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Lectin histochemistry of the interdigital gland in the Japanese serow (*Capricornis crispus*) in winter

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INTRODUCTION

The interdigital gland in the Artiodactyla varies in its morphology: in some animals the gland contains secreted material in a pouch and the hairs around its opening are wet with viscous fluid, but in others it appears only as a furrow (Pocock, 1910). The skin of a well-developed interdigital gland usually contains a mass of sebaceous or apocrine glands. The function of this specialised skin gland has not been established, in contrast to the scent-marking function of the infraorbital gland (Gosling, 1985), although it is speculated that the gland leaves a scent in trails (Quay & Müller-Schwarze, 1970; Mainoya, 1976; Langguth & Jackson, 1980).

The Japanese serow (*Capricornis crispus*) has well-developed interdigital glands in all four legs (Pocock, 1910). Unfortunately this author's observations were made on dried skin and his description of the glands of this animal was brief. Information on their detailed structure is needed in order to decide whether the interdigital glands of the Japanese serow function in scent-marking.

Histochemical studies of the interdigital glands of deer have used methods of lipid staining for the sebaceous gland component, periodic acid–Schiff (PAS) for glycoproteins and Ziehl–Neelsen for lipofuscin granules of the apocrine gland component (Quay, 1955; Chapman, 1985). Ookusa (1984) and Tsukise, Meyer & Ikeda (1985) have reported the presence of specific sugar residues in the sebaceous and apocrine glands of the skin by the use of lectin histochemistry. Atoji & Suzuki (1987) used this method to observe a secretory cycle of the apocrine gland and secretory contents in the infraorbital gland of the Japanese serow and suggested that carbohydrates may make an important contribution to the secretory fluid from the gland.

In the present study, we first examined the morphology of the interdigital gland and then applied lectin histochemistry to it in order to investigate the distribution and staining pattern of sugar-binding sites in the gland. A further aim of the study was to examine the gland in a fetus.

MATERIALS AND METHODS

A number of Japanese serows were killed each winter from 1980 to 1984 as part of a study on the conservation and control of this animal by the Agency of Cultural Affairs in Japan. Thirty two adult Japanese serows (16 males and 16 females) and a fetus (male) were selected; all the specimens were fresh or well-preserved until

dissection. The fetus was 29·5 cm in crown–rump length and estimated to be about 150 days of gestation on this basis (Sugimura *et al.* 1983).

After gross dissecting, the interdigital glands were photographed, drawn, and fixed in 10% formalin for microscopic observation. The skin around the middle phalanx was removed for comparison with the interdigital gland. Paraffin sections 5 μm thick were stained with haematoxylin and eosin, Azan or PAS.

Lectin histochemistry was conducted as follows. Seven biotinylated lectins, *Arachis hypogaea* (PNA), *Ricinus communis* I (RCA), *Dolichos biflorus* (DBA), *Glycine max* (SBA), *Triticum vulgaris* (WGA), *Canavalia ensiformis* (Con A), and *Ulex europaeus* I (UEA), were obtained from Vector Laboratories (USA). Paraffin sections were prepared and endogenous peroxidase was blocked by incubation with methanol containing 0·3% H_2O_2 before lectin staining. Sections were pre-incubated with 1% normal goat serum and then incubated with seven biotinylated lectins for 2 hours (Con A and WGA) or overnight (PNA, RCA, DBA, SBA and UEA) at 4 °C. The optimal concentration chosen for each lectin was 10 μg/ml (Con A and WGA) or 25 μg/ml (PNA, RCA, DBA, SBA and UEA). The sections were rinsed in 0·01 M phosphate-buffered saline (PBS) at pH 7·2 and incubated with avidin-biotin-peroxidase complex (ABC) (Vector Laboratories, USA) for 30 minutes at room temperature. After rinsing in PBS, the reaction product was visualised by incubation with PBS containing 3,3′-diaminobenzidine (25 mg/100 ml) and 0·003% H_2O_2 for 5–10 minutes, then washed in tap water and mounted. Controls were as follows: (1) oxidation with 1% periodic acid for 10 minutes before lectin staining; (2) substitution of unlabelled lectins for biotinylated or horseradish peroxidase (HRP) lectins; (3) exposure to biotinylated lectins and substrate ABC solution without avidin; (4) incubation with ABC solution or HRP alone; and (5) incubation with lectins containing 0·1 M *N*-acetyl-D-galactosamine (SBA, DBA), 0·1 M *N*-acetyl-D-glucosamine (WGA), 0·1 M L-fucose (UEA), 0·1 M D-galactose (PNA, RCA), or 0·1 M α-methyl-D-mannoside (Con A).

Histoplanimetry of the sebaceous and apocrine glands between the interdigital gland and digital surface skin was performed using a pattern analyser to compare the development in both sexes (6 males and 6 females). Each area of the glandular components was calculated in one mm length of skin surface on transverse sections.

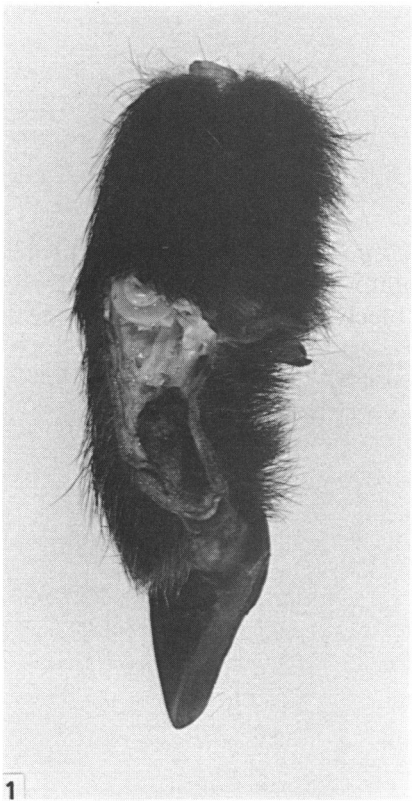
RESULTS

Gross anatomy

The interdigital gland was teapot-like in form and located between the digits of the fore- and hind-feet (Fig. 1). It was slightly larger in the former than the latter. The gland consisted of a large pouch (2·5–4·0 cm long, 1·4–2·0 cm wide) with a narrow duct (1·0 cm long). The duct started at the bottom of the pouch, bent dorsocranially and opened at the dorsal surface of the interdigital cleft which was covered with long hairs (Fig. 2). The wall of the gland was thin and folded, and a few scattered white short hairs grew on it. Brown or black mud was always found adhering to the inner surface of the interdigital gland and small twigs frequently accumulated in the pouch. No obvious secretion was observed in the pouch. There were no sex differences in the interdigital gland.

Microscopy

The skin of the interdigital gland was thin and was composed of the epidermis and subjacent connective tissue containing the sebaceous gland, apocrine gland, and hair



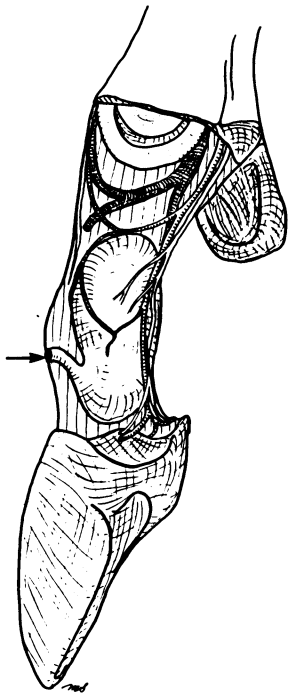


Fig. 1. Sagittal section of forefoot. The interdigital gland is bean-shaped and located at the level of the middle phalanx. A duct opens on the dorsal surface.

Fig. 2. Schematic drawing of the interdigital gland of forefoot. Arrow indicates the opening of the duct.

follicles (Fig. 3). The sebaceous gland was located more superficially than the apocrine gland and opened into the hair follicles. The volume of the sebaceous and apocrine glands in the interdigital gland was significantly less than those in the digital surface skin in both sexes ($P < 0{\cdot}01$) (Fig. 13), but there was no difference between fore- and hind-feet or between the sexes. The glandular tissue of the duct was basically similar in structure to that of the pouch but was slightly more abundant (Figs. 4, 5). Arrector pili muscles were rarely found under the epidermis.

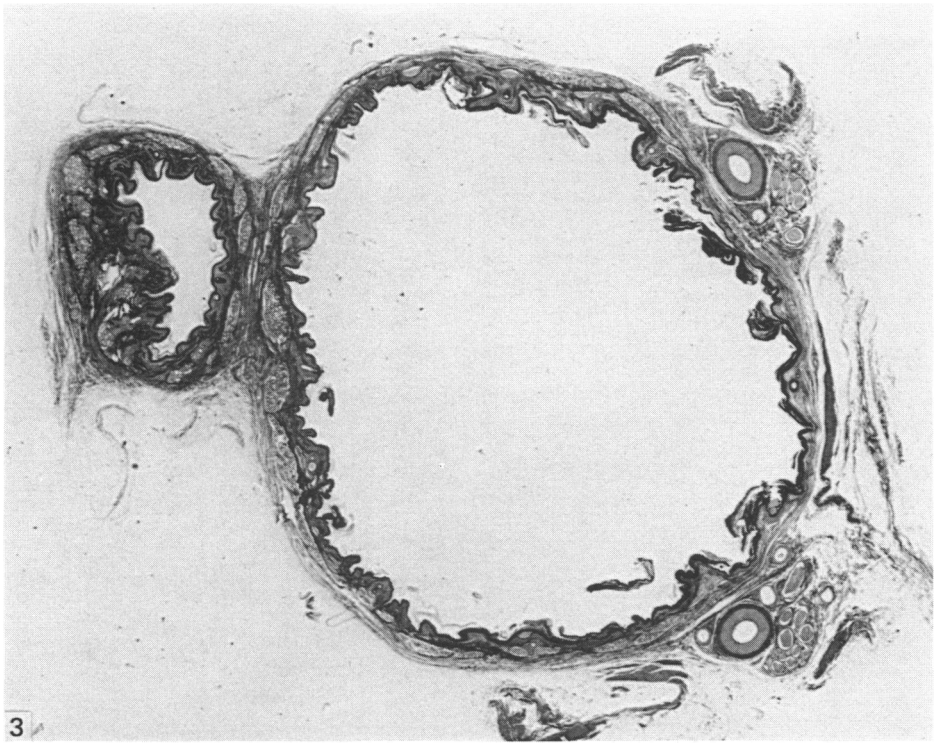
Lectin staining of the interdigital gland was similar to that of the digital surface skin except for staining with UEA (Table 1), and there was no staining difference between the duct and pouch. The staining sites of SBA lectin were located in the cytoplasm of the apocrine gland and epidermis and in the cell boundaries and the sponge-like cytoplasm of the sebaceous gland (Fig. 6). With Con A, nuclear envelopes were also stained. Differential staining that showed negative, partial or fully positive staining in the cytoplasm of different cells was clearly observed in the sebaceous glands with Con A and in the apocrine glands with PNA, SBA, DBA and Con A. With PNA, the apocrine gland showed two secretory modes, namely apocrine secretion (Fig. 7) and exocytosis (Fig. 8).

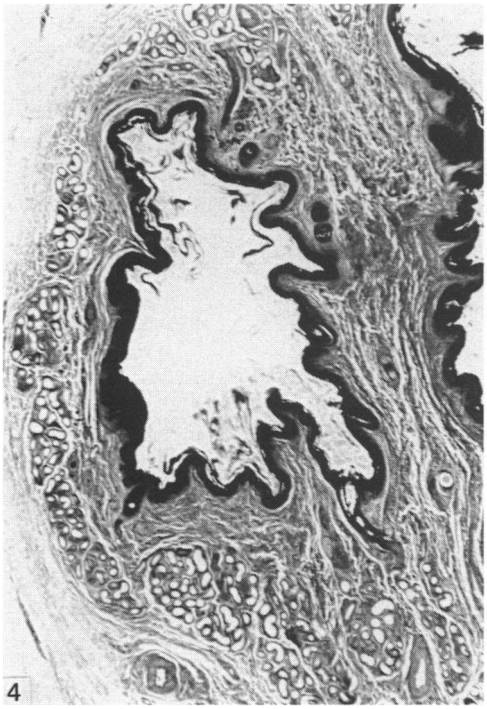
The apocrine gland in the interdigital gland was stained with UEA, but that in the

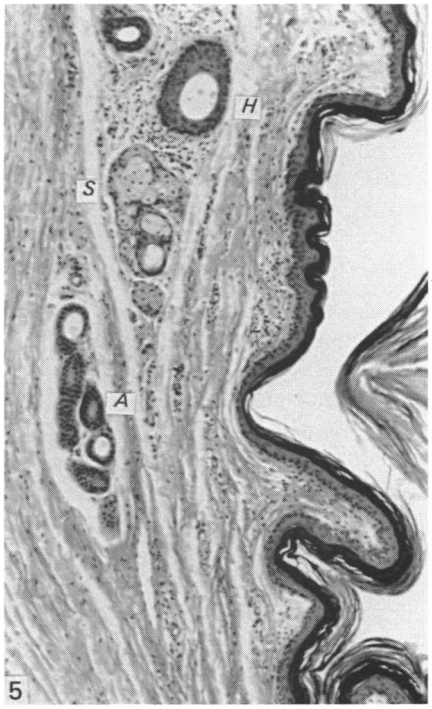
Table 1. *Lectin histochemistry of the interdigital gland and skin of the Japanese serow*

	Adult							Fetus			
	Interdigital gland				Skin			Interdigital gland		Skin	
	SG	AG	Secretion in AG tubule	Content in IDG	SG	AG	Secretion in AG tubule	SG	Anlage of AG	SG	Anlage of AG
PNA	1	1–3*	3	3	1	0–3*	3	1	3	1	3
RCA	1	2	2	2	1	1	1	3	3	3	3
SBA	1	0–3*	3	2	1	0–3*	3	1	3	1	3
DBA	0	0–3*	3	3	0	0–3*	3	0	0	0	0
WGA	1	1	1	1	1	1	1	3	3	2	3
Con A	0–2*	0–2*	1	2	0–2*	0–2*	1	2	2	2	3
UEA	0	0–3*	0	2	0	0	0	0	0	0	0

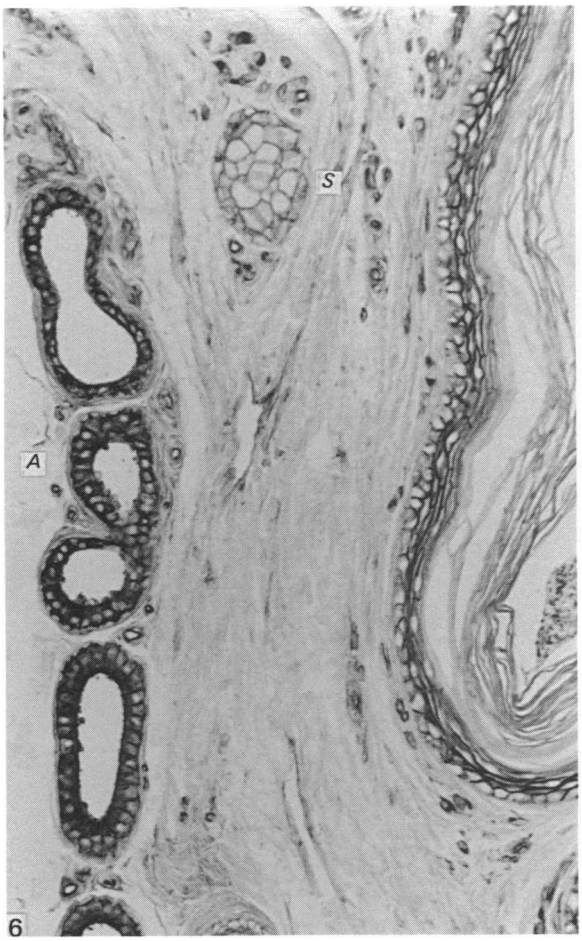
Numbers represent intensity of staining on a scale of 0–3: 0 = negative, 1 = weak, 2 = moderate, 3 = strong.
AG, apocrine gland; IDG, interdigital gland; SG, sebaceous gland.
*, Secretory cells show various degrees of staining.

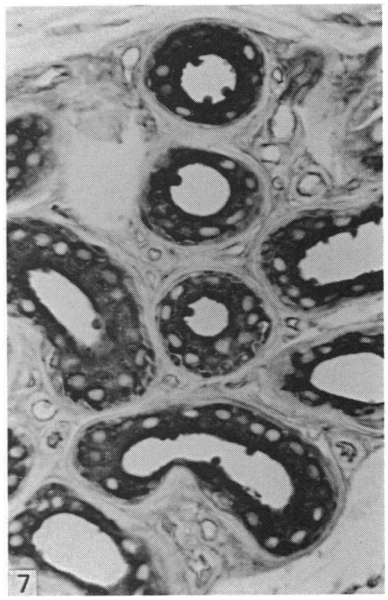






Figs. 3–5. For legends see p. 164.





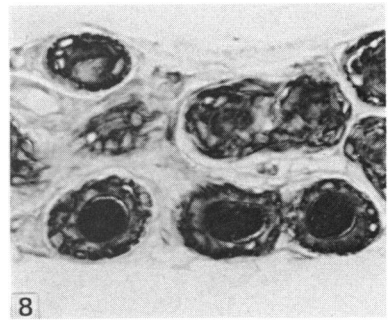


Fig. 6. Epidermis, apocrine gland (*A*) and sebaceous gland (*S*) associated with the skin in the pouch stained with SBA. × 180.

Fig. 7. Tubules stained intensely show apocrine secretion. Myoepithelial cells are also stained. PNA. × 250.

Fig. 8. Glandular lumens are full of secretion probably discharged by exocytosis. Glandular cells of the tubules are stained strongly. PNA. × 280.

digital surface skin was not (Figs. 9, 10, 11). Eight different patterns of staining with UEA were distinguished in glandular tubules of the apocrine gland in the duct. (1) Only the Golgi area of the secretory cells in tubules was positive (Fig. 12*a*); (2) the cytoplasm of some cells in tubules was found to stain weakly (Fig. 12*b*); (3) the cytoplasm of most secretory cells was stained and cell boundaries were strongly positive (Fig. 12*c*); (4) all secretory cells clearly showed weakly positive cytoplasm and

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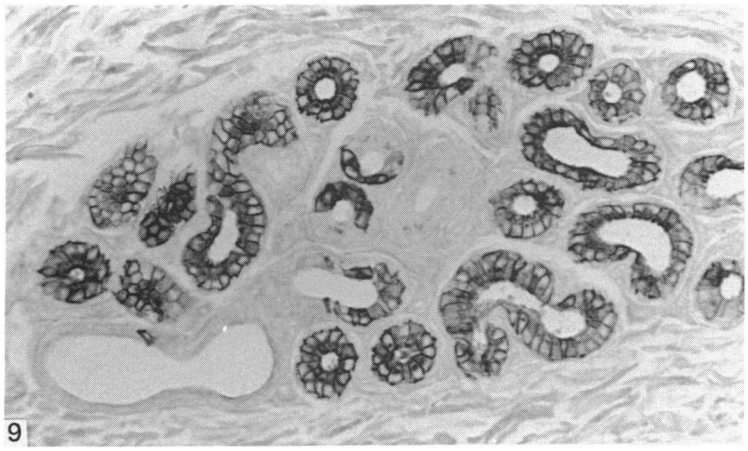
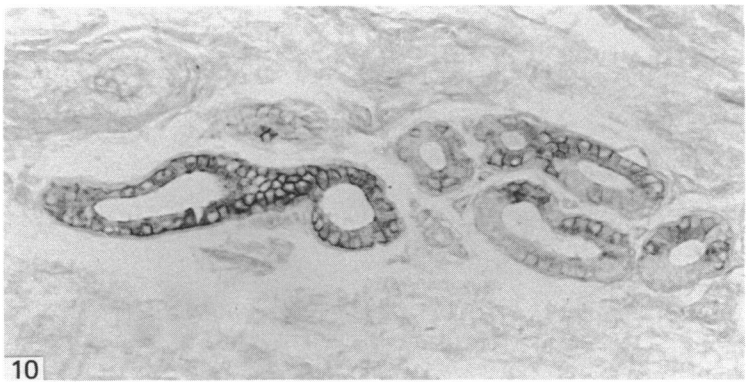
Fig. 3. Horizontal section through the duct and pouch of the interdigital gland. The wall of the gland is lined with thin folds. Two palmar proper digital arteries are situated near the pouch. Haematoxylin and eosin. × 7.

Fig. 4. Transverse section of the duct. There are numerous apocrine glands in the vicinity. Haematoxylin and eosin. × 18.

Fig. 5. Cross section of the pouch. The pouch contains detached keratinised debris. *A*, apocrine gland; *H*, hair follicle; *S*, sebaceous gland. Haematoxylin and eosin. × 74.





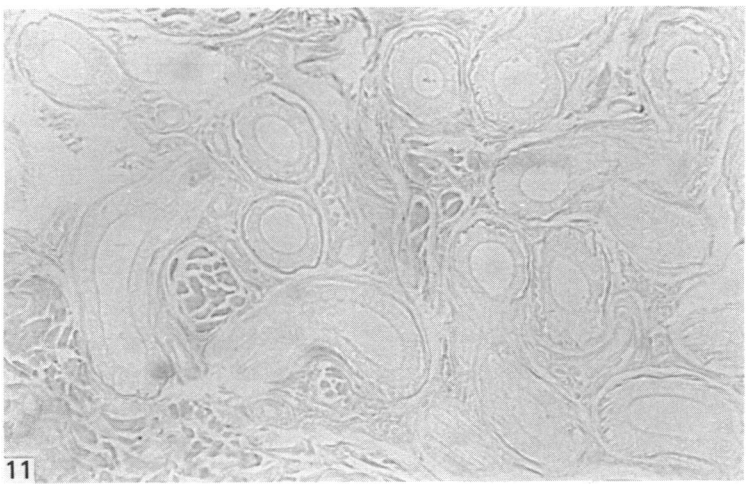


Fig. 9. Apocrine tubules in the duct show variable staining. UEA. × 160.
Fig. 10. Cells of apocrine glands in the pouch vary in staining. UEA. × 160.
Fig. 11. No reaction is seen in apocrine gland of the digital surface skin. UEA. × 160.

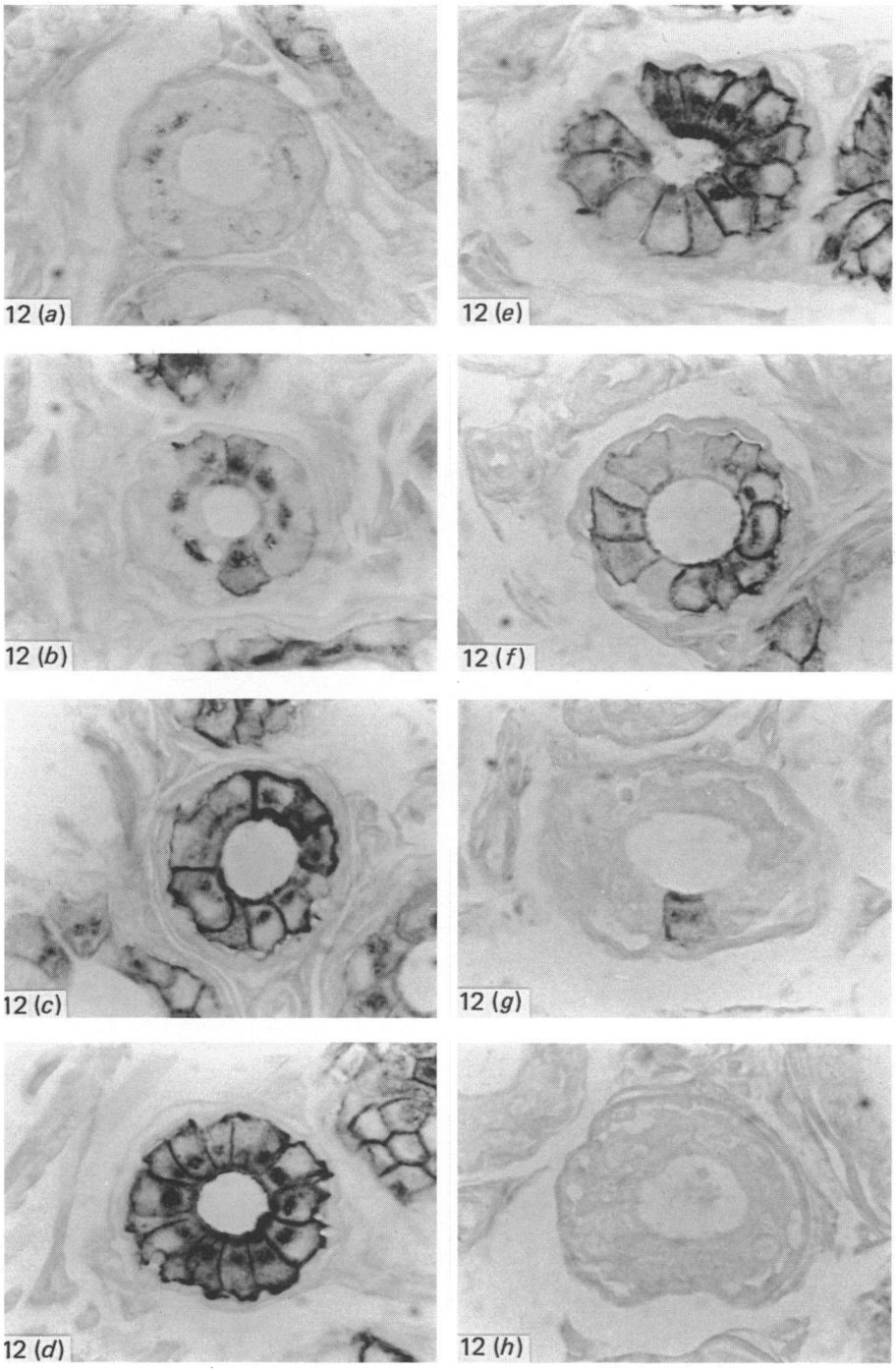


Fig. 12 (*a–h*). Eight figures represent different UEA staining patterns of apocrine tubules in the duct. The positive reaction may be seen in the supranuclear Golgi area (*a*) of glandular cells, dispersed in the cytoplasm (*b*, *c*), reaching to the cell membrane (*d*), or may be sparse or absent (*e–h*). Neither secretion nor myoepithelial cells are stained. × 500.

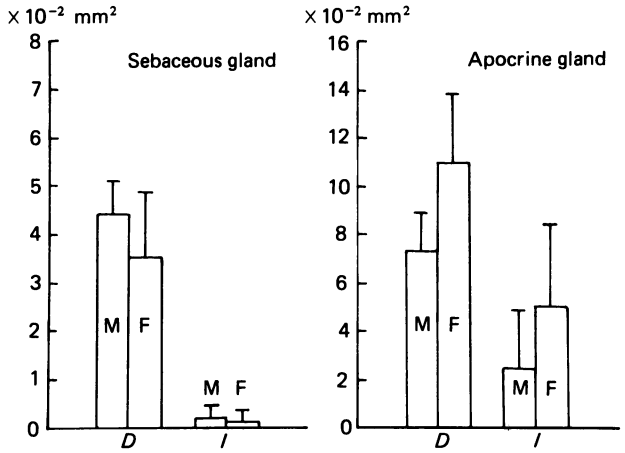


Fig. 13. Area of apocrine and sebaceous glands in the interdigital gland and digital surface skin. The area of each of the two types of gland is less in the interdigital gland than in the skin ($P < 0{\cdot}01$). This is particularly noticeable in the case of the sebaceous glands. *D*, digital surface skin; *I*, interdigital gland; F, female; M, male. Bar represents standard deviation.

strongly positive cell membranes (Fig. 12*d*); (5) some cells in tubules were negative (Fig. 12*e*); (6) the majority of secretory cells were negative (Fig. 12*f*); (7) very few cells were stained (Fig. 12*g*); (8) none of the cells was positive (Fig. 12*h*). Secretory cells of the apocrine gland in the pouch were moderately positive but the staining pattern was less conspicuous owing to the small amount of glandular tissue (Fig. 10). Myoepithelial cells of the apocrine gland in the interdigital gland and digital surface skin were stained with PNA, RCA, SBA or DBA (Fig. 7). In the single fetus that was examined (CRL 29·5 cm), a primordium of the apocrine gland was detectable in the interdigital gland and in the digital surface skin. However, no staining with UEA was observed in either region. Control sections did not show specific staining.

DISCUSSION

Conventional histology demonstrates that the skin of the interdigital gland in the Japanese serow consists of apocrine and non-specialised sebaceous gland units. Both gland types were conspicuously less well-developed than those in the digital surface skin, although a certain amount of glandular tissue was prominent in the duct. Histoplanimetry shows that the estimated relative volumes of apocrine and sebaceous components of the interdigital gland are one half and one tenth respectively of those in the digital surface skin. It seems that glandular tissues gradually decrease from the digital surface skin toward the pouch. The glandular elements in the interdigital gland of the Artiodactyla are, in general, well-developed; the sebaceous gland component may be prominent (Quay, 1959; Quay & Müller-Schwarze, 1970; Moy, 1971), the apocrine gland may be prominent (Quay, 1955; Mainoya, 1978), or both gland types may be well-developed (Kozlowski & Calhoun, 1969; Mainoya, 1976; Adams & Johnson, 1980; Langguth & Jackson, 1980; Mossing & Kallquist, 1981; Chapman, 1985).

Up to now the main difference between the apocrine component of the interdigital gland and the apocrine glands found elsewhere in the body has been based on purely morphological differences, that is the larger diameter of the glandular tubules

(Mossing & Kallquist, 1981), the larger amount of glandular tissue (Mainoya, 1976) and the seasonal variation in the thickness of the glandular tissue (Mossing & Kallquist, 1981). However, the functional differences between the apocrine glands remain unknown. In the present study it was found that the secretory cells of the apocrine part of the interdigital gland are stained with UEA although the glands in the digital surface skin are not. Ookusa (1984) reported that the apocrine glands of human skin (head, face, trunk, upper and lower limbs, and sole) were not stained with UEA. Tsukise, Meyer & Ikeda (1985) found no UEA binding in the apocrine gland of American opossum scrotal skin. Atoji & Suzuki (1987) found no binding sites for UEA in the apocrine glands in the infraorbital gland in the Japanese serow.

There are two possible explanations for these differences in binding of UEA. The apocrine gland may have become differentiated from the glands in the digital skin or the apocrine gland may have remained undifferentiated because the glandular tissue in the interdigital gland is poorly developed. If the latter is the case, positive staining of UEA would be expected in an early developmental stage of the apocrine gland. In the fetus, however, the primordium of the apocrine gland in both the interdigital gland and digital surface skin was not stained with UEA. It is therefore suggested that the binding of UEA to the apocrine gland component of the interdigital gland indicates that it has differentiated as a specialised organ.

Evidence that eight staining patterns of UEA were detected in apocrine tubules suggests a cyclic activity. Atoji & Suzuki (1987) found a functional relationship between cyclic staining pattern and secretory activity of the apocrine gland in the infraorbital gland of the Japanese serow with PNA staining. It is well known that newly synthesised proteins in the rough endoplasmic reticulum of cells are transported to the Golgi apparatus and elaborated into secretory granules or plasma membrane (Fleischer, 1983; Griffiths & Simons, 1986). In the secretory cells of the apocrine gland in the interdigital gland (Fig. 12*a–d*), it may be assumed that fucose-linked glycoproteins are synthesised in the Golgi apparatus and transported to the cytoplasm and cell membranes. The absence of staining in secretory cells (Fig. 12*e–h*) seems to represent an inactive stage. Therefore it is possible that the different staining reactions of the apocrine tubules of the interdigital gland may represent such a cycle of activity.

Secretions in apocrine tubules were stained with PNA, RCA, SBA, DBA, WGA and Con A. This finding indicates that the secretion contains a large amount of glycoconjugates mixed with some different sugar residues – *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, galactose or mannose. An important component of the secretion from the specialised skin glands is thought to be a fragrant or volatile substance (Brown, 1979) and several kinds of hydrocarbons have been isolated from the glands and suggested as likely candidates for scent materials (MacDonald, Kranz & Alpine, 1984; Yokohata *et al.* 1985; Saldern, Schliemann, Kayanja & Jacob, 1987). However, the evidence in the present study indicates a possibility that the glycoconjugates may have other important functions. This idea is further supported by the fact that secretion in the infraorbital gland of the Japanese serow is moderately or strongly stained with PNA, RCA, SBA, DBA, WGA and Con A (Atoji & Suzuki, 1987).

The function of the interdigital gland has been suggested to be scent marking in trails (Quay & Müller-Schwarze, 1970; Mainoya, 1978; Langguth & Jackson, 1980). Conversely, Chapman (1985) stated that the interdigital gland of the moose might not act as a scent gland. Gosling (1985) doubted that the location of the opening of the interdigital gland on the dorsal surface is of great advantage to active marking during

locomotion. It therefore seems unlikely that the interdigital gland of the Japanese serow has such a function, even though it apparently differentiates into a specialised skin organ.

SUMMARY

The interdigital gland of the Japanese serow was examined by histological and lectin histochemical techniques. The gland is composed of a thin-walled pouch and a duct. Both regions contain sebaceous and apocrine glands, but the development of each component was significantly less marked than those of the skin in the region. In particular, only a small amount of sebaceous and apocrine glandular elements was found in the pouch, although they were more abundant in the duct. Histochemical staining of the sebaceous and apocrine glands showed similar reactions to six lectins except for UEA in the interdigital gland and digital surface skin. UEA reacted with the apocrine part of the interdigital gland, but not with the gland in the digital surface skin. In addition, tubules in the apocrine gland revealed eight different staining patterns with UEA. These stainings possibly represent a cyclic activity of glandular tubules and suggest that the apocrine portion of the interdigital gland has a different function from that of the body skin.

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