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Labyrinth Cells, a New Cell Type in Vertebrate Olfactory Organs

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Summary. Light microscopy and transverse electron microscopy has been employed to study the olfactory organs in 82 specimens of freshwater adapted young and homing adult Baltic sea trout *Salmo trutta trutta* L. In both sensory and indifferent epithelium the olfactory mucosa has scattered cells of a type that has not been described in any olfactory organ before. They are called labyrinth cells and are characterized by an extensive, turtuous, interconnected tubular system of smooth endoplasmatic reticulum intimately connected with numerous mitochondria. This cell type is similar to chloride and other cells which probably are involved in electrolyte transport in fish gills and pseudobranch, the rectal gland in elasmobranchs and the nasal gland in reptiles and birds. It is suggested that the olfactory organ in fish is serially homologous with the pseudobranch.

Key words: Olfactory organs (Fish)-Labyrinth Cells — Ion transport — Evolution — Light and electron microscopy.

Introduction

Extrarenal structures responding to salinity have been reported from gills in stenohaline and euryhaline fishes (Holliday, 1971), the pseudobranch in teleosts (Holliday and Parry, 1962), the rectal gland in elasmobranchs (Doyle, 1962), the lachrymal gland in reptiles (Schmidt-Nielsen and Fänge, 1958), the sublingual gland in marine reptiles (Dunson *et al.*, 1971), and the nasal gland in birds (Schmidt-Nielsen, 1960). In all these structures there is a single cell type which seems to be the basis for their function. In gills it has been called chloride secreting cell (Keys and Willmer, 1932), eosinophilic cell (Liu, 1942), chloride cell (Copeland, 1948, 1950), acidophilic cell (Holliday and Parry, 1962) or mitochondria-rich cell (Conte, 1969). The term chloride cell seems to be the most common, but it is a physiological misomer because this cell may be involved in the transportation of more than one type of ion (Conte, 1969).

The characteristic feature of this cell type is a well developed tubular smooth endoplasmic reticulum (SER) closely associated with numerous mitochondria. This information by itself constitutes only circumstantial evidence in support of its ion secretory function and some workers have taken issue with this interpretation (Datta-Munshi, 1964; Doyle and Gorecki, 1961; Holliday and Parry, 1962; Parry *et al.*, 1959; Strauss, 1963).

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The present study is part of an investigation of the structure of the olfactory mucosa in young and adult Baltic sea trout (Bertmar, 1972a–e). During the analysis of the ultrastructure of the cell population it was found that some cells differ very much from the normal population, and that these cells are instead similar to chloride cells. As the function is unknown this new cell type will be called labyrinth cell. It has never been described in any olfactory organs before.

Material and Methods

Material came from 82 specimens of Baltic sea trout *Salmo trutta trutta* L. of River Umeälven. The total length varied from 12.1 to 55 cm, and the age between $1^1/_2$ (1+) and $4^1/_2$ (4+) years. The youngest were parr taken from a pond at the Norrfors' fish hatchery, 15 km West of Umeå, and the adult fish were caught at Norrfors when they were homing in their home river. Smolt were taken in May–June, a little more than 2 years old. Adult fish were caught in September 30, and by this time they should be fresh water adapted (Mills, 1971).

The technique used for light microscopy (LM) studies has been described before (Bertmar, 1972a). For electron microscopic examination (TEM) the right olfactory organ of the same specimens used for LM was fixed in 2% glutaraldehyde (Sabatini *et al.*, 1963) in phosphate buffer for 1–24 hrs. They were then postfixed in 1% phosphate buffered osmium tetroxide for 1–2 hrs. After dehydration they were embedded in Epon and cut on a LKB ultrotome III. Finally they were stained with lead citrate (Reynolds, 1963) or with uranylacetate and lead citrate and examined with a Philips 300M.

Observations

The olfactory organs of freshwater adapted sea trout have a few labyrinth cells. They are scattered both in sensory and indifferent epithelium and without any special arrangement. In transverse section of the mucosa they can be found in all zones (Fig. 1). They do not have any significant association to blood or lymph vessels. The labyrinth cells are isolated from each other and are flanked by any cell type present in the population.

The form and size of the labyrinth cells differ with their location. In the surface zone they are relatively large (about 15 μm long and 10 μm wide) columnar cells

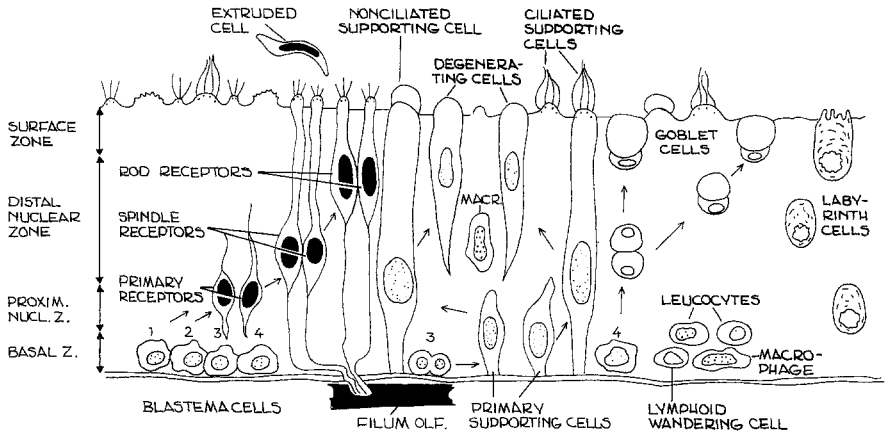

Fig. 1. Diagram of the sensory epithelium in adult freshwater adapted sea trout. The cell population contains scattered labyrinth cells lying in different zones of the mucosa

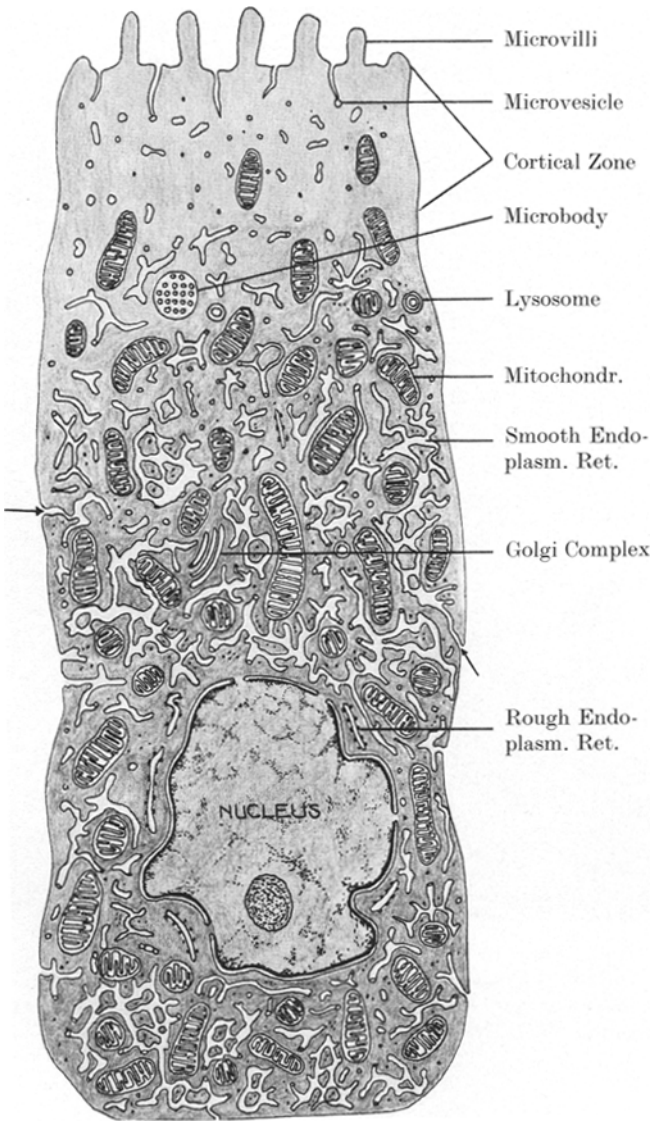

Fig. 2. Diagram of a labyrinth cell in the surface zone of the olfactory mucosa in adult sea trout. Arrows indicate some pores where the plasma membrane is continuous with the labyrinth of the smooth endoplasmic reticulum tubules

with microvilli (Figs. 1, 2). Below that zone they are smaller (about 10 μm long and 7 μm wide) and more oval in form (Figs. 1, 7). They are weakly acidophilic, and have granular cytoplasm and oval-sphaeric nucleus located basally in the cell (Bertmar, 1972c, Fig. 1 mc). This cell type is similar to the type B chloride cell of freshwater adapted eel (Shirai and Utida, 1970) and the chloride cell of freshwater adapted parr and smolt of Atlantic salmon (Threadgold and Houston, 1964).

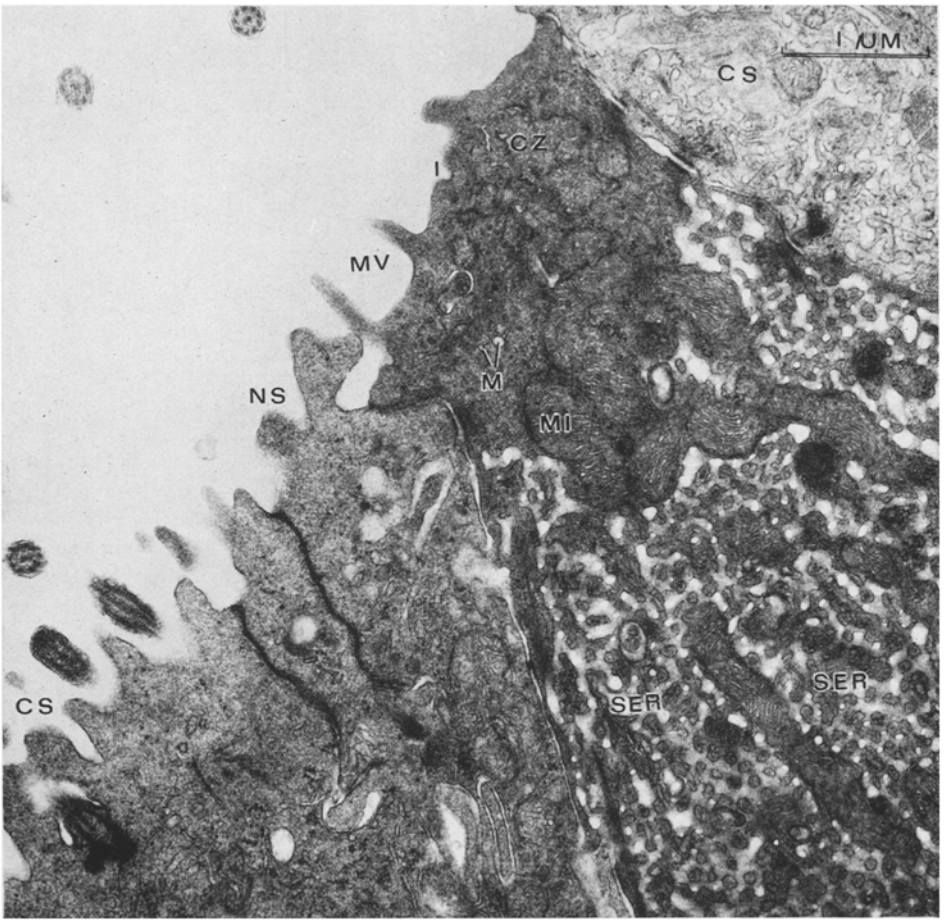

Fig. 3. Apical part of a labyrinth cell in the surface zone of sensory epithelium in adult sea trout. The cell is loosely surrounded by ciliated (*CS*) and nonciliated (*NS*) supporting cells. It has a cortical zone (*CZ*) with microvilli (*MV*), microvesicles (*M*), invaginations (*I*) and mitochondria (*MI*). The subcortical part is filled with mitochondria and smooth endoplasmic reticulum (*SER*)

So far the labyrinth cells have not been found in the TEM material of parr and smolt of sea trout but only in adult fish. The surface zone labyrinth cells have a *cortical zone*, and the rest of the cell is densely packed with SER and mitochondria (Figs. 2, 3). These cells are exposed to the external medium, but they are not in any part overlain by adjacent epithelial cells (Fig. 3). The free surface carries microvilli and invaginations. The formers are about 500 mµ long and 100 mµ wide. The invaginations are about 100 mµ deep and 50 mµ in diameter. They are connected with microvesicles and these may be continuous with SER vesicles further down in the cortical zone (Fig. 6). Some cells have a cortical zone with mitochondria (Fig. 3), others have more glycogen granula instead

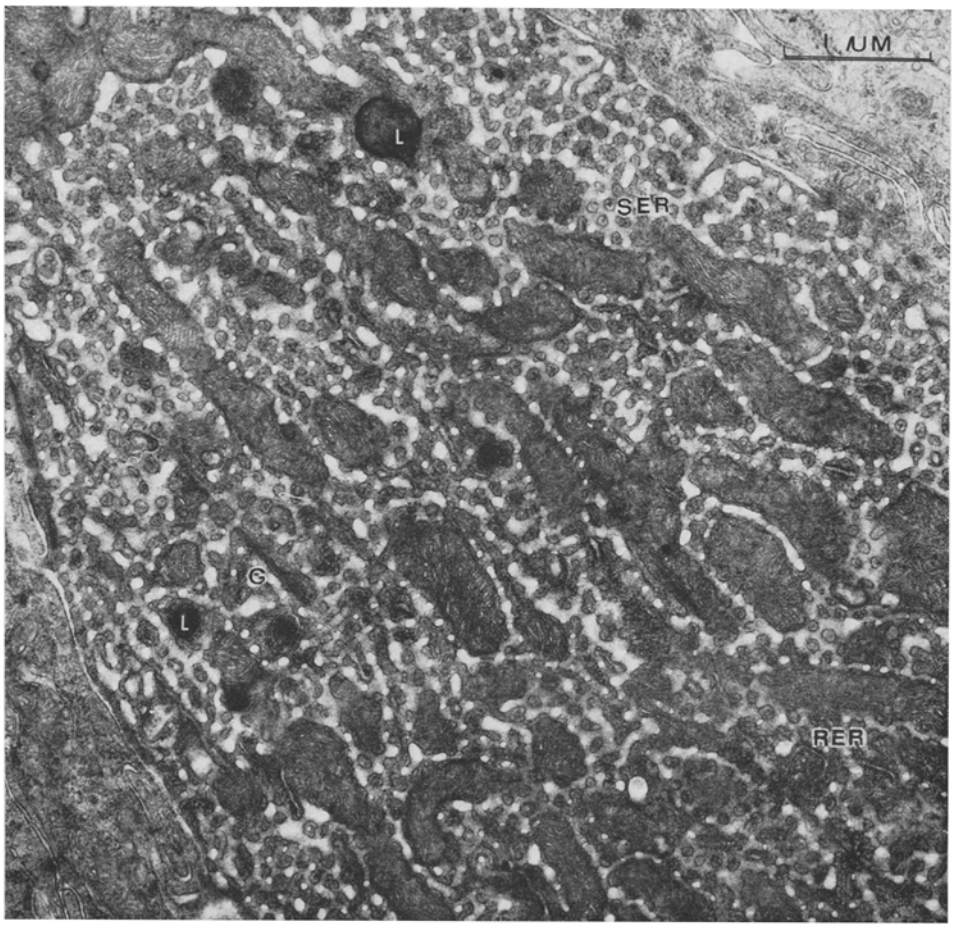

Fig. 4. Supranuclear part of the same cell as in Fig. 3. *G*, Golgi complex; *L*, lysosome; *RER*, rough endoplasmic reticulum; *SER*, smooth endoplasmic reticulum

(Fig. 6). The latter cells have few and shorter microvilli, and have a thinner cortical zone (1 µm).

This apical part of the cell is connected to other cells with a relatively short or no zonula occludens and a short zonula adhaerens (Fig. 3). The rest of the cell may be anchored with interdigital processes (Fig. 4) and a few desmosomes (Fig. 3).

The *nucleus* lies in the basal part of the cell and has a diameter of 4–6 µm. It has a high content of euchromatin indicating high metabolic activity (Fig. 5). The nuclear membrane is irregularly folded and has many nuclear pores. Nucleoli have a size of 0.5–0.8 µm. Typically they are a more or less compact, irregularly shaped element which is morphologically heterogenous.

The *mitochondria* lie close to the nucleus (Fig. 5) but also in large number in the rest of the cell below the cortical zone. They usually have the long (about

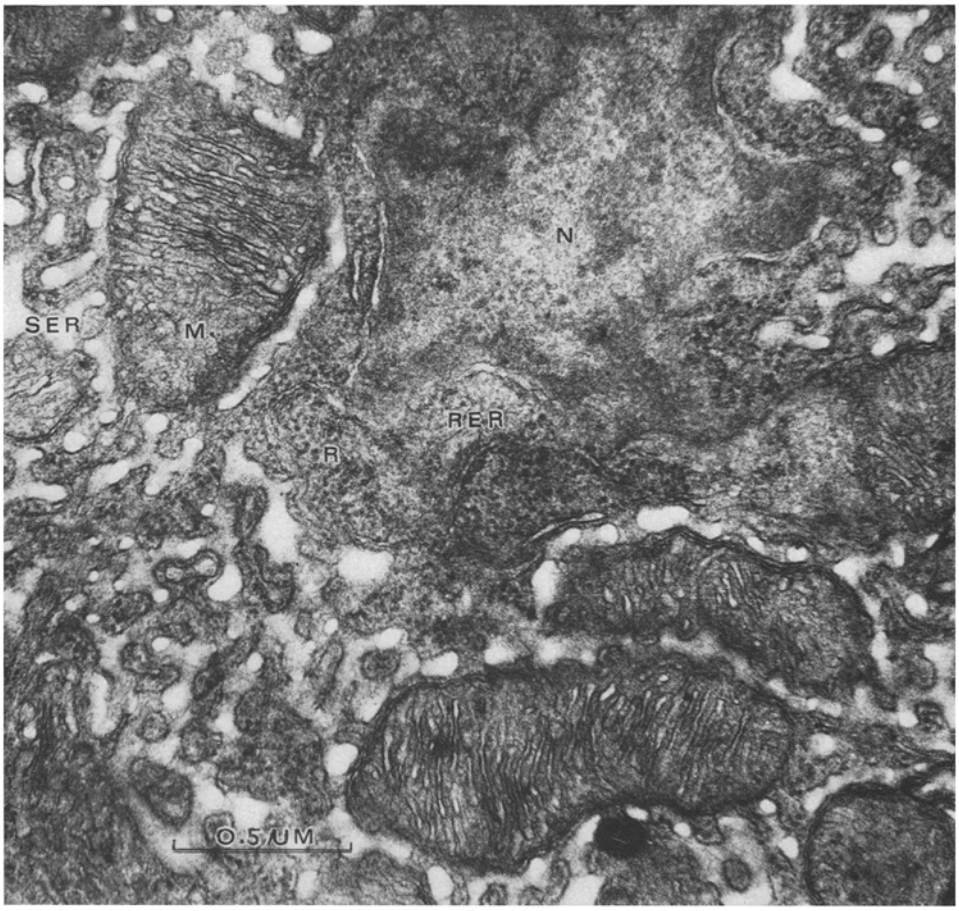

Fig. 5. Perinuclear part of a lymphatic cell in the indifferent epithelium in adult sea trout. The mitochondria (*M*) lie close to the nucleus (*N*) and are intimately surrounded by the labyrinth of smooth endoplasmic reticulum (*SER*). There are numerous free ribosomes (*R*) and a few rough endoplasmic cisternae (*RER*) around the nucleus

2 µm), slender form of typical mitochondria and a basoapical orientation in the cell (Fig. 4). Their cristae are mainly transversely arranged (Fig. 5). But their are also atypical characters (Figs. 4–6): the cristae are numerous and closely packed; some cristae are longitudinally or obliquely arranged; the matrix is sometimes denser close to the inner membrane and dense strips may run from it; the granula are small and few.

The *SER* tubuli surround the mitochondria very tightly which erronously gives the mitochondrial outer membrane a wavy appearance (Fig. 5). Most of the SER is disposed as a very extensive tubular net, which gives the cell a characteristic labyrinth-like appearance (Fig. 4). The tubuli are irregular and branched. They contain an inclusion of slightly or much lower electron opacity than the surrounding cytoplasmic matrix. The light matrix "holes" are distinctly demar-

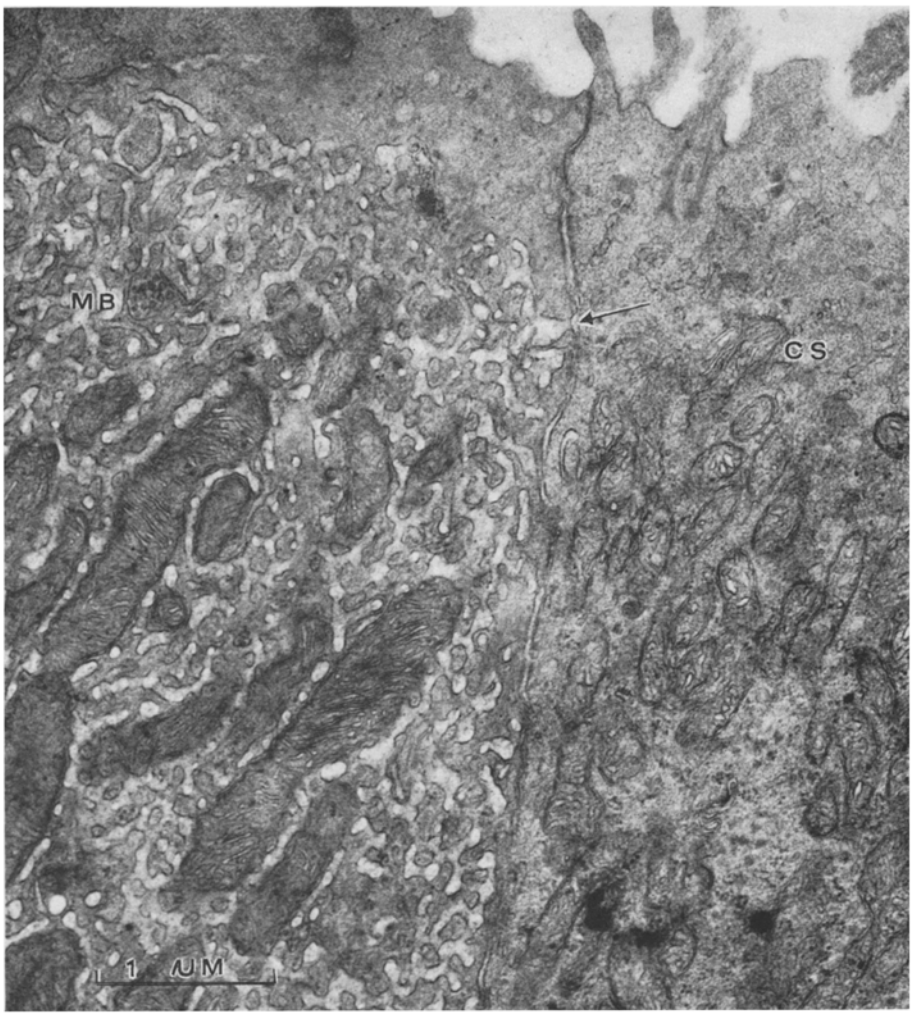

Fig. 6. Apical part of a labyrinth cell in the surface zone of sensory epithelium in adult sea trout. Note the thin cortical zone with glycogen granula but no mitochondria, and the microbody (*MB*) and basoapical orientation of mitochondria in the subcortical part of the cell. Arrow indicates a pore in the plasma membrane. *CS*, ciliated supporting cell

cated and may either be transversely cut tubuli or represent a matrix of another composition. At certain points the SER tubuli have connections with the intercellular space via pores in the plasma membrane (Figs. 2, 4, 6).

A few cisternae of *rough endoplasmatic reticulum* (RER) are scattered in the cytoplasm (Figs. 2, 4). Some of them may be closely associated with the nuclear membranes (Fig. 6). Free ribosomes are distributed in the cytoplasm. They are often arranged in circles or other patterns (Fig. 5).

The *Golgi elements* are few and have densely packed lamellae (Fig. 4). They have no special arrangement or distribution in the cell.

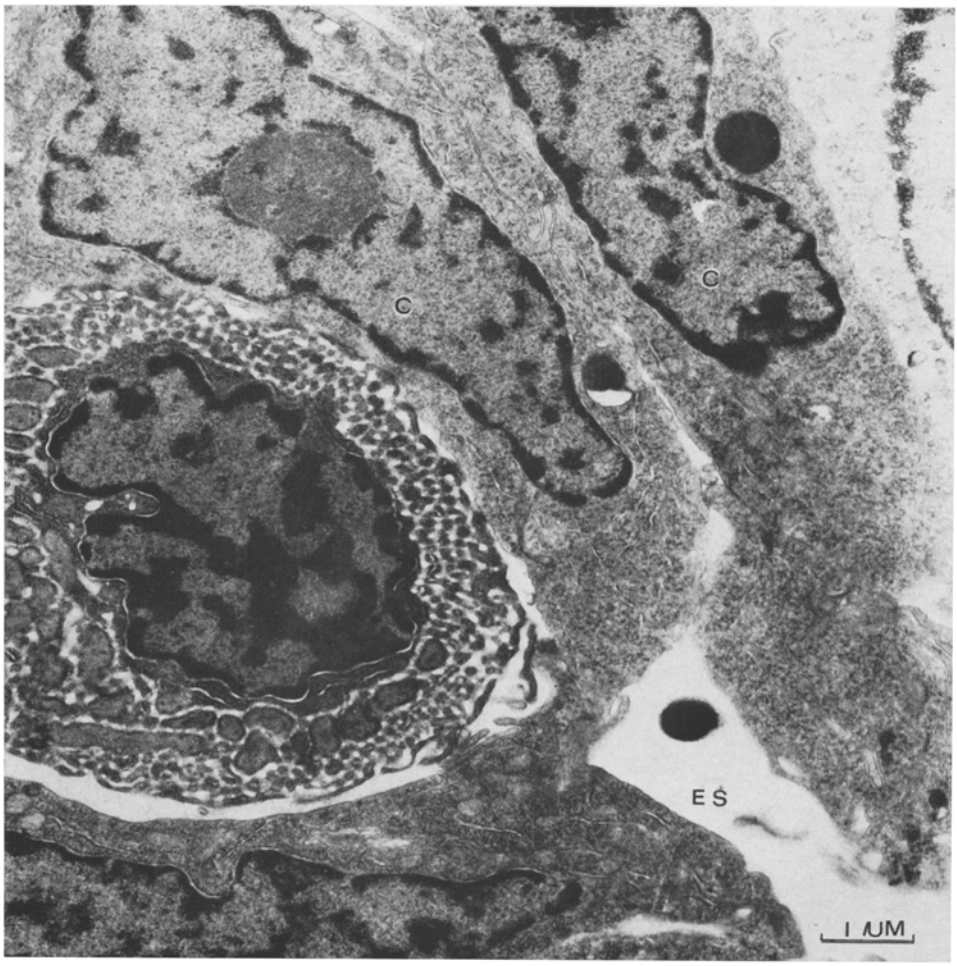

Fig. 7. Labyrinth cell in indifferent epithelium of adult sea trout. The cell is loosely anchored to ciliated nonsensory cells (*C*) and surrounded by large intercellular space which is continuous with the extracellular space (*ES*) of the basal zone

Lysosomes are located mainly in the supranuclear region (Fig. 4). The small ones contain myelin-like concentric systems of membranes sometimes intermingled with granules. Occasionally there are also *multivesicular bodies* in the supranuclear cytoplasm (Fig. 6). These are about 300 mμ in diameter, and many small vesicles are embedded in a moderately dense matrix surrounded by a single membrane.

The cells which do not lie in the surface layer have no cortical zone. Instead they have numerous villous extensions all around the cell surface (Fig. 7). These interdigitate with those of other cell types. The cells are surrounded by extensive intercellular spaces.

Discussion

Morphologically, the labyrinth cell is no modified sensory, supporting, goblet, ciliated nonsensory, microvilli or blastema cell, that is, any modified normal mucosa cell. These cells have quite another fine structure (Bertmar, 1972e). It is instead similar to a gill chloride cell adapted for fresh water (no apical cavity) or an acidophilic cell of the pseudobranch.

A transitional stage of the typical chloride cell has been reported from freshwater adapted eels (Shirai and Utida, 1970). It has some structural differences with the mature cell (poorly developed mitochondrial granules, a large amount of RER). Such a stage of labyrinth cells has not been found in this material. The subsurface labyrinth cells certainly differ in size and form from the surface labyrinth cells but their fine structure is identical as regards mitochondria and RER.

Functionally, the labyrinth cell is more difficult to identify. The most notable feature of the labyrinth cell is an extensive, turtuous, interconnected tubular system of SER which fills the cytoplasm. As this is also connected with and has the same thickness as the plasma membrane it may also be considered as a highly developed extension of the plasma membrane. In this regard it is analogous to the extensive infoldings of the plasma membrane found in such high-transport cells as those of the proximal and distal convoluted tubules of the kidney and the nasal salt glands of birds.

Demonstration that physical continuities exist between the SER and the plasma membrane in labyrinth cells reinforces the idea that the role of the tubular labyrinth is to allow electrolytes, which accumulate in the epithelial intercellular space, to gain entrance and be guided through the cytoplasmic matrix. Philpott (1966) has even shown that large molecules are able to penetrate the gill chloride cell from the blood by way of the labyrinth system. The labyrinth cells are certainly not aggregated in those parts of the olfactory epithelium underlain by blood vessels, but they are loosely anchored in the mucosa and surrounded by a relatively wide intercellular space. There may therefore be an electrolyte transport between the extracellular fluids and the the olfactory cavity. Intimately connected with the transporting tubules of the SER are numerous mitochondria. These provide the energy required by the active transport process involved.

For the outflux theory speaks also the fact that there is a similar apical structure in labyrinth cells and gill chloride cells of freshwater adapted parr and smolt of Atlantic salmon: both have microvilli, microbodies and microvesicles but no apical pit. From structural evidence it was concluded that the microvesicles in chloride cells accumulate chloride which is discharged via extrusions at the free cell surface (Threadgold and Huston, 1964). And Philpott (1966) has found a large concentration of chloride in the apical cavities and suggested that this polyanionic material is a type of acid mucopolysaccharide which serves as the electrolyte carrier acting similar to an ion exchanger. If the tubular labyrinth is the transporting system, then differences in electron density would suggest that the polyanionic material and ions are combined in the basal region of the cytoplasm and then concentrated during transportation to the apical cavities where release to the environment occurs (Conte, 1969).

On the other hand, the surface labyrinth cells also, have an apical structure (microvilli, invaginations, microvesicles, no pit) that may indicate an influx from the olfactory cavity to the intercellular space. And there are many lysosomes in the subcortical part of the cells that may take care of large molecules when they have passed the cortical zone. Philpott and Copeland (1963) demonstrated the increase of the free surface of the chloride cells in freshwater *Fundulus* and suggested the dual role (active absorption and secretion) of the chloride cell. In eel, however, it is unlikely that the chloride cells are concerned with active absorption of ions in fresh water (Shirai and Utida, 1970).

Ecologically, an appreciable difference in number and cell components of the chloride cells in *Fundulus* is not observed between sea water and fresh water adaptation, although the apical cavity disappears in fresh water (Copeland, 1948, 1950; Kessel and Beams, 1962; Philpott and Copeland, 1963). Pettengill and Copeland (1948) suggested that the chloride cells do function also in fresh water, since the alkaline phosphatase activity of *Fundulus*' chloride cells is stronger in fresh water than in sea water. Chloride cells appear to change in ultrastructure and activity in different salinities and their presence appears necessary for the survival of fish in some salinities (Conte, 1969). Whether or not their primary function is ion regulation, or whether their changes reflect a response to one or more of other variables associated with the change in salinity still awaits final elucidation (Holliday, 1971). This might also be said about labyrinth cells. Furthermore, it would be interesting to know if they are involved in the ion balance regulation *per se*, or if they also have influence on the mixed secretion that covers the olfactory mucosa (Bertmar, 1972c) and/or the function of the receptors. This is especially interesting for a species which may rely in part on olfactory cues for successful homing navigation.

Finally, from an evolutionary point of view it is interesting to note the similarity between the labyrinth cells of the olfactory organ and the chloride cells of the pseudobranch and gills. The pseudobranch is vascularized by mandibular vessels (Bertmar, 1962, 1965) and supported by the dorsal part of the modified mandibular arch (Bertmar, 1959). And the olfactory organ is in some fishes supplied by premandibular vessels (Bertmar, 1962, 1965) and supported by skeletal elements that belong to the dorsal part of the modified premandibular arch (Bertmar, 1963). The pseudobranch is a mandibular gill which in actinopterygians is often modified to a gland with mainly secretory function (salt, ammonia). And the olfactory organ may be a modified premandibular gill, serially homologous with the pseudobranch and having some secretory function too. Furthermore, normal gill arches often have taste buds, and the olfactory organ (premandibular gill) also has chemosensory capacity but in this case via primary receptors.

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