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The Morphology of the Adhesive Organ of the Sisorid Fish, *Glyptothorax pectinopterus*

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Abstract In the sisorid fish, *Glyptothorax pectinopterus*, the adhesive organ located on the ventral side of the thorax consists of a number of longitudinal ridges and grooves that alternate with each other around a triangular furrow lying in the centre. Adhesion of the fish to the rocky substratum in a hill stream habitat is brought about by the hooked and keratinized epidermal spines borne by the longitudinal ridges of the adhesive organ as well as those on the under surface of the pectoral and pelvic fins. The secretion of a surface coat of mucopolysaccharides by the mucous cells and the goblet cells is a device to protect the adhesive organ from mechanical abrasion.

Fishes inhabiting hill streams require special modifications in their body forms to prevent themselves from being washed away by the fastly flowing current of water. Such modifications are chiefly manifested in the form of adhesive structures usually located on the ventral side of the body at the anterior end and on the fins. The adhesive organs are integumentary modifications and serve as highly specialized structures.

Hora (1922, 1923, 1930) studied large number of torrential fishes with respect to their adaptive modifications in response to a life in the swift current and rocky substratum. He described the adhesive apparatus of *Garra annandalei* (Cyprinoidei) and *Glyptothorax madraspatanus* (Siluroidei, Sisoridae). Rauther (1928) dealt with the structure of the adhesive disc of *Discognathus lamta*. The adhesive apparatus of the Chinese sisorid fish, *Glyptosternum* (=*Glyptothorax*) was studied by Wu and Liu (1940) and that of the Indian hill stream sisorids, *Glyptothorax telchitta* by Bhatia (1950) and *Glyptothorax pectinopterus* by Lal et al. (1966). A considerable amount of work on the adhesive organs of hill stream fishes was done by Saxena (1959, 1961, 1966). He explained the mechanism of adhesion in *Garra mullya* and *Pseudoechinus sulcatus*.

From the foregoing literature, it is evident that whatever the work on the adhesive organ of the hill stream fishes has been done is limited to the light microscopy only. The surface morphology of the adhesive organ under scanning electron microscope (SEM) has not been studied so far. Moreover, the histochemical nature of the adhesive organ remains as yet to be investigated. The present study was conducted on the assumption that both morphological and physiological adjustments to the functional and mechanical problems associated with the adhesive organ in relation to the habitat of the fish might have occurred. This investigation therefore aims at describing the morphological and histochemical adaptations occurring in the adhesive organ of *Glyptothorax pectinopterus* (McClelland) with the aid of light and scanning electron microscopy.

Material and methods

Living specimens of *Glyptothorax pectinopterus* were collected from the locality near Hithora, Napal from the Rapti river. The adhesive organs were fixed in Bouin's fluid, Helly's fluid and 10% neutral formalin for histological examination. After fixation, the tissue was dehydrated in a graded ethanol series, cleared in benzene and embedded in paraffin wax. 5–6 μ paraffin sections of the adhesive organs were subjected to routine histological staining procedures. Ehrlich's haematoxylin and eosin, Mallory's triple stain (Jones, 1950) and Papanicolaou EA 36 stain for keratin (Gurr, 1958) were employed to study the general organization of the epidermis. The periodic acid Schiff (PAS) method (McManus, 1946) and alcian blue (AB) stainings at pH 0.5 and 4.5 (Steedman, 1950) were used to identify acid mucopolysaccharides. AB and PAS or

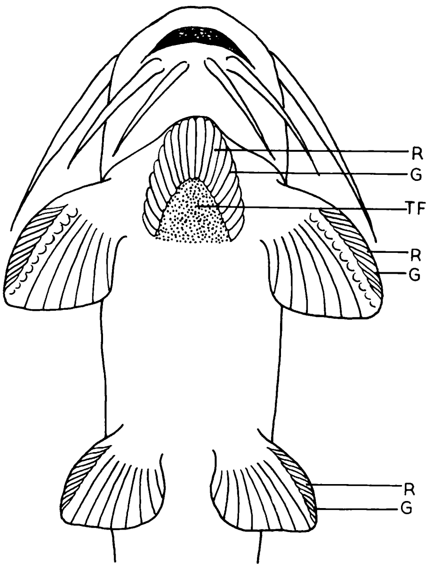


Fig. 1. Ventral view of *Glyptothorax pectinopterus* showing the ridges (R), grooves (G) and a triangular furrow (TF) of the adhesive organ. Ridges and grooves are also present on the pectoral and pelvic fins.

PAS and AB sequence was used to distinguish acid mucopolysaccharides from neutral mucopolysaccharides. The protein end groups and residues tested were amino and thiol groups, arginine, tryptophan and basic proteins. The method used were mercury-bromophenol blue for basic proteins (Mazia et al., 1953), ninhydrine for amino groups (Serra, 1946) and ferric-ferricyanide for thiol groups (Pearse, 1968).

For SEM study, tissues were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 24 hours. The fixed tissues were then washed in 2–3 changes of 30 min each in phosphate buffer and dehydrated in a graded alcohol series. The tissues were immersed in amyl acetate before putting them to critical point drying using CO_2 as the transitional fluid. The dried tissues were mounted on brass stubs with the aid of double sides tape. The tissues were then sputter coated with gold in a gold coating unit and were examined in P SEM 500. Photographs were taken at 25 KV with a spot size of 320 A.

Results

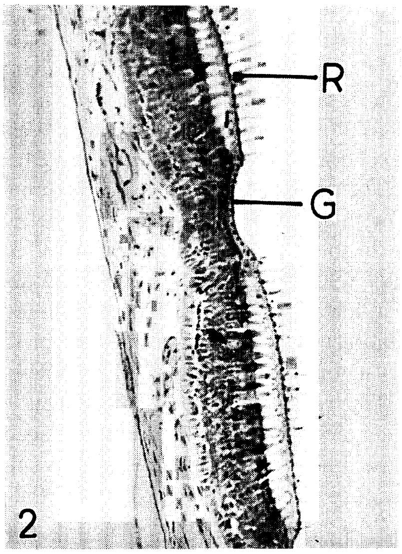
Besides the adhesive papillae on the rostral hood, lower lip and the maxillary barbels, a well defined area of the skin on the ventral surface of the thorax is modified to form an adhesive organ (Fig. 1). The skin becomes folded and forms a series of longitudinal ridges and grooves. The ridges and grooves comprise an inverted 'U' shaped structure. The anterior extremity of this structure falls short of the isthmus and occupies a large area extending from the isthmus to the level of the last pectoral ray. There is a central pit or furrow in the middle of the adhesive organ towards the posterior end. The number of ridges and grooves vary in relation to the size of the fish. The ridges and grooves as found on the adhesive organ also occur on the under surface of the pectoral and pelvic fins which add up to the adhesion in the fish.

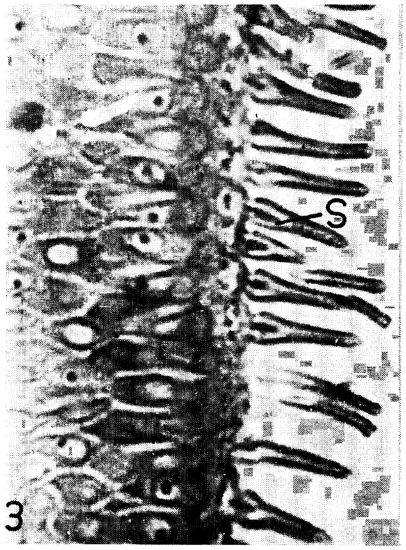
Histological examination of the adhesive organ shows that the epidermis covering the longitudinal ridges is relatively thicker but it remains thinner and devoid of modifications in the region of the grooves (Fig. 2). Sagittal sections through the adhesive organ show ridges (spinous areas), grooves between adjacent ridges and a triangular furrow lying in the centre of the organ.

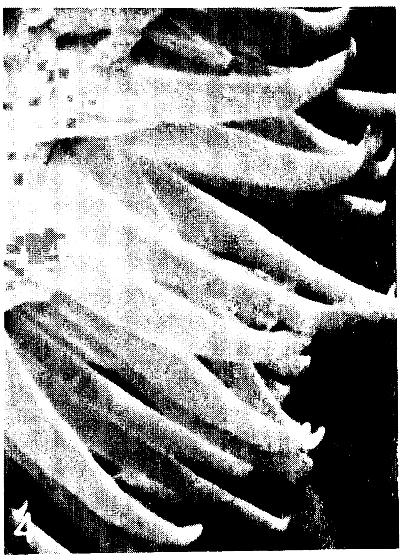
The epidermis covering the ridges is made up from inside out of stratum germinativum, the middle layer and the outermost layer. The stratum germinativum layer is composed of a single layer of columnar epithelial cells. The cytoplasm of these cells is finely granular. Each cell has a centrally located spherical nucleus. The columnar epithelial (=basal) cells rest on a wavy basement membrane. The intercellular spaces formed by the basal cells are almost negligible. The cells remain in active state of mitotic division and give rise to cells which move towards the outer region of the epidermis. The thin basement membrane is PAS positive and shows strong reaction for the thiol groups.

The cells of the middle layer show polygonal, columnar or spherical shapes and contain round nuclei. The cells are arranged in such a way that a large number of intercellular spaces exist. Few pigment cells and lymphocytes are present in this layer of the epidermis.

The outermost layer of the epidermis consists of 2 tiers of cells. The upper layer of epithelial







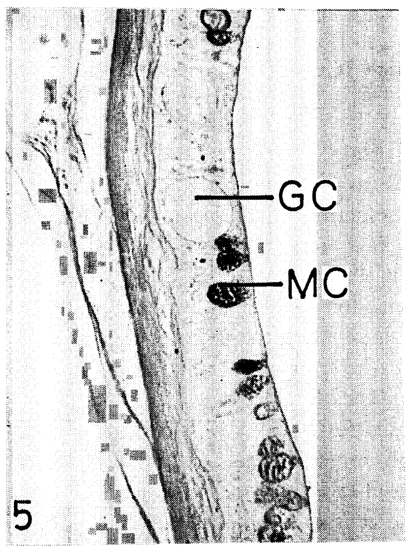


Fig. 2. Sagittal section of the adhesive organ showing ridges (R) alternating with grooves (G), H/E stain. ×200.

Fig. 3. The same magnified to show spinous layers (S) of the epidermis and two tiers of cells of the outer layer, H/E stain. ×700.

Fig. 4. SEM of the spines borne by the ridges. ×2,600.

Fig. 5. Sagittal section through the triangular furrow showing goblet (GC) and mucous (MC) cells, PAS/AB stain. ×350.

cell is modified into curved spines. The apical portion of the epithelial cell remains pointed and the basal portion is broadened (Fig. 3). There is a prominent nucleus or basal granule at the base of the epidermal spine. Surface studies of the ridges under SEM revealed the epidermal spines to be elongated, tapering and hooked structures (Fig. 4). While the bases of the spines are broadened, their apical portions are curved only in one direction. The second tier of cells also shows spine-like projections and interspinous spaces but does not depict a colour reaction with

Fig. 6. SEM showing the smaller opening of the mucous cell (MC) and larger opening of the goblet cell (GC) on the triangular furrow. ×350.

Papanicolaou stain. The interspinous spaces allow the cells to move towards the outermost region of the epidermis and replace the epidermal spines when cast off. Though the spine-like cells were not PAS positive but they showed strong reactions for keratin and basic proteins.

The epidermis covering the longitudinal grooves lying between the ridges is composed of 4–5 tiers of cells. The thin basement membrane on which these cells rest is PAS positive and shows strong reaction for the thiol groups. The cells forming the basal layer are compact and columnar in shape. The cells of the middle layer occur in various shapes and arrange themselves in such a way that intercellular spaces exist among them. The cells of the outermost layer are rectangular in shape and contain centrally located prominent nuclei. They do not show keratinization as they fail to stain with Papanicolaou stain. Mucous cells present in this layer are of two types, the smaller ones producing weak acid mucopolysaccharides and the larger ones strong acid mucopolysaccharides. These cells react differently when stained with PAS alone or in combination with AB. In PAS, while the larger type of mucous cells remain unstained, the smaller ones become purple coloured. In PAS/AB, the larger type of mucous cells are stained blue and smaller ones violet.

The epidermis covering the triangular furrow lying in the centre of the adhesive organ is composed of 4–5 tiers of cells. A single layer of columnar epithelial cells with centrally located spherical nuclei rests on the basement membrane. The middle layer consists of 3 or 4 tiers of cells with intercellular spaces and lymphocytes. The conspicuous feature of this layer is the presence of numerous ovoid or spherical goblet cells and a few tastebuds. The secretion product of the goblet cells presents a net-like picture formed of vacuolated structures in the whole of cell interior. The cells are so much distended with secretory material that the nuclei are pushed towards the peripheral region and appear smaller and inconspicuous in comparison to the cell size. In PAS, the cells remain unstained and in PAS/AB, they stain faintly with alcian blue (Fig. 5). The nature of their secretion product is weak acid mucopolysaccharides. The outermost layer of the epidermis shows two types of mucous cells as found in the grooves. Both mucous cells and goblet cells open at the surface epithelium by round pores (Fig. 6).

Discussion

The adhesive organ of *Glyptothorax pectinopterus* differs from that of another related species *G. telchitta* (Bhatia, 1950) in shape, size and structural organization. While this organ is in the form of an inverted 'U' in the former, it is oval in the latter. Moreover, there is almost a triangular furrow in the middle of the adhesive organ in *G. pectinopterus* but it is absent in *G. telchitta*. These differences are intimately connected with their different ecological habitats. *G. pectinopterus* is adapted to a comparatively more torrential habitat than *G. telchitta*.

The epidermis covering the longitudinal ridges is characterised by the presence of keratinized spines which project from the surface epithelium to the outside. The spinous cells possess prominent nucleus at their bases. The presence of the nucleus at the base of the spine was not reported by Bhatia (1950) in *G. telchitta*. However, Hora (1922) reported the occurrence of the basal granules in these species.

The underlying cells which form a thin continuous platform contain sulfated mucopolysaccharides. The cells remain busy in synthesizing protein. The protein synthesized is rich in sulphydryl groups. It is well known that the sulphydryl groups of proteins form the main constituents of the keratin chain. Evidently these cells are undergoing the process of keratinization but are not yet fully keratinized to produce a colour reaction with Papanicolaou stain. It is interesting to note that while the outermost layer of spines is keratinized, the underlying layer is kept in the process of keratinization. This layer therefore acts as a reserve and replaces the outermost layer of spines when cast off.

The most important question remains as to how adhesion is affected. Hora (1922) and Bhatia (1950) were of the view that friction alone is responsible for the adhesion process. Saxena (1966) considered that a vacuum is produced in the grooves which alternate with the ridges. The vacuum results due to contraction of the muscles attached to the adhesive organ. The present study reveals that the ridges of the adhesive organ are the main structures involved in the adhesion of the fish to the substratum. The ridges are provided with numerous spiny projection which are generally curved in one direction and form some sort of pegs or anchors as pointed out by Lal et al. (1966). It seems logical that these innumerable pegs or anchors get attached to the organic growth on the rocks easily and provide adhesion to the fish. As the spiny projections are keratinized, they are not easily worn out.

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ナマズの一種 *Glyptothorax pectinopterus* における固着器の形態

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ナマズの一種 *Glyptothorax pectinopterus* の胸部腹側に存在する固着器官は，中央に形成された三角形のくぼみの周辺に交互に配列した多数の縦走する隆堤と溝とから構成されている．渓流域の岩石などの付着基物に対する固着は，胸鰭と腹鰭の下面とこの固着器の縦走堤に生えている鉤状で角質化した表皮性の棘によって行われる．粘液細胞および杯細胞が産生した粘液多糖類からなる表面を被覆する分泌物は，機械的磨滅から固着器を保護している．