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ELECTRON-MICROSCOPICAL STRUCTURE OF GILL LAMELLAE OF THE IDE (LEUCISCUS IDUS), WITH PARTICULAR REGARD TO THE CHLORIDE CELLS AND H_2S POLLUTION

I. HORVÁTH and ARANKA STAMMER

Department of Zoology, Attila József University, Szeged
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Abstract

The respiratory epithelial cells of the gill lamellae of the ide form a double layer. Under the superficial respiratory epithelium, chloride cells are to be found in great numbers. The structural elements of the two types of cells are very different. In the chloride cells, in addition to the few rough endoplasmic reticula, there are many smooth endoplasmic reticula which probably carry out the tasks of osmoregulation. The exocytotic activity of the superficial respiratory epithelium indicates the secretory role of the gill epithelium. The damage caused by H_2S is connected with the multiplication of lysosomes and dense bodies and, at a higher concentration, H_2S results in a large amount of membrane accumulation.

Introduction

We have investigated the effect of water polution on the gills of fresh-water bony fishes for some years now. The gill apparatus of sea-fishes has also been investigated by STAMMER and VÁMOS (1973), STAMMER, HORVÁTH and CSOKNYA (1977), the gill innervation mainly by STAMMER (1966). Lately, we have also much studied the fresh-water fishes of the Tisza, using material chosen for comparison, not only with a light microscope but also with an electron microscope (STAMMER, 1972; STAMMER and HORVÁTH, 1975). The gill epithelium seems to be particularly suitable for measuring water pollution. The natural pollution, and artificial pollution with hydrogen sulphide, as well as the effect of Dikonirt 4, were examined on the organelles of epithelial cells of the gill. As a result of the harmful impact, changes in the appearance and number of lysosomes, as well as the occurence of membrane configurations and membrane fusions can be shown, connected mainly with the destruction of mitochondria.

Recently investigation into an interesting fish species, the ide, became important. Its highly developed chloride cells indicate the marine origin of the species.

Materials and Methods

8–10 cm long selected individuals of 7–9 g weight of the ide have been used for as the material for investigation. The ide is rather rare in our rivers. Thus in the Tisza it can only be fished in large quantity at the time of migration. Its most characteristic external feature is its colour. This changes greatly in the individual, depending on its age, the season and the habitat. Among our lakes, it can only be found in those of deep water, and then only exceptionally. As its main nourishment consists of insects, snails, shell-fish, and other tiny animals living on the bottom, as well as plankton, we may assume its gill respiration is satisfied by the oxygen present in deep waters. Here the water has a higher pressure and a lesser pollutin effect.

1 cm pieces of the laminae from the dissected gill apparatus were studied, in longitudinal sections, after being fixed by Bouin's method or in formalin, with routine tissue staining and impregnation. The material for the electron-microscopic examinations was cut into pieces 0.6 mm in diameter, fixed in osmium and embedded, in araldite. The sections were made with a Tesla BS 478 ultramicrotome and were examined visually with a Tesla BS 500 electron microscope.

After studying normal histological structure, by light and electron microscopy, we examined the damaging effect of H_2S on the organelles of the respiratory epithelial cells animals that were kept in water filled of 1, 2 and 3 ppt H_2S. We were able to observe reversible damage only in the case of 1 ppt H_2S.

Results

The well-developed branchial arches (four and a greatly reduced fifth arch) of the ide, *Leuciscus idus* like those in a number of other species belonging to its family — are furnished with 2 lateral gill plates with many respiratory lamellae regularly opposite one another. The gill plates having supporting elements rounded connective tissue with blood vessels and nerve fibres. In the base of the branchial arch arteries can be found in the lateral plates, running in the middle with copious primary and secondary ramifications divided into capillaries (Fig. 1). The capillaries are covered by gill epithelial cells.

Respiratory epithelial cells

Under a light microscope, we see that the respiratory epithelial cells seem to be in only a single layer under a light microscope, unlike those of the fishes examined previously. But it becomes clear even from semi-thin sections (Fig. 1) that this in only a pseudo-single layer. Under the very thin epithelium layer cuboidal epithelial cells of comparatively large size are visible. Beside the round and frequently indented nuclei of the latter cells, the longish nuclei of the superficial flattened respiratory epithelial cells are scarcely distinguishable. In the course of our comparative studies on the gill plates of fresh-water fishes, we could never distinguish (under the superficial, flattened, respiratory epithelial cells) chloride cells (acido-or osmophilic cells) in as great a number as in this species.

Chloride cells have already been described from both invertebrates and vertebrates. Cells of this type were described in publications by HUGHES and GRINSTOM (1959), DOYLE and GORECKI (1966), MORGAN (1968), NEUSTEAD (1972), PHILPOTT and COPELAND (1960), HAUSTON (1964) and TREADGOLD (1972).

The flattened respiratory endothelial cells which cover the surface adhere close together in a line of waves, without any desmosomal connection. In the cell, the endoplasmic reticulum is very weakly developed but some scattered ribosomes can be observed free in the cytoplasm. No Golgi apparatus was found. In the cell, there are few mitochondria and these are of the cristate type. The limiting membrane of the cell surface is larger than the thickness of a unit membrane (120–230 Å) and several exo-or endocytotic vesicles are connected with it. The nucleus of the surface cells is longish and never indented.

The nucleus of the chloride cells is round with a deep intussusception and sometimes one small one in addition. The number of mitochondria is fairly large; their form is round. It is noteworthy that they belong to the mitochondrion with tubular type (Figs. 2–3). Apart from the rough endoplasmic reticulum of the chloride cells,

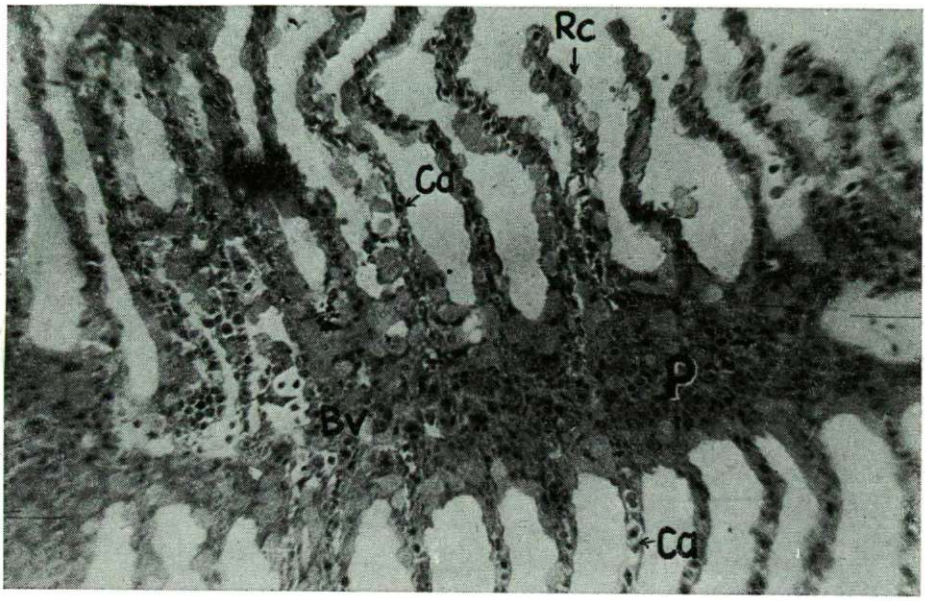

Fig. 1. *Leuciscus idus:* Section of the first gill plate with respiratory lamellae. H_2S damage came from below and removed the respiratory epithelial cells. Bv=arterioles of the lateral plates Ca — capillaries, Rc = respiratory epithelial cells, P = section of gill plate. Toluidine-blue staining, semi-thin section: x800.

a very large number of pieces of smooth endoplasmic reticulum can be observed. It is probable that the ide, as strongly euryhaline organism, transferred its habitat from seawater to fresh water. Accomodating itself easily to the change in salt concentration, it has become adapted to the new conditions, retaining the large number of chloride cells which presumably play, an important role in osmoregulation and, possibly, also in transporting oxygen to its blood vessels. Particular attention should be paid to the large space between the chloride cell and the arteriole of the gill, to be seen in the picture (Fig. 2). The steep gap filled cytoplasmic matrix between the membrane of the chloride cell and the wall of the arteriole may serve for draining of the osmotic products of the chloride cell.

The tubuli of the mitochondrion and the cisternae of the endoplasmic reticulum become wider as a result of the effect of low H_2S. The increase in membrane density can be seen particularly well in Fig. 3, and the tubular structure of the mitochondria in Fig. 4, which are electron micrographs from high magnification.

Apart from the role of the superficial respiratory epithelium in absorbing the oxygen and forwarding it to the blood vessels, the excretory function of the cell is to be particularly emphasized. It has been known since Krogh's investigations (1941) that the hyperosmotic fluid leaves bony sea-fishes through the gill epithelium. It is possible that this phenomenon cannot be observed in fresh-water species. In the present species, however, the respiratory epithelium has retained this capacity. This is proved by the detached vesicles of remarkably large size (300 Å in diameter). Vesicles like this may remove excretory matter, in solution as well as osmotic fluids.

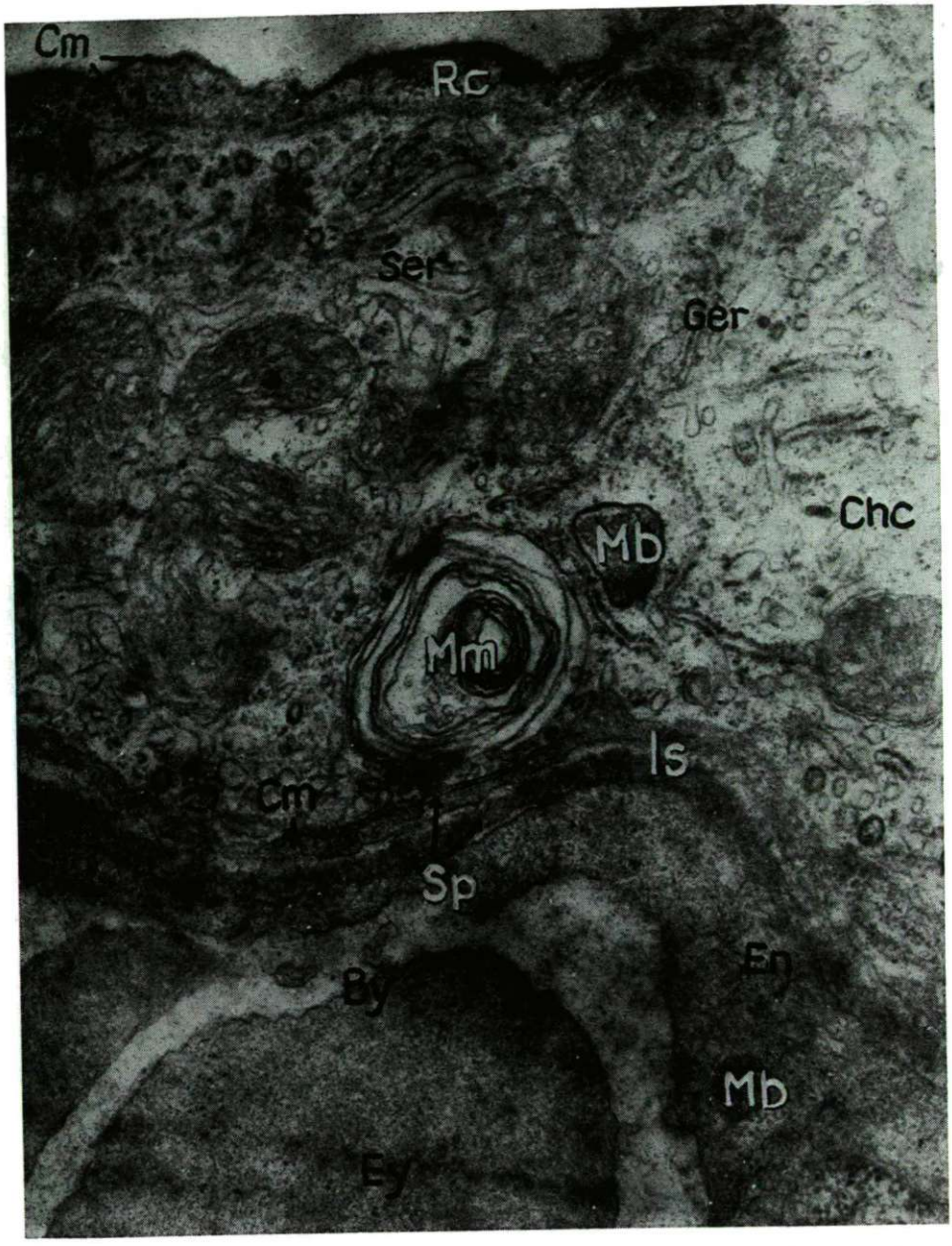

Fig. 2. *Leuciscus idus:* Cross-section of gill lamellae. Rc = respiratory epithelial cell, Chc = chloride cell, By = capillary, Ey = part of erthrocyte, En = part of endothelial cell, Ser = smooth endoplasmic reticulum, Ger = rough endoplasmic reticulum, Mm = membrane configuration, Cm = cell membrane, Is = cytoplasmic matrix, Sp = medial gap, Mb = granular body. Electron micrograph: x28 000.

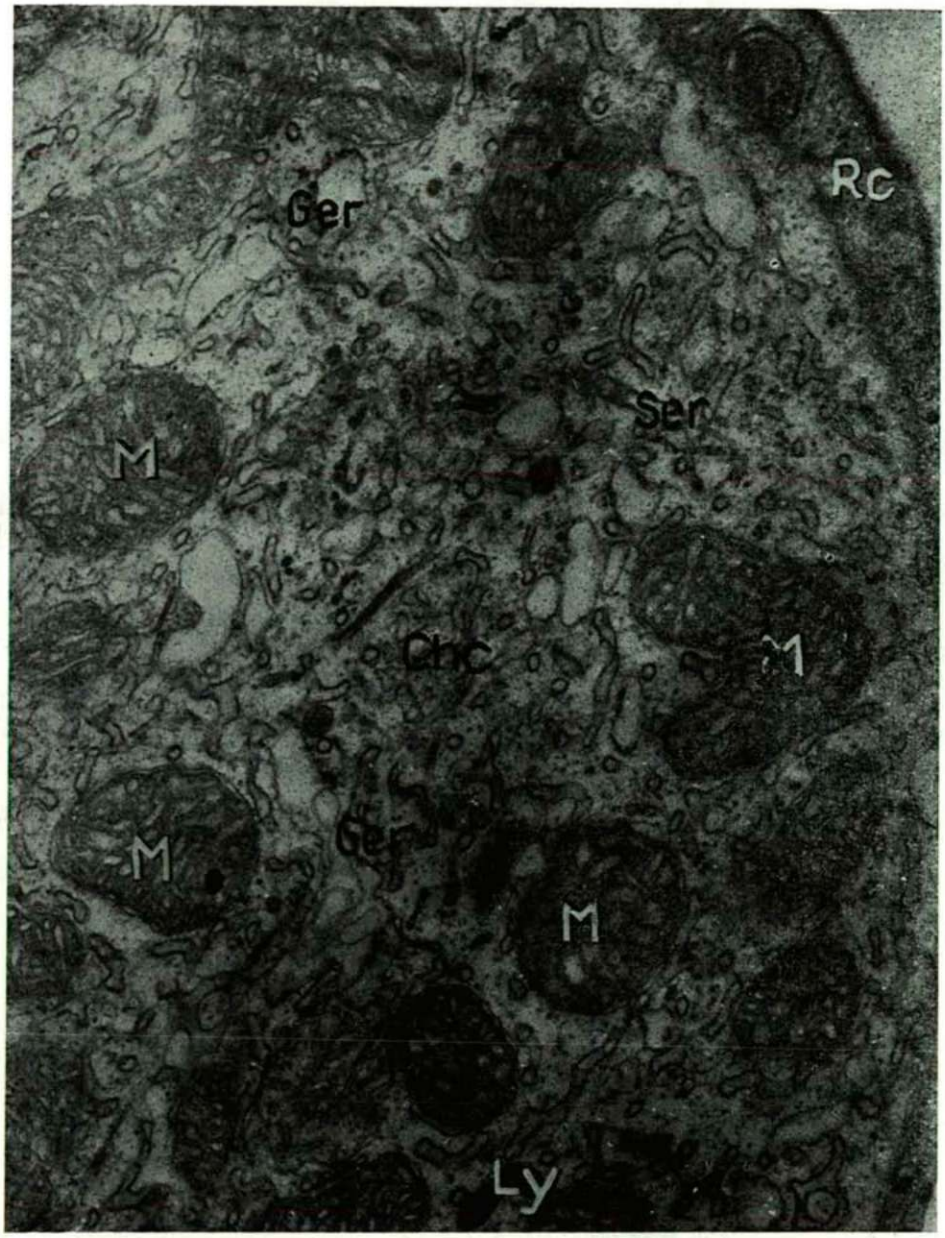

Fig. 3. *Leuciscus idus:* Cross-section of a gill lamellae. Respiratory epithelial cells. Rc = respiratory epithelium, Chc = chloride cell, Ser = smooth endoplasmic reticulum, M = tubular mitochondrion, Ly = lysosome. Electron micrograph: x28 000.

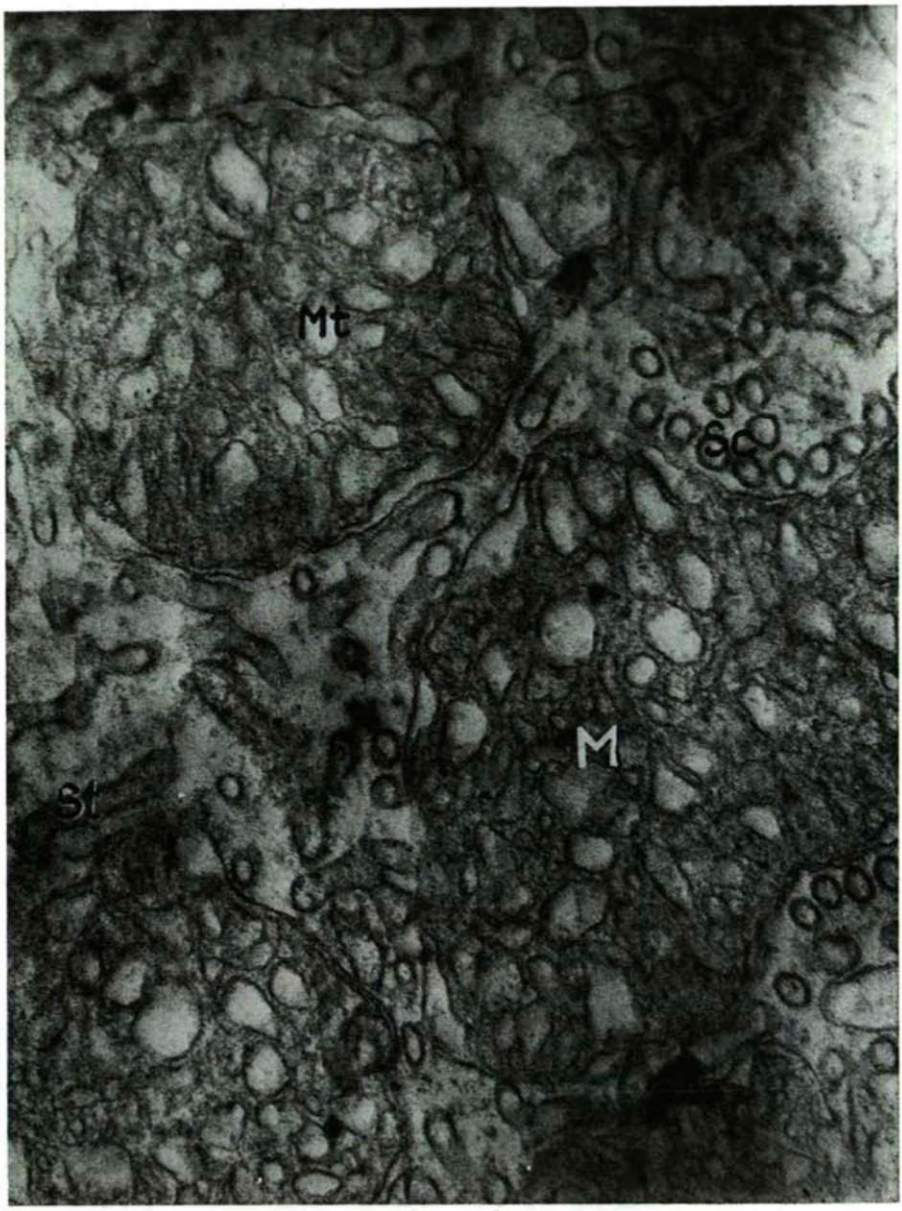

Fig. 4. Mitochondria with cross sected tubuli in the chloride cell. M = mitochondrion, Mt = mitochondrial tubulus, St = cisterna of the smooth endoplasmic reticulum, Sc = cross-sections of the smooth endoplasmic reticulum. Electron micrograph: x64 000.

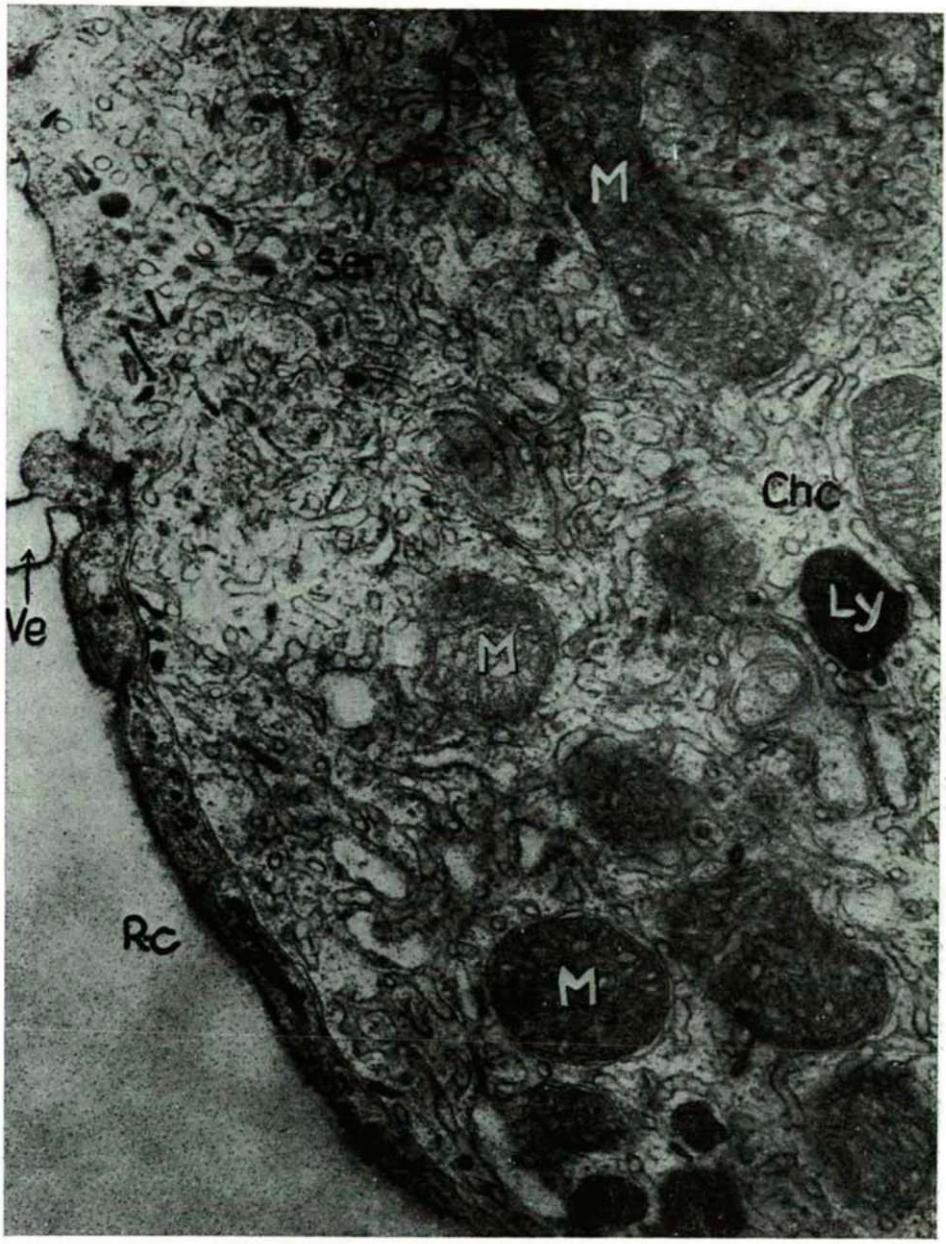

Fig. 5. *Leuciscus idus:* Respiratory epithelium with exocytotic veiscles and the structure of a chloride cell. Rc = respiratory epithelium, Chc = chloride cell, Ve = detached vesicle, M = mitochondrion, Ser = smooth endoplasmic reticulum, Ly = lysosome. Electron micrograph: x28 000.

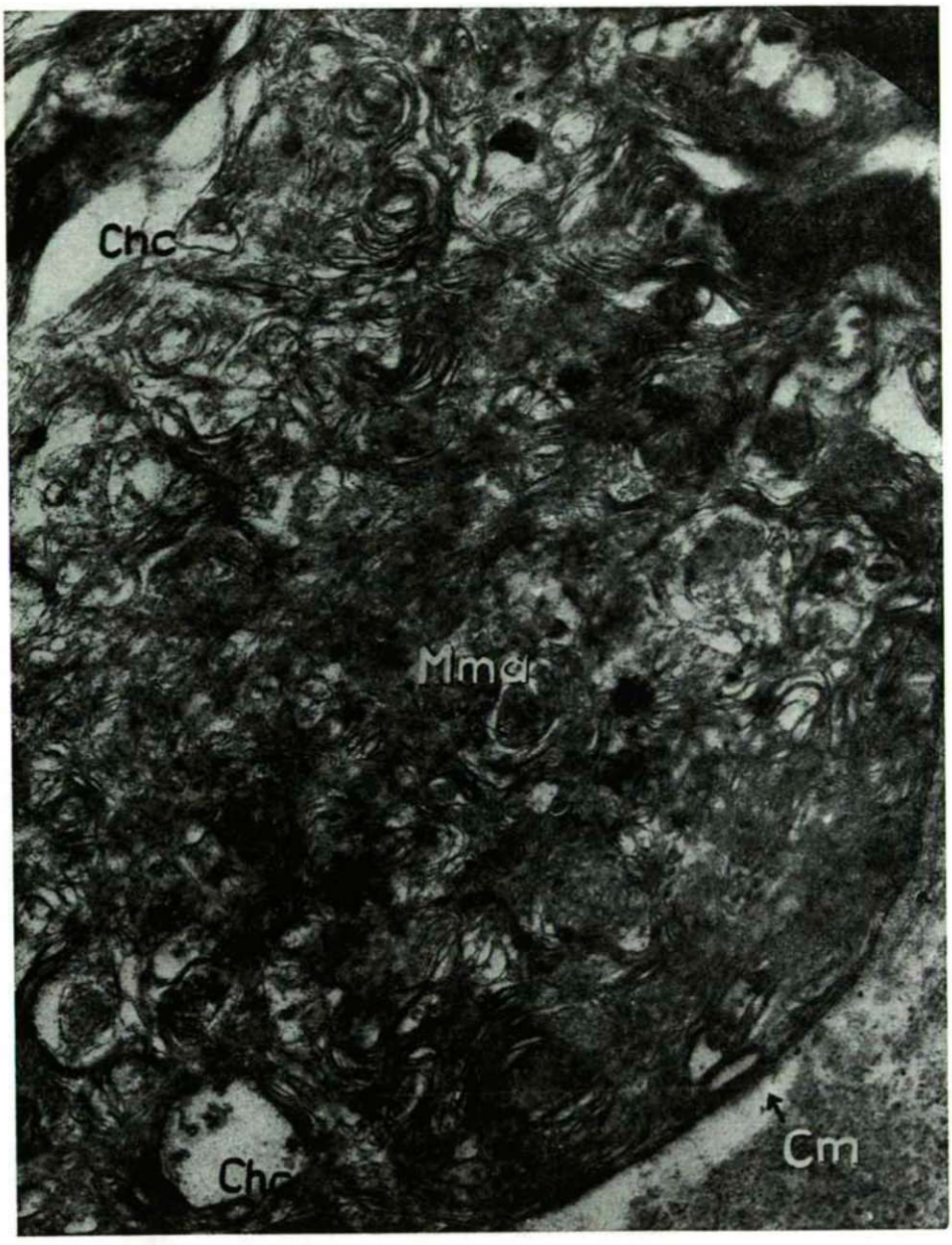

Fig. 6. *Leuciscus idus:* Irreversible damage to a chloride cell. Damage caused by 3 ppt H_2S. Chc = chloride cell cytoplasm, Mma = membrane agglomerate, Cm = cell membrane. Electron micrograph: x28 000.

Damaging effects of H_2S

The effect of H_2S used in different concentrations caused different changes in the organelles of the gill epithelial cells and their physiological function, as demonstrated by the photomicrographs. H_2S left hardly any marks at the lowest concentration used. At higher concentration (0.6–1 ppt) the membrane density of the endoplasmic reticulum becomes darker, the cysterna of endoplasmic reticulum larger, the mitochondrial tubules or cristae become more visible caused by stronger electron-density. As a result of exposure, the membrane configuration (Fig. 2) and a smaller or larger functioning lysosome appeared (Figs. 3–5).

As a result of exposure to 2 ppt H_2S, both types of respiratory epithelial cell become detached. The detachment can apparently be seen in our semi-thin section (Fig. 1). In this, the hydrogen sulphide, coming from below entirely detached, from one side, the epithelial cells covering the surface of capillaries. Among the gill cells, the respiratory epithelial cells in particular are very prone to detachment. This in also proved by our figure (Fig. 5) in which respiratory epithelial cells can no longer be observed in the upper part of the picture. The chloride cells perish and become detached from the outer surface of the capillary only after the detachment of the respiratory epithelial cells. Influenced by H_2S, the double cell membrane between the respiratory epithelial cell (and the chloride epithelial cell) becomes denser and the gap between the membrane of the chloride cell and the capillary becomes wider.

The effect of H_2S, tested at the highest concentration (3 ppt), caused the irreversible merging of all the membrane systems of the chloride cell. The bulk of these membranes derive from the mitochondrial membranes (Fig. 6).

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Address of the authors:
Dr. I. HORVÁTH
Dr. ARANKA SAMMER
Department of Zoology, Attila József University,
H—6701 Szeged, P. O. Box 428, Hungary