Histol Histopath (1993) 8: 305-316

Histology and Histopathology

Lectin histochemistry of salivary glands in the Giant Ant-eater *(Myrmecophaga tridactyla)*

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Summary. The submandibular and buccal glands of the Giant Ant-eater *(Myrmecophaga tridactyla)* have been studied by means of a series of carbohydrate histochemical methods, including a broad spectrum of PO-lectin procedures. The seromucous cells (Gl. submandibularis) and mucous cells (Gl. buccalis) of the glandular acini, as well as the secretion in the excretory duct system exhibited very strong to strong reactions for neutral and acidic glycocongugates. The serous cells of the buccal glands and the excretory duct cells reacted rather weakly. The different controls applied particularly emphasized that sialoglycoconjugates are the predominant ingredients of the saliva secreted.

Lectin histochemical differentiation demonstrated a varying pattern of saccharide residues in these substances. In the submandibular glands the glycocongujates (mostly proteoglycans) of the seromucous cells and the luminal secretion normally contained terminal ß-galactose and minor contents of terminal α-N-acetylglucosamine. After sialidase digestion this cell type exhibited distinct amounts of sialic acid-ß-galactose and sialic acid-α-N-acetyl-galactosamine. Sialic acid was also clearly present in the tough interlobular connective tissue. The buccal glands showed a similar distribution of saccharide residues in the mucous cells. In the serous cells, however, acidic glycoproteins with sialyl residues were observed, also containing terminal α-D-mannosyl, α-N-acetyl-galactosaminyl, and ß-D-galactosyl residues. The cells of the excretory duct system of both gland types reacted weakly to moderately for terminal sugar residues (N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, ß-D-galactose).

The results obtained are discussed in view of the specific feeding mode of the Giant Ant-eater, whereby high contents of sialoglycoconjugates (proteoglycans, glycoproteins) produced by the salivary glands warrant for the main function of the non-sticky saliva; i.e., to act as an effective lubricant during tongue movement.

Key words: Lectin histochemistry, Salivary glands, Carbohydrates

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Introduction

The salivary glands of Mammalia exhibit outstanding diversity at gross, light microscopical and electron microscopical levels. They also vary in number, size and location among the different mammalian groups and/or species, respectively (Fahrenholz, 1937; Shackleford and Wilborn, 1968; Wilborn and Shackleford, 1969; Pérez Clavier, 1976; Young and van Lennep, 1978). This diversity seems to be phylogenetically inherent and emphasizes the comprehensive biological functions of salivary glands throughout the amniote groups, whereby classification of the different gland types becomes difficult and somewhat confusing (see e.g. Fahrenholz, 1937; Tucker, 1958).

Important biological functions of mammalian salivary glands are closely connected with the capacity to produce large amounts of glycoconjugates in the major types of secretory acini, which include mucous, serous as well as seromucous cells. Secretion type, however, is not restricted to specific cell types, so that the whole population of secretory cells probably offers a remarkable capacity of transformation when related to the biological needs of each species. Thus, a classification of mammalian salivary glands according to their contents of neutral and acidic complex carbohydrates, previously quite common (see e.g. Shackleford and Klapper, 1962; Quintarelli, 1963; Shackleford, 1963; Leppi and Spicer, 1966; Quintarelli and Dellovo, 1969; Pal and Chandra, 1979), seemed to be a rather general way to differentiate typical functions, until it became possible to use more sensitive methods, such as those warranted by lectin histochemistry. Earlier carbohydrate histochemical procedures only allowed the

localization and differentiation of acidic from neutral glycoconjugates, on the basis of selective demonstration of vicinal diol groups by virtue of their reactivity with the periodic acid-Schiff (PAS) stain and the affinity of the anionic functional groups of the former for cationic dyes. Lectins (agglutinins) coupled to fluorescent probes or enzyme markers, such as peroxidase (PO), in conjuction with the enzymatic cleavage of sialic acid now provide more detailed and selective information on the structure of carbohydrate-containing macromolecules (glyco-proteins, proteoglycans) at specific histological sites (Culling and Reid, 1982; Lis and Sharon, 1986; Alroy et al., 1988; Spicer and Schulte, 1988, 1992). With special regard to the latter methodological approach, several studies on the glycoconjugates of salivary glands of non-human mammals (Schulte and Spicer, 1983, 1984; Schulte et al., 1985) as well as man (McMahon et al., 1989) have recently been performed, emphasizing the heterogeneity of the complex glycoconjugates present in these structures.

The current study is the first to assess the various complex carbohydrates in the major salivary glands of the Giant Ant-eater *(Myrmecophaga tridactyla)* using a broad spectrum of lectins. In this way earlier histochemical knowledge on this subject (Quintarelli and Dellovo, 1969) is reinvestigated to some extent, and the attempt is made to better differentiate the specific nature of the secretional ingredients of the enormous amounts of saliva produced. The results obtained may be of interest in view of the highly specialized feeding mode of this toothless animal, which relies exclusively on such prey as ants and termites.

Materials and methods

Tissue samples of the Gll. submandibularis and buccalis were obtained directly after death from an adult, 5-year-old male of the Giant Ant-eater *(Myrmecophaga tridactyla)*. The animal had been reared in the Tierpark Dortmund, and had to be sacrificed because of its suffering from heavy arthrosis.

One part of the tissue specimens was fixed in 10% formalin containing 2% calcium acetate (Leppi, 1968), another part in Bouin's fluid. Both were maintained for 48 h at 4 °C or room temperature. The material was then carefully dehydrated in graded series of ethanol, and embedded via Histosol (Shandon) in paraffin wax (Paraplast, Shandon), or directly in the plastic embedding resin Technovit 7100 (Kulzer) to avoid shrinkage (Hanstede and Gerrits, 1983). 3-5 µm plastic sections were stained with haematoxylin and eosin (H.E.) as well as for the PAS reaction. Additionally, several plastic sections of both gland types were silver impregnated for connective tissue fibres according to Leong and Pulbrook (1989).

8 µm paraffin sections were deparaffinized in Bio-clear (Bio-optica), hydrated through descending concentrations of ethanol and stained with various methods for the demonstration of complex carbohydrates, including combined control procedures. In general, paraffin tissue processing as described above seems to be quite appropriate for the lectin binding to tissue sections (Rittman and MacKenzie, 1983; Allison, 1987; Alroy et al., 1988).

For the demonstration of glycoconjugates the following histochemical stainings were employed: periodic acid Schiff (PAS) (Spicer et al., 1967; Schiff's reagent after Barger and DeLamater from Culling, 1974); alcian blue (AB pH 1.0) (Lev and Spicer, 1964), AB pH 2.5 (Pearse, 1968); AB pH 2.5-PAS (Mowry, 1963); coupled tetrazonium procedure (TZ) (Pearse, 1968); and different lectins labelled with horseradish peroxidase (PO) (purchased from E.Y. Labs./Medac and Sigma) in concentrations of 10-20 µg/ml in 0.1 M PBS (pH 7.2) for 30 min at 20 °C and 2h at 4 °C. The lectins used were concanavalin A (Con A), peanut agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA), *Ricinus communis* agglutinin-I and -II (RCA-I and -II), *Maclura pomifera* agglutinin (MPA), *Ulex europeaeus* agglutinin-I (UEA-I), soya bean agglutinin (SBA), wheat germ agglutinin (WGA), *Griffonia simplicifolia* agglutinin-I and -II (GSA-I and -II), *Limulus polyphemus* agglutinin (LPA) and *Limax flavus* agglutinin (LFA) (for lectin specificity see e.g. Collard and Temmink, 1974; Kiernan, 1975; Yamada and Shimizu, 1977, 1979; Stoward et al., 1980; Pearse, 1985; Miller, 1987; Alroy et al., 1988; Spicer and Schulte, 1988, 1992). The activity of the peroxidase employed for labelling was revealed by a diamino-benzidine-hydrogen peroxide system (DAB, purchased from Sigma) (Yamada and Shimizu, 1977).

Lectin controls: a) the following saccharides were added at a final concentration of 0.01M to the respective lectin solutions: α-methyl-D-mannoside for Con A, lactose for PNA, N-acetyl-D-galactosamine for SBA, DBA and RCA-II, ß-D-galactose for RCA-I, α–D-galactose for MPA and GSA-I, L-fucose for UEA-I, N-acetyl-D-glucosamine for WGA and GSA-II, and N-acetylneuraminic acid for LPA and LFA; b) substitution of unconjugated lectins for lectin-PO-conjugates; c) exposure to PO and DBA system without lectins. To detect endogenous peroxidase activity in tissue, certain control sections were reacted with DAB only.

Enzyme digestion with α-amylase (from *Bacillus subtilis*, Sigma; 1 mg/ml in 0.1M phosphate buffer pH 7.0 at 37 °C for 3h) was conducted on some sections, prior to PAS staining. For this enzyme digestion experiment, two types of controls were done: some sections were incubated in buffer solutions without the enzyme under identical conditions of temperature and duration; and other sections were kept intact without any incubation procedures (Tsukise and Meyer, 1983). Sialic acid was cleaved enzymatically in a solution of sialidase (from *Clostridium perfringens*, Sigma; 0.2 IU/ml in 0.5M sodium acetate containing 4.0mM $CaCl_2$ at pH 5.5, at 37 °C for 18h) (see Schulte et al., 1985); for specific staining of sialic acids see also Culling and Reid (1982) and Spicer and Schulte (1992).

Results

General morphology

The Giant Ant-eater *(Myrmecophaga tridactyla)* is endowed with a variety of salivary glands, including the Glandula submandibularis (submaxillaris), Gl. parotis, Gl. buccalis, and the Gl. lingualis (Fig. 1). The present study is concerned exclusively with the largest ones; i.e., the Gll. submandibularis and buccalis. In this connection, it has to be emphasized, that contrary to several wrong interpretations, the parotid glands retain their common proportional size and relative position, and are not involved in specific enlargement as is the Gl. submandibularis.

The submandibular glands were immensely developed and together exhibited a typical horse-shoe shape. They spread from the angle of the jaws across the neck to the chest, with a length of about 40 cm, and a thickness of 5-6 cm. The sheet of glandular tissue of each gland extended caudally along the sides of the neck and ventrally over the chest, where both glands formed a compact mass from shoulder to shoulder (Fig. 1). Their large excretory ducts (ducts of Wharton) dilated, thus giving rise to a reservoir or «salivary bladder». The glands were intensely penetrated by blood vessels, in that the anterior part was supplied by the Arteria carotis externa, whereas the posterior part received blood from branches of the A. cervicalis. The glands were drained by the Vena jugularis externa and its branches. The submandibular glands were innervated by branches of the Ganglion submandibularis, located near the fusion point of the excretory ducts (for specific morphological details see especially Pouchet, 1874).

The buccal glands formed a continuous layer of variable length, extending caudally between the mandible and the Musculus genioglossus to the angle of the jaws (Fig. 1). They were approximately 35 cm in length and 3-10 mm thick. The blood supply was provided by the A. and V. lingualis. The glands were traversed by the excretory ducts of the submandibular glands, and innervated by branches of the N. lingualis.

General histology

The Gl. submandibularis was a branched tubulo-acinar gland with secretory portions containing predominantly round or oval-shaped seromucous cells

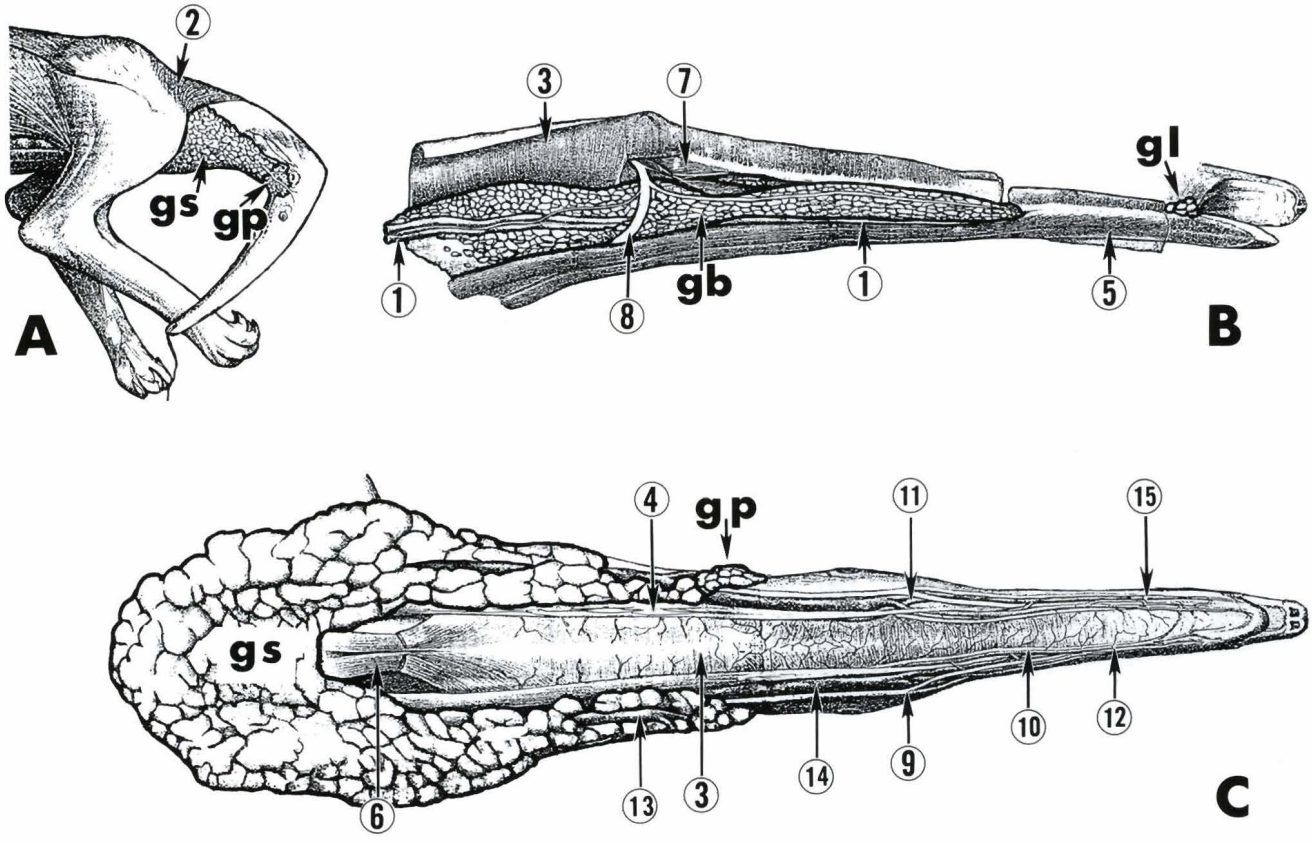

Fig. 1. Position and shape of salivary glands in the Giant Ant-eater *(Myrmecophaga tridactyla)*; **A** - lateral view of musculature of the anterior body half with salivary glands, **B** - lateroventral view of the head and neck region, **C**- ventral view of the head, neck and rostral thorax; gb = glandula buccalis, gl = gl. labialis, gp - gl. parotis, gs = gl. submandibularis; 1 - duct of gl. submandibularis, 2 - M. trapezius, 3 - M. mylohyoideus, 4 - M. sternomandibularis, 5 - M. genioglossus, 6 - M. geniohyoideus, 7 - M. temporalis, 8 - N. lingualis, 9 - N. facialis, 10 - N. mylohyoideus, 11 - A. facialis, 12 - A. submentalis, 13 - V. jugularis ext., 14 - V. facialis, 15 - V. submentalis modified from Pouchet (1874).

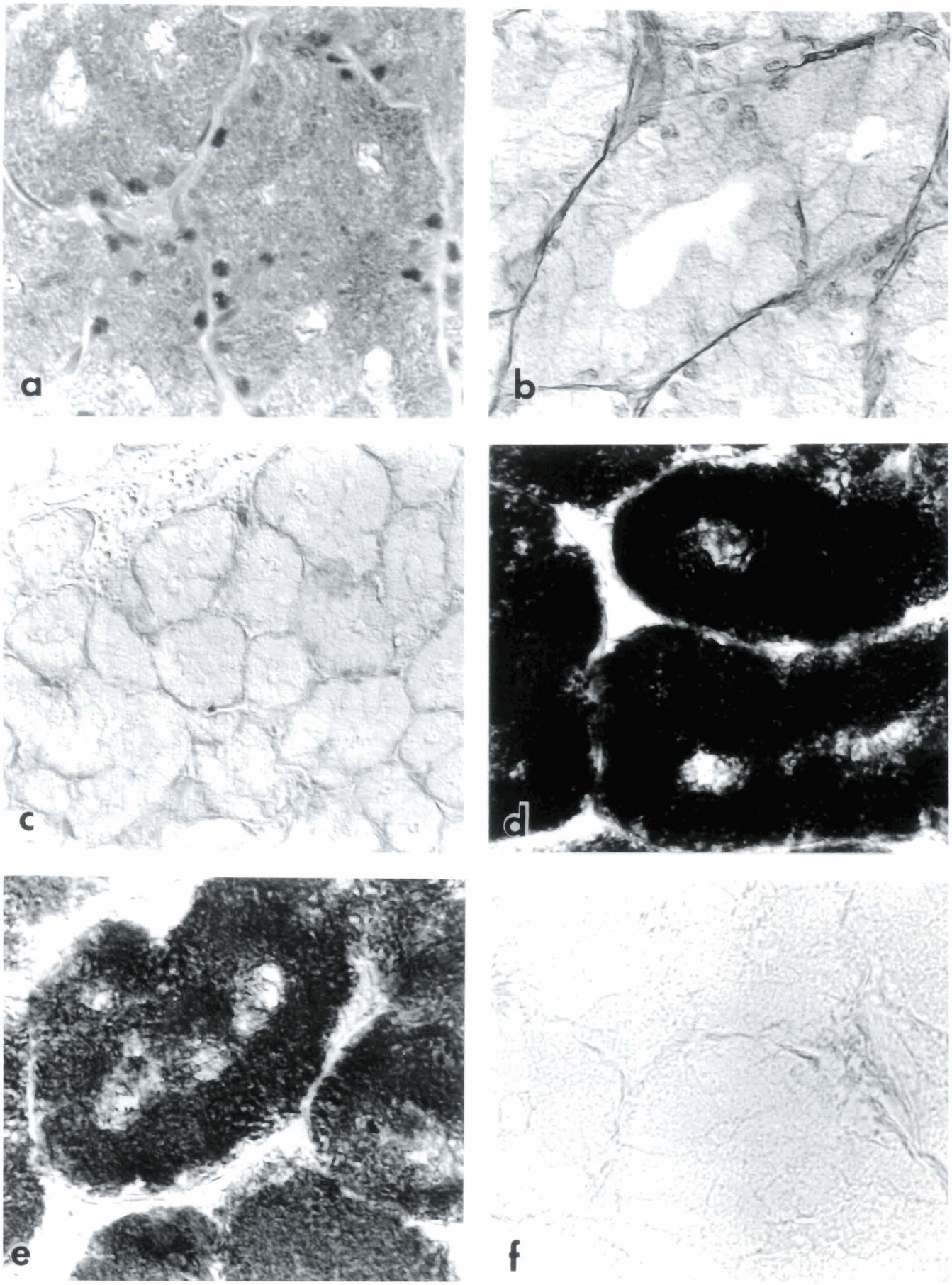

Fig. 2. Gl. submandibularis; **a** - homogeneous cytoplasm of seromucous secretory cells, H.E./Technovit, x 600; **b** - connective tissue sheath of secretory acini, silver impregnation/Technovit, x 580; **c** -tetrazonium reaction for proteins, x 240; **d** - AB (pH 2.5)-PAS, x 580; **e** - AB (pH 2.5), x 570; **f** - sialidase/AB (pH 2.5) x 520

Table 1. Carbohydrate histochemical reactions in the Glandula submandibularis of the Giant Ant-eater.

REACTIONS	SECRETORY ACINI		INTERCALATED DUCTS		INTRALOBULAR DUCTS		INTERLOBULAR DUCTS		CONNECTIVE TISSUE	VASCULAR ENDOTHELIUM	INHIBITORY SUGAR
	Seromucous cells	Luminal secretion	Epithelial cells	Luminal secretion	Epithelial cells	Luminal secretion	Epithelial cells	Luminal secretion			
PAS	4	3-4	3-4	3	2-3	3	2-3	3	2-3	2-3	
AB (pH 1.0)	1-2	1	0-1	1	0-1	1	0	1	2	0	
AB (pH 2.5)	3	3	2-3	3	2	3	2	3	0-1	0	
AB (pH 2.5)-Sial	0	0	1-2	0	0	1	0	0	0	0	
AB (pH 2.5)-PAS	5	3-4	3	3	2-3	3	2-3	3-4	2-3	2-3	
Amyl-PAS	4	3-4	3-4	3	2-3	3	2-3	3	2-3	2-3	
TZ	1	1	2	1-2	2	1-2	2	2	2-3	2	
PO-ConA-DAB	0-1	1	0-1	1	0	0-1	2-3	2-3	3-4	3	D-Man
PO-WGA-DAB	2	3*	1-2	3*	1-2	3*	3	3	3-4	3	D-GlcNAc
PO-WGA-DAB-Sial	3	3	1	2	2	3	2-4	3-4	4	3	
PO-GSA-II-DAB	1	2-3*	1	2-3*	1	2*	2-3	2-3	3	2-3	D-GlcNAc> D-Glc=D-Man
PO-DBA-DAB	1	3-4*	0-1	3	0-1	3	0-1	3	3-4	2	D-GalNAc
PO-DBA-DAB-Sial	2-3	4	2	3	1	3	1	3	4	3	
PO-RCA-II-DAB	2-3	4	2	3	0-1	3	3	3-4	4	3-4	D-GalNAc
PO-SBA-DAB	1	2*	1	2*	1	2*	2-3	2-3	2-3	2	D-GalNAc= D-Gal
PO-SBA-DAB-Sial	1-2	3-4	1	3	1	3	2	3	4	3	
PO-MPA-DAB	2	2-3*	1-2	2-3*	2-3	3	3-4	3	3	2	D-Gal
PO-MPA-DAB-Sial	2	4	1	3	2	3	3	4	4	4	
PO-GSA-I-DAB	0	0-1	0	0-1	0	1	1-2	1-2	1-2	1-2	D-Gal
PO-PNA-DAB	0-1	2-3*	0-1	2-3*	0-1	2-3*	0-1	2-3	3	3	D-Gal
PO-PNA-DAB-Sial	1	4	1	3	1	3	1	3	3-4	4	
PO-RCA-I-DAB	1-2	2-4*	1	2-4*	1	2	2-3	2	3	2-3	D-Gal
PO-UEA-I-DAB	0	0	0	0	0	0	0	0	1	0	L-Fuc
PO-LPA-DAB	0	0	0	0	0	0	0-1	0-1	1-2	1	NeuNAc> D-GlcNAc
PO-LPA-DAB-Sial	0	0	0	0	0	0	0	0	0	0	
PO-LFA-DAB	1-2	3-4*	1-2	2-3	1-2	2-3	1-3	2-3	3-4	1	NeuNAc= NeuNGc
PO-LFA-DAB-Sial	0	0	0	0	0	0	0	0	0	0	

0 = no reaction, 1 = very weak, 2 = weak, 3 = moderate, 4 = strong, 5 = very strong, * : luminal surface.

filled with numerous fine cytoplasmic granules (Fig. 2a); thus the structure was rather uniform. The glandular duct system varied considerably from very short intercalated ducts, small intralobular and wider interlobular ducts, to main excretory channels of enormous size, exhibiting a gradual change in epithelial structure from simple columnar to a stratified columnar type with a thick envelope of connective tissue. In addition, the glands showed a distinct system of interseptal connective tissue to serve as a tough frame-work of support for the otherwise very soft, pliable glands (Fig. 2b).

The Gl. buccalis was a compound tubulo-acinar mixed gland; i.e., it contained seromucous acini. Here, the serous cells, showing several cytoplasmic granules and a round nucleus, lay at one end of the acinus as demi-lunes between the basal membrane and the pyramidal mucous cells, with a light cytoplasm and a very flat nucleus (Fig. 4a). The excretory duct system was comparable to that of the submandibular gland, although clearly less voluminous in the inter-lobular/excretory part. The connective tissue system was of a very fine type.

Carbohydrate Histochemistry

The series of selected carbohydrate histochemical methods used made it possible to demonstrate varying distribution patterns of glycoconjugates in both types of glands studied. One of the few common features found was that the luminal secretions exhibited distinctly positive reactions in the glandular acini as well as in the duct system. Additionally, the secretory cells and the luminal secretion were generally strongly coloured with the PAS and AB-PAS procedures, whereas the reaction for the different PO-lectins employed offered a broader spectrum of reaction intensities.

Gl. submandibularis

The results obtained for the submandibular glands are summarized in Table 1 and Figs. 2, 3. The seromucous cells were strongly and uniformly stained deep red or purple after PAS and AB (pH 2.5)-PAS (Fig. 2d), respectively, moderately after AB (pH 2.5) (Fig. 2e), or only weakly with AB (pH 1.0) procedures. Sialidase digestion abolished any staining of the seromucous cells (Fig. 2f), whereas amylase digestion failed to alter the PAS-reaction intensity in these cells. A similar but somewhat weaker reaction pattern was observed in the luminal secretion of the secretory acini. The TZ method produced a very weak staining through all the acini (Fig. 2c).

The different PO-lectin procedures caused a varying pattern of reaction intensities in the seromucous cells.

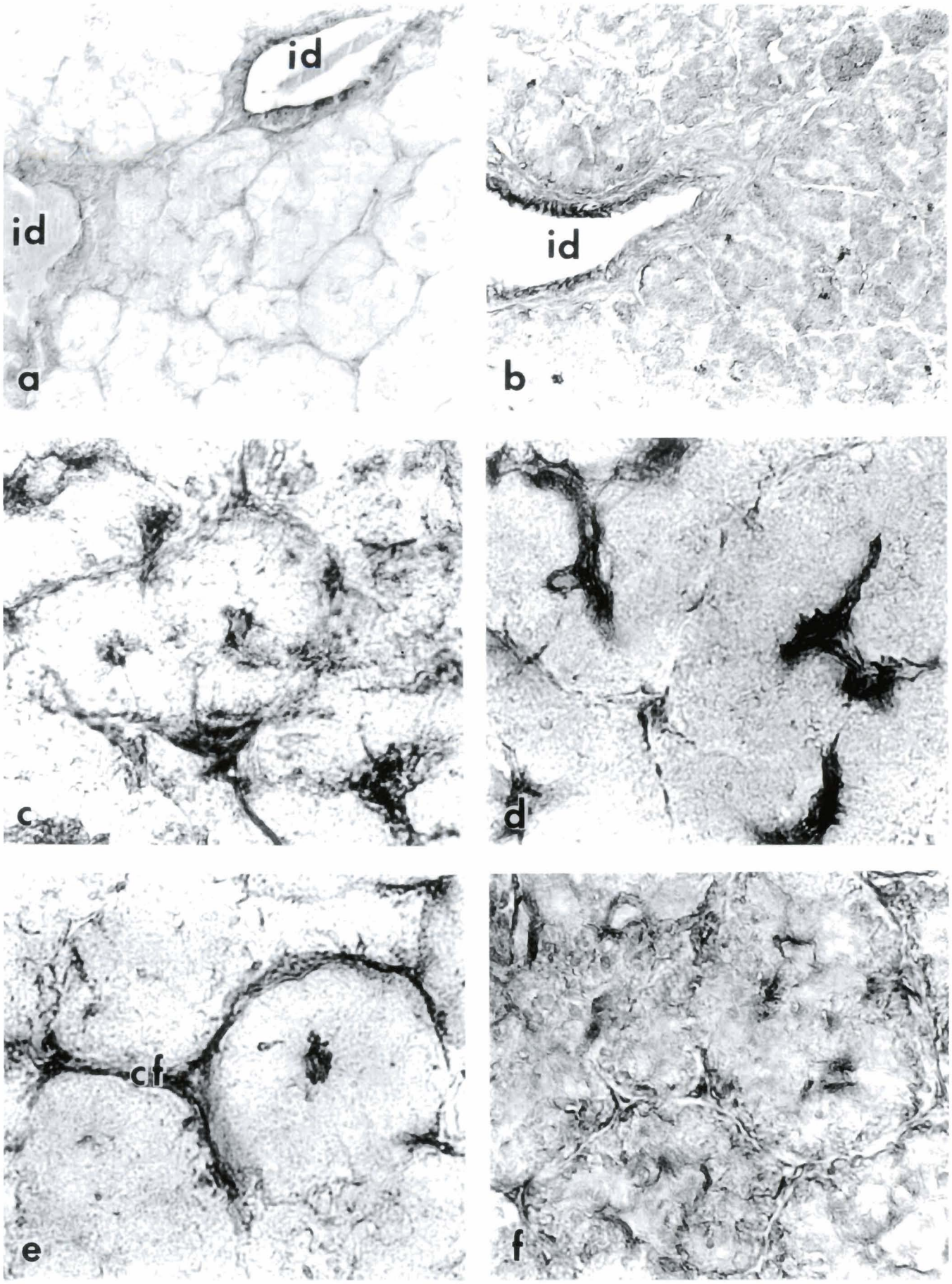

Fig. 3. Gl. submandibularis, reactions for peroxidase-coupled lectins (PO-lectins); **a** - Con A, x 220; **b** - sialidase/WGA, x 230; **c** - RCA-II, x 580; **d**- sialidase/SBA, strong reaction in acinar luminal secretion, x 590; **e** - sialidase/PNA, strong reaction in connective tissue fibres, x 570; f -LFA, x 570; id = interlobular duct, cf = connective tissue fibre.

Table 2. Carbohydrate histochemical reactions in the Glandula buccalis of the Giant Ant-eater.

REACTIONS	SECRETORY ACINI			INTERCALATED DUCTS		INTRALOBULAR DUCTS		INTERLOBULAR DUCTS		CONNECTIVE TISSUE	VASCULAR ENDOTHELIUM	INHIBITORY SUGAR
	Mucous cells	serous cells	Luminal secretion	Epithelial cells	Luminal secretion	Epithelial cells	Luminal secretion	Epithelial cells	Luminal secretion			
PAS	5	2-3	4	3	4	2-3	3-4	2-3	3-4	3	2-3	
AB (pH 1.0)	1-3	0	2	1-2	0-2	1-2	2	1-2	2	2-3	1-2	
AB (pH 2.5)	3-4	0	2-4	2-3	2-4	0-1	2-4	0-1	2-4	1	0	
AB (pH 2.5)-Sial	1-2	0	1	2	1-2	0	1-2	0	1-2	0	0	
AB (pH 2.5)-PAS	5	2-3	3-4	2-3	3-4	2-3	3-4	2-3	3-4	3	2-3	
Amyl-PAS	4	2-3	4	2-3	4	2-3	3-4	2-3	3-4	3	2-3	
TZ	1	3	1-2	2-3	2-3	2-3	3	2-3	3	2-3	2-3	
PO-ConA-DAB	0	3	0-3*	0-2	1-2*	1-2	1-2*	2-3	2-3	3	3	D-Man
PO-WGA-DAB	1-2	1-2	1-2*	1	1	1	1	2-3	3-4	4	2-3	D-GlcNAc
PO-WGA-DAB-Sial	2-3	1-2	3	1	2	1	2	3	4	3-4	3	
PO-GSA-II-DAB	0	1	0-1	0-1	1*	0-1	0-1*	2	2-3*	2-3	2	D-GlcNAc> D-Glc=D-Man
PO-DBA-DAB	0	2-3	1-2	1	1-2	1	1-2	2-3	2	3-4	2-3	D-GalNAc
PO-DBA-DAB-Sial	1	2	3	2	3	2-3	3	3	3-4	3	3	
PO-RCA-II-DAB	2	3	3*	1-2	2-3*	1-2	2-3*	2-3	2-4	3-4	2-3	D-GalNAc
PO-SBA-DAB	1	1	0-1	0-1	0-1	0-1	0-1	1-2	2-3*	2	1-2	D-GalNAc= D-Gal
PO-SBA-DAB-Sial	1	2	3	3	3	3	3	3	4	3-4	3	
PO-MPA-DAB	2	2-3	2-3*	1-2	1-3*	1-2	1-3*	3	2-4	3-4	2-4	D-Gal
PO-MPA-DAB-Sial	1	4	4	3	3	3	4	3	4	4	3	
PO-GSA-I-DAB	0	1	0-1	0	0-1*	0	0-1	1-2	0-1	2-3	2-3	D-Gal
PO-PNA-DAB	2	3	3-4*	1-2	2-3*	1-2	3*	2-3	3-4	2	2	D-Gal
PO-PNA-DAB-Sial	1	3	4	2	4	3	4	3	4	3	3	
PO-RCA-I-DAB	2	1	1-2*	1-2	2*	1-2	2*	1-2	2	2-3	2	D-Gal
PO-UEA-I-DAB	0	1	0-1	0-1	0-1*	0-1	0-1*	0-1	1*	1	1	L-Fuc
PO-LPA-DAB	0-1	1	0-1	0-1	0-1*	0-1	0-1	1	1	1-2	1	NeuNAc> D-GlcNAc
PO-LPA-DAB-Sial	0	0	0	0	0	0	0	0	0	1	0	
PO-LFA-DAB	0-1	2-4	2-3	1-2	2	1-2	2	1-2	2-4	1-2	1	NeuNAc= NeuNGc
PO-LFA-DAB-Sial	0	0	0	0-1	0	0	0	0	0	0	0	

0= no reaction, 1 = very weak, 2 = weak, 3 = moderate, 4 = strong, 5 = very strong, *: luminal surface

Clearly positive reactions were only obtained for RCA-II (Fig. 3c), and for WGA after sialidase digestion (Fig. 3b); the other lectins reacted very weakly to weakly (Fig. 3a). After RCA-II staining the cellular cytoplasm contained very prominent positively reacting granules. In the stored luminal secretions, especially at the luminal surface, somewhat stronger staining could be discerned, in particular for WGA, GSA-II, DBA, RCA-II, MPA, PNA, RCA-I, and LFA (Fig. 3f). Here sialidase digestion prior to staining intensified the reactions for WGA, DBA, SBA, MPA, and PNA (Figs. 3b,d,e). Sialidase digestion generally abolished colouring with LFA.

The epithelial cells and the luminal secretion of the excretory duct system normally showed a distinct colouring only in the intercalated ducts (PAS, AB (pH 2.5)-PAS); positive PO-lectin reactions were of weak to moderate intensity. Stronger lectin staining was confined to the luminal secretion and/or the luminal surface, as, for example, with RCA-I (intralobular ducts) or RCA-II (interlobular ducts).

The tough connective tissue between the glandular lobules and around the glands was stained with several PO-lectins, such as Con A, WGA, DBA, RCA-II, and LFA. Sialidase digestion increased the reaction intensities for WGA, DBA, MPA, PNA (Fig. 3e), and eliminated LFA staining.

The vascular endothelium in vessels of every calibre only stained intensely with RCA-II, and after sialidase digestion with MPA and PNA.

Gl. buccalis

The different histological components of the buccal glands also reacted somewhat differently, depending on the carbohydrate histochemical procedures employed. The results for this gland type are summarized in Table 2 and Figs. 4, 5. In the mucous cells the most prominent feature was a very strong and homogeneous PAS-staining, comparable to the very intense magenta colour after the AB (pH 2.5)-PAS procedure (Fig. 4c). AB (pH 2.5) also produced a distinctly positive colouring (Fig. 4e), while the AB (pH 1.0) reaction varied from very weak to moderate intensity (Fig. 4d). After sialidase digestion, the positive AB colouring was clearly weaker (Fig. 4f), whereas amylase digestion did not generally diminish the PAS staining intensity. The TZ method resulted in only very weak reactions (Fig. 4b). The PO-lectin procedures obviously failed in the differentiation of the mucous cells, in that only very weak to weak stainings could be obtained (Figs. 5a,c,d). Only after sialidase digestion was the WGA reaction somewhat stronger (Fig. 4b).

The serous cells (demi-lunes) showed weak to moderate colouring after the PAS, or AB (pH 2.5)-PAS

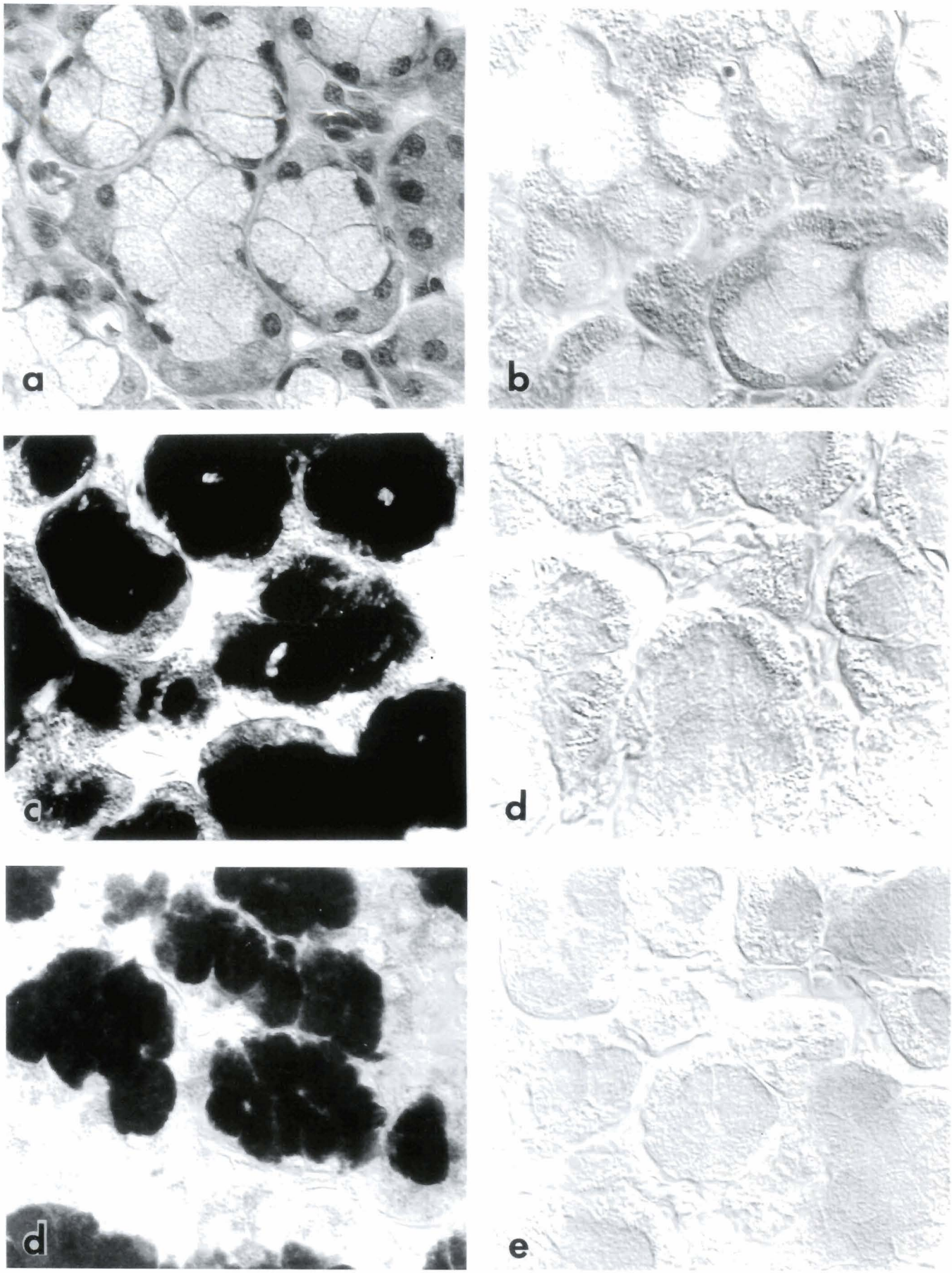

Fig. 4. Gl. buccalis; **a** - clear differentiation of peripheral serous demi-lunes and central mucous cells in secretory acini, H.E./Technovit, x 590; **b** - tetrazonium reaction for proteins, x 610; **c** - AB (pH 2.5)-PAS, x 590; **d** - AB (pH 1.0), x 580; **e** - AB (pH 2.5), x 550; **f** - sialidase/AB (pH 2.5), x 600

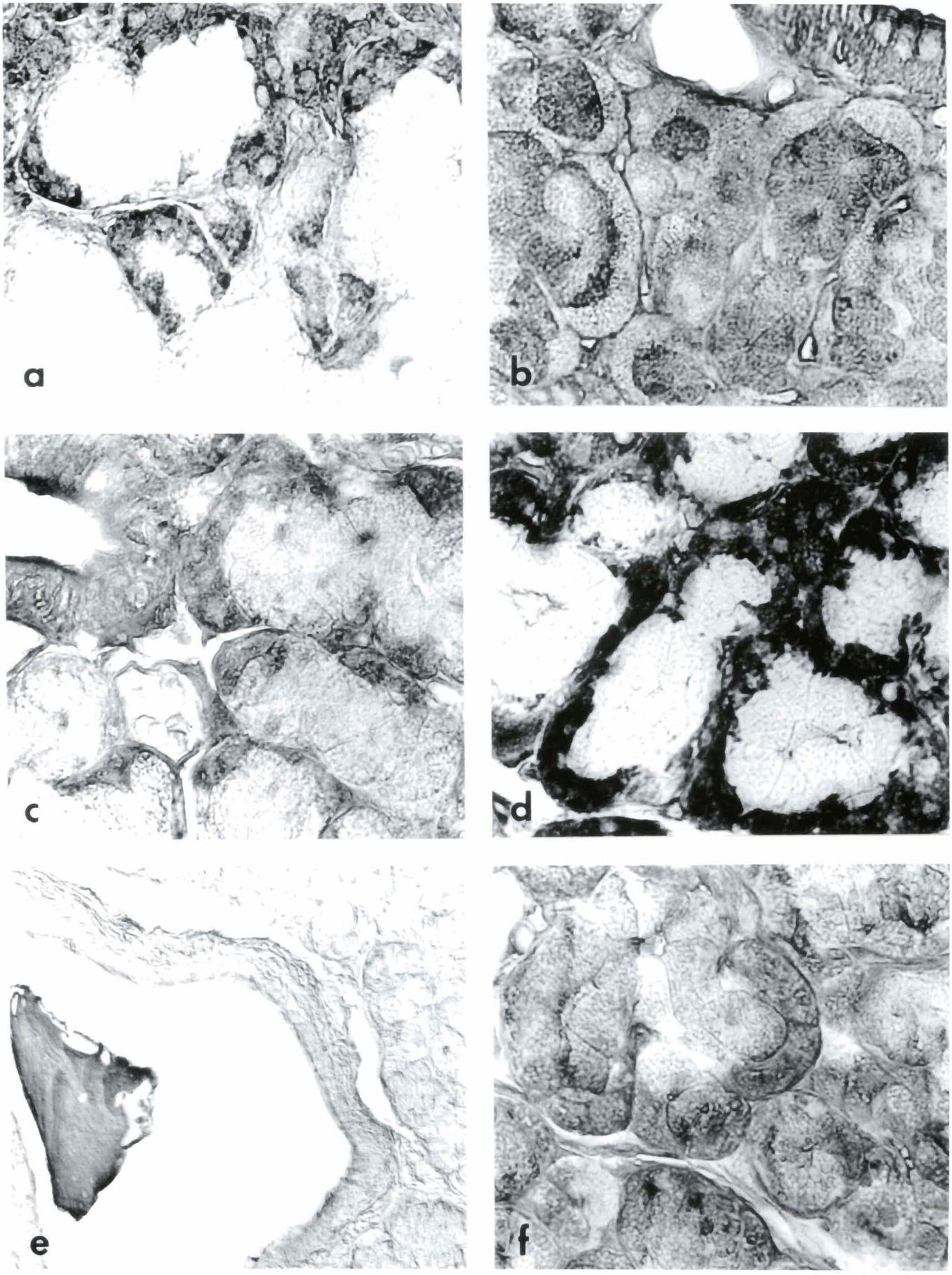

Fig. 5. Gl. buccalis, PO-lectin reactions; **a**-Con A, distinct reactions only in serous cells, x 510; **b** - sialidase/WGA, clear reaction in mucous cells, x 570; **c** -RCA-II, x 580; **d** - sialidase/MPA, strong reaction in serous cells, x 560; **e** - PNA, clear reaction in luminal secretion of interlobular duct, x 230; **f** - LFA, reaction in serous cells, x 640

methods; no staining was evident after AB (pH 1.0) and AB (pH 2.5) (Figs. 4 c-e). The amylase digestion reduced the PAS reaction staining slightly, and the TZ method generally coloured the demi-lunes clearly (Fig. 4b). The PO-lectin reaction pattern here was characterized by a more intense staining for several of the lectins used, especially for Con A (Fig. 5a), DBA, RCA-II (Fig. 5c), PNA, and LFA (Fig. 5f). Sialidase digestion slightly intensified the PNA and MPA reactions (Fig. 5d), and eliminated LFA staining.

The luminal secretions of the secretory acini offered a varying reaction pattern according to the different substances elaborated by the two secretory cell types present. Most of the material was concentrated at the luminal surface, as could be observed after the PO-lectin procedures for Con A, WGA, RCA-II, MPA, PNA, and RCA-I.

The excretory duct system exhibited distinctly positive staining, but with the excretory ducts cells showing very weak to only moderate reaction, and a more intense colouring of the luminal secretion which reacted like that found in the secretory acini. Stronger staining was visible, however, in the luminal substances of the interlobular ducts, probably caused by a concentration of the material present (Fig. 5e).

The fine connective tissue system between the glandular parts showed positive but weak to moderate reactions for PAS, AB (pH 1.0), and AB (pH 2.5)-PAS. The PO-lectins used were especially distinct in colouring with WGA, DBA, RCA-II, and MPA. Sialidase digestion caused a slight intensification of the staining for DBA, SBA, or MPA, and abolished the weak LFA reaction.

The vascular endothelium reacted only weakly to moderately in all glandular parts. A stronger colour was only visible after the MPA procedure.

Discussion

The submandibular as well as the buccal salivary glands of the Giant Ant-eater *(Myrmecophaga tridactyla)* exhibited very strong to strong reactions for both neutral and acidic glycoconjugates in the seromucous (Gl. submandibularis) and mucous (Gl. buccalis) secretory cells, as demonstrated by the well established properties of the PAS, AB (pH 2.5)-PAS, and AB (pH 2.5) stainings. The serous cells of the buccal glands reacted rather weakly with these procedures. The results obtained for the submandibular glands, thus, were generally comparable to the findings of Quintarelli and Dellovo (1969) for the ant-eater, and to those of Schulte et al. (1985) for the ovine salivary glands. In connection with the TZ reaction, it was obvious that the serous cells contained highly concentrated glycoproteins. The different controls applied, generally emphasize the predominance of sialoglycoconjugates in the glandular cells and secretion, whereas glycogen was rarely found. This latter aspect probably indicates high metabolic activities throughout all the secretory acini.

Lectin histochemical differentiation of the glycoconjugates present in the secretory portions of the submandibular and buccal glands of the Giant Ant-eater corroborates, to a certain extent, the view discussed above. For example, in the submandibular glands of the species, removal of sialic acid imparted weak to strong affinity for PNA, demonstrating a variable content of the terminal disaccharide sialic acid-ß-Gal. Similar findings were reported from the ovine submandibular glands, but were seen, however, only in a minority of mucous cells and in serous cells (Schulte et al., 1985). Sialidase digestion also imparted strong staining of the seromucous cells with DBA, indicating the uniform presence of penultimate α-GalNAc. In mammalian submandibular salivary mucus, many saccharides seem to be O-linked to serine and threonine by N-acetyl-galactosamine, and may also contain - in addition to traces of mannose - galactose, fucose, N-acetylglucos-amine, and, in particular, sialic acids. With regard to these observations, and the fact that the proportion of residues of N-acetylgalactosamine and sialic acid is much greater in mucous than in serous saliva (Kornfeld and Kornfeld, 1980; Nakai et al., 1985; McMahon et al., 1989), the secretional properties of the seromucous acini of the submandibular glands of the Giant Ant-eater are clearly characterized. It has to be added that the relatively strong reactions for AB (pH 2.5) and AB (pH 2.5)-PAS, as well as the reactions for lectins binding to N-acetylglucosamine and N-acetylgalactosamine (WGA, GSA-II, DBA, SBA) at the luminal surface of the secretory cells of the submandibular glands of the Giant Ant-eater may be connected with proteoglycan secretion (hyaluronic acid, chondroitine sulphate ?), as demonstrated for the submandibular glands of rats (Cutler et al., 1987; Gremski and Cutler, 1991) or in humans (Harrison and Auger, 1991).

With regard to the buccal glands, the lectins employed confirmed results obtained from human salivary glands (Laden et al., 1984; Nakai et al., 1985), with a spectrum of saccharide residues (N-acetylglucosamine, fucose, ß-galactose) as typically found, however, exclusively in the serous cells of this salivary gland type in the Giant Ant-eater. These cells also exhibited remarkable reactions for sialo-glycoconjugates and sialic acids (N-acetylneuraminic acid, N-glycolylneuraminic acid, according to LFA, binding, see Miller, 1987; Spicer and Schulte, 1992), probably as part of acidic glycoproteins. In the mucous cells of the buccal glands, N-linked saccharides were weakly expressed, the alcian blue staining generally emphasized, as in the seromucous cells of the submandibular glands, the presence of proteoglycans (see also Spicer and Schulte, 1992).

The apical surface and cytoplasm of all cells lining the intra- and interlobular ducts showed a strong affinity for GSA-I, indicating terminal α-Gal residues. This lectin histochemical feature was also detected in ovine salivary glands (Schulte et al., 1985). In contrast to findings in the major salivary glands of other mammals, such as rodents (Schulte and Spicer, 1983, 1984), the intercalated duct cells, especially in the submandibular glands of the Giant Ant-eater, obviously contained

sialoglycoconjugates. This aspect correlates with the fact that fucose - a relatively hydrophobic sugar - seems to be lacking in these cells. Both observations may be related to an extraordinarily high fluid transport in the production of large amounts of saliva during the typical feeding mode of the animal (see below). Similar results are known from studies on the lectin pattern of bovine nasolabial glands, which also show high production rates of aqueous secretion (Meyer and Tsukise, 1989). Thus, possible relationships between glycoconjugate structure and cellular functions, and transepithelial transport of fluids and electrolytes are indicated (Davenport, 1971; Schulte et al., 1985).

Primarily, the submandibular glands of the Giant Ant-eater are the gland type producing enormous, or better, by far the largest amounts of saliva throughout all the mammalian groups. In this respect, the high contents of sialoglycoconjugates in the saliva may be of specific interest. Sialoglycoconjugates are typically present in the submandibular glands of several mammalian species (Quintarelli and Dellovo, 1969; Corfield and Schauer, 1982; for ungulates see Quintarelli et al., 1961; Tsuiki et al., 1961; Shackleford and Klapper, 1962; Pal and Chandra, 1979; Gottschalk, 1964; for insectivores and carnivores see Shackleford and Klapper, 1962). The submandibular glands and the saliva of the Giant Ant-eater, however, contain the relatively highest concentrations of sialic acids (about 8 mg/g, and 1.7-1.8 mg/ml, respectively) when compared to other mammals (Junqueira et al., 1967), but only low amounts of digestive enzymes (Junqueira et al., 1973). This may cause high viscosity, including a highly hydrated gel structure of the saliva (Gottschalk, 1960; Strous and Dekker, 1992), and is completed by the fact that neuraminic acids provide a considerable steric hindrance for the activity of proteolytic enzymes (Gottschalk, 1964). In this way, together with the high water-binding capacities of the neutral glycoconjugates present (Silberberg and Meyer, 1983), the typical saliva functions in the Giant Ant-eater may be aided (see below).

The tongue of the Giant Ant-eater forms a capture apparatus of approximately 50 cm in length, and is not typically sticky but just moistened by saliva (see e.g. Moeller, 1988). This aspect was already indirectly mentioned by Waterton more than 150 years ago (cited by Pouchet, 1874), who stated that «upon being dried, (the saliva) loses these qualities, and you can pulverise it between your finger and thumb...!». In view of the results of this carbohydrate histochemical study discussed previously, the high contents of salivary sialoglycoconjugates (proteoglycans, glycoproteins) are responsible for the non-sticky but moist appearance of the tongue. Taking into consideration that the tongue moves up to 160 times per minute in entrapping insects such as ants and termites (Moeller, 1988), it is reasonable to assume that the main function of saliva in the Giant Ant-eater must be one of an effective lubricant. This lubricant has to be produced in large amounts, since the Giant Ant-eater requires about 35,000 insects per day during normal feeding (Moeller, 1988).

Acknowledgements. The skillful technical assistance of Mrs B. Haßfurther and Miss C. Schmidt is gratefully acknowledged. We are also greatly indebted to Dr. W. Bartmann (Director, Tierpark Dortmund) for kindly supplying the animal studied.

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Accepted December 1, 1992