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Ultrastructure of the kidney of the marine teleost *Sparus auratus*: The renal corpuscle and the tubular nephron

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Summary. The ultrastructure of the renal corpuscle and tubule of *Sparus auratus* is described. The parietal epithelium in Bowman's capsule is flattened with occasional cilia; podocytes are large with bundles of perinuclear microfilaments, a large vacuole and occasional cilia; a filtration slit membrane can sometimes be identified; mesangial cells are placed peripherally and among the walls of the capillaries. The neck segment is short and ciliated; it lacks the mucous cells which appear in some teleosts. The first proximal segment has columnar cells with a well developed brush border, and some cilia, large light vacuoles and many lysosomes appear in the apical zone; the second proximal segment has taller cells than the former, which appear with a less dense brush border, containing numerous multivesicular bodies; the third proximal segment, which has cells similar to the previous ones, possesses a less developed brush border and numerous mitochondria scattered all over the cytoplasm. No distal tubule is present. There is a collecting tubule with columnar cells with few microvilli and some apical mucin granules which empty into the collecting duct.

Key words: Kidney, teleost (*Sparus auratus*) – Ultrastructure – Renal corpuscle – Tubular nephron

Some authors have studied teleost nephrons by electron microscopy (Bulger and Trump 1968; Olsen 1970; Worsmann et al. 1971; Dobbs and de Uries 1975; Hentschel 1977; Ottosen 1978). These studies have demonstrated that the nephrons show an extensive diversity in their morphology, probably associated with the widely varied environments and different needs with respect to salt and water balance of the various species. The ultrastructure of the kidney of the marine teleost, *Sparus auratus*, was not been studied, and the aim of the present study was to examine and compare the nephron ultrastructure with that of other teleost species.

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Materials and methods

Specimens of *Sparus auratus* were caught in Mar Menor, Murcia (Spain), where the sea has a high salinity of about 44‰. Fish were maintained in recirculating salt water aquaria for about a day, then anaesthetized in salt water containing ME 222 (Sandoz AG, Basel). The kidneys of 50 fishes were fixed by immersion for 2 h with 3.5% glutaraldehyde buffered at pH 7.2–7.4 (Millonig 1961), postfixed for 1 h in 1% OsO_4, and embedded in Epon. Ultrathin sections obtained with a LKB Ultratome III, were stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963), and examined in an JEOL T-8 electron microscope.

Results

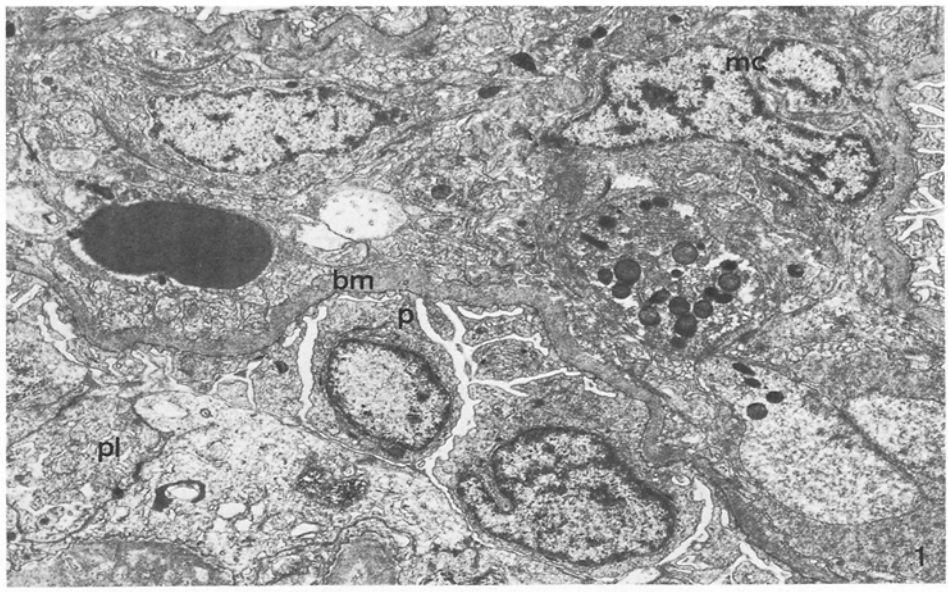
The nephron of *S. auratus* consists of a renal corpuscle, a neck, three proximal segments with basal labyrinth and brush border, and a collecting tubule which connects with the collecting duct.

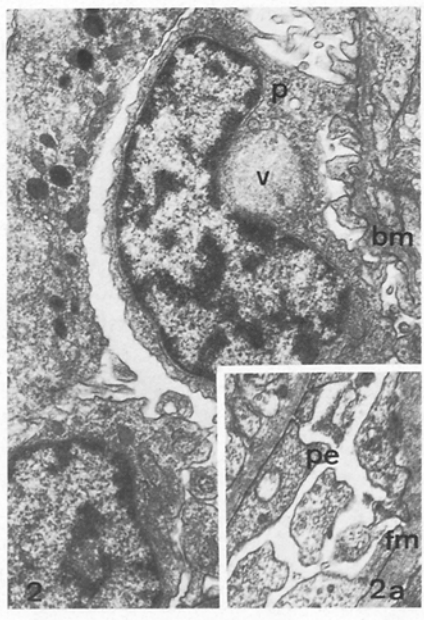
Renal corpuscle. The renal corpuscle consists of a parietal layer of Bowman's capsule, a visceral layer, Bowman's space between these two layers, capillary loops and an abundant mesangial area (Fig. 1). The parietal layer rests on a relatively thin basement membrane having a fine fibrillar structure. The parietal cells are flattened and increase in height near the urinary pole. Interdigitations and junctional complexes exist between the cells. The elongated nuclei have few shallow indentations. Occasional cilia can be found protruding from depressions in the surface. Few large and filamentous mitochondria are present containing a pale matrix and few cristae (Fig. 1). The Golgi apparatus is inconspicuous. Endoplasmic reticulum is scarce and there are few free ribosomes. The cytoplasm contains bundles of filaments peripherally near the plasma membrane.

Podocytes of the visceral layer appear as large irregular cells with several large processes, which form pedicels resting on the basement membrane of the capillaries (Fig. 2), or against which an extensive part of the surface of the podocytes sometimes lies flat. The pedicels are connected by a high filtration slit membrane (Fig. 2a). Adjacent podocytes are often connected by junctional complexes. The nuclei are rather large, sometimes showing an extensive invagination, and have homogeneous chromatin and a single nucleolus. Mitochondria are irregular in shape with a pale matrix and a few short cristae. A moderate number of cisternae of granular endoplasmic reticulum and free ribosomes could be seen. The Golgi apparatus is frequently extensive consisting of many cisternae and vesicles. There are multivesicular bodies, bundles of filaments and one, or even two, large vacuoles of moderate density (Fig. 2). A cilium is sometimes observed.

There are three to seven capillary loops, each bounded by a fine fibrillar basement membrane of about 1400 Å which somewhere shows a lamina rara externa, lamina densa and lamina rara interna. The endothelium forms an attenuated layer on the basement membrane and contains fenestrae of about 600 Å which are covered with a diaphragm. The nuclei are quite large and indented. The cytoplasm contains some ribosomes, granular reticulum and a few mitochondria. There are numerous small vesicles and some dense bodies.

The mesangial region is extensive, being composed of mesangial cells situated axially between the clusters of capillary loops or touching the walls. The mesangial cells are extremely electron-dense and contain rather large amounts of ribosomes, some mitochondria and a few dense bodies.





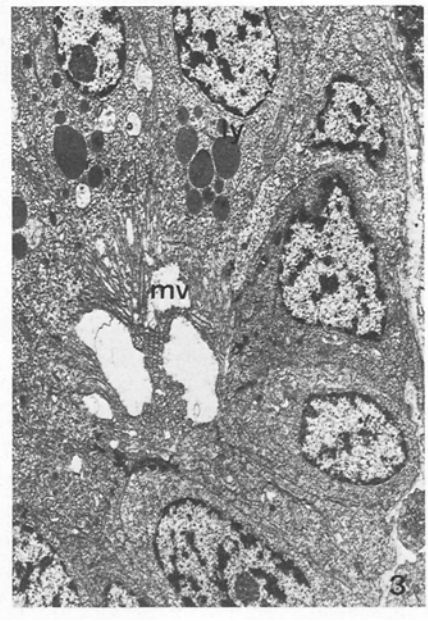


Fig. 1. Electron micrograph showing a portion of a renal corpuscle of *Sparus auratus*. The podocytes (*p*) rest on the basement membrane (*bm*) and surround the capillaries. The mesangial cells (*mc*) are numerous. An increase in height of the parietal layer (*pl*) of Bowman's capsule is observed. × 6000

Fig. 2. Electron micrograph showing a portion of a renal corpuscle. The podocytes (*p*) have small processes wich rest on the basement membrane (*bm*). A large vacuole (*v*) stands out in the cytoplasm of the podocyte. × 13 065. **a** Electron micrograph of the filtration slit between two epithelial foot processes. The pedicels (*pe*) are connected by a filtration slit membrane (*fm*). × 17 420

Fig. 3. Electron micrograph showing the transition from the neck segment to the first proximal segment. The cells on the left are typical neck segment cells. The first proximal segment cells have apical microvilli (*mv*), endocytic vacuoles (*v*) and lysosomes (*ly*). × 4000

Neck segment. The neck segment is short without brush border but with some microvilli and cilia, and tight junctions in the apical part of the cells (Fig. 3). There are very few lateral infoldings and no basal ones. The nucleus is irregular and basally located. Mitochondria are few, irregular and large. The granular endoplasmic reticulum is well developed, and there are numerous free ribosomes. The extensive Golgi apparatus, adjacent to the nucleus, consists of several parallel cisternae and associated groups of vesicles. Occasional multivesicular bodies, lysosomes and bundles of filaments near the nuclei are seen. Wandering cells are seldom found in the neck region.

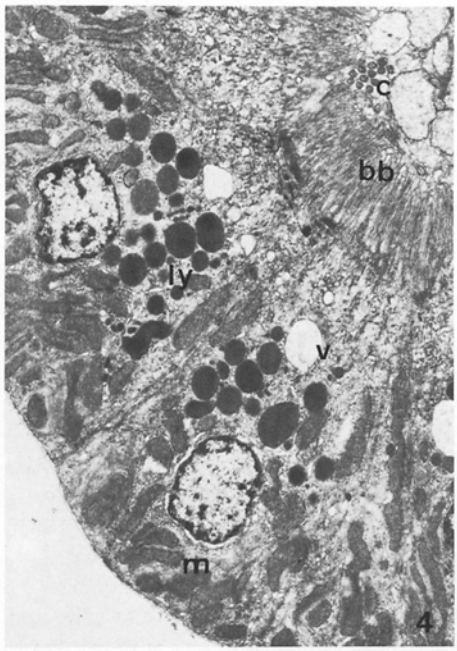
First proximal segment. There is a sudden transition from the neck segment to the first proximal segment (Fig. 3). The columnar cells of the first proximal segment have a well developed brush border and some cilia. The lateral surface is straight with extensive junctional complexes in the apical zone. The basal cell membrane is extensive and regularly infolded forming narrow compartments with very elongated mitochondria. The oval nucleus, sometimes with small invaginations, is in the basal third of the cell. The cells are characterized by numerous large and small endocytic vacuoles as well as apical tubular invaginations and dense, rounded lysosomes of various size. There are numerous ribosomes and a well developed granular reticulum. A Golgi apparatus is often identified. The filamentous mitochondria contain abundant cristae and a moderately dense matrix (Fig. 4).

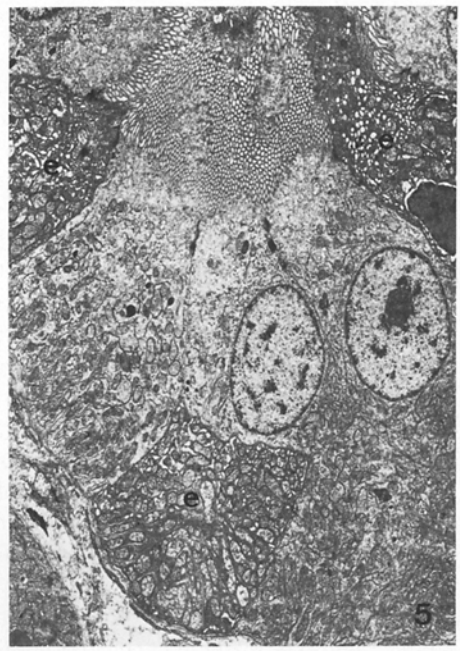
Second proximal segment. This is the longest segment in the nephron with taller cells and less tightly packed tall microvilli than those in the first proximal segment. The cell membranes are straight with tight junctions and desmosomes in the lateral surface and with many basal infoldings. Oval nuclei are centrally located (Fig. 5). There are tubular invaginations, large vacuoles, small vesicles and numerous multivesicular bodies in the apical cytoplasm where basal bodies of cilia with long striated transversal ciliar roots are seen. The Golgi apparatus are usually seen next to the nucleus in the lateral cytoplasm. Scattered rough-surfaced endoplasmic reticulum and numerous round or filamentous mitochondria with a dense matrix and scant lamellar cristae are also observed.

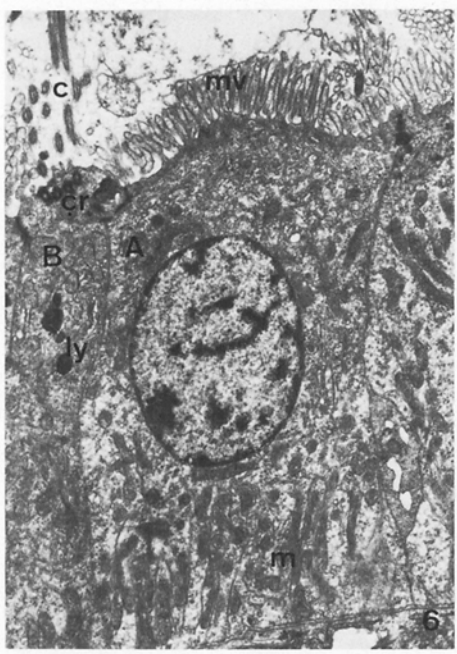
Some electron-dense cells, which appear between the cells described above in the distal portion of the second proximal segment (Fig. 5), have microvilli and junctional complexes and oval nucleus occasionally observed as two fragments. The apical cytoplasm contains numerous small endocytic vacuoles and tubular invaginations. The cell membrane forms many basal perpendicular infoldings, often with numerous mitochondria consisting of scant cristae, clear matrix and matrical granules.

Third proximal segment. This is formed by two types of columnar cells: numerous A-cells and few B-cells (Fig. 6).

The former have an apical convex surface and scant microvilli. The cell membrane is straight in the lateral border, with apical junctional complexes and basal infoldings. The centrally located nucleus is oval. Scattered filamentous mitochondria with a dense matrix containing few short cristae are observed. Granular endoplasmic reticulum is scarce. The Golgi apparatus laterally located







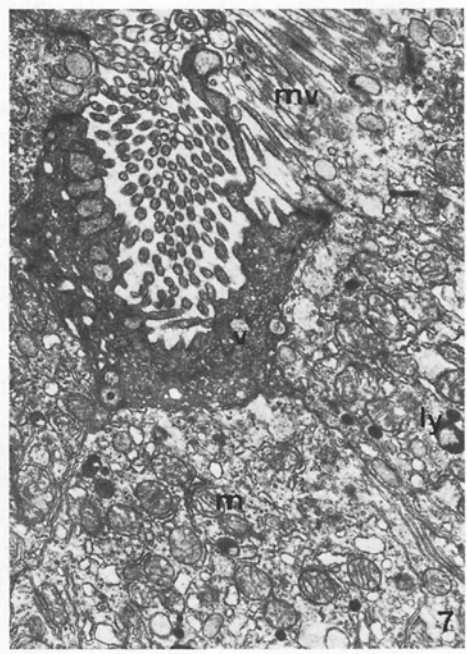


Fig. 4. Electron micrograph of the first proximal segment. The brush border (*bb*) consists of tightly packed microvilli with cilia (*c*). There are large endocytic vacuoles (*v*), numerous lysosomes (*ly*), and mitochondria (*m*) apical and basal to the nucleus. ×4200

Fig. 5. Electron micrograph of the distal portion of the second proximal segment showing electron-dense cells (*e*). ×2800

Fig. 6. Electron micrograph of the third proximal segment. The A-cells display nuclei located in a midposition, apical microvilli (*mv*) and basal mitochondria (*m*). The B-cells showing cilia (*c*), ciliary rootlets (*cr*) and lysosomes (*ly*). ×5600

Fig. 7. Electron micrograph of the apical region of the collecting tubule and the collecting duct where apical microvilli (*mv*), vacuoles (*v*), mitochondria (*m*) and lysosomes (*ly*) can be seen. ×10 500

near the nucleus, multivesicular bodies, and some apical vacuoles are also seen. The B-cells are thinner than the A-cells, with numerous cilia with ciliary rootlets and scant microvilli (Fig. 6). The cell membrane, straight in the lateral and basal surface, forms tight junctions in the apical zone. The oval nucleus has some indentations. Numerous mitochondria stand out in the apical cytoplasm.

The collecting tubule. The transition from the third proximal segment to the collecting tubule is sudden. The lumen is wide, and some cilia and microvilli are identifiable inside. The oval nucleus is usually in the basal third zone. The membrane basal infoldings are adjacent with occasional mitochondria. There are mucous granules in the apical cytoplasm and a single basal body occasionally found (Fig. 7). Numerous small filamentous mitochondria with abundant cristae and frequently partially vacuolated are seen throughout the cytoplasm. The Golgi apparatus is extremely large. The granular endoplasmic reticulum have some displays cisternae. The agranular reticulum is extensive.

Collecting duct. This is surrounded by several layers of smooth muscle cells and connective tissue elements. The epithelial cells are tall and columnar with few short microvilli and occasional cilia (Fig. 7). The oval nucleus has several indentations and a patent nucleolus. The perinuclear cytoplasm contains numerous mitochondria and agranular and granular endoplasmic reticulum. The well developed Golgi complex is next to the apical pole of the nucleus. There are small vacuoles and scant lysosomes. In the distal zone of the collecting duct the cell cytoplasm is filled with small mitochondria containing numerous cristae.

Discussion

The ultrastructure of the renal corpuscle of *Sparus auratus* is characterized by several morphological features which seem to reflect the low glomerular filtration rate, as can be expected in a medium of high saline concentration such as the Mar Menor. These features include few capillary loops, relatively thick capillary walls displaying pores with membrane, and an extensive system of mesangial cells.

The parietal layer cells of Bowman's capsule are similar to those seen in *Parophrys vetulus* (Bulger and Trump 1968), and the numerous cytoplasmic filaments are similar to other teleost species (Bulger and Trump 1968; Olsen 1970; Heath-Eves and McMillan 1974; Hentschel 1977). Cilia have occasionally been seen also in other teleost fishes such as *Parophrys vetulus* (Bulger and Trump 1968) and *Pleuronectes platessa* (Olsen 1970), in cyclostomes such as *Entosphenus japonicus* (Miyoshi 1978), and in many elasmobranch species (Bargmann and Von Hehn 1971). Large podocytes in close contact with the basement membrane have also been recorded in *Entosphenus japonicus* (Miyoshi 1978). The pedicels of *S. auratus* are connected by a filtration slit membrane, similar to that described in other species (Pack Poy 1958; Bulger and Trump 1968; Youson and McMillan 1970; Olsen 1970; Elger and Hentschel 1981); the cilia have previously only been found in the podocytes of *Entosphenus japonicus* (Miyoshi 1978). The presence of one or two large, round shaped, vacuolated podocytes near the filtration area is similar to that described in the euryhaline teleost *Gasterosteus*

aculeatus, where it is recorded exclusively in immature males in freshwater (Ruiter 1980). The filamentous basal membrane separating these cells from the endothelial cells in the glomerulus may be homogeneous, similar to *Parophrys vetulus* (Bulger and Trump 1968), or arranged in three layers as in some other fish species (Pack Poy 1958; Olsen 1970; Youson and McMillan 1970; Heath-Eves and McMillan 1974; Hentschel 1977). The numerous fenestrae with a diaphragm noted in the endothelial cells of capillary loops contrast with some marine teleosts such as *Parophrys vetulus* (Bulger and Trump 1968), *Myxine glutinosa* (Heath-Eves and McMillan 1974; Kühn et al. 1975) and *Spinachia spinachia* (Hentschel 1977), where some pores are without a membrane, and *Entosphenus japonicus* (Miyoshi 1970), where they are without pores.

The numerous electron-dense mesangial cells seen in *S. auratus* and some other species of marine fishes (Bulger and Trump 1968; Miyoshi 1970; Heath-Eves and McMillan 1974; Kühn et al. 1975), are scarce in freshwater fishes where there is abundant filtration (Pack Poy 1958; Anderson and Loëwen 1971).

Cilia occur in all the segments of the tubule nephron and are seen as well as a brush border. Cells of the first major segment of the nephron are characterized by a broad apical zone containing numerous tubular and vesicular profiles and large vacuoles and lysosomes. This apical system is little developed in the proximal segment of aglomerular fishes (Bulger and Trump 1965; Trump and Bulger 1967), but is well developed in the first proximal segment of agnate fishes (Gritzka 1963; Bulger and Trump 1968; Hickman and Trump 1969; Olsen 1970; Anderson and Loëwen 1971; Trump and Bulger 1971; Maunsbach et al. 1972; Hentschel 1977; Ottosen 1978).

The numerous multivesicular bodies in the second proximal segment cells of *S. auratus* and other teleost species (Bulger and Trump 1968; Hentschel 1977; Ottosen 1978) seem to be related to absorptive activity in many cell types (Farquhar and Palade 1962). Some electron-dense cells, not observed in any other species, appear in the distal portion of the second proximal segment.

The A-cells in the third proximal segment are similar to those seen in the third proximal segment of other teleosts (Bulger and Trump 1968; Ottosen 1978), but the B-cells located between them have not been mentioned by other authors.

The collecting tubule next to the third proximal segment, noted in the present study, is not present in the nephron of myxinoids and petromyzontids (Youson and McMillan 1970; Kühn et al. 1975), but is well developed in elasmobranchs and other teleosts (Bulger and Trump 1968; Worsmann et al. 1971; Hentschel 1977; Ottosen 1978). The cells are characterized by apical mucous granules (Hentschel 1977). The numerous small mitochondria indicate that the nephrons of *Sparus* play an active role in kidney function, and are not just a passive system of conducting tubules. The smooth muscle cells surrounding the collecting ducts may play a role in propulsion of the filtrate along the nephron by a systaltic action (Townsley and Scott 1963). The beating of ciliated cells in the neck region and the single long cilia from cells of the remaining tubular regions may also be involved in fluid movement.

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