Nephrol Dial Transplant (1999) 14: 1982–1990

Nephrology
Dialysis
Transplantation

Original Article

The kidney during hibernation and arousal from hibernation. A natural model of organ preservation during cold ischaemia and reperfusion

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Abstract

Background. During hibernation the kidney is in a hypothermic condition where renal blood flow is minimal and urine production is much reduced. Periodical arousal from hibernation is associated with kidney reperfusion at increasing body temperature, and restored urine production rate.

Methods. To assess the degree of structural preservation during such extreme conditions, the kidney cortex was investigated by means of electron microscopy in the dormouse *Muscardinus avellanarius* during winter hibernation, arousal from hibernation and the summer active period.

Results. Results show that the fine structure of the kidney cortex is well preserved during hibernation. In the renal corpuscle, a sign of slight lesion was the focal presence of oedematous endothelial cells and/or podocytes. Proximal convoluted tubule cells showed fully preserved ultrastructure and polarity, and hypertrophic apical endocytic apparatus. Structural changes were associated with increased plasma electrolytes, creatinine and urea nitrogen, and proteinuria. During the process of arousal the fine structure of the kidney cortex was also well maintained.

Conclusion. These results demonstrate that dormice are able to fully preserve kidney cortex structure under extreme conditions resembling e.g. severe ischaemia or hypothermic organ storage for transplantation, and reperfusion. Elucidation of the mechanisms involved in such a natural model of organ preservation could be relevant to human medicine.

Key words: hibernation; kidney; preservation; rodent; ultrastructure

Introduction

Structural integrity of the kidney is a pre-requisite for proper function, and preservation of renal morphology is crucial in a number of clinically relevant conditions such as renal ischaemia and organ storage for transplantation. Reduced blood flow to the kidney, and reperfusion are two key factors affecting renal integrity in these conditions. Under suitable conditions, hibernating animals are able to lower body temperature close to 0°C and they are able to reduce body functions to a minimum. Accordingly, hibernating animals undergo profound seasonal changes of renal function [1] involving drastic reduction of urine production. During periodical arousal, hibernating animals show transient increase of body temperature and restore normal urine production. Therefore, hibernating animals could represent an interesting natural model for investigation of kidney structure and function under conditions of cold ischaemia and reperfusion.

The present work analyses the ultrastructure of the kidney cortex in a true hibernator of small size, the dormouse *Muscardinus* avellanarius, during hibernation at low temperature, arousal from hibernation, and full activity (euthermia). Our aim was to define, in a mammal, the degree of structural preservation associated with sustained 'physiological' hypothermic ischaemia and subsequent reperfusion at increasing body temperature.

Subjects and methods

Muscardinus avellanarius is protected by the law, therefore only a limited number of dormice was made available for the purpose of multiple morphological investigations upon permission from the local authorities. Dormice ($n=9$) weighing 20–25 g were maintained in an external animal house as previously described [2]. They were sacrificed by decapitation during spontaneous hibernation in January ($n=3$, body temperature range 6–11°C), arousal induced by daylight exposure in March ($n=3$, body temperature about 26°C), and under light ether anaesthesia in the euthermic state in June ($n=3$). The limited number of available animals prevented investigation of other relevant periods, e.g. the pre-hibernation or the entry-into-hibernation phase as well as morphometric, quantitative analysis of the kidney.

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Urine samples (500–1000 µl) were obtained by puncture from the bladder in five dormice (two hibernating, three euthermic). In the case of euthermic dormice, urine spontaneously emitted by animals before sacrifice was pooled with the bladder urine where needed. Mixed blood samples were obtained immediately after decapitation by collecting blood dripping from the trunk. Electrolyte concentrations were measured by an ion-selective electrode technique. Other blood and urine analyses were performed utilizing commercially available kits with an automated multiparametric analyser (BM/Hitachi 917, Boehringer Mannheim, Milan, Italy).

Kidneys were rapidly removed from all dormice after sacrifice, cut in small fragments with a razor blade and immersion-fixed for 1.5 h in 2% glutaraldehyde in 0.1 M cacodylate buffer at 4°C, pH 7.4. Post-fixation was done in 1% osmium tetroxide and 1% potassium ferricyanide (final concentrations) in distilled water for 1 h at 4°C. Tissue fragments were then dehydrated through ascending concentrations of acetone and embedded in a mixture of epon and Araldite. Toluidine-blue-stained semithin sections were used for selection of cortical areas of interest and ultrathin sections were obtained on a Reichert Ultracut E ultramicrotome. Ultrathin sections were stained with lead citrate and viewed and photographed in a Zeiss EM 10 electron microscope operated at 60–80 kV.

Results

In all the hibernating and arousing dormice the urinary bladder was extremely dilated with urine and both kidneys appeared of normal size in comparison with euthermic individuals.

Urine and blood analyses

The results of these are shown in Table 1. The plasma concentrations of electrolytes were obviously higher in hibernating than in euthermic dormice. Similarly, the concentrations of creatinine and urea nitrogen were twofold and fourfold respectively, in hibernating individuals. In the urine of hibernating dormice, pH values were lower and specific gravity was higher than in euthermic. The urine of hibernating dormice was characterized by the appearance of proteinuria, which was not detectable in euthermic individuals.

Table 1. Blood and urine analyses in euthermic ($n=3$) and hibernating ($n=2$) dormice

	Euthermic	Hibernating
Blood		
Urea nitrogen (mmol/l) of urea	7.5 ± 1.03	25.9 ± 6.63
Glucose (mmol/l)	6.8 ± 1.15	7.5 ± 0.44
Sodium (mmol/l)	528 ± 97.7	934 ± 4.4
Potassium (mmol/l)	4.8 ± 1.19	39.7 ± 1.27
Chloride (mmol/l)	508 ± 83.2	925 ± 19.8
Creatinine (mmol/l)	26.5 ± 7.96	53.0 ± 15.91
Urine		
pH	8.0 ± 1.73	6.5 ± 0.71
Specific gravity	1011.6 ± 2.89	1022.5 ± 3.54
Protein	–[a]	+++[b]

Means ± SEM.
[a] not detectable.
[b] > 300 mg/dl.

The renal corpuscle (Figures 1–6)

In active, euthermic dormice (Figure 1), the renal corpuscle showed the usual structural components of the mammalian kidney. Glomerular vessels contained variable amount of blood and showed a flat, fenestrated endothelium. Pedicels of podocytes contacted the glomerular basal lamina (BL) and were separated by filtration slits. The BL comprised an internal and external lamina rara and a lamina densa in between. The average thickness of BL was 1500 nm.

In dormice hibernating at low temperature, the ultrastructure of most renal corpuscles was quite similar to that of euthermic animals (Figure 2). Focally, endothelial cells of glomerular capillaries were enlarged with abundant, electron-lucent perinuclear cytoplasm (Figure 3). In these corpuscles, podocytes were prominent, due to different degrees of cell swelling. In these cells (Figure 5) the Golgi apparatus was conspicuous, sometimes multiple, and it was surrounded by many small vesicles. Vesicles were also found scattered in the cell. Pedicels of podocytes were regularly spaced along the BL. The BL was continuous and appeared of regular texture and thickness (Figure 6). However, areas of BL wrinkling could be found. Mitochondria were of regular shape and size. The cytoskeleton was obvious and consisted of bundles of filaments and tubules. In renal corpuscles of hibernating dormice the capsular space was usually closed.

In arousing dormice (body temperature about 26°C), capillary loops in a large number of glomeruli were dilated and engorged with blood. Here, endothelial cells were often swollen, with a clear 'empty' cytoplasm. The urinary and capsular spaces were well appreciable. Podocytes were often frankly oedematous with a clear cytoplasm and the usual complement of organelles. The ultrastructure of the renal ultrafilter was regular. In many other instances, the ultrastructure of podocytes resembled the euthermic (Figure 4).

The renal tubules (Figures 7–10)

In active dormice (not shown), proximal tubule cells showed prominent microvilli and an obvious apical endocytic apparatus; the basal labyrinth was well developed and mitochondria had a few electron-dense granules. In the distal convoluted tubule, epithelial cells were provided with short microvilli and prominent endoplasmic reticulum. Dilated cisternae of the endoplasmic reticulum containing floccular material were a regular finding in many of these cells (Figure 10, inset).

In hibernating dormice (Figure 7), cells of the proximal convoluted tubules were regularly provided with packed, long microvilli occupying the tubular lumen. At the base of microvilli an impressive endocytic apparatus (Figure 7, inset) consisting of coated pits, coated vesicles, apical dense tubules and endosomes was found together with large clear vesicles and dense

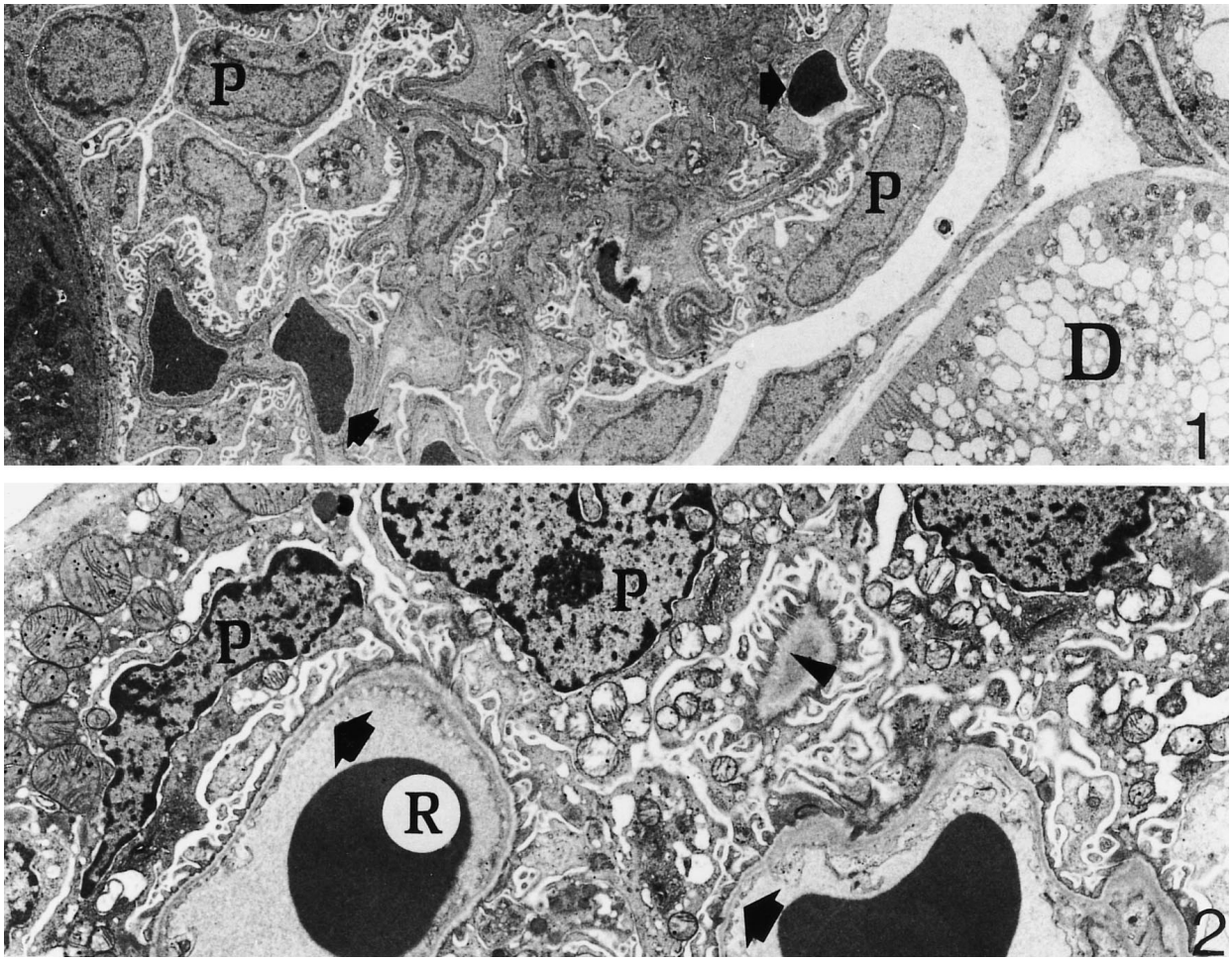

Fig. 1. Renal corpuscle of an euthermic, active dormouse. Note podocytes (P) and glomerular capillary vessels (arrows). Part of a distal convoluted tubule (D) and a proximal convoluted tubule (left bottom corner) are also shown $\times 3000$.
Fig. 2. Renal corpuscle of a dormouse hibernating at a body temperature of about 10°C. The ultrastructure of podocytes (P) and fenestrated endothelial vessels (arrows) is very well preserved. Arrowhead: regularly spaced pedicels of podocytes reach the basal lamina on a tangentially sectioned capillary vessel. (R) red blood cell $\times 6300$.

bodies. A tall membranous labyrinth (Figure 8) containing many oval to elongated mitochondria was found in the basal portion of the tubular epithelial cell. Mitochondria had homogeneously dense internal matrix with electron-dense granules. Distal convoluted tubule cells (Figure 10) showed very short microvilli and a modest basal labyrinth. In many cells, the cytoplasm was mainly occupied by cisternae of the endoplasmic reticulum filled with flocculent material.

In arousing dormice, epithelial cells of the proximal convoluted tubules (Figure 9) showed a well-developed apical endocytic apparatus albeit less prominent than in hibernating dormice. Mitochondria had shorter cristae, a clearer internal matrix and fewer electron-dense granules. In the distal convoluted tubules the endoplasmic reticulum showed fewer dilated cisternae (not shown).

Discussion

Hibernation is a complex condition involving dramatic changes in the function of organ systems and individual tissues. During hibernation, body temperature decreases close to ambient temperature, body functions are reduced to a minimum, metabolism is depressed, and animals enter a lethargic state lasting days or weeks (for a recent review of metabolic and biochemical adaptation during dormant state, see Ref. [3]). The mechanisms responsible for mammalian hibernation have not been fully elucidated. Hibernation is a seasonal torpor involving neurophysiological and neuro-endocrine adaptation leading to 'the coordinated reduction of the activity state of a specific subset of key regulatory enzymes or proteins in the cell and the activities of the processes that they control' [3]. In general, entry in (and maintenance of) the depressed metabolic state does not require extensive gene expression, albeit structural changes in nuclear constituent are found in hibernation [4–6]. As far as the renal function during hibernation is concerned, it has been shown in some of the smaller hibernators that urine production is much reduced, in association with striking reduction of renal blood flow, reduction or cessation of the glomerular filtration rate [7] and reduction

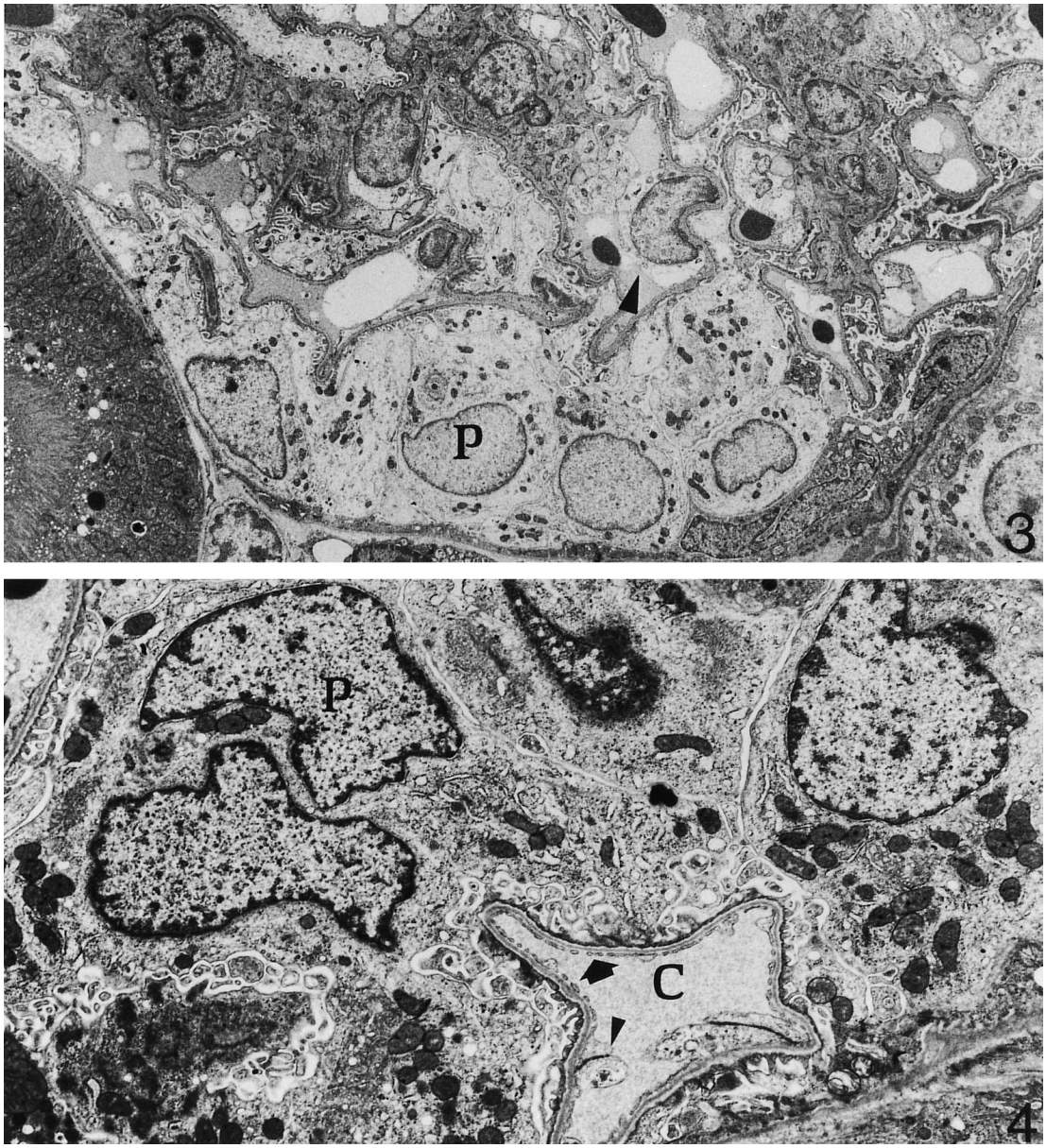

Fig. 3. Renal corpuscle of a hibernating dormouse. Some podocytes (P) are oedematous as well as capillary endothelial cells (arrowhead). However, the general ultrastructure of renal corpuscle cells is well-maintained. Bottom left corner: portion of a proximal convoluted tubule $\times 3000$.

Fig. 4. Renal corpuscle of an arousing dormouse. Both podocytes (P) and a capillary vessel (C) show very good ultrastructural preservation. Arrow: fenestrations of endothelial cell. Arrowhead: portion of endothelial cell blebbing in the capillary lumen $\times 6300$.

of the free water reabsorption rate ($^{TC}H_2O$) [1]. These changes are similar to those induced by hypothermia in non-hibernating mammals [1]. However, renal blood flow can continue during hibernation, albeit greatly reduced [8]. During periodical arousal, hibernating animals show transient increase of body temperature and restore normal blood flow to the kidney, glomerular filtration rate, and urine production [9]. In the present study we investigated the kidney cortex of the dormouse *Muscardinus avellanarius* during hibernation and arousal from hibernation by means of transmission electron microscopy. Results were compared with findings in fully active, euthermic individuals. The following points were demonstrated:

(i) The ultrastructure of the kidney cortex is well preserved during both hibernation (i.e. a condition where renal blood flow and glomerular filtration rate are permanently reduced) and arousal from hibernation (i.e. a period of rapidly resuming body temperature and renal blood flow).

(ii) The most apparent ultrastructural changes during hibernation take place in glomerular endothelial cells and podocytes. Changes extend to a larger number of such cells during arousal.

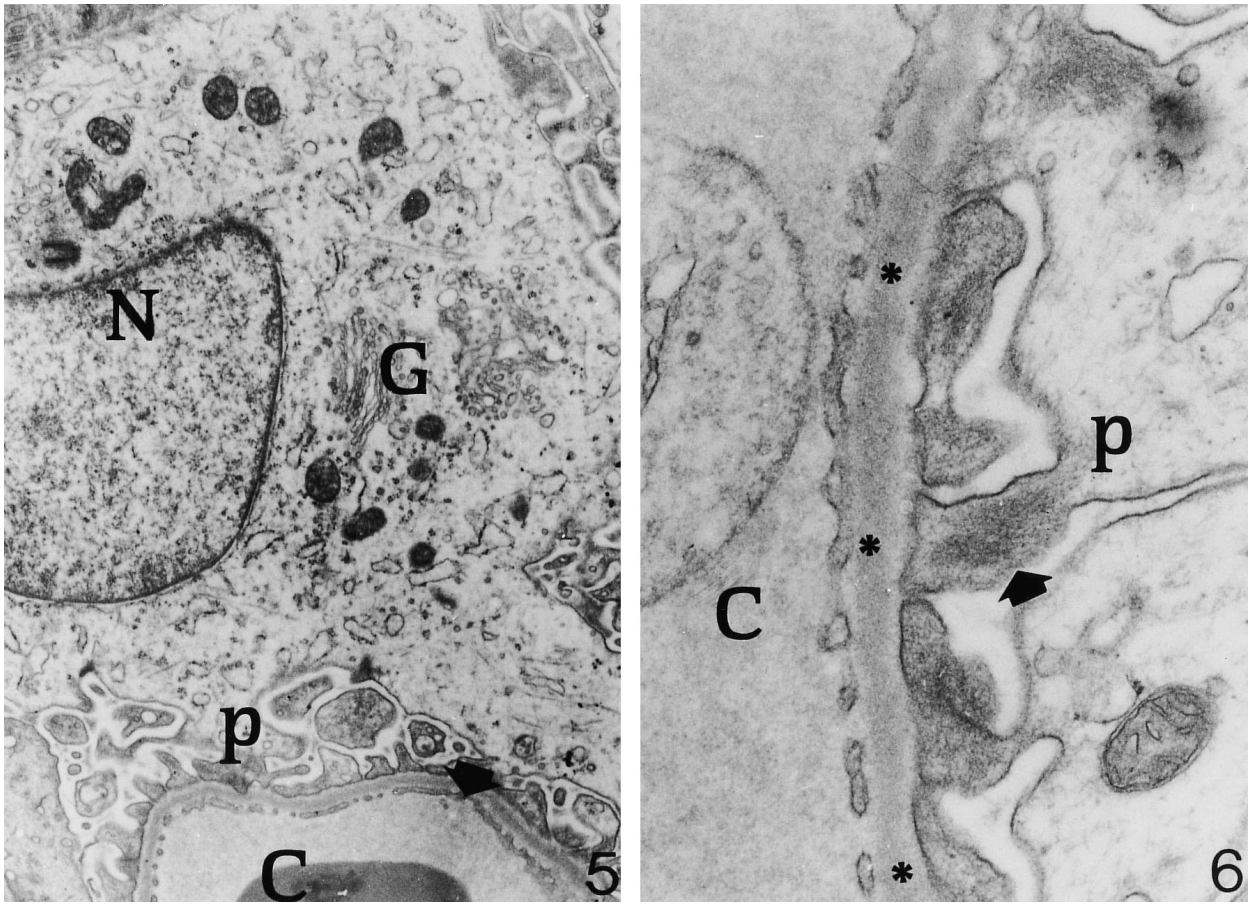

Fig. 5. Renal corpuscle of a hibernating dormouse. The fine structure of an oedematous podocyte is shown. Note the well-organized Golgi apparatus (G) and pedicels (p). N, nucleus of podocyte; C, capillary vessel; arrow, fenestration of endothelial cell ×11 700.
Fig. 6. Renal corpuscle of a hibernating dormouse. At high magnification the renal ultrafilter appears to be morphologically intact. C, glomerular capillary vessel. p, pedicel of podocyte containing packed filaments (arrow). Asterisks: basal lamina ×41 400.

(iii) In the proximal tubule, the apical reabsorbing zone of epithelial cells is activated during hibernation, in association with proteinuria.

Measurement of GFR or urine output were not performed in this study; however, the regular finding of urine-filled bladder in hibernating and arousing dormice [see also Ref. 10] strongly suggests persistent, albeit greatly reduced, urine production in the kidney during hibernation. Accordingly, blood analyses (Table 1) showed marked increase of the plasma concentrations of electrolytes, urea nitrogen and creatinine in hibernating animals. Despite such functional changes, electron microscopy showed that the general organization and fine structure of the renal ultrafilter is preserved during hibernation. Specifically, both the BL and the pedicels/filtration slits complex appear to be essentially unaffected during hibernation. Earlier studies in different species showed, in osmium or formaldehyde/osmium fixed material, that hibernation was associated with BL thickening [11] or irreversible structural and permeability changes [12] similar to those found in proteinuria or protamine sulphate administration [13]. Probably, improved fixation procedures explain the above discrepancy. A reduction of the endothelial pore number and epithelial filtration slits during hibernation have been consistently reported both qualitatively [11] and quantitatively [14] in different species. This would imply a reduced permeability of the ultrafilter. Due to the limited number of available animals, we did not perform morphometric analyses of our material but, from a qualitative point of view, the density of endothelial pores was found to be similar in hibernating and euthermic dormice. However, any quantitative evaluation of endothelial pores should be considered with caution because of their uneven distribution along the capillary endothelial cell [15]. Qualitatively, a tendency of pedicel number to increase was appreciable in hibernating dormice; this could be an adaptive response to reduced glomerular filtration rate. Podocytes often showed a certain degree of oedema during hibernation, which was more frequent in arousing dormice. This could be related to reduced metabolic activity of these cell, associated with entry of sodium and leakage of potassium; chloride would then enter the cell and an isotonic gain of water occurs. During arousal, podocyte oedema is even more

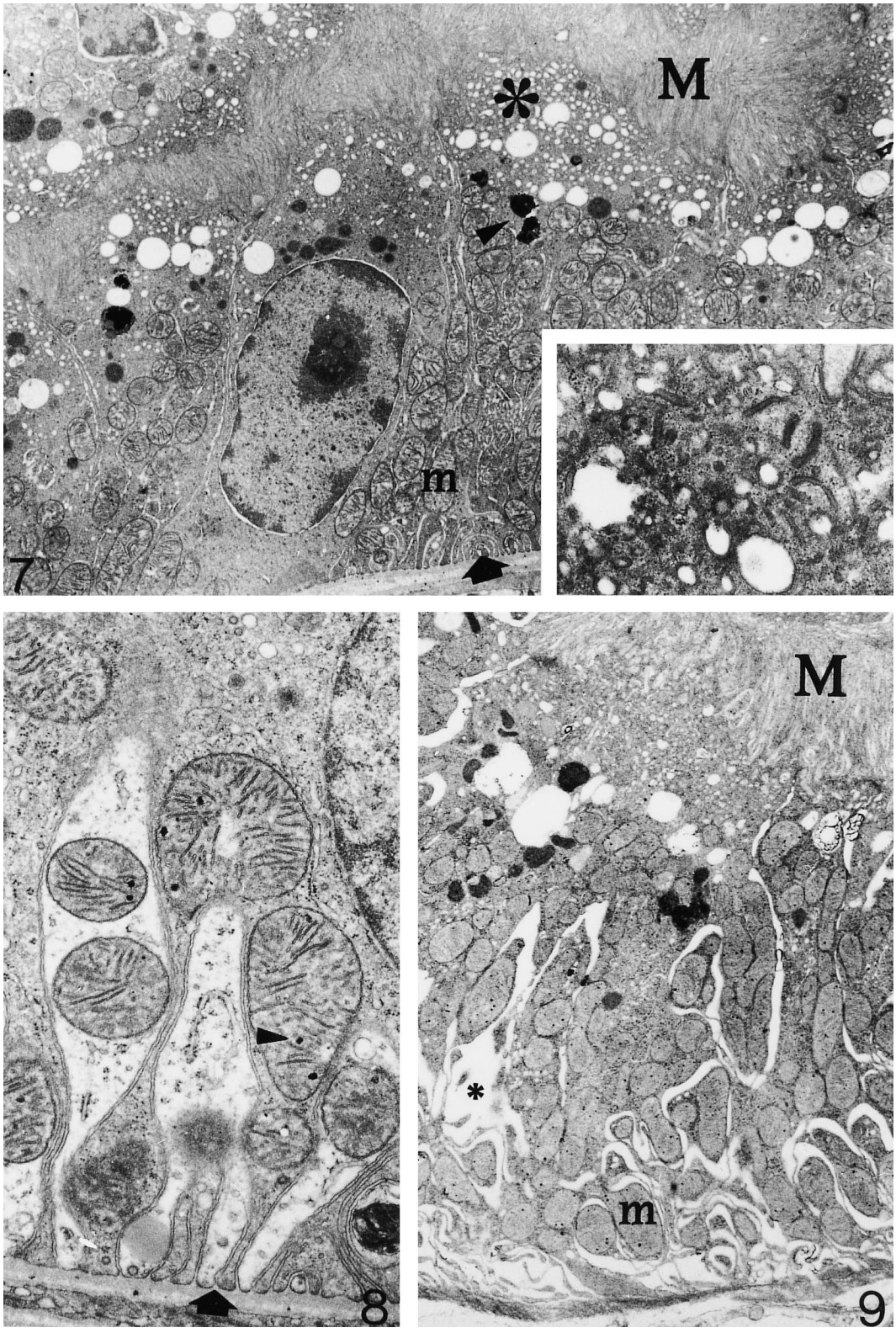

Fig. 7. Hibernating dormouse. Portion of a proximal convoluted tubule showing a fully preserved ultrastructure as well as an impressive apical endocytic apparatus (asterisk). M, long, packed microvilli in the tubular lumen; m, mitochondria; arrow, basal labyrinth; arrowhead, lysosome-like structures. Inset, detail of the apical endocytic apparatus $\times 7500$, $\times 75\,000$.

Fig. 8. Hibernating dormouse. Basal labyrinth in a proximal convoluted tubule cell. Arrow, infolding of plasma membrane; arrowhead, electron-dense granule in the inner mitochondrial matrix $\times 20\,000$.

Fig. 9. Arousing dormouse. Epithelial cells of the proximal convoluted tubule are morphologically well preserved. Note the slightly dilated intercellular space among folds of the basal labyrinth (asterisk). M, long, packed microvilli in the tubular lumen; m, mitochondria containing many electron-dense granules $\times 6300$.

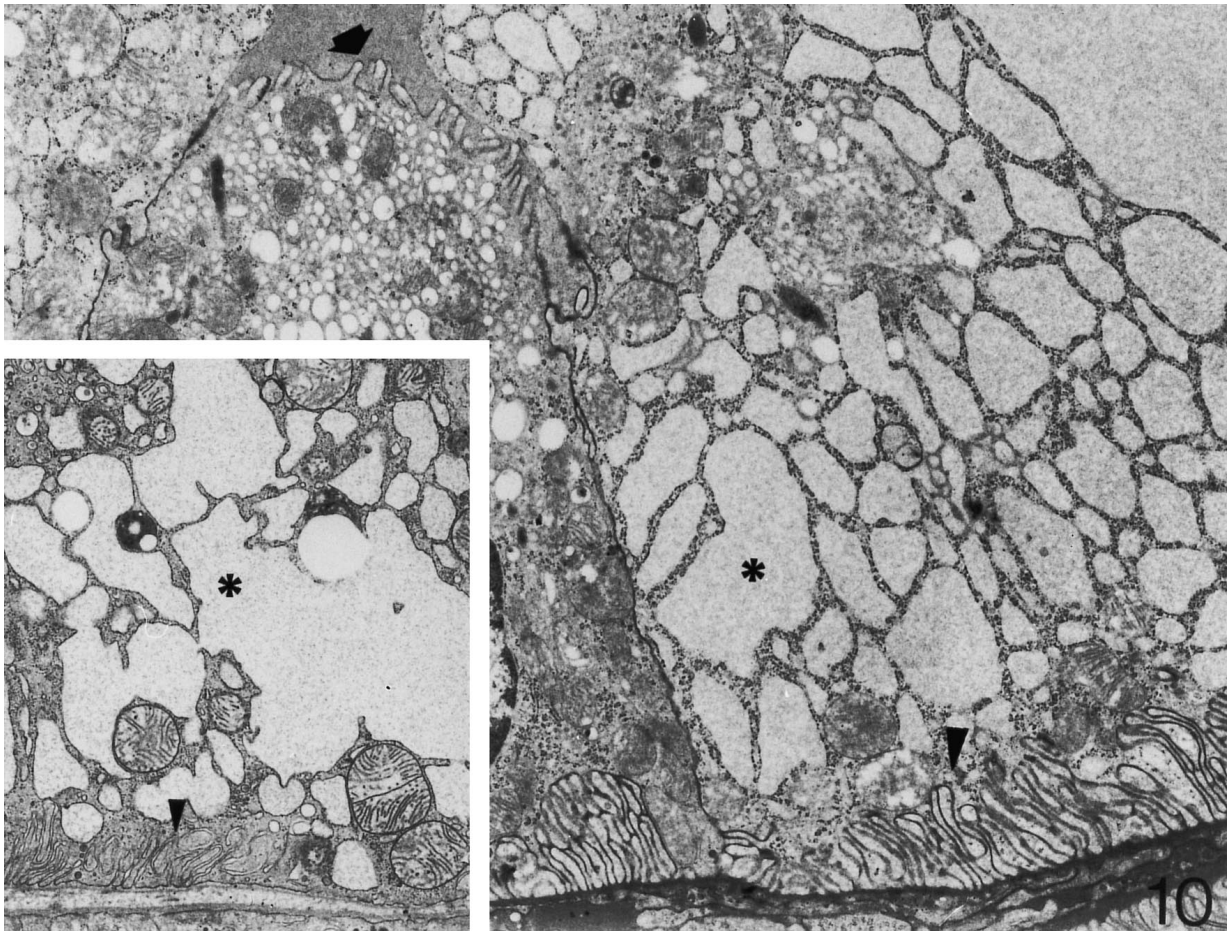

Fig. 10. Distal convoluted tubule of hibernating dormouse. Many epithelial cells show dilated cisternae of the endoplasmic reticulum (asterisk) and a small basal labyrinth (arrow head). This pattern is quite similar to the euthermic condition (inset). Arrow, short microvilli projecting in the tubular lumen $\times 8000$, $\times 8000$.

appreciable, possibly in association with the increasing blood flow in the presence of still metabolically depressed podocytes.

On the basis of reduced arterial pressure and glomerular filtration rate found in hibernation, it could be inferred that morphological changes in proximal tubules of hibernating dormice would be striking. In, for example, experimental ischaemic acute kidney failure early ultrastructural alterations of tubules are loss of microvilli and intense vacuolization [16]. Moreover, the kidney tubule is a major site of damage during ischaemia associated with organ storage for transplantation as well as following reperfusion in the host [17] and continuing efforts are made to improve the structural preservation of organs procured for transplantation since functional dependence is placed on the preserved kidney after transplant. Renal tubules in the cortex of hibernating dormice were exposed for days to very low blood flux in deep hypothermia and showed excellent preservation in comparison with euthermic individuals. Interestingly, disruption of microfilaments as well as fragmentation of microtubules occurring in epithelial cells of the proximal tubule during ischaemia [18,19] did not apparently take place in hibernating dormice. These changes contribute to loss of cell polarity and altered function in experimental renal ischaemia. Instead, tubular cells of hibernating dormice show a fully preserved brush border and apical endocytic apparatus, thereby indicating that they possess a preserved cytoskeleton. Further, the more evident ultrastructural change in proximal tubule cells of hibernating dormice was a well organized, expanded endocytic apparatus. This finding suggests that epithelial cells are able to increase reabsorption during hibernation, possibly in response to increased protein content of the tubular fluid. In accordance with this hypothesis, marked proteinuria was found in hibernating dormice (Table 1), which was not detectable in euthermic individuals. Increase of protein in the tubular fluid could be a consequence of the slow blood flow in the glomerulus, which would allow in turn a longer filtration time. The reported changes in proximal tubule morphology during hibernation are generally consistent with findings in bats [20], *Eliomys quercinus* [21], and *Spermophilus lateralis* [14]. Therefore, a mechanism of increased reabsorption at the

proximal convoluted tubule could be active in many different species of hibernators.

From the above discussion, it appears that re-setting of kidney function at low systemic arterial pressure, GFR, and temperature only involve slight structural changes in the renal corpuscle and cortical tubules. The finding of only minor ultrastructural changes in the kidney of arousing dormice undergoing 'reperfusion' further suggest that the whole process is finely regulated to prevent lesion. Although renal ischaemia, kidney storage for transplantation, and hibernation are not strictly comparable, due for example to the presence of very slow, constant kidney blood flow in the latter [8], these conditions share features such as intracellular acidosis, hypothermia, and hypoxia. Therefore, understanding of the mechanisms involved in kidney preservation during hibernation could be relevant to human kidney pathology and transplantation. For example, kidney transplantation would be improved by both extending the time that kidneys may be stored and the quality of kidney structural preservation. Unfortunately, little is known about the specific biochemical and cellular mechanisms which allow maintenance and preservation of cell and organ structure in hibernation. It has been shown that erythrocytes have a greatly extended life span during the periods of low body temperature of hibernation [22]. In general, tissues and cells of hibernators are better able than those of non-hibernators to withstand long-term storage at low temperature, possibly because of their better regulation of cations [23,24]. At the organ level, the renin–angiotensin–aldosterone system seems to be functioning during hibernation in the presence of greatly reduced mean arterial pressure [25,14], together with increased urinary excretion of ADH [26]. Indirect support to this view came from the finding of a morphologically active glomerular zone (producing aldosterone upon stimulus of renin through angiotensin) in the adrenal gland of hibernating dormice [27]. In the last years there has been a dramatic explosion of knowledge on local and systemic factors influencing blood flow and renal tubule control mechanisms (e.g. atrial natriuretic factors, nitric oxide, prostaglandins, endothelins etc.). The relevance of such factors in the kidney of hibernating animals has not been assessed. Only a short report appeared in the literature showing decreased plasma concentrations of atrial natriuretic factor in hibernating marmots [28]. Therefore, there is clearly a need for new, integrated structural and biochemical investigation of the hibernating kidney.

Acknowledgements. CZ and MM were the recipients of a fellowship in the frame of the exchange programme between the Consiglio Nazionale delle Ricerche (Italy) and the Swiss National Science Foundation.

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Received for publication: 31.8.98
Accepted in revised form: 15.2.99