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Lectin Histochemistry of the Brunner's Glands in Three Species of Monkeys (*Tupaiidae glis, Nycticebus cocang* and *Callithrix jacchus*)

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Abstract: The lectin histochemistry of the Burunner's glands of the non human primate monkeys, common treeshrew (*Tupaiidae glis*), slow rolis (*Nycticebus cocang*) and common marmost (*Callithrix jacchus*), were investigated with a battary of nine different lectins as a probe and horseraddish peroxidase or fluorescen isothiocyanate as visulant. The Burunner's glands showed great overall lectin affinity with all the lectins used. This indicates the existence of oligosaccharides with terminal galactose, N-acetylgalactosamine, N-acetylglucosamine, α-D-glucose, α-D-mannose, α-L-fucose and neuroaminic acid in the secretion of the Burunner's glands. The biological role of each sugar at the surface of the lining epithelium was suggested on the base of the results of this study and discussion with the avilable litertues.

Key wards: Brunner's gland · Carbohydorates · Lectin histochemistry · Monkey · Non human primate

INTRODUCTION

Non human primates present a unique animal model for human diseases because of their phylogenetic closeness to the human as well as their convenient size, rather than omnvorous nature of diet and their ability to stay healthy in captivity. The Burunner's glands are tubuloalveolar gland located in the submucosa of the mammalian duodenum. The morphological nature of secretory cells of the Burunner's glands shows species variation as well as variation within the species. These variations are clear in the human [1], monkey [2, 3], African elephant [4], horse and cat [5], guinea pig [6], rat [7, 8], mouse [9], ferret [10], camel [11, 12] and other mammalian species [13, 14]. In non human primates, classical carbohydrate histochemical studies show that the Burunner's glands contain neutral mucopolysaccharides [2, 3, 15, 16]. By lectin histochemistry, high levels of carbohydrate residues, i. e., galactose, galactosamine and fucose, are found in the Burunner's glands in two New World monkeys, Saimiri sciureus and Saguinus fusciocollis [2] and in the rhesus macaque and Japanese macaque [3]. However, there is difference in lectin afinity in these non human primates. Secretory cells of th Brunner's glands react with lectins of Concanavalia ensiformis (Con-A) and Lotus tetragonolobus (LTA), while those in Saimiri sciureus and Saguinus fuscisllis do not [2].

The aim of this study was to analyze the carbohyderate residues in the secretory cells of Burunner's gland in three species of monkey; treeshrew monkey (Tupaiidae glis), slow rolis monkey (Nycticebus cocang) and common marmost monkey (Callithrix jacchus) by lectin histochemistry in order to compare the mucus comosition of monkeys with those of the human.

MATERIALS AND METHODS

Animals: The samples were collected from three species of non human primates monkeys obtained from Department of Anatomy, United Graduated School of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan. All investigations were completed in the Department of Anatomy and Histology, Faculty of Veterinary Medicine, Kafr El-Sheikh and Department of Histology, Faculty of Medicine Tanta University, Egypt. A total of nine animals were used for this experiment. Among these animals, three were of common treeshrew monkeys (*Tupaiidae glis*), three were of slow rolis monkeys (*Nycticebus cocang*) and three were of common marmost monkeys (*Callithrix jacchus*).

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Table 1: Lectins used in this study and their major sugar specifications

Taxonomic name and abbrivations	Common name	Labled used	Concentration used (µg/ ml)	Major sugar specification	Sugarbindinginhibitor
Glycin max (SBA)	Soybean	FITC	10	∝-D-GalNAc, ∝-D-Gal	∝-D-Gal NAc
Dolichous biflorus (DBA)	Horse germ	FITC	100	∝-D-GalNAc	∝-D-Gal NAc
Ulex europeus UEA-1)	Gorse	FITC	100	∝-L-Fucose	∝-L-Fucose
Wisteria floribounda (WFA)		FITC	100	GalNAc	GalNAc
Arachis hypogea (PNA)	Peanut	HRP	100	Gal-β-l(1-3)-GalNAc	Lactose
Concanavalia ensiformis (Con-A)	Jak bean	HRP	20	∝-D-Man, ∝-D-Glc	∝-D-methyel Man.
Helix pomatia (HPA)	Roman snail	HRP	9	∝-D-GalNAc	∝-D-GalNAc
Triticum vulgaris (WGA)	Wheat germ	HRP	6	(β-(1-4)D-Gal-NAc)2,NeuNAc	Neu NAc
Banderia simplicifolia (BSA-1)	Girffonia	HRP	20	∝-D-GalNAc	GalNAc

Symbol: Gal= Galactose; Glc=Glucose; GalNAc=N-acetylgalactosamine; GlcNAc=N-acetylglucosamine; Man=Mannose; NeulNAc= Acetylneuraminic acid (Sialic acid). HRP= Hoursraddish peroxidase; FITC= fluorescein isothiocianate

Sample Collection and Tissues Preparation: Following the strict animal welfare regulations and the rules of the animal ethics committee in Japan, the monkeys were sacrified under deep anesthesia with an intravenous injection of sodium pentobarbital (50 mg/kg). The samples were rapidly removed and pieces of the proximal duodenum were fixed in phosphate buffered-saline (PBS) contain 4% paraformaldehyde for 72 hr and thoroughly rinsed in the same buffer. Sections of 4µm thickness were deparafinized in xylene. For detection of the binding sites of the sugar residues, sections were subjected to lectin staining using a baterry of lectins summarized in Table 1.

HRP or FITC-Lectin technique: The processing and staining protocol used with the various lectins was similar to that described by Fayed and Makita [17]. Briefly, after hydration, the sections were treated with 0.3% hydrogen peroxide (H_2O_2), rinsed in distilled water and washed in 0.1 M PBS, pH 7.4. The sections were incubated for over night at 4°C in lectins conjugated either to horsraddish peroxidase (HRP) or fluorescen isothiocyanate (FITC) (Sigma Chemical Co. St. Louis, Mo, USA), dissolved in 0.1 M PBS, pH 7.4 (contain 0.1 M NaCI, 0.1 mM $CaCI_2$, $MnCI_2$) and then rinsed three times in PBS. The optimal concentration used with each lectin which allowed maximum staining with mininum background is as listed in Table 1. Visualization of the binding sites of HRP-lectin was obtained by incubating the slides in PBS containing 3.3'diaminobenzidine tetrahydrochloride (DAB) (25 mg/ 100 ml) and H2O2 (0.003%) for 10 min at room temperature. Slides were rinsed in distilled water, dehydrated using gradual ethanol solutions, cleared in xylene and mounted in DPX. By contrast, FITC-lectin sections were rinsed in distilled water and directly examined with a fluorescence-microscope.

Histochemical and Enzymatic Treatment

Sialidase Digestion: Some sections were digested with 0.2 IU/ml sialidase enzyme from *Vibrio cholerae* (Cal. Biochem. La Jolla, USA) for 18 hr at 37úC. Sialic acid residues with O-actyel substitution which resist the enzymatic cleavage by sialidase, were treated by saponification before sialidase treatment. Removal of sialic acid either by sialidase digestion or by saponification (KOH treatment)-sialidase sequences was confirmed on an adjacent section stained by alcian blue at pH 2.5 (Ab 2.5) or Ab 2.5/ periodic acid-Schiff (AB 2.5/ PAS).

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

Periodate Oxidation: In order to identify the stable class III mucins, periodate oxidation or periodate oxidation-borohydride sequense was carried out perior to staining with Con-A.

Hapten Sugars Inhibition: Every lectin was preincubated with the corresponding hapten sugars listed in Table 1. The hapten sugar inhibitors were employed from 0.05 M to 1 M. The staining was completely eliminated at 0.2 M.

Does the Conjugation to HRP or FITC Affect the Lectins Specificity?: An experiment was carried out to determine whether the conjugation of lectins with HRP or FITC affects the specificity of the lectin to binding sites in the tissues. To the treated sections with the HRP or FITC conjungated lectins additional different concentrations of the corresponding unconjugated lectins were added. The additional unconjugated lectins at the appropriate concentrations (Table 1) caused an uniform decrease in

the staining intensity, but did not affect the staining pattern; that is, there were no additional stained cells. These observations mean that the conjugations of the lectins with HRP or FITC did not affect the specificity of the lectin because the conjugated and unconjugated lectins compete for the same sites in the tissues.

RESULTS

There were no significant variations in the staining pattern of the Brunner's glands among the three species with either FITC or HRP conjugated lectins.

The staining patterns of secretory cells of the Brunner's glands showed overall intense staining, while the other structures, such as interstitial connective tissuse, blood vessels, or enterochromaffine cells were negative with all the lectins used.

Glycin Max Agglutinine (SBA): Secretory cells of the Brunner's glands were uniformally and intensily stained with SBA (Fig. 1), indicating the existence of galactose (α-D-Gal) and N-acetylgalactosamine (α-D-GalNAc). The staining with SBA was not abolished with ether sialidase digestion or saponification-sialidase sequence.

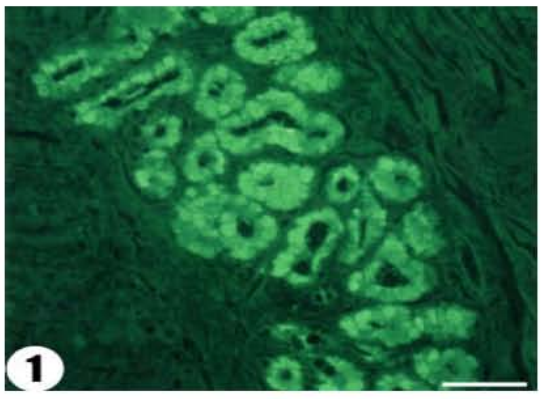
Dolichous Biflorus Agglutinine (DBA): Secretory cells of the Brunner's glands showed a great staining affinity to DBA demonestrating the existence of α-anomer of GalNAc (Fig. 2). This sugar resisted the cleavage by sialidase enzyme. The same results obtained with KOH treatment prior to sialidase digestion.

Ulex Europus Agglutinine (UEA-1): Secretory cells of Brunner's glands showed intensily positive reaction with UEA-1 (Fig. 3) demonestrating the presence of α-L-fucose sugar. No effect of the sialidase digestion and also with KOH treatment prior to the enzyme digestion.

Wisteria Floribounda Agglutinine (WFA): The existence of the terminal GalNAc in the secretory cells of the Brunner's glands confirmed by the deep positive reaction with WFA (Fig. 4). This sugar resisted the cleavage with sialidase enzymes even with piror KOH treatment.

Arachis Hypogea Agglutinine (PNA): Secretory cells of the Brunner's glands were stained deeply with PNA (Fig. 5). This indicate that these cells secrete the β-anomer of the Gal and GalNAc. Secretory cells of the Brunner's glands were positive with either sialidase digestion or KOH/ sialidase sequence when it used perior to PNA staining. (Figs. 6 and 7).

Helix Pomatia Agglutinine (HPA): With the HPA staining, secretory cells of the Brunner's glands showed intensely positive reaction (Fig. 8) indicating the existance of α-D-GalNAc. No changes in the staining could be detected with sialidase digestion (Fig. 9).



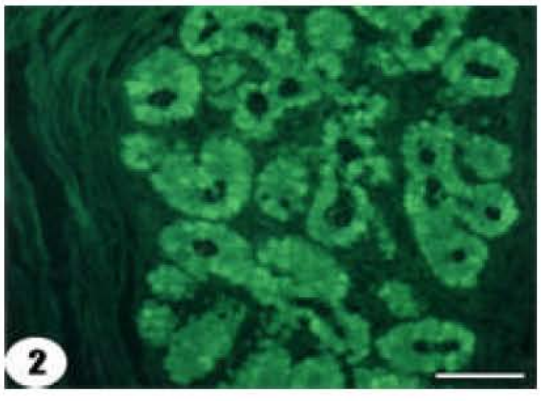


Fig. 1: Brunner's gland of common treeshrew monkeys *(Tupaiidae glis)* uniformally and intnsely stained with SBA. This indicated the existance of Gal and GalNAc. SBA-FITC; Bar=200 μm.

Fig. 2: Brunner's gland of slow rolis monkeys *(Nycticebus cocang)* showing great staining affinity to DBA demonestrating the existance of the α-anomer of the GalNAc. DBA-FITC; Bar= 200 μm

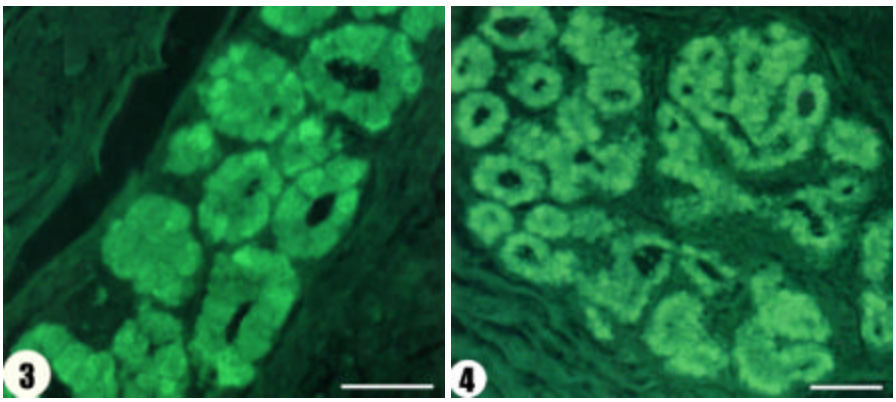


Fig. 3: Brunner's gland of common marmost monkeys (*Callithrix jacchus*) showing intensely positive reaction with UEA -1 demonestrating the presence of α-L-fucose sugar. UEA -1 FITC; Bar=200 µm.

Fig. 4: Brunner's gland of slow rolis monkeys (*Nycticebus cocang*) showing deep positive reaction with WFA revealing the presence of the terminal GalNAc. WFA -FITC; Bar= 200 µm.

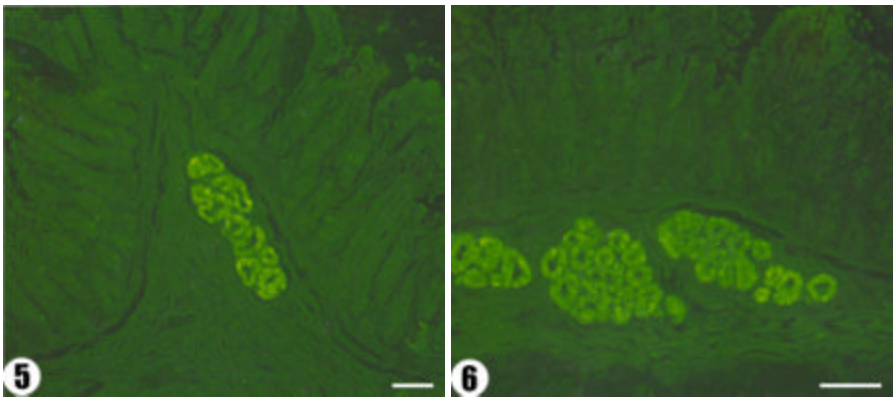


Fig. 5: Brunner's gland of common marmost monkeys (*Callithrix jacchus*) staind deeply with PNA. This indicated that, it secreat the β-anomer of the Gal and GalNAc. PNA - FITC; Bar=200 µm .

Fig. 6: Brunner's gland of common treeshrew monkeys (*Tupaiidae glis*) stained positive with sialidase digetion prior to the staining with PNA. Sialidase / PNA – FITC sequence; Bar=200 µm.

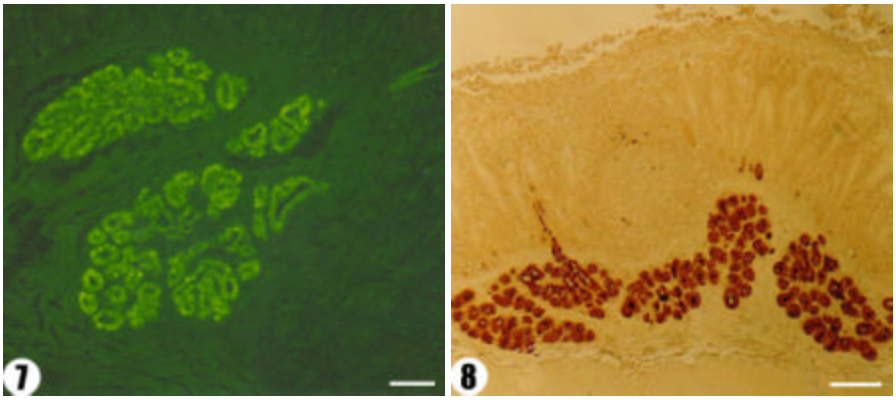


Fig. 7: Brunner's gland of slow rolis monkeys *(Nycticebus cocang)* stained positive with PNA even after treatment with KOH perior to the sialidase digestion. KOH/ sialidase/PNA -FITC sequence; Bar=200 µm.

Fig. 8: Brunner's gland of common marmost monkeys (*Callithrix jacchus*) stained positive with HPA indicating the existance of α-D-galNAc. HPA -HRP; Bar= 200 µm.

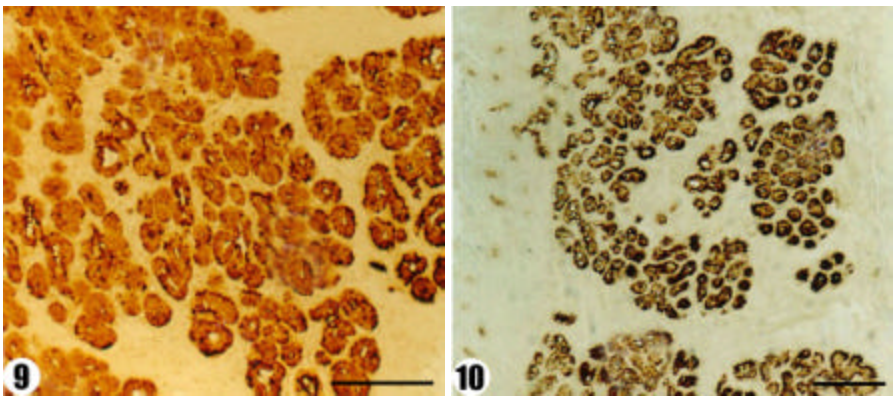


Fig. 9: Brunner's gland of slow rolis monkeys (*Nycticebus cocang*) stained positive with sialidase digestion prior to HPA staining. Sialidase/ HPA-HRP; Bar=200 µm.

Fig. 10: Brunner's gland of common treeshrew monkeys (*Tupaiidae glis*) stained positive with WGA indicating the presence of GalNAc and/or NeuNAc residuse. WGA-HRP; Bar=200 µm.

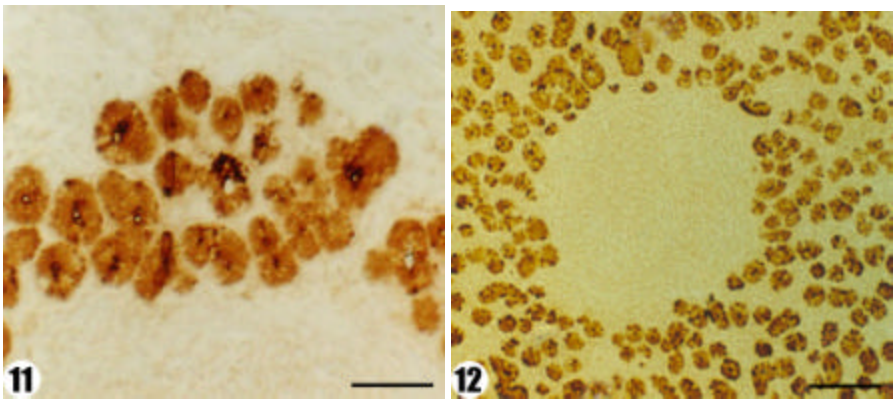


Fig. 11: Brunner's gland of common marmost monkeys (*Callithrix jacchus*) show positive staining with Con-A revealing the presences of the α-D-glu. And α-D-Man sugars. Con-A-HRP; Bar=200 µm.

Fig 12: Brunner's gland of slow rolis monkeys *(Nycticebus cocang*) showing positive staining with periodate oxidation-borohydride sequence. PO / Borohydride/ con-A HRP; Bar=200 µm.

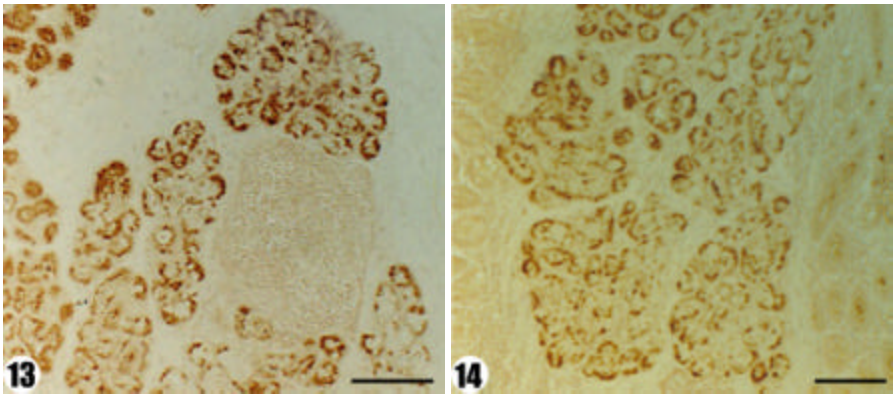


Fig. 13: Brunner's gland of common treeshrew monkeys *(Tupaiidae glis*) showing moderat positive staining with BSA-1 indicating the existance of ∝-anomer of the Gal and GalNAc. BSA-1-HRP; Bar=200 µm.

Fig. 14: Brunner's gland of common treeshrew monkeys (Tupaiidae glis) staind moderet positive with KOH treatment prior to sialidase BSA-1 sequence. KOH / Sialidase/ BSA-1-HRP; Bar=200 µm

Triticum Vulgaris Agglutinine (WGA): Secretory cells of the Brunner's glands showed intense staining with WGA (Fig. 10) indicating the presence of GlcNAc and/or NeuNAc residues. Sialidase digestion failed to change the staining pattern of the WGA even after saponification indicating that the secretory cells of the Brunner's glands have sialidase resistance glycoprotein with O-acetyel substitution.

Concanavalia Ensiforms Agglutinine (Con-A): With Con-A secretory cells of the Brunner's glands showed deep staining (Fig. 11) revealing the presence of α-D-glu and α-D-man sugars. Wihle the staining pattern did not changed (Fig. 12) with either periodate oxidation or periodate oxidation-borohydride sequense prior to the staining with Con-A indicating the exsistance of class III glycoconjugate.

Banderia Simplicifolia Agglutinin (BSA-1): Moderate staining (Fig. 13) obtained with BSA-1 indicating the existance of the α-anomer of the Gal and GalNAc. Secretory cells of the Brunner's glands remain unchanged with sialidase digestion or even saponificaton / sialidase/ BSA-1 sequence (Fig. 14).

DISCUSSION

In the present study, GalNAc, Gal, GlcNAc. α-L-fuc, α-D-gluc and/or α-D-Man were found to be the main sugar componant in secretory cells of the Brunner's glands in three species of non human primates monkeys: Tupaiidae glis, Nycticebus cocang and Callithrix jacchus. Secretory cells of the Brunner's glands of two New World monkeys, i.e. Saimiri sciureus and Saguinus fuscicollis, posses nealy the same mixture of the sugar residues (GalNAc, Gal, GlcNAc. α-L-fuc) [2], but they do not react with Con-A, LTA, or Solanun tuberosum (STA) that bind D-gluc, α-D-Man or glucosamine. However, Con-A and LTA bind secretory cells of the Brunner's glands in the Japanese macaque or rhesus macaque [3]. Secretory cells of the Brunner's glands of humans react wiith Con-A [18].These findings give clear indication about the species varations even among the primates and emphasis on needs for careful comparisons. These species difference probably attributes to the composition of diet.

Some species difference in the lectin staining pattern were noticed, for example secretory cells of the Brunner's glands of the human stained more intensily with UEA-1 comparing to that of dog, cat and guinea pig [7, 14]. In the present study, secretory cells of the Brunner's glands showed intensily staining with UEA-1, while it was completely negative in one humped camel [12].

Similarly to those of the human, cat and pig [14] and one humped camel [12], secretory cells of the Brunner's glands of the non human primate monkeys had greater overall lectin affinity. Earlier lectin histochemical investigations showed that gastrointestinal mucous carbohydrate side chain is composed of four sugars, Gal, GalNAc, GlcNAc and α-L-fucose in very constant molecular ratio [19]. This study confirmed the existance of all these sugars in secretory cells of the Brunner's glands.

This study suggests that glycoproteins with neutral sugars as Gal, α-D-glu, α-D-Man, α-L-Fuc. and α and β form of the GalNAc may play a role in the protection aganist mechanical insults caused by harsh food at the surface of the lining epithelium of the duodenum. Also it could play a role aganist the chemical insults as hyperacidity of the principal acidic glycoprotein of the duodenal mucous secretion. These suggestion is supported by Warren and Spicer [20], Fox, [21] and Hafez[22], The latter hypothsized neutralizing role of the neutral sugars to control the acidity of the principal glycoproteins. Jonas and his college [23] supposed a role for the Gal, α and β anomer of GalNAc in the polarity of the cell surface and so could play a role in protection of the lining epithelium aganist the effect of the luminal fluid bathing the cell apices.

Histophysiologically, sialic acid residues of carbohydrates are known to coat the mucosal surface to provide a hydrophilic enviroment designed to preserve hydration [24, 25]. A role in the protection of the mucous membrane of the duodenum from pathogenic agent can be hypothesied for NeuNAc. This suggestion was supported by the hypothesis [26, 27].

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