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SEM and carbohydrate histochemical aspects of the glands in the anal region of the pig

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Abstract

Investigated SEM structure and carbohydrate histochemistry of the perianal tubular skin glands and the anal glands (proctodeal glands) of 4 juvenile domestic pigs (30–40 kg). The tubular skin glands showed a secretory epithelium with apical cytoplasmic protrusions and large dilatations of the excretory ducts. The anal glands had no corresponding protrusions but the plasma membranes of the secretory cells were fenestrated.

The secretory cells and the luminal secretions of the glands contained neutral and acidic glycoproteins, with small amounts of sialic acid in the tubular skin glands and greater amounts of sialic acid in the anal glands. The reaction intensities of the PO-lectin-DAB procedures demonstrated varying amounts of particular saccharide residues, especially in neutral glycoproteins.

The results obtained indicate an apocrine secretory mechanism in the tubular perianal skin glands and an eccrine or a holocrine-apocrine mechanism in the anal glands. Both gland types obviously contain different spectra of mucus glycoproteins. The observations are discussed in relation to the territorial scent marking behaviour of the pig.

Introduction

In contrast to several other mammalian groups, the anal region of the pig does not show prominent glandular areas (e.g. circumanal gland, paranal sinus) (see e.g. Schaffer 1940; Ortmann 1960; Calhoun and Stinson 1976; Neurand and Meyer 1982; Starck 1982).

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The only glands found are the compound tubulo-alveolar anal glands (proctodeal glands), which can be detected in the whole zona columnaris ani, reaching proximally below the rectal lamina mucosa, and caudally near to the apocrine glands of the anal skin. The anal glands form small or large groups in the submucosa and between the bundles of the internal anal sphincter muscle. The short alveolar intercalary ducts lead into a longer excretory duct that shows several larger saccular dilatations (reservoirs) when passing through the muscle layers. The glandular alveoli have a thick membrana propria, and the secretory cells are iso- or highprismatic in shape, a feature which is related to their functional development. The alveoli are often surrounded by lymphatic tissue (Mladenowitsch 1907; Schaffer 1940; Ortmann 1960). Because of the paucity of glands in the porcine anal region, the morphology and function of the relatively large tubular apocrine glands of the sparsely haired perianal skin (zona cutanea ani) are also included in this paper.

The present study uses SEM as well as carbohydrate histochemical methods to define the type of secretion and the function of both gland types. The results are discussed in view of the fact that odours produced by the glands found in the mammalian anal region may be important in intraspecific communication (see e.g. Ralls 1971; Eisenberg and Kleiman 1972; Mykytowycz and Goodrich 1974; Albone 1977; Neurand and Meyer 1982).

Materials and methods

The skin specimens of the anal region were dissected from four juvenile domestic pigs (30–40 kg; German landrace). The tissue was fixed in Bouin's solution and 10 % formalin containing 2 % calcium acetate (Leppi 1968) for 48 hours at room temperature or 4 °C, embedded in paraffin (Histoplast), and cut at a thickness of 50–100 µm for SEM examination, and 6 µm for histochemical stainings.

For examination by scanning electron microscopy the thick (50–100 µm) sections were deparaffinized in several changes of 100 % xylene. Before further processing, the material was then air dried very carefully. Finally, the specimens were mounted on copper stubs using double sided adhesive tape, coated with a thin layer of gold-palladium in a sputter coater (Balzers BAE 120, FN 102) and viewed in a JEOL JSM-35L scanning electron microscope operated at 25 kV.

For histochemical purposes the 6 µm sections were stained with the following procedures: hematoxylin eosin (H.-E.); periodic acid-Schiff (PAS) (from Pearse 1968; Culling 1974); alcian blue (AB) (pH 1.0 and 2.5) (from Pearse 1968; Culling 1974); dialyzed iron-ferrocyanide (DI-FCY) (Yamada 1973); AB (pH 2.5)-PAS (from Culling 1974); peroxidase-labelled (PO)-lectin (LT) [concanavalin A (Con A), peanut agglutinin (PNA), Ricinus communis agglutinin (RCA-1), Ulex europaeus agglutinin (UEA-1), Dolichos biflorus agglutinin (DBA), wheat germ agglutinin (WGA), and Limulus polyphemus agglutinin (LPA)] diaminobenzidine (DAB) (Collard and Temmink 1974; Kiernan 1975; Yamada and Shimizu 1976, 1977, 1979; Stoward et al. 1980); and coupled tetrazonium (TZ) (from Pearse 1968).

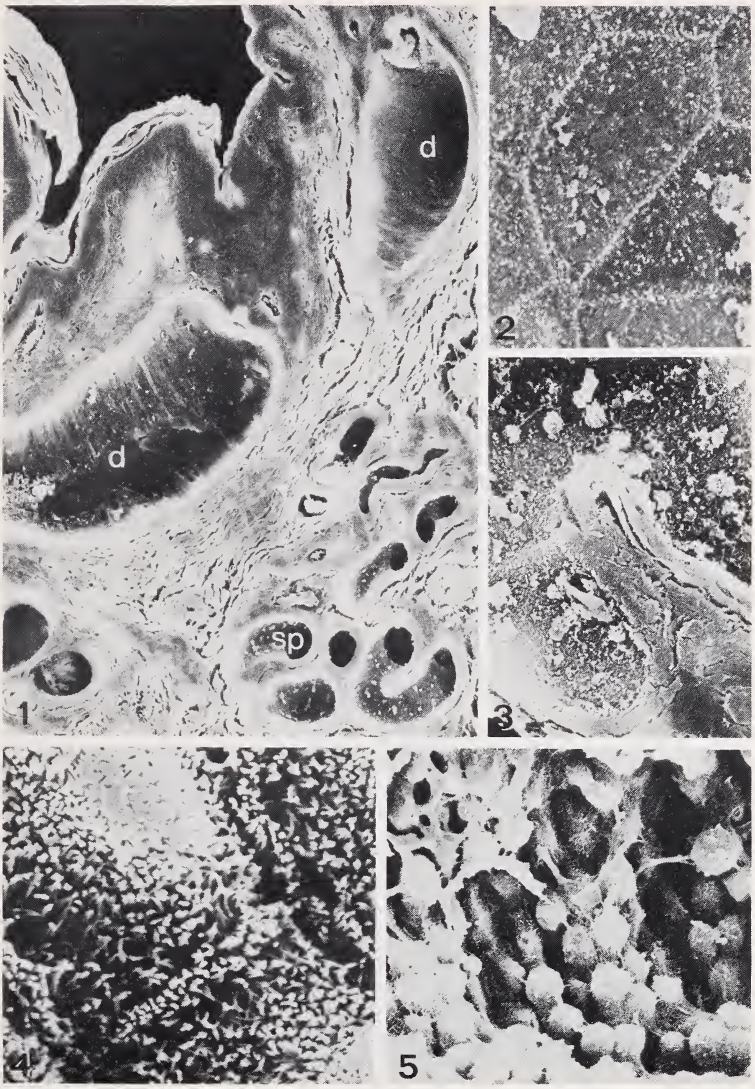
Histochemical control experiments: chemical modification procedures [active and mild methylations (AM, MM), saponification (AM–S, MM–S), sulphation (SUL)] and enzyme digestion procedures [with α-amylase, sialidase, and hyaluronidase (AMYL-, SIAL-, HYAL-)] were performed as described by Pearse (1968), Culling (1974), Tsukise and Yamada (1981), and Meyer and Neurand (1981); lectin controls were performed according to Tsukise and Yamada (1981) and Tsukise and Meyer (1983).

Results

SEM-observations

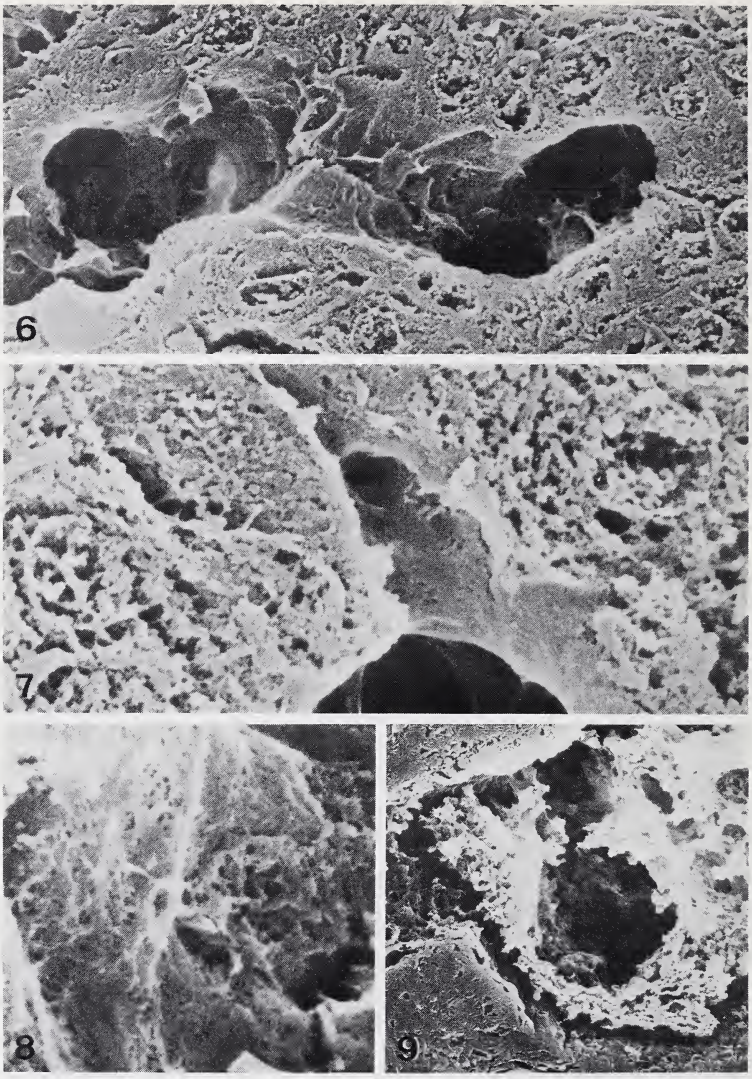
The tubular glands of the perianal skin of the pig (see figs. 1–5) showed a secretory epithelium whose cells had well-rounded apical cytoplasmic protrusions. The luminal plasmalemma of these cells was always densely studded with microvilli. The glands normally had large saccular dilatations of the excretory ducts which often lay closely beneath the epidermis. The dilatations were lined by flat luminal cells of a pentagonal or hexagonal shape, and the surface of these cells exhibited numerous short microvilli.

The epithelial cells of the secretory portions of the anal glands (proctodeal glands) of the pig (see figs. 6–9) did not show definite apical cytoplasmic protrusions. Their luminal



Figs. 1–5. SEM of the tubular apocrine glands of the perianal skin of the pig. *1:* overall view with secretory portions (sp) and saccular dilatations (d) (× 130); *2:* luminal surface cells of the dilatations (× 270); *3:* secretory portion (× 670); *4:* luminal surface of secretory cells with microvilli, beginning of the macro-apocrine secretion (× 600); *5:* macro-apocrine protrusion of secretory cells (× 900)

plasmalemma presented only a few short microvilli, but was generally distinctly fenestrated. Sometimes the secretory cells had lost large parts of the cytoplasm, and only the basal nucleus remained. In addition, the secretory epithelium showed empty pits, as if single cells had been discharged completely. The dilatations of the excretory ducts were lined by flat cells covered with several short microvilli.



Figs. 6–9. SEM of the anal glands of the pig. *6:* overall view of alveolar secretory portion (× 1650); *7:* secretory epithelial cells with empty cavity of discharged cell (× 4200); *8:* fenestrated luminal plasmalemma of secretory cells (× 8000); *9:* saccular dilatations with secretion (× 400)

Histochemical observations

The tubular apocrine glands of the perianal skin and the anal glands of the pig generally showed vivid positive reactions when tested for different carbohydrate groupings (table, figs. 10–17 and 18–24). These reactions were strongest in the luminal secretion, especially in that of the saccular dilatations (reservoirs) of the excretory ducts, but also in the epithelial cells, where the cytoplasm contained several stained granules. Strongly positive staining could also be seen in the secretion found between the lamellae of the stratum corneum and on the skin surface. The connective tissue sheath of both gland types reacted only very weakly to moderately for complex carbohydrates.

The secretory cells and the luminal secretion reacted positively for PAS as well as AB (pH 1.0 and 2.5), DI-FCY, and AB (pH 2.5)-PAS. Enzyme digestion controls showed that the intensity of the AB (pH 2.5) reaction was weaker after sialidase, but was not noticeably weaker after hyaluronidase digestion. α-amylase digestion did not diminish the intensity of PAS staining. Mild and active methylations weakened all AB stainings, while sulphation increased the intensity of the AB (pH 1.0) reaction.

The secretory epithelial cells and the luminal secretions showed positive but varying staining intensities following the application of lectins. In these cells and secretions of the apocrine perianal skin glands, relatively strong reactions were confined to PO- Con A-, -RCA-1-, -WGA-, and -DBA-DAB, while in the anal glands moderate to strong reactions were only recognized after PO- Con A-, -RCA-1-, -WGA-, and -LPA-DAB reactions. In the control experiments for PO-lectin-DAB staining procedures, the addition of particular saccharides to the PO-lectin solution diminished greatly or nearly abolished the positive PO-lectin reactions throughout all the tissue structures examined.

The tetrazonium reaction was strongest in the secretory cells and was somewhat weaker in the luminal secretions of both gland types.

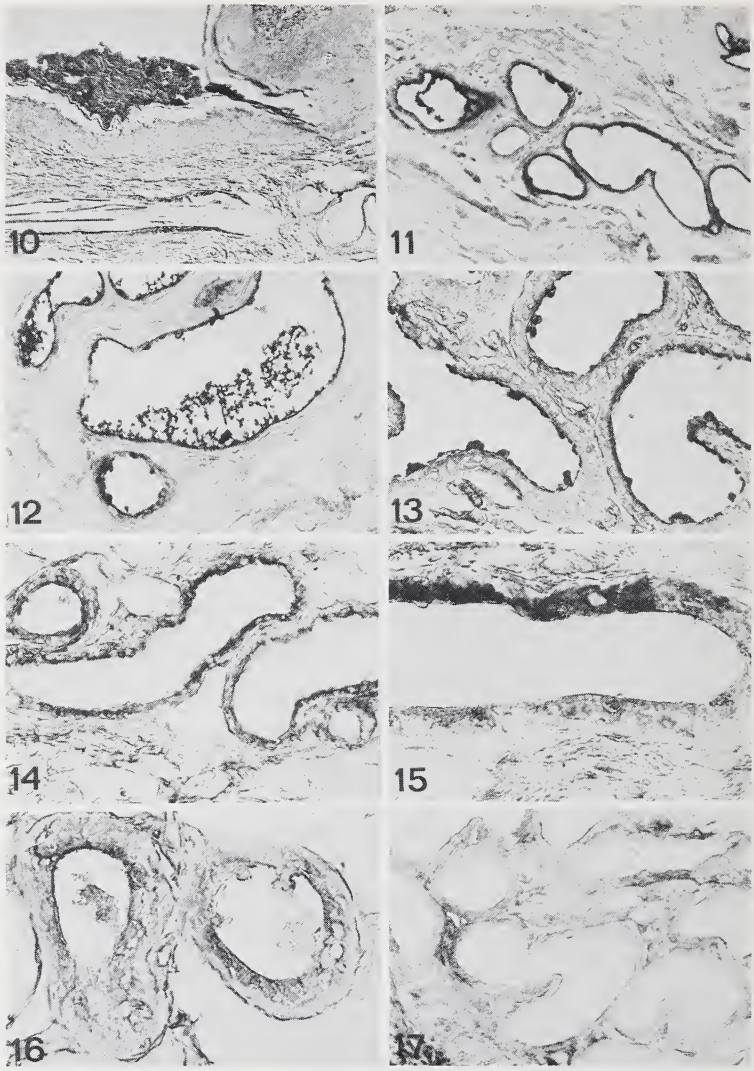
The epithelial cells of the saccular dilatations (reservoirs) of the excretory ducts generally reacted only very weakly to weakly for the histochemical procedures applied.

Table

Carbohydrate histochemical reactions in the glands of the anal region of the pig

Staining reactions	Apocrine perianal skin glands – secretory portion		Anal glands – secretory portion	
	secretory epith. cells	luminal secretion	secretory epith. cells	luminal secretion
PAS	+/++	++/+++	+++	++/+++
AB (pH 1.0)	+/++	++/+++	+/++	++/+++
AB (pH 2.5)	+/++	++/+++	++/+++	++/+++
DI-FCY	++	+++	++/+++	+++
AB (pH 2.5)-PAS	++/+++	++/+++	++/+++	++/+++
AMYL-PAS	+/++	++/+++	++/+++	++/+++
SIAL-AB (pH 2.5)	+	++	+	+
HYAL-AB (pH 2.5)	+/++	++	++/+++	++/+++
MM-AB (pH 1.0)	+	+	+	+/++
MM-AB (pH 2.5)	+	++	+	(+)
AM-AB (pH 1.0)	(+)	(+)	(+)	(+)
AM-AB (pH 2.5)	+	+	+	+
MM-S-AB (pH 1.0)	+	++	+/++	++
MM-S-AB (pH 2.5)	+	++/+++	+	+/++
AM-S-AB (pH 1.0)	−	+	−	(+)
AM-S-AB (pH 2.5)	+	++	+	++
SUL-AB (pH 1.0)	++	+++	++	+++
PO-Con A-DAB	++/+++	++/+++	++	++
PO-PNA-DAB	+	+	+	+
PO-RCA-1-DAB	+/++	+/++	++/+++	++/+++
PO-UEA-1-DAB	(+)	(+)	+	++
PO-DBA-DAB	+/++	+/++	(+)	(+)
PO-WGA-DAB	+/++	++	++/+++	++/+++
PO-LPA-DAB	+	+	++	++
TZ	++/+++	++	++/+++	++/+++

Reaction intensities: (+) = very weak, + = weak, ++ = moderate, +++ = strong, − = no reaction visible. For abbreviations of staining reactions see Materials and Methods.

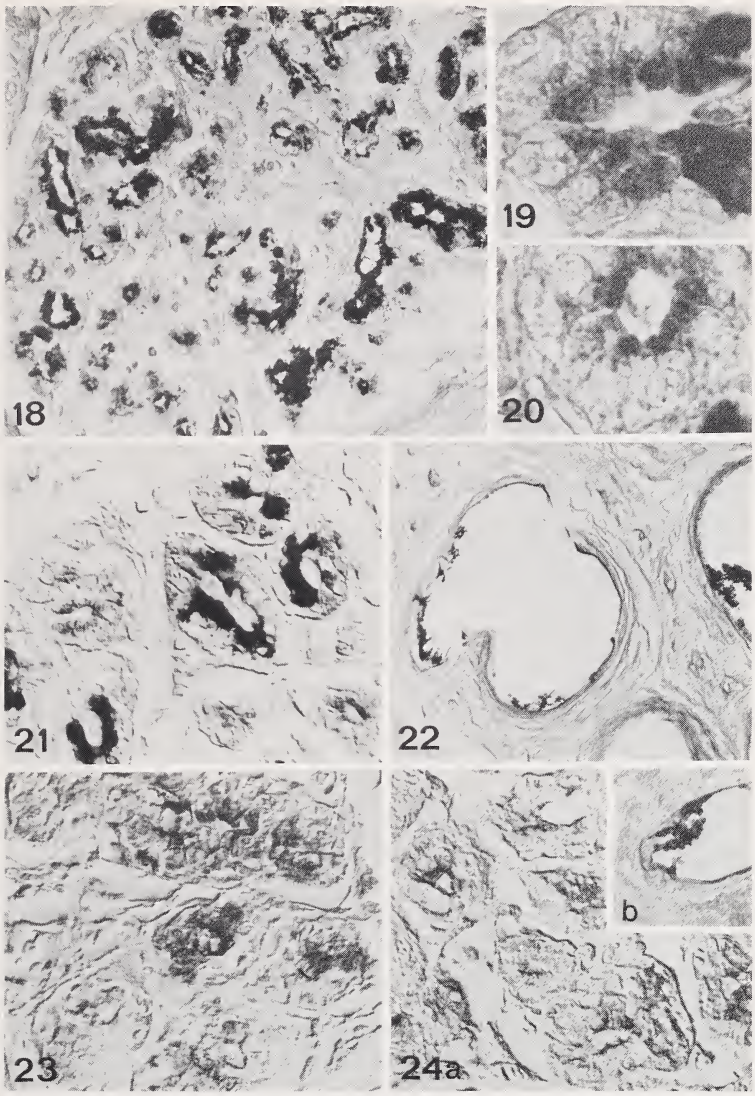


Figs. 10–17. Reactions for complex carbohydrates in the tubular apocrine glands of the perianal skin of the pig. *10:* strong positive reaction of secretion on the skin surface, DI-FCY (× 130); *11:* PAS reaction (× 120); *12:* AB-PAS reaction (× 170); *13:* PO-Con A-DAB (× 260); *14:* PO-RCA-1-DAB (× 200); *15:* PO-PNA-DAB (× 440); *16:* PO-DBA-DAB (× 450); *17:* PO-LPA-DAB (× 230)

Discussion

The SEM-methods used in the course of this study yielded satisfactory results where the surface morphology of the glandular epithelia was concerned. It was not necessary to apply critical point drying, but it was important that the material was allowed to air dry very slowly after xylene immersion (see also Meyer and Neurand 1982). Comparative observations have been reported by Liepins and De Harven (1978) using single cell layers.

Scanning electron microscopy of the tubular glands of the perianal skin of the pig



Figs. 18–24. Reactions for complex carbohydrates in the anal glands of the pig. *18–20:* strong positive PAS reactions in apical cytoplasm of secretory cells and in glandular secretion, *18:* × 170, *19–20:* × 1700; *21:* strong AB (pH 2.5) reaction (× 410); *22:* AB-PAS reaction in secretion of dilatations (× 260); *23:* PO-UEA-1-DAB (× 280); *24:* PO-WGA-DAB, a: gland (× 270), b: dilatation (× 250)

revealed typical cytoplasmic protrusions of the macro-apocrine type in the cells of the secretory epithelium. This type of secretion has also been observed in human axillary apocrine glands (Wilborn and Montes 1978; Inoue 1979) and in human ceruminous glands (Kurosumi and Kawabata 1976; Testa-Riva and Puxeddu 1980). In contrast to the findings on human apocrine sweat ducts (Kurosumi 1977), however, the luminal cells of the saccular dilatations of the tubular gland ducts clearly showed numerous microvilli.

Typical apocrine protrusions were not found in the secretory cells of the anal glands. Here the fenestrated luminal cellular plasma membrane probably indicates an eccrine

secretory mechanism while the loss of large amounts of cytoplasma and/or of whole cells may indicate a holocrine secretory mechanism. Thus, the SEM-results on the anal glands of the pig partly confirm the TEM observations of Kühnel (1971b) on the proctodeal glands of the rabbit. In our opinion, a final conclusion on the method of secretion of the anal glands seems to be very difficult to arrive at. The relatively high secretion rates of these glands also support the view of a very intensive apocrine secretion of holocrine character and/or the possibility of several secretory mechanisms occuring in one gland, with a change of mechanism with change in secretory rate (see also Braun-Falco and Rupec 1968; Schaumburg-Lever and Lever 1975).

The well established histochemical properties of PAS, AB (pH 1.0), AB (pH 2.5), DI-FCY, AB (pH 2.5)-PAS, TZ, and the controls applied, indicate that both neutral and acidic glycoproteins are present in the secretory cells and the luminal secretion of the gland types investigated. The acidic glycoproteins include small amounts of sialic acid in the apocrine perianal skin glands, and greater amounts of sialic acid in the anal glands. The controls digested with α-amylase reveal that the glands probably do not contain glycogen. This agrees with findings for the tubular apocrine glands of the hairy skin of the pig (Tsukise and Meyer 1983) as well as with those for the anal glands of other mammals (Bertolini et al. 1970; Kühnel 1971a; Meyer and Neurand 1981).

The distribution of complex carbohydrates in perianal apocrine glands is, to some degree, different from that of the hairy skin and snout of the pig, the glands of the perianal skin containing somewhat higher amounts of acidic glycoproteins (Tsukise and Meyer 1983; Tsukise et al. 1983). However, while scrotal skin glands contain no sialic acid (Tsukise and Yamada 1981), there are otherwise clear parallels with the results for perianal apocrine glands. Our results for pig anal glands are very similar to those reported for these glands in the dog (Meyer and Neurand 1981), though the pig glands show greater amounts of neutral and acidic mucus glycoproteins. In contrast to these observations, it was not generally possible to demonstrate these complex carbohydrates in the proctodeal glands of the rabbit (Bertolini et al. 1970; Kühnel 1971a).

The reaction intensities of the PO-lectin-DAB procedures demonstrated varying amounts of particular saccharide residues in the secretory cells and the luminal secretion of both gland types investigated. These findings confirm the presence of neutral glycoproteins, which usually contain: α-D-mannose, α-D-glucose, β-D-galactose, and N-acetyl-D-glucosamine. The positive reaction for LPA proves that sialic acid is also present. When the two gland types are compared it appears that there is a difference in the proportions of the saccharide components above mentioned, a result which may indicate that slightly different types of glycoprotein occur in the secretion of the different glands.

Our study supports the view that the apocrine glands in the perianal skin as well as the anal glands (proctodeal glands) of the pig produce considerable quantities of mucus containing both neutral and acidic glycoproteins. In relation to the apocrine perianal skin glands it seems important to note that this mucus is distributed as a distinct layer on the surface of this body region. Here it probably functions to prevent drying of the relatively sparsely haired perianal skin, and eventually opposes the proliferation of pathogenic microorganisms (see e.g. Jarrett 1980; Silberberg and Meyer 1982). The secretion of the anal glands may be of specific importance for the mucous moistening of the faeces (see also Mladenowitsch 1907; Grau 1935; Meyer and Neurand 1981).

It is possible that the mucus of both gland types is partly degraded by microorganisms in the saccular dilatations (reservoirs) of the glands. In this way volatile odorous substances can be released onto the anal skin and the faeces, which may have specific meanings in intraspecific communication, e.g. for territorial behaviour, as has been demonstrated by observations on the structure and function of glands in the anal region of other mammalian species (see e.g. Ralls 1971; Eisenberg and Kleiman 1972; Mykytowicz and Goodrich 1974; Mykytowicz et al. 1976; Hesterman et al. 1976; Albone 1977; Albone et al. 1978;

Meyer and Neurand 1981; Neurand and Meyer 1982). Territorial scent marking by deposition of faeces has been regularly observed in wild pigs (Meynhardt 1978). Domestic pigs obviously still show this behaviour, though in a somewhat irregular and less distinct manner (Altmann 1969; Signoret et al. 1975).

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Zusammenfassung

SEM und kohlenhydrathistochemische Aspekte der Drüsen in der Analregion des Schweines

Es wurden die rasterelektronenmikroskopische Struktur und die Kohlenhydrathistochemie der tubulösen Hautdrüsen des Perianalbereiches sowie der Analdrüsen (Protodealdrüsen) von 4 juvenilen Hausschweinen (30–40 kg) untersucht. Die tubulösen Hautdrüsen zeigten ein sekretorisches Epithel mit apikalen Zytoplasmaausstülpungen und große Erweiterungen der Ausführungsgänge. Den Analdrüsen fehlten entsprechende Ausstülpungen der sekretorischen Zellen, deren lumenständige Zellwände jedoch deutlich gefenstert waren.

Die sekretorischen Zellen und das Sekret im Lumen der untersuchten Drüsen enthielten neutrale und saure Glykoproteine mit einem geringen Anteil an Sialinsäuren in den tubulösen Hautdrüsen und einem höheren Anteil an Sialinsäuren in den Analdrüsen. Die Reaktionsintensitäten der PO-Lektin-DAB-Verfahren zeigten unterschiedliche Anteile an spezifischen Zuckerresten in den neutralen Glykoproteinen.

Die Resultate verweisen auf einen apokrinen Sekretionsmodus in den tubulösen Hautdrüsen der Perianalregion und auf einen ekkrinen oder holokrin-apokrinen Sekretionsmodus in den Analdrüsen. Die beiden Drüsentypen enthalten offenbar unterschiedliche Spektren an Mukusglykoproteinen. Die Beobachtungen werden in Beziehung zur Duftmarkierung im Rahmen des Territorialverhaltens der Schweine diskutiert.

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BEKANNTMACHUNG

Field Research in Madagascar

On 1 and 2 February, 1983, 26 biologists and conservationists from six different countries gathered in Jersey (British Isles) to discuss how foreign scientists could contribute to nature conservation in Madagascar. Also present was Mme. Berthe Rakotosamimanana, Director, Department of Scientific Research in the Ministry of Higher Education and Scientific Research of The Malagasy Republic.

The wishes of the Malagasy government are that individual scientists should not make application to conduct research directly to the Department of Scientific Research, but rather that applications be made through an external advisory group and that, although proposals on a wide and varied range of topics are welcome, each should be partially oriented towards nature conservation in Madagascar.

On the advice of the Malagasy authorities the participants to the meeting produced a formal document in conjunction with the Representative of World Wildlife Fund-International in Madagascar (whose on site conservation efforts are now official governmental policy), which describes how research by foreigners should aid nature conservation. The participants to the meeting compiled an international list of consultants with diverse fields of interest in Madagascar. From that list they elected seven biologists to form the International Advisory Group of Scientists (IAGS), whose duties (also described in the formal document) are to review and send on research proposals to Madagascar for final deliberation by the Department of Scientific Research.

The IAGS consists of established scientific investigators who are in a position to cooperate effectively in identifying the urgent conservation needs of Madagascar. The group is constituted for a period of three years from 1st March, 1983, the date that the Malagasy authorities signed this document. The IAGS does not provide financial assistance for research projects.

If you want more information about the constitution and duties of the IAGS or about how to apply to conduct research in Madagascar, please write to a member of the IAGS from your region:

Dr. Roland Albignac, Faculté des Sciences, Laboratoire de Zoologie et Ecologie Animale, Universite de Besançon, F-25000 Besançon, France.

Dr. Lee Durrell, Jersey Wildlife Preservation Trust, Les Augrès Manor, Trinity, Jersey, Channel Islands (via U. K.).

Dr. Alison Jolly, The Rockefeller University, 1230 York Avenue, New York, N. Y. 10021, USA.