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CHARACTERISATION OF SUGAR RESIDUES IN GLYCOCONGUGATES OF DUODENUM OF ONE HUMPED CAMEL (CAMELEUS DROMEDARIUS)

(With 5 Tables and 17 Figures)

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دراسات الخصائص الهستوكمائية للسكريات المقترنه مع الكربوهيدرات الموجودة في إثنى عشر الجمل وحيد السنام

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عنيت هذه الدراسة بمقارنة توزيع السكريات المختلفة في إفرازات الخلايا الكأسية مع توزيعها في إفرازات الغدد الموجودة في الطبقة تحت الطلائية في إثنى عشر الجمل وحيد السنام. ووجد أن إفرازات كلا من الخلايا الكأسية والغدد الموجودة في الطبقة تحت الطلائية تحتوي على خليط من الجلايكوبروتينات ذوات الوحدات المتعادلة القابلة للأكسدة والجلايكوبروتينات ذوات مجموعات الكبريتات وكذلك تلك المتحدة مع مجموعات الكربوكسيلات وكانت الجلايكوبروتينات ذوات المجموعات المتعادلة القابلة للأكسدة أكثر إنتشاراً في الغدد الموجودة في الطبقة تحت الطلائية بينما كانت الجلايكوبروتينات ذوات المجموعات الكبريتية منتشرة في الخلايا الكأسية. وقد تم تحديد أماكن توزيع السكريات المختلفة بواسطة اللكتينات. ووجد أن إفرازات الغدد الموجودة في الطبقة تحت الطلائية تحتوي على الجلاكتوز أمين والجلوكوز والجلوكوز أمين والمانوز وكذلك حمض النيورومين بينما إفرازات الخلايا الكأسية تحتوي على حمض النيورومين وخالية من الجلوكوز والمانوز. وبالرغم من أن الفوكوز يعتبر واحداً من أهم السكريات المرتبطة بجزئ الكربوهيدرات الموجود في القناة الهضمية فقد أكدت هذه الدراسة غيابه من كل من الغدد الكأسية والغدد الموجودة في الطبقة تحت الطلائية في إثنى عشر الجمل وحيد السنام.

SUMMARY

Sugar residues in glycocongugates of camel duodenal glands was histochemically compared with those of the goblet cells. Both duodenal glands and goblet cells contained a mixture of glycoproteins (GPs) with oxidizable vicinal diols, GPs with O-sulfate ester and GPs with carboxyl groups. The GPs with oxidizable vicinal diols were predominant in duodenal glands while those with O-sulfate ester were predominant in

duodenal glands while those with O-sulfate ester were predominant in goblet cells. Oligosaccharides with terminal galactose (Gal), N-acetylgalactoseamine (GalNAc), N-acetylglucosamine (GlcNAc), α-D-glucose (α-D-Glc) and α-D-mannose (α-D-Man) and neuraminic acid residues were rich in duodenal glands while GlcNAc and neuroaminic acid residues were rich in goblet cells but α-D-Man and α-D-Glc were devoid from it . The α-L-fucose could not be localized neither in duodenal gland nor in goblet cells of the camel.

Key words: *One-humped Camel, Duodenum, Glycoprotein, Lectin, Histochemistry.*

INTRODUCTION

The duodenal mucous-secreting cells include both goblet cells and the submucosal glands. The later are branched tubuloalveolar glands located in the submucosa of the proximal part of duodenum. Various studies dealing with the carbohydrate histochemistry of the duodenal glands and goblet cells in large number of mammals, showed a marked species variations as well as variations even within the same species. These studies include the mucous secreting cells of man (Crescenzi *et al.*, 1988), monkey (Posel *et al.*, 1988), African elephant (Coetzee *et al.* 1995), horse and cat (Odour-Okelo 1976 and Ticano *et al.*,1974), ginnea pig (Cochrance *et al.*, 1964), rat (Geleff and Böck 1984 amd Suzuki *et al.*, 1981), mouse (Obuforibo 1975), ferret (Poddar and Jacob 1979), two-humped camel (Takehana *et al.*, 2000) and comparatively in other mammalian species (Daniel and Helen 1976, Madrid *et al.*, 1989 and Skutelsky *et al.*, 1989). A review of the literature presented many references on the duodenal gland of different mammals including two humped camels but non is referred to that of the one-humped camel. The advent of lectin methods have been increased the capacity for histochemical characterization of terminal or internal sugars (Alroy *et al.*, 1984 and Rhodes and Milton 1998). Further to the previous reports on the glycoproteins of gastric mucosa of the one-humped camel (Fayed and Makita 1996 and 1997) in this report the general classes of the glycoproteins as well as the cellular distribution of the lectin binding sites in the duodenal mucosa of the one-humped camel had been studied.

2-Materials and Methods:

2.1. Sample collection and tissue preparation

Samples from seven camel's duodenum were collected from slaughter houses in Cairo, Pelpies, and Kafer El-Sheikh. The samples were cut into small pieces then fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 72 hours. Besides Gill's hematoxylin (Gill *et al.*, 1974) and eosin, sections of 4 µm thickness were stained with the histochemical staining procedures listed in Table 1 for identification of the general classes of the glycoproteins, and with the lectin staining methods listed in Table 2 for detection of the sugar residues.

The scientific and common names, abbreviations, sugar specificity and inhibitors of the lectins used in this experiment were listed in Table 3.

2.2. Histochemical and enzymatic treatment :

2.2.1. Sialidase digestion:

Sections were digested with 0.2 IU/ml sialidase enzyme from Vibrio cholerae (Cal. Biochem. La Jolla, USA) for 18 hr at 37^0C. Sialic acid residues with O-acyl substitution, which resist the enzymatic cleavage by sialidase, were removed by saponification before sialidase treatment. Removal of sialic acid either by sialidase digestion or by KOH-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).

2.2.2. Saponification (KOH-treatment):

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

2.2.3. Periodate oxidation:

Periodate oxidation blocks the enzymatic removal of N-acetylneuraminic acid (NeuNAc) as judged by prevention of the staining with sialidase/PNA and sialidase/DBA sequence. Periodate oxidase was carried out by immersing the slides in 44 mM periodic acid for 20 min at room temperature.

2.2.4. Hapten sugars inhibition:

Every lectins was preincubated with the corresponding hapten sugars listed in Table 3. The hapten sugar inhibitors were employed from 0.05 M to 1 M and complete elimination of the staining was obtained at 0.2 M.

2.3. Does the conjugations to horseradish peroxidase (HRP) or fluorescein isothiocyanate (FITC) affect the lectins specificity?

An experiment was carried out to determine wither the conjugation of the lectins with HRP or FITC affect the specificity of the lectin to binding sites in the tissues. To the treated sections with the HRP or FITC conjugated lectins, additional different concentrations of the corresponding unconjugated lectins were added. The additional unconjugated lectins at the appropriate concentrations (Table 3) caused a 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 do not affect the specificity of the lectin because the conjugated and unconjugated lectins compete for the same sites in the tissues.

RESULTS

3.1. Histology:

The duodenal mucosa of the camel is characterized by numerous intestinal villi which appear as minute finger like projections having a 1.5 - 2 mm length (Fig. 1). The surface of the epithelium is increased also by invaginations to form tubular glands (crypts of Lieberkühn) which open in the area between the bases of the villi. The epithelium covering the surface is represented by absorptive columnar epithelial cells intermingled with goblet cells (Fig. 2). The absorptive cell is columnar with ovoid nucleus situated in the lower part of the cell. The free surface shows striated border. The submucosa contain branched tubuloalveolar glands called Brunner's glands or duodenal glands. The product of the glandular epithelium was secreted into the duodenal lumen through a duct open at the base of the crypts of Lieberkühn.

3.2. Histochemistry:

3.2.1. Duodenal glands:

Without previous oxidation in periodic acid, the duodenal glands were negatively stained with Schiff reagent (method 1), and deep magenta with PAS (method 2) (Fig. 3), indicating the existence of GPs with oxidizable vicinal diols and/or glycogen. No significant changes were noticed with prior α-amylase digestion (method 3), indicating that the mucous secretion of the duodenal glands contains GPs with oxidizable vicinal diols not glycogen. These cells show clear alcianophilia by AB 2.5 and AB1.0 (methods 4 and 7) (Figs. 4 and 5). This alcianophilic reaction was completely abolished by prior active

methylation (methods 5 and 8) and could not be restored by the subsequent saponification (methods 6 and 9) indicating the presence of GPs with AB 2.5 and AB 1.0 positive O-sulfate esters. In combination of AB 2.5 with PAS (method 10) these cells show purple coloration with magenta and feeble turquoise reaction (Fig. 6). Only magenta reaction was prominent with prior active methylation or active methylation-saponification procedures (methods 11 and 12). No significant changes were obtained by sialidase digestion (methods 14 and 15) on alcianophilic reactions of these cells either with or without prior KOH treatment. This indicates the presence of sialidase resistance GPs or absences of sialic acid residues at all.

These cells were deeply stained with galactose oxidase/Schiff sequences (method 16) (Fig. 7) indicating the presence of Gal and GalNAc residues in the mucus of these cells. Concanavalia ensiformis agglutinin (Con-A) (method 17) showed intensely positive reaction in the duodenal glanad cells (Fig. 8) indicating the existence of α-D-Glc and α-D-Man in the duodenal glands secretion. Camel's duodenal gland cells were intensely stained with Triticum vulgaris agglutinin (WGA) (method 18) (Fig. 9) indicating the presence of GlcNAc and/or NeuNAc residues. Sialidase digestion (method 19) failed to abolish the WGA staining (Fig. 10). With prior saponification (method 20), sialidase digestion partially inhibited the staining of this lectin in the duodenal gland cells indicating that, these cells have sialidase-resistant sialic acid residues with O-acyl substitution. Duodenal gland cells were moderately stained with Helix pomatia agglutinin (HPA) (method 21) (Fig. 11). The results of sialidase/HPA (method 22) and KOH/sialidase/HPA (method 23) was similar to that of methods 19 and 20 (Figs. 12 &13). The results of staining degree with ConA, WGA and HPA is listed in Table 4.

Camel duodenal glandular cells were uniformly and intensely stained with Arachis hypogea agglutinin (PNA) (method 24) (Fig. 14) and Dolichos biflorus agglutinin (DBA) (method 29) (Fig. 15). This indicates that these cells secrete both α and β anomer of Gal and GalNAc. The staining of these cells with PNA and DBA was abolished by periodate oxidation (methods 25 and 30) (Fig. 16) demonstrating the existence of α and β anomer of Gal and GalNAc respectively. The Duodenal gland cells were positive with PNA and DBA with prior sialidase digestion (methods 26 and 31) or periodate oxidation/sialidase sequence (methods 28 and 33) and negative with KOH/sialidase sequence (methods 27 and 32). This indicates the presence of terminal sialic acid with O-acylated polyhydroxyl side chain. However, the

duodenal glandular cells were negatively stained with Ulex europaeus agglutinin (UEA-I) (method 34) (Fig. 16) indicating the absences of α-L-Fuc. When stained with Wisteria floribunda agglutinin (WFA) (method 35), these cells showed very strong positive reaction (Fig. 17) demonstrating the existence of GalNAc residues in the stored secretion of these cells. The results of staining degree with PNA, DBA, UEA-I, and WFA is listed in table 4.

3.2.2. Duodenal goblet cells (Dgc):

The results of staining of Dgc with methods 1-9, and 13-15 were similar to that of the duodenal gland cells. With double staining of AB 2.5/PAS (method 10), the reaction was strong turquoise and weak magenta (Fig. 6) and pure magenta with sections subjected to prior active methylation with or without subsequent saponification (methods 11 and 12). Both strong turquoise and week magenta were obtained with AB 2.5 / PAS, without (method 13) or with sialidase digestion and either with (method 14) or without (method 15) prior saponification. This showed no effect of sialidase digestion on alcianophilia of these cells due to the presence of sialidase resistance GPs or the absence of sialic acid residues.

Camel Dgc were deeply stained with galactose oxidase/Schiff sequences (method 16) (Fig. 7) indicating the presence of Gal and GalNAc residues in the mucous secretion of these cells. These cells showed complete negative reaction with all the lectins used except with HPA and WGA. With WGA (method 18) these cells were intensely stained (Fig. 9) indicating the presence of GlcNAc and/or NeuNAc residues. Sialidase digestion (method 19) failed to abolish the WGA staining (Fig. 10). With prior saponification (method 20), sialidase digestion partially inhibited the staining of this lectin in the goblet cells indicating that these cells have sialidase resistant sialic acid residues with O-acyl substitution. With HPA (method 21) These cells were moderately stained (Fig. 11). The results of sialidase/HPA (method 22) and KOH/sialidase/HPA (method 23) were similar to that of methods 19 and 20 (Figs. 12 and 13).

DISCUSSION

The used selective staining procedures enabled us to study *in situ* the histochemistry of the mucous-secreting cells of the duodenum of camel. In the present study the general classes of the GPs as well as the cellular distribution of the lectin binding sites of the mucous

glycoconjugates in the duodenal mucosa of the one humped camel were established. These results suggested that the mucous secretion of both the duodenal glands and goblet cells contains a mixture of GPs with oxidizable vicinal diols, O-sulfate ester and carboxyl groups. The GPs with oxidizable vicinal diols was predominant in the duodenal glands while that with O-sulfate ester was predominant in the goblet cells. Oligosaccharides with terminal galactose (Gal), N-acetylgalactoseamine (GalNAc), N-acetylglucosamine (GlcNAc), α-D-glucose (α-D-Glc) and α-D-mannose (α-D-Man) and neuraminic acid residues were rich in duodenal gland while GlcNAc and neuroaminic acid residues were rich in goblet cells but α-D-Man and α-D-Glc were devoid from it. The α-L-fucose could not localized neither in the duodenal glands nor in goblet cells of the camel.These findings are similar but not identical to those observed in guinea pigs (Cochrance *et al.*, 1964), and in two species of the new world monkeys (Posel *et al.*, 1988) in which the duodenal glands were stained intensely with WGA, HPA. Some species differences in the staining with the UEA-1, which recognize the non-reducing α-L-fucosyl residues, were noticed (Pereira *et al.*, 1978). The duodenal glands of human stained more intensely with UEA-1 comparing to that of the macaque, dog, cat and guinea pig (Geleff and Böck 1984 amd Skutelsky *et al.*,1989). Both the duodenal glands and goblet cells in two newly world monkey species stained with UEA-1 (Posel *et al.*, 1988). Furthermore, the absorptive columnar cells and goblet cells in humans and macaques did not stain with UEA-1. In the present study neither duodenal glands nor goblet cells were stained with UEA-1. Alroy *et al.* (1986) observed similar species difference and attributed it to the nature of the species. The finding that both goblet cells and duodenal glands of the camel showed diffuse positivity with WAG can be explained by the fact that WGA binds D-glcNAc even if these residues are located within the core of glycoproteins molecules, such occurrence has been described in mucin from different species including human (Crescenzi *et al.*, 1988) and rat (Suzuki *et al.*, 1981) duodenal glands. GPs with similar compositions were described in other areas of the alimentary tract, such as the vesiculated columnar epithelial cells of the rabbit colon (Reid *et al.*, 1988), glandular part of the gastric mucosa of the camel (Fayed and Makita 1996). GPs with similar compositions but lacking O-sulfate ester was also identified in the mucous cells of the distal colon of the rat (Park *et al.*, 1987). The detection of this mixture of glycoproteins in the mucous cells of the rat (Park *et al.*, 1987), in vesiculated columnar cells of the rabbit (Reid *et*

al., 1988) and in the camel gastric mucosal cells (Fayed and Makita, 1996) and camel duodenal glands in the present work suggested that such glycoproteins might be a normal constituent of the gastrointestinal mucin.

Similarly to those of the human, monkey, cat and dog (Skutelsky *et al.*, 1989), the duodenal glands of the one humped camel had greater overall lectin affinity than the absorptive and goblet cells. Earlier lectin histochemical investigations showed that the gastrointestinal mucous carbohydrate side chain is composed of four sugars, Gal, α-L-Fuc, GlcNAc, and GalNAc, in very constant molecular ratio (Hotta and Gosa 1981). All these sugars were localized in the duodenal glands of the one humped camel except α-L-Fuc. The existence of Gal and GalNAc residues in the camel's duodenal glands were confirmed in this study by various staining with galactose oxidase/Schiff sequence, WGA, HPA, PNA, and DBA.

It has been suggested that GPs with oxidizable visinal diols control the acidity of the principal acidic Gps of the mucous secretions (Warren and Spicer, 1961) especially those containing high sulfated Gps (Degraef and Class, 1968). The predominant amount of GPs with oxidizable visinal diols secreted by duodenal glands may control the acidity of the mucous secretion (Hafez , 1977 and Fox, 1979). Since only poor quality, salty and thorny food is available in the hot desert, the elaboration of sulfomucin with the camel goblet cell's secretion lubricates the duodenal mucosa to assist onward movement of food and protect the mucosa against the mechanical insults of this harsh food. The function of duodenal glands secretion is poorly understood. However, similarity between the pyloric glands and duodenal glands has been proposed based on the staining characteristics (Skutelsky *et al.*, 1989). This study and the study of Fayed and Makita (1997) may support the association between these two cell types, as the duodenal gland cells stained intensely with all lectins stained the gastric glandular cells.

In conclusion, this study suggested that, depending upon the sugar residues, the mucous play a different role at the duodenal epithelium surface, either lubrication of the duodenal mucosa to assist onward movement of food, protect the mucosa against the mechanical insults of the harsh food or control the acidity of mucous secretions.

REFERENCES

Alroy, J.; Ucci, A. and Pereira, M. (1984): Lectins: Histochemical probes for specific carbohydrate residues. In: Diagnostic immunohistochemistry (RA Delellis, eds) vol. 2, Masson, New York, pp 67 – 81.

Alroy, J.; Ucci. A.; Goyal, S. and Aurilio, V. (1986): Histochemical similarities between human and globoid cells in Krabbeis disease : A lectin study. Acta Neuropathol 71, 26 – 31.

Cochrance, W.; Davies, D.; Palfrey, A. and Stockwell, R. (1964): The histochemistry and electronmicroscopy of duodenal gland in guinea pig. J. Anat 98, 1 - 10.

Coetzee, H.; Kotze, S. and Lourens, N. (1995): Characterization of mucus glycoproteins in the intestinal mucosa of the African elephant (Loxodonta africana) following lectin histochemistry. Onderstpoort J. Vet..Res. 62, 187 - 192.

Crescenzi, A.; Barsotti, P.; Anemona, L. and Marinozzi, V. (1988): Carbohyderate histochemistry of human Brunner's glands. Histochemistery 90, 47 - 49.

Daniel, S. and Helen, R. (1976): Comparative histochemistry of gastrointestinal mucous glycoprotein. Am. J. Physiol. 240, 176 - 182.

Degraef, J. and Class, G. (1968): Chondrotin sulfate A and sulfated glycoprotein in dog gastric secretion from the founds. Gastroentrology 55 : 584 - 593.

Fayed, M. and Makita, T. (1996): Histochemistry of gastric epithelial glycoprotein of the glandular stomach of the one humped camel (Camelus dromedarius). Pathophysiology 4, 143 - 153.

Fayed, M. and Makita, T. (1997): Lectin histochemistry of the glandular part of the gastric mucosa of the one humped camel (Camelus dromedarius). Acta Histochem. Cytochem. 30, 423 – 431.

Fox, R. (1979): Membrane glycoprotein shed in defense of the gastrointestinal tract . Med. Hypothesis. 5, 669 - 582.

Geleff, S. and Böck, P. (1984): Panceratic duct glands. II. Lectin binding affinities of ductular epithelium, ductular gland and Brunner's glands. Histochemistry 80, 31 - 38.

Gill, G.; Frost, J. and Müller, K. (1974): A new formula for half oxidized hematoxylin solutions that neither over stain nor require differentiation. Acta Cytol. 18; 300 - 311

Hafez, E. (1977): Functional anatomy of mucous secreting cells. Adv. Exp. Med. Biol. 9, 19 - 38.

Madrid, J.; Ballesta, J.; Castells, M.; Marin, J. and Pastor, L. (1989): Characterization of the glycoconjugates in the intestinal mucosa of vertebrates by means of lectin histochemistry. Acta Histochem. Cytochem. 22, 1 - 12.

Odour-Okelo, D. (1976): Histochemistry of the duodenal glands of the cat and hourse. Acta Anat. 94, 449 - 456.

Obuforibo, A. (1975): Mucosubstances in Brunner's gland of the mouse. J. Anat. 119, 287 - 294.

Park, C.; Reid, P.; Owen, D. and Dann, W. (1987): Histochemical studies of epithelial cell glycoproteins in normal rat colon. Histochem. J. 19, 546 - 554.

Pereira, M.; Kiseilus, E.; Gruezo, F. and Kabat, E. (1978): Immunochemical studies on the combining site of blood group H-specific lectin 1 from Ulex europeus seeds. Arch. Biochem. Biophys. 185, 108 - 115.

Poddar, S. and Jacob, S. (1979): Mucosubstance histochemistry of Brunneris glands, pyloric gland and duodenal goblet cells in the ferret. Histochemistry 65, 67 - 81.

Posel, P.; Schumacher, U.; Frick, H. and Welsh, U. (1988): Histochemistry of mucosubstances in Brunner's gland cells and dudenal goblet cells of two new world monkey species (Saimiri sciureus) and (Saguinus fusciellis). Histochemistry 88, 327-332.

Reid, P.; Walker, D.; Terpin, T. and Owen, D. (1988): Histochemical studies of the colonic epithelial glycoprotein of the normal rabbit. Histochem. J. 20, 533 - 550.

Rhodes, M. and Milton, J. (1998): Lectin methods and protocols. Humana Press. Totowa, New Jersey.

Skutelsky, E.; Moore, R. and Alroy, J. (1989): Lectins histochemistry of mammalian Brunner's gland. Histochemistery 90, 383 - 390.

Suzuki, S.; Tsuyama, T.; Yamamoto, N. and Murata, F. (1981): Postembedding staining of Brunner's gland with lectin-ferritin conjugates. J. Histochem. Cytochem. 29, 946 - 952.

Takehana, K.; Eerdunchaoluo, O.; Ueda, H.; Kobayashi, A.; Iwasa, K. and Sou, K. (2000): A histochemical study of the camel (Camelus bactrianus) duodenal glands. J. Vet. Med. Sci. 62, 449-452.

Ticano, G.; Marco, J.; Marcos, S. and Neize, A. (1974): Mucopolysaccharides histochemistry of duodenal glands (Brunner's glands) and goblet cells of Equus caballus. Arq. Cent. Est. Fac. Odont. 11, 77 - 84.

Warren, L. and Spicer, S.S. (1961): Biochemical and histochemical identification of sialic acids containing mucins of rodent vagina and salivary glands. J. Histochem. Cytochem. 9; 400 - 406.

LEGENDS

Fig. 1: A camels duodenal mucosa stained with hematoxlin and eosin. Submucosal duodenal glands (small arrows) and duodenal goblet cells (large arrows). Bar = 100 μ m.

Fig. 2: A camel's duodenal mucosa stained with hematoxlin and eosin. Duodenal goblet cells (arrows). Bar = 100 μ m.

Fig. 3: A camel's duodenal mucosa stained with PAS. Duodenal goblet cells (large arrows) and duodenal glands (small arrows) are deep magenta. Bar = 100 μ m.

Fig. 4: A camel's duodenal mucosa stained with AB 2.5. Duodenal goblet cells (large arrows) and duodenal glands (small arrows) are heavy turquoise. Bar = 100 μ m.

Fig. 5: A camel's duodenal mucosa stained with AB 1.0. Duodenal goblet cells (arrows) are heavy turquoise. Bar = 100 μ m.

Fig. 6: A camel's duodenal mucosa stained with AB 2.5 / PAS. Duodenal goblet cells (large arrows) are strong turquoise and weak magenta but duodenal glands (small arrows) are purple colour with magenta and feeble turquoise. Bar = 100 μ m.

Fig. 7: A camel's duodenal mucosa stained with GO/Schiff sequences. Duodenal goblet cells (large arrows) and duodenal glands (small arrows) are deep magenta. Bar = 100 μ m.

Fig. 8: A camel's duodenal mucosa stained with Con-A/HRP. Duodenal goblet cells (large arrows) are negative while duodenal glands (small arrows) are deep brown. Bar = 100 μ m.

Fig. 9: A camel's duodenal mucosa stained with WGA/HRP. Duodenal goblet cells (large arrow) and duodenal glands (small arrows) are deep brown. Bar = 100 μ m.

Fig. 10: A camel's duodenal mucosa. Sialidase digestion fails to abolish the WGA/HRP staining from duodenal goblet cells (large arrows) and duodenal glands (small arrows). Bar = 100 μ m.

Fig. 11: A camel's duodenal mucosa stained with HPA/HRP. Duodenal goblet cells (large arrows) are moderately stained brown while duodenal glands (small arrows) is deep brown. Bar = 100 μ m.

Fig. 12: A camel's duodenal mucosa. Prior sialidase digestion fails to abolish the staining with HPA/HRP from the duodenal goblet cells (large arrows) and duodenal glands (small arrows). Bar = 100 μ m.

Fig. 13: A camel's duodenal mucosa. KOH/Sialidase procedure are partially abolish the staining with HPA/HRP from duodenal glands (arrows). Bar = 100 μ m.

Fig. 14: A camel's duodenal mucosa stained with PNA/FITC. Duodenal goblet cells (large arrows) are negative while duodenal glands (small arrows) are intensely stained. Bar = 100 μ m.

Fig. 15: A camel's duodenal mucosa stained with DBA/FITC. Duodenal goblet cells (large arrows) are negative while duodenal glands (small arrows) are intensely stained. Bar = 100 μ m.

Fig. 16: A camel's duodenal mucosa. The staining with PNA is abolished from the duodenal glands (small arrows) by periodate oxidation/PNA/ FITC. Duodenal goblet cells (large arrows) still negative. Bar = 100 μ m.

Fig. 17: A camel's duodenal mucosa stained with WFA/FITC. Duodenal glands (arrows) are intensely stained. Bar = 100 μ m.

Table 1: Histochemical staining procedures to differentiate GPs of the duodenal glands and duodenal goblet cells of the one humped camel (CAMELUS DROMEDERIUS)

Procedures	Reaction shades	Interpretation of staining reaction
A- Periodic acid based procedures		
1. WO/S	M	Free aldehydes
2. PAS	M	GPs with oxidizable vicinal diols and/or glycogen
3. α-amylase/PAS	M	GPs with oxidizable vicinal diols
	O(M)	glycogen
B- Cationic dyes based procedures		
4. AB2.5	T	GPs with carboxyl groups and /or with O-sulfate ester
5. AM/AB2.5	O(T)	GPs with carboxyl groups and /or with O-sulfate ester
6. AM/KOH/AB2.5	T	GPs with carboxyl groups
	O(T)	GPs with O-sulfate esters
7. AB1.0	T	GPs with O-sulfate esters
8. AM/AB1.0	O(T)	GPs with O-sulfate esters
9. AM/KOH/AB1.0	O(T)	GPs with O-sulfate esters
C-Combined cationic dyes based procedures and periodic acid based procedures		
10. AB2.5/PAS	M	1-GPs with oxidizable vicinal diols and/or glycogen
	T	2-GPs with carboxyl groups and/or with O-sulfate sugar
	P	Mixture of 1 &2
11 AM/AB/ 2.5/PAS	M	GPs with oxidizable vicinal diols and/or glycogen
	O(T)	GPs with carboxyl groups and/or with O-sulfate sugar
12. AM/KOH/ AB2.5/PAS	M	GPs with oxidizable vicinal diols and/or glycogen
	T	GPs with carboxyl groups
	O(T)	GPs with O-sulfate sugar
13.KOH/Buffer/ AB2.5/PAS	M	1-GPs with oxidizable vicinal diols and/or glycogen
	T	2-GPs with carboxyl groups and/or with O-sulfate sugar
	P	Mixture of 1 &2
14. KOH/Sialidase/ AB2.5/PAS	M	GPs with oxidizable vicinal diols and/or glycogen
	T	GPs with O-sulfate esters and/or non-sialic carboxyl groups
	O(T)	GPS having sialic acids with and/or without O-acyl substitutions
15. Sialidase AB2.5/ PAS	M	GPs with oxidizable vicinal diols and/or glycogen
	T	GPs with O-sulfate esters and/or GPS having sialic acids with O-acyl substitution
	O(T)	GPS having sialic acid without O-acyl substitutions

Symbols :AB: Alcian blue, Ac/Acetylation, DAc: Deacetylation,AM: Active methylation, Bl: Black,Br: Brown, Brsh: Brownish black, Buffer: Phosphate-buffered saline pH 7.4, Gps: Glycoconjugates, HID: High diamine, HID: High iron diamine, LID: Low iron diamin, M: Magenta, O : Negative reaction, P: Purple, PAS: Periodic acid Schiff, Plu: Purplish brown, PD: Physean development, S: Schiff, SP: Silver proteinate, T: Turquoise, TDH: Thiosemihydrazide, WO: Without oxidation

Table 2: The histochemical methods to localize sugar residues in the duodenal glands and duodenal goblet cells of the one humped camels (CAMELEUS DROMEDERIUS)

Staining Methods	Remarks	Control stainings
1- Enzyme histochemistry		
16- GO/S	for direct visualization of the Gal and GalNAc	1-Sections were incubated with 0.1M PBS 2-GO enzyme was denaturated by heating for 30 min. at 100 °C 3-Incubation of the section with GO in the presence of 0.1% H_2O_2
2- HRP-conjugated lectin histochemistry		
17-Con A- HRP 18-WGA- HRP 19-Sialidase/WGA-HRP 20-KOH/ Sialidase/WGA-HRP 21-HPA- HRP 22-Sialidase/HPA- HRP 23-KOH/ Sialidase/WGA-HRP	The endogenous peroxidase reaction was blocked by 0.3 % hydrogen peroxide for 5 min, then the section was incubated for 24 hr at 4°C with Con A /HRP, WGA/HRP, HPA/HRP. The peroxidase reaction was finally developed with 0.05% 3,3' diaminobenzidine tetrahydrochloride and 0.015 % hydrogen peroxide .	1- HRP- conjugated lectins were substituted with unconjugated ones. 2- Incubation with HRP or substrate medium without lectins 3-Hapten sugars shown in Table 3 were added.
3- FITC-conjugated lectin histochemistry		
24-PNA/FITC 25- Po/PNA 26-Sialidase/PNA/FITC 27-KOH/Sialidase/PNA/FITC 28-Po/Sialidase/PNA/FITC 29-DBA/FITC 30-Po/DBA 31-Sialidase/DBA/FITC 32-KOH/Sialidase/DBA/FITC 33-Po/Sialidase/DBA/FITC 34-UEA-I/FITC 35-WFA/FITC	The lectin binding sites were demonstrated by direct histochemical labeling in which sections were incubated with FITC-labeled lectins for 18 hr at 4°C and examined directly under a fluorescence microscope.	1-FITC conjugated lectins were substituted with unconjugated ones. 2-Incubation of the section in HRP or substrate medium without lectins. 3-Hapten sugars shown in Table 3 were added.

Symbols : GO = galactose Oxidase; PO. = Periodate oxidation in 44 mM periodic acid, for 1 hr at room temperature; S = Schiff sequences
Symbols for lectins as those in table 3.

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Table 3: Carbohydrate binding specificity of lectins used in this study.

Taxonomic name and Abbreviations	Common name	Label	Concentration µg/ml	Major sugar specification	Sugar binding inhibitor
Arachis hypogaea (PNA)	Peanut	FITC	100	Gal-β-(1-3)-GalNAc	Gal
Canavalia ensiformis (Con-A)	Jake bean	HRP	20	α-D-Man, α -D-Glc	α-methyl-D-Man
Dolichos biflorus (DBA)	Horse gram	FITC	100	α- D-GalNAc	α- GalNAc
Helix pomatia (HPA)	Roman snail	HRP	6	α-D-GalNAc	α-D-GalNAc
Triticum vulgaris (WGA)	Wheat germ	HRP	6	$(\beta\text{-}(1\text{-}4)\text{-D-GlcNAc})_{2,3}$ NeuNAc	NeuNAc
Ulex europeus (UEA-1)	Gorse	FITC	100	α- L-Fucose	α- L-Fucose
Wisteria floribunda (WFA)		FITC	100	GalNAc	GalNAc

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

Table 4 : Cytochemical properties of the one humped camel duodenal glands and duodenal goblet cells as stained by galactose Oxidase/Schiff sequences & HRP-labeled lectins with or without prior sialidase digestion.

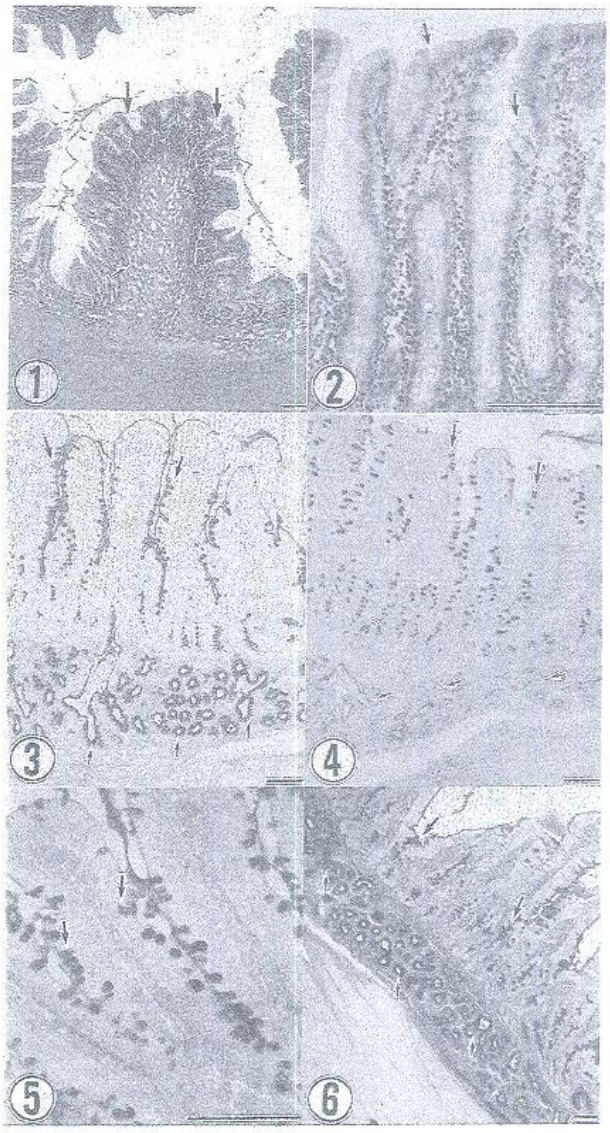
Staining Methods	duodenal Glands			Goblet Cells		
	N	S D	KOH/SD	N	S D	KOH/S D
A-Enzymatic methods						
GO/S	3-4 M	(-)	(-)	2-3 M	(-)	(-)
B- HRP Lectin labled methods						
1-Con A/HRP	0	(-)	(-)	0	(-)	(-)
2-WGA/HRP	3-4B	3-4B	0-1B	3-4B	3-4B	3-4B
3-HPA/HRP	1-2B	1-2B	0-1B	3-4B	3-4B	3-4B

Symbols: B = Brown; GO/S = galactose Oxidase/Schiff sequences; KOH = Saponification in 70% ethyl alcohol; M= Magenta; N= Normal lectin staning; 0 = No staining ; SD= Sialidase digestion; 1- 4 = weak to very strong staining ; (-) = step not carried out.
Symbles for lectins are as those in Table 3.

Table 5 : Cytochemical properties of the one humped camel duodenal glands and duodenal goblet cells as stained by FITC-labeled lectins with or without prior sialidase digestion or perioxidation/sialidase sequences.

Staining Methods	duodenal Glands				Goblet Cells			
	N	S D	KOH/SD	PO	N	S D	KOH/S D	PO
1-PNA/FITC	4	4	0-1	4	2-3	2-3	0-1	2-3
2-DBA/FITC	4 (60%)	4	0-1	4	2-3	2-3	0-1	2-3
3-UEA-I/FITC)	0-1	(-)	(-)	(-)	0-1	(-)	(-)	(-)
4-WFA/FITC	2-3 (50%)	(-)	(-)	(-)	1-2 (70%)	(-)	(-)	(-)

Symbols: PO = Periodate oxidation in 44 mM periodic acid. for 1 hr at room temperature; SD = Sialidase digestion
* Numbers indicate the staining intensity based on semiquantitative scale , 0 = No staining ; 1- 4 = weak to very strong staining ; (-) = step not carried out.
** Numbers in parentheses indicate the approximate percentage of cells showing the positive staining.
Symbols for lectins are as those in Table3.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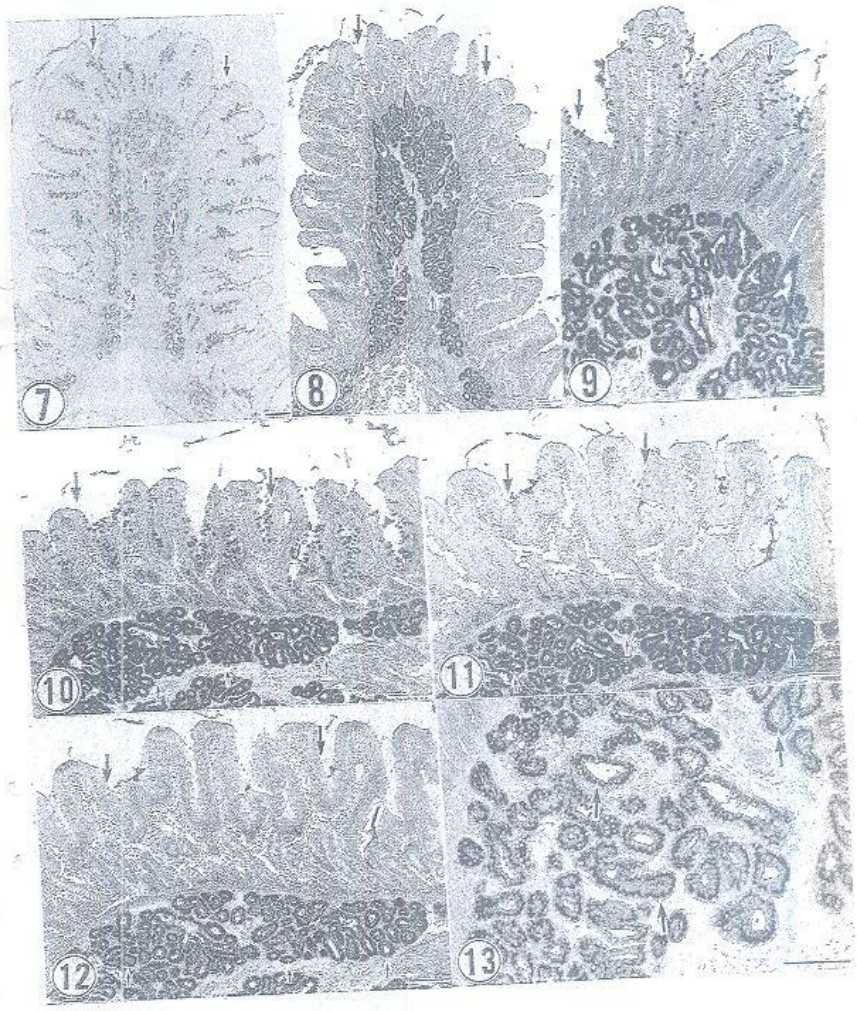
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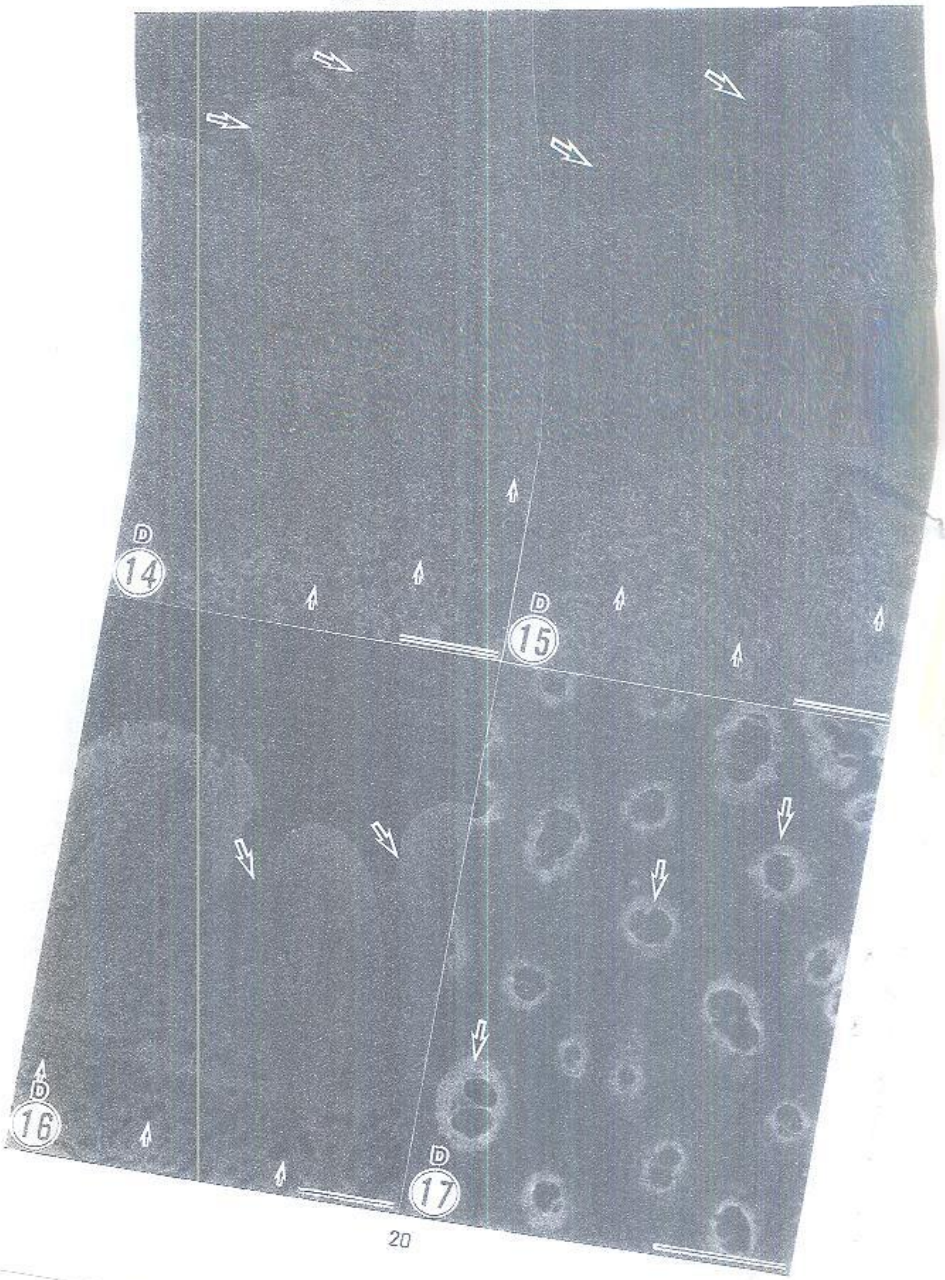
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