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Dynamic structure of glomerular capillary loop as revealed by an in vivo cryotechnique

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Abstract Morphological studies using immersion or perfusion fixation methods do not reveal the ultrastructure of functioning kidneys with normal circulation. A simple apparatus was developed for freezing the kidneys in vivo without stopping the blood supply, and the ultrastructure of the glomerular capillary loops was examined under different haemodynamic conditions. Mouse kidneys were frozen under normal blood flow conditions; others were frozen in the same way after ligation of the abdominal aorta at a point caudal to the renal arteries. They were then processed for the freeze-substitution or deep-etching method. Good ultrastructural preservation was obtained within about 5 μm depth from the frozen tissue surface. Functioning glomeruli with normal blood flow possessed open capillary lumens, different shapes of foot processes and atypical basement membranes with low density. Moreover, heterogeneity in width between foot processes was identified on the replica membranes. Under the acute conditions used to increase blood supply into the kidneys, the spaces between the flat foot processes became more widely dilated and the basement membrane was seen to be three-layered. The ultrastructure of glomeruli in functioning kidneys has been demonstrated for the first time by this "in vivo cryotechnique".

Key words Quick-freezing · Glomerulus · Basement membrane · Freeze-substitution · Deep-etching

Introduction

It is generally accepted that the glomerular basement membrane constitutes a major part of the filtration barrier that borders the capillary lumen and the urinary space [3, 18, 19, 26]. The glomerulus consists of intricate networks of capillaries, through which the blood passes under the influence of pressure and it is reasonable to assume that haemodynamic factors such as the blood pressure and flow exert an important influence on the glomerular ultrastructure [12, 18, 20]. Changes in glomerular haemodynamics may also affect the driving force that modulates the permeability properties of the filtration barrier [2, 3, 26, 29, 30]. Extensive molecular sieving of plasma proteins usually occurs under normal hydraulic pressures across the glomerular basement membrane and the distribution of albumin proteins in rat glomeruli has been reported to be changed in different haemodynamic conditions [26, 29]. Thus the functional role of the normal circulation is definitely important in maintenance of the glomerular barrier function. However, the significance of glomerular ultrastructures revealed by conventional preparation methods has been difficult to evaluate [4, 5, 33] because the chemical fixation of tissues takes a considerable time during which morphology is changed. It is generally accepted that renal ischaemia produces ultrastructural changes in glomeruli [12]. The ultimate goal of glomerular study is that all features to be examined should reflect the physiological function under investigation, and no artifact should be introduced by the preparation method.

A promising approach is a quick-freezing method by which tissues are brought into contact with copper or silver cooled in liquid helium or nitrogen [8, 13, 14, 21, 23, 27]. It means quick resolution of dynamic morphology, sufficient to fix rapid ultrastructural changes [14, 23]. It is accepted that cryofixation is the best way to preserve antigenecity and chemical composition of tissues [15, 34] and morphological changes produced by chemical fixation and dehydration steps are avoided [4, 5, 10, 16, 23, 24, 33]. Over the last decade, it has become a popular tool for the ultrastructural study of biological specimens. There is a wealth of information on cryofixation techniques, which include plunging tissues into liquid cryogen, jetting liquid cryogen against the specimen or slamming it against a precooled metal surface [7, 8, 10, 11, 13, 17, 21, 27, 31, 32].

Small specimens were commonly frozen within several seconds, following the excision of tissues [5, 10, 23]

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and most cryotechniques have been based on the use of previously excised tissues. When the kidney is isolated from the circulation it is immediately exposed to severe ischaemic condition. Quick-freezing with such specimens has some limitations, as it is well known that the ultrastructure of the kidneys is easily changed by stopping the blood supply [12]. As it is impossible to obtain natural morphology by using conventional cryotechniques there is a need for a new method capable of freezing tissues in vivo and obtaining acceptable morphology in fuctioning organs [6, 34]. In this paper, we will describe the morphological aspects of functioning kidneys, whose ultrastructures are reassessed, with regard to the haemodynamic conditions operating at the time of cryofixation. Some parts of this work have been presented in abstract form [25].

Materials and methods

The system of this in vivo cryotechnique is schematically represented in Fig. 1. The cryoknife consists of an aluminized funnel and an organ-cutting knife. The disposable knife edge is precooled by liquid nitrogen (−196° C) in a styrene-foam box, which is the main functioning part of the cryoknife apparatus (Fig. 1a). The funnel reservoir has about 100 ml liquid capacity, which is sufficient to keep the whole mouse kidney at low temperature during the in vivo freezing (Fig. 1b). The funnel itself is taped for attachment to the cryoknife holder. A small hole with a diameter of 5 mm is created through the lower tip of the funnel and poured liquid cryogen floods into the cut tissue surfaces, maintaining their coldness. Actually the precooled knife edge cuts into and instantly freezes the kidney tissue, which is simultaneously covered by liquid isopentane-propane mixture (Fig. 1c). This is followed by liquid nitrogen and then retained at liquid nitrogen temperature (−196° C). The cooling rate of the tissue surface in contact with the precooled knife edge probably depends on the sliding speed for cutting, which determines the time exposed to the edge. Filling the funnel with the liquid isopentane-propane mixture is achieved by pouring it into the wide upper opening of the funnel (Fig. 1b, c). As mentioned above, the in vivo cryotechnique with the cryoknife apparatus is a series of processes which combine the metal contact freezing method with liquid cryogen freezing method.

Preparation of the isopentane-propane mixture is described in this paragraph. About 15 ml of liquid isopentane was first loaded into a 50 ml beaker, which was then cooled in the liquid nitrogen. A magnet bar was allowed to rotate in the beaker under the influence of externally generated rotating magnetic field. The cooled isopentane was immediately added with commercially available propane gas, which was stirred with the magnetic bar. The propane gas was liquidized in the cryogen mixture cooled by liquid nitrogen. Within several minutes, the isopentane-propane mixture reached a volume of about 45 ml and at temperature of −193° C [17] and was used immediately to avoid frost contamination.

A total of 14 male ddy mice, each weighing 20–30 g, were used for the present experiment. They were kept in an air-conditioned room and fed on commercial diet and water ad libitum. For the control experiment they were anaesthetized with sodium pentobarbital via the peritoneal cavity. The abdomen was exposed through a pararectus incision and the left kidney was isolated (Fig. 1). The renal adipose capsule was gently stripped from the kidney suface with fine forceps. The cleaned kidney, in situ, was carefully put on a wax plate without disturbing the vessels or ureter. Great care was taken to avoid interference with the renal hilus and vascular pedicle and displacement of the kidney from its normal position. The "in vivo cryotechnique" was then performed under normal blood flow conditions. For another experimental protocol, during sodium pentobarbital anaesthesia, the abdominal aorta was ligated at the region caudal to the point where the renal arteries branched. The kidneys in these animals showed a temporary increase of blood supply and ballooning. Such a gross change of size was usually judged as an index of tight aortic ligation. The same "in vivo cryotechnique" was performed under the experimental conditions described below.

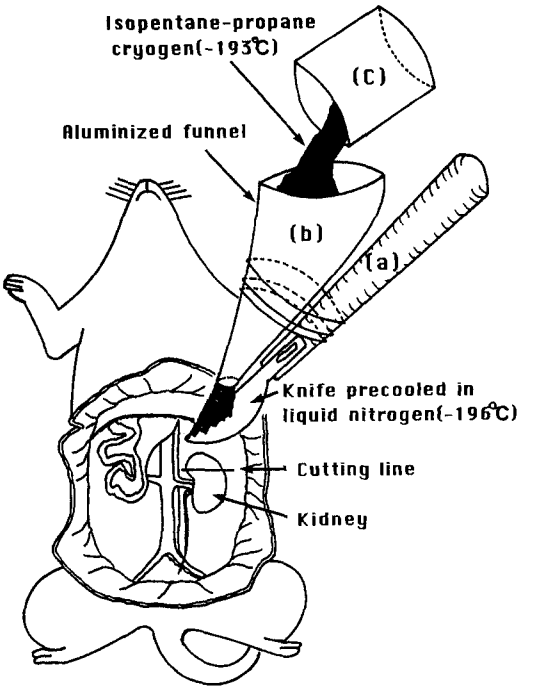

Fig. 1 Schematic representation of the "in vivo cryotechnique". Cryoknife (*a*), aluminized funnel (*b*) and 50 ml beaker (*c*)

First, the cryoknife edge was precooled in liquid nitrogen and allowed to reach the liquid nitrogen temperature (−196° C), and then it was positioned over the kidney in vivo (Fig. 1). While the heart was beating normally, the renal cortical tissues were incised and simultaneously frozen with the aid of the cryoknife edge. The cryoknife was pushed into the kidney at high speed and the tissue was immediately covered by the liquid isopentane-propane cryogen. Some of the poured cryogen remained along the knife edge and took part in the freezing process. Liquid nitrogen was poured on the frozen tissues to avoid their rewarming. The liquid nitrogen was needed to promote heat flow from the tissues and prevent the formation of gas bubbles due to boiling of propane at the specimen-cryogen interface. The metal knife edge was withdrawn after completely freezing the whole kidney with liquid nitrogen. The frozen kidney was then excised from the remaining abdominal tissues and transferred into liquid nitrogen, in which the well frozen parts were identified and trimmed off with cooled nippers.

The frozen specimens were then submitted to freeze-substitution as follows [24]. They were transferred into absolute acetone containing 2% osmium tetroxide at −80° C and kept for 24 h. They were put into a deep-freezer at −20° C for 2 h and to a refrigerator at 4° C for 2 h. They were briefly washed in pure acetone at room temperature and embedded in Epok 812. After routine polymerization, ultrathin sections were prepared perpendicularly to the frozen specimen surface, stained with uranyl acetate and lead citrate, and observed in Hitachi HS-9 and H-600 electron microscopes. Morphometric data were analysed with Student's *t*-test. Some thick sections were also prepared, stained with toluidine blue and observed by light microscopy.

Replica membranes were prepared as follows. The previously frozen kidney tissues were put on a special holder with glycerin and the cut tissue surfaces were carefully freeze-fractured with a scalpel in liquid nitrogen as described before [31, 32]. They were deeply etched under vacuum conditions of $2–6\times10^{-7}$ Torr at a tem-

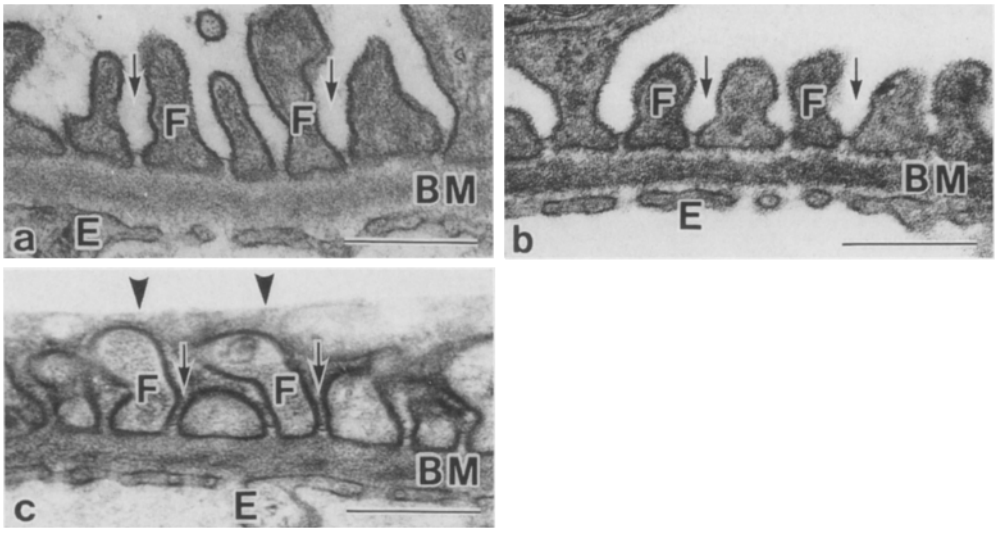

Fig. 2 Electron micrographs of glomerular capillary loops prepared by conventional methods such as glutaraldehyde-osmium tetroxide (GO) fixation (**a**), tannic acid-GO fixation (**b**) and the quick-freezing and freeze-substitution (QF-FS) method (**c**). ×34,900. *Bar*=0.5 μm. In **a** and **b** the spaces between foot processes (*F*) are dilated (*arrows*). Three layers in the basement membrane (*BM*) are clearly identified. **c** The thick basement membrane is not composed of typical lamina rara externa, lamina densa and lamina rara interna, in the resected fresh specimen as revealed by the QF-FS method. The spaces between foot processes are narrow (*arrows*). *Arrowheads* indicate tissue surface attached to cooled metal, *E* endothelium

perature of –95° C for 20–30 min. After deep-etching, the specimens on a rotary stage were first shadowed with platinum at an angle of 35° for several seconds and then rotary shadowed up to the total thickness of about 2 nm as reported before [24]. They were additionally coated with carbon at an angle of 90°. A drop of 2% collodion in amylacetate was put onto the replicas as soon as they were taken out from the machine. The replica membranes coated with dried collodion were floated on household bleach to dissolve the tissue components. They were washed in distilled water, cut into small pieces with scissors, picked up on Formvar-filmed copper grids and immersed in amylacetate solution to dissolve the collodion film. They were then observed in Hitachi HS-9 and H-600 electron microscopes at an accelerating voltage of 75 kV. Electron micrographs were printed from the inverted negative films.

Some other kidney specimens were routinely treated by conventional fixation procedures [10]. For tannic acid glutaraldehyde-osmium tetroxide (TGO) fixation, the kidneys were perfused with 1% tannic acid and 1% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.2, for 15 min at a hydrostatic pressure of 150 cm of water via the abdominal aorta [10, 24]. Then the cortical tissues were cut into small pieces and postfixed with 1% osmium tetroxide in PB for 1 h at 4° C. For GO fixation, the renal cortices were prefixed with 2.5% glutaraldehyde in PB for 2 h and postfixed with 1% osmium tetroxide in PB for 1 h at 4° C. Both specimens were routinely dehydrated in a series of ethanols and embedded in

Table 1 Morphometric data obtained from the measured distances as shown in Fig. 10

Widths of slit diaphragm and foot processes

	Conventional quick-freezing of resected fresh specimen (Mean ± S.D., N=50)	In vivo freezing under normal blood flow (Mean ± S. D., N=50)	In vivo freezing after ligation of aorta (Mean ± S.D., N=50)
A	32.2 ± 3.3 nm *	36.5 ± 5.7 nm	41.3 ± 7.2 nm (** from 32.2; * from 36.5)
B	35.7 ± 7.2 nm *	50.7 ± 13.3 nm **	84.2 ± 25.9 nm (** from 35.7)
C	257.3 ± 92.5 nm	289.2 ± 84.1 nm	297.4 ± 105.4 nm
D	246.1 ± 90.2 nm	240.7 ± 62.5 nm	175.9 ± 94.5 nm
H	460.9 ± 115.0 nm **	294.3 ± 91.5 nm	224.8 ± 44.6 nm (** from 460.9)

* P<0.1 ** P<0.05

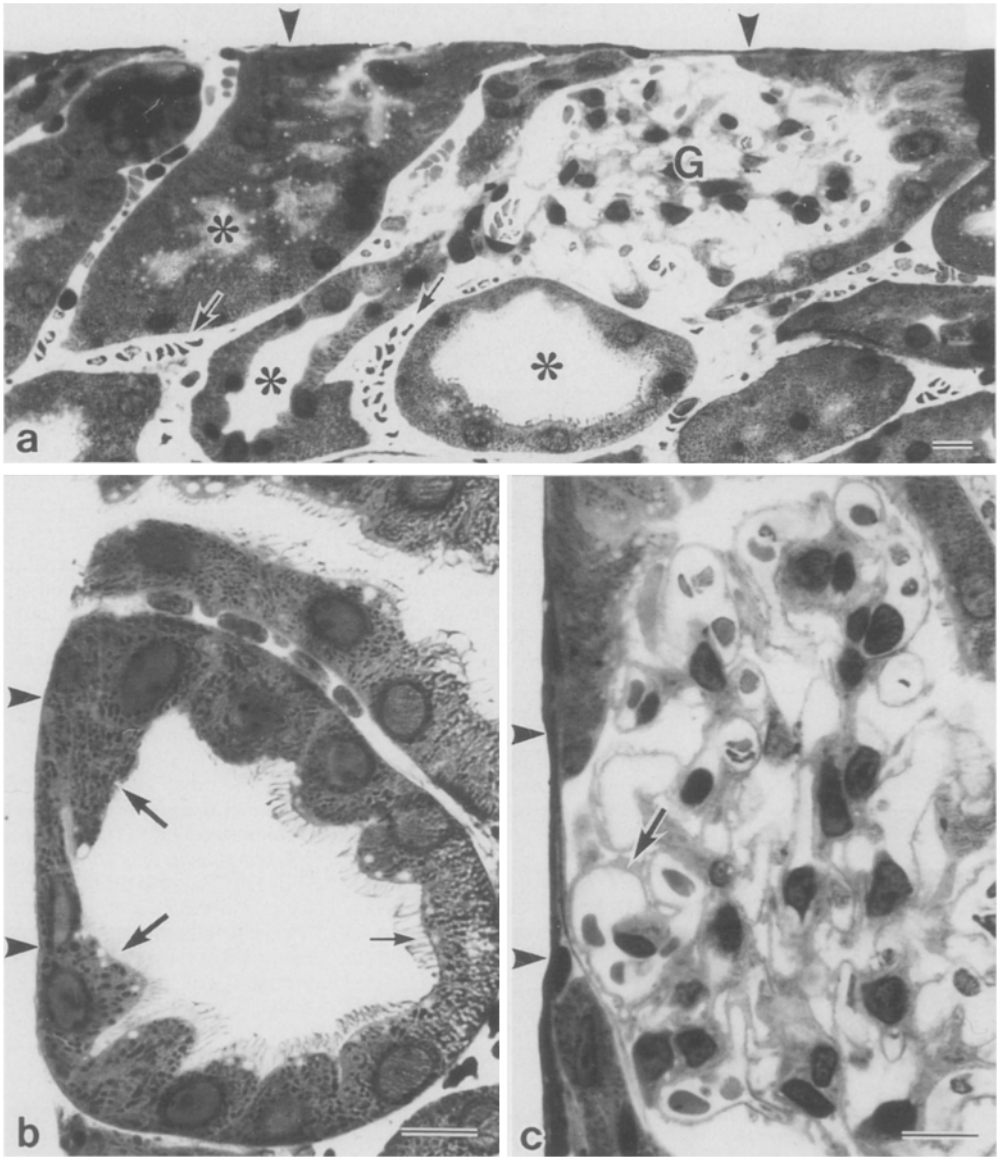

Fig. 3a–c Light micrographs of toluidine blue-stained thick sections of normal kidney cortex, prepared by the freeze-substitution method after the "in vivo cryotechnique". *Bar*=10 μm. **a** The plane of contact between the tissue and the cryoknife is seen in the upper part (*arrowheads*). Note the open tubular lumen (*asterisks*) and peritubular space (*arrows*). *G* glomerulus, ×500. **b** Well preserved proximal tubular cells are seen at a light microscopic level (*large arrows*). In contrast, epithelial cells in deep tissues show large ice crystals near the right edge of this figure (*small arrow*). *Arrowheads* indicate tissue surface attached to the cryoknife. ×1,000. **c** Higher magnification of a part of a glomerulus shown in **a**. Well preserved capillary loops are seen (*arrow*). *Arrowheads* indicate tissue surface contact with the cryoknife. ×1,000

Epok 812 as reported before [10]. Ultrathin sections were cut with an ultramicrotome, stained with uranyl acetate and lead citrate, and examined by the electron microscopes. Moreover, for comparison, fresh unfixed kidneys were taken out, quickly frozen and freeze-substituted, as described in a previous paper [24].

Results

The kidney tissues conventionally prepared with TGO or GO fixation showed the typical three-layered basement membrane composed of lamina rara interna or externa and lamina densa (Fig. 2a, b), as previously reported [10]. Foot processes of fixed glomeruli showed shrunken cell surface contours. Widely opened clefts were formed between the glycocalyx layers of adjacent foot processes. Where ultrathin sections cut foot processes vertically, some slit diaphragms could be distinguished as single lines between the foot processes. In addition, the slit diaphragms were pulled apart, their width averaging 47 nm (n=50, Fig. 2b), as reported before [10]. In the glomerular basement membrane of fresh unfixed kidneys prepared by quick-freezing and freeze-substitution (Fig. 2c), the obscure lamina densa has been reported to be directly adherent to the cell membranes of foot processes and endothelium. Their width (the distance between foot processes) had a mean value of about 32 nm (n=50, Table 1). A detailed description of the morphometric data to determine the widths of slit diaphragms has already been published in a separate paper [10]. It is thus clear that the conventionally fixed specimens possessed altered ultrastructures of foot processes or basement membranes caused by fixation or the dehydration step, as discussed before [10, 24].

Light micrographs of 1 μm-thick sections with freeze-substituted specimens indicate that some tubular lumens were widely open in large areas (Fig. 3a, b). The normally functioning kidney was also seen to have an obvious peritubular space which was extensive in volume (Fig.

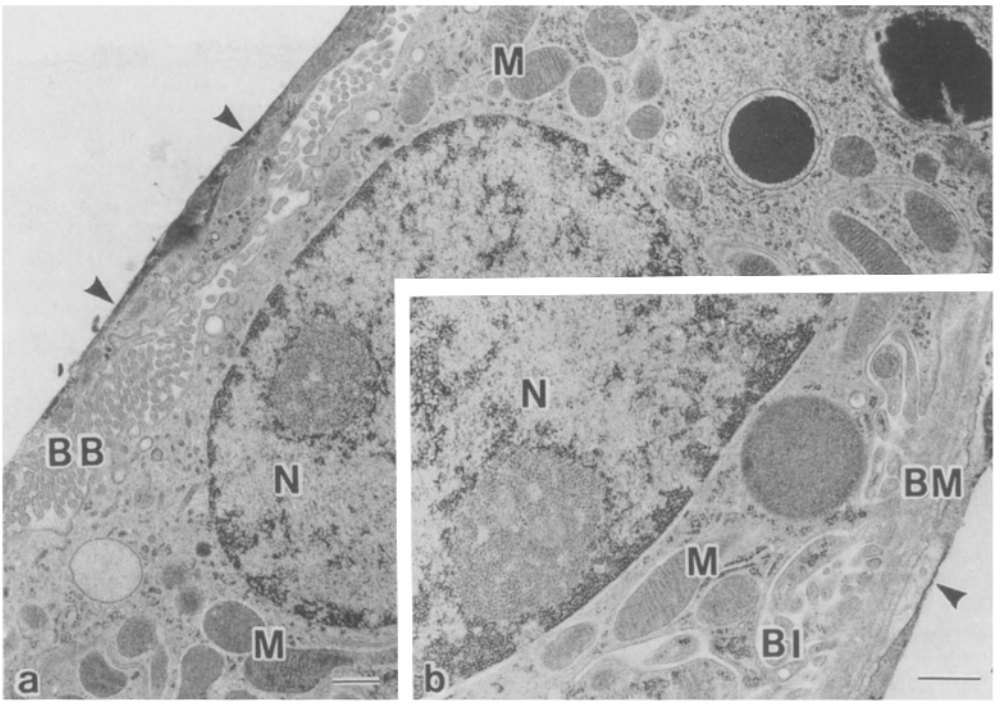

Fig. 4a, b Electron micrographs of tubular epithelial cells under normal blood flow, prepared by the FS method after the "in vivo cryotechnique". *Bar*=0.5 μm. **a** Well frozen areas in apical cytoplasm are obtained within 5 μm depth from the cut tissue surface (*arrowheads*). *BB* Brush border, *M* mitochondria, *N* nucleus. ×11,600. **b** The ultrastructures including the nucleus in basal cytoplasm of a tubular cell are well preserved. The cutting line is identified at the right lower part of this micrograph (*arrowhead*). The basal infolding (*BI*) and basement membrane are observed. ×16,300

3a). Several micrometres of depth from the cut tissue surface were probably kept in contact with the cooled knife edge. The kidney tissues showed fairly good preservation of morphology in areas close to the frozen tissue surface (Fig. 3b). The distribution of ice crystals was clearly detected in deep areas of the specimen. At the light microscopic level, there were no visible ice crystals up to 30 μm from the tissue surface, though a reticular appearance characteristic of ice crystal formation could be observed at distance from the tissue surface. In our study with the in vivo cryotechnique, glomerular morphology in areas near the tissue surface was also in an acceptable condition at the light microscopic level (Fig. 3c). The capillary lumen was kept open in glomeruli frozen in vivo during normal blood supply into kidneys.

Electron micrographs of tubular epithelial cells demonstrate good preservation of ultrastructures within about 5 μm from the cut tissue surface (Fig. 4). So a surface band layer of approximately 5 μm depth was frozen sufficiently to prevent the formation of visible ice crystals (Fig. 4a). The ice crystal size increased exponentially, depending on the depth of the tissue (Fig. 5). After processing by the in vivo cryotechnique and freeze-substitution, the tubular basement membrane was composed of a thick lamina densa, which filled the entire space between basal infoldings and extracellular matrix (Fig. 4b). Figure 5 illustrates schematically the relationship between ice crystal size and distance from the tissue surface. Tiny ice crystals were firstly identified at a distance of 5 μm.

In areas of glomeruli which were judged to be relatively free from ice crystals (Fig. 6), their ultrastructure was remarkably different from that revealed by the conventional preparation method, as shown in Fig. 2. The basement membrane consisted of a thin lamina densa

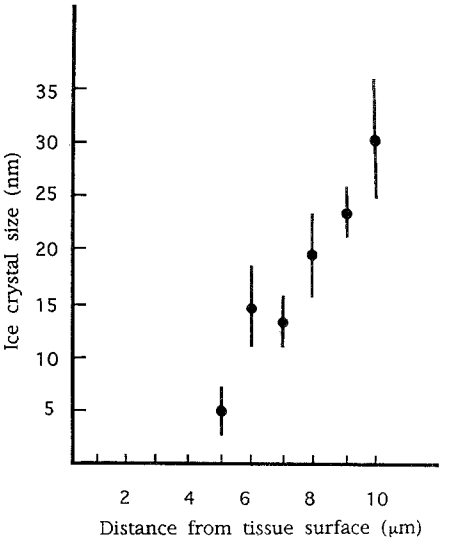

Fig. 5 Relationship between ice crystal size and distance from the tissue surface that was cut and immediately frozen in the precooled knife and the cryogen (−193° C)

and obscure lamina lucida in contact with endothelial cells or foot processes (Fig. 6a). The shapes of some foot processes became more round. Erythrocytes in the capillary loop showed little ice crystal damage, displaying a darkly contrasted homogeneous matrix. Under normal haemodynamic conditions, the spaces between adjacent foot processes were heterogeneously narrow at the level of the slit diaphragms (Table 1; Fig. 10). On morphometric analysis, their width had a mean value of 36.5 nm (n=50). At higher magnification, the lamina densa was composed of irregular cords with high electron dense

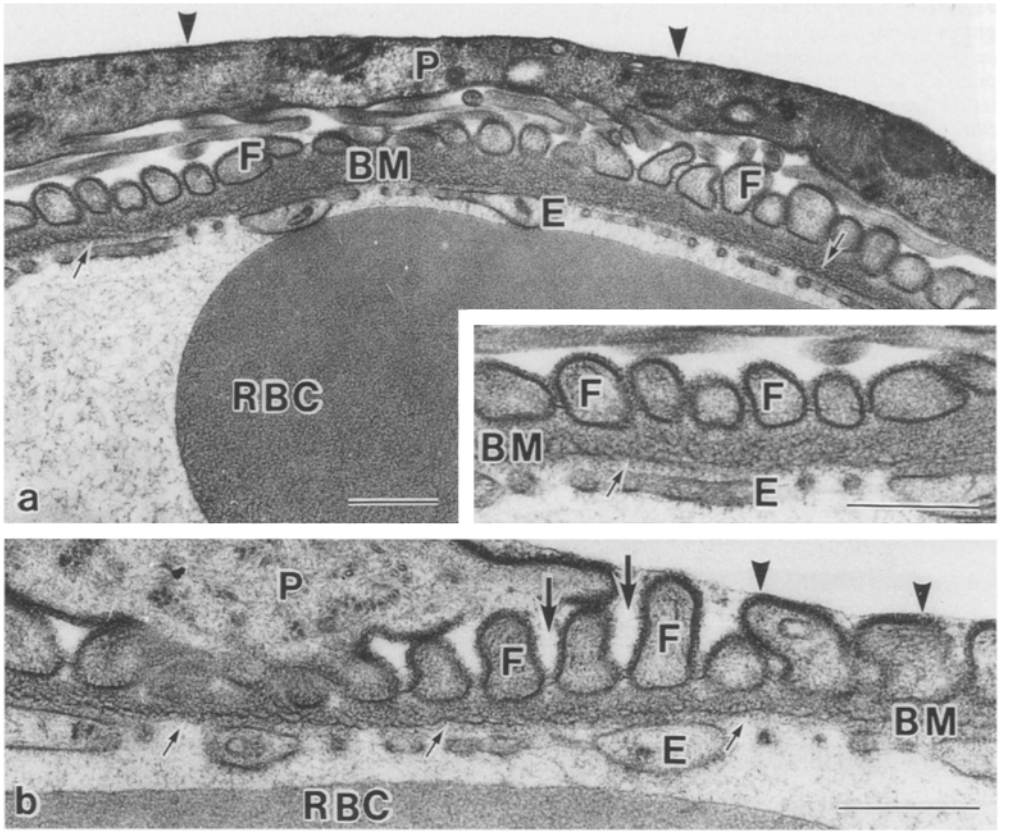

Fig. 6a, b Electron micrographs of glomerular capillary loops under normal blood flow, prepared by the FS method after the "in vivo cryotechnique". *Bar*=0.5 μm. **a** The cutting line (*arrowheads*) passes through the cytoplasm of podocytes (*P*), and well frozen foot processes and basement membrane are observed. The thin layer resembling lamina densa (*arrows*) can be identified. *E* endothelium, *RBC* red blood cell. ×23,300. [*Inset* At higher magnification, foot processes are a little flattened in some parts and thin lamina densa (*arrow*) is observed in the basement membrane. ×34,900]. **b** The cutting line (*arrowheads*) passes through the top of the foot processes. The ultrastructures are well preserved in the surface area. The spaces of foot processes appear a little dilated (*large arrows*) and thin lamina densa is also identified (*small arrows*). ×37,200

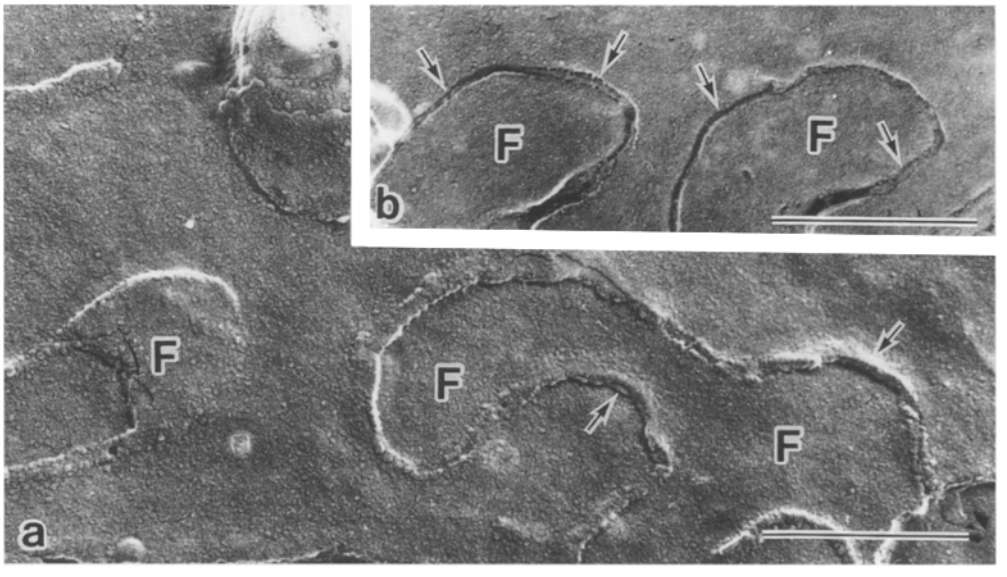

Fig. 7a, b Replica electron micrographs of horizontally freeze-fractured capillary loops under normal blood flow into kidneys, as revealed by the "in vivo cryotechnique". **a** and **b** are different glomeruli. The distance between the foot processes is narrow and uneven (*arrows*). ×53,100. *Bar*=0.5 μm

line (Fig. 6a, *inset*). A fibrillary network at both sides of the thin lamina densa was also observed in another glomerulus (Fig. 6b).

The replica membranes of slit diaphragms, which were freeze-fractured parallel to the basement membrane, revealed that interdigitating foot processes were heterogeneously in close apposition (Fig. 7). The width of the slit diaphragms, as measured from the outer surface of one foot process to the next, amounted to 20–45 nm (n=50).

The glomerular ultrastructure changed, depending on the renal haemodynamic state at the time of freezing in vivo (Fig. 8). After the acute higher blood supply induced by ligation of the aorta, there was an increased width of slit diaphragms (Fig. 8a). Their width had a mean value of 41.3 nm (n=50, Table 1). Moreover a probably transient increase in intravascular pressure caused stretching of some epithelial foot processes (Fig. 8b).

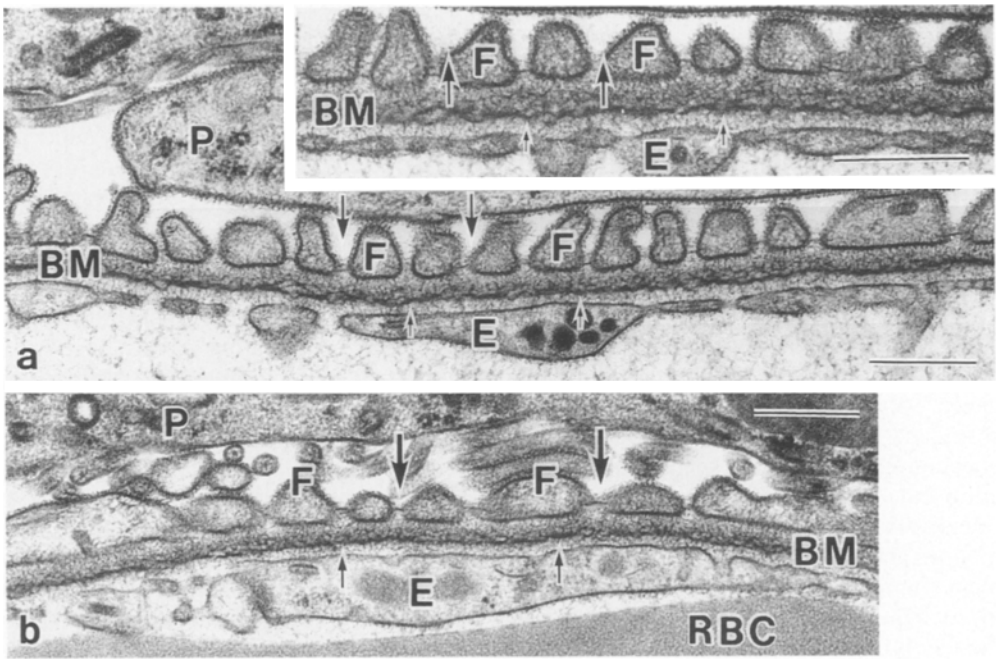

Fig. 8a, b Electron micrographs of glomerular capillary loops under higher blood supply into kidneys after ligation of the aorta, prepared by the FS method following the "in vivo cryotechnique". *Bar*=0.5 μm. **a** The spaces between foot processes are more widely dilated (*large arrows*). The lamina densa (*small arrows*) is clearly identified in the basement membrane. ×27,900. *Inset* Higher magnification of another part. ×34,900. **b** Some foot processes in another glomerulus are flattened over the basement membrane. The spaces between foot processes are more widely dilated (*large arrows*). The lamina densa is also clearly identified (*small arrows*). ×27,900

Figure 9 indicates a schematic representation of glomerular capillary loops prepared by various electron microscopic procedures, such as the conventional double fixation (Fig. 9a), quick-freezing method with fresh unfixed kidneys (Fig. 9b), in vivo cryotechnique under normal blood pressure (Fig. 9c) and in vivo cryotechnique under higher blood supply into kidneys (Fig. 9d). In summary, the shapes of the foot processes and the ultrastructure of the basement membranes were clearly changed, depending on the various techniques (Fig. 9a, b) and the experimental conditions in vivo (Fig. 9c, d). Morphometric data also indicates the wider distances between foot processes and the flattening of foot processes in functioning glomeruli (Table 1; Fig. 10).

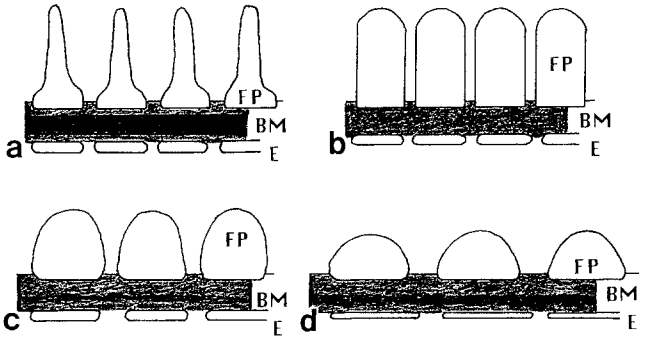

Fig. 9a–d Schematic representation of glomerular capillary loops prepared by various electron microscopic procedures. **a** Conventional double fixation method. **b** QF method with fresh unfixed kidneys. **c** The "in vivo cryotechnique", under normal blood pressure. **d** The "in vivo cryotechnique", under higher blood supply into kidneys

Discussion

The in vivo cryotechnique, directly performed in the animal, was quick to arrest transient physiological processes allowing morphological study of the kidneys. The changes created by stopping blood circulation were avoided in the outermost tissues due to the simultaneity of cutting and freezing [34]. The time interval betweeen excising and freezing was dramatically shorter than that used in the conventional preparation methods. However, one of the possible drawbacks of our cryoknife method was the tissue damage caused by the force of the knife edge. The superficial thin layer, where the impact usually takes place, is slightly compressed as shown in Fig. 4. There might be a relationship between cutting velocity and cooling rate in the present case which is not unexpected, because the precooled knife edge slides through the compact tissue. The in vivo cryotechnique has brought electron microscopy considerably closer to its ultimate aim

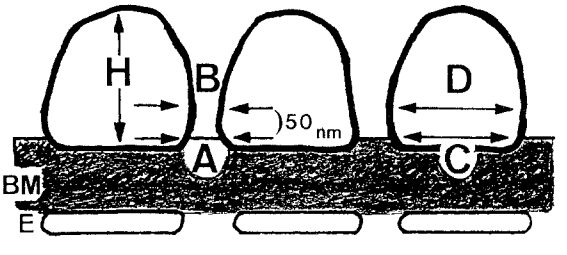

Fig. 10 Schematic representation of various measured parts in the glomerular capillary wall, which are shown in Table 1. *A* Widths of slit diaphragm, *B* distances between neighbouring foot processes at 50 nm from the slit diaphragm, *C* widths of foot processes at the level of the slit diaphragms, *D* widths of foot processes at 50 nm from the slit diaphragm, *H* heights of foot processes

of examining glomerular ultrastructures in vivo. It was clear that the surface tissue, except for the outermost damaged layer, was frozen rapidly enough to prevent the formation of ice crystals.

When kidney tissues are processed according to conventional electron microscopic methods, their glomerular basement membrane displays a lamina densa separated from epithelial or endothelial cells by laminae rarae, as shown in Fig. 2a, b. The first fixation step with chemicals such as glutaraldehyde and osmium tetroxide has been known to extract some of their materials and occasionally disturb the native ultrastructure [16, 28, 33]. Moreover, a rapid dehydration at room temperature in organic solvents has also been found to induce their shrinkage [5, 10]. The occurrence of the lamina lucida in conventionally prepared specimens is not only due to fixation but also to rapid dehydration [5, 28]. In contrast, freeze-substitution of tissues has been reported to result in slow and gentle dehydration, indicating that the basement membrane is simply composed of a lamina densa [4, 5, 10]. Therefore, it is concluded that the laminae rarae easily appeared as artifacts in conventional preparation methods. Such ultrastructural changes were also minimized by the in vivo cryotechnique, followed by freeze-substitution. It is tempting to propose that glomerular tissues have no typical basement membrane in their living state, as shown in Fig. 6. It is apparent during normal blood flow, that the glomerular ultrastructure differs from that in conventionally fixed kidneys, as summarized in Fig. 9. A functional interpretation of glomerular morphology is valid only if haemodynamic factors are taken into consideration.

A common technique of cryofixation like slam freezing is characterized by a sudden exposure of excised tissues to copper or silver metals at a low temperature [8, 13, 14, 23]. However, in the present study, isopentane-propane was selected as a liquid cryogen, because it remains liquid even at liquid nitrogen temperature and possesses good thermal conductivity [7, 10, 17, 22, 32]. All living processes in kidney organs were stopped instantly and their components were maintained in situ. There was no need to isolate the kidney tissue from the blood circulation system as reported for hearts and livers [34] and ischaemic influence on the kidney tissues was minimized using our cryofixation device. The glomerular basement membrane in living kidneys is constantly stretched by the hydraulic pressure in capillaries, whereas that in excised kidneys does not receive such stretching forces, as summarized in Fig. 9. The space between foot processes of podocytes is maintained widely open to facilitate passage of the glomerular filtrate in vivo [20]. It has been reported that the foot processes are rich in contractile proteins, such as actin, myosin and α-actinin, and are capable of such actin-mediated movement [1]. Through this active movement, the podocytes may regulate the glomerular filtration rate, thereby influencing hydraulic pressure across the glomerular basement membrane, as reported recently [20].

In the normally functioning glomerulus, the filtration slits are probably kept open by hydraulic pressure and polyanionic charge repulsion, so blood pressure must be maintained steadily before exposure to freezing. The filtration slits were found to be narrowed in the present study. It has been reported that glomerular hydraulic pressure might be regulated by the relative width of the filtration slits, the total width of the epithelial slits playing an important role in providing a porous theory that controls hydraulic conductivity and water flow [9, 20]. The present study provides ultrastructural confirmation, indicating that the passage of solutes is possibly affected by glomerular haemodynamics under normal blood flow.

It has been generally accepted that kidney morphology and function are dependent on maintenance of normal blood pressure [12, 20]. Stopping the blood flow into the kidneys resulted in notable changes in glomeruli, most of which were probably due to rapid loss of the fluid volume after interruption of the blood supply. Under normal blood flow, structural pores in the basement membrane may be too small to allow proteins to penetrate [29]. Dilation of the pore sizes may occur as the blood flow stops by loosening a compact network in the basement membrane. Normal haemodynamic conditions are definitely required for optimal retention of protein molecules [26, 29, 30] and as shown in the present study with a specially designed cryoknife, cryofixation in situ has helped to resolve the morphological problem. In addition to the structural permeability of the glomerular capillary wall, renal haemodynamic factors may also affect the molecular sieving of the ultrafiltration unit, as reportd before [2, 3, 18, 26, 29, 30].

The morphological dependence of kidneys on an intact blood supply has been demonstrated by the present study in which glomeruli in living states were observed before and after ligation of the aorta. The ultrastructural changes can be explained, in a large part, by haemodynamic factors. The greater hydrostatic pressure at the glomerular level leads to an acute increase in filtration rate. It has been reported that some changes in glomerular passage are due to the consequence of hormonal alteration in the permeability of the capillary wall [2, 3, 26]. However, with ligation of the aorta, the glomerular filtration rate may temporarily increase, depending on the acute changes of the renal blood flow. The increase in width of filtration slits probably reflects an increase of the collective slit areas, which represent the exit pathway for the glomerular filtrate [20]. It is concluded that the transcapillary passage of materials is dependent on haemodynamic factors and also structural changes in the capillary loop.

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