HISTOCHEMICAL STUDIES OF BOVINE SALIVARY GLAND MUCINS

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When certain cationic dyes such as toluidine blue or azure A, normally utilized for studies on connective tissue mucins, are employed for the staining of submaxillary and sublingual glands in different mammals, the color reaction is always orthochromatic (Quintarelli, 1960). These results suggest that such epithelial mucins do not contain chondroitin sulfates or hyaluronic acid which, if present, would produce a purple metachromatic color (Pearse, 1960). When special stains for acid mucopolysaccharides such as alcian blue or colloidal iron are utilized, these glands are intensely stained and thus appear to have acidic components. The utilization of a different metachromatic dye, safranin O, revealed an intense color reaction in the mucous acini (Quintarelli and Chauncey, 1960). Blix (1936) and Blix et al. (1956) demonstrated that sialic acid is found in high concentrations in submaxillary mucin of different mammals. Nisizawa and Pigman (1960a) established that the concentration of sialic acid in purified bovine submaxillary mucin is about 32%. The sialic acid in these tissues seems to provide a probable explanation for the positive staining reactions.

It is fairly well established that sialic acid occupies a terminal position (Heimer and Meyer, 1956) and that mild acid hydrolysis can easily split it off without further cleavage of the mucin molecule (Gottschalk, 1957). Specific enzymes found in Vibrio cholera and influenza virus remove sialic acid only partially (Gottschalk, 1958).

This investigation is a report of the use of histochemical techniques to

show that the special staining of salivary gland sections probably arises from the presence of sialic acid in sialomucins. This was done by studies of the effects of mild acid hydrolysis, proteolysis, and the use of influenza virus neuraminidase on tissue sections known by chemical analysis to contain high amounts of sialic acid.

Materials and Methods

Bovine submaxillary glands, fixed in alcohol-formalin or neutral buffered formalin, embedded in paraffin and routinely cut, were treated as follows. Serial sections were incubated in buffer solutions, pH 2.5, 3.5 and 4.5 for 1, 2, 3, 4, 5 or 6 hrs. at 37°, 55°, 65° and 85°. In other instances sections were incubated at 37° in Walpole buffer solution, pH 2.5, containing 2 mg./ml. of crystalline pepsin or in 20 ml. of 0.2 M acetate buffer, pH 5.4, containing 0.2 g. of crude papain, 6.4 mg. of KCN and 33.4 mg. of EDTA (tetrasodium salt). Control sections were incubated in the buffer solutions without enzymes.

Purified influenza virus neuraminidase solution was supplied through the courtesy of Dr. L. Robert, Dept. of Biological Chemistry, University of Ill., College of Medicine. At 37° and in one hour, one tenth ml. of this solution split 100 gamma of sialic acid from orosomucoid which had a total of 200 gamma of sialic acid. A stock buffer solution was prepared as follows: 30 g. of Na_2HPO_4, 2.72 g. of KH_2PO_4 and 170 g. of NaCl were dissolved in distilled water, and the total volume was made up to one liter. Before use one part of the stock solution was diluted with 19 parts of distilled water. Four ml. of this diluted buffer was added to 1 ml. of the neuraminidase solution, and several drops of this final solution were placed at six hour intervals on tissue sections mounted on slides. The sections were incubated at 37° for 24 hrs.; control sections were incubated in buffer solutions without enzymes. The stains used were alcian blue (Steedman, 1950), safranin O (Lillie, 1954) and periodic acid-Schiff reagent (PAS) (McManus, 1948).

Results and Discussion

Sections incubated for 2 hrs. in buffer solutions (pH 2.5 at 55°) lost all safranin O positive material from the mucous acini but only a portion of the

PAS positive material. When sections were incubated in proteolytic enzyme solutions prior to staining, the PAS, the alcian blue and safranin O positive material were entirely removed. Control sections incubated in buffer solutions without enzymes were intensely colored by all three stains.

Tissue incubated in neuraminidase solutions for 24 hrs. completely lost the metachromatic reactivity with safranin O and most of the alcianophilia, but showed only a reduction of the intensity of the PAS reaction.

The loss of metachromasia to safranin O and of staining properties to alcian blue as a result of the mild treatment with acids and with neuraminidase, suggests strongly that the metachromasia and alcianophilia of the submaxillary gland resulted from the presence of sialic acid primarily in the mucin component. The sialic acid is the only acidic material known to be removed under these conditions. The reduction in PAS staining supports this interpretation. Chemical studies (Heimer and Meyer, 1956; Nisizawa and Pigman, 1960a) showed that sialic acid in bovine submaxillary glands is found in equimolar amounts with N-acetylgalactosamine. After removal of the sialic acid the residual mucin should show PAS activity if, as is probable, a 3,4-glycol unit is present in the hexosamine component.

After incubation with the proteolytic enzymes, the sections were not stained by safranin O, alcian blue or PAS. This finding suggests that proteolysis fragmented the mucin molecule to soluble components. This interpretation is supported by the results of Nisizawa and Pigman (1960b) who demonstrated that when submaxillary mucin was treated with papain a considerable amount of the carbohydrate components became dialyzable.

The use of mild acid hydrolysis may have considerable value for the detection of tissue components containing sialic acid. Work now in progress will further broaden the methods and their application in histochemistry.

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