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A histocytochemical study of mucus glycoproteins or mucins in the intestinal tract of the African elephant *(Loxodonta africana)*

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ABSTRACT

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The distribution of neutral mucins, sialomucins and sulphomucins was determined histochemically in the duodenum, jejunum,ileum and colon of the African elephant (*Loxodonta africana*). The techniques used were periodic acid-Schiff (PAS), alcian blue/periodic acid-Schiff (AB-PAS), high-iron-diamine/alcian blue (HID-AB), alcian blue at varying pH solutions and alcian blue at high temperature after methylation and saponification. Acid mucins appear to dominate neutral mucins, the latter decreasing toward the large intestine. Sulphomucins and sialomucins occurred in almost equal amounts throughout the intestinal tract, with a slight decrease of sialomucins toward the colon.

INTRODUCTION

Mucus glycoproteins (or mucins) are high-molecular-weight compounds with large numbers of carbohydrate side chains attached to a protein core which occur, *inter alia*, in the gastro-intestinal epithelium (Filipe 1979). The functions of mucins in the gastro-intestinal tract are well documented (Neutra & Fostner 1987) and aber-rations in their secretion have often been incriminated in the aetiology of gastro-intestinal pathology, especially in humans (Reid, Owen, Dunn, Ramey, Lazosky & Clay 1985; Dohi, Sutton, Frazier, Nakamori, McIsaac & Irimura 1993; Siddiki, Ho, Huang, Byrd, Lau, Yuan & Kim 1993). Mucins may be classified as neutral mucins and acidic mucins (Lev & Spicer 1965). The latter are further differentiated into sialic-acid-containing mucins (sialomucins) and sulphate-containing mucins (sulpho-mucins), according to their histochemical properties (Filipe 1979).

The distribution of the various types of mucin has been determined histochemically in the following mammals, namely mouse, rat, hamster, gerbil, guinea pig, rabbit, cat, dog, rhesus, baboon and man (Sheahan & Jervis 1976). Filipe & Fenger (1979) and Lev & Spicer (1965) reported similar studies in humans, while Ferri & Liquori (1992) described the mucin distribution in the ruin lizard. Similar studies in the African elephant are lacking in literature, and prompted the present study to determine the distribution of neutral, sialo- and sulphomucins in the intestinal tract of the African elephant by means of histochemical methods.

MATERIALS AND METHODS

The intestinal tracts of three adult elephants and one recently weaned elephant calf were collected during a culling expedition in the Kruger National Park. Samples

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for light microscopical mucosubstance histochemistry were taken from the duodenum, jejunum, ileum, caecum and colon of each animal. Specimens were fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. Sections were cut at 6 μm and the following histochemical staining techniques (Cook 1990) were employed (Table 1).

Specimens were subsequently examined with the use of a light microscope and results were photographed.

RESULTS

The results obtained by the various staining techniques are given in Table 2.

TABLE 1 Histochemical methods applied to tissue sections

Method	Interpretation
Periodic acid-Schiff	Neutral mucins—magenta
Alcian blue/periodic-acid Schiff (AB-PAS)	Acid mucins—blue Neutral mucins—magenta
High iron-diamine/Alcian blue (HID-AB)	Sulphomucins—black-brown Sialomucins—blue
Alcian blue for acid mucins at varying pH solutions:	
pH 0,2	Strongly sulphated mucins—blue
pH 1,0	Weakly sulphated mucins, sialidase resistant sialomucins—blue
pH 2,5	Sialidase labile sialomucins—blue
Alcian blue at high temperature after:	
methylation and saponification	Only sialomucins—blue
methylation	Negative
saponification	Sialomucins and sulphomucins—blue

Adult elephants

Duodenum

Mucus goblet cells in the crypts of the duodenal mucosa reacted strongly for acid mucins with AB/PAS (Fig. 1) and reacted strongly for sulphomucins with HID/AB (Fig. 2), while showing only a weak positive reaction with PAS (Fig. 3). Goblet cells in the villi of the duodenal mucosa reacted strongly to PAS (Fig. 3), while some cells reacted for neutral mucins and others for mixed neutral and acid mucins with AB/PAS (Fig. 1). The HID/AB stain revealed some villous goblet cells to react only for sialomucins and others for mixed sulphomucins and sialomucins.

Most mucus cells in Brunner's glands reacted for acid mucins, while just a few cells reacted for only neutral or mixed neutral and acid mucins with AB/PAS (Fig. 1). A light staining reaction was observed with PAS (Fig. 3), while more or less equal numbers of cells reacted for sialomucins and sulphomucins when HID/AB was used (Fig. 2). Mucus cells in both the mucosa and Brunner's glands reacted weakly with AB at pH 0,2 and 1,0, but strongly with AB at pH 2,5 (Fig. 4). A weak staining reaction was observed in goblet cells in the villi, with high temperature methylation and subsequent saponification, indicating only sialomucins. After only methylation no staining reaction was observed.

Jejunum and ileum

Goblet cells in the mucosa of those areas reacted similarly to all the stains mentioned for the duodenal mucosal goblet cells.

Caecum and colon

Goblet cells in the caecal and colonic mucosa reacted positively for acid mucins, while dispersed cells, reacting for neutral mucins and mixed acid and neutral mucins occurred in the bases of the crypts, when AB/PAS was used. The PAS reaction was light throughout the

TABLE 2 Results obtained with the different histochemical stains

Site		PAS	AB/PAS		HID		AB at varying pH's			COMB.METH/SAPON		
			AM	NM	SI	SU	0,2	1,0	2,5	A	B	C
DUODENUM	Brunner	+–	++	+	++	++	+–	++	++	+–	–	++
	villi	++	+	+	++	+	+–	++	++	+–	–	++
	crypts	+–	++	+	+	++	+–	++	++	+–	–	++
JEJUNUM	villi	++	+–	+	++	+	+–	++	++	+–	–	++
	crypts	+–	++	+	+	++	+–	++	++	+–	–	++
ILEUM	villi	++	+–	+	++	+	+–	++	++	+–	–	++
	crypts	+–	++	+	+	++	+–	++	++	+–	–	++
CAECUM	crypt-base	++	++	+	+	++	+–	++	++	+–	–	++
	crypt-lumen	+–	++	+–	++	+	+–	++	++	+–	–	++
COLON	crypt-base	++	++	+	+	++	+–	++	++	+–	–	++
	crypt-lumen	+–	++	+–	++	+	+–	++	++	+–	–	++

++ = strongly positive
\+ = positive
+– = weakly positive
– = negative

AM = acid mucin
NM = neutral mucin
SI = sialomucin
SU = sulphomucin

A = methylation and saponification
B = only methylation
C = only Alcian blue pH 2,5 staining

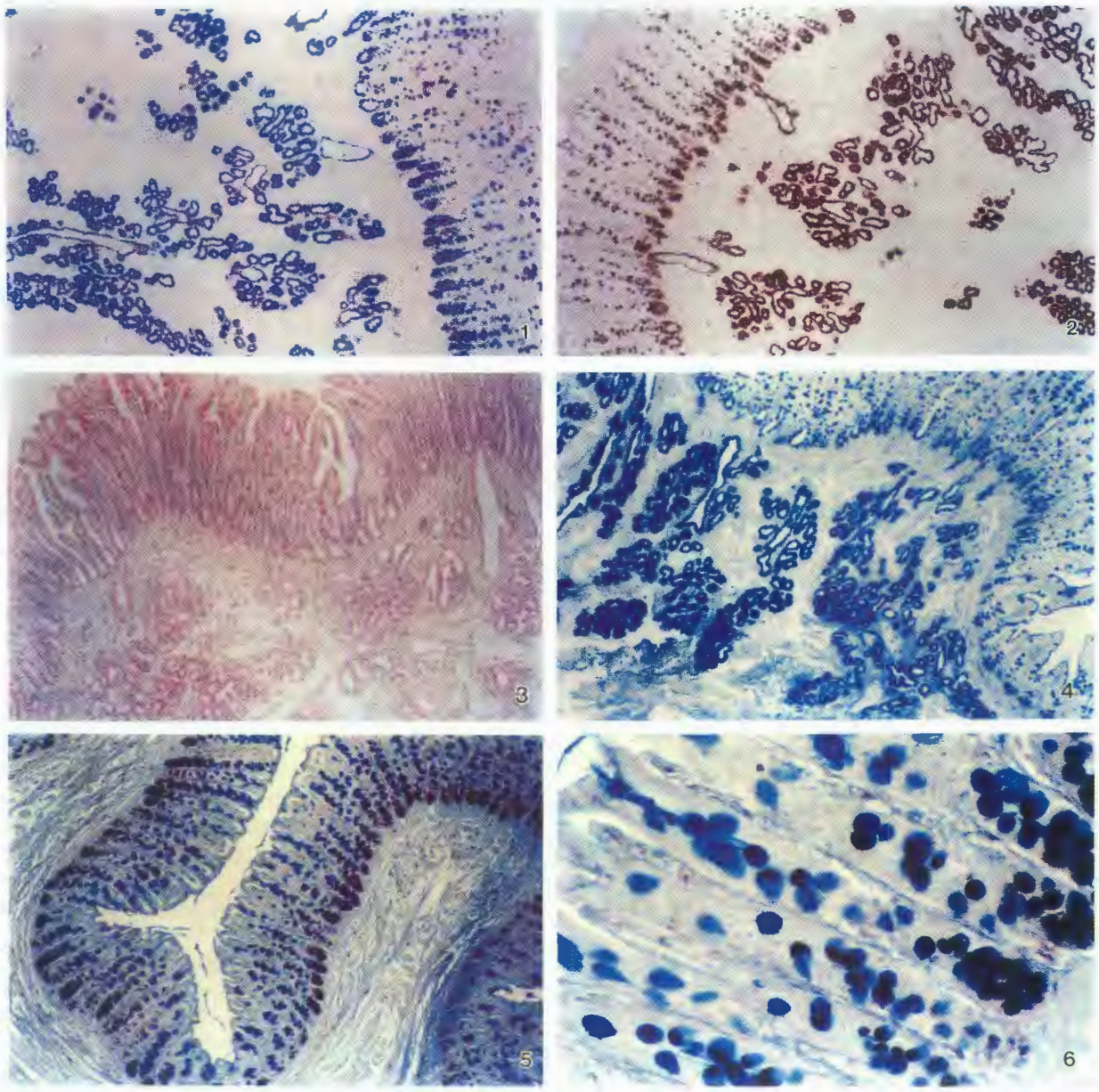

FIG. 1 AB/PAS Duodenum 45x. Acid mucins (blue) are dominant in crypts and Brunner's glands, neutral mucins (light magenta) in the villous tips and scattered in the Brunner's glands
FIG. 2 HID Duodenum 45x. Sulphomucins (black-brown) mainly in crypts and Brunner's glands (not counterstained with AB)
FIG. 3 PAS Duodenum 45x. Neutral mucins (magenta) in goblet cells in villous tips, weak PAS reaction in crypts and Brunner's glands
FIG. 4 AB pH 2,5 Duodenum 45x. Both sialo- and sulphomucins reacted strongly (blue)
FIG. 5 HID/AB Colon 45x. Sialomucins located luminally (blue), sulphomucins in crypt bases (black-brown)
FIG. 6 HID/AB Colon 224x. Goblet cells reacting for sialomucins (blue), mixed sialo- and sulphomucins located in same cell (blue/black-brown), sulphomucins in crypt base (black-brown)

mucosa with dispersed, strongly reacting goblet cells, mainly in the bases of the crypts. HID/AB stain demonstrated sialomucins to occur luminally in the crypts, while sulphomucins occurred more in goblet cells situated in the bases of the crypts (Fig. 5). HID/AB stain also revealed cells that reacted only for sialomucins and others that reacted for mixed sialo- and sulphomucins (Fig. 6).

Goblet cells reacted with AB at pH 0,2, reacted slightly more strongly at pH 1,0, and reacted strongly positive at pH 2,5. A light reaction was observed in the goblet cells after high temperature methylation and

sub-sequent saponification, indicating the presence of only sialomucins. All goblet cells reacted strongly after they had been stained with alcian blue at pH 2,5, showing the presence of sialomucins and sulphomucins. After only methylation, no staining reaction was observed.

Elephant calf

The results obtained from the intestinal tract of the elephant calf were very similar to those observed in the adult animals.

DISCUSSION

In the present study of the African elephant, acid mucosubstances appeared to dominate neutral mucosubstances in the entire small and large intestinal tract, with neutral mucosubstances decreasing slightly toward the large intestine. These findings correlate with a study carried out by Sheahan & Jervis (1976) on 11 different mammalian species. In their study, however, the concentration of sulphomucins in the small and large intestine, was greater than that of sialomucins, particularly in the distal part of the colon. In the ruin lizard colon a predominance of sulphated muco-substances was also observed (Ferri & Liquori 1992). In the normal human small intestine, Filipe & Fenger (1979) found goblet cells containing neutral and sialomucins, but no sulphomucins. Similar findings in humans were reported by Lev & Spicer (1965). In the present study sulphomucins and sialomucins occurred in more or less equal amounts right through the intestinal tract of the African elephant, with a slight decrease in sialomucins toward the large intestine, and therefore only a relative increase in sulphomucins. The sulpho-mucins in the present study were mostly weakly sulphated, while the sialomucins were mostly sialidase labile throughout the intestinal tract, in contrast to the findings in pigs (Moré, Fioramonti, Bénazet & Buéno 1987) and humans (Filipe & Fenger 1979), where the sialidase-resistant sialomucins were found to be predominant.

Filipe (1979) reported that human Brunner's glands contain only neutral mucins. Sheahan & Jervis (1976) reported similar findings in Brunner's glands of mouse, rat, cat, dog and man. In addition to neutral mucins, they reported sulphomucins in the hamster and gerbil Brunner's glands, and both sialo- and sulphomucins in the mouse, rabbit, guinea pig, baboon and Rhesus monkey. In the present study of the African elephant, the Brunner's glands contained almost exclusively acid mucins comprising equal proportions of sialomucins and sulphomucins, with very few cells staining for neutral mucins.

A variation in the distribution of mucin composition in the goblet cells located in the crypts and villi was observed in the small intestine of the elephant, as well as in humans (Filipe & Fenger 1979). Contrary to their findings, where neutral mucins occurred in the crypts of the human small intestine, neutral mucins occurred in the tips of villi in the elephant, while acid mucins occurred more in the crypts. In the caecum and colon of the African elephant, the pattern of distribution of neutral and acid mucins was opposite to that found in the small intestine. Here, neutral mucins occurred more in the base of the crypts, while acid mucins occurred more luminally, in agreement with the results of Filipe & Fenger (1979) in the human small intestine. These authors postulate that the goblet cells containing supposedly more immature neutral mucosubstance (normally found in human foetal small intestine), occur in the crypts and "mature" as they move toward the villi. The findings of the present study are in agreement with those of Filipe & Fenger (1979), in that sialomucins were found mostly in the small intestinal villi.

Sheahan & Jervis (1976) reported that the distribution of various mucins in the intestinal tract is unrelated to diet as well as to the order of mammals. However, diet may affect the amount of mucin secretion. Vahouny, Le, Ifrim, Satchithanandam & Cassidy (1985) concluded that dietary fibre in the form of wheat bran caused an increase in goblet cell secretory activity in rats. Monsma, Vollendorf & Marlett (1992) stated that gum arabic in the diet of colectomized rats increased the amount of mucin secreted from the upper gut. Moré *et al.* (1987) reported modifications in the type of mucin secreted in pigs even after short-term dietary alterations, especially alterations to dietary fibre, but found that the differences could not be linked to a specific diet. Normal colonic mucin is usually highly sulphated and has an important function as a protective barrier against luminal contents and bacterial enzymes (Raouf, Tsai, Parker, Hoffman, Walker & Rhodes 1992). Previous histochemical studies using high-iron-diamine stains show a decrease in mucin sulphation in mucosa of patients with colonic polyps, ulcerative colitis and colonic carcinoma (Raouf *et al.* 1992). The elephant, like the Equidae, is able to ferment high-cellulose-content diets at a pH varying between 6,0 and 6,8, while intestinal microorganisms play an important role in this regard. The colon is a site of higher fermentation and relative increase of protozoal numbers (Eloff & Van Hoven 1980). This necessitates a high level of mucus protection of the epithelial lining, which may be offered by the relatively high concen-tration of sulphomucins observed in the distal part of the African elephant intestinal tract.

The results of the present study shed light on the hitherto unknown distribution of the neutral and acid muco-substances found in the intestinal tract of the African elephant. The study therefore provides a basis for further investigations, such as lectin-binding techniques into the mucosubstances found in the elephant intestinal tract.

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