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Lectin histochemistry of the submandibular and sublingual salivary glands in rats

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Summary. Tissue sections from rat submandibular and sublingual glands were studied with lectin probes to identify terminal sugars of the glycoconjugates in various cell types of the salivary glands. The lectins used in the study were *Canavalia ensiformis* (Con A), *Triticum vulgaris* (WGA), Succinyl WGA (S-WGA) *Ricinus communis* I (RCA-I), *Arachis hypogaea* (PNA), and *Ulex europeaus* (UEA-I). The cytoplasm and cell membrane of both the serous and mucous acinar cells present high similarity in the distribution of some sugar residues, but differ considerably in the expression of specific sugars which appear either in the serous or in the mucous cells. The cytoplasm and cell membrane of the serous and mucous acinar cells express Mannose (Man) and Glucose (Glc), but lack Galactose (Gal), and N-acetylgalactosamine (GalNAc). Fucose (Fuc) is present only in the mucous acinar cytoplasm. The moderate to intense binding of WGA to the acinar and ductal cells and the lack of binding of S-WGA, indicate the presence of sialic acid rather than N-acetyl-glucosamine (GlcNAc). These sialic acid residues are not associated with PNA-binding sugar sequences as pretreatment with neuraminidase is not associated with exposure of additional PNA receptors.

Key words: Lectins, Rat salivary gland

Introduction

Mucins are an important secreted constituent of submandibular and sublingual glands (Roukema et al., 1976). A variety of glycoproteins have been demonstrated in the saliva from the major salivary gland. Mucous secretions contain O-glycosylated glycoproteins of high and low molecular weight (Tabak et al., 1982). The monosaccharides induced in the glycans main chain are L-fucose (Fuc), D-galactose (Gal), N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc), acetyl neuraminic acid (Sialic acid) and a structural diversity - up to 50 different carbohydrate side chains.

The heterogeneity in structure of the glycoprotein carbohydrate chain may be the result of variations in relative content of different secretory granule constituents, such as proteases or glycohydrolases, and thus may reflect differences in cell function. The incorporation of sugar moities into glycoproteins involves the sequential action of genetically determined sugar transferase (Schachter and William, 1982; Spicer and Schulte, 1992). Therefore, the differences in glycoprotein synthesis by different salivary gland cells may be the result of the heterogeneity of their carbohydrate content.

Lectins are sugar-binding proteins or glycoproteins of non-immune origin, which agglutinate cells and/or precipitate glycoconjugates having saccharides appropriate complementary sequence (Goldstein et al., 1980). The affinity of different lectins to specific sugars make them useful as histochemical probes (Alroy et al., 1984, 1988; Goldstein and Poretz, 1986; Danguy et al., 1994).

Several lectin histochemistry studies of normal and pathologic conditions of salivary glands have been reported (Naito et al., 1983; Schulte and Spicer, 1983, 1984; Laden et al., 1984; Tolson et al., 1985; Jezernik and Pipan, 1986; Takai et al., 1986). Menghi and Materazzi (1994), studied lectin binding combined with exo-glycosidase digestion.

In spite of the extensive research on the glycobiology of salivary glands using lectin histochemistry, differences still exist regarding lectin binding patterns in the rat salivary gland and their relationship to the functional differences between various cell types of salivary glands.

In the present study, tissue sections from rat submandibular and sublingual glands were studied with lectin probes to identify terminal sugars of the glycoconjugates in various cell types and in duct

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content. Data were analysed to relate specific lectin binding patterns in various cell types of the salivary glands with the functions of the cells.

Materials and methods

Submandibular and sublingual salivary glands were obtained from 12 Wistar rats, weighing approximately 200 g. All tissues were fixed in 10% buffered formalin and embedded in paraffin. With a microtome, 5 μm sections were cut and mounted on glass slides. One slide of each rat was stained with hematoxylin and eosin, and an additional six sections were deparaffinized by incubation at 60 ºC for 18 h, followed by immersion for 5 min period in xylene, absolute alcohol and twice in 95% alcohol. Sections were then washed in distilled water for 5 min and rehydrated by incubation in phosphate buffered saline (PBS) pH 7.4 for 20 min. Blocking of endoperoxidase activity was performed by incubation in 0.2% hydrogen peroxide for 10 min at 37 ºC, followed by wash in PBS for 20 min.

Histochemical procedures

Biotin derivatized lectins derived from *Canavalia ensiformis* (Con A), *Triticum vulgaris* (WGA), Scuccinyl WGA (S-WGA), *Ricinus communis* isolectin I (RCA-I), *Arachis hypogaea* (PNA), and *Ulex europeus* isolectin I (UEA-I), were purchased from Vector Laboratories (Burlingame, CA, USA) (Table 1).

Vectastain ABC standard kit (Vector), consisting of a freshly prepared complex of avidin and biotinylated peroxidase, as used to visualize the absorbed biotinylated lectins. The peroxidase substrate solution consisted of (0.01%) H_2O_2 and 0.5 mg/ml diaminobenzidine hydrochloride (Sigma, St. Louis, MO) in PBS. The histochemical reaction was conducted by covering the sections with substrate medium for 5-8 min in a moisturized chamber at room temperature. Sections were rinsed in distillated water for 5 min and counter-stained with Meyer's hematoxylin.

Control procedures included incubating the sections with biotinylated lectins in the presence of the proper complementary sugars at a concentration of 0.1 M, before and during labelling with the lectins.

All sections were analyzed separately by two of the researchers (AH, HN) using a light microscope and the results compared.

Intensity of deposition of reaction products was tabulated as: 0: no color, 1-3: faint, 3-5: moderate, 6-8: high. Counting was carried out in five arbitrary selected fields, magnification x 200.

The staining pattern and the intensity of the histochemical reaction were measured in the acinar cell cytoplasm and membrane, ductal cell cytoplasm and membrane, granular tubule cells in the submandibular gland and duct luminal content. In both the submandibular and sublingual glands, measurements were carried out separately on mucous and serous acini. The number of the labelled structures was calculated as the sum of the labelling scores divided by the total number of the specific structure measured.

Table 1. Lectins used for histochemical characterization of carbohydrate residues in the mucous and serous salivary glands in rats.

LECTIN NAME (COMMON NAME)	ACRONYM	SACCHARIDE-BINDING SPECIFICITY
Canavalia ensiformis (Jack bean)	ConA	αMan, αGlc
Triticum vulgare (wheat germ)	WGA	GlcNAc, NeuNAc
Succinyl WGA (wheat germ)	S-WGA	GlcNAc
Ricinus communis (Castor bean)	RCA-I	ßGal
Arachis hypogaea (peanut)	PNA	ßGal(1-3)GalNAc
Ulex europaeus	UEA-I	α-L-Fuc

Man: mannose; Glc: glucose; Gal: galactose; GlcNAc: N-acetylglucosamine; GalNAc: N-acetylgalactosamine; Fuc: fucose; NeuNAc:

Results

Routine histologic examination of hematoxylin and eosin stained sections revealed that the submandibular and sublingual glands were located in close proximity, separated by thin fibrous connective tissue. Most of the submandibular acini were serous, whereas most of the sublingual acini were mucous.

Figure 1 summarizes the average intensity of the lectin-binding for each type of salivary gland tissue. A broad comparison can be made between the different structures and between the lectins, with significant differences.

Most salivary gland elements react with Con-A, and to a lesser extent, with WGA. No binding reaction was seen with succinilated WGA. RCA-I and PNA reacted mainly with the tubular cells and desialation with neuraminidase only slightly increased the binding intensity of PNA (Fig. 2).

Staining with UEA-I disclosed binding only to the mucous acinar cytoplasm. The binding of UEA-I was detected only in 30% of the acini (Fig. 3).

According to the results of the lectin-binding patterns, the specific salivary gland structures are as followed:

Acinar cell cytoplasms

In both the mucous and serous acinar cells the cytoplasm stained moderately to intensely with WGA and Con-A (Figs. 4, 5). UEA-I staining was detected in 30% of the mucous acinar cells (Fig. 3). Only traces of RCA-I were seen, whereas, PNA, with or without neuraminidase treatment did not react with the acinar cell cytoplasm.

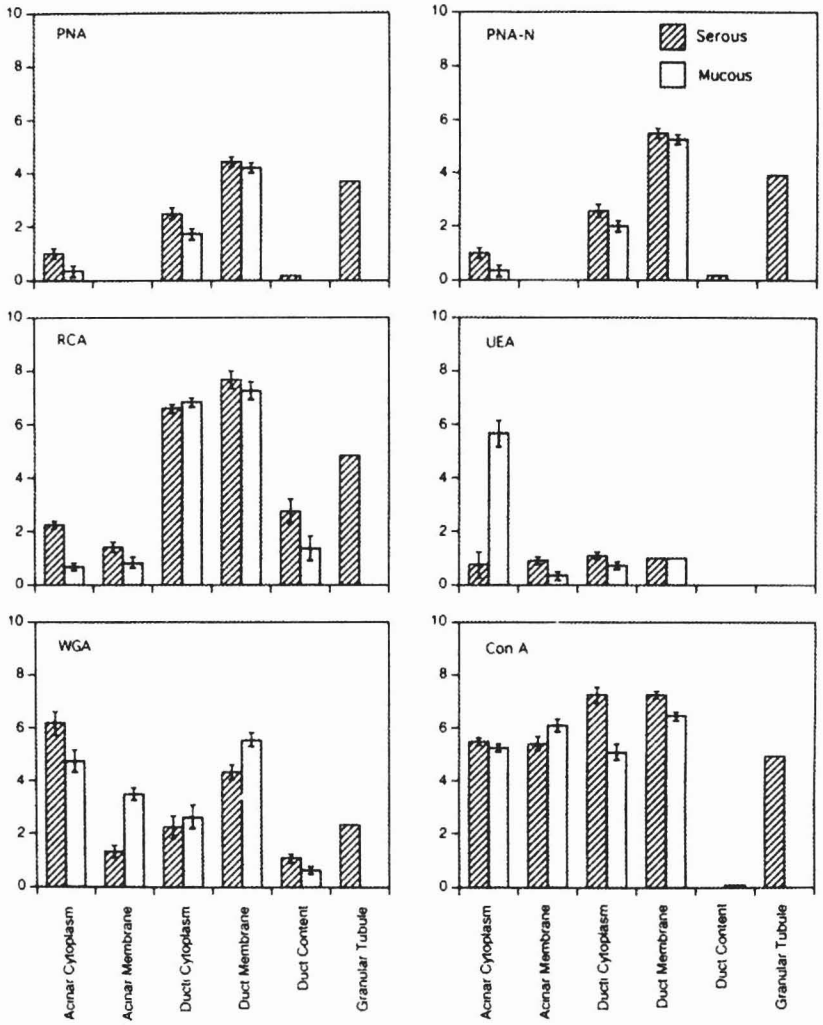

Fig. 1. The average intensity of the lectin-binding in the various cell types of rat submandibular and sublingual salivary glands.

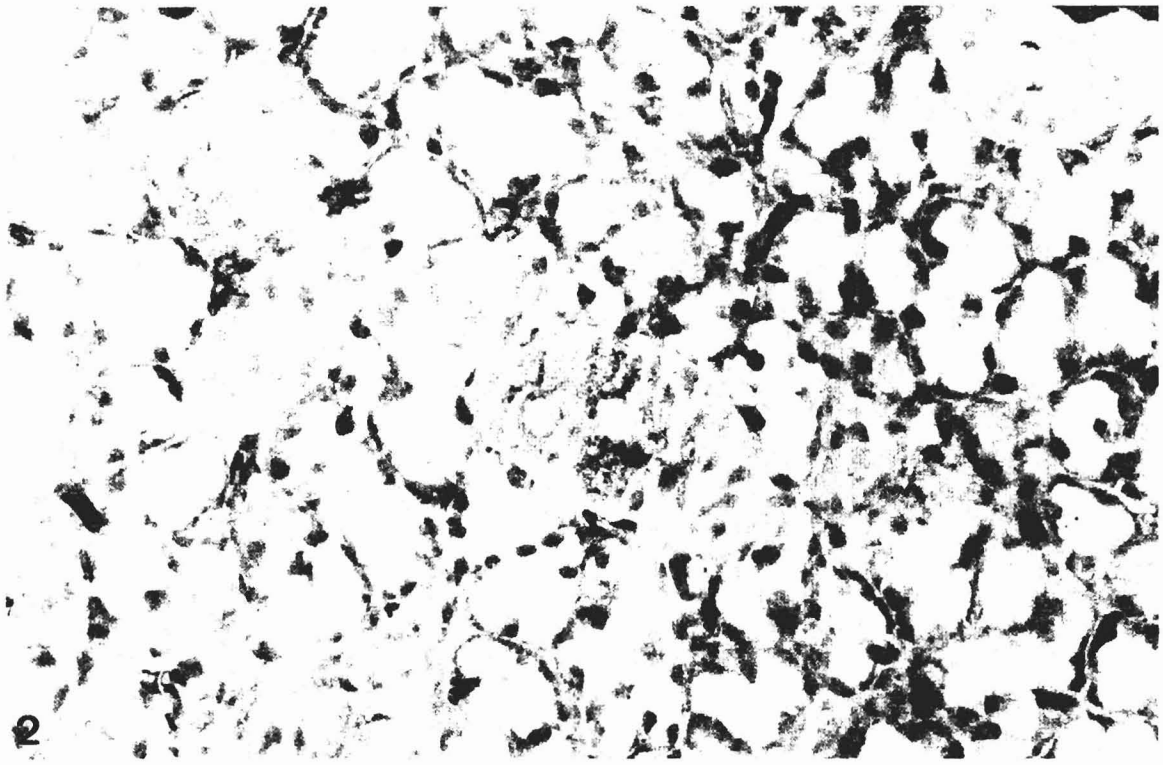

Fig. 2. Photomicrograph of PNA staining following desialation of the serous acini, showing staining only of the granular tubules and blood vessels. ABC immunostaining. x 400

Acinar cell membranes

Con-A intensely stained both the mucous and serous acinar cell membranes, while WGA moderately stained only the cell membranes of the mucous acinar cells.

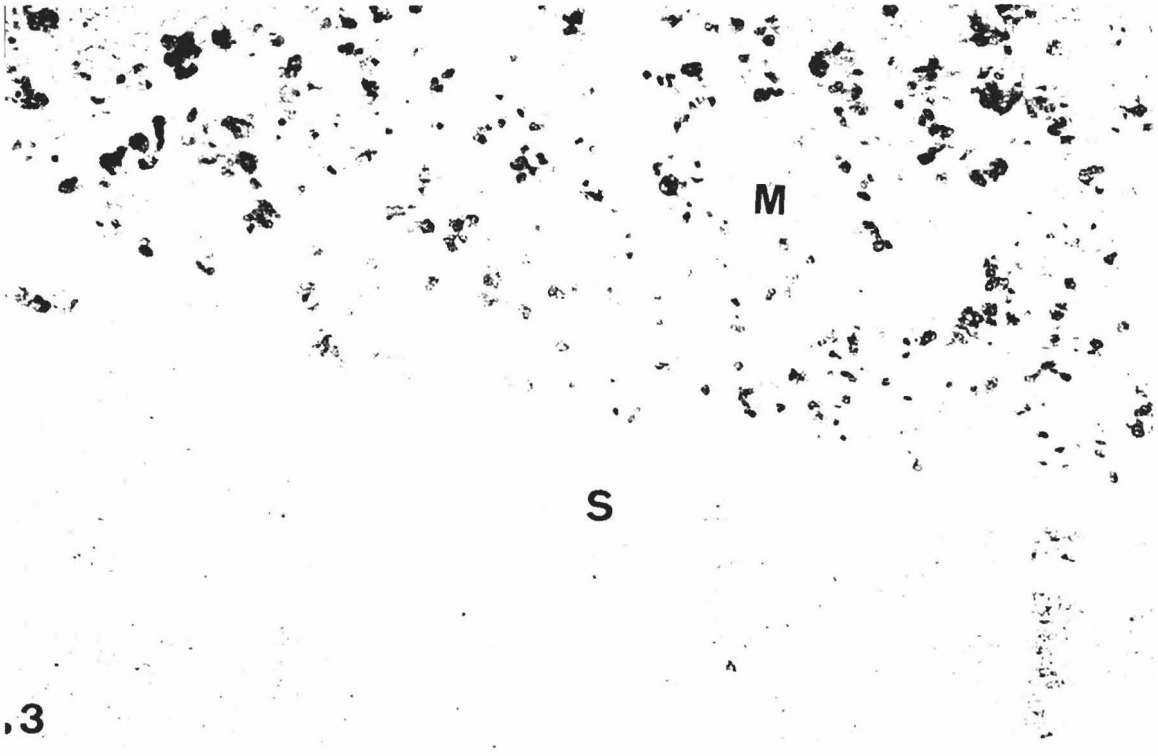

Fig. 3. Photomicrograph of UEA-I staining of mucous (M) and serous (S) acini showing high positive staining of mucous acinar cell cytoplasm. ABC immunostaining. x 100

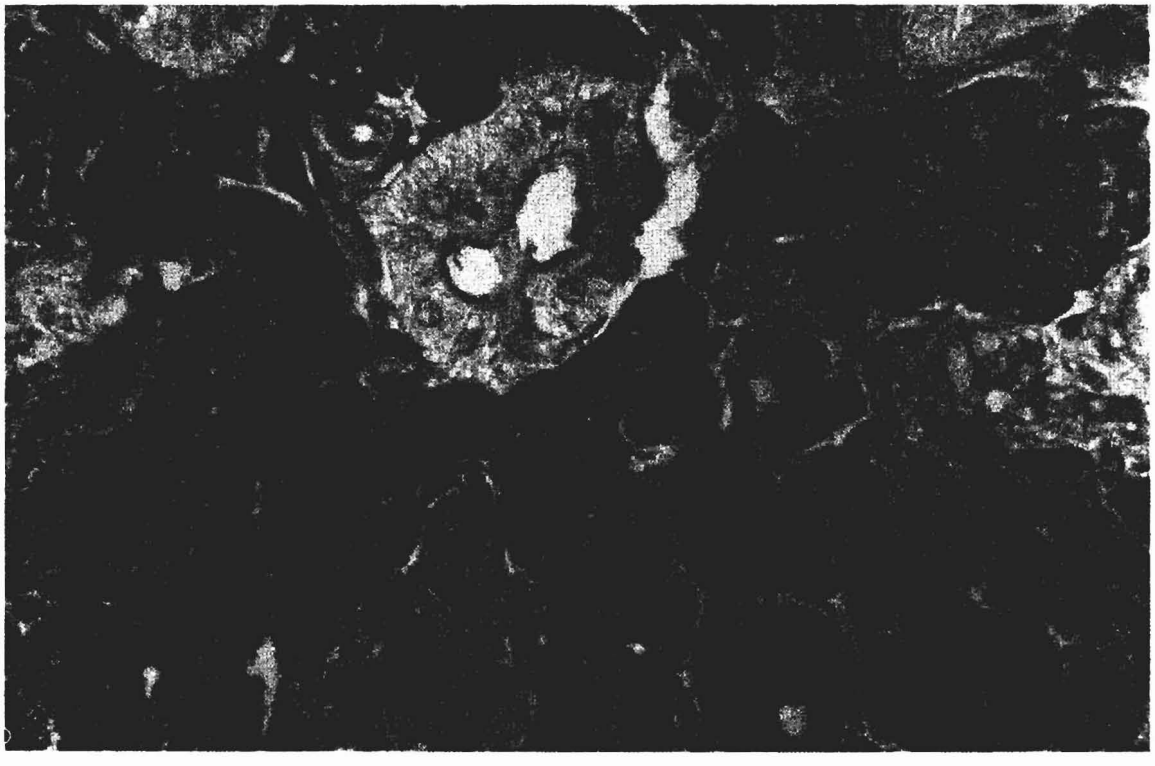

Fig. 4. Photomicrograph of WGA staining the serous acinar cytoplasm and tubular cell membrane facing the lumen. ABC immunostaining. x 400

Duct cell cytoplasms

The lectin-binding pattern of ductal cell cytoplasms was practically similar in both the serous and mucous glands. The ductal cells stained intensely with RCA-I and Con-A, and mildly with WGA and PNA.

Duct cell membranes

In both the serous and mucous glands, the membranes of the ductal cells bound intensely with RCA-I and Con-A, and moderately with WGA and PNA. Pretreatment with neuraminidase only slightly increased PNA binding.

Granular tubule cells

Moderate staining of the granular tubule cells with PNA, RCA-I and Con-A was noticed. WGA only mildly stained the granular tubule cells.

Discussion

According to the lectin-binding pattern observed in the present study, the cytoplasm and membrane of both the serous and mucous acinar cells present high similarity in the distribution of some sugar residues, but differ considerably in the expression of specific sugars. While both cell types express sugars, such as Man and Glc, the sugar Fuc is present only in the mucous cells (Table 2).

WGA is known to bind both to sialic acid (SA) and GlcNAc (Monsigny et al., 1980). However, the intense binding of WGA to the acinar and ductal cells, and the lack of binding of S-WGA indicate the presence of high density of SA sites, rather than GlcNAc (Monsigny et al., 1980). In many mammalian epithelial and endothelial cells (Skutelsky et al., 1977; Alroy et al., 1987), the SA residues appear as terminal sugar residue in an oligosaccharide containing penultimate PNA-binding sugar sequence ßGal-1,3-GalNAc (Lotan et al., 1975). These sites can be exposed only after removing the terminal sialyl residues by neuraminidase (Skutelsky et al., 1977, 1985). However, the results of the present study indicate, that despite of the high content of SA in the salivary glands, neuraminidase treatment is not

Table 2. Sugar residues in the mucous and serous cells of the rat submandibular and sublingual salivary glands.

	SEROUS		MUCOUS	
	Cytoplasm	Membrane	Cytoplasm	Membrane
Gal	-/+	-	-	-
Man	+	+	+	+
GalNAc	-	-	-	-
Fuc	-	-	+	-
NeuNAC	+	-	+	+

Gal:galactose; Man: mannose; GalNAc: N-acetylgalactosamine; Fuc: fucose; NeuNAc: N-acetylneuraminic acid (sialic acid).

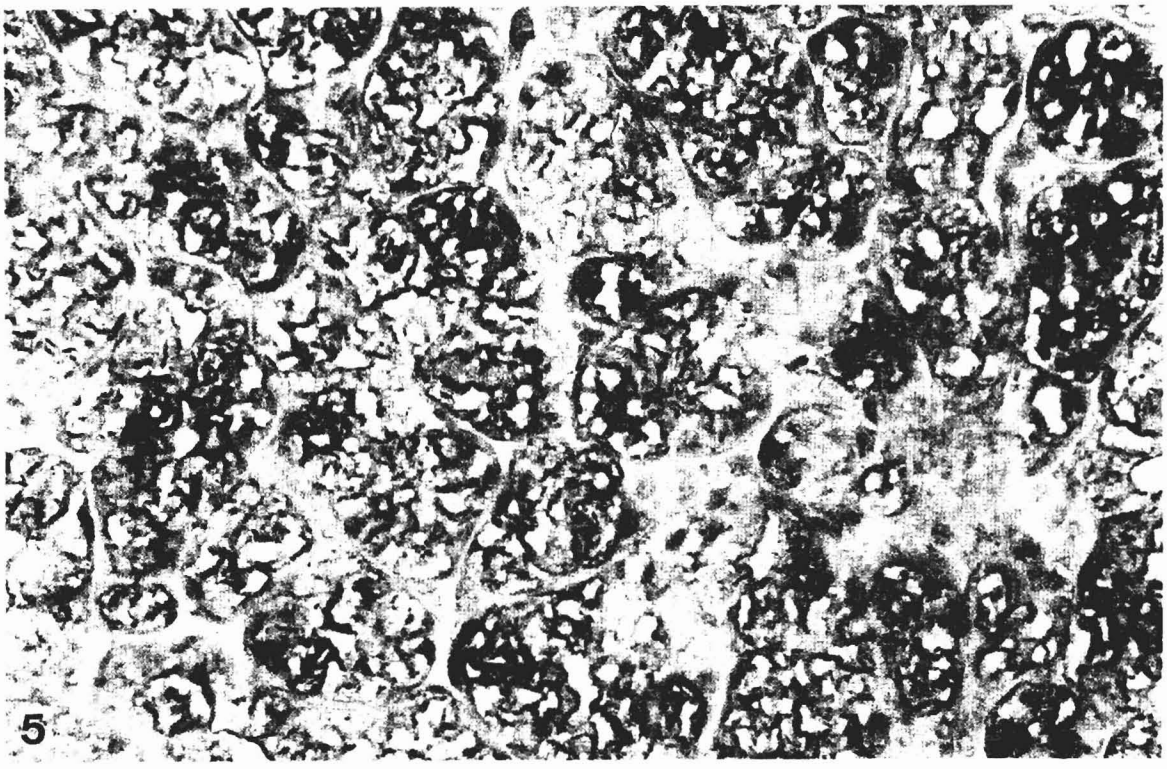

Fig. 5. Photomicrograph of Con-A staining the mucous acinar cytoplasm (ABC immunostaining. x 200

associated with exposure of additional PNA receptors. Unlike many other mammalian tissues, most SA residues in the salivary gland cells are not associated with PNA-binding sugar sequence.

The present results support earlier studies showing heterogeneity in the glycoprotein structure obtained from these cells (Keryer et al., 1973; Schulte and Spicer, 1984; Takai et al., 1986). The finding which indicates the presence of Man in the saliva (Berger et al., 1982; Reddy et al., 1982), support our observation regarding the presence of Man in the cytoplasm and cell membranes of both the mucous and serous cell types.

The Man and sialic acid in the serous and mucous acinar cells and Gal in the tubular cells have also been shown to be major constituents of the secreted mucous glycoproteins (Tabak et al., 1982). These glycoproteins bind to the tooth surface forming part of the acquired enamel pellicle and have been shown to agglutinate specific strains of oral microorganisms (Rolla et al., 1975; Hogg and Embery, 1979; Kornfeld and Kornfeld, 1985).

The high intensity of UEA-I binding observed in the mucosal acinar cells indicates a high content of Fuc-containing glycoconjugates. This observation is supported by Fleming et al. (1982), who isolated a fucose-containing mucin from rat submandibular glands. The fact that the binding of UEA-I was seen only in 20-30% of the mucous acinar cells, can be the result of the existence of more than one population of histologically similar cells. Schulte and Spicer (1984) note that rat salivary gland cells are characterized by three distinct cell populations in the sublingual mucous acinar cells. They suggest that all mucous acinar cells contain glycoconjugates with terminal SA, but only 10-25% of these cells contain Fuc, and another distinct cell population is characterized by a high GalNAc content. The heterogeneity of the lectin binding pattern can also be explained as expressing differences in the secretory phase among the acinar cells.

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