Z. Zellforsch. 137, 563—588 (1973)

Morphometrical Analysis with the Light and Electron Microscope of the Kidney of the Anadromous Three-spined Stickleback *Gasterosteus aculeatus*, form *trachurus*, from Fresh Water and from Sea Water

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Received November 29, 1972

Summary. The renal corpuscles, juxtaglomerular cells, nephronic tubules, and ureters of female sticklebacks were studied.

In fresh water fishes, the diameter of the renal corpuscles is similar to that found in fishes obtained from the sea, whereas the diameter of the glomeruli and the nuclei of the podocytes are slightly larger. Furthermore, in fresh water the podocytes produce secretory globules, which show some of the histochemical characteristics of the substance constituting the glomerular basement membrane. In sea water animals, secretory phenomena are absent. Mesangial cells, which are scarce in fresh water fishes, are numerous in marine animals. Similarly, juxtaglomerular cells, hard to find in fresh water fishes, are prominent in specimens from the sea.

The development of the epithelia of the nephronic tubules and of the ureters is better in fresh water. The cells and the nuclei are larger. In the first proximal tubule, which is involved in the reabsorption and the digestion—by lysosomes—of macromolecules, "micropinocytosis vermiformis" occurs.

The results of stereological analysis of the fractional volume of the mitochondria and of the relative extent of the infoldings of the basal cell membranes—the location of the ion transport mechanisms—in the three different segments of the nephronic tubule and in the ureter, point to the existence of a structural gradient along the kidney tubules. In fresh water fishes the mitochondrial volume, per surface unit of basal cell membrane, is low in the first proximal segment and is increasingly higher in the other segments, while the highest value is found in the ureter. This structural gradient may be functionally linked with osmotic and ionic gradients, which exist in the renal tubules in fresh water. In the kidney tubules of marine sticklebacks, which do not show a major osmotic gradient, the structural gradient is small.

The results are discussed on the basis of the known physiological differences in the function of the kidney of euryhaline teleosts in fresh water and in the sea.

Key words: Juxtaglomerular cells — Kidney — Teleost (*Gasterosteus*) — Migration — Kidney structure — Morphometry.

Introduction

The *trachurus* form of the three-spined stickleback *Gasterosteus aculeatus* migrates in the spring, before spawning, from the sea into fresh water. In summer and autumn, after the reproductive period, the surviving adults and the young return to the sea. Complicated physiological adjustments are known to accompany

* The author is indebted to Mr. J. Cappon and Mr. M. Veenhuis (Laboratory for Ultrastructural Biology, University of Groningen) for technical assistance.

the migratory movements of euryhaline teleosts (Maetz and Skadhauge, 1968; Hickman and Trump, 1969; Holmes and Donaldson, 1969). In fresh water, the blood-osmotic value is highly hypertonic compared to the value of the environment. Maintenance of a steady state involves compensation of an osmotic inwards waterflow and an outwards diffusion of ions. In the sea, the blood is hypotonic. Drinking of sea water and the subsequent secretion of divalent ions are important regulatory processes for maintaining equilibrium.

Several organs, like the gut, the gills, and the kidneys, are involved in osmoregulation. The function of the kidney has been analyzed in several euryhaline teleosts. In general, the glomerular filtration rate is high in fresh water and low in sea water (Holmes and McBean, 1963). In fresh water large volumes of urine are produced, which are highly hypotonic to the blood due to intensive reabsorption of monovalent ions by the kidney tubules. In sea water, divalent ions are secreted by these tubules, while water and some monovalent ions are reabsorbed, which leads to the production of small volumes of urine, which is almost isotonic with the blood (Chester Jones *et al.*, 1969).

It may be expected that the study of the renal changes occurring in euryhaline teleosts during migration will give valuable information on the relation between structure and function in the vertebrate kidney. Available data are scarce, however, and almost exclusively limited to light microscopic observations (Ogawa, 1968; Lam and Leatherland, 1969). There are, on the other hand, many reports on kidney morphology, including ultrastructural studies, of fresh water and marine teleosts (Hickman and Trump, 1969). A survey of the literature concerning species of both habitats shows a great similarity in the ultrastructure of the kidneys, which suggests that the structural changes occurring during migration will be of a quantitative rather than of a qualitative nature. Therefore, morphometrical techniques were used in this study of the kidney of sticklebacks from fresh water and from the sea.

Attention was paid to the renal corpuscles, to the juxtaglomerular cells, to the nephronic tubules, and to the ureters. The renal corpuscles were examined for studying the effect of a high glomerular filtration rate, as in fresh water, and of a low glomerular filtration rate, as in sea water, on size and ultrastructure.

The juxtaglomerular cells, assumed to produce renin in teleosts as well as in other vertebrates, have been reported to be more numerous in sea water fishes than in fresh water species (Bohle and Walvig, 1964). If renin is especially needed in marine conditions, as has been suggested by Lagios (1968), the juxtaglomerular cells in sticklebacks should show an increase in cellular development during and after migration into the sea. An opposite result, however, should be expected on the basis of pharmacological data showing that renin content is higher in the kidneys of fresh water teleosts (Capelli and Wesson, 1970).

The nephronic tubules and the ureters have been studied for analyzing morphological changes which may accompany the changes in the transport of ions which occur during migration.

The data presented here were obtained by histochemical techniques, by light microscopic morphometry and by the application of stereological methods (lineal analysis) at the ultrastructural level (Loud *et al.*, 1965; Sitte, 1967).

Materials and Methods

Fresh water dwelling adult specimens (yearlings) of the marine form (*trachurus*) of *G. aculeatus* were obtained from canals and kept in aquaria in running tap water for at least 4 months, at 8L16D. They were fixed in November at a water temperature of 7°C.

Sea water adapted fish of the same race were caught by trawl off shore in the Wadden Sea on December, 12th, 1971, at a water temperature of 5°C. The body length of all animals used varied from 64 to 73 mm for the females, and from 55 to 60 mm for the males.

After destruction of the brain, the kidneys were rapidly excised and their weight determined. For light microscopy the tissues were immersed in Bouin for 24 hours. Paraffin sections of 5 μ thickness were normally stained with Periodic-Acid-Schiff (P.A.S.) after McManus (Pearse, 1961), with Mayer's haemalum as a counterstain, or, incidentally, with Bowie's stain after Wilson (1952), paraldehyde-fuchsin and chrome-haematoxylin after Gomori, or alcian blue after Steedman (Romeis, 1968).

Electron Microscopy: the kidneys were fixed for 2 hours in a freshly prepared mixture of glutaraldehyde (3%) and osmium tetroxyde (1%) in phosphate buffer (0.2 M; pH 7.4) at 0°C. After dehydration in ethanol and propylene oxyde embedding followed in Epon 812. Ultrathin sections were examined, after staining with Reynold's lead citrate, in a Philips EM 100 or EM 300 electron microscope.

The uptake of macromolecules by the kidney cells was studied after intraperitoneal injection of trypan blue (0.05 ml per animal of a 1% solution of the dye in 0.6% NaCl) at 4, 8, or 16 hours prior to fixation.

For the demonstration of acid phosphatase activity the kidneys were handled as described before (Wendelaar Bonga, 1971). Alkaline phosphatase activity after Gomori, and peroxydase activity after Mitsui and Ikeda, were demonstrated on kryotome sections for light microscopy (Romeis, 1968). Diastase was used for localizing glycogen, by incubating paraffin sections for 2 hr in a 1% solution.

For establishing the diameter of Bowman's capsule and of the glomerulus, 50 renal corpuscles per animal were measured. To obtain maximal diameters per corpuscle, measurements were taken in those sections showing the vascular pole of the glomerulus as well as the neck segment of the nephronic tubule. The mean of two measurements, taken at angles of 45° in respect to the glomerular axis, was used. For determining the epithelial height of the nephronic tubule and of the ureter, circular cross sections were selected. Per animal 50 determinations were made. Nuclear surfaces were calculated on the basis of length and width of 100 nuclei per animal. Measurements refer to nuclei having their maximal dimensions in the plane of sectioning. All measurements were made on paraffin sections. Per animal 5 tissue sections were used, cut at distances of about 400 μ.

Morphometrical data of the renal epithelia at the ultrastructural level were obtained by lineal integration (Loud *et al.*, 1965). A square grid of sampling lines (the distance of the lines normally being 10 mm, for the sampling of lysosomes 5 mm) was projected on electron micrographs with a final magnification of 20000×. The sampling lines made an angle of 19° (or 71°) with the basis of the cells, as suggested by Sitte (1967) for the analysis of non-randomly distributed cellular structures. Only circular cross sections of the tubular epithelia were used for ultrastructural analysis. These were selected in 1 μ thick Epon sections in the light microscope. In electron micrographs, a length of 1000 μ per animal was sampled for determining the mitochondrial volume and the extent of the basal membrane infoldings, and a length of 2000 μ for establishing the volume of the lysosomes. The nuclei were excluded from the sampling area. The extent of the basal membranes was expressed as surface of membrane per volume of cytoplasm, by making use of the equation given by Loud *et al.* (1965). Student's T-test was applied for statistical analysis ($\alpha = 0.05$).

Observations

The kidney of *G. aculeatus* shows the morphological characteristics of the type III kidney in the classification of Ogawa (1961). The left and right half of the trunk kidney are fused caudally. The cranial parts are connected by slender strands to the

left and right head kidney, which contain haematopoietic tissue and interrenal cells. Throughout the trunk kidney clusters of 10 to 20 renal corpuscles are found. A small artery is running to each cluster and supplies every glomerulus with a short afferent arteriole. The nephronic duct is made up of a short neck segment, a first and a second proximal segment, and a collecting tubule. The collecting tubules originating from a cluster of renal corpuscles fuse and eventually open into the short branches of the two ureters. The ureters are running ventrally in the trunk kidney and terminate in the urinary bladder. The bladder opens by a small duct at the urinary papilla.

The trunk kidneys of two groups of 10 adult females, one group obtained from fresh water, the other from the sea, were quantitatively analyzed. Body weights are given in the table. Five fishes of each group were studied light microscopically, the other specimens with the electron microscope. Exclusively females will be dealt with, since in adult males the epithelium of the second proximal tubule and of the ureter is transformed into slime producing cells from the start of the reproductive period until late autumn.

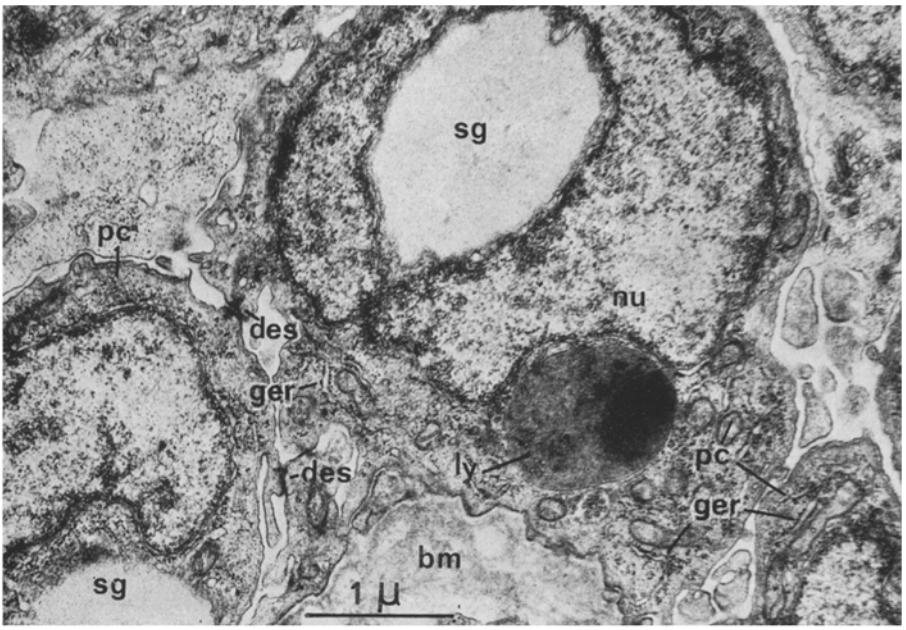
The wet weights of the kidneys of the females were similar in both groups (Table 1). The value of this result is limited, since the weight of the kidneys is influenced by greatly varying amounts of haematopoietic tissue which are distributed between the nephrons.

I. Renal Corpuscles

Fresh Water. The renal corpuscles show the familiar vertebrate structure. The glomerulus consists of capillary loops, lined by endothelial cells and underlined by a basement membrane, and mesangial cells irregularly distributed between the endothelium and the basement membrane. Bowman's capsule is made up of a visceral layer of podocytes, contacting the basement membrane, and a parietal layer of flattened epithelial cells (Simon and Chatelanat, 1969). The endothelial cells show some fenestrations, closed by a single membrane, the diaphragm. The fenestrated areas are limited in extent when compared with mammalian glomeruli. The cells are connected by tight junctions and desmosomes. Some mitochondria and pinocytotic vesicles are usually found (Fig. 1, 4A).

Mesangial cells appeared to be very scarce in fresh water specimens. They occur almost exclusively near the vascular pole. These oval cells have small cytoplasmic processes. A few membranes of the granular endoplasmic reticulum, a Golgi system characterized by small transparent saccules and many small clear vesicles, and low numbers of mitochondria and lysosomes are normally present.

The visceral layer of Bowman's capsule is composed of podocytes which contain, when compared to mammalian podocytes, a limited number of cytoplasmic processes (pedicels), interconnected by membranes (filtration-slit-membranes). The cells are connected by tight junctions, intermediate junctions and desmosomes, occasionally combined in a junctional complex. Although these junctions were common, they were not found between all podocytes. It is, therefore, improbable that the glomerular filtrate has to pass by these junctions before reaching the capsular lumen. The cells show signs of considerable metabolic activity. Indentations in the cell membrane suggest pinocytotic processes. The nuclei contain prominent nucleoli. In the cytoplasm surrounding the nuclei, strands of



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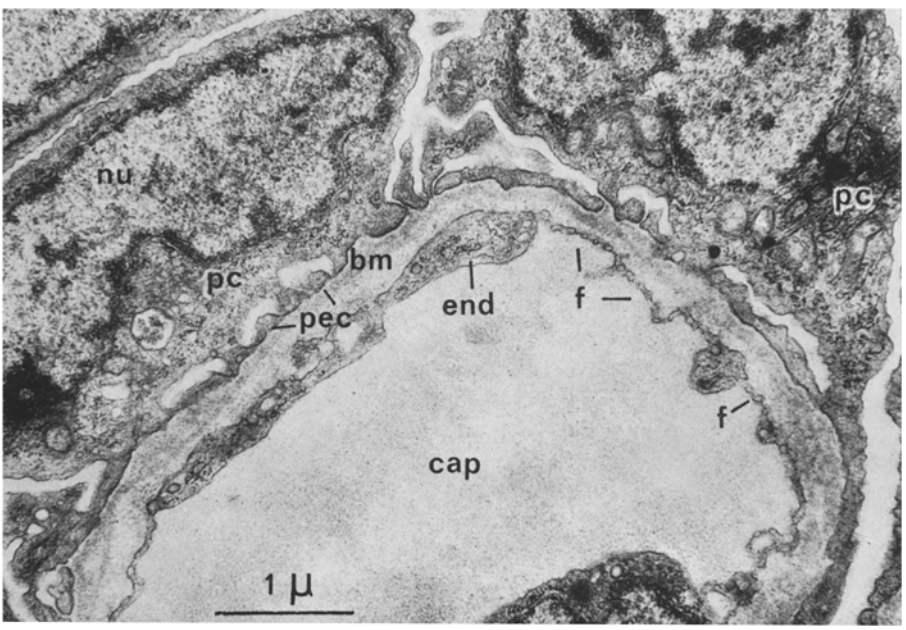

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Fig. 1. This part of the glomerulus of a fresh water specimen shows podocytes (*pc*) containing large secretory globules (*sg*); the upper one is located in an infolding of the nucleus (*nu*); *ger* granular endoplasmic reticulum; *ly* lysosome; *des* desmosomes; *bm* basement membrane

Fig. 2. Glomerulus of a marine specimen; in the podocytes (*pc*) secretory globules are absent and granular endoplasmic reticulum is scarce; *end* endothelial wall; *f* fenestrations; *nu* nucleus; *pec* pedicels; *bm* basement membrane; *cap* capillary lumen

Table 1

	Fresh water	Sea water	Significance
Body weight (g)	3.27 ± 0.28	3.54 ± 0.52	$-$ ($p > 0.1$)
Wet weight of kidney (mg)	18.8 ± 3.9	17.9 ± 4.3	$-$ ($p > 0.1$)
Diameter of renal corpuscles (μ)	43.36 ± 0.90	46.01 ± 0.86	$-$ ($p > 0.1$)
Diameter of glomeruli (μ)	43.48 ± 0.54	41.32 ± 1.51	$+$ ($p < 0.05$)
Nuclear surface of podocytes (μ^2)	12.80 ± 0.67	10.47 ± 0.55	$+$ ($p < 0.001$)
Nuclear surface of juxtaglomerular cells (μ^2)	12.31 ± 1.03	10.05 ± 1.13	$+$ ($p < 0.05$)

Wet weight of bodies and kidneys ($n = 10$); diameter of renal corpuscles and of glomeruli, and maximal nuclear surface of podocytes (100 measurements per animal, respectively; $n = 5$); maximal nuclear surface of juxtaglomerular cells (50 measurements per animal, $n = 5$). Means ($\pm$ S.E.) and statistical significance are given for fresh water and sea water sticklebacks.

granular endoplasmic reticulum are common. Large cisterns, containing a homogeneous substance of low electron density, are present in most cells. This substance probably represents prosecretory material. In most podocytes one or more large ($\varnothing$: 1–3 μ), electron transparent, globules are found (Fig. 1), which can be seen even with the light microscope. The substance of the globules has some histochemical characteristics in common with the material constituting the basement membrane. It reacts positively to P.A.S., paraldehyde fuchsin and alcian blue. which is indicative of acid carbohydrates. The saccules of the Golgi apparatus are occasionally dilated, enveloping an electron transparent material. They are probably involved in the secretory process. Dense bodies ($\varnothing$: 0.2–0.4 μ) occur frequently. As they proved to react weakly positive to the acid-phosphatase test, they are considered to be lysosomes.

The parietal layer of Bowman's capsule is made up of flat cells, connected by junctional complexes. At the luminal side of the cells there is a well-developed terminal web, consisting of bundles of microfilaments which are inserted into the desmosomal plaques and which are running parallel to the apical cell border. Microtubules and an occasional cilium are present. As in the podocytes, membranes of the granular endoplasmic reticulum are common. No secretory granules were, however, found.

Quantitative data on the size of the renal corpuscles and of the glomeruli, and on the surface of the nuclei of the podocytes are presented in Table 1.

Sea Water. In the glomerular structure of marine animals marked differences were found in two cell types which make up part of the filtration barrier the mesangial cells and the podocytes. Mesangial cells, scarce in glomeruli of the fresh water group, are numerous (Fig. 3, 4B) Their cytoplasmic processes are longer, and projected between the endothelium and the basement membrane for long distances Occasionally they even surrounded the capillary loops completely. The cell bodies are found in small groups, which are especially numerous near the

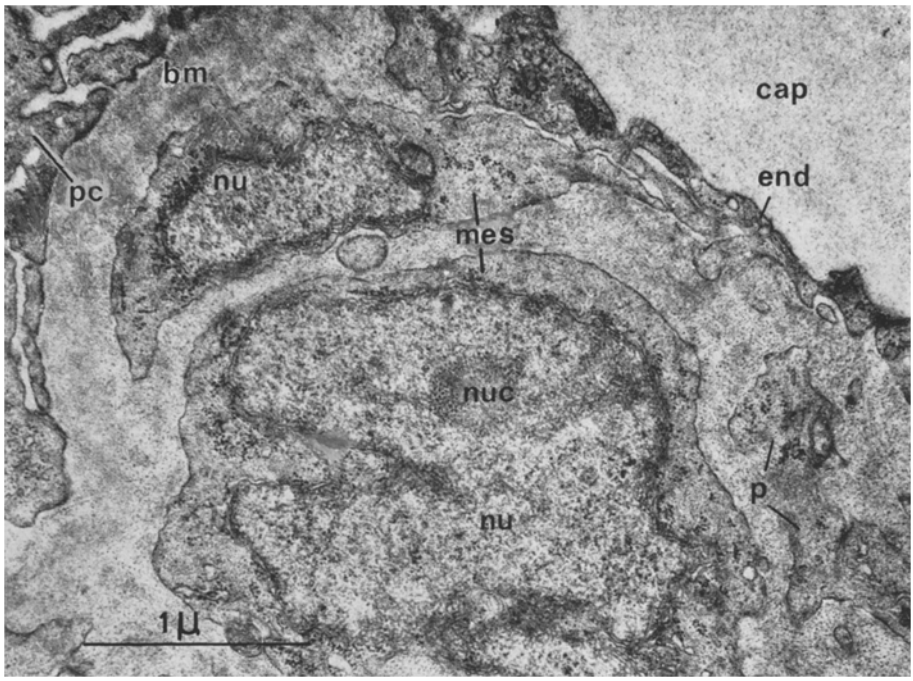


Fig. 3. Glomerulus of a marine specimen; two mesangial cells (*mes*) and processes of mesangial cells (*p*) are located between the layer of podocytes (*pc*) and the endothelium (*end*); *nu* nuclei; *nuc* nucleolus; *bm* basement membrane; *cap* capillary lumen

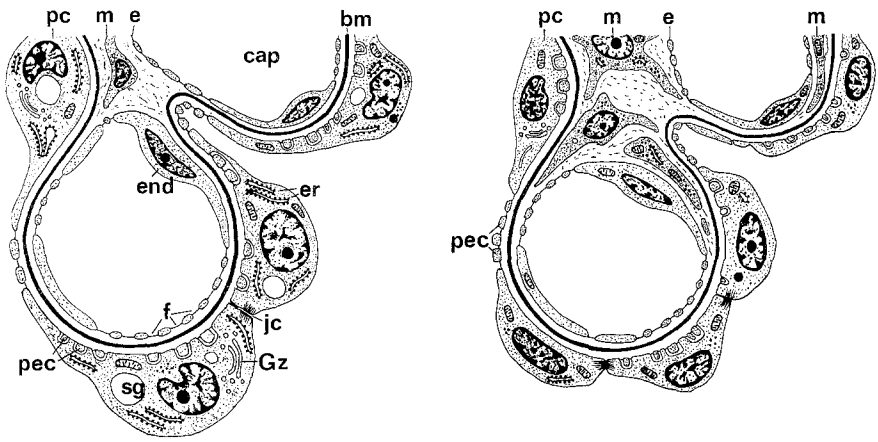


Fig. 4. A and B. Diagrams of glomeruli of sticklebacks from fresh water (A) and from the sea (B); *pc* podocytes; *pec* pedicels; *Gz* Golgi zone; *bm* basement membrane; *m* mesangial cells; *end* endothelial cells; *f* fenestrations; *cap* capillary lumen; *jc* junctional complex. In fresh water, the nuclei of the podocytes are larger, whereas the cytoplasm, containing numerous strands of granular endoplasmic reticulum (*er*) and some secretory globules (*sg*), is more developed. In sea water the mesangial cells are more numerous and better developed. Long cytoplasmic processes of the mesangial cells extend between the basement membrane and the endothelial cells

vascular pole. As the cells could not be clearly distinguished in the light microscope, no data on cell number or nuclear size are presented. Ultrastructural estimates based on small samples indicate that these cells are at least four times as numerous in marine specimens when compared to fresh water fish. A high cytoplasmic activity is indicated by the occurrence of large numbers of free ribosomes.

In the podocytes cytological changes are also obvious (Fig. 2). In contrast with the mesangium, a lower rate of cellular activity is indicated. Granular endoplasmic reticulum, prominent in fresh water fishes, is scarce and there are no apparent signs of secretory acitvity. The P.A.S.-positive mucous globules are absent. The amount of cytoplasm surrounding the nuclei is apparently smaller in the sea water group. Furthermore, the results of the measurements of the nuclei (Table 1) show that the mean nuclear surface is reduced by 18% when compared to that of the former group, a statistically significant difference. From Table 1 it also appears that the difference in the mean size of the renal corpuscles is minimal. The diameter of the glomeruli is significantly smaller, although the difference is not impressive (about 5%).

II. Juxtaglomerular Cells

Fresh Water. Juxtaglomerular cells are very scarce and are located in a single cell layer around some of the arteries running to clusters of glomeruli. They are rarely found around the small branches of these arteries, the afferent arterioles which run to each glomerulus. Their distribution pattern, therefore, conforms type IV in the classification of Krishnamurthy and Bern (1969). The height of these cells varies largely, probably due to the state of contraction of the arteries after fixation. The surface of the nuclei also varies considerably (Table 1).

The secretory material shows a low affinity for Bowie's stain, as well as for Gomori's chrome haematoxylin and paraldehyde fuchsin. A clearly positive result is obtained with P.A.S.

In the electron microscope, only a few juxtaglomerular cells were found. In the cytoplasm many large secretory granules ($\varnothing$: 0.2–0.4 μ) of moderate electron density are present. A few strands of granular endoplasmic reticulum and an occasional Golgi zone occur between the granules.

Sea Water. The presence of numerous juxtaglomerular cells in sticklebacks from the sea is in marked contrast with the low number observed in fresh water specimens. The cells are found in continuous layers around the renal arteries, often extending over long distances. They occur occasionally around the afferent arterioles. The cytoplasm stains intensely with P.A.S. and appears to be crowded with grey granules if examined in the electron microscope. Indications of release of the secretory substance were, however, not observed. The surface of the nuclei is larger than in the fresh water group by about 14% (Table).

III. Nephronic Tubules

1. Neck Segment

Fresh Water. In contrast to many other teleosts, and to the specimens of *G. aculeatus* studied by Ogawa (1967) and Mourier (1970), a clearly delineated

and well ciliated neck segment has not been encountered. The neck region consists of a short tubule, 10–20 μ in length, of cells connecting the flattened epithelium of Bowman's capsule with the cylindrical cells of the first proximal segment. Cilia are occasionally present, but in the same low number as found on the cells of the proximal segments. Unlike those latter cells, the neck cells have no brush border. In the basal half of the cells mitochondria were numerous. They are located between infoldings of the basal cell membrane, forming a basal labyrinth, similar as has been described in many water and ion transporting epithelia of invertebrates and vertebrates (Ericsson and Trump, 1969; Wendelaar Bonga and Boer, 1969). The cells are connected by apically located junctional complexes, consisting of a tight junction, an intermediate junction and a desmosome, and by 1–5 desmosomes distributed rather evenly over the lateral cell membranes. Similar junctions are present between the cells of the other nephronic segments, and of the ureter, in both fresh water and sea water specimens.

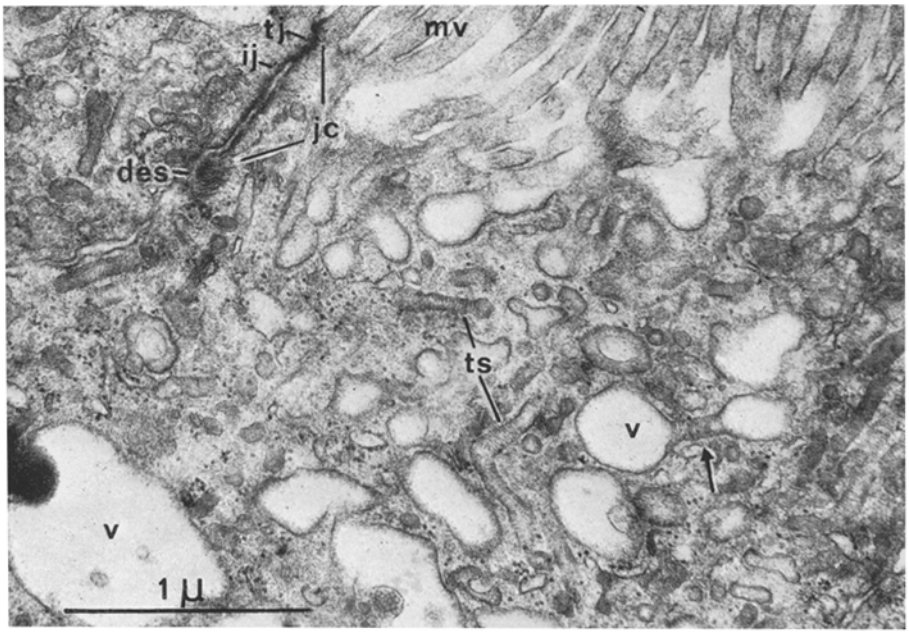
Sea Water. No qualitative differences were observed between fresh water and sea water fishes. This segment has not been quantitatively analyzed.

2. First Proximal Segment

Fresh Water. This segment is relatively short, comprising 5–10% of the length of the nephronic tubule. The microvilli react positively for alkaline phosphatases, enzymes known to be involved in transport processes (Moelbert *et al.*, 1960). An indication that glucose reabsorption takes place in these cells is the presence of a P.A.S.-positive material in the brush border and in the most apical part of the cytoplasm. As the substance is removed by diastase treatment it probably represents glycogen.

A diastase-resistant, P.A.S.-positive substance covers the apical cell membranes of this segment as well as those of the other nephronic tubules and of the ureter. It probably represents a glycocalyx, described in other vertebrate kidneys (Longley, 1969).

With the electron microscope small invaginations, suggesting the occurrence of pinocytotic phenomena, are regularly found between the microvilli. Tubular invaginations of the cell membrane, with a diameter of about 600 to 800 Å and with a dense, regular coating at the inner side of the tubules, are also found in this area (Fig. 5). Tubular and vesicular structures with a similarly coated appearance are numerous in the apical part of the cells. Examination of serially cut ultrathin sections revealed that the tubules and vesicles are continuous, apically, with the tubular invaginations of the cell membrane, and, more basally, with larger, electron-transparent and uncoated vesicles and vacuoles (Fig. 5, 6). Some of these vacuoles react slightly positive for acid phosphatase activity, and, therefore, represent secondary lysosomes. More distally from the tubular lumen, above and around the nucleus, numerous lysosomes of varying electron-density (Fig. 6) and positivity for acid phosphatase were found. In this area very dense lead deposits are found after performing the acid phosphatase test at the light microscope level. Some hours after intraperitoneal injection of trypan blue, particles of this vital stain are seen as blue granules in the light microscope, and as amorphous electron dense deposits in the electron microscope. It seems likely that in the cells of the first proximal tubules of *G. aculeatus*, as in other vertebrate proximal



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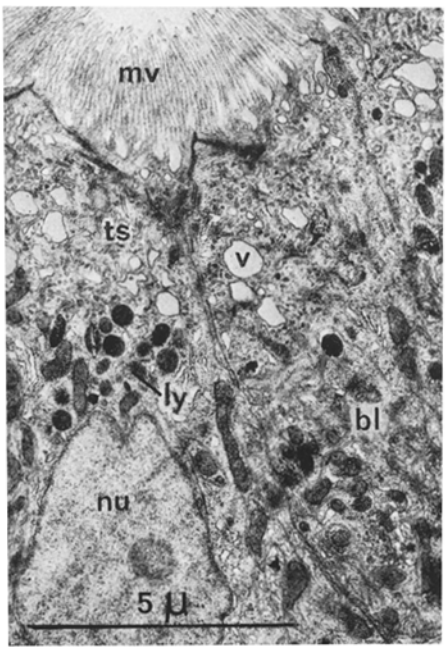

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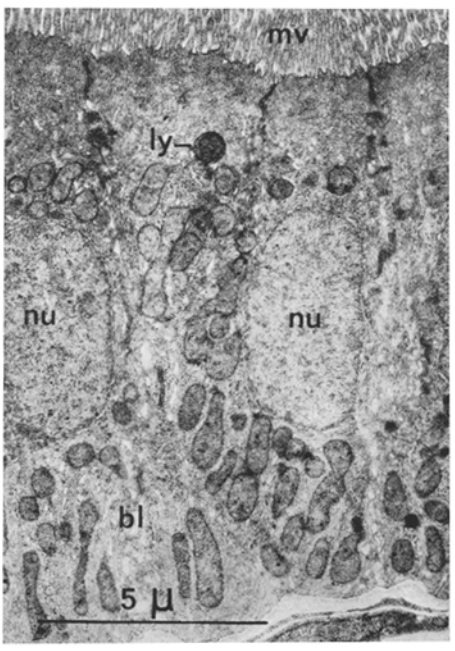

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Fig. 5. The apical part of the cells of the first proximal segment of a fresh water specimen; numerous coated tubular structures (*ts*) and coated vacuoles (*v*) are present; some of the vacuoles are connected by tubules (arrow); *jc* junctional complex; made up of a tight junction (*tj*), an intermediate junction (*ij*) and a desmosome (*des*); *mv* microvilli

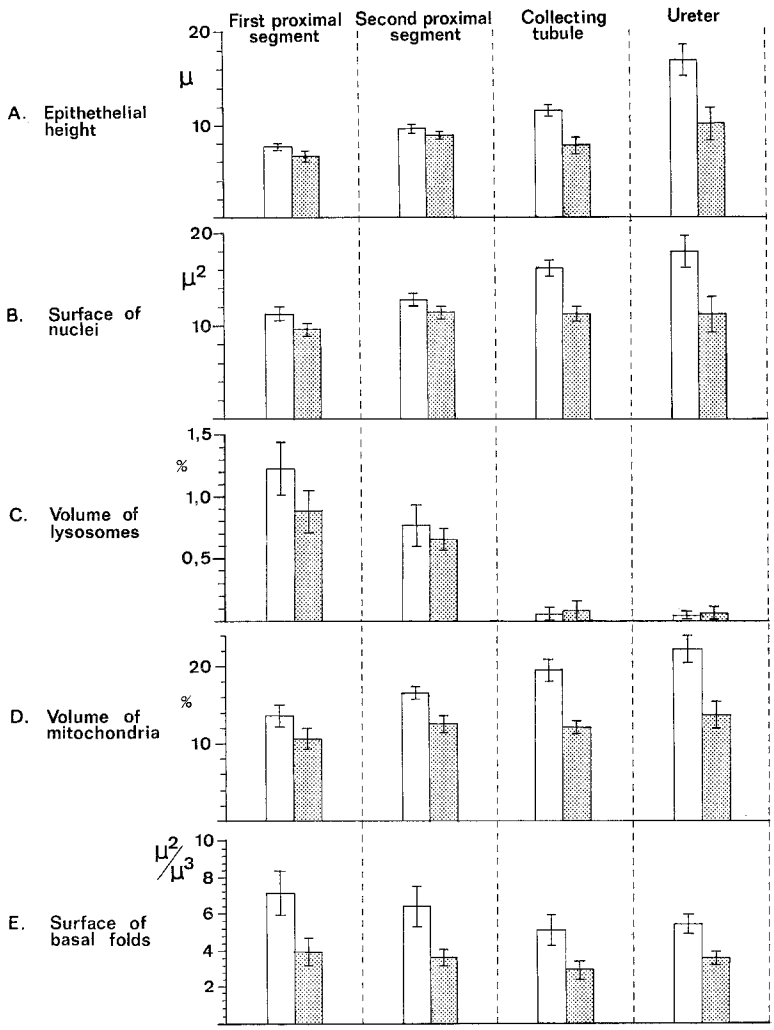


Fig. 8A–E. The results (means ± S.E.) of morphometrical analysis of the nephronic segments and ureters of 10 female specimens of *Gasterosteus aculeatus*; 5 specimens from fresh water (white bars) and 5 specimens from the sea (mottled bars). A and B: height of the cells and maximal surface of the nuclei, determined in the light microscope, C, D, and E: percentual volume of the lysosomes and of the mitochondria, and surface of the basal membrane folds per unit ($1\ \mu^3$) of cytoplasm, as determined by lineal analysis of electron micrographs

Fig. 6. First proximal segment of a fresh water specimen; the cells show microvilli (*mv*), an apical area of coated tubular structures (*ts*) and vacuoles (*v*), a region, above the nucleus (*nu*), with lysosomes (*ly*), and a basal area with a basal labyrinth (*bl*)

Fig. 7. Second proximal segment of a fresh water specimen; *mv* microvilli; *ly* lysosomes; *nu* nuclei; *bl* basal labyrinth

tubules (Ericsson and Trump, 1969), reabsorption of macromolecules and subsequent digestion of this material within the lysosomal apparatus takes place. All or part of the substances may enter the cells via the system of coated tubules, as it is interconnected between the tubular lumen and the lysosomes.

Peroxidase activity, as analyzed with the light microscope, is evident, but rather weak. Peroxisomes, known to contain this enzyme, and other enzymes known to be involved in nitrogen katabolism, are found in low numbers. The fractional volume of these organelles appeared to be too low to be reliably estimated. They are distinguished from the lysosomes by homogeneous, moderate electron dense contents and by the thickness of the limiting membrane (60–70 Å, as opposed to 90–100 Å in lysosomes).

The basal labyrinth is well developed. The mitochondria, although occurring in all but the most apical parts of the cells, are mainly (up to 80%) associated with the basal membrane folds. Quantitative data concerning this segment are presented in Fig. 8.

Sea Water. The cells of the proximal tubules of marine specimens are qualitatively similar to those of fresh water sticklebacks. However, the mean epithelial height as well as the mean nuclear surface are significantly ($p<0.05$) smaller than in fresh water fishes, by about 15% (Fig. 8A, B).

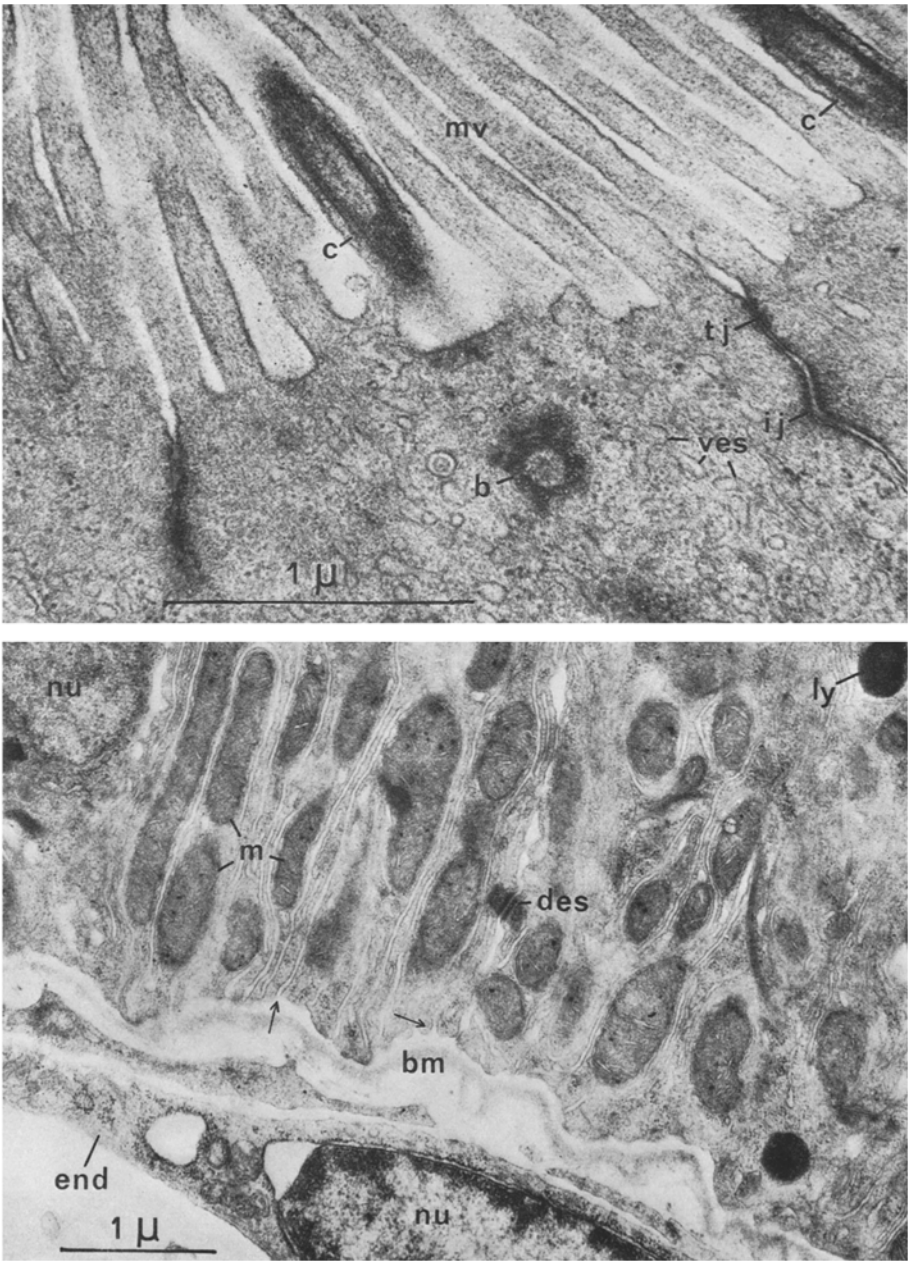
The results of the alkaline-phosphatase and peroxidase tests as well as of the P.A.S.-reaction are similar as in fresh water fishes, but deposits due to acid phosphatase activity appear to be less dense. This result suggests a lower lysosomal activity. This conclusion is confirmed by the morphometric data (Fig. 8C): the relative volume of the lysosomes is significantly lower, by about 22% ($p<0.05$). It may be concluded that the rate of lysosomal digestion and, furthermore, the amount of macromolecules absorbed from the glomerular filtrate is lower in marine animals.

Considerable differences are found in the extent of the basal labyrinth (Fig. 8D, E). The surface of the basal membrane folds, per unit of cytoplasm, is diminished by about 40% ($p<0.001$) when compared to fresh water fishes; the fractional volume of the mitochondria is also lower (22%, $p<0.01$).

3. Second Proximal Segment

Fresh Water. This is the main segment of the nephronic duct, making up about 70% of its length. It has a brush border, similar as the first proximal segment. The microvilli, however, show only a very weak reactivity with the P.A.S. and the alkaline phosphatase test. Another important difference is the absence of the apical system of coated tubules and of the large transparent apical vacuoles (Fig. 7). Uptake of injected trypan blue particles does not take place. Massive reabsorption and digestion of macromolecular substances are obviously limited to the first proximal segment. The apical cytoplasm is crowded with many small irregularly shaped vesicles (⌀: 300–800 Å), lined by smooth membranes (Fig. 9). Membrane indentations, suggestive of pinocytotic activity, are occasionally found.

The lysosomal apparatus of these cells is well developed, although less than in the first proximal segment, as appears from the mean fractional volume (Fig. 8C), Peroxisomes are found in the same low numbers as in the first segment. They



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Figs. 9 and 10. Second proximal tubule of a fresh water specimen. Fig. 9: The apical parts of the cells; *ves* small, vesicular structures; *mv* microvilli; *c* cilium; *b* basal body of cilium; *tj* tight junction; *ij* intermediate junction. Fig. 10: Basal part of the cells; the membrane folds are frequently found to be continuous with the basal cell membrane (arrows); *m* mitochondria; *ly* lysosome; *des* desmosome; *nu* nuclei; *bm* basement membrane; *end* endothelium

show a weak peroxidase activity. The basal labyrinth is well developed (Fig. 10). The mitochondria comprise about 16% of the cytoplasmic volume, slightly more than in the first proximal segment. The surface of the membranes of the basal membrane folds per unit of cytoplasm is, however, considerably lower (Fig. 8E). Up to 90% of the mitochondria is associated with these membranes.

Sea Water. The appearance of the second proximal segment is similar to that described for fresh water animals as far as histochemical and morphological aspects are concerned. The quantitative parameters, however, show that the epithelium is less developed than in fresh water (Fig. 8). Differences are small for the mean cell height, the nuclear surface, and the volume of the lysosomes. Marked differences are found in the fractional volume of the mitochondria and in the extent of the basal membrane folds ($p<0.001$).

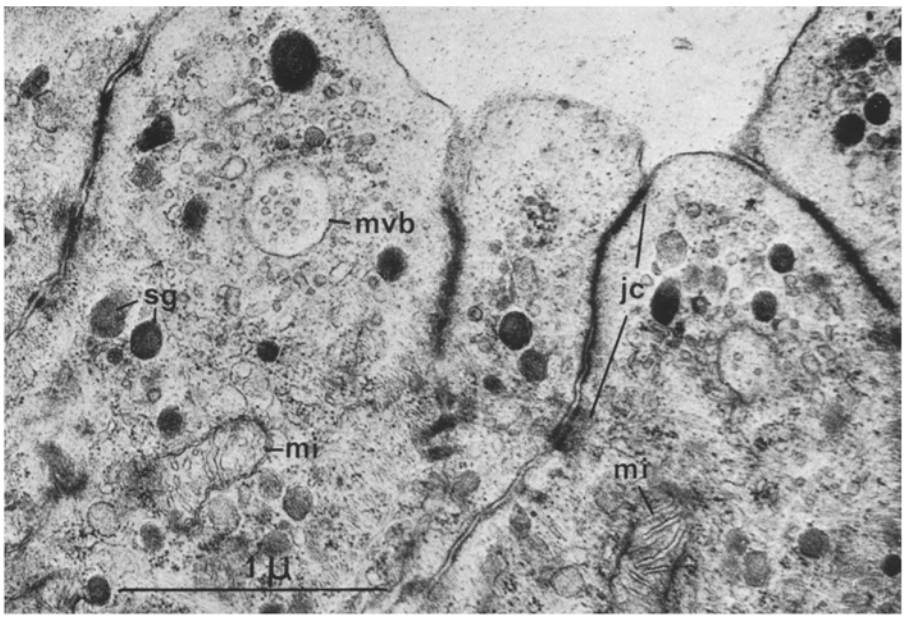
4. Collecting Tubule

Fresh Water. This segment comprises 20 to 25% of the length of the nephronic tubule. The epithelium is lined by a thin muscular layer. A brush border is absent. Small, clear, uncoated vesicles and multivesicular bodies are found in the most apical zone of the cytoplasm (Fig. 11). The cells of the collecting tubule react negatively to P.A.S., whereas tests for alkaline phosphatase, peroxidase or acid phosphatase give a very weak reaction. The low acid phosphatase activity is in line with the observed scarcity of lysosomes. The fractional volume of the lysosomes is less than 10% of that of the proximal segments. In the upper half of the cells small ($\varnothing$: 1000–2000 Å) dark granules are frequently observed (Fig. 11). They do not exhibit acid phosphatase activity, and probably arise from the Golgi system, as dense material is incidentally observed within the Golgi saccules. These supposedly secretory granules are found in all animals, but in only part of the cells.

Profiles of the smooth endoplasmic reticulum are distributed in the cytoplasm, often concentrated in the upper parts of the cells. The basal labyrinth is very extensive, comprising most of the cytoplasm, except for a small apical zone. The basal membrane folds are less densely packed than in the proximal segments; the relative volume of the mitochondria is, however, larger than in the proximal segments (Fig. 8D, E; 12).

Sea Water. The cells of the collecting tubule are considerably less developed than those of fresh water fishes (Fig. 8). Epithelial height and nuclear surface are markedly lower, by about 33 and 31%, respectively ($p<0.001$). Still greater are the percentual differences ($p<0.001$) in the extent of the basal labyrinth (43%) and the fractional volume of the mitochondria (38%). The development of the basal labyrinth often differs largely between adjacent collecting tubules or even between different areas of the same segment. In some cells basal infoldings are very scarce. Dark small granules are present in some of the cells, like in fresh water fishes (Fig. 13).

Fig. 11. Apical part of cells in a collecting tubule of a fresh water specimen; *sg* secretory granules; *mvb* multivesicular body; *jc* junctional complex; *mi* mitochondria



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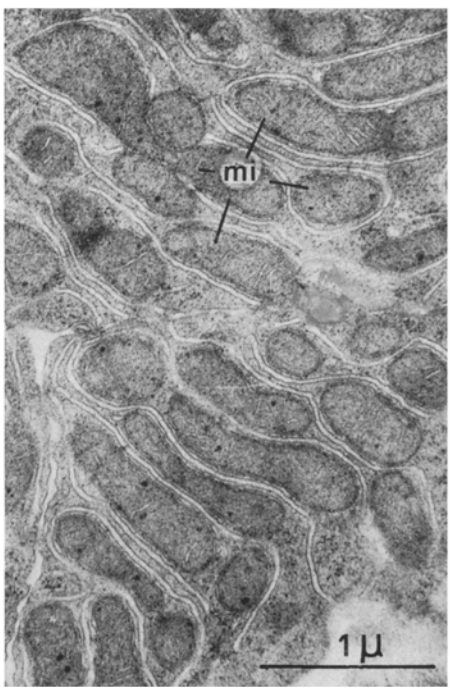

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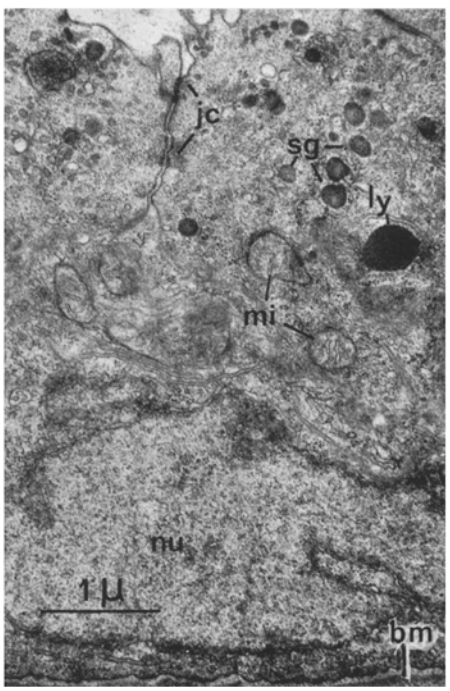

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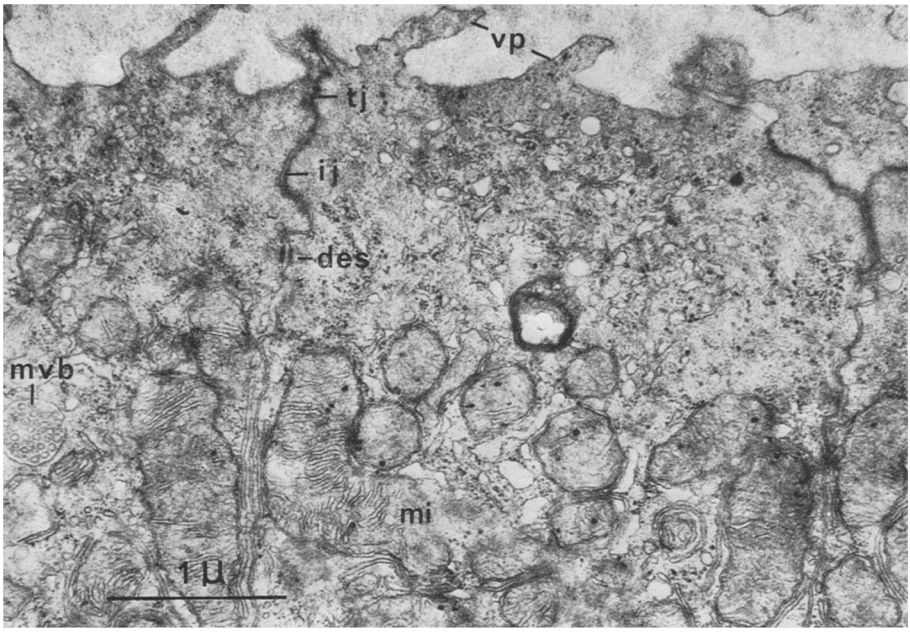
Fig. 12. Basal labyrinth of a cell in the collecting tubule of a fresh water specimen; the mitochondria (*mi*) are more densely packed when compared to those of the proximal tubules

Fig. 13. Collecting tubule of a marine specimen; *jc* junctional complex; *sg* secretory granules; *ly* lysosome; *nu* nucleus; *mi* mitochondria; *bm* basement membrane



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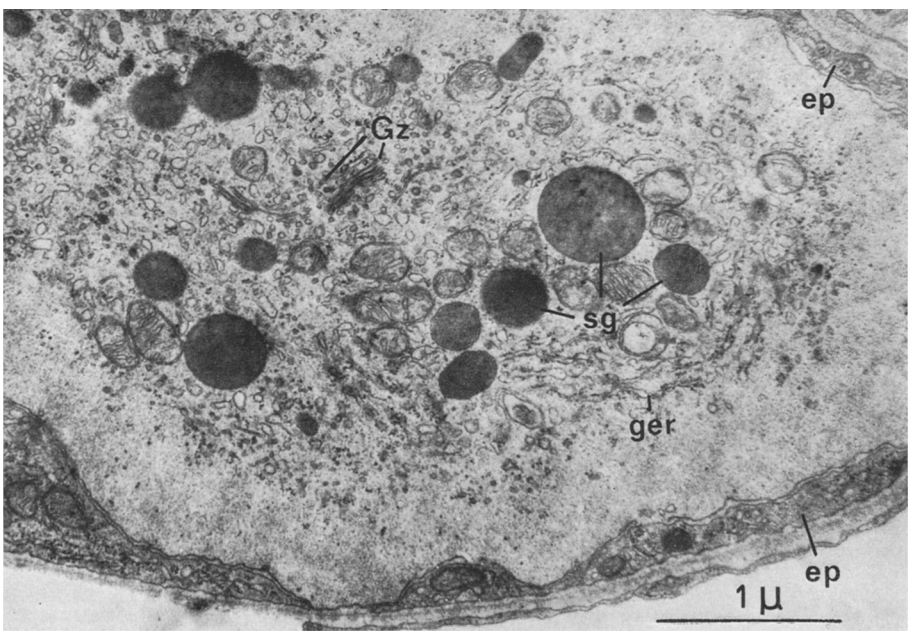

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Fig. 14. Apical part of cells in the ureter of a fresh water specimen; *vp* villous processes; *tj* tight junction; *ij* intermediate junction; *des* desmosome; *mvb* multivesicular body; *mi* mitochondrium

Fig. 15. Granular cell located between the epithelial cells (*ep*) of Bowman's capsule; *Gz* Golgi zone; *sg* secretory granules; *ger* granular endoplasmic reticulum

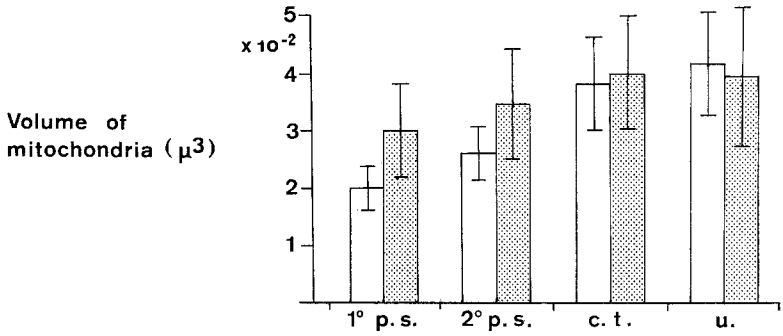

Fig. 16. Volume of the mitochondria per surface unit ($1\mu^2$) of basal membrane folds in the cells of the first (*1° p. s.*) and second (*2° p. s.*) proximal segments, the collecting tubules (*c. t.*) and the ureters (*u*) of two groups of 5 sticklebacks, one from fresh water (*white bars*) and one from the sea (mottled bars). Means $\pm$ S. E

5. Ureter

Fresh Water. The cells forming the epithelium of the ureter are histochemically and structurally similar to those of the collecting tubules. Small dark granules, however, have not been observed (Fig. 14). The ureter can be distinguished in tissue sections by the large diameter of its tubules, and by the great thickness of the muscular coat around the epithelium. Furthermore, the eosinophilia of the cytoplasm in ureter cells exceeds that of the cells of the collecting tubule.

There is a marked variation in height of the cells in different parts of the ureter. This may be partly caused by differences in the state of contraction of the surrounding muscular layer. But in general a size gradient is apparent. The lowest cells are found in the small initial ducts of the ureter. The cells have about the same height in that area as the cells of the collecting tubules. When these ducts combine to larger ducts, the height of the epithelium increases and reaches its highest value in the caudal parts of the ureters, near the bladder. Quantitative data are presented from cross sections of the main ducts of the ureters in the most caudal part of the kidney (Fig. 8).

The basal labyrinth is better developed than in the collecting tubules. The membranes are found in all parts of the cytoplasm. The fractional volume of the mitochondria is the highest found so far (22%), but the extent of the membranes per volume of cytoplasm is the lowest. Tubular and vesicular membranes of the smooth endoplasmic reticulum are frequently encountered in the apical area of the cells.

Sea Water. Compared to fresh water specimens the cell height as well as the nuclear surface are considerably reduced, by 40 and 37%, respectively ($p<0.001$). As the whole cytoplasmic area makes up part of the basal labyrinth, a marked reduction of the extent of this labyrinth is implicated by the decrease of the cell height. Since the reduction of the surface of the basal membranes per volume of cytoplasm is also considerable (about 36%; $p<0.001$), the total membrane surface per cell makes up less than 40% of the surface present in the ureter

cells of fresh water sticklebacks. The fractional volume of the mitochondria is reduced by 38% ($p<0.001$), which also means a reduction with about 60% when the volume per cell is considered.

When the volume of the mitochondria and the extent of the basal membrane folds, per unit of cytoplasm, are compared in the four types of tubular cells in fresh water fishes, two marked tendencies become apparent (Fig. 8D, E). The volume of the mitochondria increases, and the surface of the basal folds decreases, in relation to the distance of the respective tubular cell types to the glomerulus. The existence of these gradients implicates that the ratio of mitochondrial volume and membrane surface, per volume unit of cytoplasm, also shows a gradient. This ratio increases from 0.020 in the first proximal segment to 0.041 in the ureter (Fig. 16). In sea water sticklebacks the gradient is small. In these animals the mitochondrial volume as well as the extent of the membrane folds differ only slightly in the four cell types.

IV. Three Other Cell Types

Apart from the renal cells dealt with so far, three other types of cells are normally present in the kidney of fresh water as well as of marine sticklebacks.

Cells containing ovoid, electron dense granules are found in low numbers between the cells of the nephronic and the ureteral epithelia. They are identified as macrophages (Mourier, 1970).

Unicellular parasites occur in low numbers between the cells of the first and second proximal tubule and occasionally of the collecting tubules.

A third cell type, of unknown origin and function, is commonly found between the cells of the epithelium of Bowman's capsule and of the nephronic epithelia. The cells are ovoid and up to 15 μ in diameter. The cytoplasm is rather electron transparent and contains some strands of granular endoplasmic reticulum, some vesicles and short tubules of the smooth endoplasmic reticulum, and an occasional mitochondrium. Small-Golgi zones are present, and globules ($\varnothing$: 0.1–0.6 μ) with homogeneous electron dense contents (Fig. 15). These globules are negative for acid phosphatase and P.A.S. They show a high affinity for azocarmin, paraldehyde fuchsin, and Bowie's stain.

Discussion

Renal Corpuscles. In general, the renal corpuscles of marine teleosts are smaller and lower in number than those of fresh water species (Ogawa, 1961; Marshall and Smith, 1930). The degree of glomerular development has been related to the glomerular filtration rate, which is low in marine, and high in fresh water fishes (Holmes and McBean, 1963). Furthermore, the reduction of the urine flow following the migration of euryhaline teleosts into sea water has been mainly found to result from a four- to sixfold reduction of the glomerular filtration rate, e.g., in the rainbow trout *Salmo gairdneri* (Holmes and McBean, 1963), in the European eel, *Anguilla anguilla* (Sharratt *et al.*, 1964) and in the coho salmon, *Oncorhynchus kisutch* (Miles, 1971). A concommittant decrease of glomerular size may be expected. However, in the present study of the three-spined stickleback, differences in the diameter of Bowman's capsule were not observed between fishes from fresh water and specimens from the sea. The difference found in the diameter of the glomeruli,

although statistically significant, was very small. These results are not in line with the data reported by Ogawa (1968) for the same species He studied kidneys of sea water adapted sticklebacks after transfer into fresh water and observed an increase in the size of Bowman's capsule as well as of the glomeruli, with about 10% in late autumn and with about 20% in late spring. The changes were already detectable within an hour. In similar experiments, however, Lam and Leatherland (1969) were not able to confirm Ogawa's observations on *G. aculeatus*. These authors only found a transitory increase, and, like in our experiments, they did not find changes in the size of Bowman's capsules. Our results indicate that the considerable reduction of the rate of glomerular filtration during migration to the sea is accompanied by a relatively small reduction of the glomerular volume. Therefore, changes of the glomerular filtration rate are not reliably reflected in the glomerular size.

The high numbers of mesangial cells found in specimens in sea water, as opposed to those living in fresh water, point to a proliferation of these cells during seaward migration. The cell bodies and the long cytoplasmic processes of these cells may form a kind of barrier to filtration. Observations of Hickman and Trump (1969) are consistent with our results, as they concluded, after studying some fresh water and marine species of teleosts, that the mesangium is considerably more developed in marine fishes.

The nature of mesangial cells in vertebrates is not clear. These cells have been regarded as homologous to endothelial cells, to fibroblasts or to smooth muscle cells (*cf.*, Michielsen and Creemers, 1967). A relationship with juxtaglomerular cells, which are probably modified smooth muscle cells, was suggested by Dunihue and Boldosser (1963). They found that the juxtaglomerular cells. as well as the mesangial cells and the smooth muscle cells, reacted similarly to mineralocorticoid deficiency. Some kind of relationship may also exist in *G. aculeatus*. as both the juxtaglomerular cells and the mesangial cells were more numerous in marine specimens Several functions have been attributed so far to the mesangium. A role as pressure receptor controlling the secretion of renin, as supposed by Michielsen and Creemers (1967), may be a functional link with the juxtaglomerular cells. A supporting function, for maintaining the structural integrity of the glomerulus, was suggested by Kawayi and Oyama (1960). On the basis of this hypothesis, the degree of development of the mesangium may be expected to be proportional to the intensity of the filtration pressure, and accordingly, to the glomerular filtration rate. The observations on the stickleback point, however, to the opposite.

The high metabolic activity of the podocytes in fresh water specimens, as reflected by the nuclear size and the structure of the cytoplasm, is in line with the conclusion of Menefee and Mueller (1967) that these cells are actively involved in the filtration process Investigations on podocytes of several vertebrate species have revealed that the podocytes have endocytic capacities. In *G. aculeatus*, it seems likely that they have a secretory role as well (see also Mourier, 1970). Secretory activity of a similar nature as observed in the stickleback has been reported from the podocytes of man and rat (Thoenes, 1967). Secretory processes do probably not generally occur in fresh water teleosts, as they were not mentioned in other species studied like the bluegill, *Lepomis macrochirus* (Hickman and Trump, 1969) or the pike, *Esox lucius* (Linns, 1968). The staining character-

istics of the secretory globules in rat and man were, as in sticklebacks, similar to those of the basement membrane. This similarity may implicate that the podocytes take part in the formation of this membrane. There is experimental evidence in favour of this hypothesis. Kurtz and Feldman (1962) established that in rats, after labeling of the basement membrane with silver, new material was exclusively deposited on the podocytic side of this membrane. It seems possible that the rate of deposition of new material on the basement membrane is related to the glomerular filtration rate. If so, the absence of apparent secretory phenomena in the podocytes of marine sticklebacks could be interpreted as a reflection of a low filtration rate.

Juxtaglomerular Cells. The juxtaglomerular cells appeared to be much more numerous in marine sticklebacks than in fishes obtained from fresh water, which probably implicates that the production of renin is higher in sea water. Light microscopically assessed changes in the number of secretory cells—in fact, of those cells showing visible amounts of secretory material—are in general hard to interprete, as an increase of the secretion stores may reflect an increase as well as a decrease of the secretory activity (Wendelaar Bonga, 1971). For the juxtaglomerular cells, however, enhancement of the activity may be concluded from the accompanying increase in the size of the nucleus. Furthermore, the high number of these cells in marine fishes was confirmed at the ultrastructural level, where inactive cells are less likely to escape attention.

Juxtaglomerular cells have been described in many teleosts. In line with our results, several authors established that the cells are more prominent in marine species than in fresh water species (Bohle and Walvig, 1964; Meyer *et al.*, 1967; Sokabe *et al.*, 1966; Oguri and Sokabe, 1968). The report of Lagios (1968) on the euryhaline teleost *Cymatogaster aggregata* is even more convincing. He has found that transfer of the fish from sea water into lower salinities leads to cytoplasmic involution of the juxtaglomerular cells. This author, therefore, has concluded that renin is most needed in a marine environment. This conclusion has been corroborated by Kaley and Donshik (1965) as these authors have been able to demonstrate renin only in marine teleosts. However, more recent pharmacological findings are in contrast with their data. Capelli *et al.* (1970) have found a reverse relationship between renin content of the kidney, and salinity of the medium, in three euryhaline teleosts. In the carp, where juxtaglomerular cells are very scarce, a very high renin content has been demonstrated (Taylor and Davis, 1971). Therefore, the structural and pharmacological data are conflicting. It is possible, however, that renin like substances are not exclusively originating from the juxtaglomerular cells (*cf.*, Ogawa, 1967).

Nephronic Tubules and Ureters. The differentiation of the nephronic tubule, in a first and a second proximal segment and a collecting duct, was similar in fresh water and marine specimens. A distal segment, commonly found in fresh water teleosts and in some euryhaline species, as well as an intermediate segment, present in most fresh water teleosts (Hickman and Trump, 1969), were absent in the stickleback. Therefore, the nephrons of *G. aculeatus*, may be characterized as being of the marine type (*cf.*, Ogawa, 1968).

A well developed basal labyrinth is present in the cells of all renal tubules of the stickleback, as is normal for vertebrate kidneys. The architecture of this

labyrinth in the stickleback is, however, essentially different from the labyrinth described in many other vertebrate species. In mammals, three-dimensional reconstruction of tissue sections revealed that the labyrinth is formed by interdigitating cytoplasmic cell processes (Ericsson and Trump, 1969). In the stickleback, the labyrinth is constituted by infoldings of the basal cell membrane. Cytoplasmic processes penetrating adjacent cells are absent in this species, as is concluded from the rectangular profiles of the kidney cells in tissue sections.

The first proximal segment shows marked structural similarities with the first tubular segment in other glomerular vertebrates, from the myxinoid ureteric duct to the mammalian proximal tubule (Gritzka, 1963; Ericsson and Trump, 1969). These epithelia have in common an alkaline phosphatase- and P.A.S.-positive brushborder, a system of small coated tubules and vesicles, and a well developed lysosomal apparatus. A segment of this type is absent in aglomerular teleosts, which is in line with its function of reabsorption of substances like glucose and macromolecules, which have passed the glomerular filtration membrane.

Several studies have shown that the coated tubules and vesicles take part in the reabsorption of macromolecules, which are subsequently degraded in the lysosomes (Ericsson and Trump, 1969). In the stickleback, tissue reconstruction from serial sections revealed that the tubular and vesicular structures form a continuous membrane-lined system channeled between the tubular lumen and the lysosomal apparatus. A system of this kind is not limited to kidney cells, but has been described by Matler *et al.* (1968) in mammalian Kupffer cells in the liver and in other macrophage-like cells where it is also known to be involved in endocytosis. The authors suggested to call this process "micropinocytosis vermiformis".

The epithelium of the second proximal segment shares its apical differentiations—a brush border and a great number of uncoated vesicular structures—with the second segment of other glomerular teleosts (Hickman and Trump, 1969). Epithelia of this type have also been encountered in the nephrons of some aglomerular fishes (Bulger, 1965; Olsen and Ericsson, 1968). The function of the vesicles is not clear. Olsen and Ericsson have suggested that they may be involved in the uptake of substances from the lumen of the nephron. In the stickleback, the cells of the second proximal segment may also take part in the uptake of material from the primary urine. The fractional volume of the lysosomes, although lower than in the cells of the first proximal segment, is substantially higher than in the collecting tubule and the ureter. It seems too high to account exclusively for the autophagous digestion of worn out cell organelles. Heterophagic digestion is therefore indicated. Whether the clear vesicles are engaged in the uptake of substances or in the secretion of materials (as suggested by their occurrence in aglomerular teleosts) remains to be established. Lee (1970) attributed a role in ammonia excretion to vesicles of a similar appearance in Henle's loop in the rat, as he demonstrated transaminase activity inside these vesicles.

The ultrastructure of the cells of the collecting tubules of the stickleback is similar to that described in some other teleosts (Hickman and Trump, 1969; Ericsson and Trump, 1969). The presence of secretory granules in part of the cells of the collecting tubule seems, however, typical for the stickleback. Granules of the same size and appearance have been described by Mourier (1971) in his

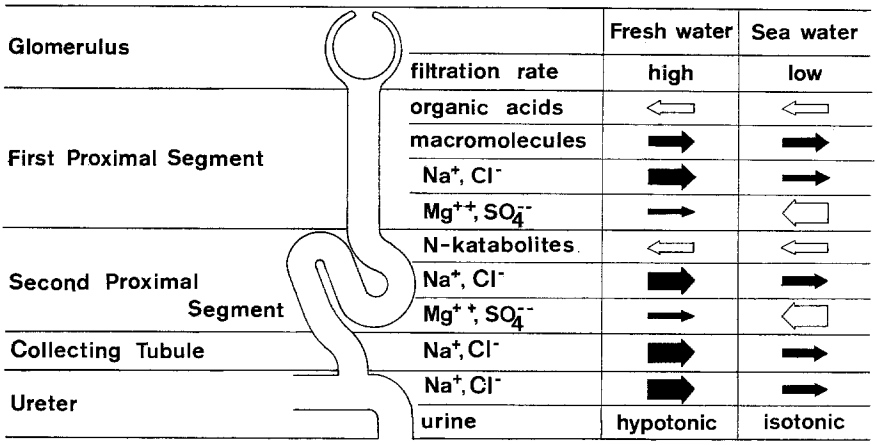

Fig. 17. Generalized model of the main functions of the epithelia of nephronic segments and ureters of euryhaline teleosts in fresh water and sea water; black arrows reabsorption; white arrows secretion; the thickness of the arrows is an indication of the intensity of the processes (after data from Hickman and Trump, 1969)

study of the transformation of the kidney of the male stickleback, during the reproductive period. They have been found in the second proximal segment, just before and during the change of the cells into slime producing cells. The relation with the present observations is not known.

The cells of the collecting tubules and of the ureter have many structural features in common. The small differences found in these study (the cytoplasm of the ureter cells is more eosinophilic and contains more membranes of the smooth endoplasmic reticulum) do not exclude that the cells of the collecting tubules and of the ureter are of the same type.

Marked quantitative structural differences were found by comparing the nephronic tubules and the ureters of sticklebacks adapted to fresh water with those of specimens caught after migration into the sea. For a functional interpretation of these structural differences, physiological information is needed. Detailed data on the function of the various tubular epithelia are absent as far as *G. aculeatus* is concerned. However, physiological investigations on a fair number of teleost species have revealed a great functional similarity between species of the same environment. This enabled Hickman and Trump (1969) to design generalized models of kidney function in fresh water, marine, and euryhalinef teleosts. The results of measurements of urine flow and of determinations of urine composition in *G. aculeatus*, obtained by Lam and Leatherland (1969) are in line with those reported for other euryhaline species. Furthermore, the ultrastructural similarities of the renal epithelia of the stickleback with those of other teleosts studied with the electron microscope, are evident. It is probable, therefore, that the general pattern of kidney function in euryhaline fishes (Fig. 17) also holds for *G. aculeatus*.

All quantitative parameters used in this study showed that the renal cells were more developed in fresh water than in the sea. This is in line with the

generally accepted opinion, based on physiological research, that osmotic and ionic regulation in the kidney is more intense in fresh water (Hickman and Trump, 1969). Morphometrical studies on renal epithelia are very scarce. Estimates of renal tubular surface, in relation to the body surface, have been made by Nash (1931). His data suggest that tubular development is better in fresh water species, but the differences are small.

The differences observed in the present study between marine and fresh water sticklebacks comprise cellular height, nuclear size, relative volumes of lysosomes and mitochondria, and the extent of the infoldings of the basal cell membrane. The measurements of epithelial height and of nuclear size reveal that general metabolic activity is lower in sea water. The data on lysosomes point to a lower rate of intracellular digestion in sea water, which may include autophagous as well as heterophagous processes.

The reduced extent of the basal labyrinth in all renal tubules indicates a lowered ion transport activity in sea water. Evidence that the ion transport mechanisms are located in the basal cell membranes has been obtained by biochemical (Kinne and Schmidt, 1971) as well as histochemical (Ericsson and Trump, 1969) techniques. The enzymes engaged in ion transport, like sodium- and potassium-activated and magnesium-activated ATPase appeared to be bound to those membranes. The basal cell membranes take probably part in both secretion and reabsorption of ions, since the basal labyrinth is well developed in glomerular as well as aglomerular teleosts (Bulger, 1965; Ericsson, 1968). A quantitative relationship between transport intensity and extent of the basal infoldings was demonstrated by Schmidt *et al.* (1970). They added folic acid *in vitro* to kidney cells of the rat and found a considerable decrease of the content of sodium-potassium-activated ATPase and a reduction of the membranes of the basal labyrinth.

The cells of the nephron and of the ureter are less developed in sticklebacks from the sea than in fresh water specimens. However, the differences varied in the respective cell types. The differences in cell height were small for the proximal segments, especially for the second, but were rather substantial for the collecting tubules and even more prominent in the ureters. The difference in the mean nuclear surface showed a similar tendency. These phenomena indicate that the reduction in metabolic activity after migration to the sea is small in the first and almost absent in the second proximal segments, but considerably more in the other ducts. This conclusion can be correlated with functional changes occurring during migration in the renal cells (Fig. 17; *cf.*, Hickman and Trump, 1969; Miles, 1971). When the fishes enter sea water, the reabsorptive activities diminish in all tubular cells. In the first and second proximal segments, however, the rate of nitrogen katabolism is assumed to be similar as in fresh water. Furthermore, the reduction in the reabsorption of monovalent ions is compensated in these segments by an intense secretion of divalent ions. Thus, a limited reduction of the cell activity in the first and second proximal segments, as indicated by our results, is in line with physiological evidence The function of the collecting tubules and the ureters is rather similar in fresh water and in the sea and concerns mainly the reabsorption of sodium and chloride. The reduced reabsorption of these ions in sea water, therefore, obviously accounts, at least partly, for the reduced cellular activity indicated by our morphometrical data.

However, the mentioned functional changes occurring during migration to the sea do not account for all morphometrical differences found between the four cell types. An additional factor is probably involved, especially as far as the basal labyrinth is involved. In fresh water specimens a marked structural gradient was found in the mitochondrial volume per surface unit of basal membrane folds. The energy needed for ion transport processes across the basal membranes is supplied by the mitochondria which are associated with these membranes. Thus, since almost all mitochondria in the kidney cells appeared to be confined within the basal labyrinth, it may be assumed that the energy needed for ion transport is proportional to the mitochondria to membrane ratio. This implies that in fresh water the energy needed for the transport of ions, per surface unit of cytoplasm, increases with the distance of the cells concerned from the glomeruli.

Due to the reabsorption of ions, osmotic and ionic gradients arise in the kidney tubules of fresh water fishes (Fleming and Stanley, 1965; Hickman and Trump, 1969). These gradients also increase in height with the distance from the glomeruli. It may be assumed that the energy needed for ion transport is related to the osmotic and ionic differences between the blood and the fluid in the renal ducts. Therefore, it is suggested that the structural gradient in the renal ducts, expressed as the mitochondria to membrane ratio, is functionally linked with the ionic and osmotic gradients. Especially the osmotic gradient may account for the structural gradient. In the renal ducts of sea water fishes, where an apparent structural gradient is absent, the osmotic gradient is very small while ionic gradients do exist (Hickman and Trump, 1969; Miles, 1971).

In many euryhaline fishes, including the stickleback, osmoregulation in fresh water is under control of prolactin (Ball, 1968). This hormone furthermore facilitates the transfer of sticklebacks from the sea to fresh water, by accelerating the osmoregulatory adjustments (Lam, 1968). The first results of our study of the effect of injections of ovine prolactin on the structural changes in the kidney, during and after transfer of sticklebacks to fresh water, have shown that the structural adjustments to fresh water conditions are also accelerated by prolactin (Wendelaar Bonga, 1973).

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