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Structure and ultrastructure of microvessels in the kidney seen by the corrosion casting method

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SUMMARY

Scanning electron microscopic observation of corrosion casts is the finest technique to describe spatial patterns of microvessels in many organs, giving a readily interpreted representation of their vascular architecture without interference from surrounding tissues. We focused on the renal cortex of guinea pigs to make an in-depth morphological analysis of structural and ultrastructural details left by the cells on the resin cast. In addition, we made a qualitative description of normal variants usually observed in glomerular disposition, arteriolar morphology or capillary arrangement in the space to shed more light on the relationship between vascular tissue and surrounding cells. The study also disclosed some examples of vascular adaption to physiological and pathological conditions occurring in renal microvessels such as many systems essential to flow regulation, filtration and excretory processes.

At lower magnification, all major vessels can be readily distinguished: interlobar, arciform and interlobular arteries and veins, along with a web of peritubular and capsular capillaries. At higher magnification, the glomeruli become visible and the afferent and efferent arteries and the tortuosity the inner vessels can be distinguished. In some of them, the resin, due to the narrowing sizes, suddenly stopped leaving a half-casted glomerulus. This helped to reveal its internal circulation characterized by thin capillaries with a high degree of bi or trifurcation.

In addition, we confirmed the close correspondence between cellular ultrastructural detail (pores, corrugations of cellular membrane, perivascular cell branches) and the impressions left on the resin visible only at high magnifications.

INTRODUCTION

The corrosion casting technique, useful in 3D visualization of vascular structures (Murakami T., 1971; Hodde K.C. et al., 1980; Lametschwandtner A. et al.,

1990; Hodde K.C. et al.,1990), finds an interesting application for teaching and scientific purposes in those organs where the peculiar morphological arrangement of their vessels is strictly connected to physiological mechanisms (Stein J.H., 1990). Hence this method yields sharp tridimensional models, readily interpreted, and clear images useful to understand many physiological and pathological conditions (Konerdig M.A., 1991; Kimura K. et al., 1993; Slizova D., 1995; Ninomiya H. et al., 2000).

The kidney is one of the most studied organs for the physiological implication of its vascular system in the excretory and secretory functions, not only from a macroscopic view but also in its finest microscopical features detectable at scanning electron microscopic analysis (Horacek M.J. et al., 1986).

The most interesting region of the kidney, from a morphophysiological point of view, is the cortex (Xu L.X. et al. 1994; Vodenicharov A. et al., 1998), because of the extreme specialization assumed by vascular tissue visible both in the capillary architecture and morphology. As a matter of fact, glomerular, peritubular and capsular capillaries can contribute to secretory and excretory functions of the kidney representing the basis of the physiological mechanism of filtration.

The glomerulus (Moore B.J. et al., 1992) is the most specialized vascular unit of the kidney and is one of the finest examples of the close connection existing between morphological and physiological aspects.

Corrosion casting is the best method for 3D visualization of this vascular structure (Kikuta A. et al., 1989; Ditrich H. et al.,1990) not only in its normal shape but also in its many variations (Evan A.P., 1996): it is possible to observe a series of differences in the afferent or efferent arterioles (Nopanitaya W., 1980) (bi/trifurcation), or double glomeruli connected in series. This technique also allows in-depth study of the glomerulus, giving precise ultrastructural information, thanks to the high resolution reached by SEM analysis.

Our aim is to make a detailed report on spatial architecture of the blood/urine filter confirming the data already known in literature and increasing, at the same time, the didactical power of corrosion casting tecnique with high definition tridimensional images at SEM.

We applied corrosion casting technique to 15 guinea pigs performing a low viscosity resin injection through the abdominal aorta, and a consequent digestion of tissues around vessels with hot alkali.

The casts were then dissected, treated for SEM analysis and mounted for observation.

MATERIALS AND METHODS

15 Guinea Pigs weighing 200-300 g were anesthetized with an intraperitoneal injection of 5 ml of pentobarbital. The abdominal cavity was opened by laparotomy, all viscera displaced and the aorta exposed under a dissecting microscope (Leica

WILD M3C). A 21 G cannula was then inserted into the abdominal tract of aorta near the iliac bifurcation and driven almost to the origin of the renal arteries. In addition, we ligated the thoracic aorta to prevent diffusion of the resin into the upper part of the body and the vena cava was sewn to permit blood outflow.

The cannula was fixed to the vessel by a vascular hemoclip.

The kidneys were perfused at first with 20 ml of heparinized solution to prevent blood clotting and then with 20 ml of saline solution to clear the vascular bed of blood, minimizing peripheral blood reservoirs.

The pressure of injection was monitored manually paying attention not to cause any mucosal oedema (valuable as a nasal exudate).

We proceeded by fixing vessels with a solution of 0.25% glutaraldehyde, 0.25% paraformaldehyde in 0.1M Na-cacodylate buffer at pH 7.2, to prevent resin leakage and to reduce the changes occurring to the endothelial cells during injection of the casting medium.

10 ml of resin (MERCOX CL-2R-5 – SPI supplies) mixed with 0.2 ml of catalyzer (benzoyl peroxide - SPI supplies) were injected through the cannula until we noted an increase in pressure and, when the reflux from the venous vessel became evident, we stopped the injection leaving the resin to freely diffuse into the capillaries.

One hour after partial polymerization of the resin, the kidneys were explanted and immersed in a warm water bath (60°C) to complete the polymerization process overnight. The kidneys were then immersed in a 15% KOH solution at 40°C to digest all the tissues around the vessels. The KOH solution was changed daily for about 1 week.

The resulting casts were dissected under a stereomicroscope (Leica WILD M3C) and the specimens obtained were then treated for SEM observation: dehydrated in graded ethanol, critical point dried in an Emitech K850 CPD apparatus, mounted on aluminium stubs on adhesive film and coated with 10 nm of gold in an Emitech K250 sputter-coater.

In some cases, because of the dimension of specimens, we used metallic bridges to maintain the conduction all over the stub.

The specimens were then observed in a Philips XL-30 FEG scanning electron microscope at 10 kV.

RESULTS

At lower magnification, we could already distinguish the normal vascular architecture made up of interlobar, arciform and interlobular arteries along with the peritubular, glomerular and capsular capillary networks (Fig. 1). We could also see some medium-sized veins enveloping the arterial vessel and characterized by irregular endothelial cell imprints, not oriented along the axis of the vessels as occurs on arterial casts (Fig. 2).

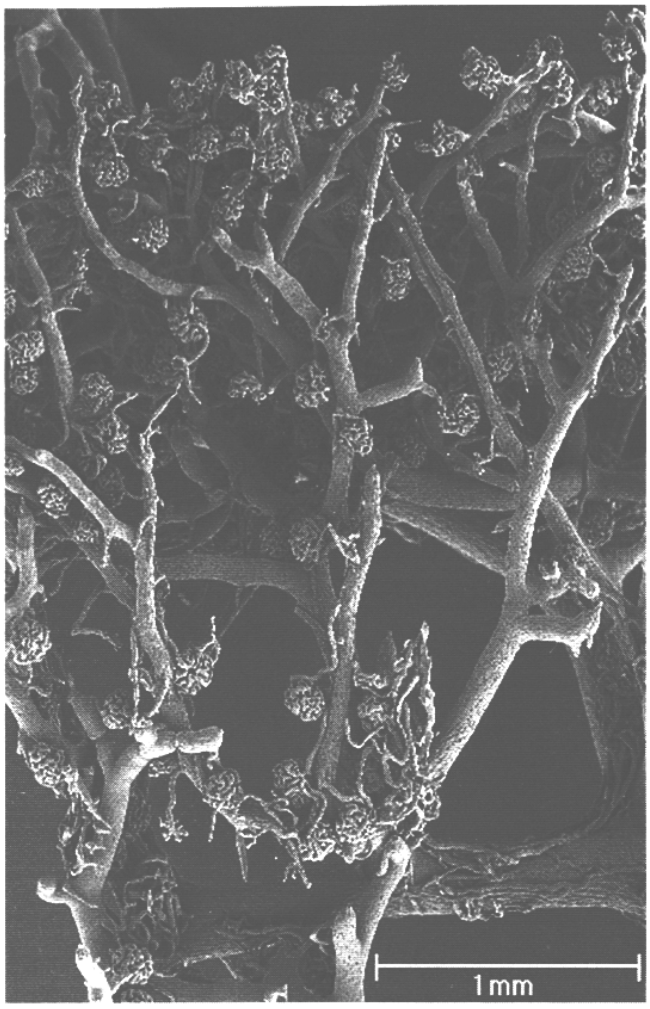

Fig. 1. — Low magnification of renal microcirculation showing the arcuate, interlobar, interlobular and recta vessels.

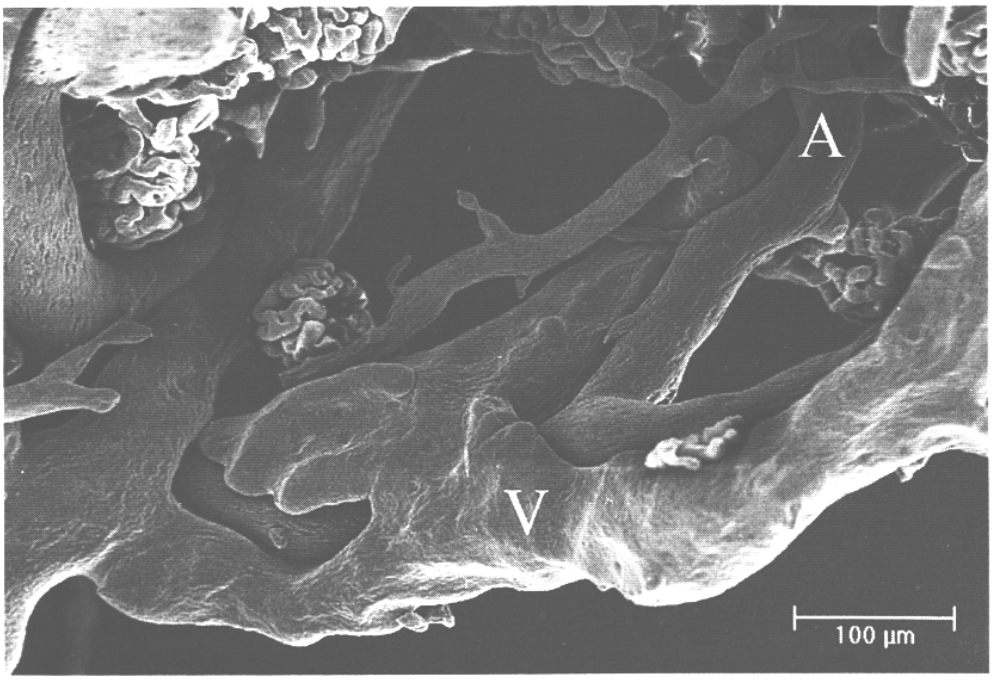

Fig. 2. — We can clearly distinguish the artery (A) from the vein (V) also looking at the nuclear impression left by the endothelial cells on the cast.

At higher magnification, we clearly observed the glomerular structure made up of tortuous anastomotic capillaries grouped in an ovoidal architecture arising from the afferent arteriole and ending in the efferent one (Fig. 3).

On the casts, we came across some impressions of various shapes and sizes: the nuclei of endothelial cells (Fig. 4) and some circular constrictions created by

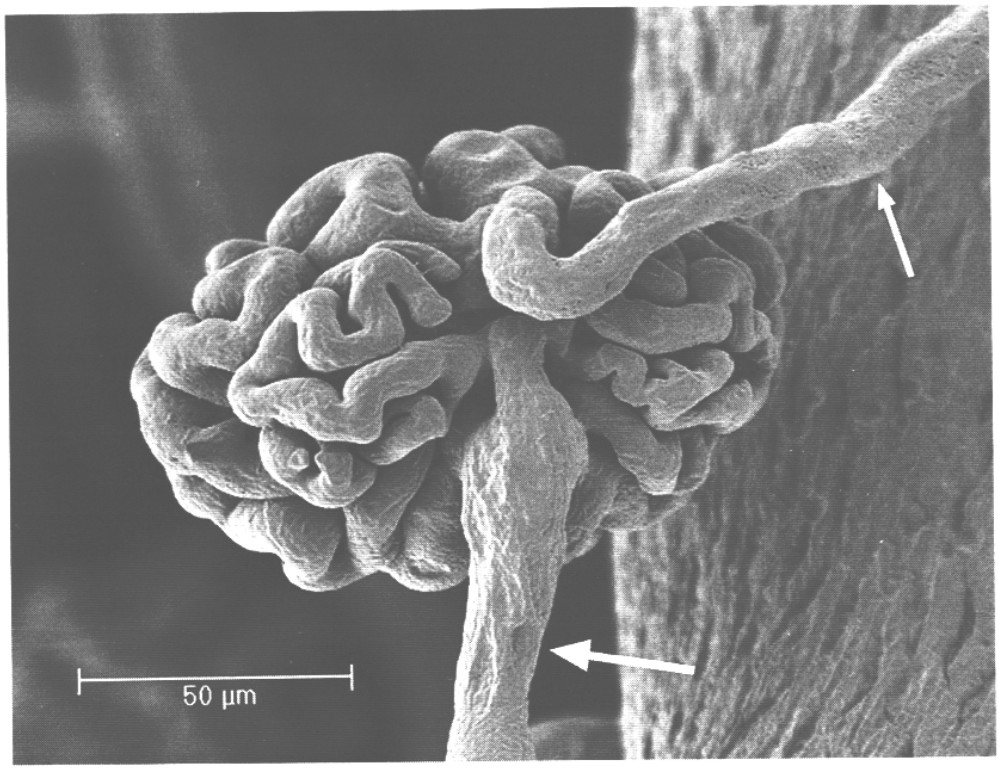

Fig. 3. — High magnification of the glomerular structure seen from its vascular pole. We clearly distinguish the afferent (thin arrow) and efferent (thick arrow) arterioles along with the tortuosity of capillaries forming the glomerulus itself. It is also possible to see some nuclear imprints on the vascular cast.

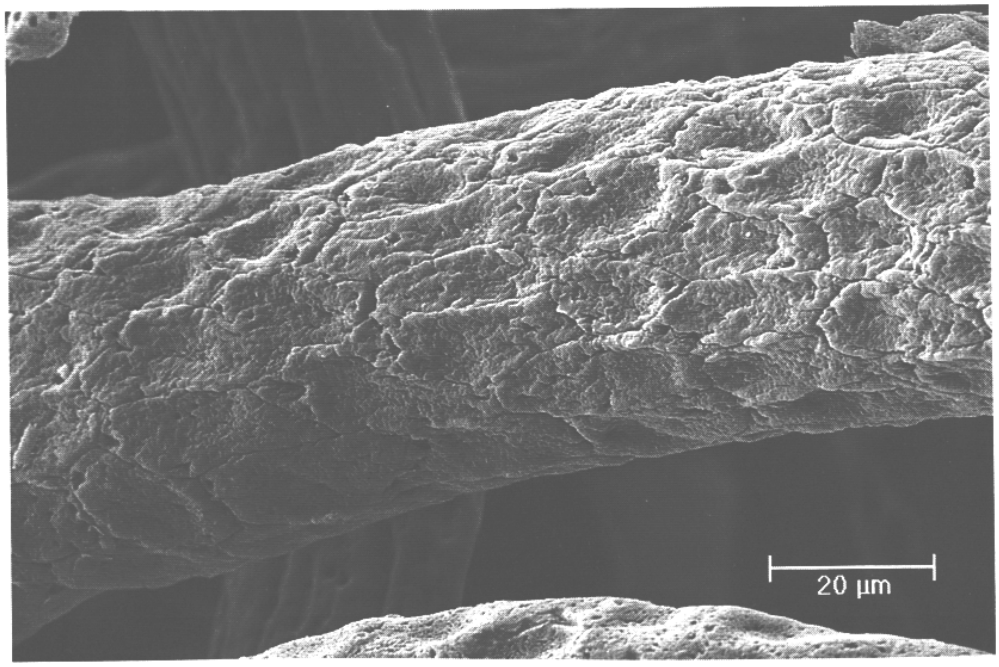

Fig. 4. — Nuclear impression on the cast of the endothelial cells.

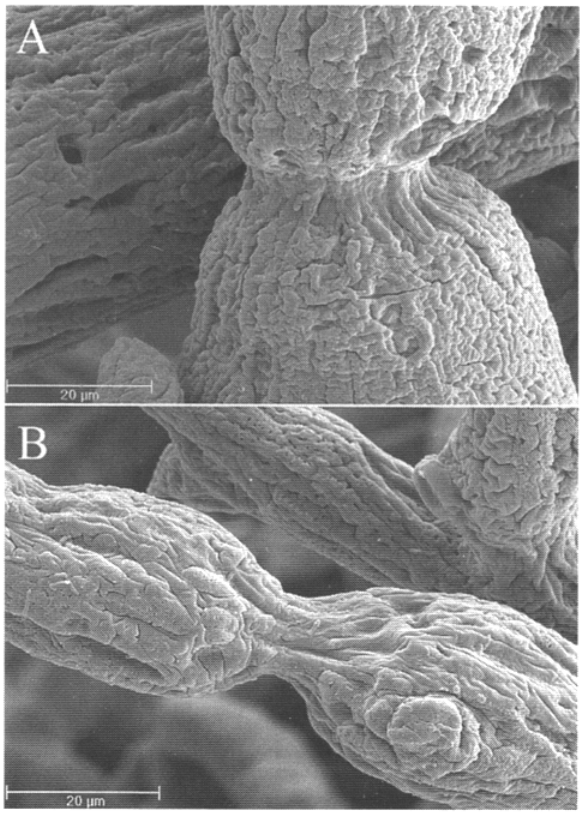

Fig. 5. — Sphincteric ring-like constrictions showing the longitudinal corrugation left by the endothelial cell membrane, stretched by external compression.

sphincteric muscular systems (Stein J.H., 1990) surrounding the adventitial membrane of medium-sized arteries and regulated by a stress-induced local activation (Fig. 5a/5b). Sometimes these systems extended along the vessel constantly reducing arteriolar size and often obstructing blood flow inside the glomerulus (Fig. 6a/6b).

Analyzing the cast, the constrictions appeared in several patterns depending on the muscular spasm.

At high magnification, the surface of the cast of glomerular capillaries appeared to be strewn with a number of dome-shaped structures measuring approx 9 nm, regular in shape and disposition (Fig. 7a). Some of them were 5 nm high, while others measured 7 nm.

On the cast it was also possible to observe a lot of deep pits located within the grooves left by endothelial cell's junctions.

There were also linear grooves evident on the cast and some deeper ones with an irregular shape (Fig. 7b). The former were left by the normal elevations of cellular borders, whereas the latter reflected the conformation of endothelial cell membranes.

Thank to corrosion casting technique we could follow the sharp disposition of glomeruli in the space, coming across some normal variations such as two glomeruli connected in series by the same vessel (Fig. 8).

On this vessels we found longitudinal impressions of endothelial cells' nuclei as we often observe on arterial vessels.

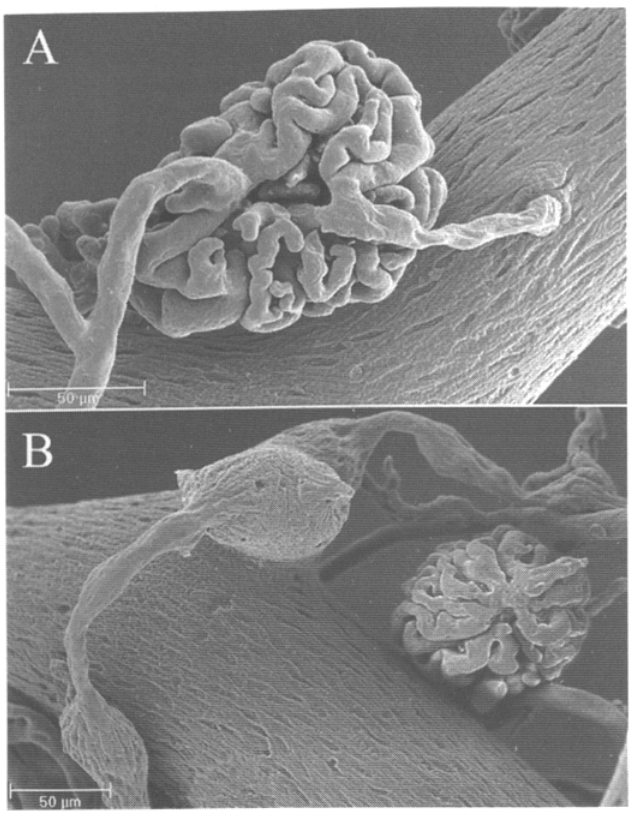

Fig. 6. — Two examples of a constant reduction in arteriolar size: in the first case the constriction is visible on an afferent arteriole that probably causes a reduction in glomerular blood flow. The second case is a medium-sized vessel longitudinally constricted by a sphincter with the consequent expansion of the vessel before it.

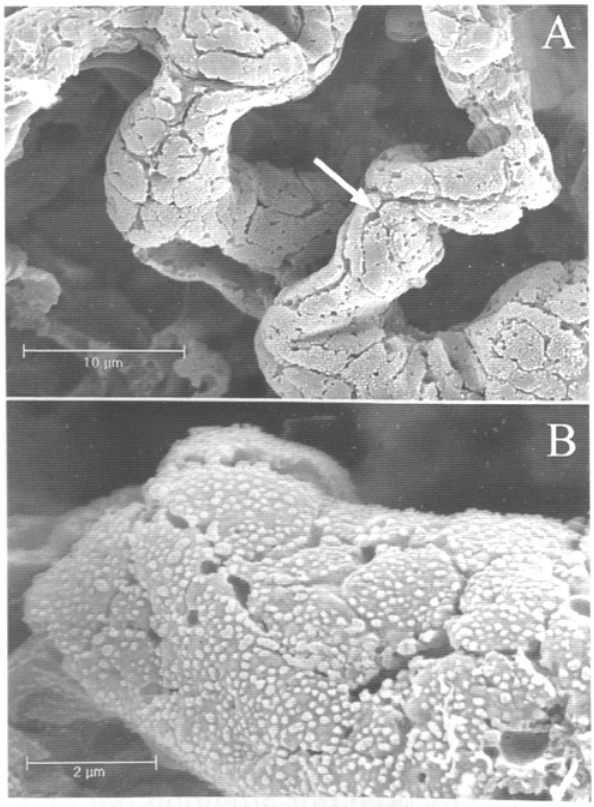

Fig. 7. — At lower magnification, the cast of glomerular capillaries appears characterized by linear grooves (arrow) left by endothelial cell junctions and probably by the corrugation of the cellular membrane. Moreover it is possible to distinguish a lot of deep pits in correspondance to these grooves. At high magnification, a number of dome-shaped structures become evident caused by the extravasation of the resin inside the thickness of cellular membranes in the pores.

In this case the vascular architecture was made up by an afferent arteriole that started from the interlobular artery and ended in a normally shaped glomerulus.

The efferent arteriole of this glomerulus gave rise to an another similar glomerular structure not directly connected to the capillary peritubular system.

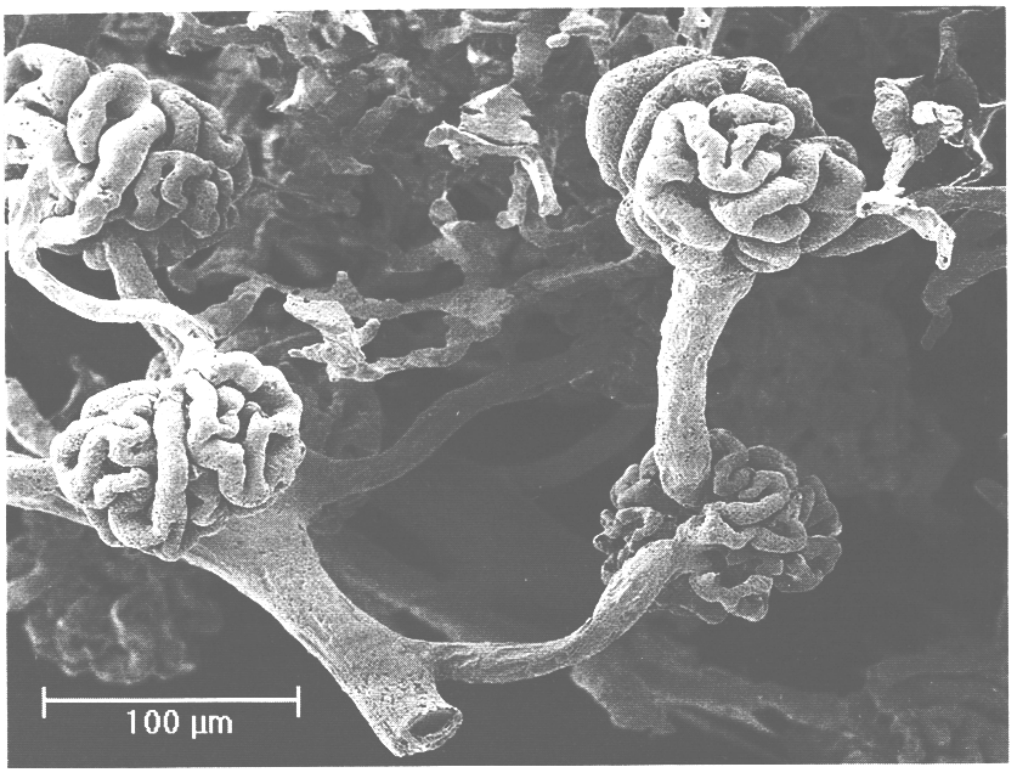

Fig. 8. — Two glomeruli are connected by the same arterial vessel.

DISCUSSION

Thank to corrosion casting and to the increasing information and details that can be revealed by SEM high defined analysis, we reached a deeper level of morphological investigation, visualizing the ultrastructural aspects of the renal microcirculation in three dimensions to demonstrate the faithfull correspondence with the data already available from TEM analysis.

The most impressive result was the accuracy reached in indirectly revealing the cellular pores of endothelial cells and the sharp mirror impression of their membranes on the casts.

In addition, it was possible to make some hypotheses on the origin of the impressions and grooves often visible, at high SEM magnification, on the cast: these are probably due to the superficial corrugation of the endothelial cell's membrane, plasmatic extensions and mesangial intravascular branches.

Moreover, on the cast, some deep pits were visible, mostly located near endothelial junctions and probably left by intraluminal projections of cellular branches from the interstitial environment: as a matter of fact is widely known that mesangial cells and podocytes may have intraluminal membrane projections that assume a physiological detector function.

Relying on the faithfulness of this method, we focused our attention not only on confirming structural details already known from literature, but also obtaining a tridimensional depiction of ultrastructural features in the blood urine filter.

This could be useful for teaching purposes, considering the easy interpretation of data provided by the cast and the tridimensional pictures obtained by SEM analysis.

Furthermore, this technique disclosed high-resolution details of the endothelium, the sphincteric structures of the arterial tree, the degree of bi and trifurcations within the glomerulus, and some vascular variations such as the presence of multiple glomeruli connected in series even if this structure seems not to be functionally useful but, however, represents an unusual morphological variant.

The spincteric constrictions visible on the cast have two different patterns: the circular one, caracterized by a single and defined circular groove, and the filiform one caused by the gradual narrowing vascular size that results in a thin blind ended cast.

The longitudinal impressions often visible correspond to the extended wrinkling caused by endothelial cell membrane's contraction.

The corrosion casting method has always been used to disclose structural features in space, overlooking its applicability at a deep ultrastructural level. For example, at high magnification, the shape of a cell's surface can be easily detected by observing the marks left on the cast.

We observed a lot of bulges on glomerular casted vessels probably due to the extravasation in the endothelial cells membrane's pores characteristic of blood-urine filter: in some case the diaphragms usually sited in this pores can limit this phenomenon and the resin penetration is not visible anymore.

On the other hand, resin is a valid medium to obtain instant three-dimensional high definition reproduction of structural but also ultrastructural features of vascular tissue, thereby representing a useful teaching device. Of course, as with film negatives, we always have to think in terms of opposites, but after the "developing process" we are able to see fine details not previously visible.

Corrosion casting, however, is prone to artefacts which must be known so as to distinguish an air bubble from a groove left by cellular components or a blind end caused by clotting from a constriction due to the muscular layer, and so on. The most common artefacts are ring structures around the vessels due to extravasation of the resin between the layers of vessel wall and wrinkling of the cast surface due to coarctation derived from the fixation injury.

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