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In Situ Identification of Sugar Residues in Monkeys' Salivary Glands by Lectin Histochemistry. II-Parotid Gland

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Abstract: The parotid salivary gland (PSG) of the monkey was histochemically analyzed by lectin histochemistry aiming to investigate the features of the available sugar residues and their suggested biology. These lectins were used as probe and the horseradish peroxidase (HRP) as visualant. The intensity of lectin binding affinity of the secretory acini and the smaller excretory ducts (intercalated, striated and interlobular ducts) showed wide variations. The reaction of the serous acini was strong with the Wheat germ agglutinin (WGA), moderate with the Ulexus europeus agglutinin-1 (UEA-1), scanty with the Concanavalia ensiformes agglutinin (Con-A) and negative with Peanut agglutinin (PNA). The lining epithelial cells of the intralobular ducts (intercalated and striated ducts) showed strong reaction to WGA, Con-A and PNA. While those of the interlobular ducts were negative to all lectins used except the moderate reaction to PNA. The goblet cells of the interlobular duct gave strong reaction with WGA only. In conclusion, the present study suggested that, the nature and composition of stored glycoproteins in monkey PSG is heterogeneous. The predominant terminal sugar residues are supposed to be N-acetylglucosamine (α-D-GlcNAc) in secretory acini and α D-glucose (α-D-glc) and α D-mannose (α-D-man) in the ducts system.

Key words: Non-human primates · Parotid salivary gland · Glycoproteins · Lectin histochemistry

INTRODUCTION

Important biological functions of mammalian salivary glands are closely connected with the ability to produce large amounts of glycoconjugates in the major types of secretory acini, which include mucous, serous as well as seromucous cells [1, 2]. The type of the secretion is not restricted to specific cell type whereas the whole population of secretory cells probably offers a remarkable capacity of transformation when related to the biological need of each species [3]. Lectins in conjugation with the enzymatic cleavage of sialic acid provide more detailed information on the structure of carbohydrate containing macromolecules at specific histological sites [4]. Several studies on the glycoconjugates of salivary glands of non-human mammals have been performed. Among these studied species is the horse[5], rodent [6, 7], dog [8], cat [9] as well as human [10, 11]. Non human primates present a unique animal model for human diseases because of their phylogenetic closeness to human, their convenient size, their omnivorous natural diet and their ability to stay healthy in captivity. Further to the previous study on the mandibular salivary gland of the monkey [12], this study aimed to investigate the distributions of the sugar residues in the PSG and their suggested biology.

MATERIALS AND METHODS

Animals: The samples were collected from three species of non-human primates, monkeys, obtained from the Department of Anatomy, United Graduated School of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan. All investigations were completed in the Department of Anatomy Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt. A total of nine animals was used for this experiment. Among these animals, three were of common tree shrew monkey (Tupaiidae glis); three were of slow rolis monkeys (Nyeticebus cocang) and three of common marmoset (Callithrix Jacchus).

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Table 1: Carbohydrate binding specificity of lectins used in this study

Taxonomic name	Common name	Abbreviations	Label	Concentration µg/ml	Major sugar specification	Sugar binding inhibitor
Arachis hypogea	Peanut	PNA	HRP	100	Gal-ß-(1-3)-GaLNAc	Gal
Canavalia ensiformis	Jake bean	Con-A	HRP	20	α-D-Man, α-D-Glc	α-methyl-D-Man
Triticum vulgaris	Wheat germ	WGA	HRP	6	(ß-(1-4)-D-GlcNAc)2,NeuNAc	NeuNAc
Ulex europeus	Gorse seed	UEA-1	HRP	100	α-L-Fucose	α-L-Fucose

Symbol: Gal=Galactose; Glc=Glucose; GalNAc=N-acetylgalactosamine; GlcNAc= N-acetylglusoamine; Man=Mannose; NeuNAc=N-acetyl neuraminic acid (sialic acid); HRP = horseradish peroxidase

Table 2: General pattern of lectin staining of the parotid salivary gland

			Duct system			
					Interlobular duct	
Lectin	CTS	Serous acinar cells	Intercalated duct	Striated duct	Lining epithelium	Goblet cells
WGA	0	4	3	3	0	3
Con-A	0	1	4	4	3	3
PNA	0	0	3	3	0	0
UEA-1	0	2	0	0	0	0

Numbers indicate staining intensity on a subjectively estimated scale from 0, un-reactive, to 4, most reactive

Samples Collection and Tissues Preparation: According to the strict animal welfare regulations and the rules of the animal ethics committee in Japan the monkeys were scarified under deep anesthesia with an intravenous injection of pentobarbital sodium (50 mg/kg). The PSG were rapidly removed and pieces of its tissue were fixed in phosphate buffer saline (PBS) contain 4% paraformaldehyde for 72 hours at room temperature and thoroughly rinsed in the same buffer after fixation. The samples are processed in routine histological techniques. Sections of 4 ìm thickness were deparafinized in xylene and stained with Gill's haematoxylin [13] and eosin (H and E) for histological assessment. For detection of the binding sites of the sugar residues other sections were subjected to the histochemical staining methods summarized in Table 1.

HRP-Lectin Technique: The processing and staining procedure with the various lectins (Table 1) was similar to that previously described [6, 14, 15]. Briefly, after hydration, the sections were treated with 0.3% hydrogen peroxide (H_2O_2), rinsed in distilled water and washed in 1% bovine serum albumen (BSA) in 0.1 M PBS PH 7.4. The sections were then incubated for 12 hours at 4°C in HRP-lectin (Sigma chemical Co. St. Louis, MO, USA), dissolved in 0.1 M PBS PH 7.4 (contains 0.1M NaCL, 0.1 mM $CaCL_2$, $MgCl_2$, $MnCl_2$) and then rinsed three times in PBS. The optimal concentration used with each lectin which allowed maximum staining with minimum background was listed in table 1. Visualization of the sites containing HRP-lectin was obtained by incubating the slides with PBS containing 3\, 3\ diaminobenzidine tetrahydrocholride (DAB) (25mg/100ml and 0.003 % H_2O_2) for 10 min at room temperature. Slides were rinsed in distilled water, dehydrated using gradual ethanol solution, cleared in xylene and mounted in DPX.

Histochemical and Enzymatic Treatment

Sialidase Digestion: Sections were digested with 0.2 IU/ml sialidase enzyme from vibrio cholera (Cal. Biochem. La Jolla, USA) for 18hr at 37oC. Sialic acid residues with O-acyl substitution which resist the enzymatic cleavage by sialidase were removed by saponification before sialidase digestion.

Saponification: Saponification was carried out in 0.5% (w/v) KOH in 70% (v/v) ethanol for 15 min.

Control Reaction: Controls for the lectin staining included either pre-incubation of the sections with the corresponding haptten sugars inhibitors listed in Table 1 or the sections were exposed to HRP and substrate medium without lectins. The sugar inhibitors were employed from 0.05 M to 1 M and complete elimination of the staining binding was obtained at 0.2 M. The aim of this control was to test the efficiency of the specific staining for each sugar.

RESULTS

In the present work, no significant difference were obtained among the three studied species of non-human primates neither in histological structure nor in histochemical staining pattern

General Morphology: The light microscopic investigations revealed that, the PSG of the monkey is formed from glandular lobules separated by interlobular connective tissue trabeculae (Figs. 1 and 2). Each lobule was consisted of purely serous glandular secretory acini mostly of compound tubulo-acinar type. They were built-up of pyramidal cells with high vesicular, spheroid basely situated nuclei surrounding a narrow central lumen (Figs. 5 and 6). The excretory duct system of the gland was composed of intercalated ducts, striated duct, interlobular ducts and main duct. The intercalated ducts were numerously detected within the glandular lobules. Each duct was lined with a one layer of low cuboidal cells with flattened vesiculated nuclei (Fig. 1 and 2). The striated ducts were clearly observed. Each duct had a relatively wide lumen and lined by a single layer of cuboidal epithelium of vesicular spheroid nuclei (Fig. 6). The interlobular ducts were sheathed with thick bundles of collagenous fibers. They had a wider lumen and lined with one to two layers of stratified columnar epithelium with clear goblet cells (Figs. 3 and 7).

Lectin Histochemistry: The reaction degree of different types of lectins is representing the levels of certain sugars. The corresponding sugars for the used lectins were listed in Table 1.

WGA/HRP: The intensity of lectin binding affinity of the secretory acini and the smaller excretory ducts (intercalated, striated and interlobular ducts) of the studied PSG showed wide variations. The serous acini showing great staining intensity with WGA (Fig. 8) indicating the existence of GlcNAc and/or NeuNAc. The reaction was clear at the periphery of the individual lobules and diffuses throughout the cytoplasm of the individual cell. GlcNAc and/or NeuNAc sugars were detected in the cells of the lining epithelial of the intralobular ducts as evidenced by the moderate affinities to WGA. Although the lining epithelial cells of the interlobular duct showed almost negative reaction with WGA, strong staining was localized in the goblet cells of it (Fig. 9). Sialidase digestion partially abolished the reaction from the secretory acini but it failed to abolish it from the duct system (Fig. 10). This indicates that the secretory acinar cells have sialidase labile glycoprotein with N-acetyl substitution, while the duct system contains sialidase resistant glycoprotein with O-acetyl substitution. The sugar residues which resist cleavage by sialidase enzyme were removed by KOH/Sialidase sequence as evidenced by the negative reaction of the secretory acini (Fig. 11).

Con-A/HRP: The reactivity of secretory acini with Con-A was scanty (Figs. 12 and 13); it tends to be seen only at the luminal surface of the acini. In contrast, the lining epithelium of the duct system showed a strong positive reaction with Con-A (Figs. 12 and 13) revealing the presence of ∝-D-glc and ∝-D-man sugars in the lining epithelium of the duct system.

PNA/HRP: The reactivity of secretory acini with PNA was negative (Figs. 14 and 15). The lining epithelium of the intercalated and striated duct showing moderate reaction while as the interlobular duct was negative to it (Figs. 14 and 15). This indicates the existences of β-anomer of the Gal and GalNAc in the lining epithelium of the intercalated and striated duct.

UEA-1/HRP: The secretory acini of the PSG showed moderate reaction with the UEA-1 (Fig. 16) demonstrating the presence of ∝-L-fucose sugar, while the duct system was completely negative to it (Fig. 16).

DISCUSSION

The present study, revealed that the PSG of the monkey is of compound tubulo-acinar type and their secretory acini is pure serous in its nature. These findings were in agreement with the findings in goat [2, 15], in camel [17, 18] and in human [3]. On the contrary, such acini were seromucoid in its nature as described in camel [18], dogs and cats[1] and [20] in human [20]. Moreover, it was added that the PSG is a compound acinar type [1]. The structure of the duct system in the present study was closely similar to that described in goat [16], sheep[22] and human [20]. With regards to the histochemical finding the PSG acinar cells possess a considerable amount of glycoconjugates. More precisely composed of GlcNAc, α-D-glc, α-D-man and α-L-Fuc. The reactivity of acinar cells, intercalated duct and striated duct varied markedly with the different lectins. These results were in line with those mentioned by [6, 7, 23].

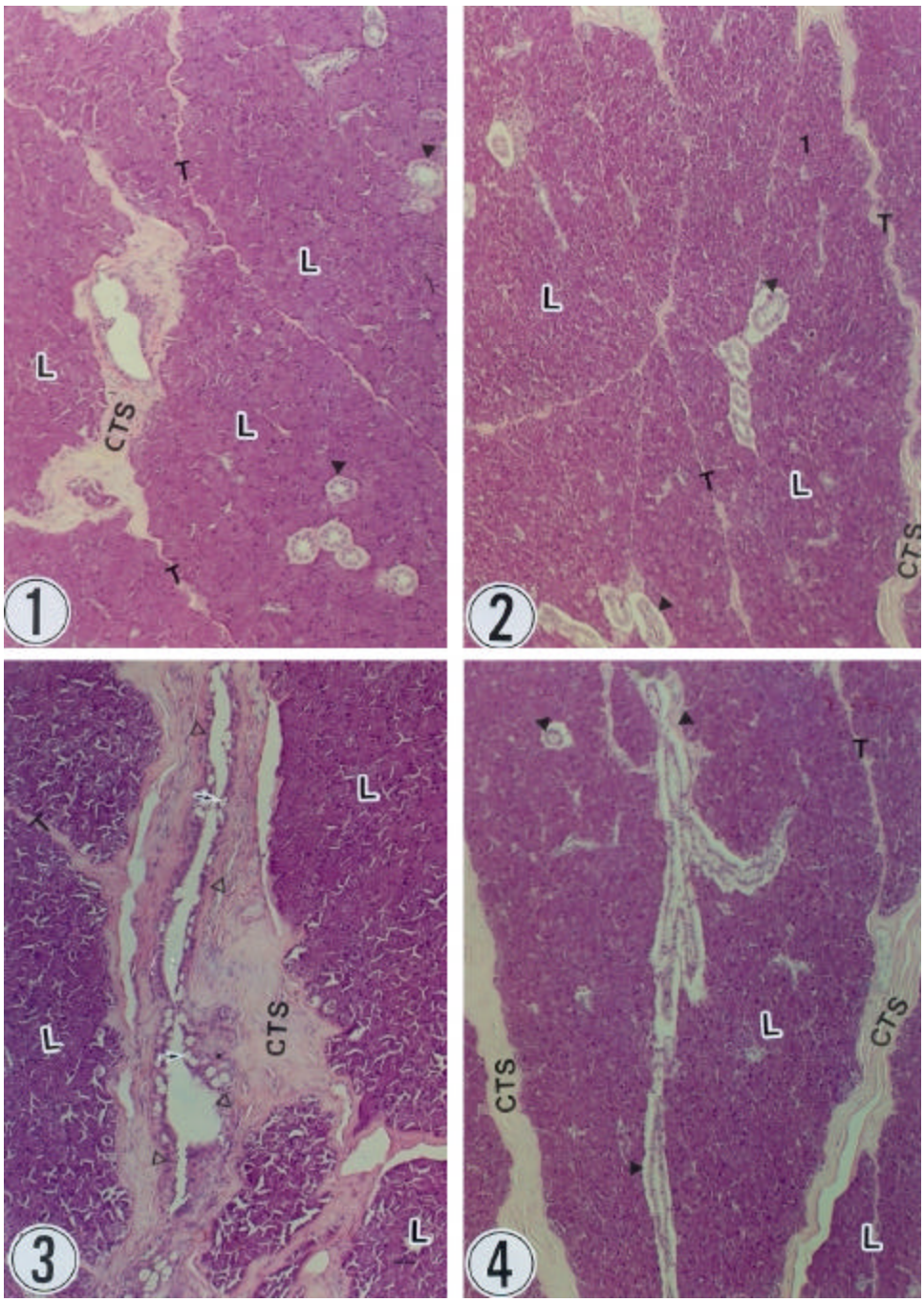

Fig. 1: A photomicrograph of the monkey PSG showing, the glandular lobules (L); the connective tissue septa (CTS); interlobular trabeculae (T); and intercalated duct (arrow head). H&E. stain, Bar = 100µm.

Fig. 2: A photomicrographs of the monkey PSG showing the central part of the glandular lobules (L); the connective tissue septa (CTS); interlobular trabeculae (T); and intercalated duct (arrow head). H&E. stain, Bar = 100µm.

Fig. 3: A photomicrograph of the monkey PSG showing, the lining epithelium of the interlobular duct (arrow head); the goblet cells (arrow); the glandular lobules (L); the connective tissue septa (CTS); interlobular trabeculae (T); H&E. stain, Bar = 100µm.

Fig. 4: A photomicrograph of PSG showing the glandular lobules (L); the connective tissue septa (CTS); interlobular trabeculae (T); and striated duct (arrow head). H&E. stain, Bar = 100µm.

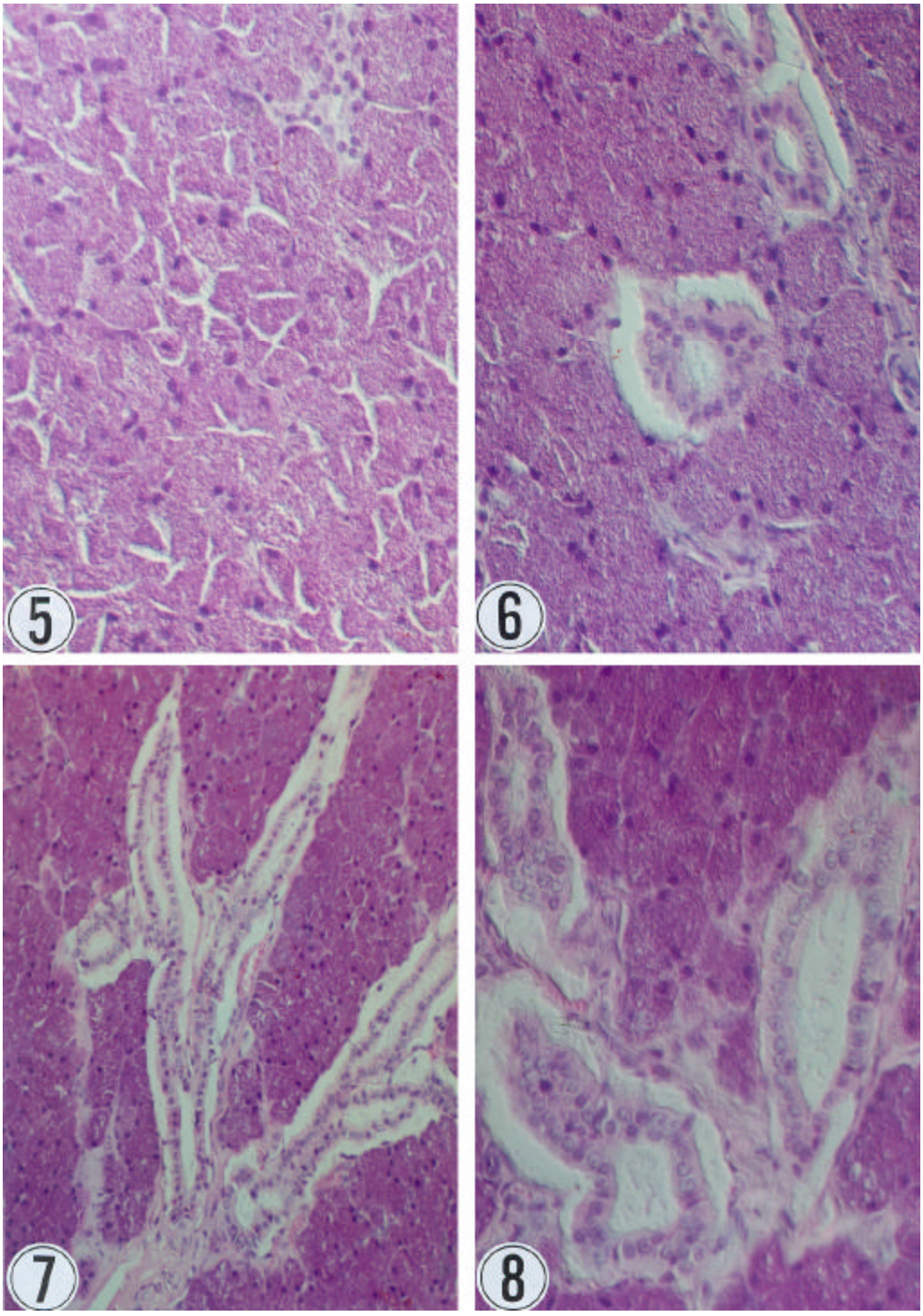

Fig. 5: A photomicrograph showing, the serous secretory acini H&E. stain, X25.
Fig. 6: A photomicrograph showing the serous secretory acini & the striated duct. H&E. stain, Bar = 100µm.
Fig. 7: A photomicrograph showing the interlobular duct H&E. stain, X 50.
Fig. 8: A photomicrograph showing the interlobular duct H&E. stain, Bar = 100µm.

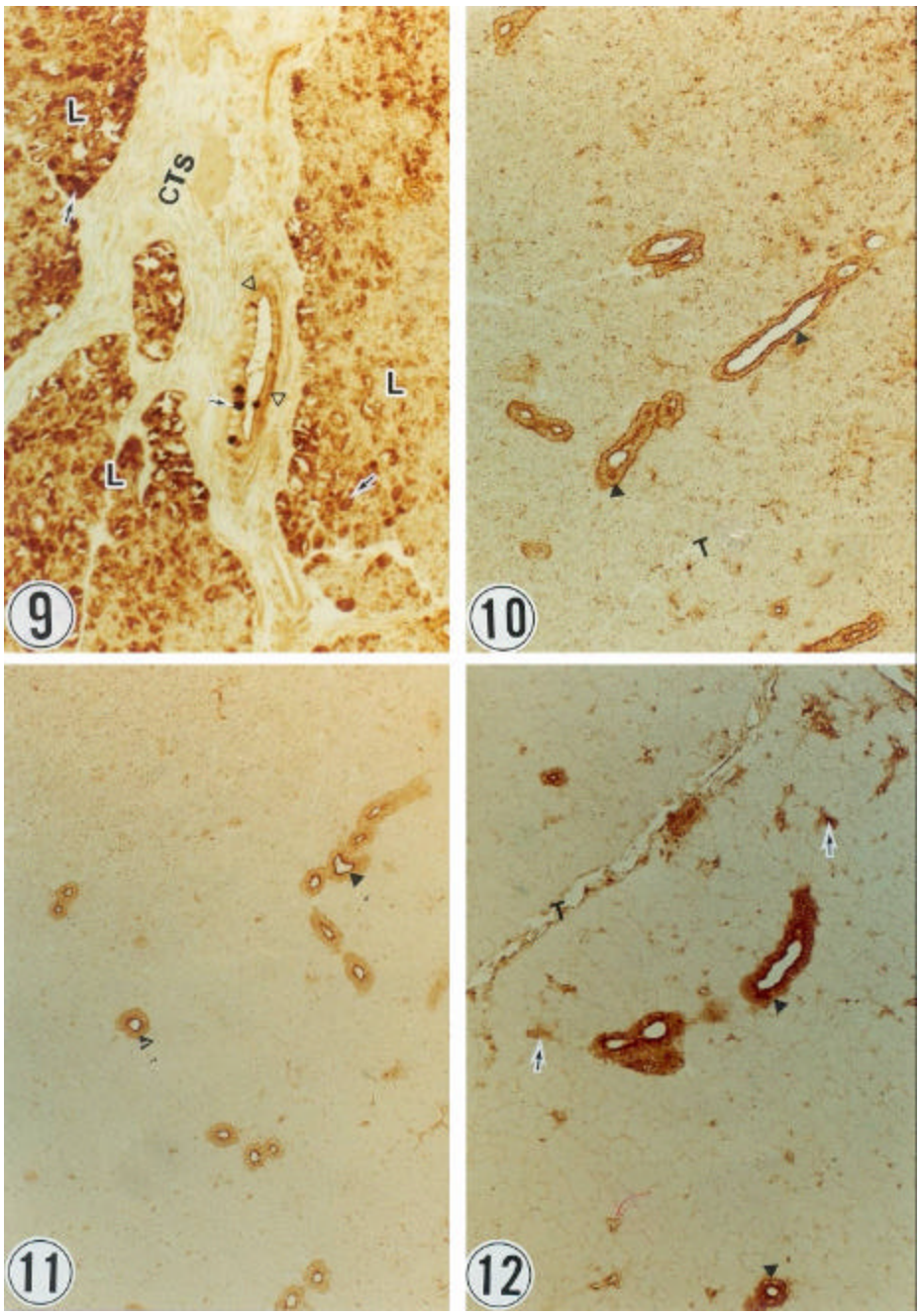

Fig. 9: Stored apical secretory granules in parotid serous cells (arrow) show intensely positive reaction that tends to be localized in the periphery of the lobules (L). Striated ducts are un-reactive (arrow head), while the goblet cells are positively reacted (arrow). WGA stain, Bar = 100µm..

Fig. 10: Serous acini digested by sialidase enzyme showing little affinity with WGA while the epithelial cells of the intercalated and striated ducts showing moderate reaction (arrow). WGA stain, Bar = 100µm.

Fig. 11: Serous acini that resist cleavage by sialidase enzyme were removed by KOH / sialidase sequence showing negative reaction with WGA. The apical surface of the intercalated duct showing positive reaction (arrow). WGA stain, Bar = 100µm.

Fig. 12: Serous cells showing little reaction with Con-A that seen at its luminal surface (arrow). The epithelial lining of the intercalated and striated duct showing intense positive reaction (arrow head). Con-A stain, Bar = 100µm.

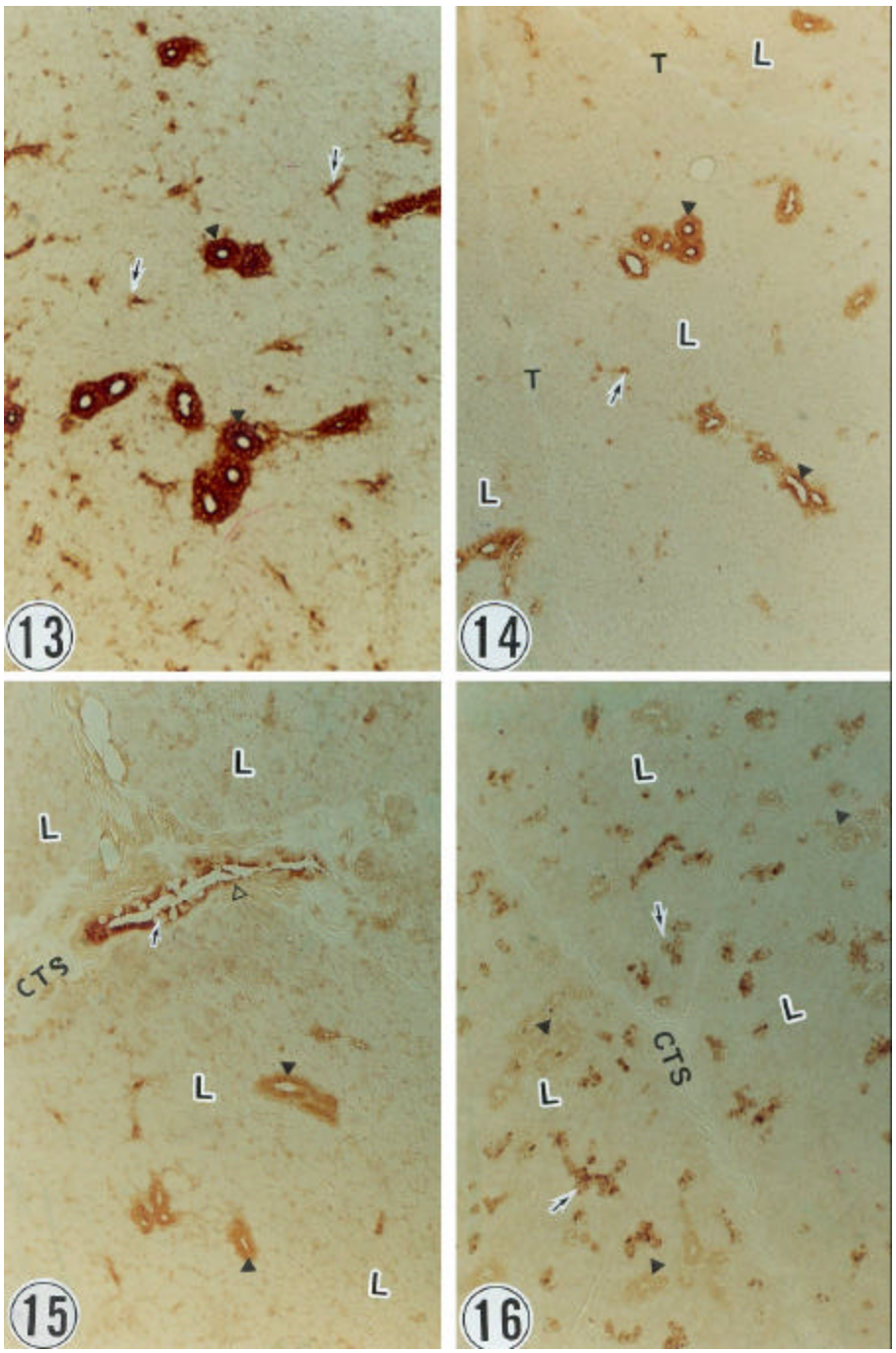

Fig. 13: Serous cells showing little reaction with Con A in the form of sporadic foci (arrow). The epithelial lining of the intercalated and striated duct showing intense positive reaction (arrow head). Con-A stain, Bar= 100µm.

Fig. 14: Serous cells show negative reaction with PNA (arrow), the epithelial lining of the intercalated and striated duct showing positive reaction (arrow head). PNA stain, Bar = 100µm.

Fig. 15: Serous cells showing negative reaction with PNA (arrow). The epithelial lining of the striated and interlobular ducts showing moderate reaction (arrow head) and the goblet cells showing negative reaction. PNA stain, Bar = 100µm.

Fig. 16: Serous cells showing moderate reaction with UEA-1 in the form of sporadic foci (arrow). The epithelial lining of the striated duct showing negative reaction (arrow head). UEA-1 stain, Bar = 100µm.

The present study showed that, the secretory acini and the interlobular duct failed to stain with PNA. This result was in agreement with finding in horse [5]. On contrast, secretory acini and the interlobular duct of rat and mouse showed moderate to strong staining with PNA [6, 7]. The lining epithelium of the intralobular ducts showed moderate reaction with PNA in the current study. In the same context, the intralobular ducts were un-reactive towards PNA [23].

We reported here that the secretory acini of the PSG showed a moderate reaction with the UEA-I, while, the lining epithelium of the duct system of the gland, was negative. In an accord, all serous cells of PSG evidenced moderate to strong staining with UEA-I, [7]. Moreover, a heterogeneous binding pattern with UEA-I and variation of the acinar cells in their affinity to this lectin ranging from negative to strong positive reaction was reported [23]. In marked contrast, Gargiulo and his college [5] Recorded a negative reaction of the serous cells with the UEA-I. These differences in morphologically similar cells may be related to activation and /or deactivations of glycosidases and glycosyltranseferases during the anabolic and catabolic phases of secretory glycoconjugates metabolism at different times in the different cells. This explanation was supported by the finding of [9]. Concerning the duct system of the gland, our result was in disagreement with that of [6, 23, 24], the latter authors mentioned that the apical cytoplasm in intralobular duct cells were heavily stained with UEA-I.

In the present work, the reactivity of secretory acini with Con-A was scanty and tends to be seen only at the luminal surface of the acini. At the same time the lining epithelium of the duct system showed an intense reaction with Con-A. In partial agreement, [11] reported a distinct supranuclear granular reactivity within the ductal epithelium. Meanwhile, [23], documented moderate and uniform staining with Con-A in the secretory acini together with a slight reaction at the luminal surface, but not with the apical cytoplasm of ductal cells. These results suggest a homogenous presence of glycoconjugates containing α-D-man in the ductal epithelium of the monkey PSG.

Our result showed that, the serous acini gave intensive reaction with WGA. This indicates that, the predominant terminal sugar residues in the monkey PSG is GlcNAc. This result was in agreement with that reported in deer [25]. On the contrary, in horse all serous cells of the PSG were un-reactive with the WGA [5]. This could be explained on the basis of species variations and the nature of the food in both species. Concerning the duct cells, our result was in line with that of many authors [22, 25, 26], However, it was recorded strong staining reaction localized in the apical cytoplasm and surface epithelial cells lining striated duct with WGA [26].

In conclusion, the nature and composition of stored glycoproteins in monkey PSG is heterogeneous. The predominant terminal sugar residues are supposed to be α-D-GlcNAc in secretory acini and α-D-glc and α-D-man in the ducts system.

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