a classification of resistance arteries in cast preparations.

V.H. Gattone: Are there differences in the intramural (lamina propria) vessel pattern from upper, middle and lower segments of the ureter?
Authors: We found no differences.

V.H. Gattone: Did "anastomoses" between "second-order venules" differ between different segments of the ureter? They appeared more prominent for the upper segment than in the middle segment (figures 3 and 4).
Authors: There are no significant differences in the "anastomoses" between second order venules of upper and middle ureter segments.

V. Gattone: While I agree that it is convenient to use size to separate the first and second order arterioles (likewise for the venules), there are also ultrastructural characteristics of arterioles and venules, which need to be used. I feel, that the authors need to use TEM to convince the readers that some of the thicker walled vessels of the lamina propria are not arterioles and some of the thinner walled vessels of the adventitia are not venous (other than the first order venules expected to be in the adventitia). A major point of the paper is the presence of the arterial cascade in the adventitia, while the venous plexus is basically confined to the lamina propria. Rhodin (1974) describes, that the arterioles and venules lie in the adventitia with the capillary network in the lamina propria. Please clarify this point in the literature with correlative TEM. SEM alone is insufficient to clarify this point.
Authors: It is the branching rather than the size which leads us to term vessels first and second order vessels. Characteristic endothelial cell nuclei imprint patterns again clearly differentiate arterial and venous vessels; correlative light microscopy enables to attribute vessels to tissue layers (see figure 1). We therefor think that transmission electron microscopy can be omitted.

Additional reference

Rhodin JAG (1974). Histology. A textbook and atlas. Oxford University Press, New York, Toronto, 803 pp.