HISTOLOGY AND HISTOCHEMISTRY OF SPECIALIZED INTEGUMENTARY GLANDS IN EIGHT SPECIES OF NORTH AMERICAN SHREWS (MAMMALIA, INSECTIVORA).

JAMES W. BEE, DUMITRU MURARIU, ROBERT S. HOFFMANN

Integumentary glands which are in immediate contact with the external environment perform important roles, some of them quite specialized, in the life of most mammals. Unlike common skin glands which function in thermoregulation, elimination of wastes or lubrication of hair follicles, specialized integumentary glands are seasonally variable in function and structure and their secretions regulate intra- and interspecific interrelationships.

Quay (1952, 1953, 1954 a—b, 1955, 1959, 1962, 1965, 1968) studied skin glands from some rodents and cervids. Clarke and F r e a r s o n (1972) studied sebaceous glands on the hindquarters of vole Microtus agrestis, S t o d d a r t (1972), the lateral scent glands (organs) from Arvicola terrestris, and S o k o l o v and S k u r a t (1975) studied glands in other microtines. Other investigators have reported on pheromones and olfactory communication in mammals (B r o n s o n, 1971; E i s e n b e r g and K l e i m a n, 1972; G l e a s o n and R e y m i e r s e, 1969; J o h n s o n, 1973; M y k y t o w y c z, 1970; and R a l l s, 1971). In S o k o l o v’s book (1973) regarding the skin glands of all mammals, 43 pages are dedicated to Insectivora.

There is a paucity of information about specialized integumentary glands of shrews, both as to their structure and physiology of secretions. Side glands morphology in shrews has been described (J o h n s o n, 1914; S t a m m, 1914; E a d i e, 1938; H a m i l t o n, 1940; S c h a f f e r, 1940; C r o w c r o f t, 1957; O r t m a n n, 1960; N i e t h a m m e r, 1962). Recently the specialized skin glands in some European shrews have been studied by one of us (M u r a r i u, 1971, 1972, 1973, 1974, 1975, 1976).
Hawes (1976) alludes to the poverty of histological information on side glands of shrews stating: "the glands of *Sorex vagrans* and *S. obscurus* are probably a mixture of highly vascularized sweat tubules and large sebaceous glands, as described for other species (*Eadie, op. cit.; Pearson, 1946)."

**MATERIALS AND METHODS**

The materials are catalogued alcoholic specimens of *Sorex cinereus* Kerr, *S. vagrans* Baird, *S. nanus* Merriam, *S. palustris* Richardson, *S. merriami* Dobson, *Blarina brevicauda* (Say), *Cryptotis parva* (Say) and *Notiosorex crawfordi* (Coues), (Table 1), from the Museum of Natural History at University of Kansas and from the National Museum of Natural History (Smithsonian Institution), and livecaught *Blarina* and *Cryptotis*. A male and female each of eight North American species of shrews were chosen for study (Table 1). The alcoholic specimens were used for general comparative histological purposes. Not all alcoholic specimens may have been fixed in 10 per cent formalin, as some show postmortem changes, but some specimens were properly fixed and preserved in satisfactory condition since 1888 (Table 1). The fresh material of *Blarina* and *Cryptotis* was fixed in Lillie's neutral buffered formalin, recommended by McManus and Mowry (1964) for histochemical work.

On the basis of published results we expected to find specialized integumentary glands in shrews from the following body regions: point of the muzzle, lips and corners of mouth, external auditory meatus, palmo-plantares surfaces, sides of body, region of anus, prepuce and vulva, and the mammae (nomenclature of Schäffer, *op. cit.*). We excluded the ventral glands of *Blarina* (*Eadie, op.cit.*), and the undertail glands occurring in some European shrews (*Niemann, op.cit.*). Fixed skin samples from the above regions, were dehydrated, embedded in paraffin and sectioned 10 µm thick. For histology we stained with Harris' hematoxylin and azan methods. For histochemistry we used Millon tyrosine reaction, Sudan black B and periodic acid Schiff (PAS) reaction; the ninhydrin reaction and the tryptophane-histidine method were also tried, without conclusive results. For several measurements of each tissue we derived an approximate dimension expressed in micrometers or millimeters. Glands were measured with an ocular micrometer calibrated with a stage micrometer.

**RESULTS**

**HISTOLOGY**

**Point of the muzzle.** — *Sorex palustris*, the only semi-aquatic species studied, lacks skin glands which are found in the other species (Table 1), except small sebaceous acini attached to the follicles of vibrissae. The most distal part of the muzzle (in serial sections of male *Sorex vagrans*, the first 0.4 mm (Fig. 1), has thick keratinized epidermis (100 µm), an exceptionally thin dermis, and nasal cartilages lined with thin keratinized epithelium.
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*NMNH = National Museum of Natural History, Smithsonian Institution, Washington, D.C.
**KU = The University of Kansas, Museum of Natural History, Lawrence, Kansas.
All photographs are cross sections; diagramatic representations are longitudinal sections through the side "organs".

Fig. 1. — Distal point of the muzzle of Sorex vagrans, without skin glands. Note the high keratinization of stratum corneum in this region. Azan stain. Fig. 2. — Muzzle of S. cinctus. In the hair-covered skin, are small, elongated sebaceous glands. Azan. Fig. 3. — Upper lip of S. palustris showing apocrine sweat glands; the secretory part is surrounded by myoepithelial cells with elongated nuclei parallel to the longitudinal axis of the gland. H. and E. Fig. 4. — Upper and lower lip of Cryptotis parva. Note the presence of compound sebaceous glands in both lips. PAS.
Hair on the external surface of the skin is associated with common sebaceous glands in the dermis; no sweat glands were found. These sebaceous glands are relatively small, being 50–200 μm in length and 20–75 μm in breadth. Sebaceous glands of the male of *Sorex cinereus* have a length to width ratio of 3:1 (range 2:1–6:1) with some oval sebaceous glands between them (Fig. 2). Sebaceous glands in the female of *S. cinereus* are larger overall (Table 2) and wider at the proximal end, near the excretory duct at the hair follicle. In both sexes of *S. vagrans* sebaceous glands average ca. 20 μm greater in length and width than in *S. cinereus* whereas in *S. nanus* they are smaller than in *S. cinereus*, and those of females are smaller than males (Table 2). Glands on the dorsal side of the muzzle, moreover, are oval, elongation being only on the lateral sides, in the lip region. In the female of *S. merriami* those glands, although variable, tend to be elongated on the dorsal surface of muzzle, whereas in the male they are small and round (Table 2). In contrast, sebaceous glands of the male of *Cryptotis* (ca. 30 ×200 μm) are elongated on the dorsum of muzzle, although some are more typical of common sebaceous glands (ca. 50 ×90 μm); those of female *Cryptotis* are similar, but average larger. Sebaceous glands of the dorsal muzzle skin in both sexes of *Blarina* are ca. 100 μm in length and their shape is aciform, being narrow at the level of the excretory duct, and expanded to a rounded oval (45 μm in breadth) at the distal end. Smaller sebaceous glands extend from the dorsal to the midventral area of the muzzle. In *Notiosorex crawfordi* the sebaceous glands also are small and rounded (50 μm ×40 μm) in both sexes.

**The oral region.** — Most of the shrews studied possessed only compound sebaceous glands in upper and lower lips, and corners of the mouth. Variation in gland size and shape, and number of acini was evident. The male of *Cryptotis* is representative; compound sebaceous glands are well developed both in upper and lower lips (Fig. 4). Glands from the lower lips measure 500 μm in length and 200 μm or more in breadth, with 10 to 12 acini. In the upper lips they are smaller, measuring ca. 350 μm ×130 μm with only six or seven acini. In the female of *C. parva*, in contrast, only small crowded sebaceous glands measuring 120 μm in length and 30 μm or less in width are present in upper and lower lips. The male of *Blarina* is similar to the male of *Cryptotis*, but in the female, compound sebaceous glands are longer (Table 2), measuring 420 μm and there are more acini. In *Notiosorex*, too, sebaceous glands are better developed in the oral region of the female than the male. On the basis of relative size, glands from upper lips of male and female have the same physiological capacity. In the lower lips of female of *N. crawfordi* sebaceous glands have eight to ten acini, considerably more than in the same region of the male. In serial sections, shrews of the genus *Sorex* have similar variety of size and shape of compound sebaceous glands. In the male of *S. vagrans* in the upper lips acini are elongated, measuring 300 μm in length and 30 μm wide while other acini are 150 μm ×70 μm. In female of *S. vagrans* five or six acini in the lower lips (fewer in the upper lips) dominate the tissue in this area. Their acini are 80 μm in length and 50 μm wide. The entire gland is 400 μm ×120 μm. (Table 2). *S. nanus* and *S. merriami* usually have fewer acini (4–5) in the lower lips, and the glands are smaller. In *S. nanus* compound sebaceous glands in the upper lips are smaller.
<table>
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<th>Species &amp; Sex</th>
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<th>Oral Region</th>
<th>External auditory meatus</th>
<th>Palminotrichal surfaces</th>
<th>External entomoneural zone</th>
<th>Intermediate zone</th>
<th>Prepuce &amp; perineum</th>
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**LEGENDS:**
- Sch = sebaceous glands
- Sw = sweat glands
- Proct. = proctodeal glands
- Mam. = mammary glands
- E = eccrine sweat glands
- A = apocrine sweat glands
- $< = small$
- $> = large$
and more round, not exceeding 180—200 μm in diameter for the entire gland and they have fewer acini than in the lower lips.

In contrast to the previous Sorex species, S. cinereus and S. palustris, while possessing common and compound sebaceous glands in the oral region, also have sweat glands. In the upper lips of the male of Sorex cinereus are common sebaceous glands beneath which are eccrine sweat glands; the lower lips contains well-developed compound sebaceous glands of five or six acini, with length of 150 μm and breadth 50 μm; other acini are shorter (ca. 100 μm) but wider (ca. 80 μm). In these glands it is possible to see cellular membranes, and nuclei in the peripheral cells of each acinus. In the centers of the acini the nuclei and cells disappear because of the degenerative secretory process. Each acinus has an excretory duct which is connected to a larger excretory duct of the composite gland attached to the hair follicle. The upper lips of female of S. cinereus has compound sebaceous glands; their acini are smaller than those in the lower lips of the male (Table 2), but more numerous. Each acinus varies in length but their general shape is elongated. Acini penetrate over 400 μm into the dermis. In the lower lips of the female, acini of compound sebaceous glands are fewer in number but larger than from an equivalent area in the male.

In the upper lip of the male of Sorex palustris specialized sebaceous glands have five or six acini; their total length is 320 μm. Longitudinal sections through the distinctive apocrine sweat glands of this species (Fig. 3), show the opening of the excretory duct into the hair follicle sheath, at the same level as the opening of the sebaceous glands. This gland has a total length of 420 μm. The secretory unit has a single layer of epithelial cells and is 30 μm in width. Surrounding the secretory part are myoepithelial cells with elongated nuclei. These cells have their long axes tangential to the secretory duct. Nuclei of secretory cells are large (ca. 5 μm) and are located at the basilar pole of cells. Cuboidal cells mark transition between secretory units and excretory ducts, which are also composed of cuboidal cells. The excretory duct is narrower, measuring only 15 μm wide, being only half the width of the glandular part. In the lower lips of the male of S. palustris, compound sebaceous glands are smaller (250 μm) than in the upper lips. Unlike the male, the female of S. palustris lacks apocrine sweat glands in the upper lips (Table 2) and the compound sebaceous glands are larger (750 μm long and include approximately 15 well developed acini). In the lower lips, specialized sebaceous glands are of the same size and shape as in the male.

The external auditory meatus. — This region has a thin epidermis consisting of stratum germinativum and stratum corneum, beneath which is a thin corium, skin thickness being only 30 μm in female and 40 μm in male Sorex cinereus. In this thin layer of skin there are integumentary glands which produce cerumen. Most of these glands are sebaceous. Where papillae proliferate in the lumen the thickness of skin increases to 140—200 μm. Compound sebaceous glands have three or four acini (70 μm length and 40 μm breadth each) and apocrine sweat glands occurred in smaller numbers in all specimens except S. nanus and the male of Notiosorex. Their secretory units, in most cases, are flattened 90 μm × 30 μm) to almost round.
Sebaceous glands of *S. vagrans*, *S. nanus* and *S. merriami* are smaller than those in *S. cinereus*, the integument is thinner (10—20 μm), and apocrine sweat glands are scarce to apparently absent in *S. nanus* (Table 2). In male of *Sorex palustris* papillae of sebaceous glands include approximately four hair follicles, each of them being surrounded in some cases by seven acini. These acini elevate the integument to 130 μm in height, whereas elsewhere it measures only 20 μm. The acini have a 50 μm diameter and total 80 μm length. Apocrine sweat glands are few near the pinna (external end) and are absent near the tympanic membrane (inner end). Sebaceous glands in the distal part of the meatus have papillae 250 μm in length while in the proximal part of meatus they are fewer and measure only 150 μm in length. Height of the integument as a result is only 75 μm instead of 130 μm in the distal part of meatus. The greater density of skin glands, from the external end of meatus, supplies the skin surface with more protective secretion, while in the inner end, sebaceous glands are reduced and serve only for lubricating hairs and skin surfaces. Unlike the male, sebaceous glands and apocrine sweat glands in female of *S. palustris* are well represented in the external end and their papillae extend laterally more than 1 mm. Even though the length of glandular papillae is wider in females than males, the height of the integument does not exceed 130 μm as is true for males. Compound sebaceous glands have four or five rounded or slightly flattened acini. The apocrine sweat glands have their secretory units well developed (130 μm ×50 μm diameter). In the inner end of meatus the glandular papillae are less and where they appear, include only compound sebaceous glands. As in the male, the length of glandular papillae in the distal part is only 150 μm.

In *Blarina* and *Cryptotis* (Fig. 5), integument of the meatus has both sebaceous and apocrine sweat glands. The papillae are few in number in the external end, but where they occur, are well developed and measure 400 μm in width. Some sebaceous glands have five or six rounded acini. Beneath them lie the apocrine sweat glands with secretory units measuring 30—50 μm in breadth. In the females, glandular papillae are as wide as in male *Cryptotis* or even wider than in the male of *Blarina*, although fewer in number in *Cryptotis*. Unlike the male, however, the female of *B. breviceps* has apocrine sweat glands in the inner end of the meatus.

In male of *Notiosorex crawfordi* are few compound sebaceous glands in papillae which measure 100 μm in length and 110 μm in height. In the female of *N. crawfordi* in contrast, glandular papillae include both sebaceous and apocrine sweat glands. In male, papillae were few in number in the external end of meatus, whereas in female they are many and crowded in the same region but become scarce in the inner end, where apocrine sweat glands are absent (Table 2).

The palmo-plantar region. — In shrews of both sexes there are only eccrine sweat glands, somewhat variable in size, on the ventral surfaces of the front and hind feet. Unlike apocrine sweat glands, eccrine sweat glands have a sinuous ball with a thin secretory unit and a narrow lumen. In cross section the secretory unit is circular or flattened with external diameter of 15 μm to 25 μm. Their cells have large rounded nuclei which usually are situa-
Fig. 5.—External auditory meatus in C. parva. Note the presence of sebaceous and apocrine sweat glands. In the secretory units of apocrine sweat glands there are secretory products at the apical pole of glandular cells. PAS. Fig. 6.—Palmo-plantar pad of Sorex cinereus. Note the presence of apocrine sweat glands (A) and their excretory duct (B) passing between epidermis cells toward the external surface of the skin. Sudan black B stain. Fig. 7.—Finger of Cryptotis parva. Note the presence of eccrine sweat glands in the pad of the finger. H. and E. Fig. 8.—Skin from external cutaneous zone of the anal region of Sorex palustris. Note the hypertrophied circumanal sebaceous glands (A) in comparison with common sebaceous glands (B). Azan.
ted in the apical pole of the cells. Transition to the excretory duct is not
evident as is in apocrine sweat glands. The excretory duct of the epithelium
passes in a sinuous course to the stratum germinativum and then continues
through living and nonliving cells of the epidermis and thence to an opening
on the external surface of the skin (Fig. 6).

Eccrine sweat glands are found only in the metacarpal pads. There
are in some species of shrews peculiar glands in phalangeal pads (M u r a r i u,
1976.) A thick horny layer occupies more than half of the epidermis which
ranges in thickness from 50 to 90 μm.

In both sexes of Blarina and Cryptotis there are eccrine sweat glands in
the pads of the phalange and palmo-plantar pads. Secretory units are circular
or compressed and predominately are lined with a single layer of truncated
pyramidal cells. Nuclei are apical. Eccrine sweat glands are present in pads
ventral to the phalanges including pads of distal segments of the digits. In
the latter pads they are more numerous and are better developed. The
external diameter of the secretory unit is 15 μm in Cryptotis, 25 μm in Blarina.
Unlike other species of shrews, sebaceous glands in C. parva are located under
the skin between fingers. These sebaceous glands are over 100 μm in length
and 35 μm in breadth. In pads of the metatarsals of both male and female
of C. parva, eccrine sweat glands are more developed than in pads of fingers.

In Sorex palustris sexual dimorphism is pronounced. Only in male are
eccrine sweat glands present in pads beneath metacarpals or metatarsals
and beneath the phalanges (Table 2). Eccrine sweat glands are more developed
in pads of fingers than in pads of toes. In some European shrews the reverse condition is true (M u r a r i u, 1976). In metacarpal and metatarsal
pads of the male of S. palustris, eccrine sweat glands are developed extensively.

The anal region. — According to the nomenclature of S c h a f f e r
(op.cit.) and O r t m a n n (op.cit.), skin glands from the anal region have their
excretory ducts opening: a) in the external cutaneous zone, having circumanal
glands; b) in the internal cutaneous zone, having anal glands; c) in the inter-
mediate zone, having proctodeal glands.

In male of Sorex cinereus, the external cutaneous zone has only large
compound sebaceous glands and ducts (Table 2) with four or five acini (150
μm in length and 60 μm in width), surrounded by muscles and they are con-
fined within the zone. Anal glands are large elongated sebaceous glands
with six or more acini 150 μm in length and with large excretory ducts. In
the intermediate zone surrounding the anal walls, there are tubulo-alveolar
proctodeal glands in lobes 1 mm in length and 0.5 mm in breadth supported
by connective tissue. At the contact between the internal cutaneous zone and
intermediate zone, anal sebaceous glands and proctodeal glands are mixed.
All shrews examined had proctodeal glands in the intermediate zone.

In the female of S. cinereus, circumanal glands (2 mm in length) with
eight to ten acini with large excretory ducts are better developed than in the
male. These glands are between muscles isolated from the surrounding skin
by common sebaceous glands. Latter glands are smaller, measuring 100 μm
in length. Anal sebaceous glands are present and well developed. The gland
occupies 400 μm² of surface. Lobes of proctodeal glands (0.5 mm in length
and 100 \( \mu m \) in breadth) are larger in the female, forming a glandular ring around anal walls in the intermediate zone. The diameter of glandular units is not over 30 \( \mu m \).

In the male of \textit{Sorex vagrans}, circumanal glands of the external cutanate zone are compound sebaceous glands. The length of their acini is 120 \( \mu m \) and the breadth 60 \( \mu m \) while in the common skin sebaceous glands are 50 per cent smaller. In the internal cutanate zone, anal sebaceous glands are larger than circumanal glands. Their acini are over 200 \( \mu m \) in length and 90 \( \mu m \) in breadth and they have large excretory ducts. The external anal sphincter separates them from the external skin. The axes of lobes of proctodeal glands are parallel with the anal walls in the intermediate zone. Their length is 300 \( \mu m \) and usually there are two or three layers of lobes surrounding the anal walls. In female of \textit{S. vagrans}, circumanal glands are compound sebaceous with four to six acini, each gland well developed (about 200 \( \mu m \times \times 100 \mu m \)), and they are in two or three layers surrounding the anal tube. Apocrine sweat glands are not specialized and unlike previous species (\textit{S. cinereus}) and in the male of \textit{Sorex vagrans}, they are present only in the female of \textit{Sorex vagrans} (Table 2). In the internal cutanate zone there are anal compound sebaceous glands (130 \( \mu m \times 50 \mu m \)) and unlike male \textit{S. vagrans} and other species of \textit{Sorex}, unspecialised sweat glands are present in female \textit{S. vagrans} (Table 2). Apocrine sweat glands are better developed and more numerous than in the external cutanate zone. Their secretory units have a diameter of 50 \( \mu m \); some units are compressed and are wider. Cells in full secretory activity are 25 \( \mu m \) in height (in contrast to 10 \( \mu m \) at rest), pyramidal in shape and have their apex rounded towards the lumen. Nuclei are basally situated. Secretory units of apocrine sweat glands extend into the intermediate zone and lie between proctodeal glands. Proctodeal glands are well developed and lobes are in layers. One layer close to the anal wall has three rows of proctodeal lobules, each lobule with a length of 250 \( \mu m \) and breadth of 150 \( \mu m \). A second layer with proctodeal lobes is separated by a bundle of circular striated muscles with a thickness of 50 \( \mu m \). These lobes are larger, measuring 700 \( \mu m \) length and 350 \( \mu m \) in breadth. Their longitudinal axis is parallel with the anal walls. In female of \textit{S. vagrans} these glands extend 1 mm anteriorly beyond the ano-rectal junction although their excretory ducts open in the intermediate zone. The only other shrews in which proctodeal glands are so arranged are \textit{Crocidura leucodon} (Muranai, 1975 a), and \textit{S. nanus} (see below).

Male of \textit{Sorex nanus} has only compound sebaceous glands in the external cutanate zone. Acini are elongated (170 \( \mu m \) in length and 90 \( \mu m \) in breadth) and have four to six acini, each with a large excretory duct. These glands are in two layers around the anal tube. In the internal cutanate zone, anal glands are compound sebaceous glands. Their acini are 200 \( \mu m \) in length and 70 \( \mu m \) in breadth and in some places are mixed with proctodeal glands. The position of proctodeal glands are as in the female of \textit{S. vagrans}. Their lobes are elliptical (longitudinal axis 200 \( \mu m \) and the smaller axis 100 \( \mu m \)) and are in two or three layers surrounding the anal walls. Circumanal and anal sebaceous glands in the female of \textit{S. nanus} are almost the same size as in the male. Anal sebaceous glands extend into the intermediate zone and there
Fig. 9.—Internal cutanate zone of the anal region of Sorex palustris. Note the coexistence of anal sebaceous glands (A) and proctodeal glands (B) extended from the intermediate zone. Sudan black B. Fig. 10.—Perivulvar skin of S. palustris. The hypertrophied perivulvar sebaceous glands (A) are in the lateral position; median are only common sebaceous glands (B). Sudan black B. Fig. 11.—Nipple of mammary gland of S. merriami. Note the presence of compound type of mammary gland with two lactiferous ducts in nipple. Azan. Fig. 12.—Nipple of mammary gland Notiosorex crawfordi. The compound type of mammary gland here has three lactiferous ducts in nipple. Azan.
they are mixed with proctodeal glands. Proctodeal glands extend beyond the anorectal junction but not as far as in the female of S. vagnans. Their lobes are 200 μm in length and about 100 μm in breadth.

In the external cutaneous zone in male of Sorex palustris there are well developed compound sebaceous glands (1 mm diameter) in two or three layers around the anal walls (Fig. 8). In the internal cutaneous zone, all structures are dominated by two or three layers (over 2 mm in thickness) of compound sebaceous glands; each gland having eight to ten acini measuring 100 μm in length and 75 μm in breadth. In this ring of sebaceous glands are proctodeal glands; their length is 400 μm and their breadth 200 μm. In female of S. palustris, circumanal glands are noticeably more developed than in the male. Each compound sebaceous gland is the same size as the male but has three or more layers of sebaceous glands surrounding the anal canal. Some sebaceous lobes exceed 200 μm in length and 150 μm in breadth. In Fig. 9, anal sebaceous glands are being replaced by lobes of proctodeal glands. With a length of 400 μm and a breadth of 200 μm these lobes tend to have their longitudinal axis perpendicular to the anal walls, as male of S. palustris and unlike previous species, they are not circular to the anal walls. A peculiar feature of the anal sebaceous glands in female of S. palustris is that they are not completely replaced by proctodeal glands and are external to them. They meet at the ano-rectal junction, where usually proctodeal glands are missing.

In both sexes of Sorex merriami sebaceous circumanal and anal glands are few and form an interrupted ring around the anal wall; their acini are 175 μm length and 100 μm in breadth. Anal sebaceous glands of the internal cutaneous zone are similar in both sexes. Proctodeal glands do not extend over the ano-rectal junction. The proctodeal lobes are 450 μm in length and 175 μm in breadth.

In both sexes of Blarina brevicauda, both circumanal and anal sebaceous glands also are scarce and weakly developed; their acini being only 100 μm in length and 50 μm in breadth and they do not form a ring around the anal tube. Apocrine sweat glands in contrast, are well developed with large secretory units (Table 2). The proctodeal glands form one ring around the anal walls in the intermediate zone and their lobes are small measuring 150 μm in length and not exceeding 50 μm in breadth. They do not extend beyond the ano-rectal junction.

In Cryptotis parva, both in male and female, circumanal glands are better developed than in Blarina brevicauda, even though Cryptotis is smaller in body size. The sebaceous glands from the external cutaneous zone have four to six acini (150 μm length and 75 μm breadth). In the internal cutaneous zone anal sebaceous glands are fewer in number and smaller (100 μm × 60 μm) than circumanal glands. Proctodeal glands are well developed in both sexes of C. parva. As in Blarina brevicauda, the proctodeal lobes are disposed in a single layer but they are more condensed and their longitudinal axis is perpendicular to the anal walls and measure 350 μm and the breadth is 160 μm.

In both sexes of Notiosorex crawfordi circumanal and anal glands are weakly developed. The acini of compound sebaceous glands are only 50 μm in length and 30 μm in breadth. There are no apocrine sweat glands. Procto-
deal glands are well developed forming a ring 400 \( \mu m \) thick around the anal walls in the intermediate and internal cutaneous zones, where they mix with small anal sebaceous glands. The proctodeal lobes which are perpendicular to the anal walls are thin and elongated measuring about 200 \( \mu m \) in length and 40 \( \mu m \) in breadth.

**The prepuceal and perivulvar region.**—According to Schaffer (1940) and others, there are no specialized skin glands in the prepuceal and perivulvar regions of shrews. Some results of our investigation differ from the literature and are as follows:

In the prepuce of male of *Sorex cinereus*, there are specialized compound sebaceous glands with acini varying from 100 \( \mu m \) to 150 \( \mu m \) in length and 100 \( \mu m \) in breadth. These glands are on the lateral sides of the prepuceal region, and are symmetrical. Common sebaceous glands are simple and are 80 \( \mu m \) in length and 50 \( \mu m \) in breadth. Apocrine sweat glands are beneath and between the hypertrophied sebaceous glands but are missing in the prepuceal skin which have common sebaceous glands. Their diameter is over 30 \( \mu m \) and they have tall, pyramidal cells with a narrow lumen in the secretory unit. In the perivulvar skin of females of *S. cinereus* are the same kind of apocrine sweat gland as in the male.

In female of *Sorex vagrans* the perivulvar skin contain hypertrophied sebaceous glands, with acini 150 \( \mu m \) in length and 75 \( \mu m \) in breadth. Apocrine sweat glands are larger (50 \( \mu m \) diameter) but fewer. There are no hypertrophied skin glands in the prepuce of male of *S. vagrans*.

In male of *Sorex nanus*, compound and hypertrophied sebaceous glands are in the lateral sides of prepuceal skin. Their acini are 150 \( \mu m \) in length and 100 \( \mu m \) in breadth. In the perivulvar skin of female of *S. nanus* are sebaceous glands with smaller acini and they are disposed laterally.

In prepuceal skin of male *Sorex palustris*, in the lateral position, are large, compound sebaceous glands with round to oval acini.

In the perivulvar skin there are only compound sebaceous glands in the lateral sides (Fig. 10). Some acini measure 300 \( \mu m \) in length and over 100 \( \mu m \) in breadth.

In the prepuceal and perivulvar skin in both sexes of *Sorex merriami* are small but specialized integumentary glands (length 100 \( \mu m \) and breadth 60 \( \mu m \)) which are larger than common sebaceous glands (60 \( \mu m \times 25 \mu m \)).

In both sexes of *Blarina brevicauda*, *Cryptotis parva* and *Notiosorex crawfordi* there are no specialized skin glands in either the prepuceal or perivulvar regions.

**Mammary glands.**—Shrews have three pairs of mammary glands in the inguino-abdominal region. In the eight species of shrews which we prepared for study, we did not find variation of number or position of these glands.

All species had compound mammary glands (more than one glactophore duct in the top of each nipple) of two glactophore ducts except *Notiosorex crawfordi* which has three (Fig. 11 and 12).

**The side glands.**—Most shrews have prominent of specialized skin glands on the sides of their body. With some differences between species but especially depending on the season when the specimens are collected,
Fig. 13.—Secretory part of the mammary gland of Cryptotis parva. Millon tyrosine reaction stain. Fig. 14.—The end of side "organ" of Sorex vagrans. Note the greater number of hypertrophied sebaceous glands (A) in comparison with the apocrine sweat glands (B). Azan. Fig. 15.—The center of side "organ" of C. parva. Apocrine sweat glands (A) are developed only in a thin layer beneath the hypertrophied sebaceous glands. Millon tyrosine. Fig. 16.—The center of side "organ" of Blarina breviceuda. The sebaceous glands (A) are common glands while the core of the side "organ" consists of hypertrophied apocrine sweat glands (B). Millon tyrosine.
lateral "organ" consist of apocrine sweat glands and sebaceous glands. All of the shrews studied had side glands in both sexes, easy to identify in adult males but difficult to find in females, requiring in many cases a microscopical examination of the tissue.

In *Sorex cinereus*, side "organs" have large sebaceous glands (75 μm × 50 μm) surrounding apocrine sweat glands (Fig. 17). The secretory units of the apocrine sweat glands have a diameter of 75 μm.

On the edge and ends of the "organs" sebaceous glands dominate apocrine sweat glands in the ratio of 50:1 (Fig. 14). In the middle of the side "organ" the core has well developed apocrine sweat glands. The diameter of the secretory units is 50 μm and their walls are lined with pyramidal glandular cells 25 μm high with nuclei in the basal poles of the cells. Between their apical poles is a narrow lumen. Nuclei are in the basal pole of cells. Surrounding the glandular cells are myoepithelial cells. Hypertrophied sebaceous glands are interrupted and replaced by common sebaceous glands over the apocrine sweat glands.

In *Sorex nanus*, unlike the previous species, specialized sebaceous glands 160 μm in length and 90 μm in breadth, cover the sweat apocrine glands in the middle of the side "organ" (Fig. 18). Common sebaceous glands over them are more superficial in the dermis and measure 50 μm in length and 30 μm in breadth. The secretory units of apocrine sweat glands are 30 μm in diameter.

For *Sorex palustris* we do not have properly prepared material to interpret the structure of the side "organ". It is easier to find in adult males than in subadults or females.

In *Sorex merriami*, the structure of the side "organs" is as in *S. nanus*. Common sebaceous glands maintain the same size across the side "organs" but become crowded laterally.

In both male and female of *Blarina brevicauda* there are only apocrine sweat glands in the side "organs", and only common sebaceous glands (Fig. 19).

In *Cryptotis parva* the side "organs" have large, specialized sebaceous glands and beneath them, in a very thin layer lie the apocrine sweat glands (Fig. 15). Sebaceous acini have a length of 400 μm and a breadth of 100 μm. The apocrine sweat glands have large secretory units with large lumena, their external diameter is 100 μm. Between the dense and pluri-stratified layers of sebaceous acini, excretory ducts of apocrine sweat glands pass from the deepest region of the dermis to the hair follicles (Fig. 20). This structure is different in *Blarina brevicauda* where there is a prevalence of apocrine sweat glands (Fig. 16).

In *Notiosorex crawfordi* side "organs" are predominantly apocrine sweat glands covered by common sebaceous glands and in this respect resemble side "organs" of *Blarina brevicauda*.

**HISTOCHEMISTRY**

Most histochemical studies of integumentary glands refer to man. For other mammals, integumentary glands from rat, mouse, rabbit, guinea pig, cow, goat, carnivores and monkeys (Aldrich, 1896; Kelly and
To our knowledge, however, there are no general histochemical studies of the specialized integumentary glands of insectivores, except the brief account by Murariu (1976).

The present study was designed to identify carbohydrates, lipids and proteins. Most of our results are from live-trapped Blarina breviceuuda and Cryptotis parva as well as from some alcoholic specimens of Sorex cinereus and S. palustris.

Muzzle glands. — Sebaceous glands in the muzzle have a marked affinity for Sudan black B, and are colored deep blue-black; the surrounding tissue is bluish. PAS reaction is weak. and the red is confined to the centers of sebaceous glandular acini and their excretory ducts. Peripheral cells show negative PAS reaction. Connective tissue, especially collagen fibers with mucopolysaccharides, stain red while the outlines of sebaceous glands and other integumentary structures are green from methylene green used as additional staining after PAS. After Millon tyrosine reaction, proteins are weak pink in middle of sebaceous glands.

Oral glands. — Only compound sebaceous glands were tested histochemically. They are intensely sudanophilic, with acini black in comparison with the surrounding bluish connective tissue. In the cytoplasm of older cells in the middle of sebaceous acini or close to the excretory duct, there are lipid droplets, the nuclei having disappeared. PAS reaction is the same as in the muzzle. Methylene green is in small peripheral cells of glands while reddish color, which indicates presence of carbohydrates, increases in intensity from the edges of sebaceous acini to their center and in the excretory ducts (Fig. 4). Protein indicating red of the Millon reaction is visible only in the central cells of sebaceous glands.

Ear glands. — In the external auditory meatus there are both sebaceous and apocrine sweat glands, their ratio being variable from one species to another. Sudanophilic lipids are present in both kind of glands. Sebaceous glands have the same kinds and amount of lipids as those from the muzzle and mouth, the only difference being a stronger sudanophilia in the glands of the meatus. Usually it is not possible to distinguish cellular structure of Sudan stained cells. In apocrine sweat glands, secretory cells are a homogenous deep-black. In the lumen of the secretory unit there is a secretion which also has affinity for Sudan black B, but its color is lighter than in secretory cells. There is a slight PAS reaction only in sebaceous glands while in the apocrine sweat glands red is only in the cytoplasm of secretory cells. Secretion from the lumen of secretory units has only a slight tint of red (Fig. 5). This suggests that most is lipid, with some carbohydrate. Proteins, as evidenced by Millon tyrosine reaction, are scarce both in sebaceous and apocrine sweat glands of the external auditory meatus. The lumena of the apocrine secretory unit, after this reaction, are empty.

Palmo-plantar glands. — Sudanophilic lipids are prominent in glandular cells in the walls of eccrine sweat glands from the palmo-plantar surfaces but are absent from the lumena (Fig. 6). We could not distinguish an increase in staining, compared to the nuclear region, as mentioned by Montagna.
Carbohydrates are not evident, from PAS reaction, in the cytoplasm of eccrine sweat glands and the Millon tyrosine reaction for proteins was negative.

**Anal glands.**—All three zones in this region have sudanophilic, from small undifferentiated peripheral cells to cells in the center of the acinus and excretory ducts. Apocrine sweat glands from the circumanal region in *Blarina* have an evident sudanophilia, especially in the cytoplasm of the secretory cells. Cytoplasm of cells of secretory units of proctodeal glands are similar to sebaceous gland cells except the cells of excretory ducts of the former are darker (Fig. 9).

In the anal region, intensity of PAS reaction for carbohydrates is inversely related to lipid reactions in the sebaceous glands and in apocrine sweat glands of *Blarina*, but overall, the amount of carbohydrates is less than fats. In proctodeal glands the amount of carbohydrates is minimal. Red stain appearing only in excretory duct cells and irregularly in secretory cells of the glandular units. Circumanal and anal sebaceous glands do not have as much protein, as evidenced from Millon tyrosine reaction, as sebaceous glands from other regions; small amounts of protein are present in apocrine sweat glands. Proctodeal glands do not show any protein reaction.

**Prepuceal and perivulvar glands.**—Hypertrophied sebaceous glands and apocrine sweat glands from these regions are sudanophilic, and have the same distribution of staining intensity as in other regions (Fig. 10). A faint PAS reaction for carbohydrates is seen in the cytoplasm and excretory duct lumen of sebaceous glands, and in lumen of glandular units of apocrine sweat glands. Tyrosine reaction with Millon reagent is very faint in the sebaceous glands.

**Mammary glands.**—In mammary glands stained with Sudan black B, secretory cells are deep-black while the lumena of lactiferous ducts are a homogeneous blue. Cytoplasm of secretory cells does not show reddish secretion granules, but near the lumen, PAS reaction is positive. Millon reagent shows proteins, especially in the lactiferous ducts (Fig. 13).

**Side glands.**—Differing relative proportions of apocrine sweat units and sebaceous glands and the histochemical components in each type are found in side "organs" of different shrew species. Both types of glands are sudanophilic. In species in which the side "organs" has only apocrine sweat glands, the lumena of their secretory units are filled with a bluish granular secretion. In the second kind of side "organ" in which the sebaceous glands are dominant, sudanophilic granules are especially common in the cytoplasm of the gland cells. PAS positive elements are in both kinds of specialized integumentary glands, and have the same distribution of carbohydrates in the secretory cycle, as in other regions. In the cytoplasm of the supranuclear region of secretory cells and in the lumena of apocrine sweat glands the secretion is reddish. In sebaceous glands PAS reaction is especially strong in the center of the glandular acini and in excretory ducts. In peripheral cells, stain is faint. Concentration of carbohydrate granules in the lumena of glandular units and excretory ducts is below that of the homogeneous blue lipid droplets which also occupy the lumena. In those side
“organs” where sebaceous glands are dominant, PAS positive elements are more evident in the central cells and in the excretory ducts. Apocrine sweat glands in these side “organs” are fewer in number than sebaceous glands, and the secretory cells and lumena are PAS negative. Tyrosine appears positive, especially in the lumena of apocrine sweat gland ducts of the side “organs” (Fig. 16), but in some side “organs” especially where sebaceous glands are dominant, tyrosine is absent in apocrine sweat glands, their ducts being empty (Fig. 15). Millon reagent gives a positive reaction in hypertrophied sebaceous glands (Fig. 15).

CONCLUSIONS

1. Among eight species of North American shrews, only Sorex palustris lacked specialized integumentary glands in the point of the muzzle. In the other seven species sebaceous glands vary in size, shape and position.

2. In the oral region, including upper and lower lips and corners of the mouth, there are specialized sebaceous glands in all eight species, smallest in the female of Cryptotis parva and largest in the female of Sorex palustris. In the male of S. palustris apocrine sweat glands were present while in the male of S. cinereus eccrine sweat glands were found.

3. In the external auditory meatus, apocrine sweat glands are present, except in Sorex nanus and males of Notiosorex crawfordi. Sebaceous glands are present in both sexes of all eight species. This leads us to believe that in shrews, sebaceous glands produce the cerumen. These glands are more dense in the external end of the external auditory meatus than in the inner end.

4. Except in the female of Sorex palustris all specimens of the eight kinds of shrews had eccrine sweat glands in pads beneath the metacarpals and metatarsals. The male of S. palustris and both sexes of S. merriami and Cryptotis parva have in addition eccrine sweat glands in phalangeal pads. Sebaceous glands are noticeable between fingers or toes and between metacarpals or metatarsals, especially in Cryptotis parva.

5. The anal region is divided into circumanal, anal and intermediate zones. In most species the external cutaneous zone (circumanal) and internal cutaneous zone (anal) have glands which are usually in two or three layers around the anal walls. These glands are weakly developed in Sorex merriami, Blarina brevicauda and Notiosorex crawfordi. In other species and especially in Sorex nanus and S. palustris these glands are usually enlarged and their acini are in at least three layers around the anal walls. The female of S. vagrans and both sexes of Blarina brevicauda have apocrine sweat glands in this region. Proctodeal glands have lobes of different sizes and their axes are parallel or perpendicular to the anal walls. In most species secretory lobes stop at the ano-rectal junction, but in the female of S. vagrans one lobe extended cranially ca. 1 mm beyond this junction.

6. In prepuceal and perivulvar regions, especially in the lateral sides there are hypertrophied sebaceous glands in both sexes of Sorex cinereus, S. nanus, S. vagrans, S. palustris, and S. merriami. In females of S. cinereus and S. vagrans, there are also apocrine sweat glands beneath the large sebaceous glands. In Notiosorex crawfordi, Blarina brevicauda and Cryptotis parva there are no specialized prepuceal or perivulvar glands.
7. All species of shrews have a compound mammary gland in that at least two lactiferous ducts are in each nipple. Unlike some European and North American shrews, mammary glands of Notiosorex crawfordi have three lactiferous ducts which open separately on the top of each nipple. In contrast, the European hedgehog (Erinaceus europaeus L.) and mole (Talpa europaea L.) have a simple type of mammary gland (Muraicu, 1976).

8. Side “organs” occur in both sexes of shrews. They are well developed and easy to identify in males, but in females it is sometimes necessary to verify their presence by microscopical studies. In some species (Sorex vagrans, S. nanus, Cryptotis parva) side “organs” have hypertrophied sebaceous glands as the principal elements while apocrine sweat glands are weakly developed especially in the center of the “organs”. In Sorex nanus, S. merriami and Cryptotis parva, hypertrophied sebaceous glands cover the core of apocrine sweat glands while in Blarina brevicauda and Notiosorex crawfordi, only common sebaceous glands cover the hypertrophied apocrine sweat glands. Shrews with specialized integumentary glands and side “organs” are rarely eaten by mammalian predators, and we consider that these side “organs” can play a role in defense against predators as well as a role in intraspecific and interspecific interaction (Muraicu. 1973. 1976).

9. Histochemically, all specialized integumentary glands of our specimens secreted lipids and carbohydrates. Proteins are found mainly in mammary and side “organ” secretions, but other glands also produce small amounts.

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HISTOLOGIA ȘI HISTOCHIMIA GLANDELOR TEGUMENTARE SPECIALIZATE LA OPT SPECII DE INSECTIVORE (MAMMALIA, INSECTIVORA) DIN AMERICA DE NORD.

REZUMAT

Rezultatele cercetării histologice și histochimice a glandelor tegumentare la 8 specii de insectivore au evidențiat existența unor astfel de glande în: virful botului, regiunea bucală, conductul auditiv extern, suprafețele palmo-plantare, pe flancuri, regiunea anală, în cea prepuțială sau perivulvară și glandele mamare.

În virful botului numai la Sorex palustris n-au fost identificate glandele sebacee existente la celelalte specii. În jurul gurii și în conductul auditiv
extern există la toate speciile, glande sebacee cu diferențe numai în privința mărimii lor; glandele sudoripare nu sînt constant prezente.

Glandele laterale difere doar prin ponderea glandelor sebacee ori a celor sudoripare apocrine; hipertrofierea lor a fost corelată cu perioadele de reproducere. Suprafețele palmo-plantare adăpostesc numai glande sudoripare eccrine; diferențele sînt de ordin topografic. Glandele sebacee, deși atașate firelor de păr, sînt localizate în pielea dintre falange.

În regiunea anală glandele tegumentare se întind de regulă, pînă la nivelul joncțiunii ano-rectale; numai la ♀ de S. vagrans glandele proctodeale depășesc mult această limită.

Glandele mamare, cu cite două ducte lactifere în mamelon, fac excepție la Notiosorex crawfordi, la care au cite 3 conducte.

Reacțiile histochemice au evidențiat prezența considerabilă a lipidelor, cu precădere în secretiile glandelor sebacee și hidrâți de carbon în cele sudoripare. Proteinele există și ele dar în cantități reduse; numai în glandele laterale și în cele mamare cantitatea proteinelor este mai crescută.

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