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Histochemistry of the respiratory surface of the caudal gills of *Ceriagrion coromandelianum* (Fabricius), Zygopteran larvae (Odonata: Zygoptera)

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Abstract
The present study showed that mucous cells in the gills of damselfly larvae were found mainly in the surface of the gill lamellae when employing with Schiff's reagent (PAS test), and recognized the presence of muco-polsaccharides. The present cells of the epithelium of the caudal gills have been found to contain acid muco-polysaccharide. The perusal of literature pertaining to the histochemical investigation of the different zones of the caudal gills of Zygopteran Odonate larvae revealed that practically no work has been done so far on these taxa. The aim of the present work was to study histochemical characterization of the caudal gills of damselfly larvae to investigate the deposition of chemicals in respiratory epithelium of the caudal gills of Zygopteran larvae.

Keywords: *Ceriagrion*, Caudal gills histohemical, PAS method, mucous cells, muco-polysaccharide

Introduction
The gills are a complex organ which has multipurpose functions such as respiration, osmo-regulation, and excretion of metabolic nitrogen, acid-base balance, locomotion and feeding (Pears, 1950, Heisher, 1984; Randall and Daxboeck. 1984; Maina, 1990) [10, 6]. For these functions, the gill epithelium is highly specialized and consisted of at least five different cell types which are cuticle, pavement cells, chloride cells, neuro-epithelial cells and mucous cells (Asakawa, 1970 and Laurent, 1984) [1] and much has been done of their morphology (Yamada, 1964; Zaccone, 1981; Flethcer *et al.*1976) [17, 18], functions and the chemical contents of the mucin (Gona, 1979) [4]. The available literature indicate that considerable work has been done on the structure, function and nature of the chemical contents of mucous cells of gills, epidermis and digestive canal of teleost fishes.
The histochemical nature of mucous secreting cells and their secretary products has been extensively studied in mammals (Lillie, 1954, 1958, 1965; Curran, 1964; Wichard and Eisenbeis, 1979) [9, 2, 16]. These authors have adopted autoradiographic studies as well as combinations of basic dye and viz.-glycol localization methods (Alcian Blue-PAS; colloidal ion-PAS). The authors have also employed specific blockage techniques (e.g. methylation, acetylation saponification) and enzymatic digestion techniques movable with by almomidase and diastase. On the basis of these results, these mucosubstances have been recognized either as neutral or acid muco-polysaccharides.
The caudal gills of zygopteran Odonate larvae exhibit protrusive growth in which the distal zone, which has a thinner cuticle than the proximal zone, increases in length as the nymph matures (MacNeil, 1960). In additional to a direct respiratory function, tracheal gills may serve to ventilate respiratory surfaces, thus, reducing the O_2 diffusion gradient. Gills are also used for locomotion, feeding and may be modified for adaptation to current. Tracheal gills also function in osmo–regulation and ionic regulation. Thus, the histochemical and cytochemical make up of the caudal gills of the zygopteran larvae might differ in different species even the different zones of the gill lamellae of the same species.
The fine structural organization and histochemical demonstration of the caudal gills revealed that chloride cells (uni cells or cell complexes) and chloride epithelia occur as ion absorption sides in these insects. Kommick (1977) provides an excellent review of chloride cells and chloride epithelia in zygoptean larvae. Kommick (1977) presented considerable data on the fine structure of ion absorption sites, as well as details on the taxonomic distribution of the types and subtypes of chloride cells among aquatic insects. Ion absorption structures are known only from aquatic stages of amphibiotic insects. It has been found that the numbers of chloride cells are inversely proportional to the salinity of the medium. The chloride cells of plecopteran and zygopteran larvae, which were formerly interpreted as sensory or respiratory in function (Zwick, 1973) and in plecopteran larvae according to their structural variations, three types termed as caviform, coniform and tubiform chloride cells-have been distinguished (Wichard and Komnick, 1973, 1974). The fine structural organization of the chloride cells and the histochemical demonstration of chloride in the apical region of the cell complex of caudal gills point to the ion transporting function which participates in osmo-regulation by the absorption of ions.
The review of literature on histochemical studies of the respiratory epithelium indicated that the detailed work has been done on the localization of muco-polysaccharides and glycogen in the gills of the fishes. Among the perusal of these literatures concerning review on histochemical examination of

muco-polysaccharides have been principally done in fishes and mammals and information in insect lineages is very meagre. Muco-polysaccharides are compound of polysacchrides, glycoproteins and proteoglycans and collectively these compounds are known as mucosubstances. In insects these mucosubstances are the chief constituents of chitin, gill epithelium of Odonate larvae (rectal gills and caudal gills), alimentary canal specially secretory midgut epithelium) and needed during biosynthesis of secretary products and hormones.

No detailed study appears to have been carried out on the chemical nature of the mucous secreted by caudal gills of damselfly larvae. The present study was, therefore, undertaken in an attempt to elucidate the chemical nature and properties of the mucous secreted by the caudal gills of damselfly larvae.

Materials and Methods

Damselfly larvae were collected from local fish ponds. These larvae were sorted out and kept in an aquarium in the entomological research laboratory of the Department with pond water. Aquatic weeds like *Hydrilla* were supplied to help the insects in clinging to the plants. The gills were dissected out from the caudal region of the species *Ceriagrion coromandelianum* (Fabricius), and kept in specimen tube separately. The dissected gills taken on the slides and washed with distilled water 1 to 3 times. After washing the gills of species kept in aqueous Bouin's fluid and continuously kept in Bouin's fluid for about 24 hours, gills become strict and hard. After fixation both gills were processed separately for paraffin block preparation.

The 6-8 μm paraffin sections were cut by rotary microtome and subjected to various histochemical tests to evaluate the carbohydrate content of the mucous gland cells. The paraffin sections were stained with the PAS technique (Pearse, 1960, 1968) [11, 12] using a sulphite rinse after treatment with the Schiff's reagent in order to stain sulphated groups and subsequently the carboxyl groups.

The mucous cells were stained with 1% Periodic Schiff's reagent to localize the sulphated muco-polysaccharide content, an alcohalic solution for water stable mucoproteins and histochemical differentiation of acid muco- polysaccharides.

For the dilution of neutral and acidic mucins, acetylation and deacetylation for the confirmation of hydroxyl group was performed following Lillie (1954) [9]. Methylation and demethylation (Spicer, 1960) [14] were done to confirm the acidic nature of the mucins. In the PAS method sections were treated with periodic acid which oxidizes certain hydroxyl groups to aldehydes. The aldehydes are visible by reaction with Schiff's reagent.

Results

The result obtained in the histochemical tests for the presence of glycogen in the various parts of the gill sections of damselfly larvae i.e., *Ceriagrion coromandeliaum* (Fabricius) is shown in (Plate - I) respectively.

Ceriagrion coromandelianum (Fabricius)

Epiproct

The results of PAS histochemical test for the presence of muco-polysaccharide in the mucous epithelial cells. When the gills sections were stained with Schiff's reagent, most of the cells particularly epithelial cells were stained strongly and appearing as red purple colour. But in cuticle region the epithelial cells showed that darker colour in comparison to other cells. This result indicated that the epithelium cells of cuticle regions were strongly react with Schiff's reagent and showed the presence of muco-polysaccharide which was maximum in this area, but in tracheal incircaling +ve reaction was noticed (Plate –I; Fig. A1,a).

Paraproct

The results of various histochemical analyses of the caudal gills indications the composition of the mucous material. When the gills were stained with Schiff's reagent most of cells were stained strongly and appeared as red purple colour. This reaction indicated that the mucous cells in the epithelium of damselfly larvae gill were observed to contain muco-polysacchride. The (Plate - I; Fig. A1, b) epithelial cells in cuticle region was strongly ++ve with Schicff's reagent. In the tracheal region the epithelial cells were stained normally and appeared red purple colour but not showed that strongly PAS + positive.

In case of paraproct, (Plate –I; Fig A1, a, b) showed that the red purple colour in whole section to react with Schiff's reagent but in epiproct the clolour showed more darker.

Table 1: A summary of the histochemical tests performed to show the chemical nature of the caudal gills of damselfly larvae (Zygoptera: Odonata)

Species name	Material	Tissue	Fixative	Techniques with Schiff's reagent	Result obtained			
					Epithelial cell	Cuticle	Trachea	Haemocoel
Ceriagarion coromandelianum (Fabricius)	Caudal Gills	Epiproct	Bouin's	PAS Test	++ ve. Pb	++ ve.Pb	+ ve. Pb	- ve
		Paraproct	Bouin's	PAS Test	++ ve. Pr	++ ve. Pr	+ ve. Pr	- ve

Legend = Pr - Purple red, Pb – Purple brown, ++ ve = Strongly positive, + ve = Normal positive, -Ve = Negative

Plate 1

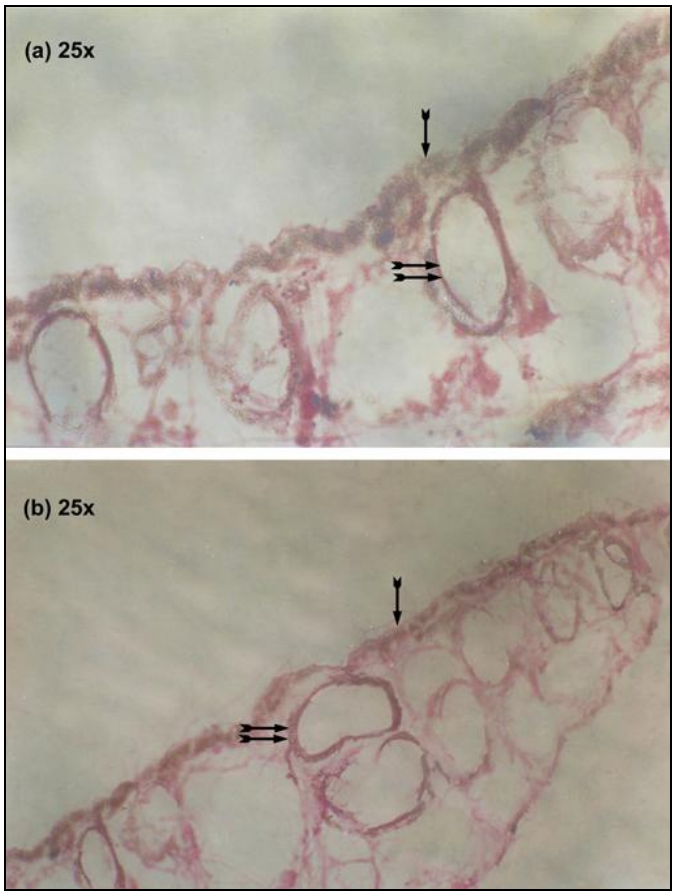

Fig 1a,b,: Histochemical studies of Epiproct and Paraproct (a,b) of *Ceriagrion coromandelianum* (F) Dark stain showing the mucupolysaccride in cuticle (single arrow) and of trachea (double arrow). (a) Epiproct 25x, (b) Paraproct 25x

Discussion

Present observations showed on the histochemistry of the respiratory surfaces of Zygopteran larvae that more muco-polysaccharide is absorbed by the cuticle and tracheal areas in the epithelial cells, when, employed Schiff's reagent (PAS test) to recognize the presence of muco-polysaccharide in epithelial cells. On this basis, therefore, cells of the cuticle and trachea have been found to contain muco-plysaccharides as revealed by staining showing red purple with PAS method.

The caudal gills of damselfly larvae i.e. *Ceriagrion coromandelianum* (Fabricius), showed the chemical nature of the mucous cells in epithelium has not been definitely determined. There is much evidence to indicate that they are largely muco-polysaccharides in nature. They were stained by Schiff's reagent and given the positive reaction with 1% periodic acid. The mucous glands were recorded as PAS positive. The PAS positive reaction was due to the presence of muco-polysaccharide contents in caudal gills (Plate - I; Fig.A. I, a, b).

Mucous cells in the epithelium of damselfly larvae gill were observed to contain muco-polysaccharide. Most of mucous cells of damselfly larvae gill mainly contained neutral mucin. The surface of damselfly larvae gills is covered with mucous cells and this substance acts as an ion exchange material absorbing, contributing to the buffering function of the gills.

The strong acidic character of the muco-polysaccharides was well demonstrated in getting red purple colouration with Schiff's reagent (pH 0.5), the colour less areas of the mucous cells denotes their weak acidity whereas the red purple reaction points to their strongly stained with Schiff's reagent and shows the presence of muco-polysaccharide and acidic character. The suppressive effect of acetylation on the Schiff's reagent staining on a large number of epithelial cells and regaining of their original colour intensity after deacetylation might be attributed to the acidic groups of muco-polysaccharides.

The large amount of trachea and tracheoles present in the epithelial cells of gills appears to be correlated with the muco-polysaccharide secretion. The failure to detect them histochemically in the present study might be accounted for their insufficiency to produce colour reactions.

The mucous gland cells of the respiratory epithelium in the gills of damselfly larvae have been found to be reactive towards Periodic acid Schiff. The carbohydrate material of the mucous cells were identified as sulphated acid muco-polysaccharides which, however, appear to possess more functional carboxyl groups than sulphate groups. The mucous cells (epithelium) also contain mucoprotein residues which were undetectable histochemically.

Present study reported that there were differences in the mucous components in mucous cells of gill epithelia of the zygopteran larvae in same species in the same environment. They are generally PAS negative though those belonging to the epithelium are PAS positive, when the muco-polysaccharides elements of the epithelial cells are extracted with 1% periodic acid Schiff's reagent. It is possible that the carbohydrate elements of the epithelial cells are masked by muco-polysaccharide molecules.

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