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Histoenzymological detection of sialic acids in the rodent salivary glands

D. Accili, M. G. Gabrielli, G. Menghi and G. Materazzi
Dipartimento di Biologia M.C.A., Università di Camerino, Camerino, Italy

Summary. Sections from the major salivary glands of rats and mice were used to locate, characterize and compare sialoglycoconjugates by means of lectin histochemistry, sialidase digestion, periodate oxidation and potassium hydroxide deacetylation. The gland sialylated macromolecules contained the terminal dimers sialic acid-ß-galactose and sialic acid-α-N-acetyl-galactosamine but differed in the varieties of sialic acids and the linkages of sialic acids to penultimate sugars. Indeed, the submandibular and parotid glands exhibited a notable occurrence of periodate labile sialic acids with C_7 and/or C_8 and/or C_9 acetyl groups in their polyhydroxyl chains. In particular, C_9 acetylated sialic acids were mostly linked α2-6 to ß-galactose. The sublingual glands, instead, were strongly characterized by a presence of C_9 acetylated sialic acids bound α2-3 to ß-galactose. Also, sialic acids with O-acetyl substituents at C_4 were evident in the mouse parotid gland and in the rat submandibular and sublingual glands. The great variety of sialoderivatives expressed by the rodent salivary glands was correlated with the differential involvement of these compounds in lubricating and defensive processes. Sex-related differences regarding the sialic acid location, acetylation degree and linkage were shown in the submandibular glands of both species.

Key words: Rodent, Salivary glands, Sialic acids, Lectins, Histoenzymology

Introduction

Salivary glands in rodents consist of organs which, analogously to other species, show differences in gross anatomy and histology (Young and van Lennep, 1978; Pinkstaff, 1980; Hand, 1986). Heterogeneity in the nature of the secretory products, which appeared to be composed of neutral and acid sugars in different quantities, was also discovered by biochemical techniques (Spiro, 1969; Gottschalk and Bhargawa, 1972; Moshera and Pigman, 1975; Roukema et al., 1976; Menghi et al., 1977, 1986; Pigman, 1977; Gallangher and Corfield, 1978; Denny et al., 1980; Fleming et al., 1982; Menghi and Accili, 1989) and histochemical procedures at optical and electronic level (Shackleford and Klapper, 1962; Spicer and Duvenci, 1964; Shackleford and Wilbron, 1968; Lima and Haddal, 1981; Denny and Denny, 1982; Bondi et al., 1984; Moreira et al., 1989). An appropriate use of lectins and exoglycosidases supported further and emphasized the different composition and sequence of carbohydrate chains elaborated by these secretory organs (Schulte and Spicer, 1983, 1984; Hosaka et al., 1986; Jezernik and Pipan, 1986, 1989; Menghi et al., 1986; Takai et al., 1986; Hennigar et al., 1987; Menghi and Bondi, 1987; Muresan and Muresan, 1987; Spicer and Schulte, 1988; Accili et al., 1992; Menghi and Materazzi, 1994). However, in spite of the numerous researches about the rodent salivary glands, contributions to the identification of sialoglycoconjugates have only been debated in the sublingual gland by histochemical methods (Reid et al., 1991), lectins recognizing sialic acid (Schulte et al., 1984) and use of oxidation and deacetylation methods combined with sialidase digestion and lectin binding (Schulte and Spicer, 1985).

Thus, starting from the supposition that comparative studies can be useful to determine and correlate similarities and differences occurring between glycoconjugates within corresponding organs of different species, this study was designed to provide information about the localization of sialic acid residues, their chemical structure and the linkages to acceptor subterminal sugars in an attempt to further characterize and discriminate the major salivary glands of mouse and rat as well as to relate the relationship between the physiological function and sialic acid content.

Materials and methods

Tissue collection

Submandibular, sublingual and parotid glands from adult Swiss mice and Wistar rats of both sexes were used (stock Morini, S. Polo d'Enza, Reggio Emilia). All

Offprint requests to: Prof. Daniela Accili, Dipartimento di Biologia M.C.A., Università di Camerino, Via Aldo Moro 10, 62032 Camerino (MC), Italy

samples were fixed for 24 h in Carnoy's fluid and postfixed for 3 h in a 2% calcium acetate-4% paraformaldehyde mixture (1:1) at room temperature. Tissue samples were then dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin block.

Lectin staining

On the basis of previous data (Menghi et al., 1992; Accili et al., 1994) the following lectins were selected: LPA from *Limulus polyphemus*, SNA from *Sambucus nigra*, PNA from *Arachys hypogaea* and DBA from *Dolichos biflorus*. The horseradish peroxidase (HRP)-conjugated lectins were purchased from Sigma Chemicals, St. Louis, Mo., USA and from USB, Cleveland, Ohio.

Tissue sections, 5 µm thick, after inhibition of endogenous peroxidase activity by 0.3% H_2O_2/methanol for 30 min, were flooded with a solution of each lectin-HRP conjugate (10-20 µg/ml) in 0.05M phosphate-buffered saline (PBS), pH 7.2, as previously detailed by Menghi et al. (1989). After exposure of each lectin-HRP conjugate for 30 min at room temperature, tissue sections were rinsed with PBS and 0.5M Tris-HCl buffer, pH 7.4, incubated for 10 min at room temperature in diaminobenzidine-H_2O_2 medium (Graham and Karnovsky, 1966), and dehydrated through graded alcohols. Controls for lectin staining included: a) substitution of lectin-HRP conjugates with corresponding unconjugated lectins at similar concentrations; b) exposure of sections to lectin-HRP conjugates added with the specific hapten sugars at concentrations of 0.2-0.4M.

Histochemical procedures and sialidase digestion

The designs of the histochemical experiments have been formulated with and without prior sialidase digestion on adjacent sections according to Schulte and Spicer (1985). In brief, sections were treated as described below:

a. 1mM-44mM PO/PNA-DBA
b. Sialidase/PNA-DBA
c. KOH/sialidase/PNA-DBA
d. 1mM-44mM PO/sialidase/PNA-DBA
e. 1mM-44mM PO/KOH-sialidase/PNA-DBA
f. KOH/lmM-44mM PO/sialidase/PNA-DBA
g. Alcian blue pH 2.5
h. KOH/Alcian blue pH 2.5

Mild and strong periodate oxidation was carried out by immersing slides in a 1mM or 44mM aqueous solution of periodic acid, respectively, for 15 min at room temperature (Schauer, 1978).

Saponification was performed by immersing sections in a 0.5% solution of potassium hydroxide in 70% ethanol solution for 30 min at room temperature (Reid et al., 1978).

Digestion with 0.5U/ml of sialidase from *Clostridium perfringens* (Type V, Sigma) was performed for 16 h at 37 °C in 0.1M acetate buffer, pH 5.5, containing 10mM $CaCl_2$ (Spicer and Warren, 1960). Controls for enzyme digestion aimed to determine the influence of enzyme-free buffer (Plendl et al., 1989), and verify the efficacy and specificity of sialidase treatment.

Alcian blue (AB), staining pH 2.5, was performed for 2 h at room temperature (Spicer et al., 1967).

Results

PNA, DBA, LPA and SNA staining intensities with and without prior enzymatic degradation and histochemical treatments were graded in a subjective scale and are summarized in Tables 1, 2. The effect of the enzyme-free buffer, not reported in tables, was found

Table 1. Comparative lectin staining in the major mouse salivary glands with and without sialidase digestion and histochemical methods.

	SM MALE			SM FEMALE			PAR		SL		
	A	CGT	SD	A	CGT	SD	A	SD	D	A	SD
PNA	0	1	1[a,b]	0	0-1	0	0-1	0-1	0	0	0-1
Sialidase/PNA	2	1	1[a,b]	2	0-1	0	1-2	1	0	3-4	1
KOH/sialidase/PNA	2	1	1[a,b]	2	0-1	0	2-3	1	0	3-4	1
1mM PO/sialidase/PNA	1	0	0	1	0-1	0	0	0	0	3-4	1
44mM PO/sialidase/PNA	0	0	0	1	0	0	0	0	0	3-4	1
1mM PO/KOH/sialidase/PNA	1	0	0	1	0-1	0	0	0	0	3-4	1
44mM PO/KOH/sialidase/PNA	0	0	0	1	0	0	0	0	0	3-4	1
DBA	1-3	0	0	0	0-1	0	2-3	0-1	0-1	0-2	1
Sialidase/DBA	3-4	0	0	0	0-1	0	3-4	0-1	0-1	0-3	0
KOH/sialidase/DBA	3-4	0	0	0	0-1	0	3-4	0-1	0-1	0-3	0
1mM PO/sialidase/DBA	0-2	0	0	0	0-1	0	0	0	0	0	0
44mM PO/sialidase/DBA	0	0	0	0	0	0	0	0	0	0	0
1mM PO/KOH/sialidase/DBA	0-2	0	0	0	0-1	0	0	0	0	0	0
44mM PO/KOH/sialidase/DBA	0	0	0	0	0	0	0	0	0	0	0
LPA	0	0	0	0	0	0	0	0	0	0	0-1
SNA	0	0	0	0	0	0	0	0	0	0	0-1

Results are expressed in arbitrary units ranging from 0 to 4 for negative to intense staining respectively. SM: submandibular gland; PAR: parotid gland; SL: sublingual gland; A: acini; CGT: convoluted granular tubules; SD: striated ducts; D: demilunes; [a]: luminal border; [b]: material inside the lumen.

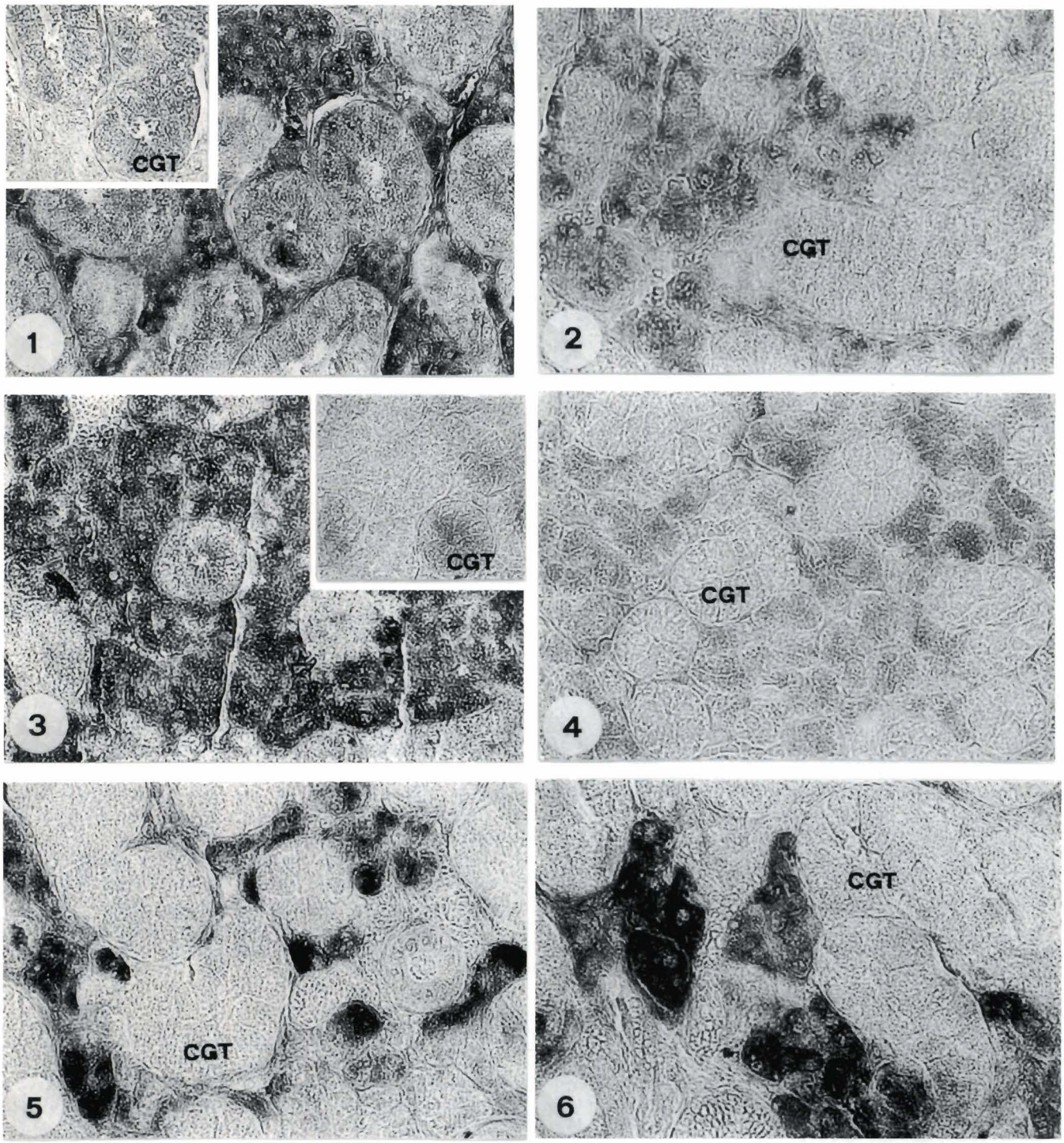


Fig. 1. Mouse submandibular gland. Sialidase/PNA/HRP staining. Male. Following the removal of sialic acid, acinar cells are intensely stained. x 370. **Inset:** sialidase-free buffer PNA-HRP staining. A weak reaction is shown in convoluted granular tubules (CGT). x 300

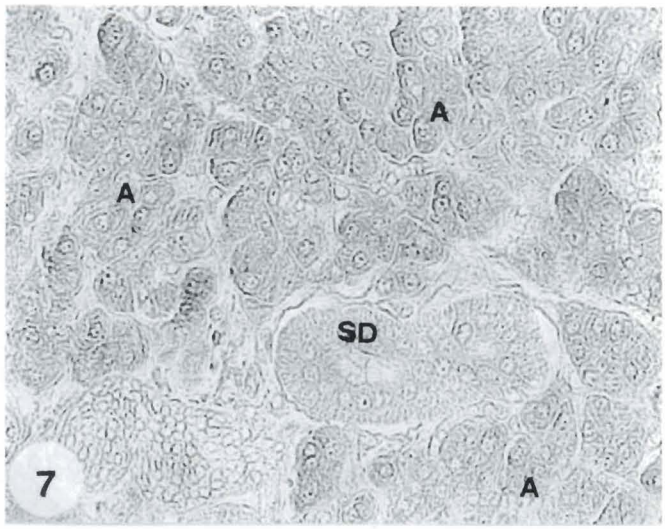
Fig. 2. Mouse submandibular gland. 1mM PO/sialidase/PNA-HRP staining. Male. A decrease of sialidase-PNA binding is evident at acinar cell level. Convoluted granular tubules (CGT). x 370

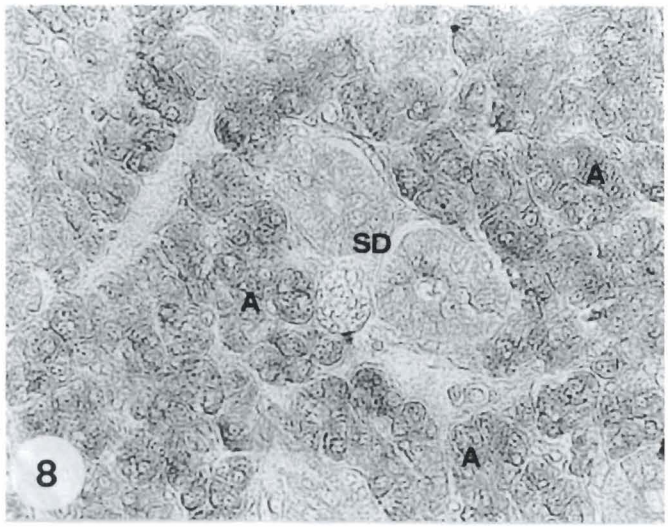
Fig. 3. Mouse submandibular gland. Sialidase/PNA/HRP staining. Female. Pretreatment with sialidase imparts an intense PNA staining to acinar cells. x 390. **Inset:** sialidase-free buffer PNA-HRP staining. Only convoluted granular tubules (CGT) are slightly reactive. x 350

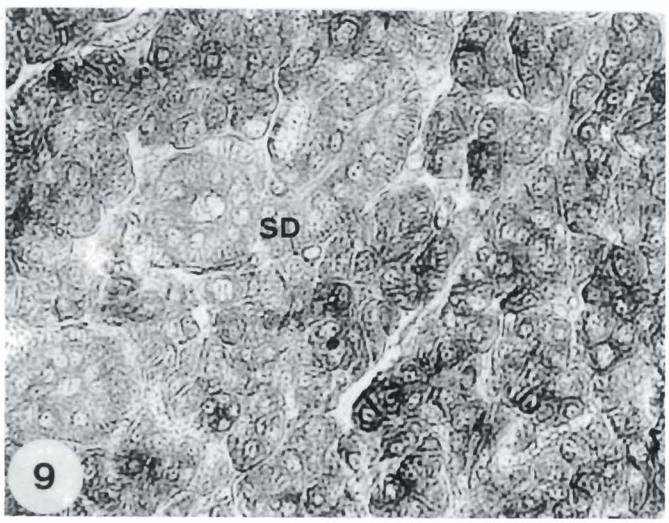
Fig. 4. Mouse submandibular gland. 44mM PO/sialidase/PNA-HRP staining. Female. Note the persistent sialidase-PNA binding of acini after strong oxidation. Convoluted granular tubules (CGT). x 390

Fig. 5. Mouse submandibular gland. Sialidase-free buffer DBA-HRP staining. Male. Acinar cells show a notable content of α-N-acetylgalactosamine, as demonstrated by the numerous DBA-positive sites. Convoluted granular tubules (CGT). x 420

Fig. 6. Mouse submandibular gland. Sialidase/DBA-HRP staining. Male. Sialidase digestion increases the DBA reactivity in acinar cells. Convoluted granular tubules (CGT). x 420







to slightly decrease PNA and DBA staining.

Mouse

The submandibular gland acinar cells contained sialoglycoconjugates with the terminal dimer sialic acid-ß-galactose (Figs. 1, 3). A codistribution of periodate-labile sialic acids, lacking in O-acyl substituents, and of C_7 and/or C_8 and/or C_9 acetylated residues was revealed by 1mM and 44mM PO/sialidase/PNA stainings (Figs. 2, 4). In addition, sialic acids which were bound to α-N-acetylgalactosamine (Figs. 5, 6) were found to be periodate labile in males.

The parotid gland was characterized by sialic acids linked to both ß-galactose (Figs. 7, 8) and α-N-acetylgalactosamine (Figs. 10, 11). The sialic acids which were bound to ß-galactose did not exhibit C_7 and/or C_8 and/or C_9 acetylated groups, since pretreatment with 1mM periodic acid abolished sialidase/PNA staining, but contained 4-O-acetylated residues, as revealed by the sialidase/PNA increased binding consequent to KOH deacetylation (Fig. 9). Also, the unchanged Alcian blue staining after KOH treatment agreed with this datum.

The sublingual gland acinar cells exhibited the strongest PNA affinity consequent to sialic acid cleavage (Fig. 12) completely resistant to 44mM oxidation (Fig. 13), thus suggesting the occurrence of C_9 acetylated sialic acids linked α2-3 to ß-galactose (Schulte and Spicer, 1985). Conversely, the sialic acids which bound to α-N-acetylgalactosamine (Figs. 14, 15) did not contain acetylated groups.

LPA and SNA lectins failed to react in any histological structure except for a very weak binding located on the sublingual gland striated ducts.

No differences occurred between males and females as regards the parotid and sublingual glands.

Rat

The submandibular gland of males showed the exposition of PNA reactive sites within acini and convoluted granular tubules only after KOH/sialidase sequence (Fig. 16). This staining and the unmodified Alcian blue positivity, following KOH treatment, indicated the presence of C_4 acetylated sialic acids having ß-galactose as acceptor sugar. The negative reaction consequent to 1mM PO/KOH/sialidase/PNA

Fig. 7. Mouse parotid gland. Sialidase-free buffer PNA-HRP staining. Male. Little PNA staining is present in acinar cells (A) and striated duct (SD). x 370

Fig. 8. Mouse parotid gland. Sialidase/PNA/HRP staining. Male. The removal of sialic acid discovers a greater number of PNA-positive sites in the acini (A). Striated duct (SD). x 370

Fig. 9. Mouse parotid gland. KOH/sialidase/PNA-HRP staining. Male. KOH treatment prior to sialidase digestion evidences additional PNA binding in the acinar cells. Striated ducts (SD). x 370

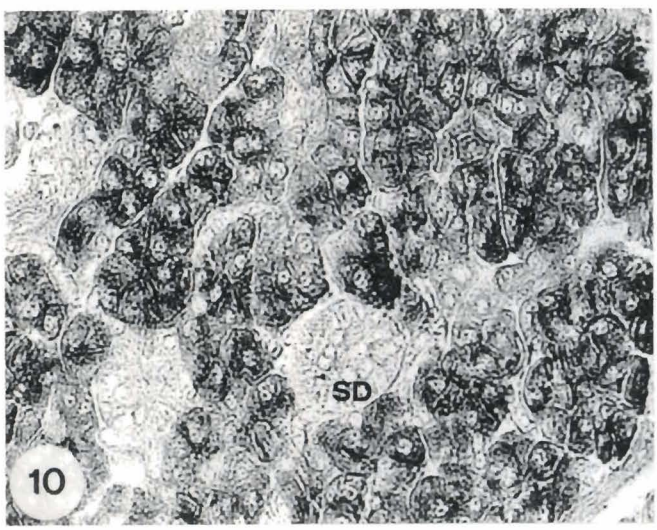
staining revealed that the sialic acids acetylated at carbon 4 of the furanose ring did not exhibit acetyl groups on the polyhydroxyl side chain. In the submandibular gland of females, a moderate KOH/sialidase/PNA binding was restricted to the convoluted granular tubules (Fig. 17). Conversely, sialic acids with penultimate α-N-acetylgalactosamine were in part periodate-labile and in part C_7 and/or C_8 acetylated, as revealed by findings from 1mM and 44mM PO/sialidase/DBA stainings (Figs. 18-21). No reactivity towards LPA and SNA was observed.

PNA- and DBA-sialidase-induced reactivity at acinar and ductal cells of the parotid gland indicated the occurrence of sialic acid-ß-galactose (Figs. 22, 23) and sialic acid α-N-acetylgalactosamine (Figs. 24, 25) dimers. Blocking of the sialidase-PNA affinity by mild oxidation demonstrated that all sialic acids were not acetylated. The reduction of sialidase/DBA positivity by mild oxidation and its abolition after strong oxidation evidenced that the sialic acids, bound to α-N-acetylgalactosamine, were either periodate labile or C_7 and/or C_8 acetylated. LPA showed a very slight affinity in both acinar and ductal cells, whereas a faint reactivity for SNA was restricted to the duct cells.

Table 2. Comparative lectin staining in the major rat salivary glands with and without sialidase digestion and histochemical methods.

	SM MALE			SM FEMALE			PAR		SL		
	A	CGT	SD	A	CGT	SD	A	SD	D	A	SD
PNA	0	0	0	0	0	0	0	0	0	0	0
Sialidase/PNA	0	0	0	0	0	0	2	2	0	0	0
KOH/sialidase/PNA	0-2	1-3	0	0	0-2	0	2	2	0	2	0
1mM PO/sialidase/PNA	0	0	0	0	0	0	0	0	0	0	0
44mM PO/sialidase/PNA	0	0	0	0	0	0	0	0	0	0	0
1mM PO/KOH/sialidase/PNA	0	0	0	0	0	0	0	0	0	2	0
44mM PO/KOH/sialidase/PNA	0	0	0	0	0	0	0	0	0	1-2	0
DBA	2-3	0-1	0	2	0-1	0	1-2	1-2	0	0	1-2
Sialidase/DBA	3-4	1-2	0	3-4	0-1	0	2-3	2	0	0	1-2
KOH/sialidase/DBA	3-4	1-2	0	3-4	0-1	0	2-3	2	0	1-2	0-1
1mM PO/sialidase/DBA	1-2	0	0	1	0	0	1	0-1	0	0	0
44mM PO/sialidase/DBA	0	0	0	0	0	0	0	0	0	0	0
1mMPO/KOH/sialidase/DBA	1-2	0	0	1	0	0	0	0	0	1-2	0-1
44mM PO/KOH/sialidase/DBA	0	0	0	0	0	0	0	0	0	1	0
LPA	0	0	0	0	0	0	0-1	0-1	0	0	1-2
SNA	0	0	0	0	0	0	0	0-1	0	1-2	1-2

Results are expressed in arbitrary units ranging from 0 to 4 for negative to intense staining respectively. SM: submandibular gland; PAR: parotid gland; SL: sublingual gland; A: acini; CGT: convoluted granular tubules; SD: striated ducts; D: demilunes.



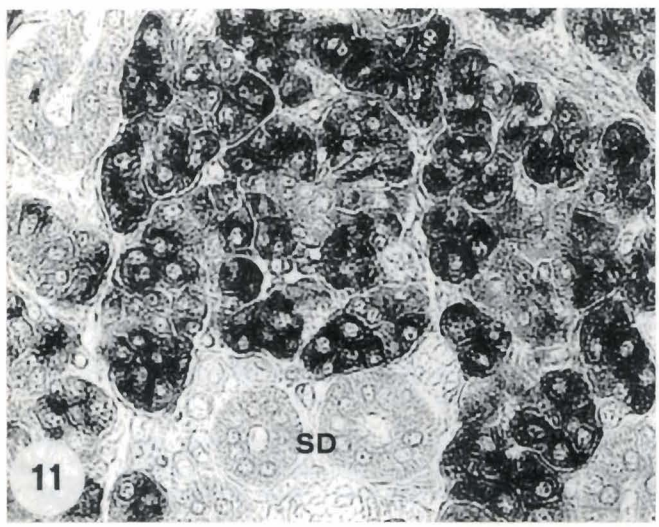
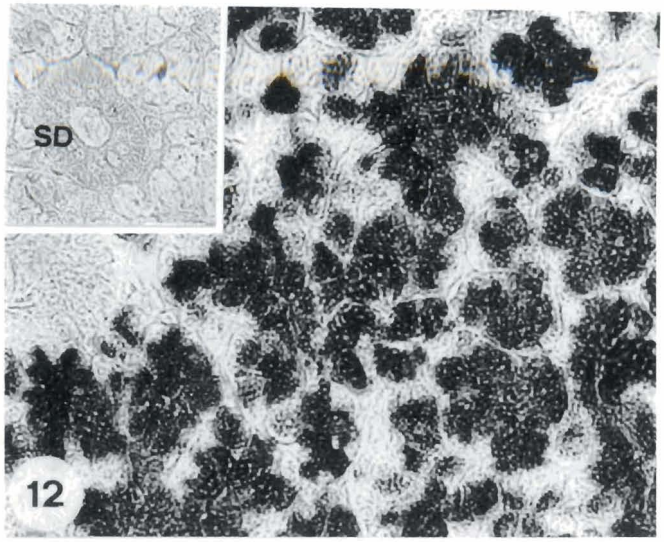
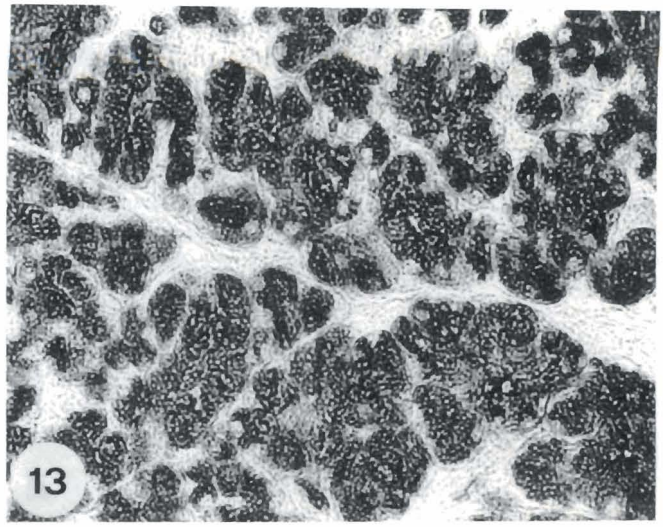
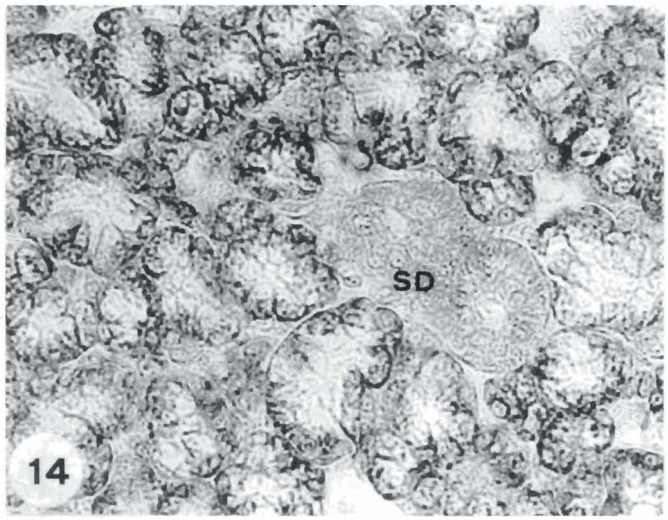


Fig. 10. Mouse parotid gland. Sialidase-free buffer DBA-HRP staining. Male. DBA reactivity is above all present in the acini (A). Striated ducts (SD). x 370

Fig. 11. Mouse parotid gland. Sialidase/DBA-HRP staining. Male. An increased reaction consequent to enzymatic cleavage of sialic acid is visible in the acini. x 370







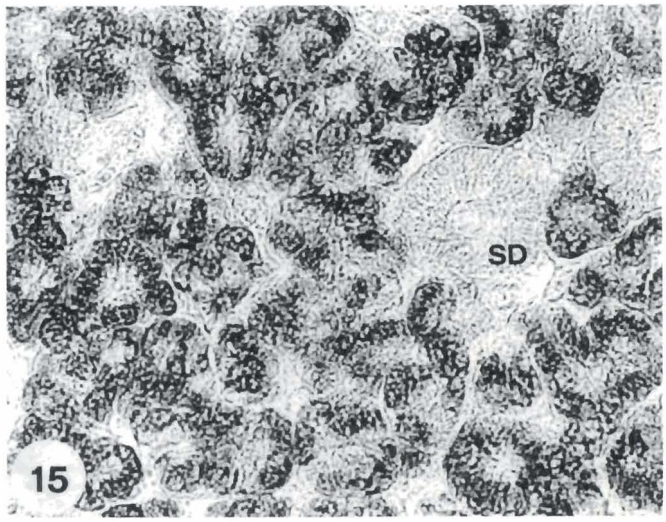


Fig. 12. Mouse sublingual gland. Sialidase/PNA/HRP staining. Male. A very strong affinity for PNA is discovered in acinar cells following enzymatic pretreatment. x 400. **Inset:** sialidase-free buffer PNA-HRP staining. No PNA staining is observed in the acini. x 300.

Fig. 13. Mouse sublingual gland. 44mM PO/sialidase/PNA-HRP staining. Male. Treatment with 44mM periodic acid has no effect on the sialidase-induced PNA positivity. x 400

Fig. 14. Mouse sublingual gland. Sialidase-free buffer DBA-HRP staining. Male. Acinar cells are heterogeneously stained with DBA lectin. Striated ducts (SD). x 400

Fig. 15. Mouse sublingual gland. Sialidase/DBA-HRP staining. Male. Sialidase digestion increases DBA binding in acinar cells. Striated ducts (SD). x 400

Fig. 16. Rat submandibular gland. KOH/sialidase/PNA-HRP staining. Male. Deacetylation with KOH prior to sialidase digestion imparts PNA reactivity to the acini and convoluted granular tubules (CGT). x 450. **Inset:** Sialidase/PNA-HRP staining. x 330

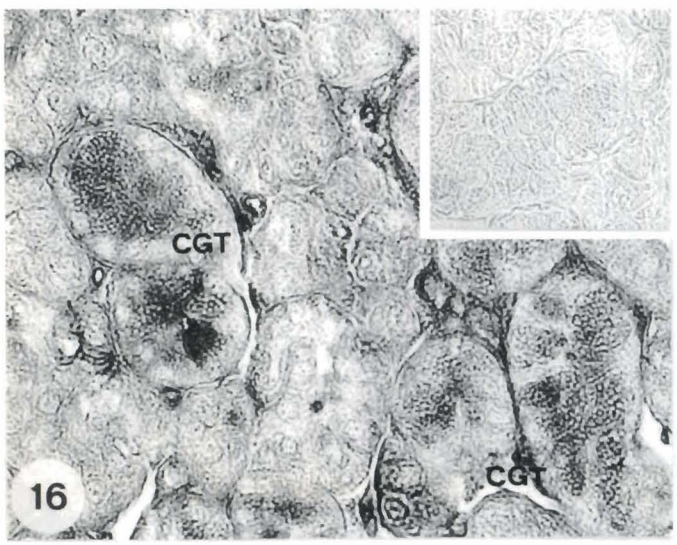
Fig. 17. Rat submandibular gland. KOH/sialidase/PNA-HRP staining. Female. Treatment with KOH evidences PNA staining after sialidase digestion only in some convoluted granular tubules (CGT). x 470. **Inset:** sialidase/PNA-HRP staining. x 330

Fig. 18. Rat submandibular gland. Sialidase-free buffer DBA-HRP staining. Male. Note the intensely reactive acini contrasting with the faintly positive convoluted granular tubules (CGT). x 400

Fig. 19. Rat submandibular gland. Sialidase/DBA-HRP staining. Male. Enzymatic treatment increases DBA binding in both acinar cells and convoluted granular tubules. x 400. **Inset:** 1mMPO/sialidase/DBA-HRP staining. Specimens treated with 1mMPO/sialidase exhibit a reduced affinity of acinar cells and a blocked reactivity of convoluted granular tubules for DBA. x 330

Fig. 20. Rat submandibular gland. Sialidase-free buffer DBA-HRP staining. Female. DBA reactivity is more intense in acini than in convoluted granular tubules (CGT). x 400.

Fig. 21. Rat submandibular gland. Sialidase/DBA-HRP staining. Female. Enzymatic treatment induces an increased DBA binding only in the acini. Convoluted granular tubules (CGT). x 400. **Inset:** 1mM PO/sialidase/DBA-HRP staining. Mild oxidation remarkably reduces sialidase/DBA-induced affinity. x 330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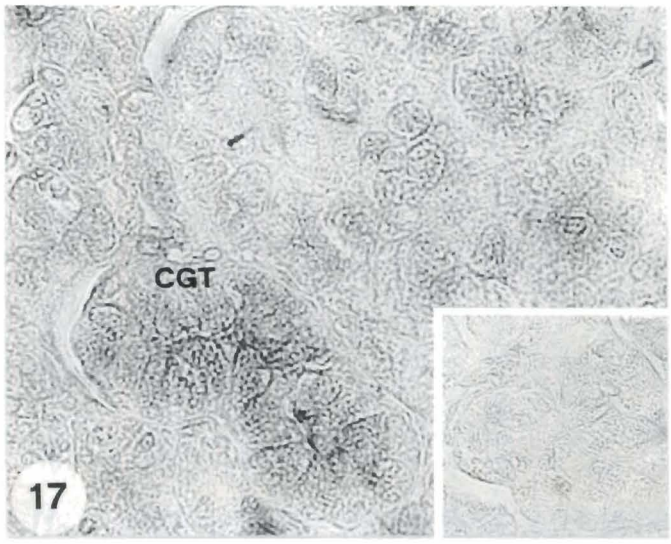
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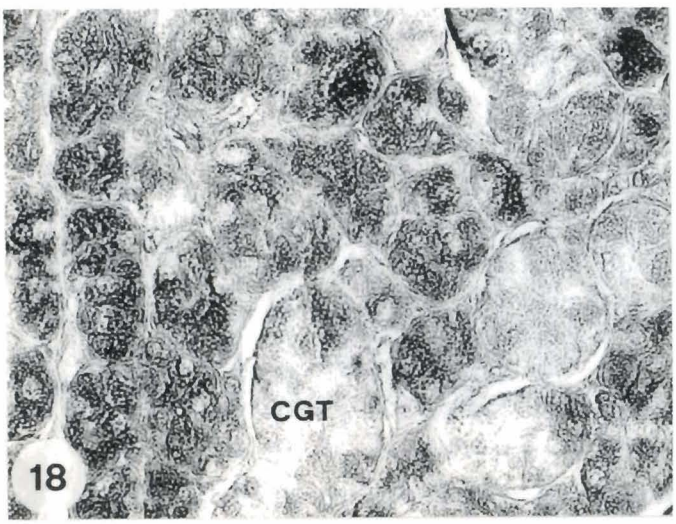
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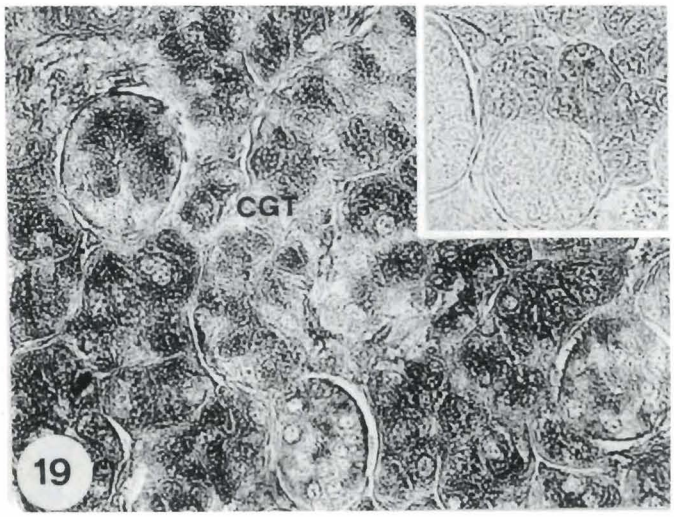
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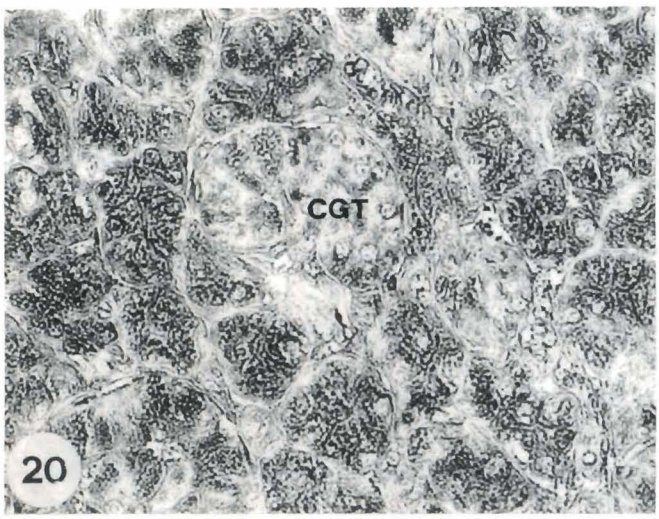
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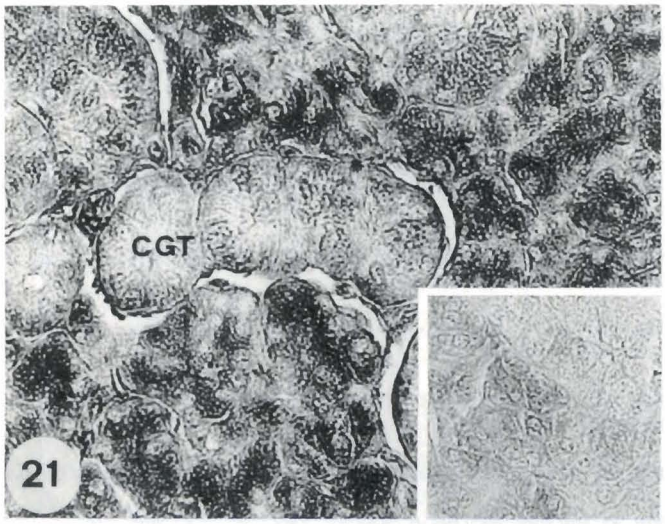
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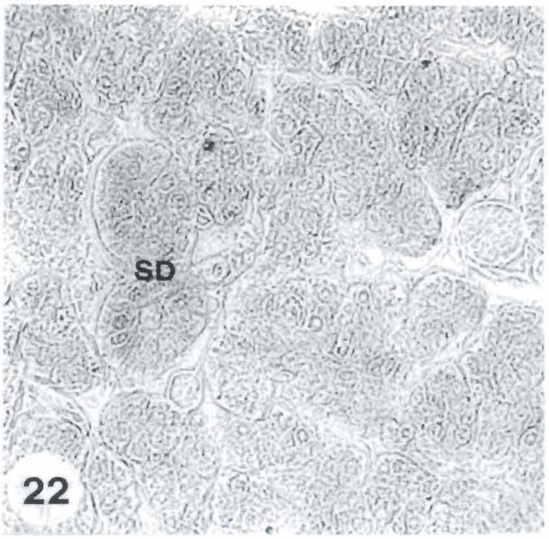
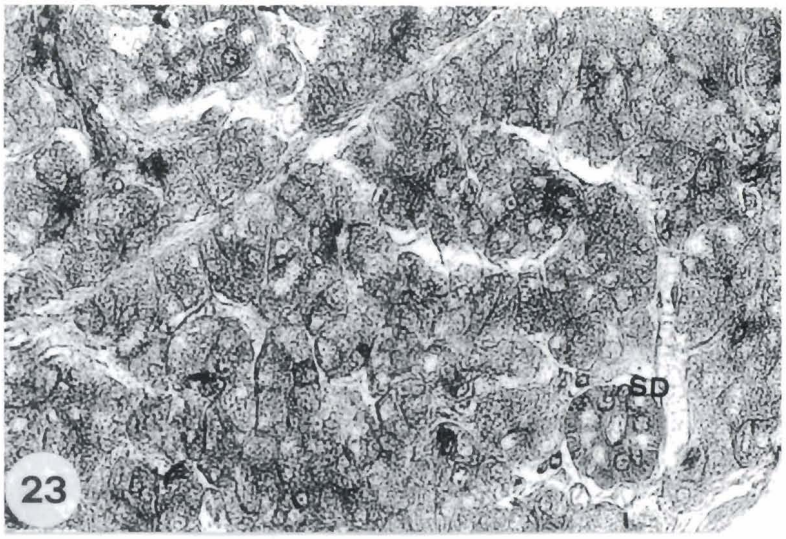
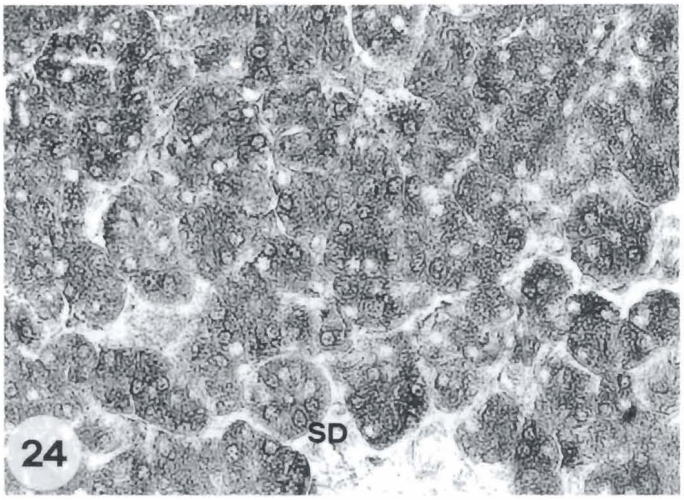






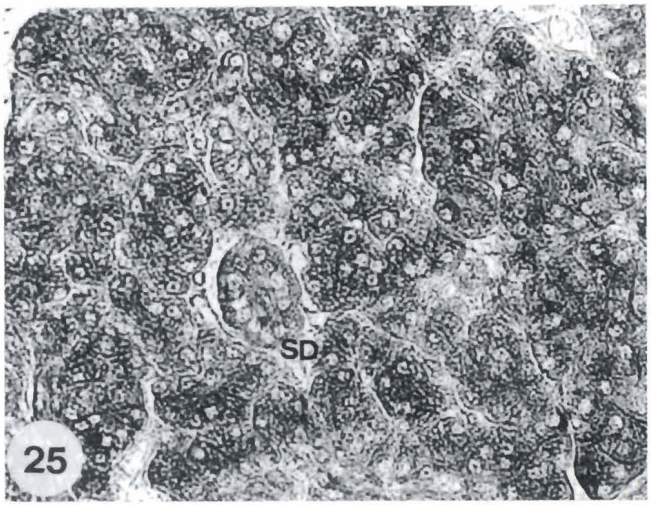


Fig. 22. Rat parotid gland. Sialidase-free buffer PNA-HRP staining. Male. No histological structures show reaction. Striated ducts (SD). x 400

Fig. 23. Rat parotid gland. Sialidase/PNA/HRP staining. Male. After sialidase digestion a rather uniform staining is observed in both acinar and ductal (SD) cells. x 400

Fig. 24. Rat parotid gland. Sialidase-free buffer DBA-HRP staining. Male. DBA bindings is moderately intense on acinar and ductal (SD) cells. x 370

Fig. 25. Rat parotid gland. Sialidase/DBA-HRP staining. Male. The cleavage of sialic acid discovers additional DBA-positive sites. Striated duct (SD). x 370

Fig. 26. Rat sublingual gland. Sialidase/PNA/HRP staining. Male. The enzymatic treatment fails to discover PNA binding in either acinar or ductal (SD) cells. x 400

Fig. 27. Rat sublingual gland. KOH/sialidase/PNA-HRP staining. Male. PNA affinity is revealed in acinar cells after saponification prior to sialidase digestion. Striated duct (SD). x 400

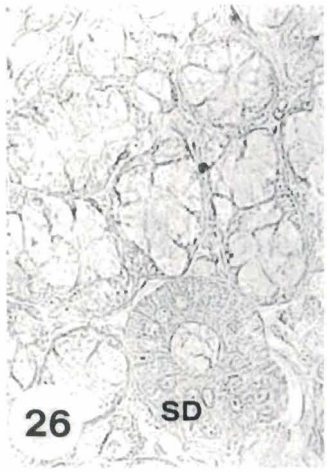
Fig. 28. Rat sublingual gland. Sialidase/DBA-HRP staining. Male. Sialidase digestion does not change the native DBA reactivity restricted to the striated ducts (SD). x 400

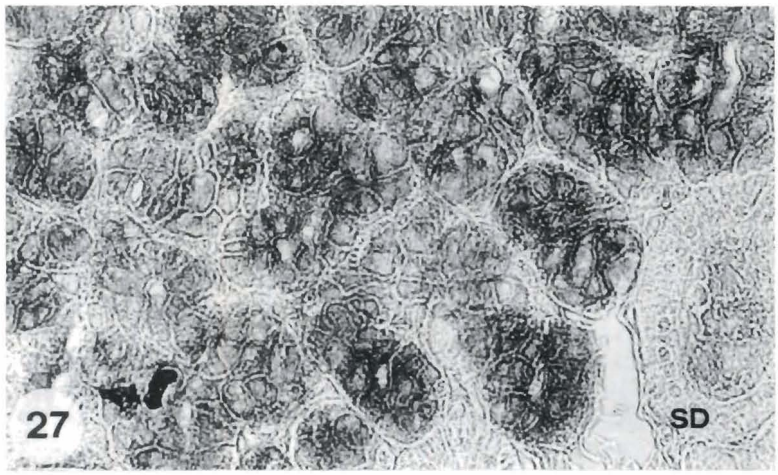
Fig. 29. Rat sublingual gland. KOH/sialidase/DBA-HRP staining. Male. KOH treatment prior to sialidase digestion induces DBA positivity in the acini. Striated duct (SD). x 400

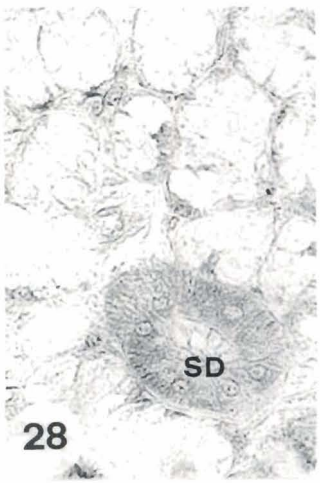
Fig. 30. Rat sublingual gland. Sialidase-free buffer LPA-HRP staining. Male. Note the moderate reaction restricted to the striated ducts (SD). x 400

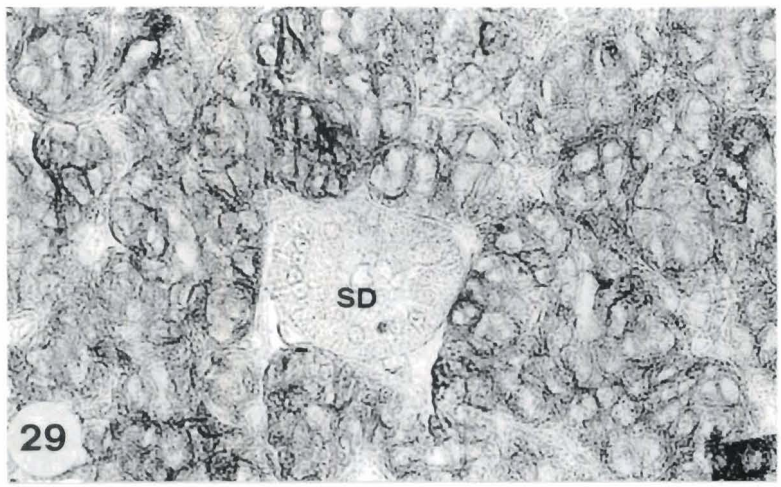
Fig. 31. Rat sublingual gland. Sialidase-free buffer SNA-HRP staining. Male. A faint staining is visible on the basal region of acinar cells. x 400

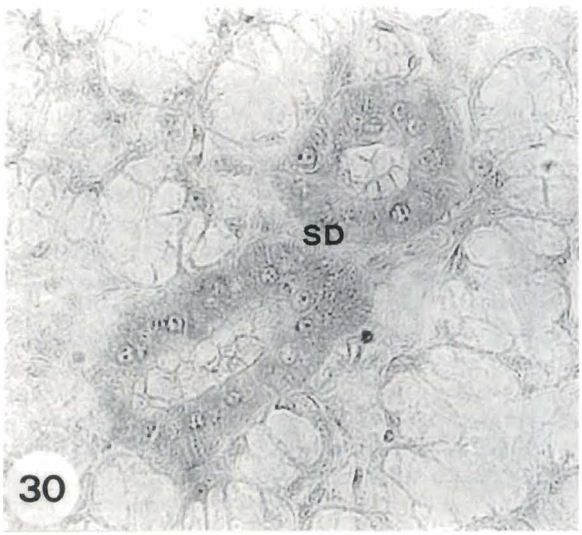
The sublingual gland acinar cells expressed C_4 acetylated sialic acids subtended by ß-galactose (Figs. 26, 27) and α-N-acetylgalactosamine (Figs. 28, 29) since deacetylation prior to sialidase digestion was required to discover PNA and DBA binding. These sialic acids were found to contain additional C_7 and/or C_8 and/or C_9 acetyl substituents, as proved by 1mM and 44mM PO/KOH/sialidase/PNA-DBA sequences. Ductal cells

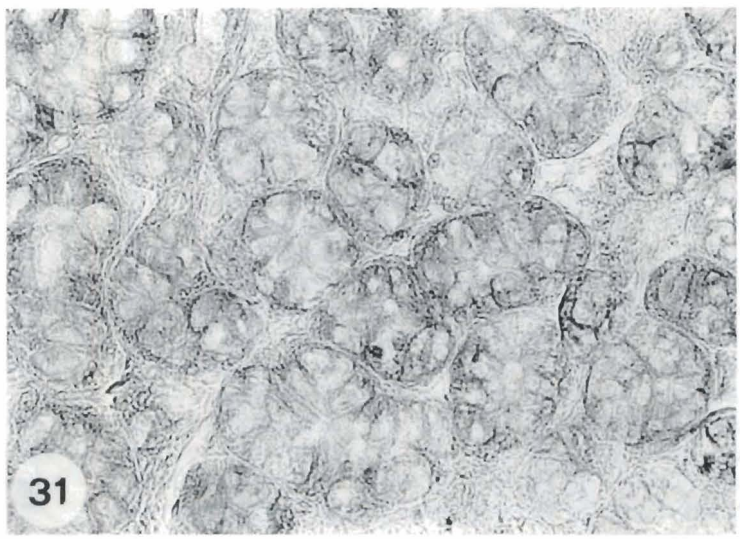












reacted moderately with LPA (Fig. 30). A weak SNA reactivity was observed in both acinar and ductal cells (Fig. 31).

Parotid and sublingual glands did not show differences between male and female subjects.

Discussion

The present comparative investigation documented the occurrence, in the salivary gland acinar cells, of sialic acids bound either to ß-galactose or α-N-acetylgalactosamine as usually found within secretory sialoglycoconjugates. Although differences in the subterminal acceptor sugars were not evidenced, the application of oxidizing and deacetylating agents, combined with sialidase digestion and lectin labelling, allowed us to point out a great variability within the molecular arrangement of sialic acids.

It was evident that sialoglycoconjugates from corresponding glands in different species do vary and that the sialoglyconjugate secretions from different glands of the same species may also vary. In fact, the mouse sublingual gland appears to be an important source of C_9 acetylated sialic acids linked via α2- 3 to ß-galactose according to previous findings (Schulte and Spicer, 1985). The corresponding gland of rat was also found to be a source of these sialoderivatives containing additional C_4 acetyl groups. Thus, the rodent sublingual glands seem to be organs of primary importance to regulate the viscous properties of the secreted mucus owing to the configuration of α2-3 linkage (Montreuil, 1980). Indeed, the physicochemical properties, mainly the viscosity, of the excreted saliva may be modified in correlation with variations of the sugar content, especially of sialic acids. In addition, the large proportion of O-acetyl groups may account for a relevant role of the sublingual gland in the defensive function achieved by formation of a protective pellicle covering tooth enamel and oral mucosa as well as by promotion of bacterial aggregation and clearance from the mouth (Tabak, 1990). Moreover, the bulky acetylated sialic acids can further potentiate their masking effect influencing, for example, the survival of biologically active glycoconjugates (Schauer, 1982).

The submandibular and parotid glands exhibited quite different kinds of sialoglycoderivatives. In particular, C_9 acetylated sialic acids were linked via α2-6 to the penultimate galactose, as visualized by enzymatic degradation and oxidizing agents, with the exception of the female mouse submandibular gland, where C_9 acetylated residues were characterized by an α2-3 linkage. In addition, the rat submandibular gland exhibited galactose-linked sialic acids with O-acetyl substituents at C_4 that can cooperate in the protective function by virtue of the complete resistance of C_4 acetylated sialic acids to bacterial sialidase (Hanaoka et al., 1989; Zimmer et al., 1992). Finally, the periodate labile non-acetylated sialic acids, found in different proportions in both glands of each species, may contribute to the functional performance of salivary mucins playing a mechanical role as lubricating agents. Thus, the numerous properties of secretory mucus tightly depend on the different functional aspects of each sialoglycoderivative type.

By examining the chemical nature of sialylated secretory products of individual glands within each species it also emerged that our findings agree with previous data about differences in the sialic acids of rat tissues correlated with differentially tissue-specific expressed sialyltranferases (Paulson and Colley, 1989; Paulson et al., 1989). Also, reports on a tissue- and species-specific characteristic O-acetylation of sialic acids (Schauer, 1982) have been here substantiated.

The location, content and structural characteristics of the submandibular gland sialoglycoconjugates differed between males and females of both species and this finding further supported the sex-related morphological (Lacassagne, 1940; Smith and Frommer, 1975; Jayasinghe et al., 1990) and biochemical differences (Keryer et al., 1973; Hatakeyama et al., 1987). In particular, periodate labile sialic acids linked to α-N-acetylgalactosamine were only visualized in males while C_9 acetylated sialic acids linked via α2-3 to ß-galactose were restricted to the mouse females. In addition, the terminal dimer sialic acid-ß-galactose was not visualized on acinar cells of the rat females.

In spite of the interesting information originating from the use of enzymatic degradation and histochemical treatments, the direct visualization of sialic acids provided scarce data. The sialic acid-specific lectins, indeed, failed to visualize the true distribution of sialoglycoconjugates as previously found with SNA and LPA in the bovine sublingual gland (Accili et al., 1994) and LFA in the hamster trachea (Schulte et al., 1984), probably due to the difficult internalization of the two lectins and recognition of the stored complex sialoglycoderivatives. However, an LPA labelling consistent with actual findings has been observed with rhodamine-conjugated LPA in the rat parotid gland where the staining was located at the periphery of the acinar cells (Muresan and Muresan, 1987).

To sum up, the present results shed light on the great variety of sialic acids expressed by the rodent salivary glands and point out that the acetylation degree, the acceptor sugars as well as the types of linkage can only be discovered by employing techniques of oxidation and deacetylation coupled to enzymatic degradation and selective use of lectins recognizing the subterminal sugars. This approach also seems to provide an adequate tool for identifying the contribution of specific cytochemical components to the various physiological aspects of the organ tissues.

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