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

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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 oxidezable vicinal diols and/or glycogen were predominates in FMC while, the GPs with carboxyel 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 α- anomer of the N-acetylgalacto-samine (α-GalNAc), N- acetyl- glucosamine (GlcNAc) and neuroaminic acid (NeuNAc). The SMC and FMC were devoid of α- D-glucose (α- D- Glc), α- D- mannose (α- D- Man), galac-Tose (Gal) and β- anomer of the N-acetylgalactosamine (β- 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 α- and β- anomer of the GalNAc and NeuNAc.

Key words

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 histo-chemical 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 α-L-Fuccose (α-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 hunan (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 rained in the same buffer. Paraffin sections of 4µm 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_2O_2), rinsed in distilled water and washed in 1% bovine serum albumen (BSA) in 0.1 M PBS pH 7.4. The sections were then incubated for 12 hours at 4ºC in HRP-lectin (Sigma Co. St. Louis, Mo,USA), dissolved in 0.1m PBS Ph7.4 (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 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_2O_2) 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 epith-elium 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 cyto-plasm 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 epithe-lium 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 cyto-plasm 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 reveled that the SMC and FMC in fundic gland of Zebra secrete a mixture of GPs with oxide-zable 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 sugges-ted 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 oxidezable 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 partic-ular 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 quanti-ties 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 histo-chemistry, the present study revealed that the SMC and FMC are highly rich in oligosaccharides with terminal β-GalNAc, GlcNAc and NeuNAc residues and lacke of α- anomer of GalNAc, α-D -Glu and α-D -.man. Similar results were detected in the gastric mucosa of adult shi drum (*Umbrina cirrosa*) (**Pedini, et al 2005).** In addition to those sugars**,** β- anomar of GalNAc also detected in the SMC and FMC of the one humped camel (Fayed and Makitta, 1997b). The presence of GalNAc con-fined 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 Zolotarev 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 mem-brane 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 oligo-saccharide 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 dioles and GPs with carboxyl group and/or with O- sulphat 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.

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

Sugar binding inhibitor	Major sugar specification	Concen- tration mg / ml	Label	Abbre- viations	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)$_2$,NeuNAc	6	HRP	WGA	Wheat germ	Triticum vulgaris

Symbol: Gal= Galactose; Glc= Glucose; GalNAc= N-acetylgalactosamine; GlcNAc= N-acetyelglucosamine; Man= Mannose; NeuNAC= N-acetyl neuraminic acid (sialic acid); HRP= horseradish peroxides
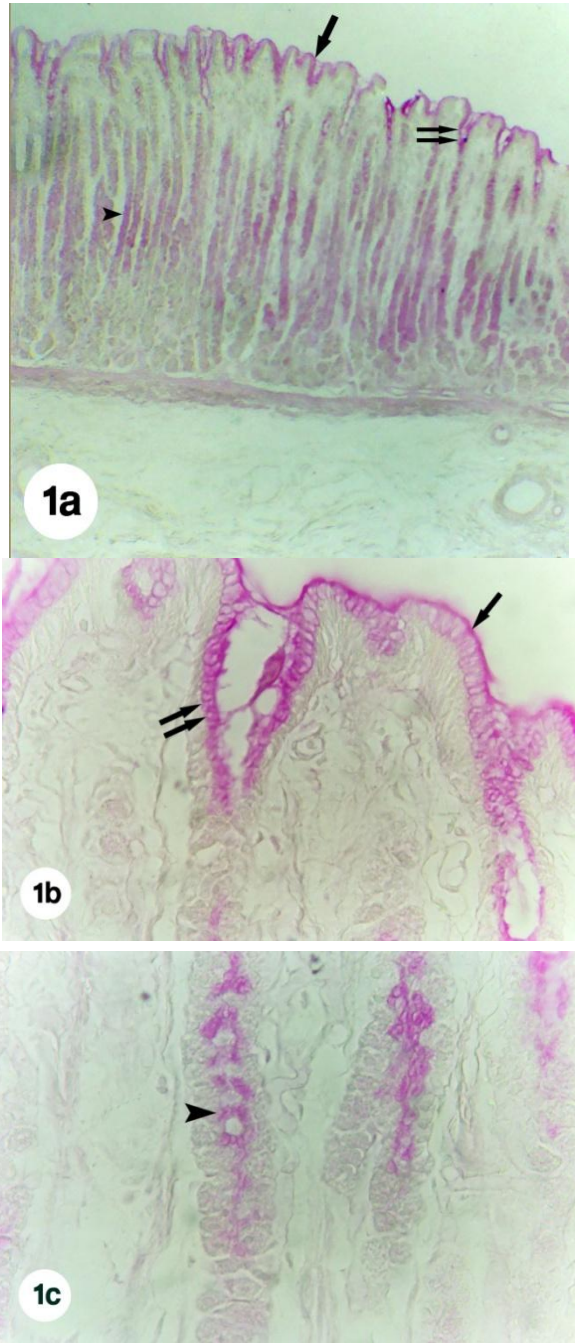
Fig (1 a, b, c): Fundic mucosa of Zebra showing the reaction of PAS stain in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head). Note that the MNC were –ve. (PAS stain, X: a, 40 b& c, 400).
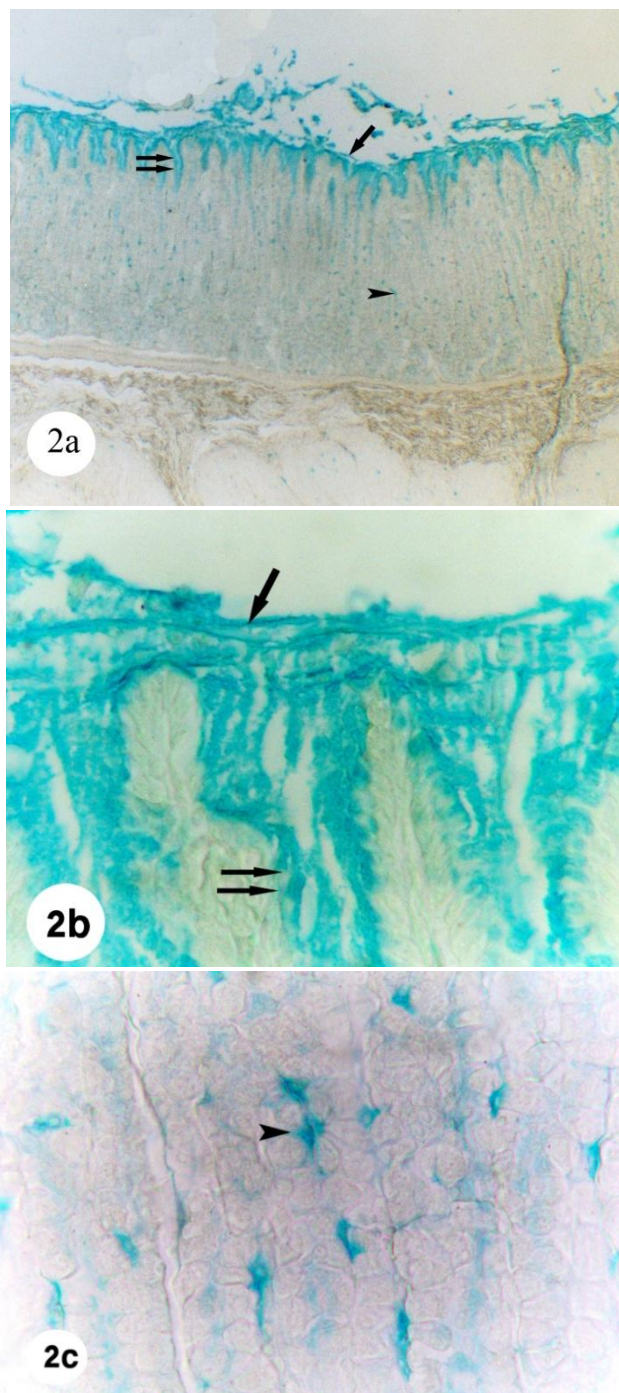
Fig (2 a, b, c): Fundic mucosa of Zebra showing the reaction of Ab2.5 stain in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head). (Ab2.5 stain, X: a; 100, b & c; 400).
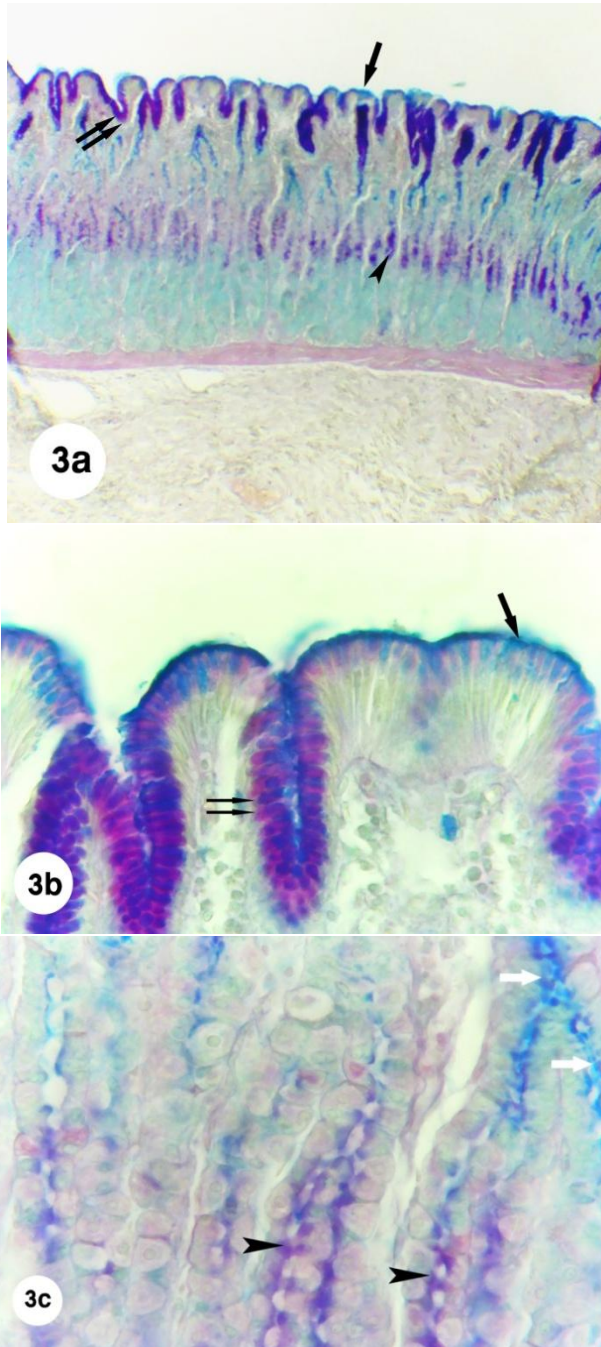
Fig (3 a, b, c): Fundic mucosa of Zebra showing the reaction of PAS/Ab 2.5 stain in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head). While Ab2.5 +ve reaction is clear only in the MNC (white arrows). (PAS/Ab2.5 stain, X: a; 40, b& c; 400)
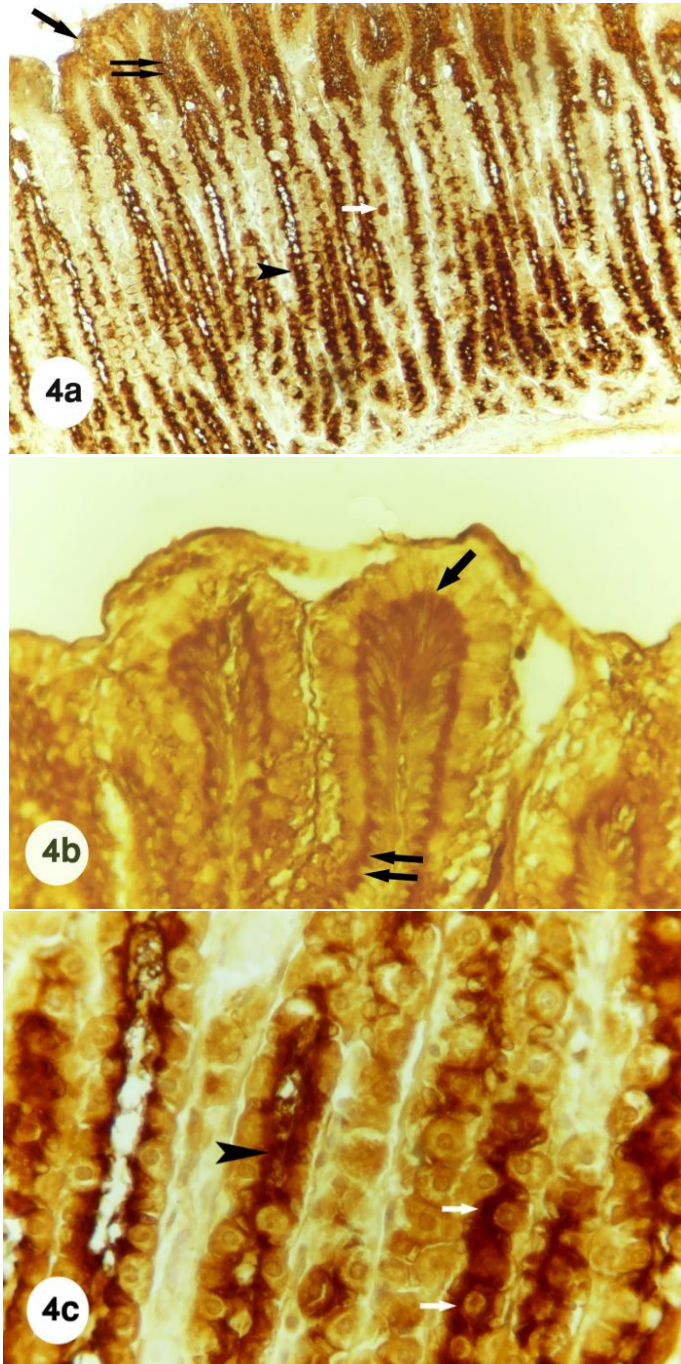
Fig (4 a, b, c): Fundic mucosa of Zebra showing the reaction of HPA/HRP reactions in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head), clear reaction in the PC (white arrows). (HPA/HRP stain, X : a; 100, b & c; 400).
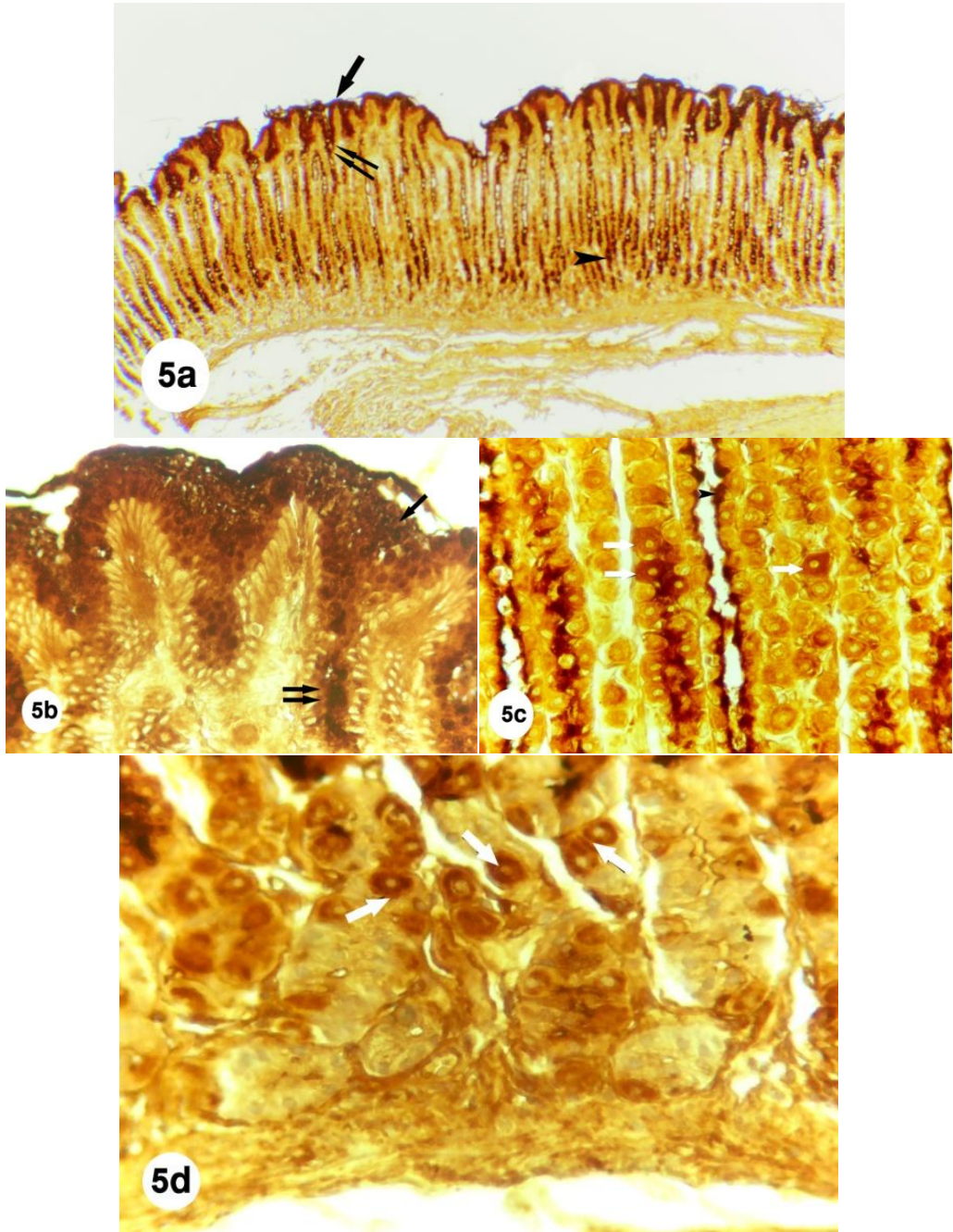
Fig (**5 a, b, c, d**): Fundic mucosa of Zebra showing the reactions of WGA/HRP in the SMC (arrow), in the FMC (two arrows) and the GMC (arrow head), clear reaction in the PC (white arrows). (WGA/HRP stain, X: a; 40, b, c& d; 400).
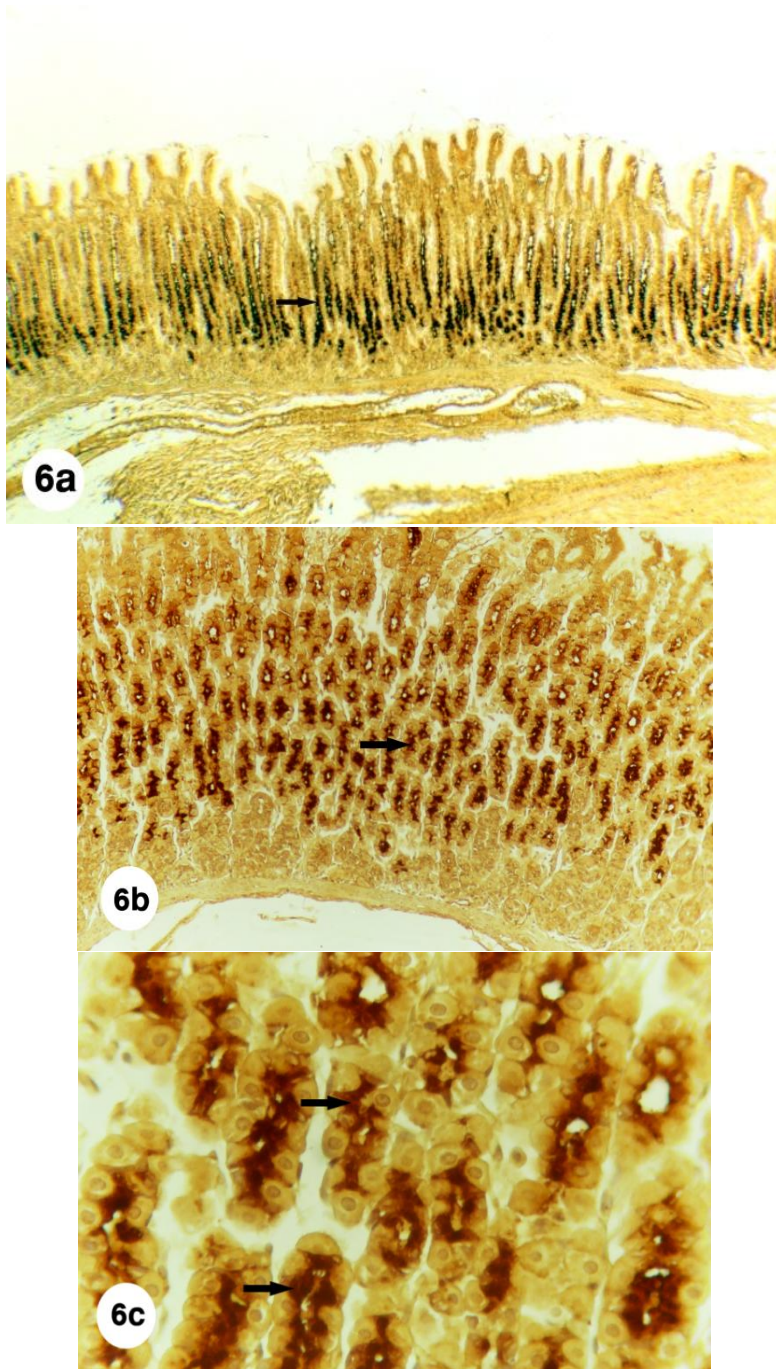
Fig (6 a, b, c): Fundic mucosa of Zebra showing the reaction of Con A/HRP in the GMC (arrow), (Con A/HRP stain, X : a; 40, b & c; 400).
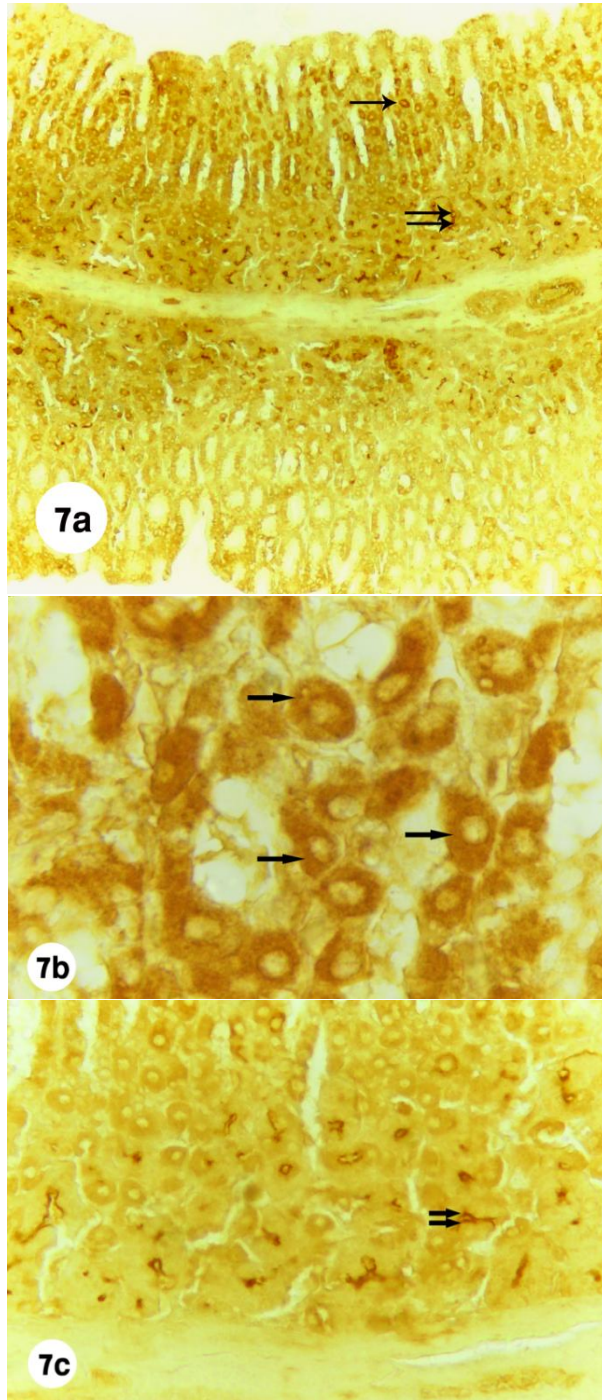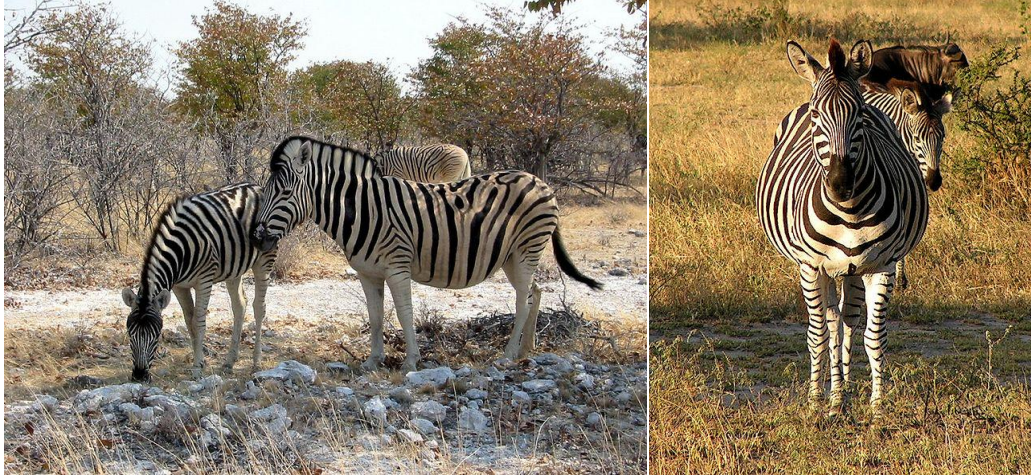
Fig (7a, b, c): Fundic mucosa of Zebra showing the reaction of PNA/HRP in the PC (arrows), in the lumen of the gastric glands (two arrows) . (PNA/HRP stain, X : a; 40, c; 1000 &d; 100).

Animal species in this issue

Zebra (*Equus burchellii*)

Kingdom: Animalia & Phylum: Chordata & Class: Mammalia & Order: Perissodactyla &
& Family: Equidae & Genus: *Equus &* Subgenus: *Hippotigris &* Species: *E. quagga & Subspecies:*
E. q. burchellii

Burchell's Zebra is the most common type of zebrid mammal with a white/black coloring. The Chapman's variety of the plains zebra can be distinguished from Burchell's zebra by the presence of black and white to confuse their predators.

Burchell's zebras are 217 to 246 cm in length, with tail lengths of 47 to 56 cm. At the shoulder, their height is 110 to 145 cm. Males are slightly larger than females and usually have thicker necks as well.

With their distinctive black and white stripes, Burchell's zebras are easily recognizable. The patterns of their stripes differ from other species of zebras. Their stripes are especially wide becoming wider and more horizontal towards the flanks and rear of the body. The stripes on the neck to the forelimbs are vertical. These neck stripes continue in the mane which is short and sticks straight up. In most populations, the stripes extend to the belly where they meet. Stripes on the limbs are narrower and horizontal and continue until reaching the hooves. Facial stripes are ordered both horizontally and vertically creating beautiful patterns. Not all stripes are distinctly black and white. Some stripes may appear a faint brown or may leave a brown "shadow" stripe in the white region.

Animal species in this issue

Nile Monitor (*Varanus Niloticus*)

Kingdom: Animalia & Phylum: Chordat & Class: Reptilia & Order: Aquamata &
Suborder: Scleroglossa & Family: Varanidae & Genus: *Varanus &* Species: *V.niloticus*

The **Nile Monitor** (*Varanus nilotictus*) is a large member of the monitor lizard family (Varanidae).

Nile Monitors grow to about 1.5 to 2 m in length. They have muscular bodies, strong legs and powerful jaws. The teeth are sharp and pointed in juvenile animals and become blunt and peg-like in adults. They also possess sharp claws used for climbing, digging, defense, or tearing at their prey. Like all monitors they have a forked tongue, with highly developed olfactory properties.

Their nostrils are placed high on the snout, indicating that these animals are highly aquatic, but are also excellent climbers and quick runners on land. Nile Monitors feed on fish, snails, frogs, crocodile eggs and young, snakes, birds, small mammals, large insects, and carrion.

In South Africa they are commonly referred to as "leguaan," from the Dutch for *iguana.*