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HISTOCHEMICAL ANALYSIS OF MUCOUS CELLS IN THE EPIDERMIS OF SNAKE EEL *PISOODONOPHIS BORO* (HAM-BUCH)

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ABSTRACT

A detailed histochemical study was made on the epidermal mucous cells of the estuarine burrowing snake eel *Pisoodonophis boro*. PAS, Alcian blue at pH 2.5 and 1.0, combined Alcian blue pH 2.5/PAS, Alcian blue pH 1.0/PAS, Aldehyde fuchsin, combined Aldehyde fuchsin/Alcian blue pH 2.5, Hale's colloidal iron, Bromophenol blue, Methylation procedures, digestion with diastase, hyaluronidase and neurominidase techniques were followed for histochemical tests.

The results showed the presence of sialic acid containing glycoproteins with a minor sulphated mucopolysaccharide. The protein content was low. The mucous cells also contained glycogen. Sialic acid is associated with viscosity. The predominant sialic acid component of mucin in *P. boro* could lubricate and reduce friction during burrowing.

INTRODUCTION

IT IS well known that the epidermal mucous secretions of marine animals are valuable to mankind. The mucous secreted by the mullet *Mugil cephalus* and the catfish *Plotosus anguillaris* possess antimicrobial properties (Lewis, 1971). The mucous secretions of the ophiuroid *Ophiocomina nigra* (Fontaine, 1964) and of the clam *Spisula solidissima* (Thomas, 1954) contain highly sulphated polysaccharides and hence possess heparin-like anticoagulant property. The mucus of the murrel *Ophiocephalus* provided extra strength to lime mortar and was used for the construction of old Churches in Cochin (Antony, 1952). As fish mucous is advantageous to man, numerous workers carried out biochemical and histochemical analyses in marine animals (Bullock and Roberts, 1974). Biochemical analysis of the chemical nature and property of fish mucous is difficult because the secretion of mucous cell and Malphigian cell origin have not been separated, whereas histochemistry offers a more sensitive method for localising the different types of glycoproteins and their intracellular combinations in mucous cells than the biochemical analysis (Fletcher *et al.*, 1976). Therefore the present investigation describes only the histochemical analysis of mucous cells in the epidermis of the snake eel *Pisoodonophis boro*.

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MATERIAL AND METHODS

Specimens of *Pisoodonophis boro* were collected by sorting out through the muddy area of the Vellar Estuary. They were transferred to the laboratory and maintained in aquarium until used for experiments.

Eels were anesthetized in MS 222 and sampling of the integument was taken from

the nape region. About 1 cm^2 area of the skin was removed from this region for histological and histochemical studies. The excised tissue was fixed in phosphate buffered formalin, which preserved structural detail with minimal distortion (Bullock *et al.*, 1976). After fixation for 24 hr, small portions of integument were dehydrated and embedded in celloidin using a standard procedure. The celloidin blocks were then embedded in paraffin and sections cut at 6 μ. A tabulated outline of histochemical methods used are given in Table 1.

TABLE 1. *Histochemical methods used to demonstrate the mucous cells of* Pisoodonophis boro

Method	Specific histochemical result	Procedure used
Periodic-acid Schiff (PAS)	Oxidation of vicinyl hydroxyl groups followed by formation of coloured complexes (red) with Schiff reagent.	MC Manus (1948)
1% Alcian blue in 0.1 N-HCl (pH 1.0)	Sulphated mucins-blue	Pearse (1968)
1% Alcian blue in 3% HAc (pH 2.5)	Acidic mucins-blue	Pearse (1968)
Alcian blue pH 1.0/PAS	Sulphate-free, sialic acid containing mucins —red.	Mowry and Winkler (1956)
Alcian blue pH 2.5/PAS	Sulphate-free, sialic acid containing mucins-blue Magenta.	Mc Manus and Mowry (1960a)
Aldehyde Fuchsin	Sulphated mucins purple or blue-purple. Non-sulphated acid mucins stain weakly or not at all.	Spicer and Meyer (1960)
Aldehyde Fuchsin/Alcian blue pH 2.5	Sulphated mucins purple or blue purple. Non-Sulphated acid mucins stain weakly or not at all.	Spicer and Meyer (1960)
Hale's Colloidal Iron	Acidic mucins-blue	Mc Manus and Mowry (1960b)
Mercury—Bromophenol blue	Proteins stain clear blue	Pearse (1968)
Diastase treatment (one hr. at 37°C subsequently stained with PAS)	Inhibits subsequent staining of glycogen with PAS	Lillie (1947)
Confirmatory test for glycogen and starch with ' Lugol's iodine solution	Glycogen-mahogany. Starch-blue	Lillie and Greco (1947)
Mild Methylation (0.1 N HCl in Methanol for 4 hrs. at 37°C)—subsequently stained with Alcian blue (pH 2.5)	The alcianophilia of both non-Sulphated and sulphated mucins is inhibited.	Spicer (1960)
Active Methylation (0.1 N HCl for 4 hrs. at 60°C) subsequently stained with Alcian blue (pH 2.5)	The alcianophilia of both non-sulphated and sulphated mucins is inhibited.	Spicer (1960)
Hyaluronidase treatment at 37°C subsequent staining Alcian blue, pH 2.5	Hydrolyses several common glycosaminoglycans	Pearse (1968)
Neuraminidase treatment at 37°C subsequent staining Alcian blue pH 2.5/PAS	Inhibits alcianophilia of sialic-acid containing glycoproteins	Spicer and Duvenci (1964)

Results

The results of the mucoid histochemistry are presented in Table 2. Intensity of the stain taken by mucous cells was visually estimated with 4 representing strongest staining activity, 3 representing strong reaction, 2 representing moderate reaction and 1 denoting weak reaction. The results of the histochemical reactions and the intensity of the colour taken are shown in Table 3.

All the mucous cells stained reddish-purple with PAS-technique, showing the presence of glycoprotein (Pl. I A). But following diastase digestion the PAS reaction was negative. Treating the sections with Lugol's iodine after diastase treatment confirmed the presence of glycogen. Mercury bromophenol blue gave weak positive reaction and thereby indicating very little amount of protein in the mucous cells.

The positive reaction of Hale's colloidal iron indicates the presence of acidic mucins in the mucous cells. Almost all the mucous cells were alcianophilic in both Alcian blue pH 2.5 and in the combined Alcian blue pH 2.5/PAS procedures (Pl. I B, C). This response shows the presence of sialic acid containing mucins in the mucous cells. Whereas at Alcian blue pH 1.0 and the combined procedure with PAS, very few cells showed weak alcianophilia (Pl. I D, II A). This indicates that sulphated mucosubstance are also secreted in small quantity. In combined Alcian blue pH 1.0/PAS procedure the intensity of colour varied from cell to cell showing a mixed mucous cell population of which most cells contained sialomucins (red colour) and a few cells contained sulphomucins (Weakly blue ; Pl. II A). with Aldehyde fuchsin, a stain for sulphated mucosubstances, only a few cells showed positive reaction and most of the cells were not stained (Pl. II B). In the combined Aldehyde fuchsin/Alcian blue pH 2.5 procedure most cells stained blue and some cells bluish purple, indicating that the major component is non-sulphated acid mucins with a little sulphated mucins (Pl. II C). Both mild and active methylation procedures inhibited alcianophilia at pH 2.5 and thus evincing support for the presence of sialic acid containing mucosubstances. This was clearly demonstrated by the enzyme neuraminidase, which virtually removed the alcianophilia of mucous cells at pH 2.5. However, the alcianophilia of the mucous cells was not inhibited by hyaluronidase digestion. This shows the absence of hyaluronic acid.

Thus various single and combined histochemical tests showed that the mucin of *Pisoo donophis boro* contains large quantity of sialic acid and a small quantity of sulphated mucopolysaccharide in addition to glycogen and little protein.

Discussion

The snake eel *Pisoodonophis boro* has large number of mucous cells occupying a major portion of the epidermis. All the mucous cells stained reddish purple with PAS technique showing the presence of glycoprotein. After diastase digestion the sections remain unstained showing the presence of either glycogen or starch (Lillie, 1947). The presence of glycogen was confirmed by using Lugol's iodine solution. Mercury bromophenol blue gave weak positive reaction in the mucous cells indicating the presence of protein in small quantity.

Hale's colloidal iron technique, Alcian blue at varying pH and combined with PAS confirm the acidic nature of the mucosubstance. Almost all mucous cells stained blue with the combined Alcian blue pH 2.5/PAS. In the case of Alcian blue pH 1.0/PAS combination, most of the cells stained red and some stained weakly blue. This suggests that the major component is non-sulphated with a minor fraction of sulphated mucins. The presence of sulphomucins cannot be excluded in the combined Alcian blue pH 2.5/PAS technique as the

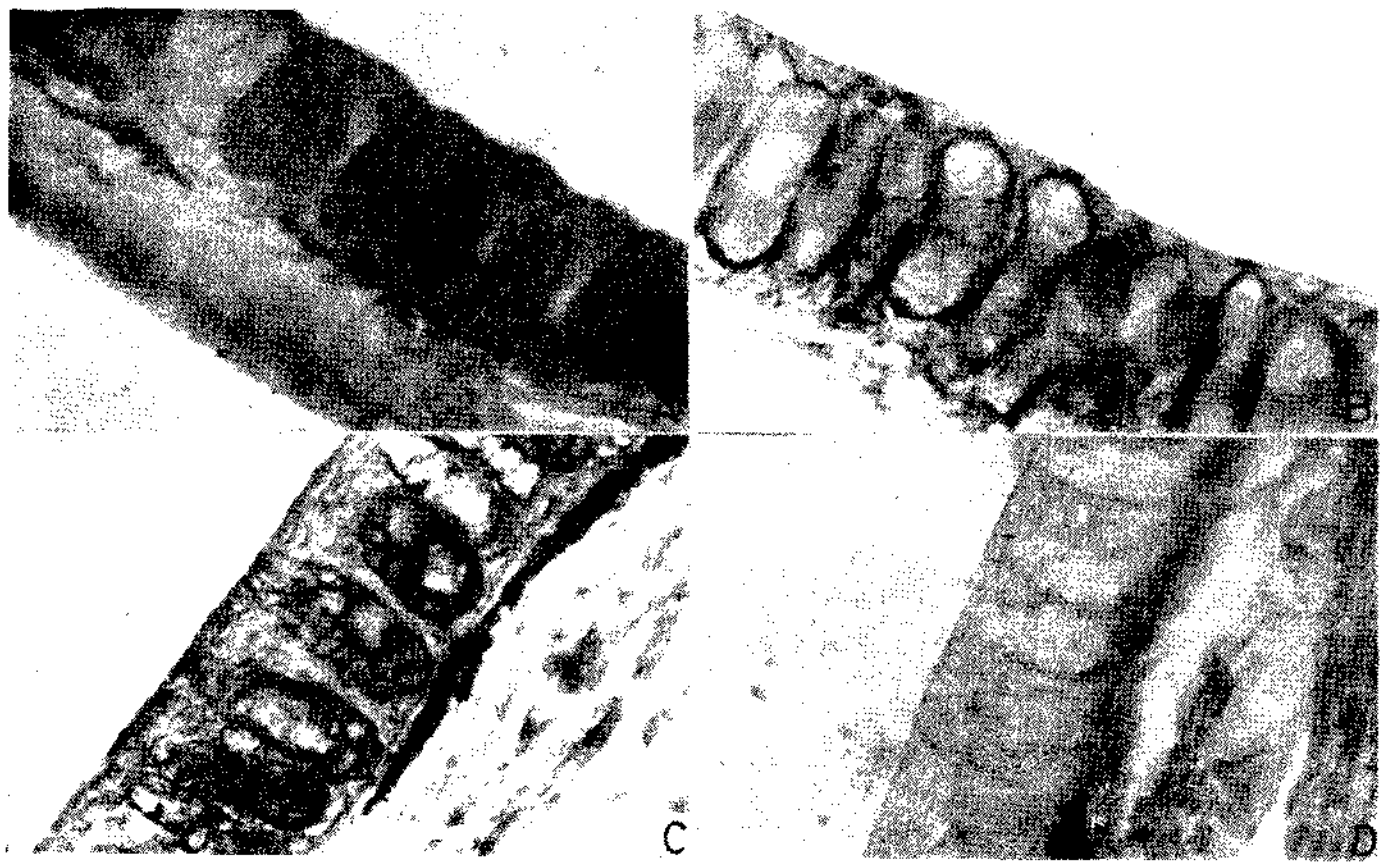

PLATE 1. T.S. of skin of *Pisodonophis boro* : A. Periodic acid Schiff reaction, B. Alcian blue pH 2.5, C. Combined Alcian blue pH 2.5/PAS and D. Alcian blue pH 1.0 (All × 400).

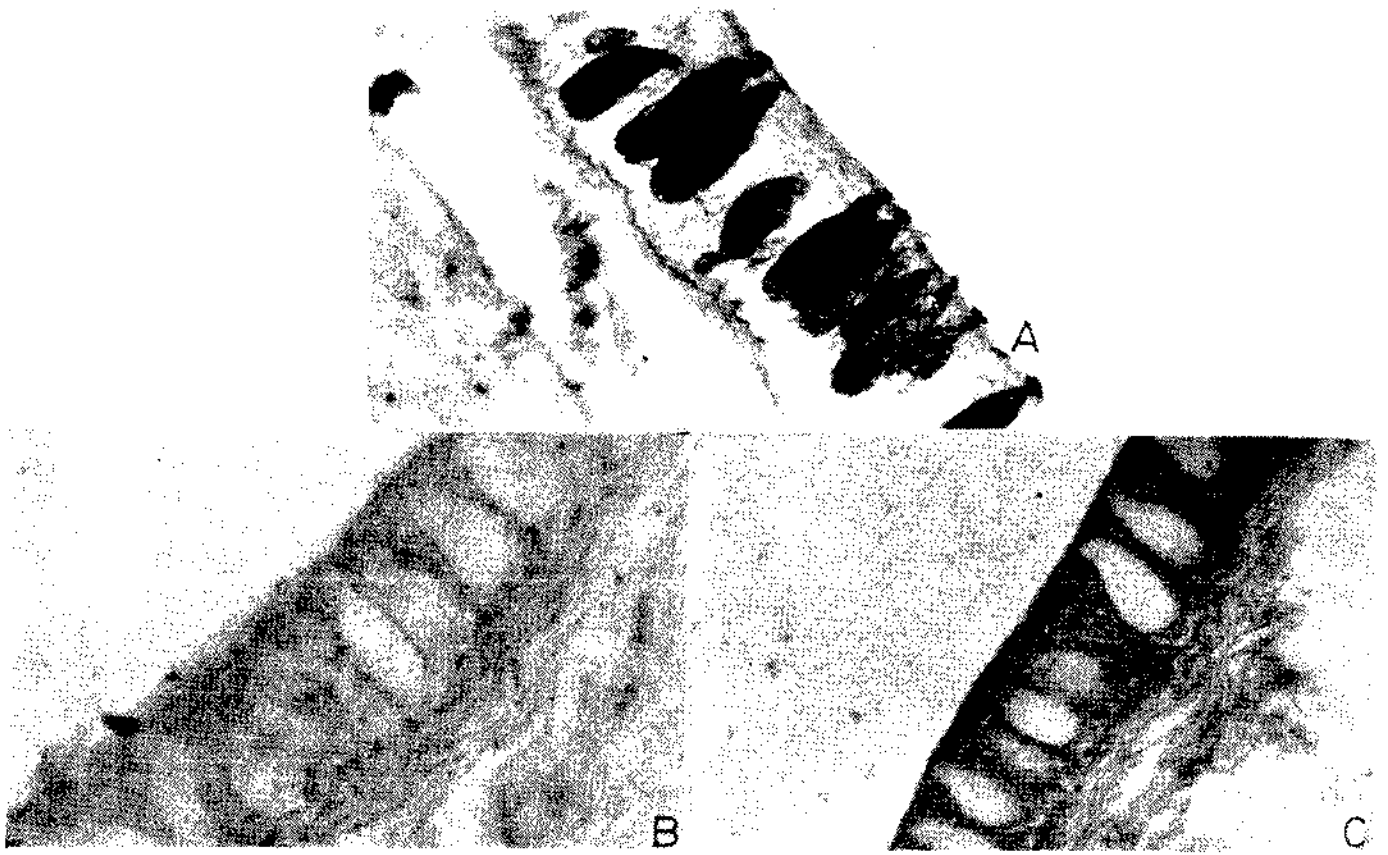

PLATE II. T.S. of skin of *P. boro*. A. Combined Alcian blue pH 1.0 PAS. B. Aldehyde Fuchsin and C. Combined Aldehyde Fuchsin Alcian blue pH 2.5 (All ×400).

TABLE 2. *Results of mucoid histochemistry on the integument*

Stain	Component demonstrated	Reaction
PAS	Glycoproteins	Strong positive
Mercury Bromophenol Blue	Proteins stain	Weak positive
Hale's Colloidal Iron	Acidic mucins	Positive
Alcian blue pH 2.5	Acidic mucins	Positive
Alcian blue pH 1.0	Sulphated mucins	Weak positive
Alcian blue pH 2.5/PAS	Sulphate-free sialic acid containing mucins-blue	Positive (blue)
Alcian blue pH 1.0/PAS	Sulphate-free sialic acid containing mucins-red	Positive (red) (some weakly blue)
Aldehyde Fuchsin	Sulphated mucins-red non-sulphated acid mucins stain weakly or negative	Few cells positive (red) most cells negative.
Aldehyde Fuchsin/Alcian blue pH 2.5	Sulphated mucins-purple	Most cells positive (blue), some cells bluish purple
Mild methylation	Alcianophilia of non-sulphated mucin is inhibited	Inhibition of Alcianophilia
Active methylation	Alcianophilia of sulphated and non-sulphated mucin is inhibited	Complete inhibition
PAS-Diastase reaction	Glycogen	Evidence of glycogen
Hyaluronidase treatment	Inhibits alcianophilia of uronic acid	No inhibition
Neuraminidase treatment	Inhibits alcianophilia of sialic acid containing glycoproteins	Inhibition of alcianophilia

TABLE 3. *Histochemical reactions of epidermal mucous cells*

Stain	Intensity	Colour
Mercury Bromophenol Blue	1	Blue
PAS	3-4	Reddish purple
Alcian blue pH 2.5	3-4	Blue
Alcian blue pH 1.0	1	Very weak blue
Alcian blue pH 2.5/PAS	2-3	Blue
Alcian blue pH 1.0/PAS	2-3 red (1 blue)	Most cells red ; some weakly blue
Aldehyde Fuchsin/Alcian blue pH 2.5	2 blue (2 bluish purple)	Most cells blue; some bluish purple
Aldehyde Fuchsin	1	Very few cells red

staining of non-sulphated mucin (major component) would mask their identification. Mild methylation of sections (4 hr at 37°C) blocks the alcianophilia of most non-sulphated acid mucosubstances through their esterification, but not the sulphated mucosubstances, whereas active methylation (4 h at 60°C) blocks both non-sulphated and sulphated mucosubstances by hydrolysing sulphate esters (Spicer, 1960). In *Pisoodonophis boro* both mild and active methylation inhibited alcianophilia at pH 2.5. Alcian blue in combination with aldehyde fuchsin stains sulphated mucins purple and non-sulphated mucins blue (Spicer and Meyer, 1960). In *P. boro* most of the cells stained blue and some cells bluish purple. The single Aldehyde fuchsin technique stains red the sulphated mucins and the non-sulphated mucins weakly or negative. Only a few cells of the eel stained red. This suggests that the predominant component is non-sulphated mucin in the epidermal mucous cells of *P. boro*.

It is well known that sialic acids are responsible for the acidic nature of the carbohydrate. In mammalian epithelia, sialomucins combine with Alcian blue above pH 1.5 (Jones and Reid, 1973). In *P. boro* the sections stained with Alcian blue at pH 2.5 whereas alcianophilia was lacking at pH 1.0. The presence of sialic acid is proved by neuraminidase digestion. Gottschalk (1960) has shown that neuraminidase specifically removes terminal neuraminic acids from polysaccharides. Neuraminidase virtually removed all the alcianophilia at pH 2.5. The hyaluronidase digestion had no effect on the staining properties of mucous cells with Alcian blue. This precludes the possibility of hyaluronic acid in the non-sulphated group. Thus the epidermal mucous cells of *P. boro* contains large quantity of sialic acid containing glycoprotein. Glycoproteins are generally heterogenous and vary in relation to the proportion of fucose to sialic acid present and no sharp demarcation can be shown to exist between acidic and neutral glycoproteins (Hashimoto and Pigman, 1962). This heterogenity may be responsible for individual difference in mucous cells in staining intensity (*e.g.* Alcian blue pH 1.0/PAS).

Glycoproteins exist with and without acid groups in the epidermal mucous cells. Of the acid glycoproteins both sialomucins and sulphomucins are widely distributed in teleosts (Bremer, 1972). The mucous cells of *Platichthys flesus* (Fletcher *et al.*, 1976) contains both acid and neutral glycoproteins of which the former being either sialomucin or sulphomucin. In the electricfish *Malopterurus electricus* (Carmignani and Zaccone, 1974), the brown trout *Salmo trutta* (Harris *et al.*, 1973) and the char *Salvelinus alpinus* (Pickering and Macey, 1977) sialomucins are the major acid component. The mucous cells of the eel *Anguilla vulgaris* and *A. japonica* contain sulphated and non-sulphated acid polysaccharides (Bolognani-Fantin and Bolognani, 1964 ; Asakawa, 1970). Leppi (1968) has detected significant amount of both components in the mucous cells of three species of hagfish *Bdellostoma stouti*, *Bdellostoma deani* and *Myxine circifrons*. In the mucous cells of the Atlantic salmon *Salmo salar* only traces of sulphate were detected (Harris and Hunt, 1973). The result of the histochemical analysis of the mucous cells of *Pisoodonophis boro* is consistent with the findings of Bolognani-Fantin and Bolognani (1964) in *Anguilla vulgaris*, Asakawa (1970) in *Anguilla japonica* and Harris and Hunt (1973) in *Salmo salar*. Carmignani and Zaccone (1975) identified glycogen in *Torpedo ocellata*. Low concentrations of proteins was identified in *Salvelinus alpinus* (Pickering and Macey, 1977). Hence the presence of glycogen and low amount of protein in the mucous cells of *P. boro* is not surprising.

The eel *Pisoodonophis boro* is found in brackishwater environment and often in burrows. It is interesting that the abundance of mucous cells in the integument especially in the head and tail region provides lubrication

and reduce friction during burrowing (Subramanian, 1978). Lopezvidriero and Reid (1978) have pointed out that increase in concentration of sialic acid has been associated with more viscosity. It is reasonable to assume that the predominant sialic acid component of mucin in *P. boro* could lubricate and reduce friction during burrowing because of its high viscosity.

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