**Integument:**

This chapter deals with *H. fossilis* and *C. batrachus* integument which, unlike mammals, is one of the major organs of mucus secretion. It forms an all-round covering over the body surface and serves as the direct interface between animal and its environment. Fish integument is essentially based on generalized mammalian pattern and performs majority of the functions associated with vertebrate skin. However, in certain ways, teleost skin is unique and histologically diverse since it reflects adaptation of the teleost to the challenges posed by aquatic environment. In this chapter, three important aspects of catfish integument have been dealt with. The first aspect relates to general morphology and histology including ultrastructural studies and also the qualitative and quantitative aspects of glycoconjugate moieties in the skin mucous cells of *H. fossilis* and *C. batrachus*. The other two aspects describe (a) salinity and seasonality induced changes in the skin mucous cells number and size and (b) circannel (seasonal) changes in the sulphomucin contents of skin mucous cells of *H. fossilis*. 

In the present study, the histological studies have shown that all the three classical layers viz. epidermis, dermis and hypodermis including that of basement membrane are clearly demarcated in both the catfish species i.e. *H. fossilis* and *C. batrachus*. However, there are finer structural differences between the two species. In *H. fossilis*, dermis is twice of the epidermis whereas in *C. batrachus* it is four times. In epidermis, the abundantly present spherical shaped mucous cells are largely confined to outer most layer in *C. batrachus* but are present both in the outer as well as middle layer in *H. fossilis*. These mucous cells show variations in shape, size, degree of granulation and vacuolation during normal seasonal variations and also under salinity stimulation. Mucous cells do not get stained with hematoxylin and eosin but are positively stained with PAS and AB singly or in combination. The middle layer of epidermis of both the catfishes is overwhelmingly occupied by large size club cells, which are characterized by cap like vacuolar area, eosinophilic cytoplasm. These cells are elongated and columnar with multiple nuclei in *C. batrachus* and round or spherical with 1-2 centrally placed nuclei in *H. fossilis*. Other cell types such as sacciform cells, swollen cells, ionocytes, cuticle-secreting cells and merkel cells as observed in some other teleosts do not exist in both these catfishes. The last layer of epidermis, stratum germinativum is a single-layered not too well-demarcated layer and unlike the mammals, is not the only multiplying layer in fishes but all the epithelial cells overlying stratum germinativum are metabolically active. There is no evidence of any upper most keratinized layer or cuticle covering area of the epidermis in these catfishes. The lymphatic spaces containing lymphocytes provide protection against microorganism and nutrition to stratum germinativum. In addition their features like taste buds, apical pit are also observed. Just beneath the basement membrane lies a continues layer of chromatophores, a characteristics of poikilotherms which also exist sporadically in the subcutis region of the skin of these catfishes. The dermis of both the catfishes correspond to
the typical teleostean plan of being formed by an upper loosely arranged stratum spongiosum and lower compactly packed stratum compactum which are well demarcated in *H. fossilis* and somewhat diffused in *C. batrachus*. Both these dermal layers are made up of collagen fibers which do not get stained with PAS and AB (2.5) & (1.0) but take up distinct stain with Masson Trichrome. The dermis of these catfishes is richly invested with blood vessels, nerve ending and sense organs. A well-defined hypodermis or subcutis, characterized by large number of adipose cells with in-between empty spaces with sporadic blood supply and nerve endings and some chromatophoresis also observed in catfishes. It is generally assumed that the amphibious fishes which are capable of cutaneous respiration are characterized by the thick epidermis due to partial O₂ uptake. However, the critical evaluation of available data on epidermal thickness in air-breathing and non-breathing fishes including that of the present study on both catfishes do not support such an assumption. Interestingly, the club cells in *C. batrachus* are much taller, than those of *H. fossilis* but epidermis thickness remains the same. This situation is explained in the catfishes by the fact that the area of club cells and mucous cells follow an inverse relationship leaving the effective thickness of the epidermis not being altered due to this compensation. The above findings clearly show that the histological features of both these catfishes conform to the basic architecture of a typical teleostean species with interesting deviation from broad generalization which makes this study significant.

The ultrastructural feature of *H. fossilis* integument have also been studied employing both SEM and TEM approaches. The SEM study of catfish *H. fossilis* integument shows the overwhelming presence of pavement cells which border the outermost epithelial layer. These cells are characterized by conspicuous concentrically arranged microridges with a single layer border. At the junctions of these PVCs, there are occasional openings presumably of the MCs which lie buried under these PVCs. These openings are clear, unblocked and are the exit points of the MCs for secretion of mucus which extensively fills the space between the microridges. Since the skin of this catfish is scale less and is also devoid of any other epidermal or dermal derivatives which may project out of the skin, the entire integument surface under SEM view shows the predominance of only PVCs and the opening of the MCs. The SEM study of the catfish integument has not revealed any other cellular or other structural details. The perusal of literature related to the SEM study on the integumentary surface structures show relatively fewer studies as compared to TEM studies on the same structures which may be largely due to the fact that SEM studies have the drawback of revealing only the limited information. Any environmentally altered condition such as ambient salinity, sudden change in pH or temperature leads to copious exudation of mucus which covers the pavement cells of skin. A similar effect has been observed in the present study when the catfish has been transferred to 25% SW which has resulted in the copious secretion of mucus which filled the microridges to the extent of blurring its contours.
Under TEM observations of the skin, almost all the different cellular components can be observed quite unlike the SEM study which largely captures the view of surface lined pavement cells and the orifices of the MCs. Understandably, this is because of the plane of visualization. However, even under TEM studies, the predominant cells are MCs and pavement cells, which are universally present in the skin of all teleost fishes. The other cell-types which have been described under TEM integument studies on other teleosts are filament cells, cuticle-secreting cells, undifferentiated cells, merkel cells and club cells. Chloride cells, in addition to gill epithelium, have also been found to occur in the skin of large number of euryhaline teleosts but are mostly absent in stenohaline fishes. Rodlet cells which are likely to form non-specific defense mechanism of the skin have also been reported in epidermis of the fish exposed to stressors. In our study on H. fossilis, we have clearly deciphered the presence of MCs, pavement cells and the club cells within the limited observations made by us. Mucous cells clearly show the intermingled mucosomes of electron lucent (pale) and electron dense vesicles in the tap water maintained catfish. Interestingly, there is a well-marked regional aggregation of these mucosomes into distinct patches of electron dense and electron light granules. While we have observed a large number of chloride cells in the gills of both FW & 25% SW maintained H. fossilis but these cells are conspicuously absent in the skin of this fish. A similar absence of these cells has been reported in the skin of C. carpio even though chloride cells have been reported in large number in euryhaline teleosts. Based on the above observations, it is tempting to suggest that the stenohalinity of the teleost may perhaps be in some way related to the absence of the chloride cells in the skin which will limit the ability of these fishes to make forays into divergent salinities.

The present study has also elaborately described the distribution of glycoprotein (GPs) moieties in the mucous cells. The H. fossilis exhibits the predominance of neutral GP whereas those in the C. batrachus, it is acidic GP. This study has also shown tissue specific GPs variations in the same species as seen in H. fossilis where skin mucous cells contain neutral GP but it is the mixture of all GPs in the gill mucous cells of same fish. The observations of present study, as also of others, have clearly established that the distribution of various types of GPs in skin mucous cells does not conform to any specific pattern of either phylogenetic kinship, salinity based habitat i.e. FW, SW or brackish water or any other morphological or structural demand of a particular species. If one probes the functional role of various types of GPs, a fair degree of apparent overlap is clearly noticed. The protection against the invasion of pathogenic microorganism has been associated with both sulpho- as well as sialomucin and similarly thick viscosity imparted by acidic mucin and somewhat more fluidic mucus produced by neutral mucin has been associated with an over all lubrication function in teleosts. Interestingly, sulphomucin has been shown to facilitate the osmoregulatory homeostasis in freshwater teleosts but not all freshwater teleosts have predominance of sulphomucin. The present study has clearly established that it
is not prudent to associate the occurrence of any specific GPs moiety in skin mucous cells to only one specific function since each such function is governed by multiple type of GPs.

The salinity-induced changes on the number and size of the skin mucous cells in *H. fossilis* show an interesting biphasic response. A significant hyperplasia and hypertrophy is observed following transfer of this catfish to 25% and 30% SW which is followed by a sudden and significant decrease in 35% SW. Our present observation on skin mucous cells are in complete contrast with those observed in gill mucous cells where no changes are observed in mucous cells area and number upto 30% SW but a significant increase is registered in 35% SW. In literature, there are conflicting reports where in certain studies a sudden decline in mucous cell number and size has been reported whereas in equally good number of species a significant increase is recorded. These apparently conflicting observations have been explained by certain authors based on the following reasoning. A changes in salinity or parasitic or any other stresses causes an initial release of mucus on the skin surface as a combative action which leads to exhaustion of mucous cells causing the reduction in their number and size. This phase is, however, followed by the generation of new skin mucous cells which requires varying time in each species and ultimately causes the increase in mucous cells number and size. It is thus clear that a clear-cut biphasic response occurs following transfer of fish to altered salinity. Hence, the conflicting reports on the apparent anomaly in mucous cells dynamics following salinity or other stimuli may possibly be due to random sampling at varying time whereas it does underline the need for a well spread time course study. But in *H. fossilis* the graded increase in 25% and 30% SW and a substantial decline in 35% SW with uniform period of acclimation points to the role of mucous cells in osmoregulatory homeostasis of this catfish.

Another interesting aspect of this study is the circannual variations recorded on monthly basis, in the size, number and sulphomucin content of skin mucous cells of *H. fossilis*. The results clearly show an almost parallel profile change in number and size of mucous cells which are marked by two major peaks—one spring peak during April and the other winter peak during December-January. The sulphomucin contents also register high values during March and June to February with highest levels during January but a near total absence during April and May. There are hardly any studies on the seasonal variations in mucous cells dynamics largely due to its arduous execution and more importantly due to multiplicity of factors operating in natural habitat and it becomes virtually impossible to quantify all of them. In the instant study, it becomes difficult to predict whether the observed changes are the result of single factor or the collective response of consortium of these factors. However, the available data suggest that the seasonal temperature variations of both high as well as low magnitude are governing the hypertrophy and hyperplasia corresponding to one peak at the onset of high temperature during April and the same peak is evident at the falling temperature during December and January. The observation on sulphomucin profile clearly suggests that this GP moiety is not required during April and May when its contents