LECTIN HISTOCHEMICAL IDENTIFICATION OF GLYCOCONJUGATE SUGAR RESIDUES IN PANCREAS OF MONKEY

M. H. Fayed and S. M. Ghattas

Department of Anatomy and Histology, Faculty of Vet. Medicine,
Kafr El-Sheikh, Tanta University, Egypt

ABSTRACT

Pancreata from nine adult healthy male and female of three monkey species were used to study the distribution of the glycoconjugaed sugar residues in situ, using a battery of lectins (Con-A, WGA, PNA, HPA and BSA-1). These lectins were used as a prob and the horsraddish peroxidase (HRP) as visualant. The results revealed, wide variations in the reactions of the different pancreatic cellular structures with the used lectins. While the apical cytoplasm of the all pancreatic acinar cells were uniformly reacted to WGA, the basal region of which were reacted to Con-A. Some pancreatic acinar cells were positively reacted to PNA and BSA-1, others were negative to them and the all were negatively reacted to HPA. The lining epithelium of the interlobular ducts reacted positively to all the used lectins except Con-A. Islets of Langerhans were negatively reacted to all the used lectins.

It was concluded that, the pancreatic acinar cells contained galactose as a terminal transitionally located carbohydrate residue, also stored N, acetylglucosamine and/or sialic acid as a terminal carbohydrate residues condensed in their zymogen granules also stored α-Dmannose and α-D glucose as terminal carbohydrate residues in their basal cytoplasm. The lining epithelium of the interlobular duct secretes a mixture of N-acetylglucose and sialic acid, while their apical cell membrane contained galactose and α & β anomer of N, acetylgalactose amine.

INTRODUCTION

Carbohydrate-binding proteins called lectins, have become useful tools in describing various cellular activity **(Liener et al., 1986; Sharon and Lis, 1989)**. The complex oligosaccharides and glycoproteins were described in a variety of cellular interactions **(Hynes et al., 1989)**. More recently methods have evolved for characterizing complex carbohydrates in situ depending on al-

finity of labelled lectins for specific sugar in pancreas of several species in rats (**Jonas et al., 1991 and Takiyama et al., 1988**); in dog and cats (**Skutelsky et al., 1987**) and in chicken (**Gherl et al., 1997**). Little information about characterization of glycoconjugates in pancreas of primates are available. This study aimed to describe specific glycoconjugate sugar residues and their distribution in situ by using lectin markers in pancreas of non-human primates.

MATERIAL AND METHODS

Animals :

The samples were collected from three species of non-human primates 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, tanta university, Egypt. A total of nine adult male and female animals were used for this investigation. Among these animals, three were of common treeshrew monkeys (Tupaiidae glis), three were of slow rolis monkeys (Nycticebus cocang) and three of common marmoset monkeys (Callithrix jacchus).

Sample collection and tissues preparation:

According to the strict animal welfare regulations and the rules of the animal ethics committee in Japan, the monkeys were saerificed under deep anesthesia with an intravenous injection of pentobarbital sodium (50 mg/kg). The pancreas was rapidly removed and pieces of its tissue were fixed in phosphate buffer saline pH 7.4 (PBS) containing 4% paraformaldehyde for 72 hours and thoroughly rinsed in the same buffer. Sections of 4 µm thickness were deparafinized in xylene and stained with Gill's hematoxylin (**Gill et al., 1974**) and cosin (H & E) for general histological assessment. For detection of the binding sites of the sugar residues, other sections were subjected to the histochemical staining summarized in table 1.

The horsraddish peroxidase (HRP) lectin technique:

The processing and staining procedure with the various lectins (table 1) was similar to that described by **Schulte and Spicer (1983b), Bancroft and Stevens (1996) and Rhodes and Milton (1998)**. Briefly, after hydration, the sections were treated with 0.3% hydrogen peroxide, for blocking the endogenous peroxidase enzyme 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 either HRP-lectin (Sigma Chemical Co. St. Louis, MO, USA), dissolved in 0.1 M PBS pH 7.4 (contain 0.1 M $NaCl_2$, 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 mini-

mum background was as listed in table 1. Visualization of the sites containing HRP-lectin was obtained by incubating the slides with PBS containing 3,3 diaminobenzidine tetrahydrochloride (DAB) (25 mg/100 ml and 0.003% hydrogen peroxide) for 10 min. at room temperature. Slides were rinsed in distilled water, dehydrated in graded ethanol solutions, cleared in xylene and mounted in DPX.

Control reaction :

Control for the lectin staining was done by pre-incubation of the sections with the corresponding haptten sugars inhibitors listed in table 1. The sugar inhibitors were employed at concentrations from 0.05 M to 1 M and complete elimination of the staining was obtained at 0.2 M. The aim of this control was to test the efficiency of the specific staining for each sugar.

RESULTS

General structure:

There were no significant variations in the morphology, as well as lectin histochemistry of the pancreata among the three studied species

Pancreas of monkey appeared as an encapsulated and lobulated compound tubuloacinar gland. The acinar cells were formed of one cell type, serous in nature, pyramidal in shape leaving narrow lumina (Fig. 1). The acinar cells rested on a distinct basal lamina and had spherical darkly stained nuclei. Their cytoplasm was characterized by basophilia at the basal region and around the nuclei, and by the presence of acidophilic zymogen secretory granules at the apical region. The ductal portions were represented by centroacinar cells, intercalated ducts and interlobular ducts. The centroacinar cells were formed of telescoping flat, palely stained cells located in between the pancreatic acini and forming the initial portion of the intercalated ducts which were lined by cuboidal cells. The interlobular ducts were present within the interlobular connective tissue septa and were lined by high columnar epithelium (Fig. 1). Islets of Langerhans were scattered in the parenchyma. They appeared as spherical clusteres of lightly stained cells. These islets were interconnected by cords of lightly staining cells (Fig. 2).

Lectin histochemistry :

1-D-Mannose and-α-D-glucose-binding lectin(concanavalin ensiformis agglutinine;(con A):

The cytoplasm of the basal region of all pancreatic acinar cells was stained intensely with con-A (Figs. 3 & 4). The lining epithelium of the intercalated ducts as well as that of

the interlobular ducts were negatively reacted to Con-A , however the pancreatic juice at their lumena were intensely reacted. The cell population in the islets of Langerhans were negatively reacted to con-A (Figs. 3 & 4).

2- N-acetyle glucosamine and sialic aeid binding lectin (Triticum vulgaris agglutinine; (WGA) :

The cytoplasm at the apices of all pancreatic acinar cells showed strong reaction to WGA (Fig. 5). The lining epithelium of the large interlobular ducts cleared an intense reaction at the apical region of their cytoplasm (Fig. 6). The cell populations of islets of Langerhans were negatively reacted to WGA.

3- Galactose and N-acetylgalactosamine binding lectin (Arachis hypogaea agglutinine; (PNA) :

The cytoplasm of some pancreatic acinar cells were intensely stained with PNA while others were negative or weakly stained (Fig. 7). The lining epithelium of the interlobular ducts showed an intense staining to PNA at their apical membranes (Fig. 8). All cell populations of pancreatic islets of Langerhans were negatively reaeted with PNA (Fig. 7).

4-N-acetylgalactoseamine binding lectin Helix pomatia agglutinine; (HPA):

The all pancreatic acinar cells were negatively reacted with HPA (Figs. 9 & 10). The lining epithelium of the interlobular ducts showed strongly reaction to HPA at their apical borders. All cell populations of islets of Langerhans were negatively reacted with HPA (Figs. 9 & 10).

5- Galactose and N, acetylgalactosamine binding lectin (Griffonia simplicifolia agglutinine; BSA-1) :

The pancreatic acinar cells varied markedly in their reactivity with BSA-1. While their cytoplasm was stained strongly at the peripheral region of the pancreatic lobule and around the interlobular ducts, they were stained weak or negative at the deepest regions (Figs. 11 & 12). The apical cell borders of the lining epithelium of the interlobular ducts showed strong reaction with BSA-1 (Fig. 12). The cell populations of islets of Langerhans have not reacted markedly with BSA-1.

DISCUSSION

Lectin methods in the present study have increased the capacity for histochemical charaeterization and differentiation of glycoproteins in the pancreas of monkey.

In the present study some pancereatic acinar cells were strongly reacted with PNA while others were reacted with BSA-I indicating the existence of galactose and both α-and β-anomer of N-acetylgalactosamine. At the same time all pancreatic acinar cells failed to react with HPA, a lectin specific to α-anomer of N-acetylgalactosamine. On the basis of the negative reaction of the acinar cells to HPA and the positive reaction of some acinar cells to PNA and others to BSA-I, we could exclude the existence of β-anomer of the N-acetylgalactosamine but only galactose and _-anomer of N-acetylgalactosamine were existed as terminal carbohydrate residues. The heterogenic reaction of PNA and BSA-I with some pancreatic acinar cells while other were not supports the suggestion of **Jeraldo et al. (1996)** who demonstrated high heterogeneous labeling of zymogen granules content. The existence of α-galactose was confirmed in pancreatic acinar cells of rat by **Schulte and spicer (1984) and Detza et al. (1998)**. The latter authors supposed that it has biological active role in digestion of fibers.

In addition to galactose and β-anomer of the N-acetylgalactosamine, the α-anomer of the N-acetylgalactosamine could be detected in the apical cell membrane of the lining epithelium of the interlobular ducts,as revealed by positive reaction to HPA. Physiologically these types of sugars were eoncerned with the polarity of the cell surface **(Jonas, et al., 1991)** and so could play a role in protection of the cells against the effect of the luminal fluid bathing the cell apices, especially in the gastrointestinal tract **(Spicer et al., 1979)**.

In the present study, the detection of PNA reaction in some pancreatic acinar cells as well as the apical cell borders of the interlobular duct was in agreement with **Stoward et al. (1980)** who reported that PNA has selectively reaeted with secretory bodies and exclusively in Golgi cisternae and the apical cell surface in various cell types. This indicates that galactose occurs, transiently as a terminal residues. **Lotan et al. (1975)** reported that the most complementary structure for PNA binding is the terminal dimer galactose and N-actylgalactosamine. **Montreuil (1980)** added that, oligosaccharide chains contalning the terminal dimer galactose N-aetylgalactoseamine form a portion of core region of O-glycosidieally linked secretory glycoproteins whieh are largely restricted to epithelial secretions.

The current investigation showed strong reaction to WGA at the cytoplasm of all panereatic acinar cell apices and intensively reacted at the cytopalsm of the apical region of the columnar epithelium lining the interlobular ducts. This reaction revealed terminal N-acetylglucoseamine and sialic acid as terminal carbohydrate residues. **Pinto et al. (2000)** demonstrated a significant

increase in WGA reaction in the pancreatic acinar cells due to fusion of zymogen granules with plasma membrane as suggested by exocytosis which observed by electron microscopy. They observed loss of N-acetylglucoseamine and/or sialic acid by examination of glycoconjugates in plasma membrane when acute pancreatitis was induced by duct obstruction. The present study revealed N-acetylglucoseamine and/or sialic acid stored in the cell apices of pancreatic acinar cells, area of aggregated zymogen granules. This result is in parallel with **Garcia Montero et al. (1997)**. They reported that, pancreatic zymogen granules contained N-aeetylglucosamine which was increased when rats were treated with hydrocortisone and decreased by adrenalectomy. **Willemer et al. (1990)** demonstrated that, Golgi apparatus of pancreatic acinar cells as well as membranes of condensed vacuoles, zymogen granules and lysosomal bodies to be increased in its affinity to WGA. The present study reported an intense reaction to WGA in the cytoplasm of apical region of the lining epithelium of the interlobular duct. These results were in agreement with the explanation of **Palade (1975)** who reported that reactive glycoconjugates in secretory granules and Golgi complex observed in the epithelial cells support the concept that secretory glyeoconjugates are elaborated in Golgi complex, packaged in the secretory granules and transported to the apical cytoplasm and released to the lumen. So seeretory glycoconjugates are released by exocytosis **(Suprasert et al., 1986)**. Histophysiologically, sialic acid residues of carbohydrates are known to coat the mucosal surface as to provide a hydrophilic environment designed to preserve hydration **(Jeanloz and Codington, 1976 and Montreuil, 1980)**. In addition these saccaride residues seem to function in proteetion of the cell from pathogenic organisms **(Schult and Spicer, 1984)**.

The present investigation showed clear intense reaction to con-A at the basal region of the cytoplasm in the all pancreatic aeinar cells, as well as in the luminal secretion of the interlobular duct. This reaction indicating α-D-mannose and α-D-glucose as terminal carbohydrate residues. **Jonas et al. (1991)**; demonstrated that Con-A strongly reacted with basolateral glyeocalyx as eonserved with junction complexes between the cells. The present study emphasizes the presence of this reaction at the basal cell region of the panereatic acinar cells, the same area of basal basophilia in (H & E) staining. This result was confirmed by **Hebert et al. (1995)** who explained trimming and reglucosylation aetivity of endoplasmic reticulum and established a direct correlation between glycosylation and folding. **Willemer et al. (1990)** reported that rough endoplasmie retieulum and the nuclear envelope of aeinar cells were selectively reacted with Con-A.

The present study revealed a laek of reactivity in the pancreatic islets of Langerhans to all types of lectin used. **Patzette and Weber (1986)** reported that, in rat pancreatic islets O-glyeosidie linkage of sugars has not been reported for prohormones. They added that the apparent absence of N-glycosidically bound sugars in proglucagon gives evidence for an unusual type

of protein glycosylation.

Finally, it was concluded that, pancreatic acinar cells of monkey contained galactose as a terminal transitionally located carbohydrate residues and N-acetylglucosamine and/or sialic acid as terminal carbohydrate residues condensed in their zymogen granules also contained α-D-mannose and glucose as a terminal carbobydrate residues in their basal cytoplasm. However, the lining epithelium of the interlobular duct functionally secretes, N-acetylglucosamine and/or sialic acid. However, their apical cell membrane contained galactose and N-acetylgalactoseamine as structural units.

Table (1):

Lectins Scientific name	Common name	Labled	Abbreviation	Concentration used (µg/ml)	Carbohydrate-binding specificity	Sugar binding inhibitor
Arachis hypogaea	Peanut	HRP	PNA	100	Gal-β-(1-3)-Gal NAC	Lactose
Concanavalin ensiformis	Jak bean	HRP	Con A	20	α-D-Man, α-D-Glc	α-d-methyl man.
Helix pomatia	Roman snail	HRP	HPA	6	α-D-Gal NaC	α-D-GalNAc
Triticum vulgaris	Wheat germ	HRP	WGA	6	(B-(1-4)-D-GLCNAC) 2,Neu NAC	NeuNAc
Griffonia simplicifolia	Banderia simplicifolia	HRP	BSA-I	20	α-D-Gal, α-D gal NAC	GalNAc

Abbreviations: Gal= Galactose; Glc=Glucose;
Gal NAC= N-acetylgalactosamine;
Glc NAC = N-acetylglucosamine; Man=Mannose;
NeuNAC = N-Acetyl neuraminic acid (Sialic acid).
HRP= Horsraddish peroxidase.

Comparative staining of monkey pancreas with horsraddish peroxidase conjugated lectins.

Histochemical staining method / Cell types	Con A	WGA	PNA	HPA	BSA-I
Acinar cells	3	2	0 ~ 3	0	0 ~ 2
Epithelium of interlobular ducts	0	3	3	2	2
Islets of Langerhans	0	0	0	0	0

Intense reaction = 3 Strong reaction = 2
Weak reaction = 1 Negative reaction = 0

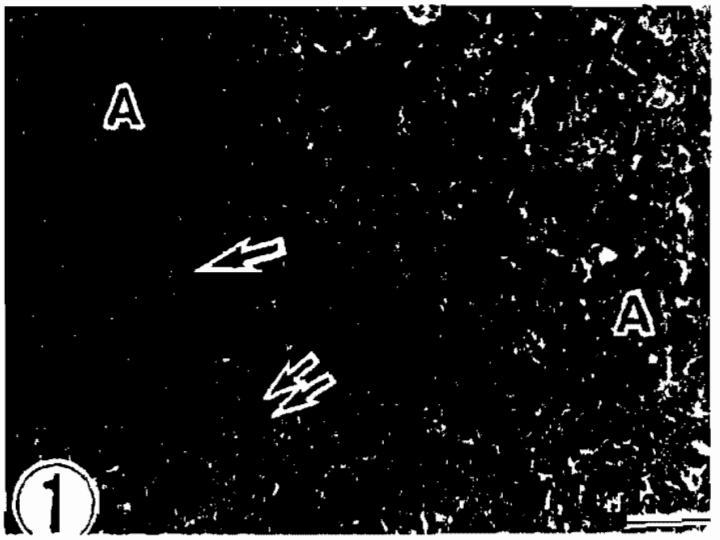

Fig. (1) : A photomicrograph of a section of monkey's pancreas showing, serus acini (A), intercalated duct (arrow) lined by cuboidal epithelium and interlobular duct (double arrows) located at the interlobular connective tissue and lined by columnar epithelium (H & E, bar=100 µ).

Fig. (2) : A photomicrograph of a section of monkey s pancreas showing, acinar cells (large arrows) having spherical nuclei surrounded by basophilic cytoplasm, flat pale stained centroacinar cells (small arrows), and islets of Langerhans (L) (H & E, bar=100 µ).

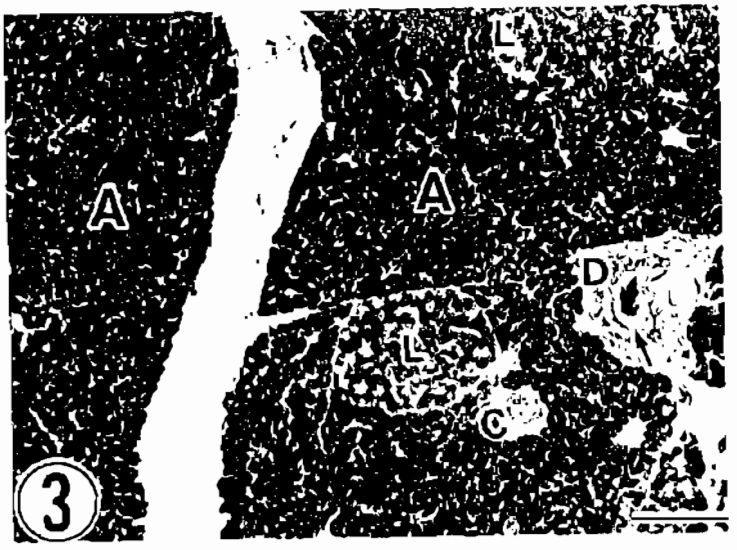

Fig. (3) : A photomicrograph of a seetion of monkey's pancreas showing, intense staining with Con-A indicating terminal α-D-mannose and α-D-glucose stored in the cytoplasm of the basal regions of all acinar cells (A) and in pancreatic juice (arrow) in the lumen of interlobular duct (D) lack of reactivity in the lining epithelium of the interealated duet (C) and islets of Langerhans (L) (HRP/Con-A, bar=100 μ).

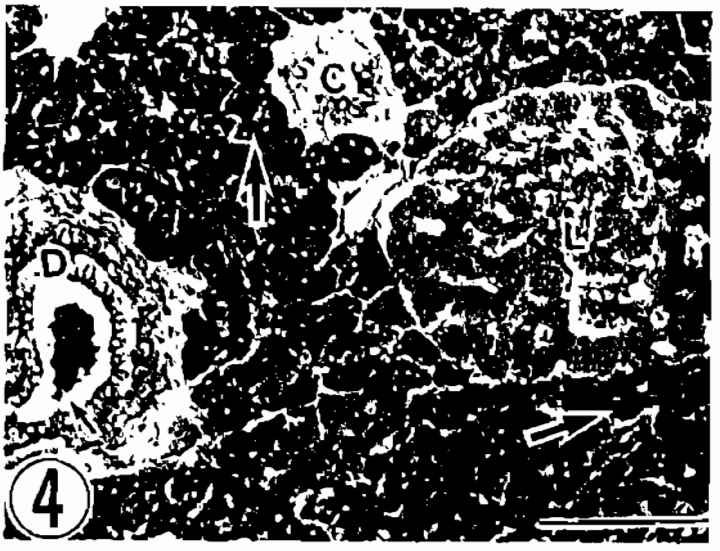

Fig. (4) : High magnification of (Fig. 3) showing intense staining at the basal cytoplasm of all acinar cells (Large arrow) and also in the pancreatic juice (small arrow) and contrasted with a lack of reactivity in islets of Langerhans (L), lining epithelium of the intercalated ducts (c) and lining epithelium of interlobular duct (D) (HRP/Con-A, bar=100 μ).

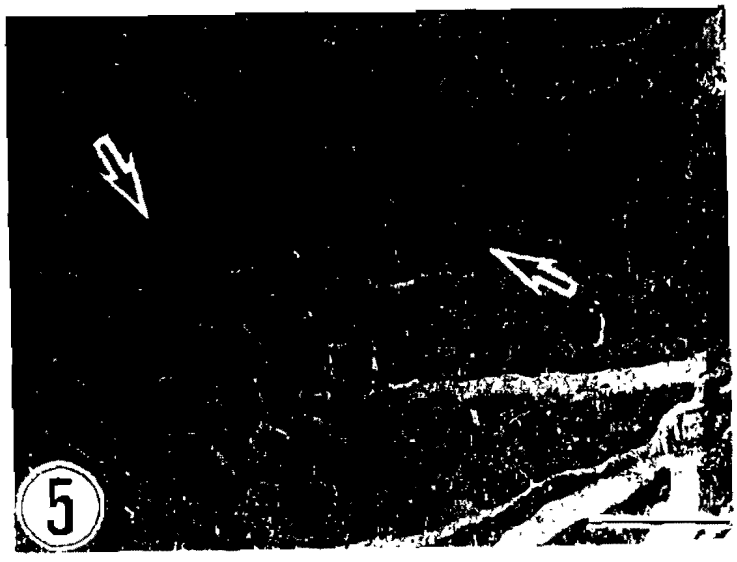

Fig. (5) : A photomicrograph of a section of monkey's pancreas showing, strong staining (dark brown) indicated terminal N. acetyleglucosamine and/or sialic acid stored in the cytoplasm of the apices of acinar cells (arrows) (WGA/HRP bar=100μ).

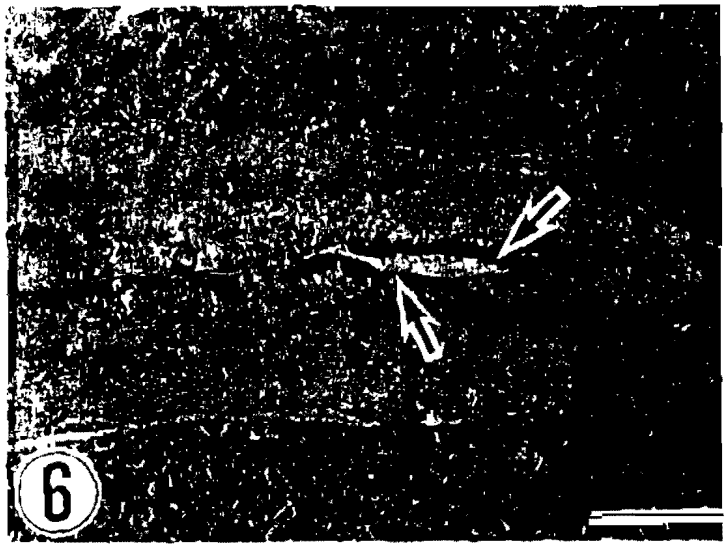

Fig (6) : A photomicrograph of a section of monkey's pancreas showing, intense staining (dark brown) indicated terminal N. acetylglucosamine and/or sialic acid stored in cytoplasm of the apical region of the lining epithelium of the interlobular ducts (arrows) (HRP/PNA, x bar =100 μ).

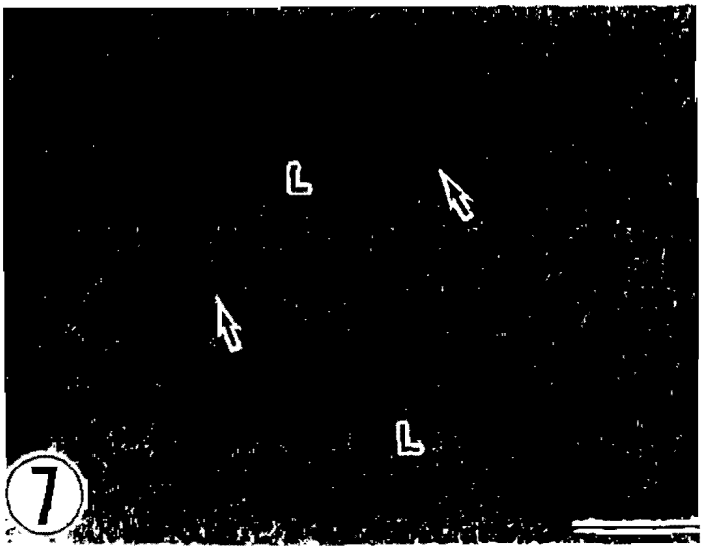

Fig. (7) : A photomicrograph of a section of monkey's pancreas showing, intense staining indicating terminal galactose and N-acetylgalactoseamine stored in cytoplasm of some acinar cells (arrows) and contrasts with a lack of reactivity in islets of Langerhans (L) (HRP/PNA, bar = 100μ).

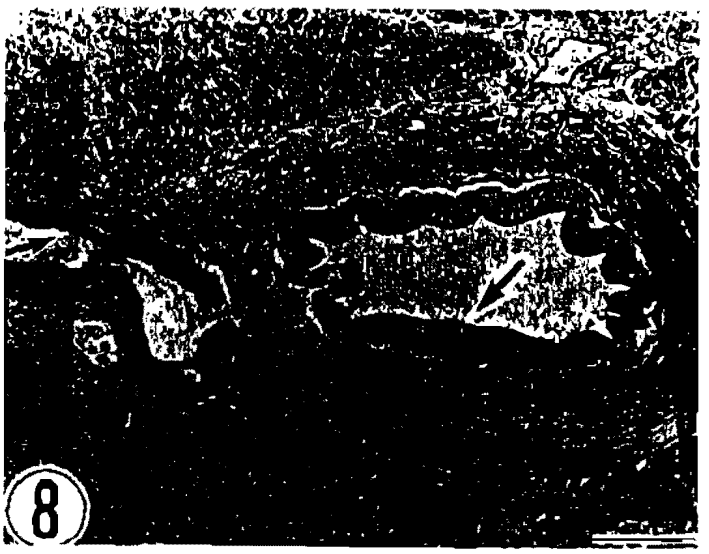

Fig. (8) : A photomicrograph of a section of monkey's pancreas showing, intense staining indicating terminal galactose and N-acetylgalactoseamine at the apical border of the lining epithelium of the interlobular ducts (arrows) (HRP, bar = 100μ).

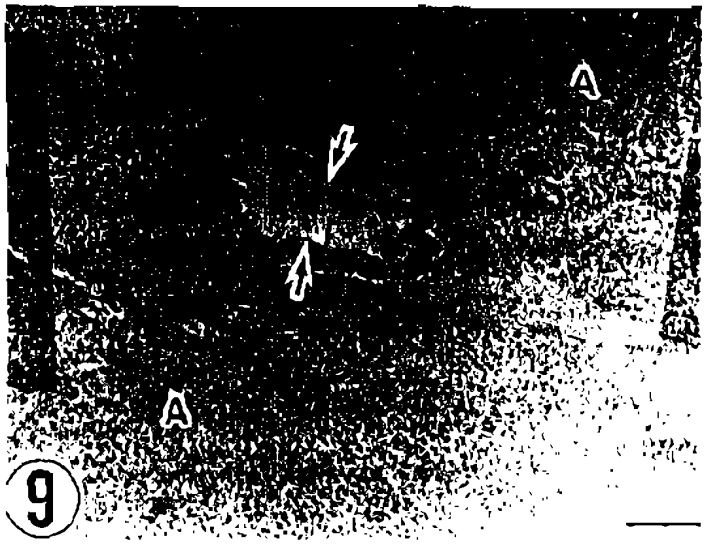

Fig. (9) : A photomicrograph of a section of monkey's pancreas showing, strong staining indicating terminal α-anomer of the N-acetylgalactosamine at the apical border of the lining epithellum of interlobular ducts (arrows) and contrasts with a lack of reactivity in all pancreatic acini (A) (HRP/HPA, bar =100μ).

Fig. (10) : High magnification of Fig. (9) showing, strong staining indicating terminal α-anomer of the N-acetylgalactosamine at the apical border of the columnar lining epithelium of the interlobular duct (arrows) (HRP/HPA, bar = 100 μ).

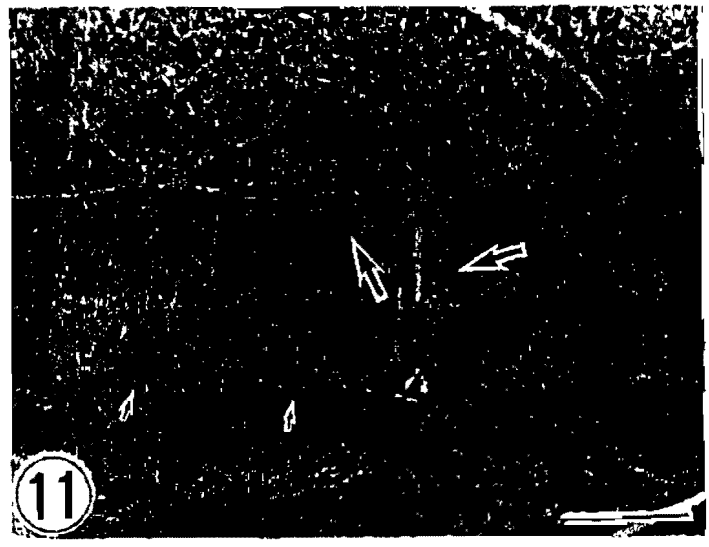

Fig. (11) : A photomicrograph of a section of monkey's pancreas showing, remarkable variation with the staining of BSA-I indicating α-D-galactose and α-D-N-acetygalactosamine. The acinar cells surrounding the interlobular ducts show strong staining (large arrows) while those in the deeper part show lack of reactivity (small arrows) (HRP/ BSA-I, bar = 100 μ).

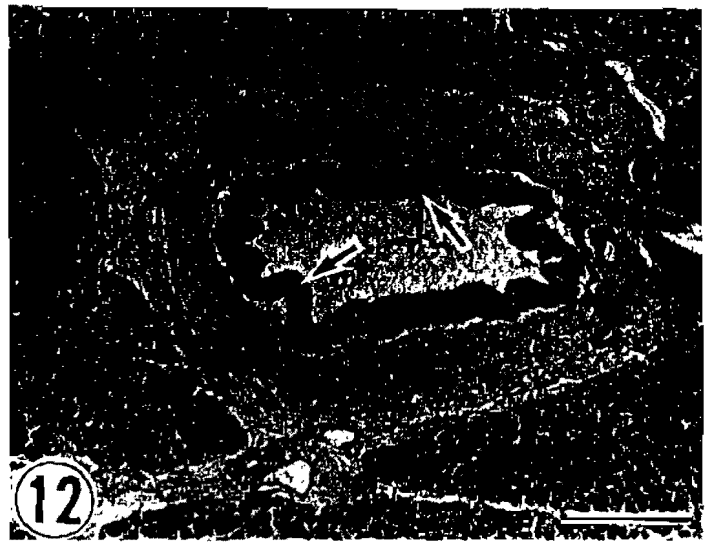

Fig. (12) : A photomicrograph of a section of monkey's pancreas showing, strong staining indicating terminal α-D galactose, α-D-N actylgalactosamine at the apical border of the lining epithelium of the interlobular duct (arrows) (HRP/ BSA-I, bar = 100μ).

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الملخص العربى

التعرف بهستوكيميائية اللكتين على الآثار السكرية للكربوهيدرات المقترنة فى بنكرياس القردة

المشتركون فى البحث

مسعود حسن فايد سوسن محمود غطاس

قسم التشريح والهستولوجيا - كلية الطب البيطرى - كفر الشيخ - جامعة طنطا

لقد أستخدم فى هذا البحث عدد تسعة من بنكرياس ذكور وإناث ثلاثة أجناس من القردة البالغة وذلك لدراسة مواقع الآثار السكرية للكربوهيدرات ومدى إنتشارها وذلك باستخدام دلالات اللكتين (con, WGA, PNA, HPA and BSA-1) كما أستخدم (HRP) كمظهر للتفاعل، وقد أوضحت النتائج أن هناك فروقاً واضحة لتفاعلات الليكتين مع التراكيب الخلوية المختلفة للبنكرياس على النحو التالى :

تفاعل السيتوبلازم فى جميع خلايا الحويصلات البنكرياسية كان تفاعلاً إيجابياً وقوياً مع WGA عند قمم الخلايا، وكان تفاعلاً إيجابياً وقوياً مع عند قاعدة الخلايا وجميع هذه الخلايا لم تظهر أى تفاعل مع WGA وقد أظهرت بعضها تفاعلاً قوياً مع PNA, BAS-1 فى بعض الأماكن دون الأخرى.

وقد أظهرت الخلايا الطلائية المبطنة للقنوات البينية تفاعلاً إيجابياً وقوياً مع كل من BSA-1, HPA, WGA, PNA, ولكنها كانت سلبية التفاعل مع Con A .

أما خلايا جزر لانجرهانز البنكرياسية فلم تظهر أى تفاعل مع أى من دلالات اللكتين المستخدمة فى هذا البحث.

والخلاصة : أن خلايا الحويصلات البنكرياسية قد إحتوت بصفة مرحلية على الجلاكتوز كبقايا سكريات طرفية بينما أختزنت استيل الجلوكوز الأمينى فى حبيبات الزيموجين الموجودة فى السيتوبلازم عند قمم هذه الخلايا كذلك اختزنت سكريات الجلوكوز والمانوز فى السيتوبلازم عند قاعدتها، أما النسيج الطلائى المبطن للقنوات البينية فقد أظهر إفراز للأستيل جلوكوز أمين وحامض السايلك بينما كان الغشاء العلوى لتلك الخلايا محتوياً على الجلاكتوز واستيل الجلاكتوز الأمينى.