

# Lectin Histochemistry of the Glandular Part of the Gastric Mucosa of Zebra (*Equus burchellii*)

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With 7 figures

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## Abstract

The Fundic glands of the stomach of Zebra were histochemically analyzed by conventional and lectin histochemistry. The surface mucous cells (SMC) and foveolar mucous cells (FMC) were highly rich with glycoprotein (GPs) with oxidizable vicinal diols and/or glycogen and GPs with carboxyl groups and/ or GPs with O-sulphate esters. The GPs with oxidizable vicinal diols and/or glycogen were predominates in FMC while, the GPs with carboxyl groups and/ or GPs with o-sulphate esters were predominate in SMC. The glandular epithelium of the base region of the fundic gland was devoid of both types of the GPs. All mucous secretig cells (SMC, FMC, mucous neck cells (MNC) and glandular mucous cells (GMS) were highly rich with  $\alpha$ - anomer of the N-acetylgalacto-samine ( $\alpha$ -GalNAc), N- acetyl- glucosamine (GlcNAc) and neuroaminic acid (NeuNAc). The SMC and FMC were devoid of  $\alpha$ - D-glucose ( $\alpha$ - D- Glc),  $\alpha$ - D- mannose ( $\alpha$ - D- Man), galac-Tose (Gal) and  $\beta$ - anomer of the N-acetylgalactosamine ( $\beta$ - GalNAc).

The parietal cells (PC) appeared to react with Helix pomatia agglutinin (HPA), wheat germ agglutinine (WGA) and peanut agglutinin (PNA) indicating

the existence of  $\alpha$ - and  $\beta$ - anomer of the GalNAc and NeuNAc.

## Key words

Lectin, Histochemistry, Zebra, sugar residues.

## Introduction

The gastric surface is protected from its own highly acidic secretion by a thin film of mucous that is being constantly produced by the SMC (Smith, 1988) Previous biochemical and histochemical investigations showed that oligosaccharides side chain of the complex carbohydrate in numerous mammalian glycoconjugates terminate with different sugar residues (Kornfed and Kornfed, 1976, Horowitzand and Pigman, 1977 and Montereuil, 1980). Those of gastrointestinal mucosa are composed of four sugars, Gal, GalNAc, GlcNAc and  $\alpha$ -L-Fucose ( $\alpha$ -L-Fuc), in very constant molecular ratio (Allen and Snary, 1972, Clamp, et al, 1981 and Forster, et al, 1982). These sugars play a key role in the normal function of these glycoconjugates. Such function includes regulation of protein-confir-mation, protection of protein from

proteolytic attach, cell-cell interaction (Montereuil, 1980), and ion transport across the membrane (Vandeheede, et al., 1972). These sugars have been histochemically localized in the mucus secreting cells of human (Aoki, et al., 1993), carnivorous (Mayer and Tsukise, 1995), in camel (Fayed and Makitta, 1997b) and in experimental animals (Ishihara, et al., 1996 and Yang, et al., 1996).

Marker-labeled lectins have been employed as selective histochemical reagents for the detection of various sugar residues in gastric mucosa of different animals with different habits as in rats (Tatematsu, et. al., 1989), in camel (Fayed and Makitta, 1997a& b) and in fish (Pedini, et al 2005). The lake of literature for the detection of various sugar residues in gastric mucosa of Zebra was the principal aim to performing this work.

## Materials and Methods

The samples were collected from the fundic region of 3 animals of Zebra obtained from the zoo in Gizza. The samples were fixed in phosphate buffer saline (PBS) contained 4% Para-formaldehyde for 72 hours and thoroughly rinsed in the same buffer. Paraffin sections of 4 $\mu$ m thickness were Deparaffinized in xylene and stained with periodic acid Schiff (PAS), Alcian blue at pH 2.5 (Ab 2.5) and PAS/Ab2.5 combination (*Bancroft and*

*Stevens 1996*). For detection of the binding sites of the sugar residues, paraffin sections were subjected to the

histochemical staining summa-rized in table 1.

### The horseradish peroxidase Lectin technique:

The processing and staining procedure with the various lectins (table 1) was similar to that described by Schulte and Spicer (1983), Bancroft and Stevens (1996) and Rhodes and Milton (1998). Briefly, after hydration, the sections were treated with 0.3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 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 Co. St. Louis, Mo,USA), dissolved in 0.1m PBS Ph7.4 (contain 0.1 M NaCl<sub>2</sub>, 0,1 mM CaCl<sub>2</sub>, MgCl<sub>2</sub>, MnCl<sub>2</sub>) and then rinsed three times in PBS. The optimal concentration used with each lectin, which allowed minimum back-ground, was listed in table 1. Visualization of the sites containing HRP-lectin was obtained by incubating the slides with PBS containing 3'. 3' diaminobenzidine tetrahydrochlorid (DAB) (25 m\100 ml and 0.003%H<sub>2</sub>O<sub>2</sub>) for 10 min. at room temperature. Slides were rinsed in distilled water, dehydrated using graded ethanol solutions, cleared in xylene and mounted in DPX.

## Results

### Conventional histochemistry:

#### PAS stain:

The apical region of the SMC and FMC showed PAS +ve reaction (Fig. 1a & b), while GMC showed PAS +ve reaction in the luminal surface (Fig. 1c). The MNC were faintly stained with PAS while, the

glandular epithelium of the basal region was negatively reacted with PAS. (fig. 1a).

#### **Ab2.5 stain:**

The SMC and FMC showed strong +ve reaction for Ab2.5. Luminal surface of few GMC showed also strong +ve reaction for Ab2.5 (Fig. 2a & b)

#### **PAS/Ab2.5**

The SMC showed +ve reaction for both Ab2.5 and PAS but, the reaction for Ab2.5 was stronger than PAS and restricted in the supranuclear region of the cells. The FMC was positive for both Ab2.5 and PAS and the reaction was shown in the whole cytoplasm of the cells. The MNC showed only Ab2.5 +ve reaction at the luminal surface of the cells. In the body region, the cells showed moderate +ve reaction for PAS and slightly +ve reaction for Ab2.5. The reaction was shown in the luminal surface of the cells. The glandular epithelium of the base region was negative for both PAS and Ab2.5 (fig. 3a, b & c).

#### **Lectins histochemistry:**

##### **Helix pomatia agglutinin (HPA/HRP):**

The SMC and FMC were strongly stained with HPA/HRP at the supranuclear region while, the cytoplasm was slightly positive (Fig. 4a & b). The glandular mucus cells showed strong reaction with it. The reaction was highly positive in the lateral cell boundaries and in the luminal surface (Fig. 4a&c). Some PC were strongly reacted with HPA (Fig. 4a & c).

##### **Wheat germ agglutinin**

##### **(WGA/HRP):**

All mucus cells (SMC, FMC, MNC and GMC) were intensely stained with WGA/HRP (Fig. 5a, b, c&d). The reaction in the SMC and FMC cells was found in the apical region of the cells above the nuclei (Fig. 5b). In the GMC, the reaction was observed in the luminal surfaces (Fig. 5c). Some WGA/HRP reactive material was observed in the lumen of some glands. Few PC were moderately reacted with WGA especially in the body and base regions. (Fig. 5 c & d).

##### **Concanavalia ensiformis agglutinin (Con-A/HRP)**

GMC were densely stained with Con-A/HRP, while SMC and FMC were negative to it (Fig.6a). The GMC in the body region showed +ve reaction in the lateral cell boundaries and luminal surfaces (Fig. 6b & c). The PC was negative to Con- A (Fig.6c).

##### **Peanut agglutinin (PNA/HRP):**

The whole cytoplasm of the PC appeared to react with PNA; the reaction was very strong in the neck region, but the staining faded away towards the basal region where, some reactive substance could be observed in the lumen of the glands while SMC and FMC were negative. (Fig.7 a & b).

## **Discussion**

The present study revealed that the SMC and FMC in fundic gland of Zebra secrete a mixture of GPs with oxidizable vicinal diols and GPs with

carboxyl groups and/or GPs with O-sulphate esters. Similar finding described in the gastric mucosa of *Camelus dromedaries* (Fayed and Makitta 1997a). It has been suggested that the GPs with oxidizable vicinal diols control the acidity of the gastric secretion (Tsukise and Yamada, 1987). So, the predominant amount of GPs with oxidizable vicinal diols produced with the gastric mucosa of Zebra could be explained on the basis of controlling the acidity of the gastric secretion.

In the alimentary tract of mammals, the mucopolysaccharides of particular importance in lubrication of the gastrointestinal mucosa, especially those containing O-sulphate esters (Fox, 1979, Hafez, 1977 and Smith, 1988). Since poor quality and thorny foods is the most available food for Zebra, the elaboration of large quantities of GPs with O-sulphate ester in the gastric secretion, lubricate the gastric mucosa to assist the inward movements and protect the mucosa from the mechanical insult of the harsh food.

On the basis of the lectin histochemistry, the present study revealed that the SMC and FMC are highly rich in oligosaccharides with terminal  $\beta$ -GalNAc, GlcNAc and NeuNAc residues and lack of  $\alpha$ -anomer of GalNAc,  $\alpha$ -D-Glu and  $\alpha$ -D-mannan. Similar results were detected in the gastric mucosa of adult shi drum (*Umbrina cirrosa*) (Pedini, et al 2005). In addition to those sugars,  $\beta$ -anomer of GalNAc also detected in the SMC and FMC of the one humped camel (Fayed and Makitta, 1997b). The presence of GalNAc confined to plasmalemmal glycol-

conjugates is indicative of the role of these molecules in the regulation of movements of ions and fluid across this membrane (Spicer and Schulte, 1992). The existence of these sugars in the apical luminal surface could be contributed to the protective function of the gastric mucosa and neutralizing the gastric acidity (Parillo et al., 2002).

The present study showed that the PC appeared to react with HPA, WGA and PNA. This finding are in agreement with the PNA binding studies of Schulte and Spicer (1983) and with the WGA binding studies of Suzuki et al. (1982) and with PNA and WGA binding studies of Baintner et al. (2000) in rats. This finding may be due to the GPs situated on the numerous invaginations (tubulovesicles) of the plasma membrane (Baintner et al, 2000). Also, the biochemical studies (Goldkorn, et al 1989) shown that, the proton pump ATPase constitutes the principal membrane GPs. Goldkorn, et al. (1989) and Zolotarey et al. (1996) stated that the GPs of the PC are desialylated exposing galactosyl units at terminal positions and therefore, allowing them to react with PNA. Fischer et al (1984) observed that the staining intensity of the luminal membrane surfaces of the mucinous parietal and chief cells of human stomach was often stronger by PNA and HPA lectins than that of the mucus secretions in the highly differentiated mucus cells. These results indicate the existence of either heterogeneous GPs components or mucus molecules with variations in the degree of glycosylation of their oligosaccharide chains in the different cells.

The ability of the various mucus cells to react with only one or more than one

lectin may be due to the particular phase of mucus synthesis they are in, although it is also possible that, different cells produce different secretory products (Etzler and Branstrator, 1974).

## Conclusion

The gastric secretion of the Zebra contains a mixture of GPs with oxidizable vicinal diols and GPs with carboxyl group and/or with O-sulphate esters, with variety of sugar residues. These sugars could play a role in, lubrication and protection of the gastric mucosa against mechanical insults of the harsh food as well as proteolytic activity of the microflora.

## References

- Allen, A. and Snary, D. (1972):* The structure and function of gastric mucus. *Gut* 13: 666-672.
- Aoki, T.; Kawano, J.; Oinuma, T.; Haraguchi, T.; Eto, T. and Saganuma, T. (1993):* Human colorectal carcinoma glycoconjugates detected by pokeweed mitogen lectin. *J Histochem. Cytochem.* 41: 1321-1330.
- Baintner, K.; Jakab, G.; Gyori, Z. and Kiss, P. (2000):* Binding of FITC-labeled lectins to the gastrointestinal epithelium of the rat. *Path. Onco.* 6 (3): 179-184.
- Bancroft, J. D. and Stevens, A. (1996):* Theory and Practice of Histological Techniques. Churchill Livingstone, Einberg, London.
- Clamp, J.; Fraser, G. and Reid, A. (1981):* Study of the carbohydrate content of mucous glycoprotein from normal and diseased colons. *Clinical Science* 61: 229-234.
- Etzler, M. E. and Branstrator, M. L. (1974):* Differential localization of cell surface and secretory components in rat intestinal epithelium by use of lectins. *J. of cell Biology.* 62: 329-343.
- Fayed, M. H. and Makita, T. (1997a):* Histochemistry of gastric epithelial glycoproteins of glandular stomach of the one humped camel (*Camelus dromedaries*). *Pathophysiology*, 4: 143-153.
- Fayed, M. H. and Makita T. (1997b):* Lectin histochemistry of the glandular part of the gastric mucosa of the one humped camel (*Camelus dromedaries*). *Acta Histochem.* 30 (5&6): 423-431.
- Fischer, J.; Klein, P. J.; Vierbuchen, M.; Skutta, B.; Uhlenbruck, G. and Fischer, R. (1984):* Characterization of glycolconjugates of human gastrointestinal mucosa by lectins. 1. Histochemical distribution of lectin binding sites in normal alimentary tract as well as in benign and malignant gastric neoplasm. *J. Histochem. Cytochem.* 32 (7): 681-689.

*Forster, G.; Wesley, A. and*

*forster, J. (1982):* Mucous in health and disease. Plenum Press, New York. 199-224.

*Fox, R. A. (1979):* Membrane

glycoprotein shed in defense of the gastro-intestinal tract, *Med. Hypothesis* 5: 582 – 669.

*Goldkorn, I.; Gleson, P.A. and*

*Toh B-H (1989):* Gastric parietal cell antigens of 60-90 KDa, and 100-12 KDa associated with autoimmune gastritis and pernicious anemia. Role of N-glycans in the structure and antigenicity of the 60- 90 KDa component. *J Biol. Chem.* 264: 18768-18774.

*Hafez, E. (1977):* Functional

anatomy of mucous secreting cells, *Adv. Exp. Med. Biol.* 9: 19 - 38.

*Horowitz, M. I. and Pigman, W.*

*(1977):* The glycoconjugates1, Academic Press, New York. 1-2.

*Ishihara, K.; Kurihara, M.;*

*Goto Y.; Ota, H.; Katsu-yama, T. and Hotta, K. (1996):* Establishment of monoclonal antibody against gastric mucins distributed in the different sites of and layer of the gastric mucosa. *Glycoconjugate J.* 13; 857- 864.

*Kornfed, R. and Kornfed, S.*

*(1976):* Comparative aspects of glycoprotein. *Annal. Rev. Biochem.* 45: 217- 224.

*Montereuil, J. (1980):* Primary structure

of glycoprotein glycans. Basis for the molecular biology of glycoprotein. *Adv. Carbohydr. Histochem. Bio-chem.* 31:157-224.

*Mayer, W. and Tsukise, A.*

*(1995):* Lectin histochemistry of snout skin and foot pads in the wolf and domesticated dog. *Anatomischer Anzeiger.* 6: 177:189.

*Parillo, F.; Fagioli, O.;*

*Ceccarelli, P. and Pedini, V. (2002):* From conventional to modern histochemistry: Away for the fine characterization of secretion glycol-conjugates. *J. Anat Em-bryol.,* 107 (2): 43-54.

*Pedini, V; Dall'Aglio,*

*C.; Parillo, F. and Scocco, P. (2005):* Glycoconjugate distribution in gastric fundic mucosa of *Umbrina cirrosa* L. revealed by lectin histochemistry. *J. Fish Biology,* 66 ( 1): 222-229.

*Rhodes, J. and Milton, J.*

*(1998):* Lectin methods and protocols. 1<sup>st</sup> Ed. Humana Press. Totowa, New Jersey.

*Schulte, B. and Spicer, S.S.*

*(1983):* Light microscopic histochemical detection of terminal galactose and N-acetylgalactosamine residues in rodent complex carbohydrates using a galctose oxidase- Schiff sequence and peanut lectin-horseradish peroxidase conjugate. *J. Histochem. Cytochem.* 31: 19- 24.



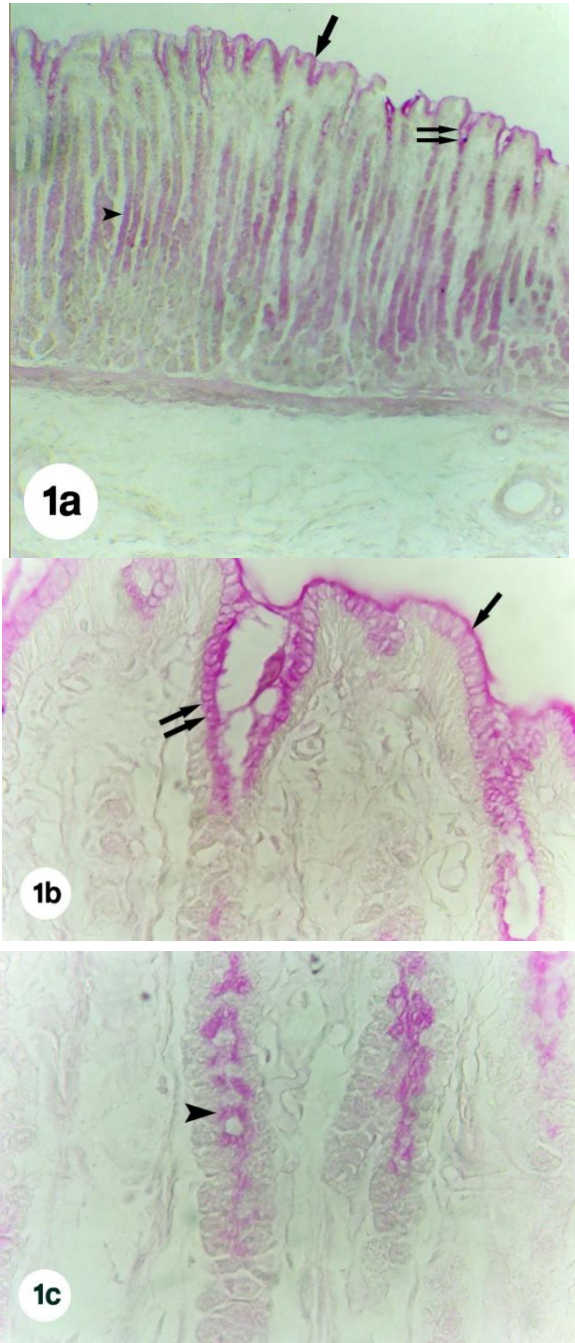
- Smith, D. E.; Paterson, C.; Scratcherd, T. and Read, N. W. (1988):* Textbook of Physiology. Churchill Living-stone, Einberg, London, 11Ed. 234.
- Spicer, S.S. and Schulte B.A. (1992):* Diversity of cell glycol-conjugates shown histochemically: a presec-tive. J. Histochem. Cytochem, 40: 1-38.
- Suzuki, S.; Tsuyama, S. and Murata F. (1982):* Post-embedding staining of rat gastric mucous cells with lectins. Histochemistry 73:563- 575.
- Tatematsu, M.; Katsuyama, T.; Mutai, M.; Asakawa, E. and Ito, N. (1989):* Pyloric gland phenotypic expression of gastric cancers developing in rat fundic glandular stomach. Carcinogenesis. 10 (6): 1033-1039.
- Tsukise, A. and Yamada K. (1987):* The histochemistry of complex carbohydrates in the scrotum of the boar. Histochem. J. 19: 546- 554.
- Vandeheede, J. R.; Ahmed A. and Feeny R. (1972):* Structural and role of carbohydrate in freezing point- depressing glycolprotein from an Antarctic fish. J. Biol. Chem. 247:7885-7889.
- Yang, D.; Karsamo, H.; Miyauchi, M.; Tsuyama, S. and Murata, F. (1996):* Ontogeny of the sulphated glycoconjugate producing cells in the fundic glands. Histochem. J. 28: 33-43.
- Zolotarey, S.; Townsend, R R.; Stuart-Tilley, A. (1996):* HCO<sub>3</sub>-dependent conformational change in gastric parietal cell AE2, a glycoprotein naturally lacking sialic acid. Am J. Physiol. 271: G311- G321

**Table (1):** Carbohydrate binding specificity of lectins used in this study.

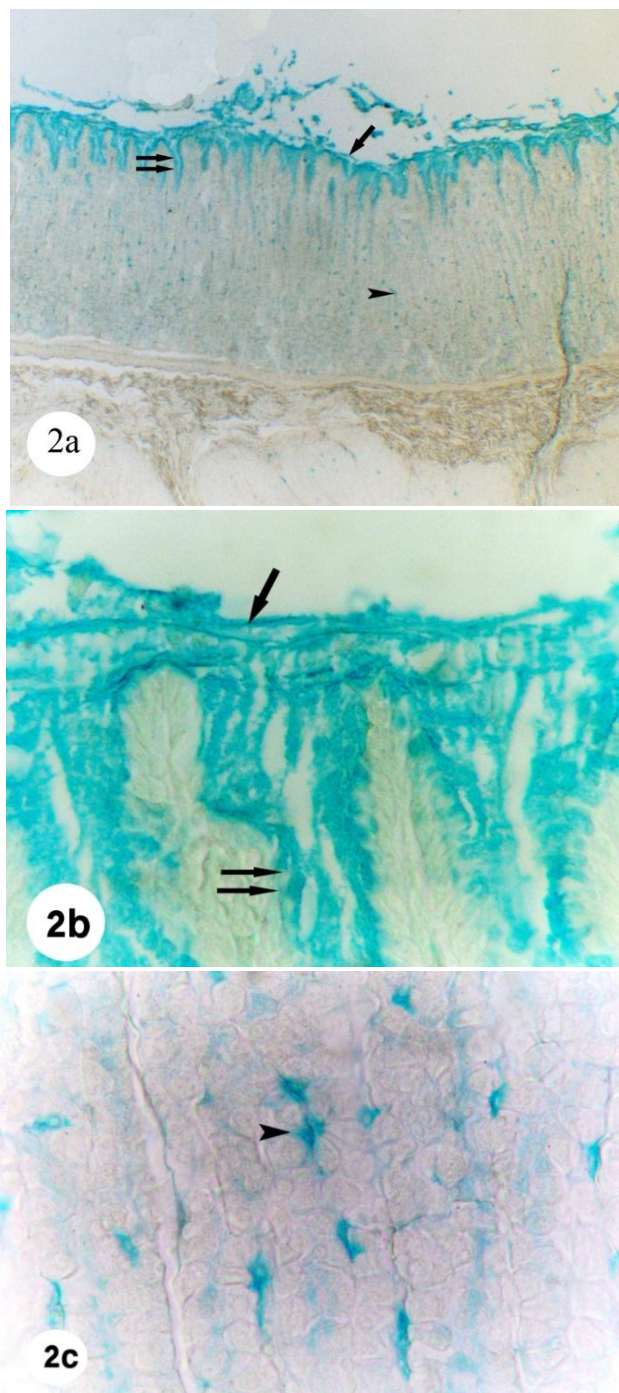
Sugar binding inhibitor	Major sugar specification	Concentration mg / ml	Label	Abbreviations	Source	Taxonomic name
Gal	Gal-β-(13-)-GalNAc	100	HRP	PNA	Peanut	Arachis hypogea
α-methyl-D-Man	α-D-Man, α-D-Glc	20	HRP	Con-A	Jake bean	Canavalia ensiformis
α-D-GalNAc	α-D-GalNAc	6	HRP	HPA	Roman snail	Helix Pomatia
NeuNAc	β-(14-)-D-GlcNAc <sub>2</sub> , NeuNAc <sup>c</sup>	6	HRP	WGA	Wheat germ	Triticum vulgaris

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

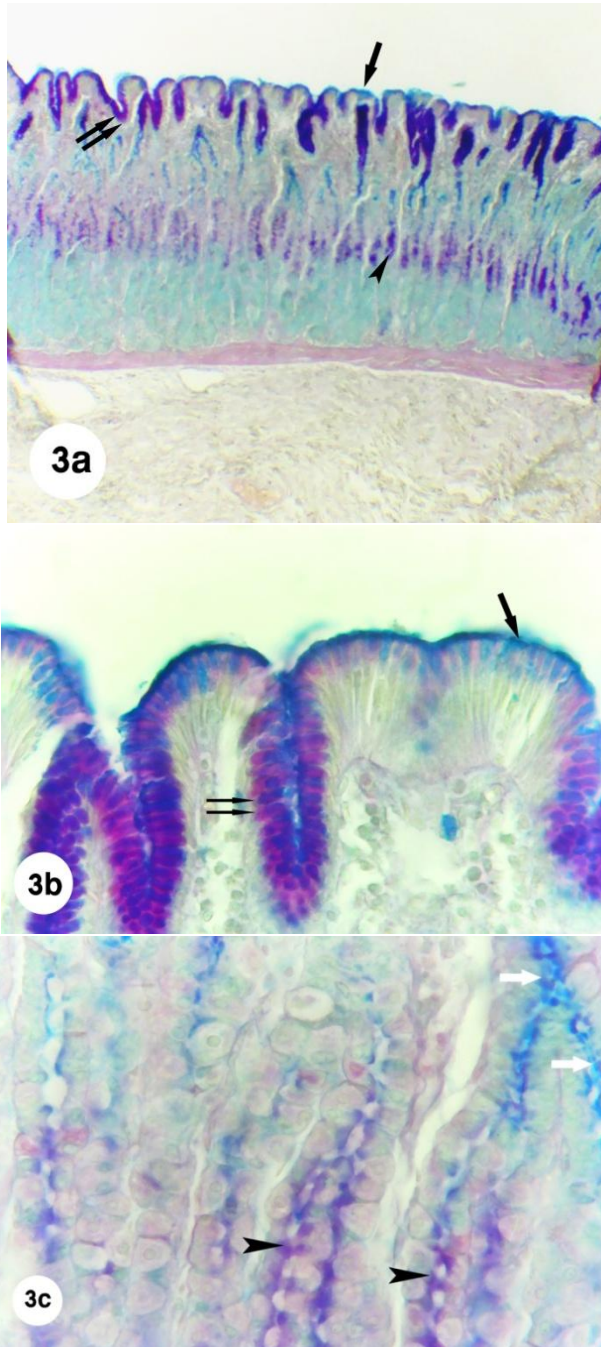




**Fig (1 a, b, c):** Fundic mucosa of Zebra showing the reaction of PAS stain in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head). Note that the MNC were -ve. (PAS stain, X: a, 40 b& c, 400).

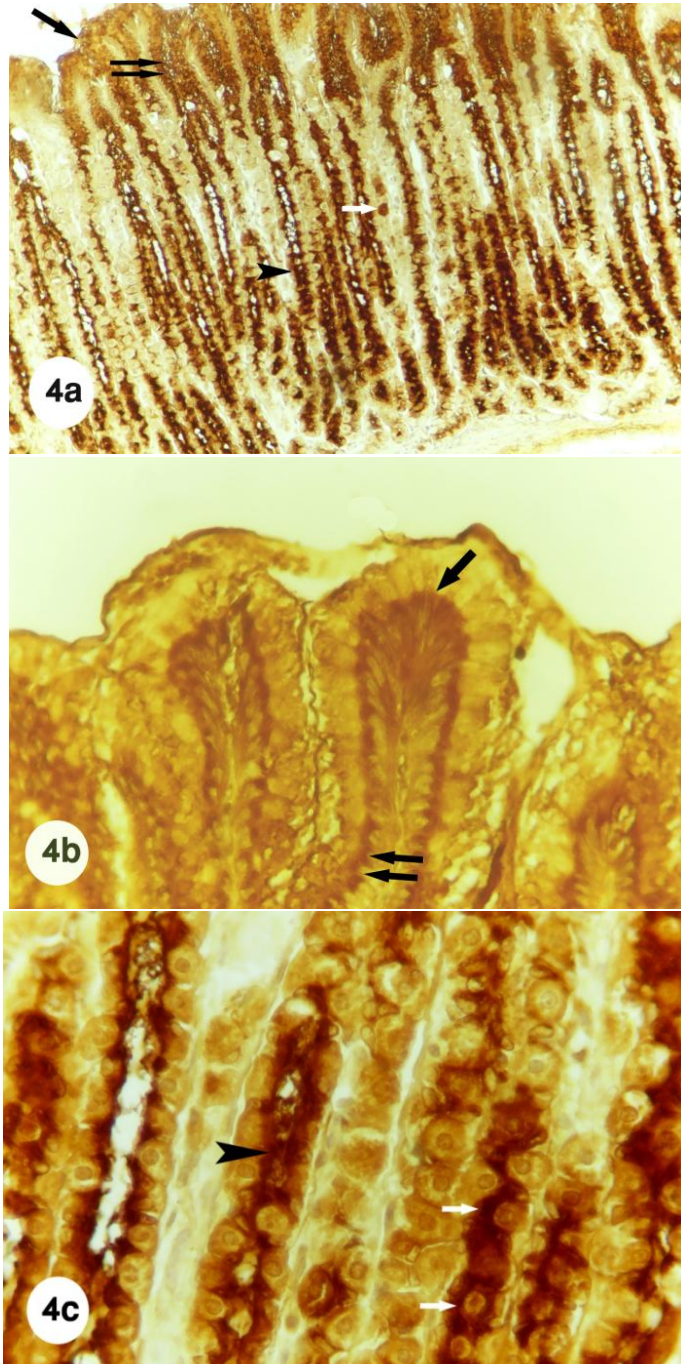


**Fig ( 2 a, b, c):** Fundic mucosa of Zebra showing the reaction of Ab2.5 stain in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head). (Ab2.5 stain, X: a; 100, b & c; 400).

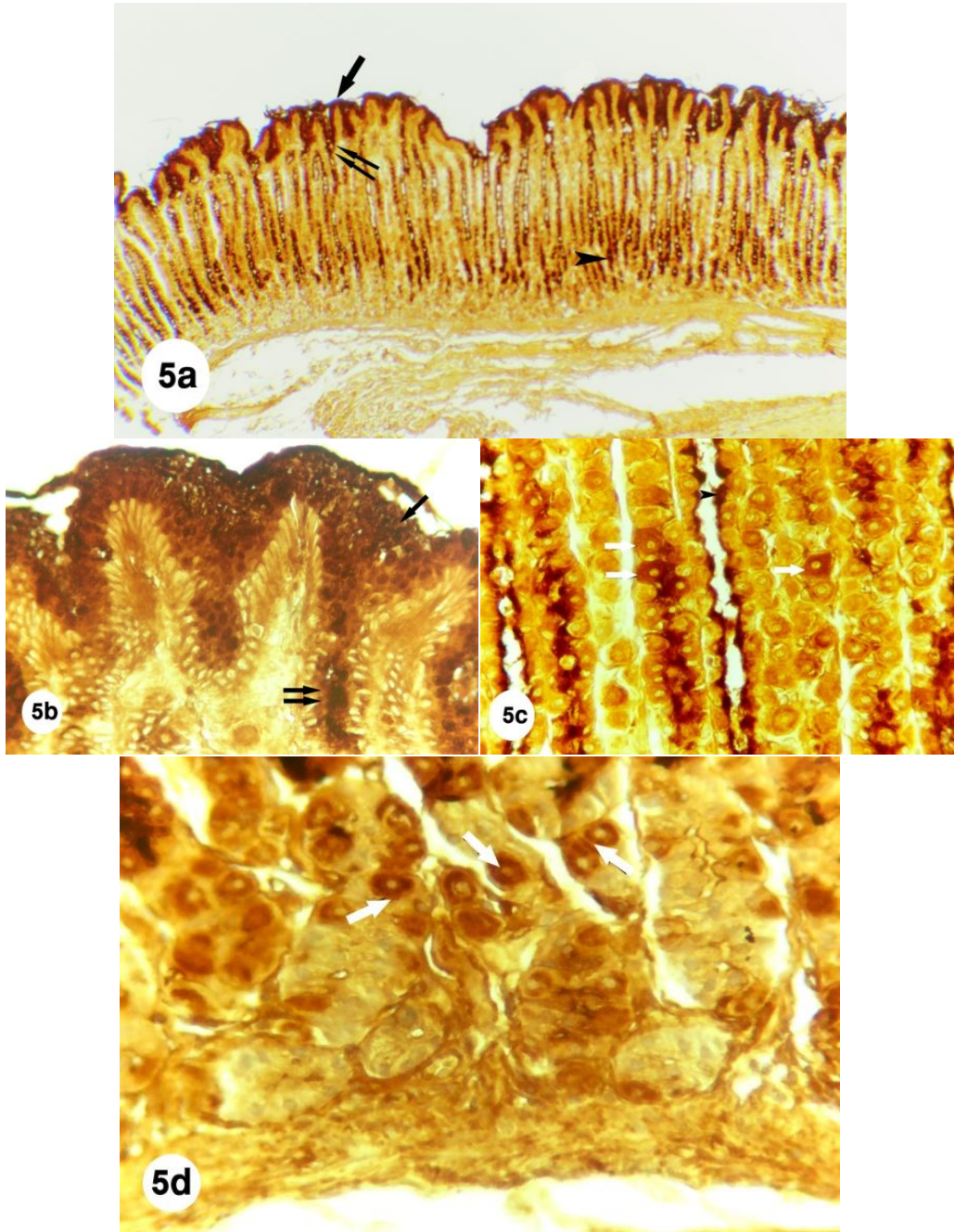


**Fig (3 a, b, c):** Fundic mucosa of Zebra showing the reaction of PAS/Ab 2.5 stain in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head). While Ab2.5 +ve reaction is clear only in the MNC (white arrows). (PAS/Ab2.5 stain, X: a; 40, b& c; 400)



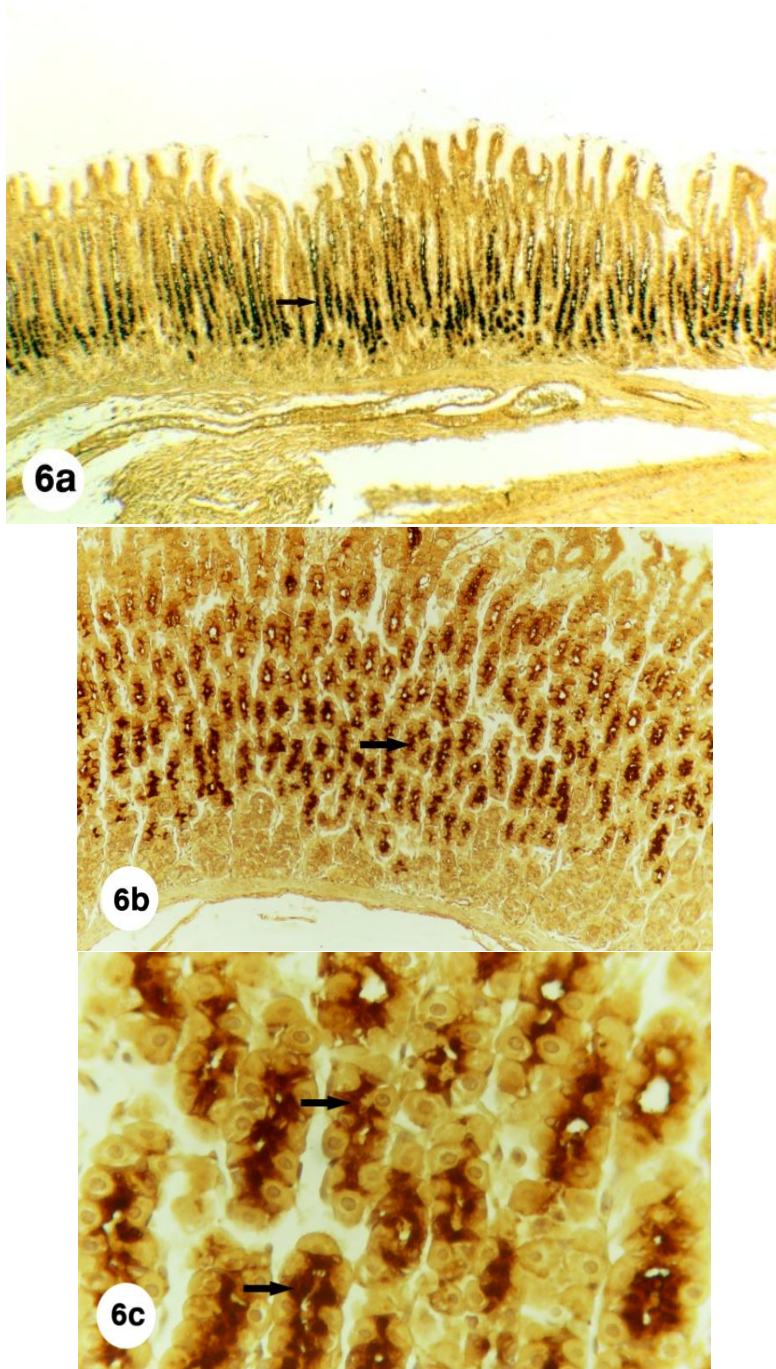


**Fig (4 a, b, c):** Fundic mucosa of Zebra showing the reaction of HPA/HRP reactions in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head), clear reaction in the PC (white arrows). (HPA/HRP stain, X : a; 100, b & c; 400 ).

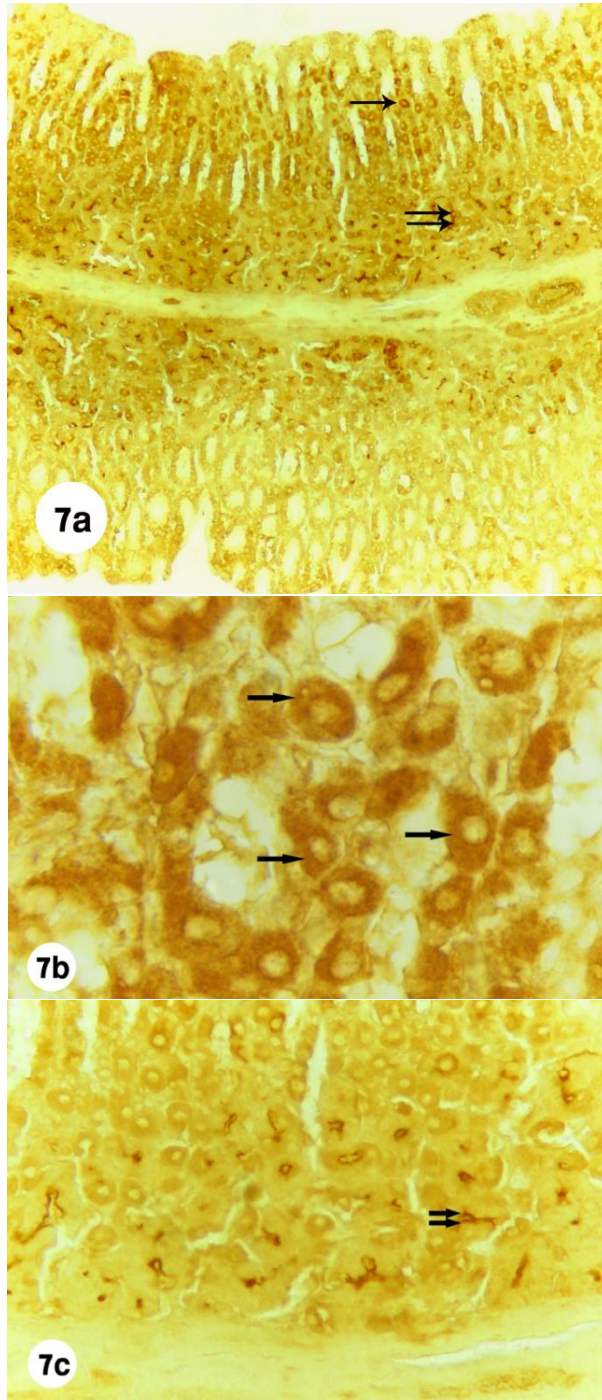


**Fig (5 a, b, c, d):** Fundic mucosa of Zebra showing the reactions of WGA/HRP in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head), clear reaction in the PC (white arrows). (WGA/HRP stain, X: a; 40, b, c& d; 400).





**Fig (6 a, b, c):** Fundic mucosa of Zebra showing the reaction of Con A/HRP in the GMC (arrow), (Con A/HRP stain, X : a; 40, b & c; 400 ).



**Fig (7a, b, c):** Fundic mucosa of Zebra showing the reaction of PNA/HRP in the PC (arrows), in the lumen of the gastric glands (two arrows) . (PNA/HRP stain, X : a; 40, c; 1000 &d; 100 ).