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ELECTRON MICROSCOPIC STUDY OF GENERAL BODY EPIDERMIS OF *HOMALOPTERA BRUCEI* FISH OF KUMAUN HIMALAYAN REGION

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ABSTRACT

The hill-stream fishes are very well adapted to some specialized conditions of their life in the torrential environment where turbulent flow over rough substratum (rocks, boulders, cobbles and pebbles) of the stream is a feature, directly related to the degree of slope or gradient and elevation. The skin is composed of three distinctive layers; epidermis, dermis and hypodermis. The epidermal cells covering the skin surface possess microridges. The openings of mucous cells were visible among the junctions of the epithelial cells.

KEYWORDS: Epidermis, Epithelial Cells, Hill-Stream and Skin

INTRODUCTION

The cyprinid fishes in the Hill-streams of India are represented by genera belonging to the families Cyprinidae, Cobitidae and Homalopteridae. These fishes show a remarkable uniformity in their body contour. Dorsally the body is slightly arched, while ventrally from snout to anus is usually flat. The remarkable abilities of fish to adapt to Hill-stream, where chiefly the strength of water current is extremely high and the general body epidermis (GBE) is modified according to its role (Joshi, 2010). The superficial epithelial cells remain metabolically active and secrete an extra cellular coat (Whitear, 1970). Various cellular components of the epidermis of fishes vary in abundance and dimensions, between species. This may be related to the mode of living of the fish and its response to the environment. Epidermis or the outer thin layer of integument situated as it is in contact with external environment, forces the assumption that the epidermis must serve as buffer between the host and the environment. Indeed, the epidermis serves many ways to protect the organism from the variety of noxious agents and to maintain the constancy of the internal milieu. It protects the body from microbes, from injury to delicate inner tissues, from the injurious effects of chemicals and from damage by ultraviolet radiation of the sun and helps as thermoregulator for normal internal physiological functions. The remarkable ability of epidermis to adopt its surrounding accounts not only for the seemingly endless structural and functional differences between the various species but for certain basic pattern common to all.

MATERIALS AND METHODS

The live fishes viz. *Botia almorhae* (Teleostei: Cobitidae), (approximately 5-7 inches in length) were collected from the Kosi river at Kakrighat of Distt. Nainital (elevation- 1200 m. above mean sea level), *Homaloptera brucei* (Teleostei: Balitoridae), (approximately 3-4 inches in length) from West Ramganga at Chaukhutia in Distt. Almora (elevation-1200 m. above mean sea level) and *Schizothorax richardsonii* (Teleostei: Cyprinidae), (approximately 6-8 inches in length) from the Kosi river at Hawalbagh in Distt. Almora (elevation- 1194 m. above mean sea level) Uttarakhand. The water current is very fast having the velocity between 0.5 to 2.0 m/sec. (Bhatt and Pathak, 1991) and the river bed is rocky.

The fishes were transferred from the site of collection to the laboratory in well ventilated plastic containers and

were kept for a period of about 5-6 days in glass aquaria having an artificially prepared rocky bed with aquatic vegetation grown therein. The aquaria were cleaned and supplied with fresh spring water on alternate days. The fishes were fed on aqua feed (tropical fish food).

To study the details of the morphological adaptations in some fishes, SEM was done. The following procedure was adopted for the preparation of the specimen for SEM.

The specimen was maintained in laboratory at 25±2^{0}C. The fishes were cold anesthetized following Mittal and Whitear, 1978, for SEM preparation. Skin fragments of about 10×10 mm were cut from their dorsal sides just behind their heads. Tissue were excised and rinsed in 70% ethanol with one change of saline solution to remove debris and then fixed in 3% Glutaraldehyde in 0.1M phosphate buffer at pH 7.4 over night at 4^{0}C in a refrigerator.

The tissues were washed with 2-3 changes in phosphate buffer and dehydrated in ascending series of ice cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30 mins.) and dried at critical point using a critical point dryer (BIO-RAD England) with liquid carbon dioxide as the transitional fluid. Tissues were glued to stubs, using conductive silver preparation (Eltecks, Corporation, India). The samples were coated with gold using a sputters coater (JFC 1600) and examined under (JEOL, JSM- 6610 LV) scanning electron microscope and the images were observed on the screen.

RESULTS

The aim of the present study is to provide a basis for better knowledge of the surface architecture of the GBE of some hill-stream fishes. The skin of the hill- stream fishes, on the dorsal surface of the body just behind the head, is densely set with scales and composed of an epidermis and a dermis supported by a hypodermis. Each scale is covered externally by the epidermis which reaches the posterior free margins transversing a short distance on its inner surface and then continue to the outer surface of the underlying scale. The epidermis is both type smooth and rough, smooth epidermis possesses epithelial cells and mucous cell apertures between the epithelial cells and the rough epidermis possesses tubercles.

The skin covering the general body surface of *B. almorhae* is rough and provided with a large number of scales. (Figure 1 and 2).

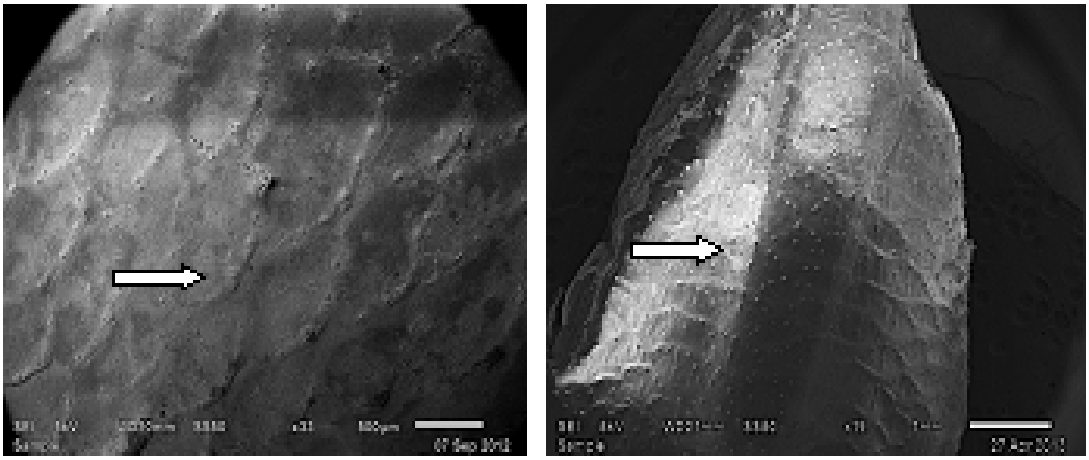

Figure 1 & 2: SEMPH of the GBE of *H. brucei* Showing the Scales (Scale Bar- 500µm and 5µm)

The surface of the mucogenic epithelium of the GBE is covered by irregular polygonal epithelial cells of varied dimensions. The polygonal epithelial cells are present in the GBE of *H. brucei* (Figure 3).

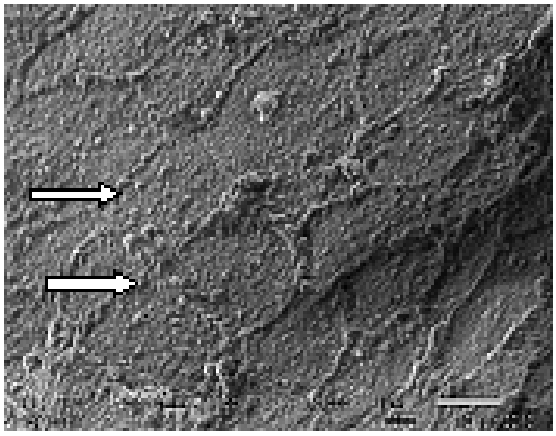

Figure 3: SEMPH of the GBE of *H. brucei* Showing Polygonal Epithelial Cells (Marked by Arrow), (Scale Bar-5µm)

The free surface of the epithelial cells is differentiated into microridges, forming characteristic patterns. The epidermal cells covering the skin surface are intricately patterned with microridges. The microridges on the surface of the epithelial cells generally have a finger print-like structure (Figure 4).

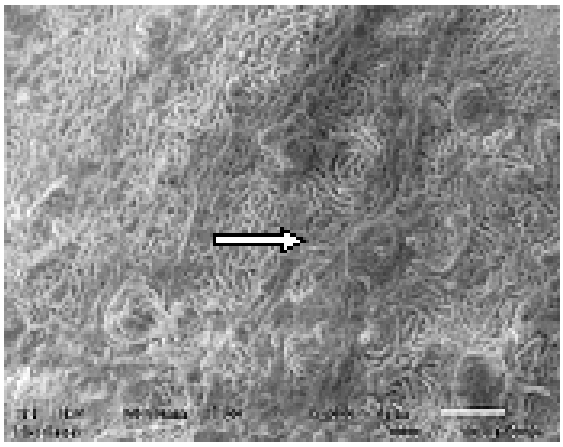

Figure 4: SEMPH of the GBE of *H. brucei* Showing the Microridges are Generally Short, Straight or Sinuous and Smooth Often Arranged in the form of Small Groups (Marked by Arrows), (Scale Bar- 5µm)

The mucous cell apertures are rare comparatively and occur at the border of three or four epithelial cells (Figure 5).

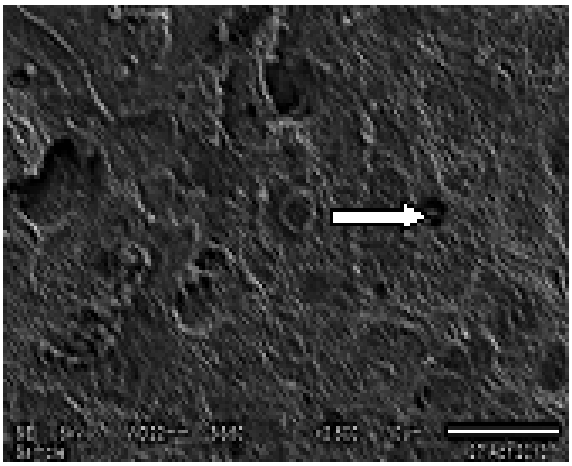

Figure 5: SEMPH of the GBE of *H. brucei* Showing the Openings of Mucous Cells (Marked by Arrows), (Scale Bar- 10µm)

A large number of tubercles are found on the epidermal surface of *H. brucei*, these tubercles exist in a well designed pattern. (Figure 6 and 7).

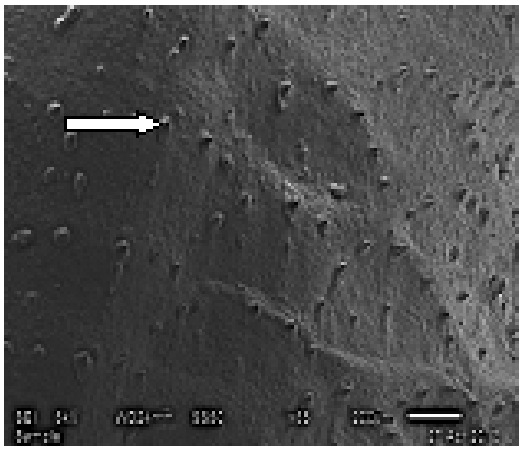

Figure 6: SEMPH of the GBE of *H. brucei* Showing the Well-Developed Tubercles (Marked by Arrows), (Scale Bar- 200µm)

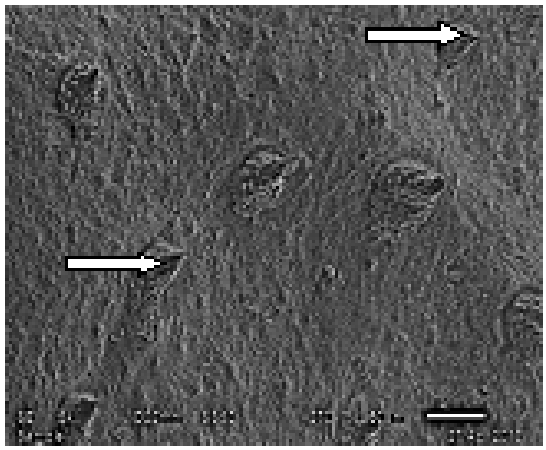

Figure 7: SEMPH of the GBE of *H. brucei* Showing the Well-Developed Tubercles at High Magnification (Marked by Arrows), (Scale Bar- 50µm)

The epithelial cells of each tubercle develop into unculi. Unculi are equidistantly placed and supported by epithelial cells, polygonal outlining of the epidermal cells are seen at the base of the unculi, indicating that unculi are modified epithelial cells (Figure 8).

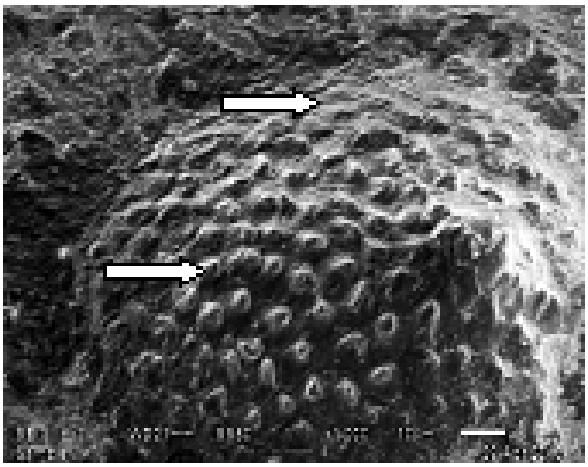

Figure 8: SEMPH of the GBE of *H. brucei* of Showing Polygonal Epithelial Cells and Unculi on the Tubercules (Marked by Arrow), (Scale Bar- 10µm)

ABBREVIATIONS

SEMPH- Scanning electron micro-photograph.

DISCUSSIONS

H. brucei is adapted to life in hill streams characterized by fast flowing streams under boulders. It is found in mountain streams (high gradient streams). The general body epidermis of *H. brucei*, exhibits compactly arranged microridges forming intricate mesh-like patterns, which are characteristic of the habitat under the boulders and stones. Furthermore, these microridges may gain a firm base and support from a dense network of fine filaments. The free surface of each epithelial cell is characterized by the presence of a series of microridges. The microridges of the cells appear smooth and uniform in width. A dense network of microridges could be interpreted as a means to retain more and more

mucus at the surface of epithelial cells because the epidermis of the general body possesses only a few mucous cells. Fishes are in constant interaction with their aquatic environment, which contains a wide range of pathogenic and non-pathogenic micro-organisms. Fish mucus is believed to play an important role in the prevention of colonization by parasites, bacteria and fungi and thus acts as a chemical defence barrier. Frictional force is less under boulder and stones; therefore, the requirement of lubrication is minimum in *H. brucei*. Fishes and other aquatic vertebrates are covered with a mucous epidermis over their entire body surface.

Virtually all fish are covered with an integumental mucus secretion that is involved in many aspects of their biology (Daniel, 1981 a, b). The epidermis is ectodermal in origin and consists of several layers of simple cells, of which the outer are being constantly worn away by wear and tear and replaced by newer ones which develop at their base. These layers of cells are composed of flattened cells, known as epithelium cells, of which the deepest layers are made up of columnar cells forming the stratum germinativum in which cells are always multiplying by mitotic division to replace the outer worn out cells. A superficial layer of dead horny cells, forming the stratum corneum is not present in fishes as an adaptation to life in water (Khanna, 1993).

The epidermis of *H. brucei* possesses a large number of elevations distributed at irregular intervals. The epidermis with elevations alternates with that of the non-elevated surface. The average thickness of the epidermis varies in the two regions of *H. brucei* (Non-elevated region: 61.7 µm, at elevated region: 85.9 µm) (Bisht, 1999). In several fish groups, breeding tubercles, or "pearl organs," develop on the head, body, or fins during the spawning season (Wiley and Collette, 1970). These are horny protuberances which are secreted in response to seasonal hormonal changes, primarily in males and presumably function in enhanced contact during spawning. These tubercles slough off after the spawning season.

CONCLUSIONS

Studies of fish skin indicated that epidermal cells follow separate pathways of differentiation in different fishes. In most of the fishes, the epidermis is related more to the deposition of slime over its surface and undergoes the process of mucogenesis and in some the epidermal cells undergo the process of keratinization forming a layer at the surface.

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