

HISTOCHEMICAL STUDIES ON THE OLFACTORY EPITHELIUM OF SOME HILLSTREAM TELEOSTS

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ABSTRACT

Histochemical localization of carbohydrates (1:2 glycol groups, glycogen, B-metachromasia and acid mucopolysaccharides), proteins and protein bound NH_2 group, bound lipids and the enzymes (acid and alkaline phosphatases) have been studied in the olfactory epithelium of some coldwater fishes. The receptor, supporting, basal and mucous cells show varying degrees of distinction and distribution of these substances. The enzymes are present on the surface of the epithelium, sensory hairs and the boundaries of mucous cells only.

INTRODUCTION

Only few studies have been made on the olfactory epithelium of fish (Singh, 1967; Ojha and Kapoor, 1972; Popova, 1972; Munshi and Singh, 1975 and Evans and Hara, 1977) and the present paper for the first time embodies the observations on the coldwater fishes from Garhwal Himalaya.

MATERIALS AND METHODS

Living specimens of *Barilius bendelisis*, *Crossocheilus latius latius*, *Torputitora*, *Glyptothorax pectinopterus* and *Pseudecheneis sulcatus* were collected from the streams and rivers of Garhwal Himalaya. Their olfactory rosettes were removed and fixed in various fixatives. Alcoholic bouin's fluid was used for carbohydrates. The tissue was fixed in cold acetone at 4°C for the localization of the enzymes. Carnoy's fluid, 10% neutral formaline and calcium formol were the other fixatives used for the lipids and proteins. It was followed by the preparation of paraffin blocks of the tissue. This sections (4-6 μ) of the paraffin embedded

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tissue were cut and then dehydrated, stained and finally mounted in canada balsam. Appropriate control procedures were also employed whenever required. Different staining techniques were adopted from Pearse (1968) with some modifications in the staining time in order to obtain satisfactory results.

OBSERVATIONS

Carbohydrates

1. *Periodic Acid Schiff's Reaction* : In the PAS test, mucous cells and the surface of the epithelium stained positive (Plate I, 1). The positive reaction was due to the presence of 1:2 glycol groups as this reaction was negative after deacetylation control but reappeared after deacetylation. The mucous cells took an intense stain showing a higher concentration of 1:2 glycol groups than the surface of the epithelium. The mucous cells as well as the surface of the epithelium gave a positive reaction after digestion with human saliva for half an hour at room temperature showing the absence of glycogen. A free aldehyde group is also absent as mucous cells and the surface of the epithelium gave negative reaction with Schiff's reagent without prior oxidation with periodic acid.
2. *Glycogen and Acid mucopolysaccharides* : The receptor, supporting, basal and mucous cells and the surface of the epithelium as well as the central core of the lamella appeared negative to best carmine test showing the absence of glycogen. The surface of the epithelium and the mucous cells took a bluish green stain with alcian blue showing the presence of acid mucopolysaccharides.
3. *Metachromasia* : Metachromasia in all the species except *P. sulcatus* was demonstrated in the nuclear membrane of the supporting, receptor and basal cells showing a granular appearance. It was also demonstrated in the proximal and distal parts of the receptor and columnar supporting cells. The violet stain obtained with toluidine blue indicates a reaction for B-metachromasia.
4. *Lipids* : It has been observed that the olfactory epithelium of the present species is quite rich in bound lipids. The receptor, supporting and basal cells including sensory hairs and the surface of the olfactory epithelium stained black with sudan black B showing their lipoid nature (Plate I. 2-4). Mild concentrations of lipids were observed in the nuclear membranes of various cell types, cilia of columnar supporting cells and cytoplasm of basal cells. The characteristic colour of sudan black B was lacking in control sections.

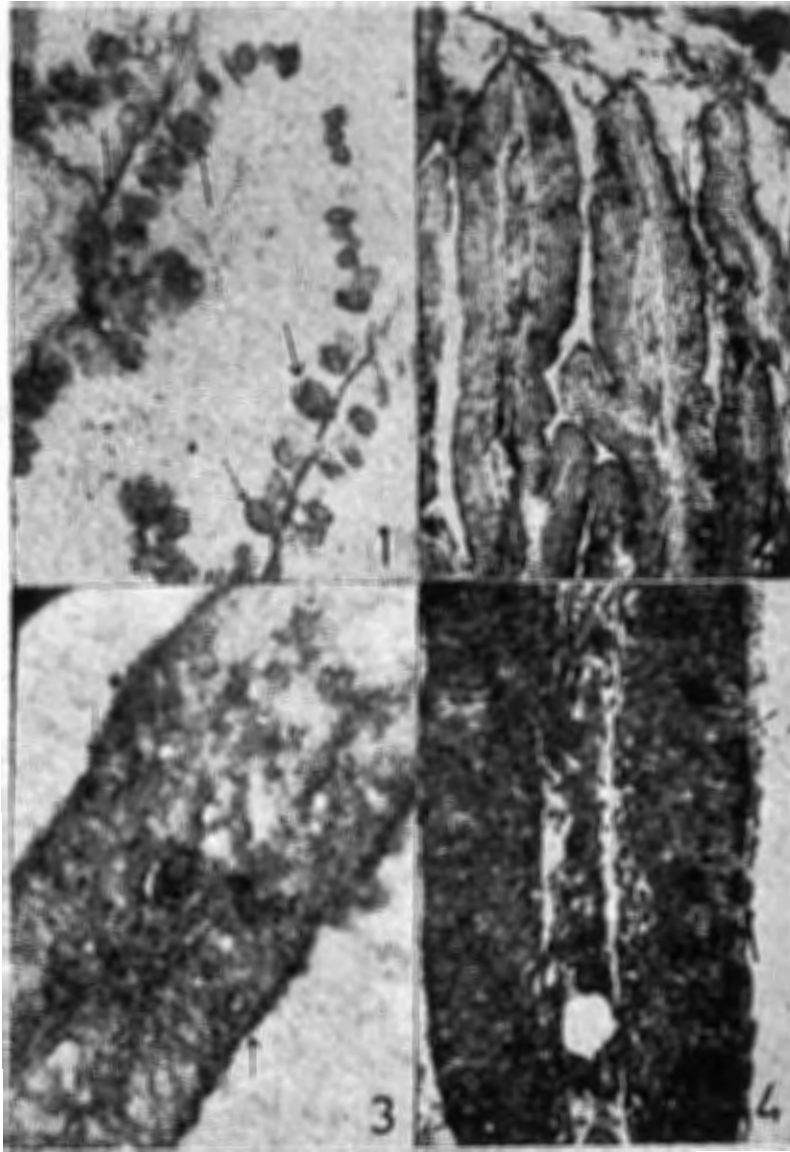


Plate I Photomicrograph of transverse sections of olfactory epithelium of (1) *T. putitora* showing the PAS positive mucous cells and the surface of the epithelium (arrows) X 225. (2) *T. putitora* showing the distribution of lipids (arrows) X 50. (3) *G. pectinopterus* showing the distribution of lipids (arrows) X 225. (4) *P. sulcatus* showing the distribution of lipids (arrows) X 225.

5. *Proteins* : The receptor, supporting and basal cells with surface of the epithelium including sensory hairs and the central lamellar space were positive to mercury bromophenol blue reaction showing a high concentration of proteins in the olfactory epithelium (Plate II. 5-7). The olfactory epithelium of all the present species gave a negative reaction with millon's reaction showing the absence of tyrosine.

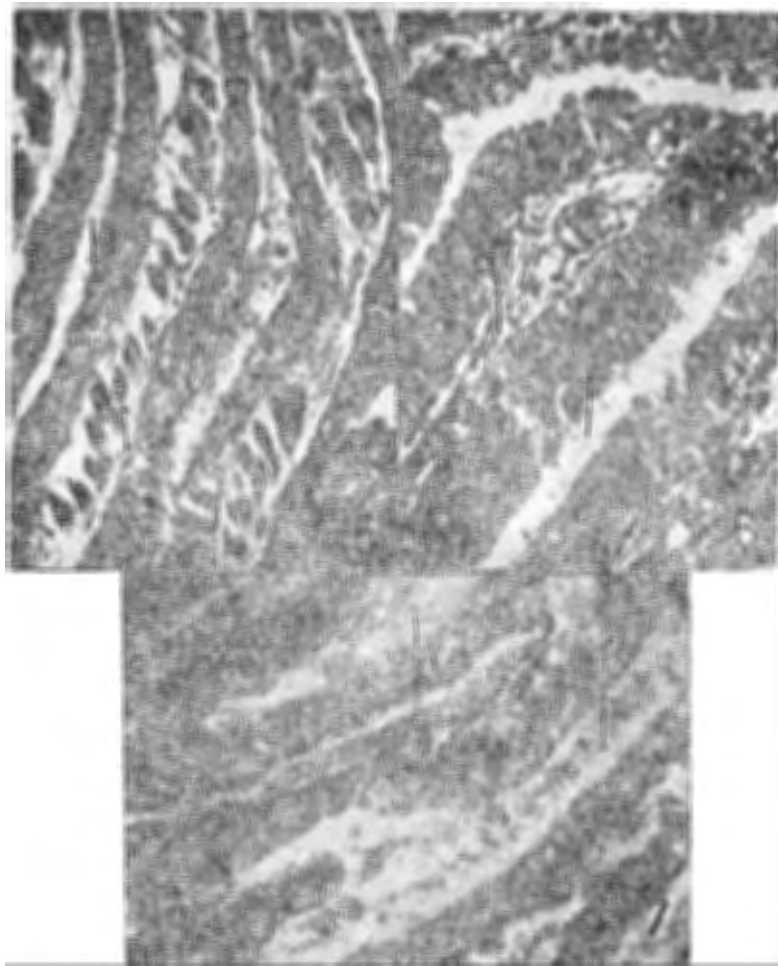


Plate II Photomicrograph of a transverse section of the olfactory epithelium of (5) *T. putitora* showing the distribution of proteins (arrows) X 225. (6) *G. pectinopterus* showing the distribution of proteins (arrows) X 225. (7) *C. latius latius* showing the distribution of proteins (arrows) X 225.

6. *Protein bound NH₂ group* : Ninhydrin Schiff's test gave a positive reaction for mucous cells and the surface of the epithelium showing the presence of protein bound NH₂ group.

7. *SS and SH groups* : The mucous cells and the surface of the olfactory epithelium stained positive with performic acid schiff and performic acid alcian blue showing the presence of SS groups in the epithelium. The epithelium gave a negative reaction with ferric ferricyanide and alkaline tetrazolium.

8. *Enzymes* : A positive reaction was noticed on the surface of the epithelium, sensory hairs and boundaries of mucous cells with lead-nitrate and calcium-cobalt showing the presence of alkaline and acid phosphatases.

DISCUSSION

The histochemical observations suggest that the olfactory epithelium of hillstream fishes contains carbohydrates (1:2 glycol groups, B-metachromasia, acid mucopolysaccharides), proteins and protein bound NH_2 group, bound lipids and the enzymes (acid and alkaline phosphatases).

The receptor, supporting and basal cells are rich in proteins, lipids and B-metachromasia in all the present species. The presence of above inclusions has also been reported by Singh (1967) in *Catla catla* and *Rita rita* and Ojha and Kapoor (1972) in *Channa punctatus*. The enzymes were observed by the authors on the surface of the epithelium, sensory hairs and boundaries of mucous cells in the present species. The presence of acid and alkaline phosphatases has also been reported in the olfactory epithelium of *C. punctatus* (Munshi and Singh, 1975; Ojha and Kapoor, 1972).

It has been observed by the authors that non-specific lipids are moderately distributed throughout the olfactory epithelium of the present species. Ojha and Kapoor (1972) reported that lipids occur in high concentration along synaptic connections in primary neurones, proximal limbs of supporting cells and secretory granules of goblet cells of *C. punctatus*. Doroshenko and Popova (1975) also reported the presence of lipids in olfactory epithelium of sharks and rays. Evans and Hara (1977) observed that in fishes the phospholipids are widely distributed along dendrites of receptor cells.

Singh (1967), Ojha and Kapoor (1972) and Popova (1972) have reported the presence of mucin and acid-mucopolysaccharides in the gland cells of olfactory epithelium of many teleostean fishes. The presence of large number of mucous cells in the present species except in *B. bendelisis* makes their olfactory epithelium highly secretory. The mucous layer provides protection to delicate sensory hairs against osmotic effects of water (Hopkins, 1926).

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