Ultrastructure of intestinal and gall-bladder epithelium in the teleost *Gasterosteus aculeatus* L., as related to their osmoregulatory function

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Summary. Intestinal and gall-bladder epithelial cells in sticklebacks have been examined in ultrathin sections and freeze-etch replicas. Enterocytes throughout the intestine appear to have a well-developed basal labyrinth similar to that of renal tubular cells, consisting of baso-lateral infoldings closely associated with numerous mitochondria. The lumen inside these intracellular membranes is continuous with the intercellular space via pores. Such a membrane system is also present in the epithelial cells lining the gall bladder, distinguishing them from gall-bladder cells of higher vertebrates. Morphometric analysis indicates that the basal labyrinth of enterocytes in the posterior part of the intestine increases markedly in both sexually mature males and androgen-treated females. This does not occur in the anterior part or gall bladder. In sticklebacks, androgens cause reduced urine excretion and enhanced fluid release via the anus. We conclude that the cells lining the intestine and gall bladder possess an extensive basal labyrinth that may function as a backward channel system, enabling fluid to be produced in the intestine of fish. The androgen-induced increase in the extent of the basal labyrinth in the posterior part of the intestine may be related to the enhanced rate of intestinal fluid excretion observed in sexually mature male sticklebacks.

Key words: Basal labyrinth – Electron-microscopical morphometry – Testosterone – Intestinal fluid – Hydromineral regulation – Teleosts

The kidneys of freshwater fish provide the main pathway by which osmotically accumulated water is excreted. They have a high glomerular filtration rate (GFR) and the epithelial cells of the renal tubules have a high ion-resorptive capacity; this enables freshwater fish to excrete large volumes of dilute urine (Hickman and Trump 1969; Hender son et al. 1978).

During sexual maturation, under the influence of testosterone, marked structural changes take place in the glomeruli and most of the renal tubule cells in the kidneys of male stickleback (Mourier 1970, 1972; De Ruiter 1981; De Ruiter and Mein 1982). This process, which accompanies the transformation of the kidneys into glands that secrete mucus used for nest-building, leads to a considerable loss of ionresorptive capacity of the renal tubules and to a reduction of urine flow (De Ruiter 1980, 1981; De Ruiter and Mein 1982). The osmotic permeability of the gills (the main route for osmotic water uptake in fish) to water is unaltered in sexually mature fish (De Ruiter 1980), and therefore the necessity for water excretion in these fish is likely to be as high as in fish with normal kidneys. Thus in sexually mature males water balance will be disturbed unless alternative routes for water elimination are employed.

We consider that the intestine can provide a compensatory route in these fish, since we have found that excretion of fluid via the anus is considerably enhanced in mature males (De Ruiter 1980). It is unlikely that intestinal fluid is secreted via ultrafiltration, as occurs in the kidneys, since structural indications of ultrafiltration sites, e.g., filtration-slit membranes, have never been observed in fish intestine. A more likely possibility is the secretion of water by solute-linked transport across the intestinal epithelium by a mechanism described in the backward channel, standing gradient/osmotic flow model (Diamond and Bossert 1967, 1968; Berridge and Oschman 1972; Dibona and Mills 1979).

The presence of a basal labyrinth and associated mitochondria are considered to be characteristic for solute-linked water transport. Such a basal labyrinth has been found in enterocytes of *Carassius auratus*, *Salmo irideus* (Yamamoto 1966), *Cyprinus carpio* (Noaillac-Depeyre and Gas 1973) and *Ctenopharyngodon idella* (Stroband and Debets 1978). Our preliminary observations on sticklebacks indicate that the enterocytes of sticklebacks also contain a basal labyrinth (De Ruiter 1978, 1980). The presence of such a labyrinth may explain the capacity of the fish to excrete fluid from the anus. The gall-bladder may also contribute to the production of intestinal fluid. It is connected to the intestine via the bile duct and in many vertebrates the gall-bladder epithelium shows ultrastructural features common to water and ion transporting membranes (Diamond and Bossert 1967, 1968; Berridge and Oschman 1972).

The aim of the present study was to investigate whether the epithelia lining the intestine and gall-bladder contain...
a basal labyrinth that can function as a backward channel system for fluid production. To this end ultrathin sections and freeze-etch replicas were examined. We also wished to determine whether a correlation exists between the extent of the basal labyrinth and the rate of fluid production. Morphometric analysis of the effects of sexual maturation and androgen treatment on the development of the basal labyrinth were therefore carried out. The androgen employed, methyltestosterone, is known to effect typical male reproductive behaviour, nuptial coloration, and the glandular transformation of the kidneys in sticklebacks (Wai and Hoar 1963; Mourier 1972; De Ruiter 1981; De Ruiter and Mein 1982); it also stimulates intestinal fluid production to a similar extent as sexual maturation under natural conditions (unpublished observations).

Materials and methods

Sexually immature, adult sticklebacks, of the *trachurus* form, were obtained from laboratory stock. Their body lengths varied from 60–71 mm in females and from 46–69 mm in males. Their diet consisted of *Tubifex*, *Daphnia* and fresh-ground bovine heart. They were raised and kept in aquaria with tap water (Ca$^{++}$ content: 1.65 mmol/l; 15 mOsm/l) for at least 2 months at 20° C on a daily photoperiod of 8 h, starting at 8 am. The numbers of animals used for the experiments are given in the Figures and Table 1.

Experimental procedures

Sexual maturity was induced by exposing immature males to a daily photoperiod of 16 h at 20° C (Baggerman 1972) and was considered to have been attained once a nest had been built. Controls were sexually immature males and females held on a light regime of 8L/16D at 20° C.

Methyltestosterone treatment

Because no immature males were available for this experiment, immature female sticklebacks were used. The experiments of Wai and Hoar (1963) and of Mourier (1972), and our pilot studies have shown that administration of methyltestosterone to either gonadectomized males or sexually immature females induces typical male secondary sex characters and renal/mucous transformation. Furthermore, from our pilot studies, we have concluded that sexually immature female sticklebacks can be considered immature males whereas methyltestosterone-treated females resemble mature males with respect to all measured parameters in these experiments. Methyltestosterone (the kind gift of Organon Laboratories, Oss, The Netherlands) was administered according to the method described by De Ruiter (1981) and De Ruiter and Mein (1982) at a concentration of 0.5 mg/l of tap water, for a period of 2 weeks.

Electron microscopy

The fish were decapitated and the intestine and gall-bladder rapidly removed. The distribution of the basal labyrinth over the intestine is unequal (Noaillac-Depeyre and Gas 1973; Stroband and Debets 1978). The results of our pilot studies on comparable control and experimental groups have shown that the distribution of the basal labyrinth in the anterior and middle intestinal segment is similar but different from that of the posterior region. Therefore we regarded the intestine as comprising two segments, the ante-
The epithelium lining the intestinal lumen consists mainly of a single layer of columnar absorbing cells. Some mucocytes, lymphocytes, granular leucocytes and macrophages are occasionally found between these cells. Underneath the epithelial layer is a basal lamina, a layer of connective tissue containing small capillaries and circular and longitudinal layers of smooth muscle cells (Fig. 1).

**Ultrastructure of the intestinal absorbing cells**

- *Immature sticklebacks.* The luminal surface of the absorptive cell is provided with numerous glycocalyx-coated microvilli. Immediately below the striated border is the terminal web, which contains many microtubules and microfilaments in addition to fibrillar structures originating from the microvilli. Cytoplasmic organelles are scarce in this apical region apart from some endocytotic vesicles and free ribosomes. Adjacent epithelial cells are connected near the lumen by junctional structures collectively known as the junctional complex. This region consists of a well-developed tight junction (zonula occludens), an intermediate junction (zonula adherens) and desmosomes (Fig. 2). In addition to characteristic absorptive cell structures such as lysosomes and residual bodies, the apical cell cytoplasm contains a
Fig. 2. Apical portion of enterocytes showing characteristic structures of absorptive cells such as microcilli (mv) and lysosomes (ly). The lateral cell membranes (lc,m) in this area show the typical structure of a junctional complex consisting of a tight junction (j), intermediate junction (ij) and desmosome (des); tw terminal web; mit mitochondria. × 25000

Fig. 3. Basal part of enterocytes showing membranes of the basal labyrinth (arrows) with closely associated mitochondria (mit) and sites (arrowheads) where membrane pairs of the basal labyrinth are continuous with the basal cell membrane (bcm); bl basal lamina; asterisk cytoplasm of a non-absorptive intestinal epithelial cell. × 16700

Figs. 4, 5. Freeze-etch micrographs of enterocytes showing details of the basal cell membrane (Fig. 4) and of the lateral cell membrane (Fig. 5). The basal labyrinth appears to be continuous with the extracellular space (arrowhead); where the cell membrane contacts the basal labyrinth, pores (arrows) can be observed on the lateral cell membrane; these are frequently arranged in rows (Fig. 5). PF protoplasmic fracture face; PS protoplasmic surface; gj gap junction; mit mitochondria. Fig. 4 × 15800; Fig. 5 × 22500

poorly developed smooth endoplasmic reticulum, mitochondria, free ribosomes, strands of granular endoplasmic reticulum, small clear vesicles and multivesicular bodies. The large oval nuclei are situated near the cell base, with the Golgi system in most epithelial cells lying in a supranuclear position. The microbodies occurring mainly in the perinuclear region are often provided with a crystalline-structured core and are probably peroxisomes (De Ruiter and Veenhuis, in preparation).

The lateral plasma membranes of adjacent epithelial cells do not show interdigitations with each other. The absorbing cells exhibit a complex system of frequently branching membrane infoldings originating from the lateral and basal cell membranes. These infoldings are generally oriented parallel to the long axis of the cells and are mainly confined to the basal two-thirds of the cytoplasm (Fig. 3). The lumina inside this labyrinth are continuous with the extracellular space via pores in the basal and lateral cell membranes, as shown in freeze-etch replicas (Fig. 4). In the lateral cell membranes, the pores are sometimes arranged in rows (Fig. 5). The infolded membrane pairs are thicker than the membranes of the granular endoplasmic reticulum, and are, in contrast to the latter membranes, asymmetric in cross section. The surfaces of these membranes are covered with a fuzzy coating that is continuous with the glycocalyx covering the outer cell membranes. The basal part of the cells contains an abundance of granular

Fig. 6. Extent of basal labyrinth membranes and mitochondrial area in the intestinal epithelium of controls [immature male sticklebacks (I) and females (C)] and experimental animals [sexually mature males (M) and methyltestosterone-treated females (MT)]. *P<0.05 when compared to control posterior segments. **P<0.001 when compared to both control posterior segments and all anterior segments.
endoplasmic reticulum, free ribosomes and mitochondria, the latter usually being closely associated with the membranes of the basal labyrinth. The morphometric data presented in Fig. 6 show that, in the controls of both experimental groups, no differences were found in the extent of the basal labyrinth or mitochondrial area between the anterior and posterior intestinal segments or between the corresponding segments in both groups.

b) Sexually mature males and androgen-treated female sticklebacks. The fine structure of the absorptive cells in these two groups of sticklebacks is similar to that of the controls.

In sexually mature males and testosterone-treated females, the extent of membranes of the basal labyrinth in the posterior intestinal segment is significantly larger than the anterior intestinal segment (Fig. 6). Moreover, the extent of basal labyrinth in corresponding intestinal segments is almost equal in sexually mature males and androgen-treated female sticklebacks.

In addition, the posterior intestine of sexually mature males and androgen-treated females exhibits significantly higher values for mitochondrial area than the same segment in the controls (Fig. 6).

Gross anatomy and histology of the gall bladder

The pear-shaped gall bladder lies on the right-hand side of the body cavity, enclosed between the liver and the stomach. The bile duct opens into the anterior intestine immediately behind the pyloric sphincter. A single layer of columnar cells, with large nuclei, constitutes the epithelium. Underneath this layer are a basal lamina, a connective tissue layer and a muscular layer.

Ultrastructure of the gall-bladder epithelium

a) Immature sticklebacks. The apex of the gall-bladder epithelial cells bears numerous microvilli, which are shorter and less regularly oriented than those of the brush border of the intestinal epithelium (Fig. 7). Filamentous rootlets of the microvilli penetrate the terminal web, which is devoid of cytoplasmic organelles. A junctional complex similar to that described for the intestinal epithelium connects adjacent epithelial cells. The apical cytoplasm contains numerous mitochondria, free ribosomes, granular endoplasmic reticulum, a poorly developed smooth endoplasmic reticulum, and occasionally lysosomes, multivesicular bodies and microbodies (Figs. 7, 10). The latter can be considered peroxisomes (De Ruiter and Veenhuis, in prep.). The large nuclei usually appear lobated in ultra-thin sections and lie near the cell base, with the Golgi system positioned supranuclearly (Fig. 7).

Below the apically located junctional complex, the lateral cell boundary remains relatively straight, with desmosomes along the length of the intercellular space; it lacks...
complex interdigitations (Figs. 7, 8, 9, 10). The gall-bladder epithelial cells exhibit a basal labyrinth comparable with that of the intestinal absorptive cells. In the basal region of the cell cytoplasm, there is an abundance of granular endoplasmic reticulum and free ribosomes, whereas the distribution of the mitochondria in the cell seems to be random (Figs. 7, 8, 9, 10).

b) Sexually mature male sticklebacks. In contrast to the basally located nuclei of the epithelial cells of the gall bladder of immature sticklebacks, the nuclei in mature animals are mostly situated near the cell apex (Fig. 11).

The mitochondria, although present throughout the cytoplasm, are more concentrated in the basal part of the cells (Fig. 11). This differs from their location in immature control fish (Figs. 9, 10). Otherwise, no differences can be observed between the ultrastructure of gall-bladder epithelial cells in immature and mature fish.

Basal labyrinth and mitochondrial area measurements do not reveal any difference between immature and mature fish (Table 1). We therefore have not examined the effect of methyltestosterone on gall-bladder cells.

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<th>Basal labyrinth ( \mu m^2/\mu m^2 ) cytoplasm</th>
<th>Mitochondria ( \mu m^2/\mu m^2 ) cytoplasm</th>
<th>(n)</th>
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<tr>
<td>immature</td>
<td>865 ± 110</td>
<td>0.11 ± 0.04</td>
<td>3</td>
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<tr>
<td>mature males</td>
<td>1040 ± 250</td>
<td>0.14 ± 0.03</td>
<td>3</td>
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<td>significance</td>
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Discussion

Our electron microscopical observations on the epithelial cells lining the intestine and gall-bladder of sticklebacks demonstrate the presence of an extensive basal labyrinth. As far as the intestine is concerned, this labyrinth appears to be more extensive than in other fish species studied so far. In sticklebacks enterocytes are provided with a well-developed basal labyrinth over the entire length of the intestinal tract. This is in contrast with other fish such as Caras-
sins auratus (Yamamoto 1966), Ctenopharyngodon idella (Stroband and Debets 1978) and Cyprinus carpio (Noaillac-Depeyre and Gas 1973) where a basal labyrinth has only been observed in the cells of the anterior part (the former two species) or the posterior part (the latter species) of the intestine. The ultrastructure of the basal labyrinth of the stickleback intestinal epithelial cells closely resembles that of its renal tubule cells, as described by Wendelaar Bonga and Veenhuis (1974). However, we have observed fewer openings from the basal labyrinth to the basal lamina than in the nephronic cells. Insect midgut epithelial cells also have only a few narrow openings from the basal labyrinth to the basal lamina (Berridge and Oschman 1972).

The basal labyrinth has been considered a channel system for solute-linked water transport, and is a characteristic feature of cells involved in fluid transport from mucosa to serosa (forward channel system) or vice versa (backward channel system; Diamond and Bossert 1968; Berridge and Oschman 1972; Dibona and Mills 1979). At the ultrastructural level, stickleback enterocytes closely resemble the cells of the insect Malpighian tube and of the agglomerular renal tubule of marine teleosts; these are known to transport fluids from serosa to mucosa by a mechanism involving osmotic filtration (Berridge and Oschman 1972). We have observed that both female and sexually immature male sticklebacks excrete substantial amounts of (an isotonic) fluid via the anus (De Ruiter 1980). We propose that the fluid production of the intestine is, at least partially, effected by enterocytes using their basal labyrinth as a backward channel system.

Two observations support this proposition. Firstly, sexually mature males excrete a larger amount of (hypotonic) intestinal fluid than female or immature male fish (De Ruiter 1980). The present finding shows that the basal labyrinth has extended markedly after sexual maturation or androgen treatment. Secondly, according to the backward channel concept, the rate of transepithelial fluid transport is controlled by the osmotic permeability of the epithelial cell membranes to water (Diamond and Bossert 1968). We have observed that the osmotic permeability of the intestinal wall to water is increased in sexually mature male sticklebacks (De Ruiter et al. 1984).

Another possible contributor to intestinal fluid secretion in sticklebacks is the gall-bladder. The ultrastructure of the epithelial cells of the stickleback gall-bladder, especially the structure of the basal labyrinth, resembles the ultrastructure of their intestinal cells and renal tubule cells. The basal structure of the basal labyrinth, resembles the ultrastructure of their intestinal cells and renal tubule cells. The basal

On the other hand, the structure of stickleback gall-bladder epithelium is dissimilar in several respects to the gall-bladder epithelium of higher vertebrates such as rabbits, guinea pigs and humans (Kaye et al. 1966; Berridge and Oschman 1972). The complex interdigitations of the lateral cell membrane in the gall-bladder epithelium of higher vertebrates are absent in sticklebacks, except for some slight interlocking. In the latter epithelium, desmosomes between adjacent cells are evenly distributed over the whole length of the lateral cell membranes, whereas in the gall-bladder of higher vertebrates desmosomes are restricted to the apical junctional complex (Diamond and Tormey 1966; Berridge and Oschman 1972).

In immature male sticklebacks, the distribution of mitochondria in the gall-bladder epithelial cells exhibits some apolarity, with a higher concentration in the apical region. This concentration of mitochondria in the apical area has also been described in higher vertebrates; this suggests that these mitochondria provide the energy supply for absorptive processes at the luminal cell borders (Diamond and Tormey 1966; Berridge and Oschman 1972). However, in sexually mature males, the mitochondria are accumulated in the basal regions of the gall-bladder cells. This phenomenon indicates that the processes occurring in the basal labyrinth of the gall-bladder cells of sexually mature males are more energy-consuming than in sexually immature control fish.

The differences in gall-bladder epithelial ultrastructure between sticklebacks (especially mature males) and higher vertebrates can be explained by assuming that, in sticklebacks, the gall-bladder epithelium contributes to intestinal fluid production by transporting water from the serosal side of the cells into the gall-bladder lumen, which is connected with the intestinal tract. This assumption is supported by our preliminary experiments with immature and mature male sticklebacks, using intraperitoneally injected aqueous amaranth solution; the contents of both the anterior intestine and the gall bladder were dyed within 30–60 min, even when the ducts from liver to gall-bladder and from gall bladder to intestine were ligated.

Studies on higher vertebrates have shown that the liver secretes iso-osmotic fluid into the bile duct (Berridge and Oschman 1972) and therefore the liver cannot be excluded as an additional pathway in intestinal fluid production.

We conclude that the cells lining the intestinal and gall-bladder wall in sticklebacks have a well-developed basal labyrinth. As far as the intestine is concerned, this labyrinth appears to be more extensive than in other fish species. Both epithelia therefore seem well equipped for solute-linked water transport from the serosal to the mucosal side. Such a mechanism explains the capacity of sticklebacks to excrete fluid via the anus, a process that may contribute to the elimination of osmotically accumulated water in freshwater fish.

There is little evidence for water excretion via gall bladder and intestine of freshwater-adapted fish other than sticklebacks, although the role of the digestive tract in maintaining water balance of seawater-adapted fish is well established (Hickman and Trump 1969; Shehadeh and Gordon 1969; Hirano and Bern 1972; Hirano and Mayer-Gostan 1976). In the male stickleback, the capacity of the intestine to excrete fluid increases markedly during sexual maturation (De Ruiter 1980). This may be interpreted as a compensatory mechanism for the elimination of osmotically accumulated water, since, in these fish, urine excretion is reduced as a consequence of the transformation of a greater part (about 90%) of the kidneys from excretory organs to mucus secreting glands (De Ruiter 1980; De Ruiter and Mein 1982). The present data show that androgens, which induce this transformation and the disappearance of the basal labyrinth from most of the renal tubule cells (De Ruiter and Mein 1982), concomitantly stimulate the expansion of the basal labyrinth in the enterocytes of the posterior intestine.

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Accepted November 22, 1984