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Peroxisomes in intestinal and gallbladder epithelial cells of the stickleback, *Gasterosteus aculeatus* L. (Teleostei)

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Summary. The occurrence of microbodies in the epithelial cells of the intestine and gallbladder of the stickleback, *Gasterosteus aculeatus* L., is described. In the intestine the organelles are predominantly located in the apical and perinuclear zone of the cells and may contain small crystalline cores. In gallbladder epithelial cells the microbodies are distributed randomly. The latter organelles are characterized by the presence of large crystalloids. Cytochemical and biochemical experiments show that catalase and D-amino acid oxidase are main matrix components of the microbodies in both the intestinal and gallbladder epithelia. These organelles therefore are considered peroxisomes. In addition, in intestinal mucosa but not in gallbladder epithelium a low activity of palmitoyl CoA oxidase was detected biochemically. Urate oxidase and L-α hydroxy acid oxidase activities could not be demonstrated.

Key words: Peroxisomes – D-amino acid oxidase – Catalase – Cytochemistry – Intestinal epithelium – Gallbladder epithelium – *Gasterosteus aculeatus* (Teleostei)

Peroxisomes are cell organelles biochemically defined by the presence of catalase and one or more H_2O_2-producing oxidases (De Duve and Baudhuin 1966). At the ultrastructural level these organelles show a fine granular matrix surrounded by a single membrane of approximately 7 nm and occasionally contain crystalline inclusions (for ref. see Hruban and Rechcigl 1969; Böck et al. 1980; Veenhuis et al. 1983). Despite their simple morphology peroxisomes show a great diversity in enzyme repertoire and, dependent on the tissue, they may be involved in different metabolic processes such as fatty acid oxidation, lipid biosynthesis or gluconeogenesis (Kindl and Lazarow 1982).

The knowledge on the occurrence and function of peroxisomes in teleost tissues is limited. Microbody-like organelles have been described in liver and kidney cells of several teleosts (Gritzka 1963; Trump and Bulger 1968; Hruban and Rechcigl 1969; Kramar et al. 1974; Goldenberg 1977; Goldenberg et al. 1978). In kidney cells of the three-spined stickleback, *Gasterosteus aculeatus*, such organelles have been characterized cytochemically as peroxisomes (Veenhuis and Wendelaar Bonga 1977).

This paper describes the presence of peroxisomes in epithelial cells lining the lumina of the intestine and gallbladder of the three-spined stickleback, *Gasterosteus aculeatus* L. It appeared that catalase and D-amino acid oxidase are important components of these peroxisomes.

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Materials and methods

Sexually immature adult female three-spined sticklebacks (*Gasterosteus aculeatus* L.) of the *trachurus* form were obtained from freshwater laboratory stock (Veenhuis and Wendelaar Bonga 1977). Part of the fish were adapted for at least six weeks to full-strength Wimex® artificial seawater (De Ruiter 1980).

Biochemistry

For D-amino acid oxidase assay, the intestinal mucosa of individual fish was homogenized in about 250 µl distilled water in an all-glass tissue grinder. For the other assays, the intestinal mucosa of 4 to 6 fish was pooled in 50 mM phosphate buffer, pH 7.4, containing 30 mM sucrose. The tissues were homogenized in a Potter Elvehjem tube, subsequently sonified at 20 KHz for 3 min at 0° C with an MSE 100 W ultrasonic desintegrator (M.S.E. Ltd., Crawley, Sussex, England) and centrifuged at 20000 *g* for 30 min to remove intact cells and debris. Gall bladders of 4–6 fish were pooled for all assays and homogenates prepared in the same way as for the pooled intestinal tissue.

Catalase was assayed by the spectrophotometric method of Lück (1963). Oxidase activities were determined using 50–100 µl of freshly prepared crude homogenates with a Clarke oxygen electrode (Biological Oxygen Monitor, Yellow Springs Instr. Co., Inc., Yellow Springs, Ohio, USA) at 30° C. D-amino acid oxidase, L-α-hydroxy acid oxidase and urate oxidase were assayed as described previously (Veenhuis and Wendelaar Bonga 1977).

Palmitoyl CoA oxidase was assayed in 50 mM phosphate buffer, pH 7.4, containing 0.7 mM palmitoyl CoA, and 3 mM KCN to inhibit mitochondrial palmitoyl CoA oxidation.

Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

Electron microscopy and cytochemistry

Catalase activity was demonstrated with the conventional diamino-benzidine (DAB)-technique (Roels et al. 1975; Veenhuis and Wendelaar Bonga 1977). Control experiments were performed in the absence of H_2O_2, in the presence of 50 mM 3-amino-1,2,4-triazole as a specific inhibitor of catalase activity or in the presence of 1.5 mM KCN to avoid mitochondrial staining (Roels et al. 1975).

For the localization of D-amino acid oxidase, urate oxidase, L-α-hydroxy acid oxidase and palmitoyl CoA oxidase the cerium technique was used (Veenhuis et al. 1976; Veenhuis and Wendelaar Bonga 1977). All incubations were performed at room temperature under continuous aeration. Control experiments were performed in the absence of substrate or under anaerobic conditions. After the incubations, the tissue blocks were washed in 0.1 M cacodylate buffer, pH 6.0, for 15 min and postfixed in 0.5% OsO_4 and 2.5% $K_2Cr_2O_7$ in the same buffer at pH 7.2 for 90 min at 0° C. The material was poststained in 1% uranyl acetate, dehydrated in a graded ethanol series and embedded in either Epon 812 or Spurr's epoxy resin. Ultrathin sections were cut with a diamond knife on an LKB-Ultratome and examined in a Philips EM 300 without additional staining.

Results

Biochemical observations

In tissue homogenates of freshwater stickleback intestinal mucosa and gallbladders, catalase and D-amino acid oxidase activities were detected (Table 1). High specific activities of D-amino acid oxidase were found in the intestine, allowing measurement in homogenates of individual fish. The enzyme was specific for D-amino acids (L-amino acids were not oxidized) and showed high preference for D-alanine as substrate. Other D-amino acids, such as D-methionine, were oxidized to a relatively low extent.

In only some of the pooled intestinal tissue a low activity of palmitoyl CoA oxidase was detected. In the gallbladder homogenates palmitoyl CoA oxidase could not be detected (Table 1). Activities of other H_2O_2-producing oxidases, such as urate oxidase and L-α-hydroxy acid oxidase, were also not detected in both tissues.

Adaptation of the animals to sea water did not result in significant alterations in the enzyme profiles presented above for freshwater fish (Table 1).

Morphology

The morphology and ultrastructure of the stickleback intestinal and gallbladder epithelial cells has been described by de Ruiter et al. (1985). To estimate the number of peroxisomes, 1–2 μm-thick sections were prepared from Epon-embedded intestinal and gallbladder tissues that were incubated to demonstrate catalase or D-amino oxidase activity. Both methods allowed detection of peroxisomes at the light-microscopical level (Fig. 1). Small-scale morphometry on longitudinally sectioned cells, showing a nucleus, indicated that the mean number of peroxisomes per cell in the intestinal epithelium was almost threefold higher than in the gallbladder epithelium. No remarkable differences in number between freshwater and seawater fish were observed.

Electron-microscopical observations on intestinal epithelial cells indicated that in these cells the peroxisomes are mainly located in the apical and perinuclear zone of the cells, frequently in close association with lipid droplets, vacuoles and lysosomes (Fig. 3). Smaller numbers were present in the basal part of the cells (Figs. 1, 3). The organelles measured from 200–500 nm in diameter, and were surrounded by a single membrane of approximately 7 nm. Infrequently, a small crystalloid was observed in the matrix of the organelles (Fig. 2).

In the gallbladder epithelial cells the peroxisomes were relatively scarce and randomly distributed in the cytoplasm (Fig. 7). These organelles had dimensions up to 400 nm and were characterized by the presence of large crystalline inclusions (Fig. 6).

Cytochemistry

The cytochemical experiments revealed that catalase activity was present in the peroxisomes of the intestinal epithelium (Fig. 4). Staining was absent in control experiments performed in the absence of H_2O_2, or in the presence of aminotriazole as an inhibitor of catalase activity (Fig. 5). Staining of the mitochondria occurred independently of the presence of H_2O_2 and was prevented by cyanide. Therefore, this staining most probably was due to the presence of the cytochrome C-cytochrome oxidase complex (Veenhuis and

Table 1. Enzyme activities in tissue homogenates of individual sticklebacks (intestinal amino acid oxidase only; $n=6$) and pools of 4–6 fish each (from sticklebacks) kept in fresh water or sea water

	Intestinal mucosa		Gallbladder	
	fresh water	sea water	fresh water	sea water
D-amino acid oxidase	50.5 ± 17.8	50.1 ± 8.9	13.0	14.1
Palmitoyl CoA oxidase	1.0	1.0	–	–
Catalase	6.0	8.1	13.8	12.2
Urate oxidase	–	–	–	–
L-α-hydroxy acid oxidase	–	–	–	–

Oxidase activities are expressed as nmol $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$; catalase activity is expressed as $\Delta E\ 240 \cdot min^{-1} \cdot mg\ protein^{-1}$; – no activity detectable

Bar = 1.0 μm unless otherwise stated

Fig. 1. Light micrograph of a 2-μm-thick Epon section of intestinal epithelium of a freshwater fish, incubated with $CeCl_3$ and D-alanine for the demonstration of D-amino acid oxidase, showing the distribution of peroxisomes (visible as dark *dots*) in the apical and basal areas of the cells. *MV* and *arrow* microvillous border

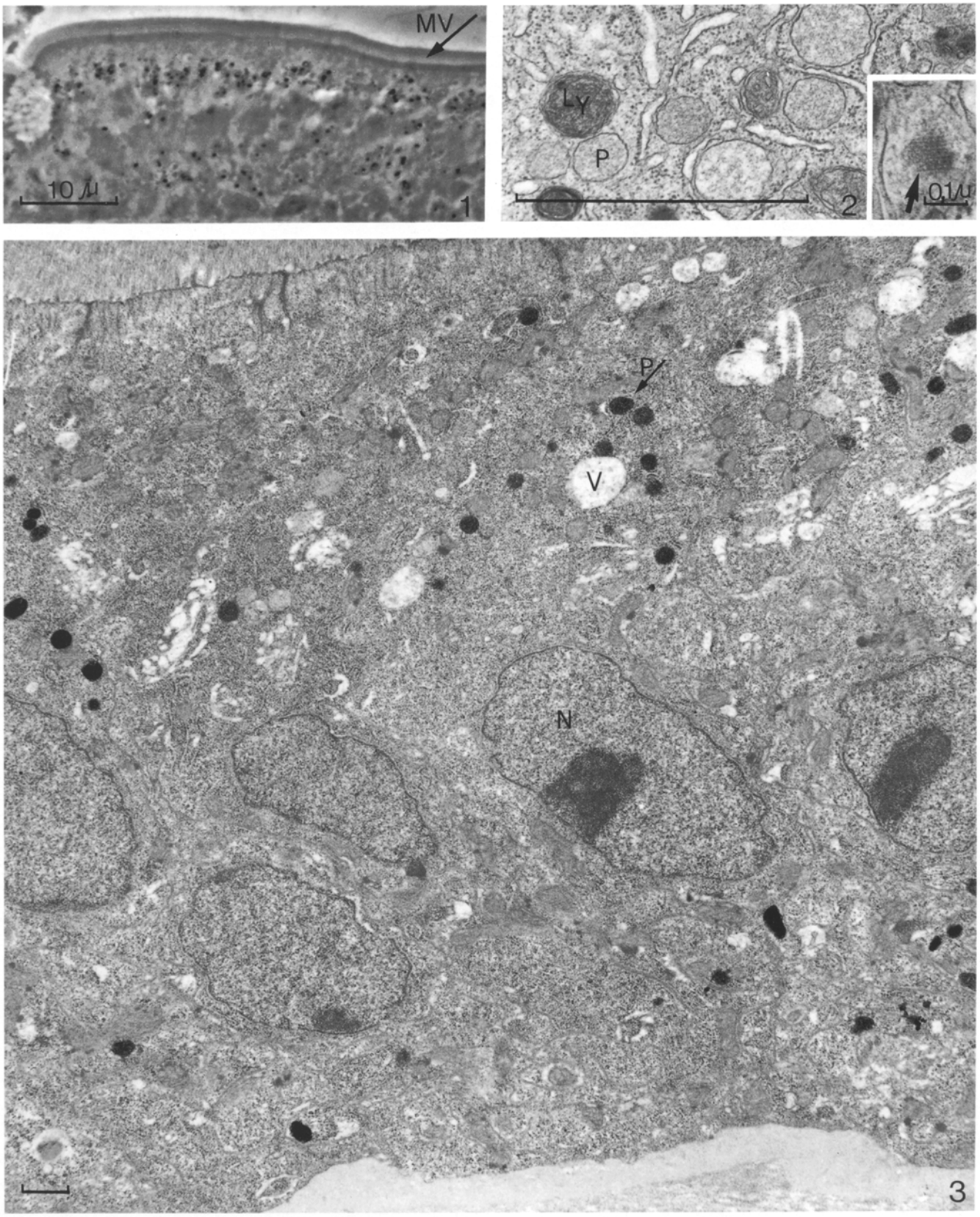
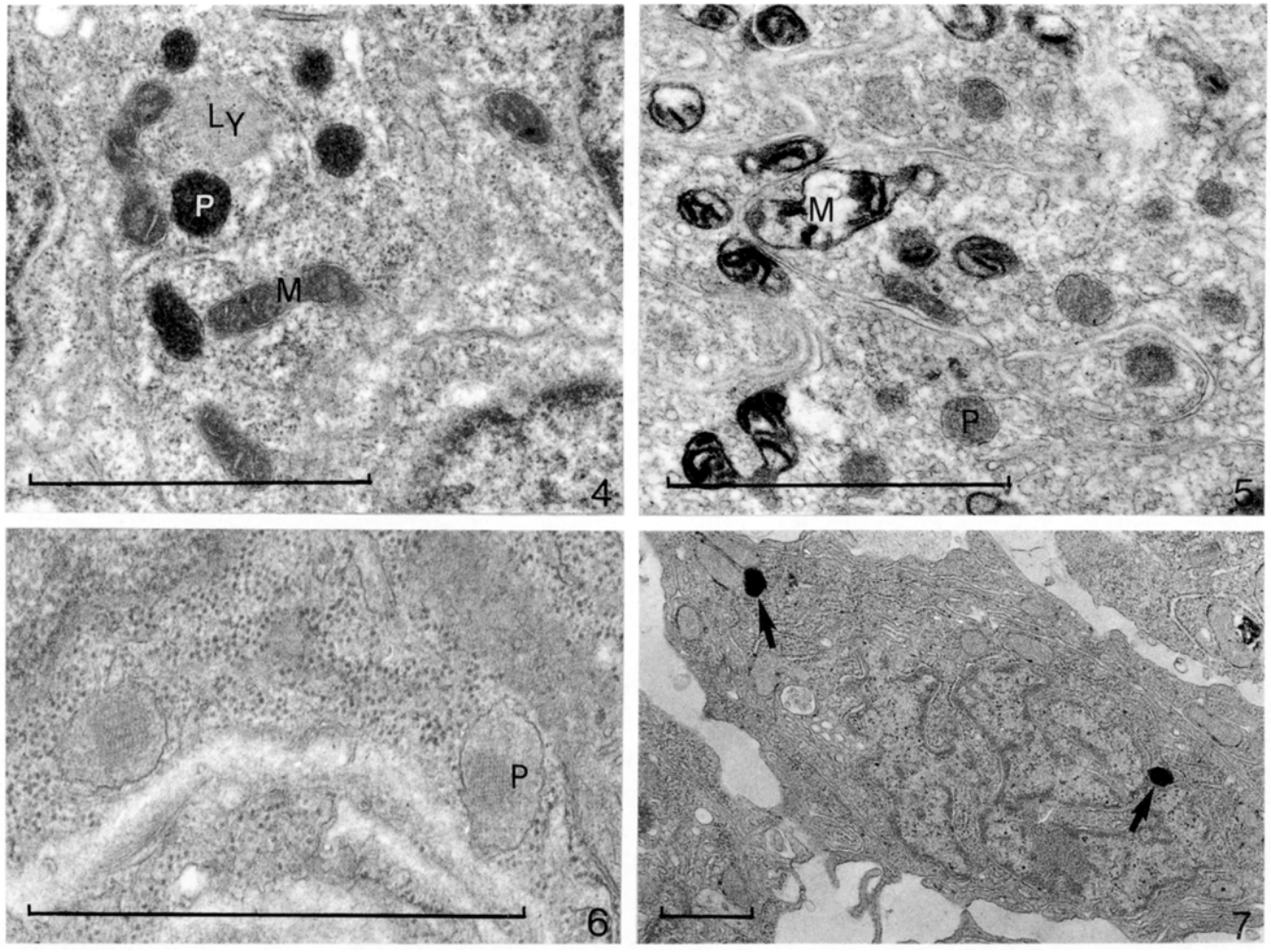


Fig. 2. Detail of an intestinal cell of a seawater fish to demonstrate the substructure of the peroxisomes in these cells. The high magnification in the *inset* shows a peroxisome, which contains a crystalline core (*arrow*); *Ly* lysosome; *P* peroxisome

Fig. 3. Electron micrograph of the tissue shown in Fig. 1. As already indicated in Fig. 1, the majority of the peroxisomes (*P*) is located in the apical part of the cells. They often are closely associated with vacuoles (*V*) and lysosomes; *N* nucleus



Figs. 4, 5. Details of cells showing peroxisomes (*P*) positively stained for catalase after incubation with DAB and H_2O_2 (**Fig. 4**). In control incubations, in the presence of the catalase inhibitor aminotriazole the peroxisomes (*P*) are only slightly stained (**Fig. 5**); *M* mitochondrion; *Ly* lysosome

Figs. 6, 7. Fig. 6. Substructure of peroxisomes (*P*) in gallbladder epithelial cell. The organelles all contain a crystalline core. The presence of D-amino acid oxidase in these organelles was demonstrated after incubations with $CeCl_3$ and D-alanine (**Fig. 7**, *arrows*)

Wendelaar Bonga 1977; Roels et al. 1975). After incubations to demonstrate the subcellular sites of D-amino acid oxidase activity, by incubating glutaraldehyde-fixed cells with $CeCl_3$ and D-alanine, the reaction products were confined to the peroxisomal matrix (Fig. 3). This staining was prevented in control experiments performed in the absence of substrate or under anaerobic conditions.

Cytochemical observations on the gallbladder epithelial cells showed that the microbodies contain both catalase and D-amino acid oxidase (Fig. 7). Other peroxisomal matrix enzymes such as palmitoyl CoA oxidase, urate oxidase and L-α-hydroxy acid oxidase could not be detected cytochemically, thus confirming the biochemical data.

Discussion

The DAB-technique for catalase – in the presence of cyanide to prevent mitochondrial staining – has been used successfully for the detection of peroxisomes at both the electron-microscopical as well as the light-microscopical level (Roels et al. 1975; Roels and Goldfisher 1979). Our results, however, show that the cerium technique is also very suitable for light-microscopical observations in lower vertebrates. In fact, this technique has distinct advantages over the DAB-method. It shows an enhanced contrast, localizes the actual sites of intracellular H_2O_2 production and avoids the utilization of toxic compounds, such as DAB and cyanide and eventually OsO_4, since the reaction product needs no osmification for visualization at the light- and electron-microscopical levels.

We have previously characterized peroxisomes cytochemically in kidneys of the stickleback and have demonstrated that the activities of catalase and D-amino acid oxidase were confined to the same cell organelles, which could thus be considered as peroxisomes (Veenhuis and Wendelaar Bonga 1977). For the same reasons, the microbodies present in the epithelial cells of the intestine and gallbladder described in this paper can be considered to be peroxisomes. The combined biochemical and cytochemical data suggest that catalase and D-amino acid oxidase are main components of the peroxisomes in both tissues.

The current knowledge on the occurrence of peroxisomes in intestinal tissue in general is mainly based on both biochemical and cell fractionation studies. Very low activities of D-amino acid oxidase (E.C. 1.4.3.3) have been reported in goldfish and guinea-pig intestine (Connock 1973; Connock et al. 1974). Fickeisen and Brown (1977) showed that D-amino acid oxidase is a common enzyme in several species of fishes. Highest activities were detected in the liver. This enzyme was also present in kidney and pyloric caecae. As in our experiments, the in vitro activity of the enzyme is FAD-dependent. In contrast to our results, the latter authors found that liver extracts of the salmon *Oncorhynchus nerka* (Walbaum) also oxidized L-alanine to a low rate, probably due to the presence of low activities of L-amino acid oxidase or a racemase, which converst L-alanine into its D stereo isomer.

Our biochemical results show that palmitoyl CoA oxidase is also present in stickleback intestinal homogenates. Small et al. (1980) detected activities of this enzyme in guinea-pig small intestinal tissue as well as in other organs, such as liver, kidneys and heart. In their opinion this enzyme may be involved in energy balance and in thermogenesis.

The relatively high activities of D-amino acid oxidase detected in the stickleback intestine and gallbladder may reflect specific physiological functions of the peroxisomes present in these cells. Apart from digestive processes the epithelial cells of the intestine and gallbladder of *Gasterosteus aculeatus* are involved in solute-linked water transport both in sea water and in fresh water (Honma and Yamada 1978; de Ruiter et al. 1985). Adaptation of freshwater fish to sea water did not noticeably influence the level of enzyme activity. Therefore, there appears to be no indication for involvement of peroxisomes in hydromineral regulation.

Peroxisomes of intestinal and gallbladder epithelium both have relatively high concentrations of D-amino acid oxidase and catalase, although the ratios of the enzymes differed significantly between the two types of epithelia.

In the intestinal epithelium the mean number of peroxisomes per cell is considerably higher than in the gallbladder epithelium. This tendency is in agreement with the biochemical data. The metabolic function of the peroxisomes in both cell types is still unclear. However, in the intestinal epithelium the frequently observed close association of peroxisomes with lysosomes or vacuoles may reflect a functional relationship. Most likely the peroxisomes are involved in digestive processes, e.g., fat metabolism/fatty acid oxidation and/or the oxidation of lysosomal products.

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