Histol Histopath (1986) 1: 139-146

Histology and Histopathology

Ultrastructure of the renal corpuscle of *Testudo graeca* (Chelonia). A comparison between hibernating and non-hibernating animals.

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Summary. The renal corpuscle of hibernating and non-hibernating *Testudo graeca* was studied by means of light and electron microscopy. Renal corpuscles are small and have a glomerular architecture similar to that found in other vertebrates with a limited glomerular filtration rate.

In hibernating animals, unlike non-hibernating, some morphological changes took place. The cells of the renal corpuscle were densely packed, podocytes and parietal cells showed a marked cytoplasmic vacuolization, there was a highly developed capillary basement membrane and the endothelial and mesangial cells showed abundant dense granules. These morphological features apparently correspond to a vacuolar degeneration. They may also be the morphological basis of the decrease in the glomerular filtration rate observed during this period.

Key words: Ultrastructure - Renal corpuscle Hibernation - *Testudo graeca*

Introduction

The reptilian kidney has mainly been considered in studies by classical histologists and physiologists (Gampert, 1866; Regaud and Policard, 1903; Huber, 1906; Bishop, 1959). More recently, several ultrastructural studies on the renal corpuscle have been published (Anderson, 1960; Roberts and Schmidt-Nielsen, 1966; Davis and Schmidt-Nielsen, 1967; Schmidt-Nielsen and Davis, 1968; Davis et al., 1976; Fernandez et al., 1978; Gabri and Butler, 1984; Solomon, 1985). However, possible morphological changes in this structure due to the hibernating period have not been considered.

Hibernation is a lethargic period which takes place when temperature falls and nutrients are scarce, the renal function decreasing 90% (Soria, 1985).

The aims of the present work are to study the renal corpuscle of *Testudo graeca* (Chelonia), a reptilian characterised by a terrestrian and Mediterranean climate, and the morphological modifications that take place during hibernation. A study of the renal morphology of this species has not previously been undertaken.

Materials and methods

Specimens of *Testudo graeca* were caught in August and February in their own habitat in Murcia (Spain). The animals were anesthetised by intraperitoneal injection of 3 mg of pentobarbital per 100 g of body weight. The kidneys of 20 turtles were exposed and immediately fixed. Samples for light microsocopic study were fixed in Bouin's solution, processed routinely and stained by H.E., P.A.S., Perl's and melanin (Masson Fontana) techniques. For electron microscopy, fixation was obtained by immersion for 2 h in 3.5% glutaraldehyde buffered at pH 7.2 - 7.4, postfixed for 1 h in 1% OsO_4, and embedded in Epon. Ultrathin sections, obtained using a LKB Ultratome III, were stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 10 C electron microscope.

Results

The renal corpuscle of *Testudo graeca* showed a similar structure to that found in mammals (Fig. 1). Each was composed of a Bowmann's capsule having an outer

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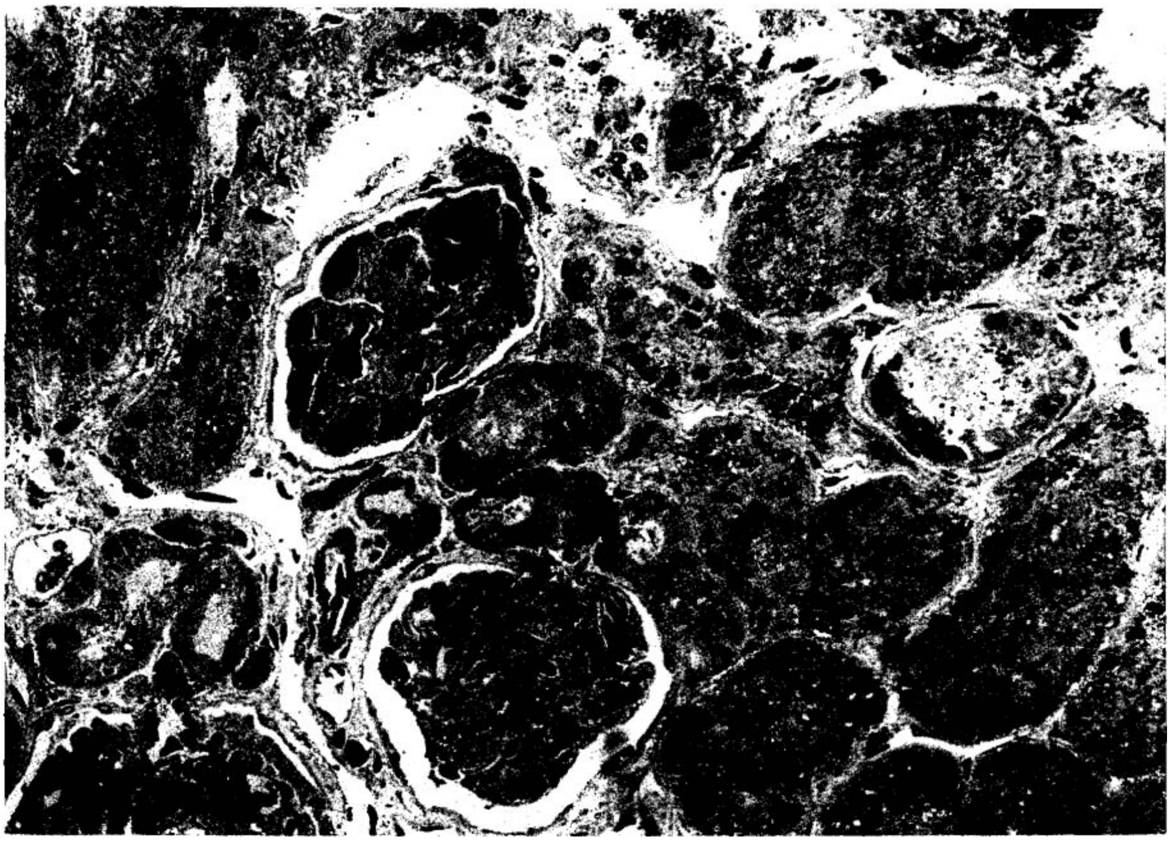


Fig. 1. Sections of renal corpuscles of non-hibernating *Testudo graeca* showing a similar structure to that found in mammals. x 312

parietal and an inner visceral layer, glomerulus and mesangium (Fig. 2).

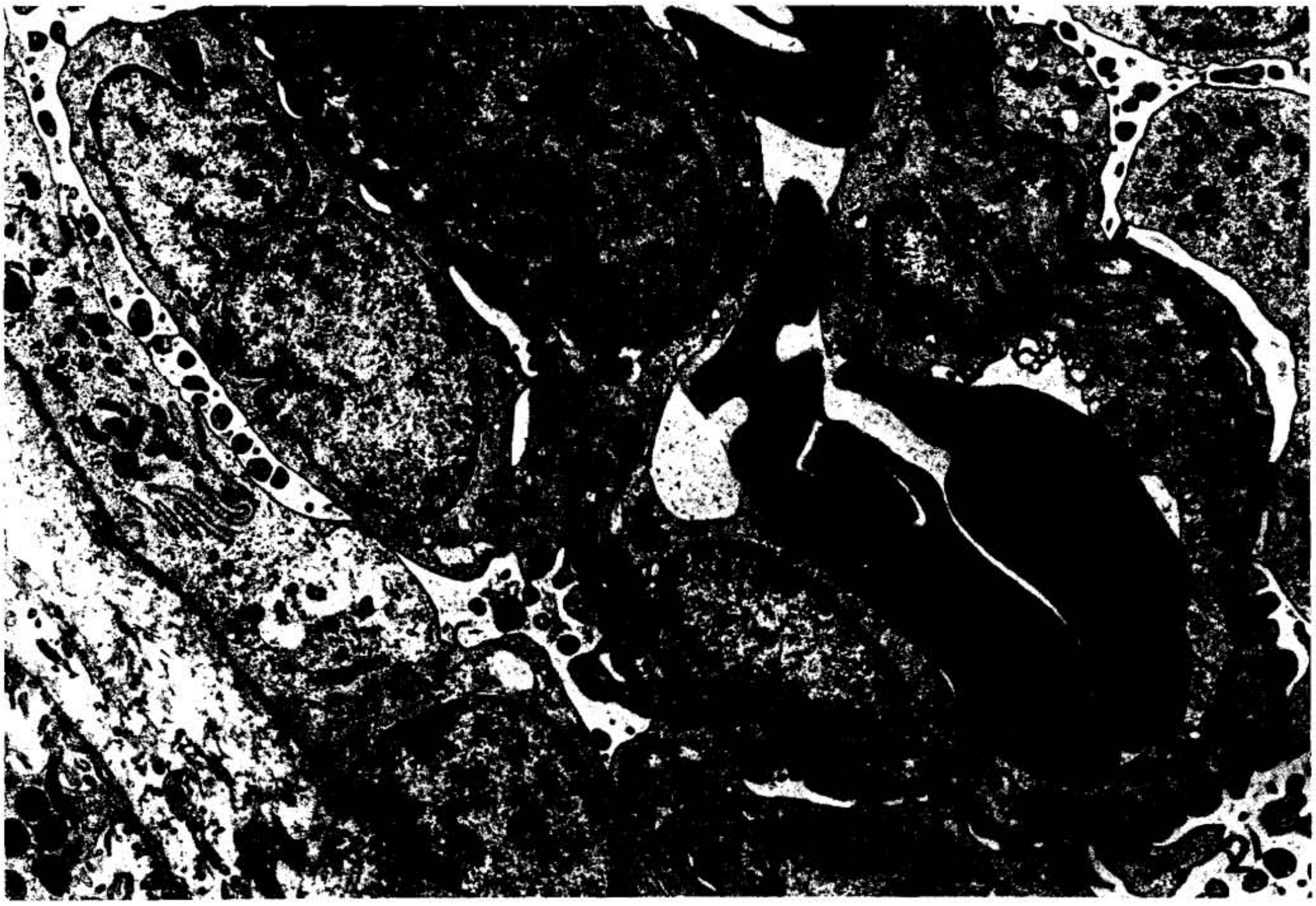
The parietal laver rested on the basement membrane which was, on average, 1.6 μ thick; parietal cells were flattened (Fig. 2). The lateral surface of adjacent cells were linked by deep interdigitations and tight junctions; a few short microvilli could also be seen on the surface. The elongated nuclei had a prominent nucleoli. Normal cell organelles were present throughout the cytoplasm (Fig. 2).

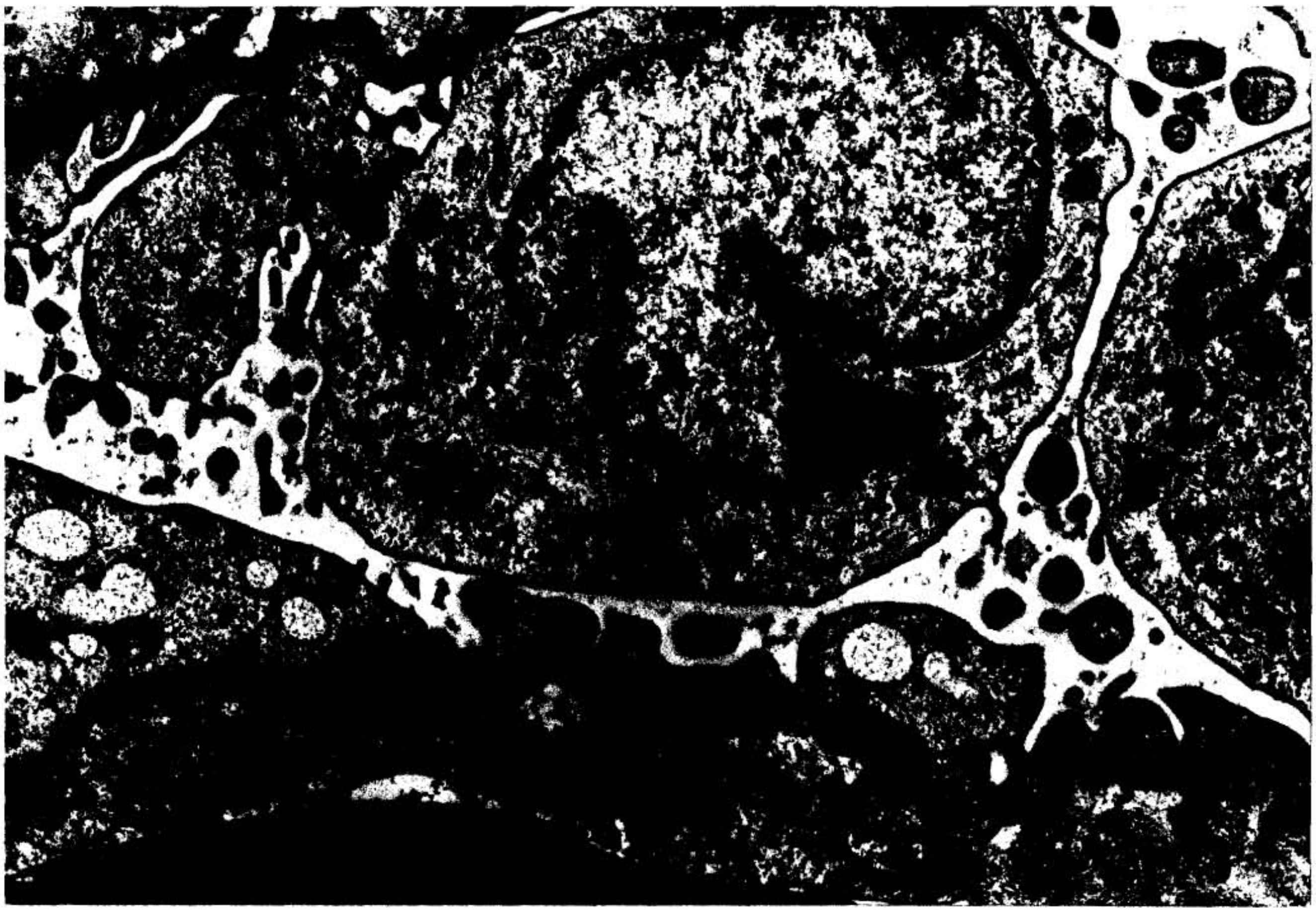
The visceral layer of Bowmann's capsule consisted of large epithelial cells having a large irregular-shaped nucleus. The cells were linked by tight junctions (Fig. 3). Free ribosomes, short cisternae of endoplasmic reticulum, Golgi apparatus and mitochondria could be observed in the cytoplasm; micropinocytotic vesicles were scarce and there were some multivesicular bodies (Fig. 3). Primary prolongations of the podocytes formed irregular pedicels; however, in some areas, neither primary prolongations nor pedicels were well-differentiated and the surface of the podocytes rested on the basement membrane. Filtration slits were not prominent. A diaphragm could occasionally be seen. Microvilli were observed in the podocytes towards the urinary space (Fig. 3).

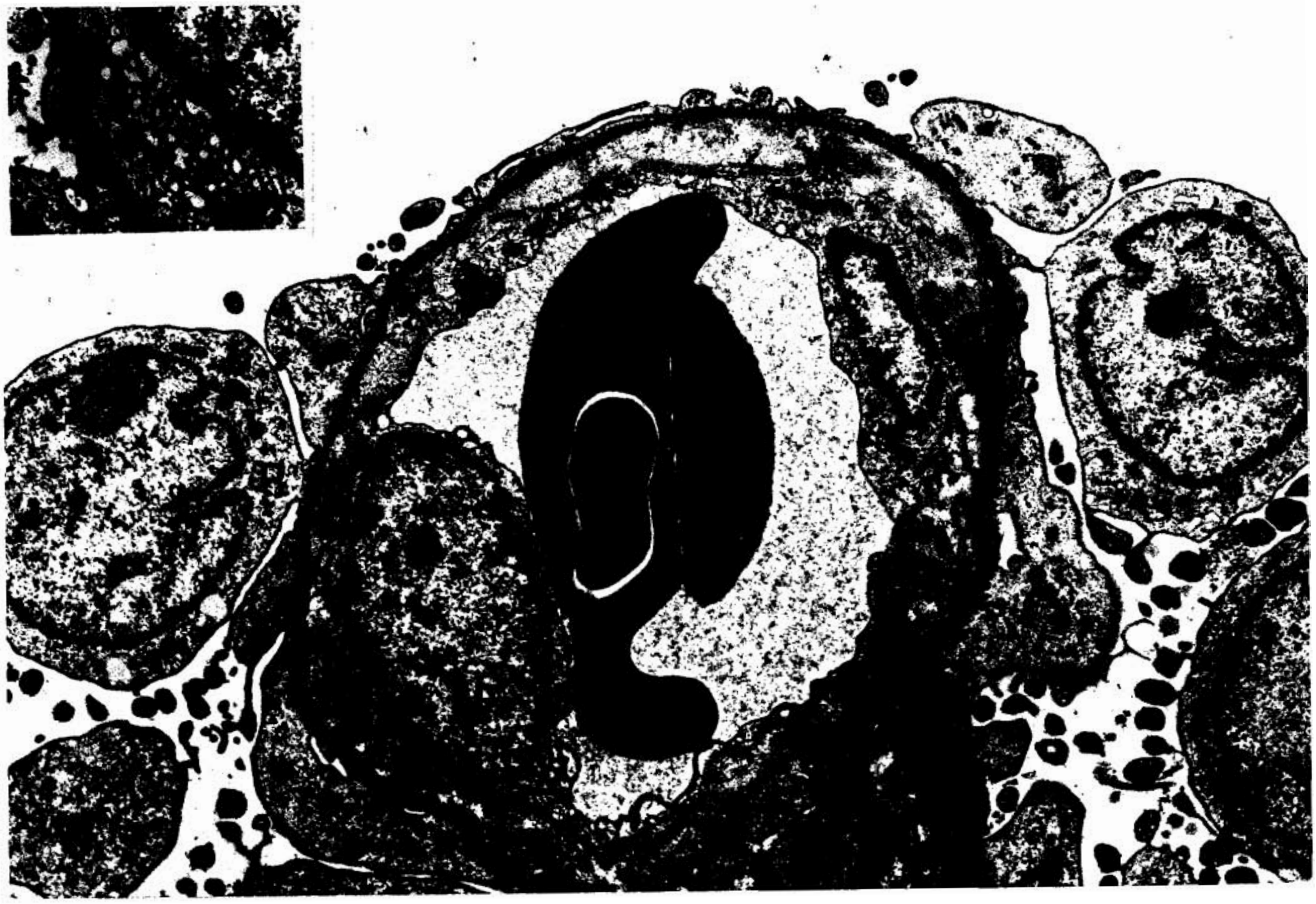
In all sections examined, the glomerulus consisted of a tuft of ten to twelve capillaries (Fig. I). The endothelial cells showed interdigitations, tight junctions and a large nucleus with a prominent nucleolus (Fig. 4). There were free ribosomes, short cisternae of rough endoplasmic reticulum, mitochondria, micropinocytotic vesicles and some dense bodies in the cytoplasm. The luminal surface was poorly fenestrated. A diaphragm could be observed

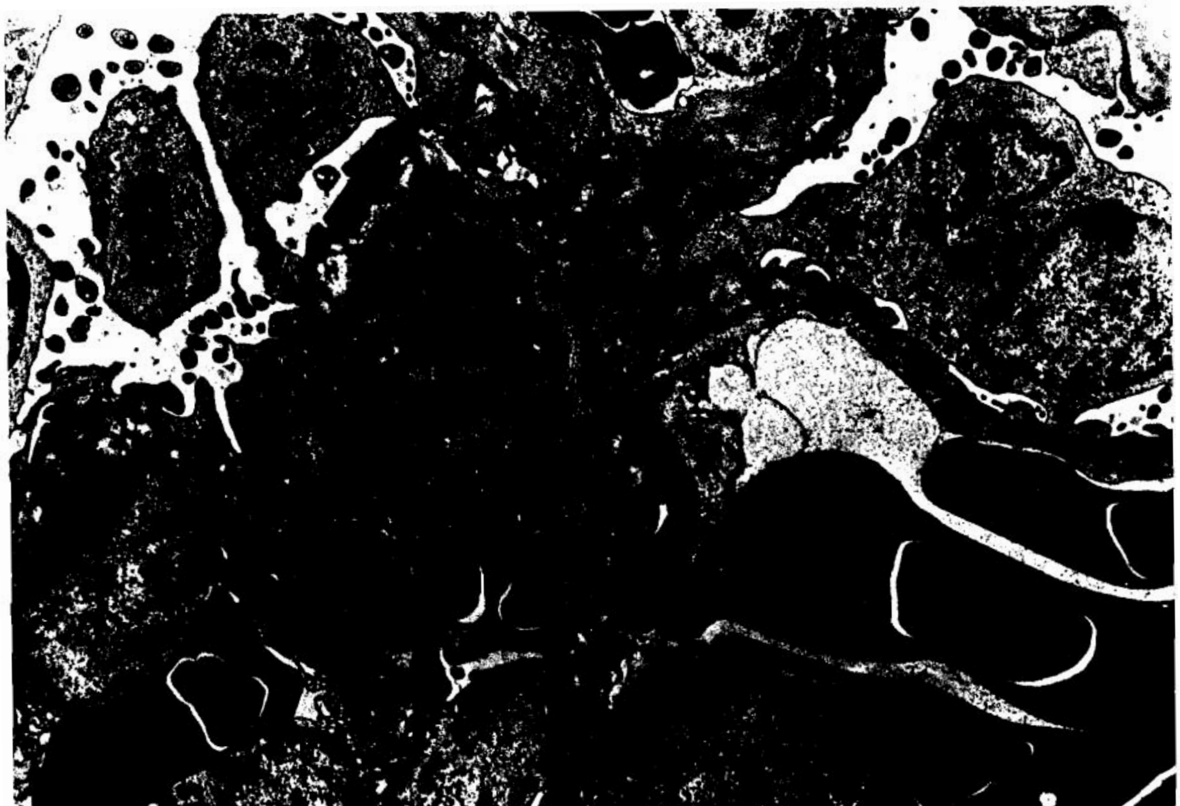
Fig. 2. Electron micrograph showing a portion of a renal corpuscle of non-hibernating *Testudo graeca.* Bowmann's capsule is composed of an outer parietal (P) and an inner visceral (V) epithelium which surrounds the glomerular capillaries lined with endothelial cells (E). x 6,250

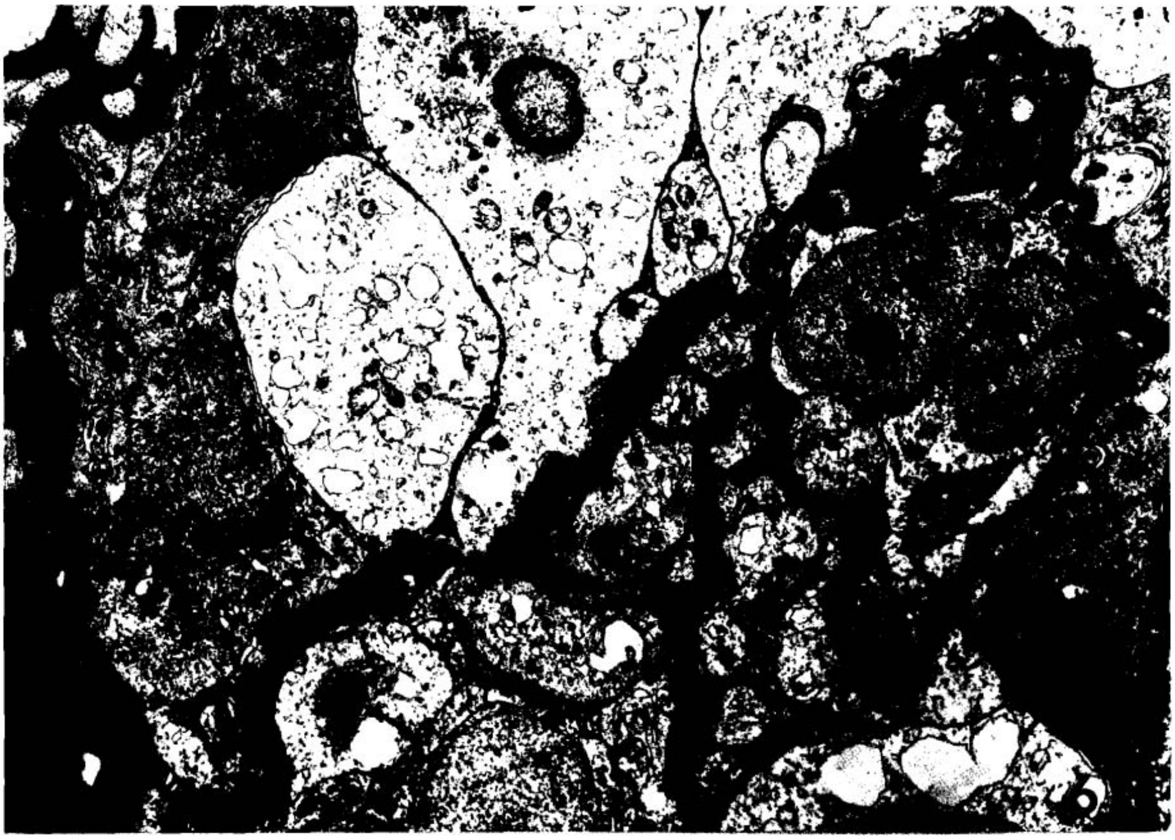
Fig. 3. Renal corpuscle of non-hibernating *Testudo graeca.* The podocytes linked by tight junctions (arrow)have irregular processes which rest on the capillary basement membrane. Microvilli arise from the cytoplasm to project into the urinary space. x 16,000

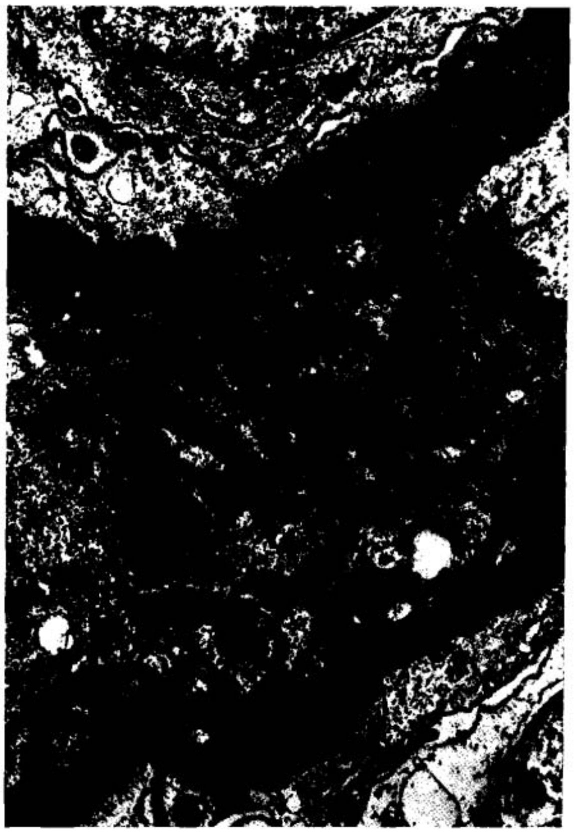












in the fenestrations. The cytoplasm had a reticular appearance in some areas (Fig. 4).

The capillary basement membrane had a trilaminar structure: lamina rara externa, lamina densa and lamina rara interna, the latter being characterised by its large size (Fig. 4).

There were abundant mesangial cells, in a dense mesangial matrix, between the glomerular capillary loops. These cells had an irregular morphology and a large indented nucleus. The cytoplasm was dense and contained abundant ribosomes, cisternae of rough endoplasmic reticulum, mitochondria and few lysosomes (Fig. 5).

Some morphological changes were observed in the renal corpuscle of hibernating *Testudo graeca.* The cells of the renal corpuscle were densely packed. The podocytes and parietal cells showed a marked cytoplasmic vacuolization (Fig. 6). The capillary basement membrane did not display three layers, as in active *Testudo graeca,* but appeared as a single, well developed, homogeneous layer (Figs. 6, 7a). Cytoplasm of the endothelial and mesangial cells showed abundant dense granules of varying shapes and sizes, these granules being intensely Perl positive and melanine negative (Fig. 7).

A characteristic cell type was found in the renal corpuscle of hibernating *Testudo graeca.* These cells had a large deeply indented nucleus and an electrodense cytoplasm containing mitochondria and abundant clear vesicles (Fig. 8).

Fig. 4. Transverse section of a glomerular capillary of non-hibernating *Testudo graeca* showing endothelial cells (E), basement membrane (B) and podocytes (P). Note a highly developed lamina rara interna in the basement membrane. Inset showing reticular appearance of the cytoplasm of an endothelial cell. x 8,500. Inset x 15,000

Fig. 5. Mesangial cells in the kidney of non-hibernating *Testudograeca.* The cells are included in a dense matrix. × 6,500

Fig. 6. Renal corpuscle of hibernating *Testudo graeca.* Note that the basement membrane is homogeneous and dense, and that some podocytes are vacuolised. × 6,500

Fig. 7. Renal corpuscle of hibernating *Testudo graeca.* Electron micrographs showing the cytoplasmic dense granules of the endothelial (a) and mesangial (b) cells. a × 8,000. b × 16,000

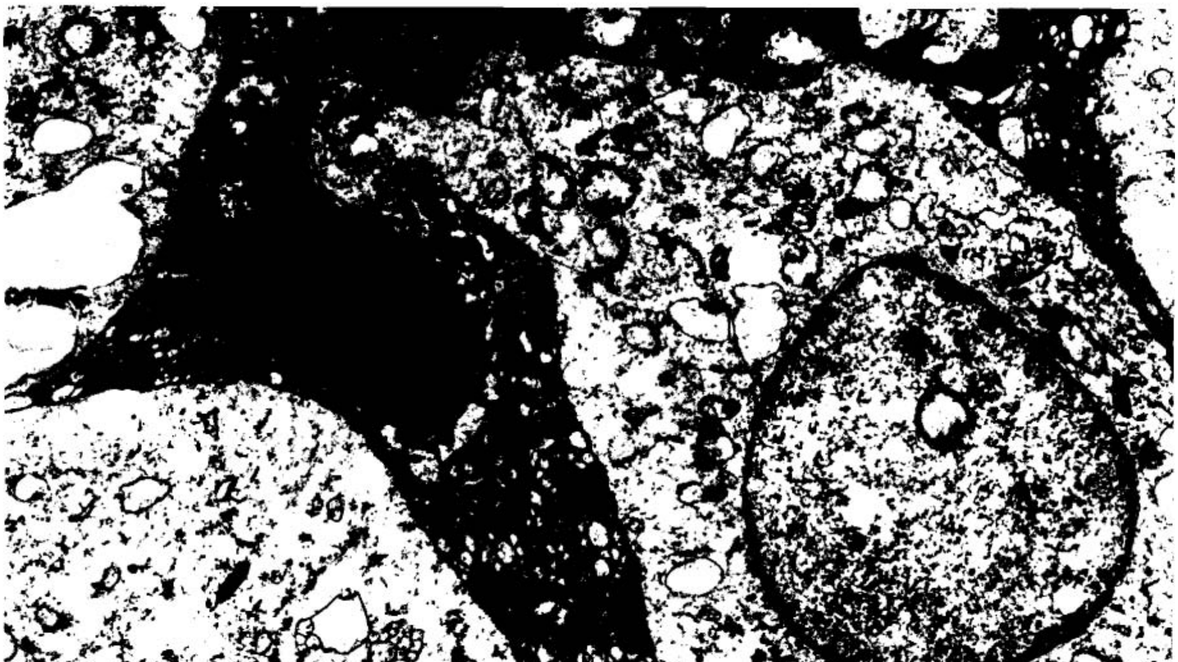


Fig. 8. Electron micrograph of the visceral layer of a renal corpuscle of hibernating *Testudo graeca* showing vacuolised podocytes and electrodense cells, with numerous clear vacuoles and mitochondria in the cytoplasm. x 9,000

Discussion

The renal corpuscles of *Testudo graeca,* like those of other reptiles, birds and some marine teleosts, are small compared to those of cyclostomes, elasmobranchs, fresh water teleosts, amphibian and mammals (Marshall, 1934; Anderson, 1960; Fernandez et al., 1978; Peek and McMillan, 1979; Zuasti et al., 1983; Gabri and Butler, 1984).

The small size of the renal corpuscle, a simple glomerular architecture and the ultrastructure of the endothelial cells may place a limitation on the glomerular filtration rate (Peek and McMillan, 1979). The endothelial glomerular cells of *Testudo graeca* show scarce fenestrations, similar to those described in some reptilian species (Anderson, 1960; Peek and McMillan, 1979). They are, however, in contrast to the finding of abundant fenestration without diaphragm in other reptilian species (Fernandez et al., 1978; Gabri and Butler, 1984). Occasionally, the glomerular endothelium has a reticular appearance similar to that found in other vertebrates (Yoshinari and Fujita, 1982; Kondo and Ushiki, 1985; Solomon, 1985). Kondo and Ushiki (1985) suggest that reticular appearance is a characteristic of the fenestrated endothelial cells and is not associated with pathological or degenerative changes.

Podocytes of *Testudo graeca* are large, have irregular pedicels and few fenestrae, occasionally having a diaphragm. These characteristics could be to limit the glomerular filtration rate (Peek and McMillan, 1979). Towards the urinary space the podocytes have abundant microvilli of unknown function. These podocytes do not show the bundles of microfilaments described in some species (Peek and McMillan, 1979). The mesangial cells of *Testudo graeca* are numerous, being situated in the axis of the renal corpuscle in a similar way to that described in other reptilian species (Davis et al., 1976; Fernandez et al., 1978; Peek and McMillan, 1979; Gabri and Butler, 1984; Soares and Fava de Moraes, 1984).

The hibernating *Testudo graeca* has abundant clear vesicles in the cytoplasm of podocytes and parietal layer cells which, apparently, correspond to a vacuolar degeneration. The capillary basement membrane appears as a single, homogeneous, dense layer. It is well-developed and could contribute to a decreased glomerular filtration rate during this period, as observed in hibernating squirrel (Zimny, 1968; Zimny and Rigamer, 1969).

An accumulation of dense granules of varying shapes and size can be observed in the cytoplasm of endothelial and mesangial cells in hibernating specimens of *Testudo graeca.* These granules are strongly Perl positive, thus indicating an iron-derivative content due to the increase in the activity of these cells during hibernation (Zimny and Levy, 1971).

The renal corpuscles of hibernating *Testudo graeca* have a few electrodense cells, similar to the dark cells described in the collecting tubules of other vertebrates (Griffith et al., 1968; Nicholson, 1981). It seems that these cells are involved in some forms of cyclic secretory activity, playing an important part in the secretion-reabsorption of potassium (Nicholson and Kendall, 1983).

Acknowledgements. We are grateful to Mrs. M. Garcia and Mrs. F. Calderon for technical assistance.

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Accepted December 27, 1985