THE SPECIALIZED MIDVENTRAL GLAND OF THE EASTERN WOOD RAT, *Neotoma floridana osagensis*

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A Thesis

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Master of Science

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by

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[redacted]

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INTRODUCTION

Specialized scent glands occur in a variety of mammalian orders, ranging from the primitive Insectivores to the highly advanced Primates. Among the rodents, a diverse number of specialized glands have evolved in various locations on the body. The most common type found in the Order Rodentia is the holocrine sebaceous gland. Since odors are of importance to rodents for communication purposes, it is of interest to study those glands that are involved in producing scent. The histology and behavioral implications of these glandular regions have become a major topic of interest in understanding the species.

Among the Nearctic cricetine genera, midventral sebaceous glands are found in Sigmodon, Peromyscus, and Neotoma. In the genus Neotoma, only casual observations of the glandular region have been recorded (Poole, 1940; Linsdale and Tevis, 1951; Rainey, 1956; and Spencer, 1968).

In view of the fact that a void exists in the knowledge of the specialized midventral gland, this study was initiated. Research was conducted in order to establish basic data concerning the development, structure, and function of the midventral gland in the Eastern Wood Rat, Neotoma floridana.

The study was resolved into the following phases:

1. Relationship of gland size to sex, age, and breeding season.
2. Description of glandular histology.
3. Influences of gonadal hormones on glandular development.
4. Basic histochemical properties of the gland region.

Literature Review

Histological studies of specialized scent glands have been

conducted on a number of different mammals. The gland location varies considerably as shown by the following examples: there is a dorsal rump gland in the peccary (Werner, et. al., 1952), a chin gland in rabbits (Mykytowycz, 1968), para-anal glands in the fruit bat (Quay, 1970), and interdigital, metatarsal, and tarsal glands in deer (Quay, 1959, 1971; Quay and Muller-Schwarze, 1970, 1971). Numerous additional examples of gland locations and uses are given by Ewer (1968).

Among the rodents, the glandular regions of a few species have been described in detail, but more often reference to these areas has been in conjunction with other studies. Quay (1965) described the possibilities of determining the taxonomic status of species in the genus Perognathus by the development and structure of the sebaceous caudal gland. Thiessen, et. al. (1968) studied the territorial marking behavior of the Mongolian gerbil which possesses an abdominal sebaceous gland.

Montagna and Noback described the preputial gland (1946) and the histochemical properties of unspecialized sebaceous glands (1947) of the laboratory rat. The effects of hormones upon sebaceous glands have been studied in the albino rat, Rattus (Ebling, 1948, 1951); the Mongolian gerbil, Meriones unguiculatus (Glenn and Gray, 1965); and the Deer Mouse, Peromyscus maniculatus (Blum, et. al., 1971; Doty and Kart, 1972).

Midventral specialized sebaceous glands have been described in Rattus exulans (Quay and Tomich, 1963), Meriones unguiculatus (Glenn and Gray, 1965) and Peromyscus sp. (Richland and Roslund, 1952; Doty and Kart, 1972) and in eleven species of Malaysian murids (Rudd, 1966). Distinct variations in glandular histology are evident but in each species gland size is dependent on sex, age, or reproductive state.

The first description of midventral glands in the genus Neotoma was by Howell (1926). He described males of N. cinerea as having

> "a thickened dermal area midventrally...In females this portion of the integument is barely thicker than over the remainder of the animal. Microscopic examination of vertical sections of this area shows many enlarged, highly specialized glandular masses of the sebaceous type. Some are engorged with secretion and appear as though they were accompanied by a progressive breaking down of the more ental cells. They are separated by trabeculae, carrying the secretion to the external surface."

He concluded by stating that this glandular area apparently acts as a scent gland.

Vestal (1938) did not mention an abdominal gland in Neotoma fuscipes but observed that in houses of adult males there was a strong musky odor which increased in intensity during the breeding season. Poole (1940) described a ventral area void of hair along the midline of the abdomen in both sexes of N. f. magister. The hair around this region was continually discolored while the animals were sexually active. He further described rubbing of the abdomen and hindquarters along the ground during the breeding season.

The staining of the venter by secretions from the abdominal gland in N. fuscipes was carefully recorded by Linsdale and Tevis (1951). They found that all adult and subadult males showed staining, while only a few females had discolored venters. Individual variation in gland activity was found, as well as marked differences due to age, sex, and breeding season.

Rainey (1956), studying N. floridana, observed that there was a seasonal intensity of the ventral staining, and that the stain was most pronounced in older males; only one adult female was found with the midventral stain. By comparison, adult males of N. micropus have a more

conspicuous glandular region than N. floridana (Spencer, 1968). Spencer noted that histological sections of the ventral gland revealed an abundance of sebaceous glands which were larger and more numerous in adult males in May and September than in December. He also observed that captive adult males spent considerable time dragging their venters over objects.

METHODS AND MATERIALS

Specimens of Neotoma floridana osagensis used in this study were collected from Lyon and Chase counties, which are in the eastern third of Kansas. The primary habitat of wood rats in this area is along the numerous Osage Orange hedgerows that border fields and roads. Thorny branches from these trees are used in construction of the houses and afford the occupants a secure shelter.

Wood rats were trapped monthly from August, 1972 through July, 1973. Live traps, baited with peanut butter, were placed at the entrances of active rat houses. Occupation of the houses was determined by the presence of fresh leaf cuttings and fresh fecal pellets around and upon the houses. Both homemade and commercially-made live traps (Fig. 1) were utilized, with about equal success.

Trapped rats were taken to the laboratory and housed individually in cages measuring 25 x 20 x 18 centimeters. Rats being held for later experiments were fed Purina Laboratory Chow and occasional tidbits of lettuce and apple, along with a constant supply of water. No attempt was made to control the light cycle in the laboratory; therefore, rats kept indoors were only used for those studies which would not be affected by a changing light cycle.

Histology

Preparations of the midventral gland were made in order to study the microscopic elements of the glandular region. Ether asphyxiation was utilized to kill the rats prior to gland removal. The ventor of the rat was shaven and gland length and width were measured to the nearest millimeter. Either the entire gland and one centimeter of surrounding

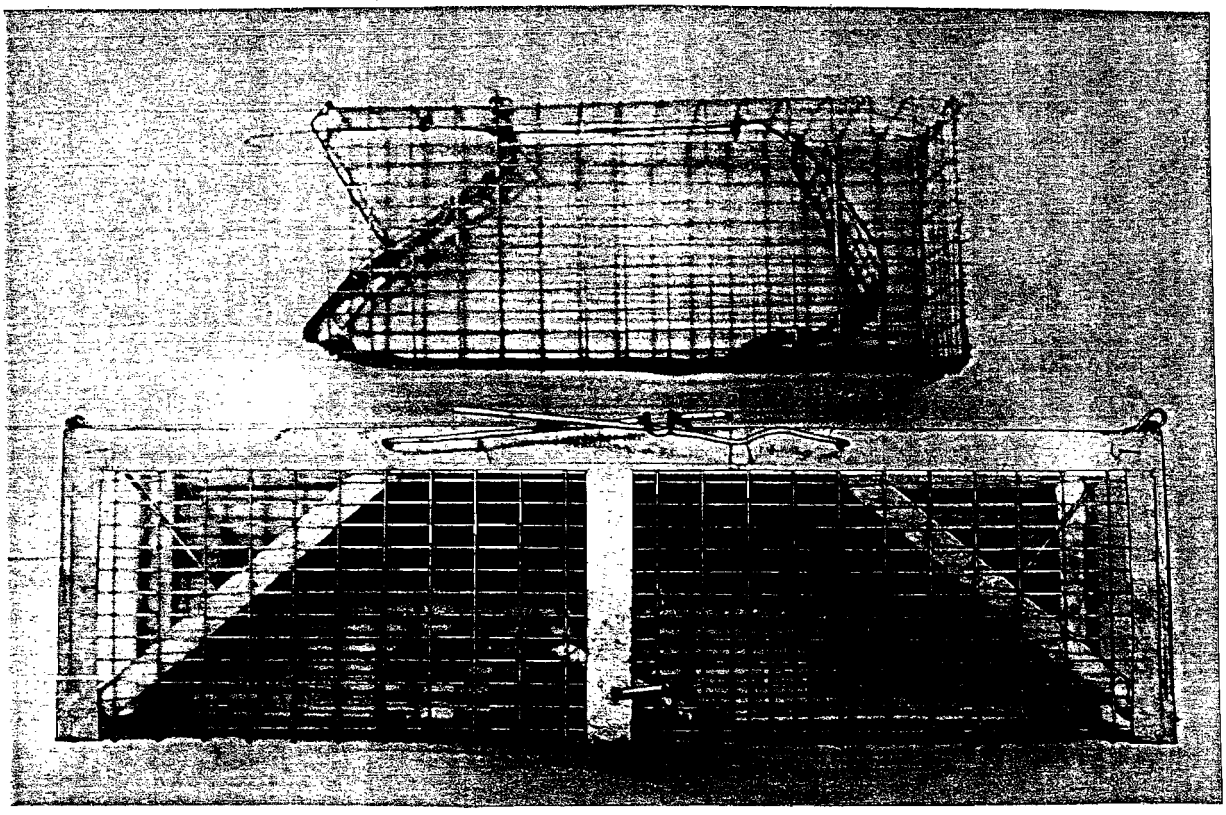

Fig. 1. Live traps utilized for the capture of wood rats in this study.

skin, or specific portions of the gland, were excised and put into Bouin's fixative for 24 hours. The tissue was then dehydrated by immersion in successive ethanol baths of 70, 95, and 100 per cent solutions, followed by xylene: alcohol (1:1), xylene, and then embedded in Tissue Prep (paraffin). Tissue blocks were either sectioned transversely or longitudinally at 10 microns and the sections stained in Delafield's hematoxylin and Eosin Y. For comparative purposes, similar sections were prepared of skin lateral to the abdominal gland.

Slides were observed with a Bausch and Lombe light microscope equipped with an optical micrometer. Each glandular alveolus was measured at its widest point by examining serial sections and using the section showing the maximum size. These diameters were then averaged in order to have a single average alveolar width for each rat. Individual cells in the alveoli were randomly chosen and measured to determine an average cell diameter for each specimen (basal cells were excluded from these measurements). To calculate the average number of cells per alveolus for each specimen, the computed volume of an average cell was divided into the computed volume of an average alveolus. Although the shapes of the glands were quite varied, many approached a spherical shape, and therefore it was decided to use the geometric formula for a sphere to compute the alveolar and cell volumes. Also, skin thickness, epidermis thickness, and the number of acidophilic cell layers in the alveoli were recorded for each individual.

Sex, Age, and Seasonal Relationships

Histologically prepared slides of 36 individuals were examined for anatomical, cytochemical, and size differences between sexes, different

age groups (juvenile, subadult, and adult), and different months of the year. Macroscopic examinations of the glandular region for condition of the skin and hair, presence of sebum globules, and presence of stained hair were made on each specimen. Midventral gland area in square millimeters was approximated by multiplying the gland length by the gland width at its widest point. Weight, body measurements, and sexual condition were recorded for all individuals; and testes size and weight were also recorded for males.

In addition to those specimens trapped, a number of museum skin specimens belonging to the Kansas State Teachers College Museum was examined for presence of midventral staining. Two juvenile males and two juvenile females, beginning at one month of age, were examined periodically to determine the onset of glandular activity.

Testicular Weight - Gland Area Relationship

To determine the relationship between testicular weights and glandular area, 15 males, ranging in weight from 17 grams to 441 grams, were castrated. The weight, length, and width of the testes were recorded, as well as the ventral gland area. Position of the testes, either scrotal or abdominal, was noted. A direct line correlation was run between the glandular area and testicular weights.

Influences of Gonadal Hormones

The influence of gonadal hormones on glandular development was examined using two littermate juvenile females. One was given 0.5 mg of testosterone propionate daily for 14 days. The other was used as a control and given a sham injection daily. Injections were subcutaneously administered to the medial side of the thigh. Daily records were kept of

the midventral region for signs of glandular proliferation. At the end of the two weeks, both specimens were sacrificed, midventral skin excised, and vertical sections prepared.

In another experiment, three adult male wood rats were castrated, the glandular area was measured, and the rats allowed to recuperate for 20 days. On day 21, a daily series of 0.8 mg of testosterone propionate injections was initiated and continued for seven days. Glandular area was measured every other day from day 0 to day 28.

Two adult female wood rats were ovariectomized and glandular area measured every other day for three weeks. This was to determine the effect of estrogen and progesterone on glandular maintenance.

Histochemistry

In order to establish some basic properties of the specialized glands, several histochemical techniques were utilized. Wood rats used for these studies were not separated according to sex, season, or age, since all possessed the gland, and the histochemical properties should essentially be identical. Both frozen sections (15μ) and paraffin sections (10μ) were used according to the type of staining technique involved. The specific fixative used (Bouin's, 10% formalin, etc.) was also dependent upon the technique.

To detect total fat content (liquid lipids), Sudan Black B was used in conjunction with frozen sections (Humason, 1967). Some tissues were extracted with xylene: glacial acetic acid (3:7) for 15 hours and then stained as before. Sudan Black B was also utilized to stai for phospholipids (Humason, 1967) using paraffin sections. For neutral lipids, Oil Red O was used on frozen sections (Gomori, 1967).

Acidic lipids and basic lipids (unsaturated glycerides) were differentiated with the Lillie Alternate Nile Blue A method (Humason, 1967). Ninhydrin was used to detect the presence of α-amino acids. Untreated frozen sections were examined with a light microscope for natural color.

Fluorescence was observed by using a Schott BG 12 emission filter and a 510 eyepiece filter in a Leitz fluorescence scope. Other filters were tried, but this combination gave the brightest fluorescence from the glands. A Mineralight short wave UV light was used to observe external fluorescence of the intact glandular region on the wood rats before dissection of the gland.

RESULTS

Sex, Age, and Seasonal Relationships

The ventral pelage of adult Neotoma floridana consists of pure white hairs on the pectoral and inguinal regions, and white hairs with gray bases across the majority of the abdomen. Along the midline of the venter, the hairs are directed mesially and then posteriorly to form a furrow along the center of the abdomen. The specialized ventral gland is positioned along the midline of the venter, beginning in the lower pectoral region and continuing on the abdomen, being tapered at both ends (Fig. 3). The gland can be easily located by rubbing the hair backward over the midline of the venter. This reveals the gland exudate that has collected at the bases of the hairs. In addition to the sebum globules, a brownish stain on the hair over the gland may be present depending upon the sex and season of the year. This stain is greasy to the touch and has a strong musky smell that is readily evident to human faculties. The gland is more highly developed in males than in females (Figs. 3 and 5). In addition, gland size increases with maturity. Ventral gland area is correlated with body weight in males ($r = 0.81$; P .01) and females ($r = 0.$; P .0). Seasonal changes occur in the proliferation of the glandular units in both sexes.

Females

In all adult females captured throughout the year, a yellowish-brown waxy exudate was found at the bases of hairs over the midventral gland. This exudate did not extend to the base of the hair shaft. There was usually a gap of 0.5 to 1.5 millimeters between the skin surface and the point at which the exudate was located on the hair shaft. The sebum globule itself varied between 0.5 to 1.0 millimeters

Fig. 2. June 12 adult male showing area devoid of hairs over ventral gland.

Fig. 3. June 12 adult male with shaved venter showing location of ventral gland.

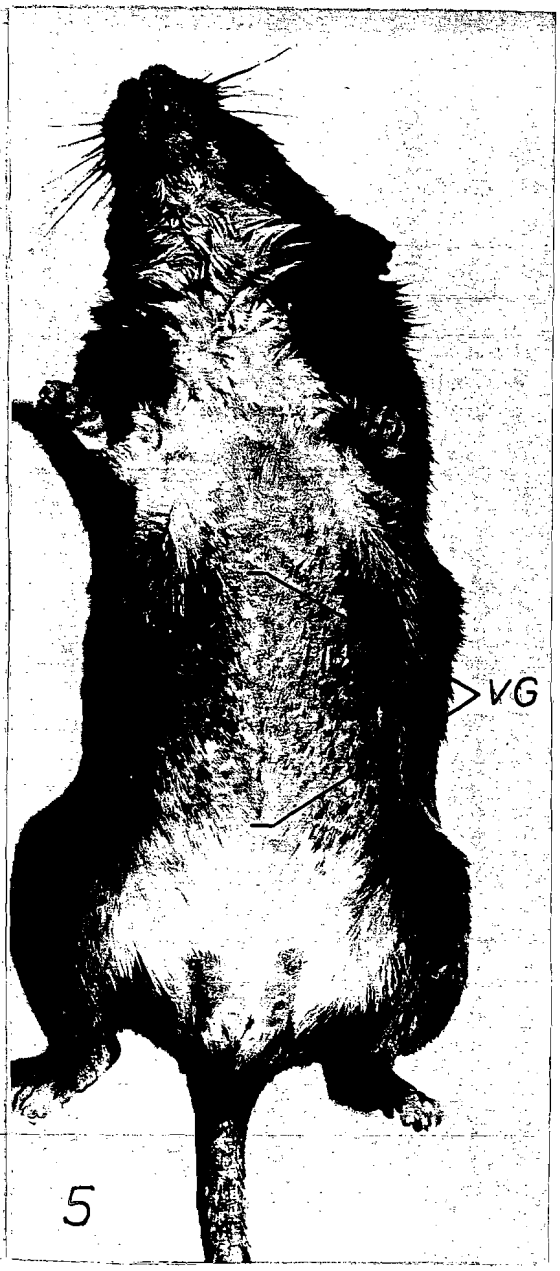

Fig. 4. Adult female with an unshaved venter.

Fig. 5. Adult female with shaved venter revealing the narrow ventral gland (VG).

in diameter. In many instances sebum globules caused clumping of several (2 to 7) hair shafts. This made the midventral hair appear sparse. Sebum globules were found in greatest density in the subpectoral region and directly down the midline of the venter. There was a decrease in number of globules lateral to the midline and also in the lower abdominal region.

In females, the largest glands were in those captured in the fall, September through November (Fig. 6). The average size of the gland during this period was approximately 300 mm^2. None of the females examined contained stained hairs on the venter, although a 6 October specimen had a faint discoloration of some midventral hairs. The sebaceous alveolar diameters did not vary to any appreciable degree during this period, ranging from 86 to 96 microns (Table I). These diameters are smaller than in spring and summer individuals. The number of cells per alveolus varied somewhat, but was mostly a matter of individual variation rather than a seasonal phenomenon.

Adult females caught in the winter (Dec. - Feb.) had the smallest alveolar size of the year, ranging from 76 to 89 microns in diameter (Table I). The glandular area decreased from the fall peak of 300 mm^2 to approximately 135 mm^2 (Fig. 6). Even though all of the females examined during this period contained sebum globules on hairs along the midventral gland, globules were not present in large numbers, indicating a period of glandular inactivity. A histological section of an inactive ventral gland is shown in Figure 7. Two December females exhibited a light brown stain on the midventral hairs.

One adult female captured on 17 February had a bare callous area on the subpectoral region of the venter which measured 28 by 10 mm.

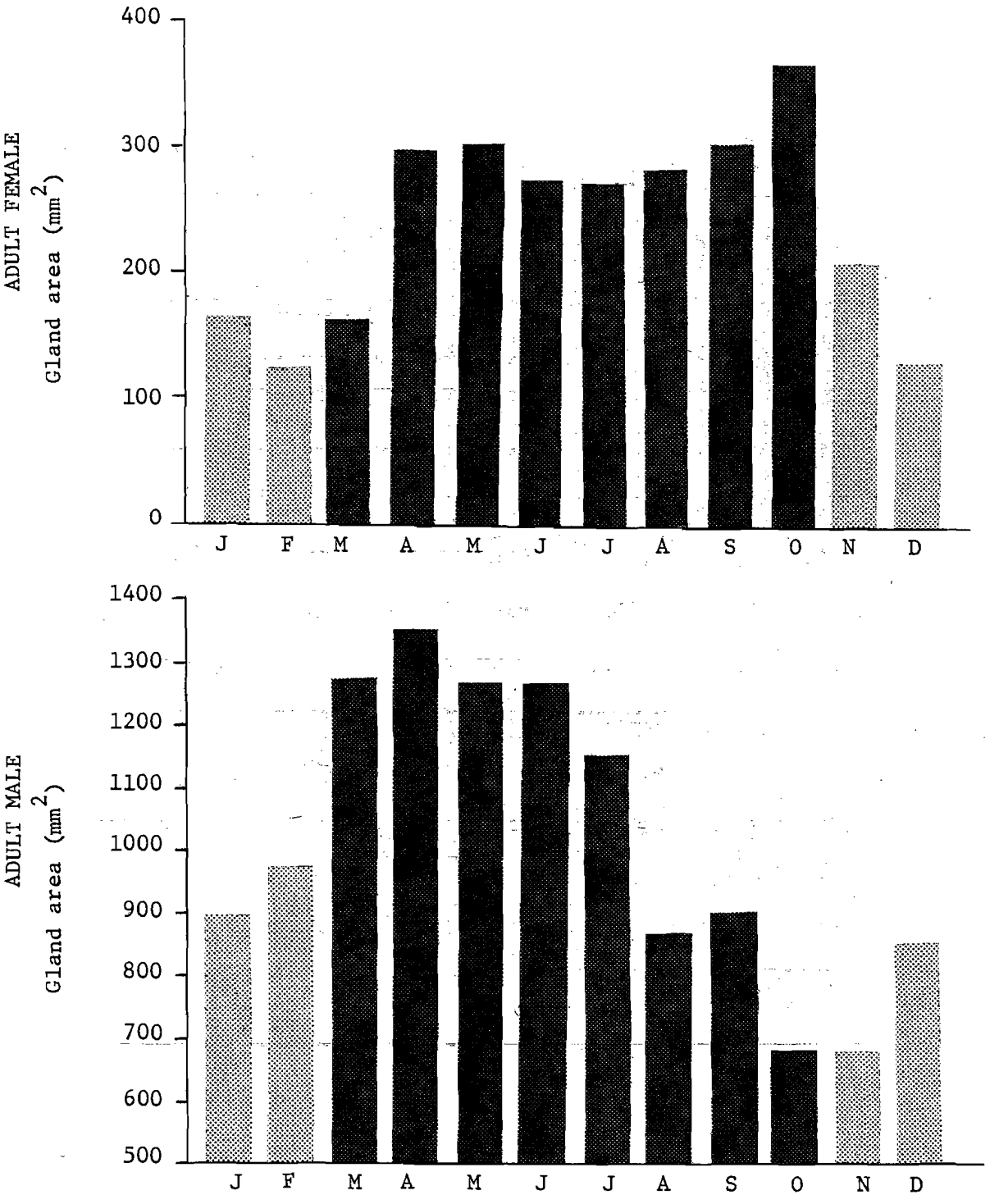

Figure 6. Relationship of midventral gland area (length X width) to months of the year for adult male and female N. floridana. Black bars indicate those months included in the breeding season.

Table I. Average alveolar size (AAS), average cell size (ACS), and number of cells per alveolus (C/A) for 20 female and 16 male adult N. floridana. Alveolar and cell measurements are in microns.

	FEMALES			MALES		
MONTH	AAS	ACS	C/A	AAS	ACS	C/A
JAN	76.3	14.8	137	243.0	20.0	1793
FEB	78.5	17.5	91	276.0	20.1	2270
MAR	65.0	14.1	99	299.0	22.0	2510
APR	163.1	17.7	782	283.6	19.6	3011
MAY	81.0	16.3	214	262.9	20.0	2271
JUN	121.0	16.7	389	258.5	20.5	2065
JUL	106.0	17.1	238	229.0	20.0	1510
AUG	94.1	15.8	211	172.3	19.6	679
SEP	92.3	16.3	182	139.4	19.2	383
OCT	96.4	13.8	347	122.8	20.0	232
NOV	86.2	15.1	186	128.6	18.8	320
DEC	88.6	18.7	106	177.5	17.9	975

Fig. 7. A transverse section of an adult female inactive ventral gland (40x). SG, sebaceous gland; HF, hair follicle.

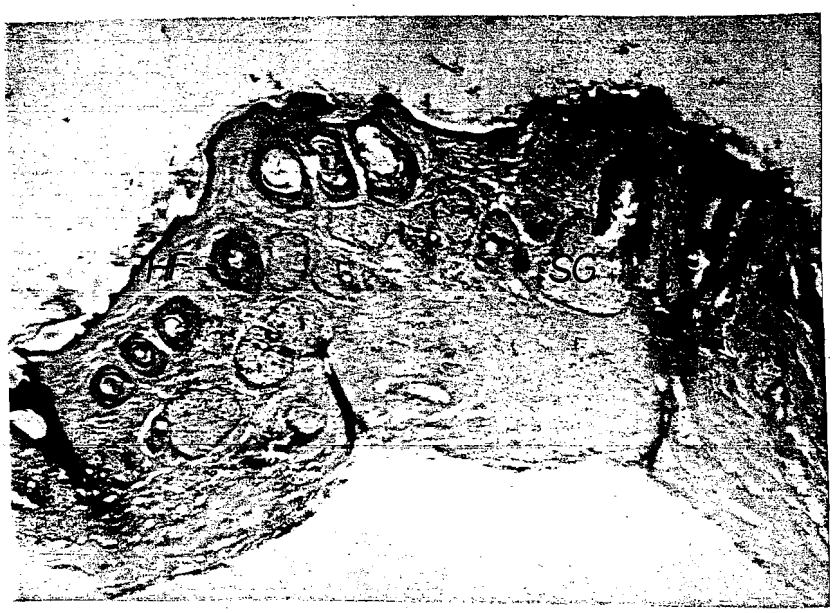

Fig. 8. A transverse section of an adult female active ventral gland (40x). SG, sebaceous gland; HF, hair follicle.

Approximately one-third of this area showed prominent glandular activity. Immediately posterior, a narrow strip of sebum globules (3 mm wide) continued down the midline of the venter. It was not ascertained whether the bare area consisted of old scar tissue or whether hair loss was associated with the gland activity.

No adult females caught from March through May had signs of midventral staining (Fig. 9). During this period, there was an increase in glandular area (Table II) and ventral gland alveolar size (Table I and Fig. 8). Proliferation of the glandular alveoli resulted in a range of alveolar diameters from 65 to 163 microns. This extensive variation was due in part to a single large female which had an unusually well developed midventral gland, but no ventral staining. Most of the spring females were either pregnant or lactating when captured.

Summer adult females maintained a midventral gland area of about 280 mm^2, somewhat smaller than for females in April and May (Table II). As summer progressed, trends toward smaller gland size and fewer cells per alveolus were noted (Table I and Fig. 10). These trends leveled off in the fall months. One female, captured in June, had light brown staining on the hairs of the venter. All the females captured during the summer were either nursing young or were pregnant.

Females, in general, did not have stiffer or coarser hair over the midventral gland than on the rest of the body. There was no indication of hair loss on the gland, except in the one instance mentioned previously. The thickness of the midventral skin in adult females was not significantly different from that of subadult females ($P>0.05$), but was significantly smaller than adult males (Table III). When midventral skin was compared with skin lateral to the ventral gland (Tables V and VI), there were no

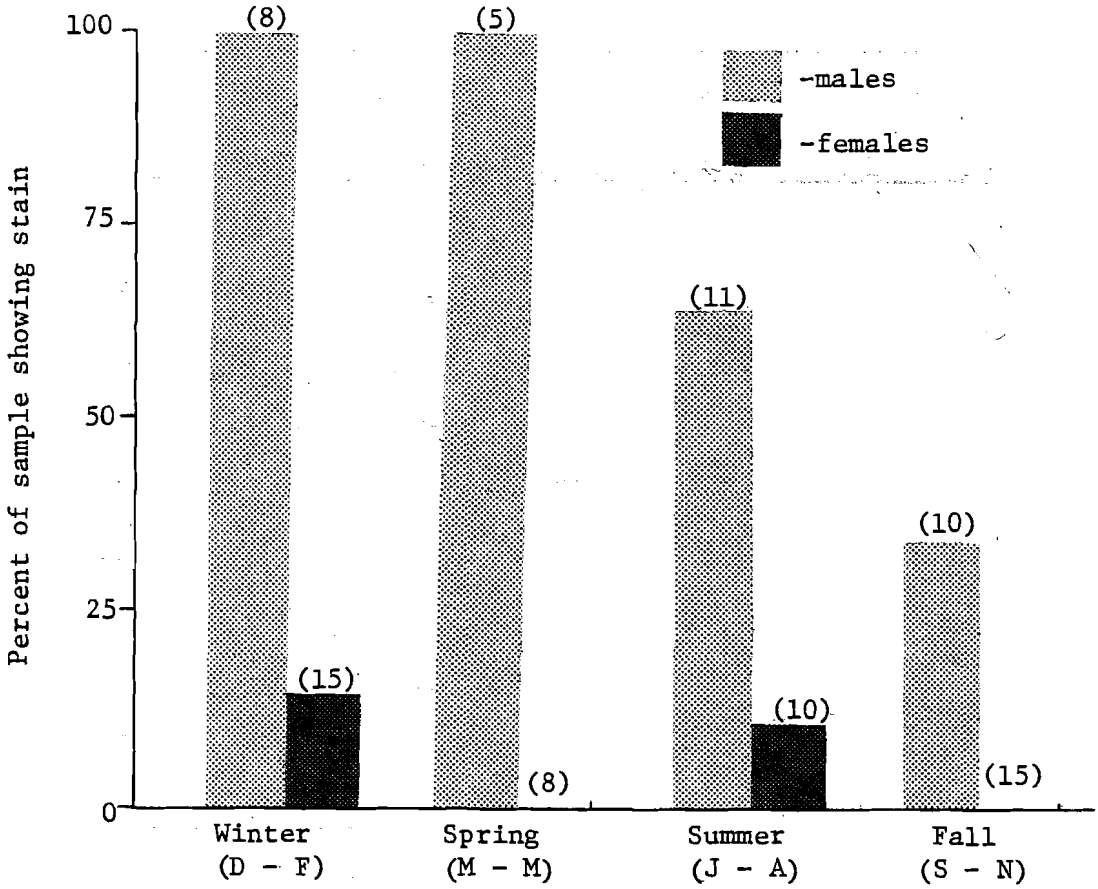

Figure 9. Seasonal variation in midventral staining in 82 adult male and female N. floridana; numbers in parentheses indicate sample size. D - F indicates December through February.

Table II. Monthly means of midventral gland area (length x width) of N. floridana. All measurements are in mm^2.

	ADULTS		SUBADULTS		JUVENILES	
MONTH	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
JAN	897	165				
FEB	963	131				
MAR	1250	162				
APR	1342	290				
MAY	1260	302	372		0	0
JUN	1260	274	278	82	0	0
JUL	1141	278	292	105		
AUG	870	281			0	0
SEP	912	304				
OCT	692	360				
NOV	686	205				
DEC	849	126				

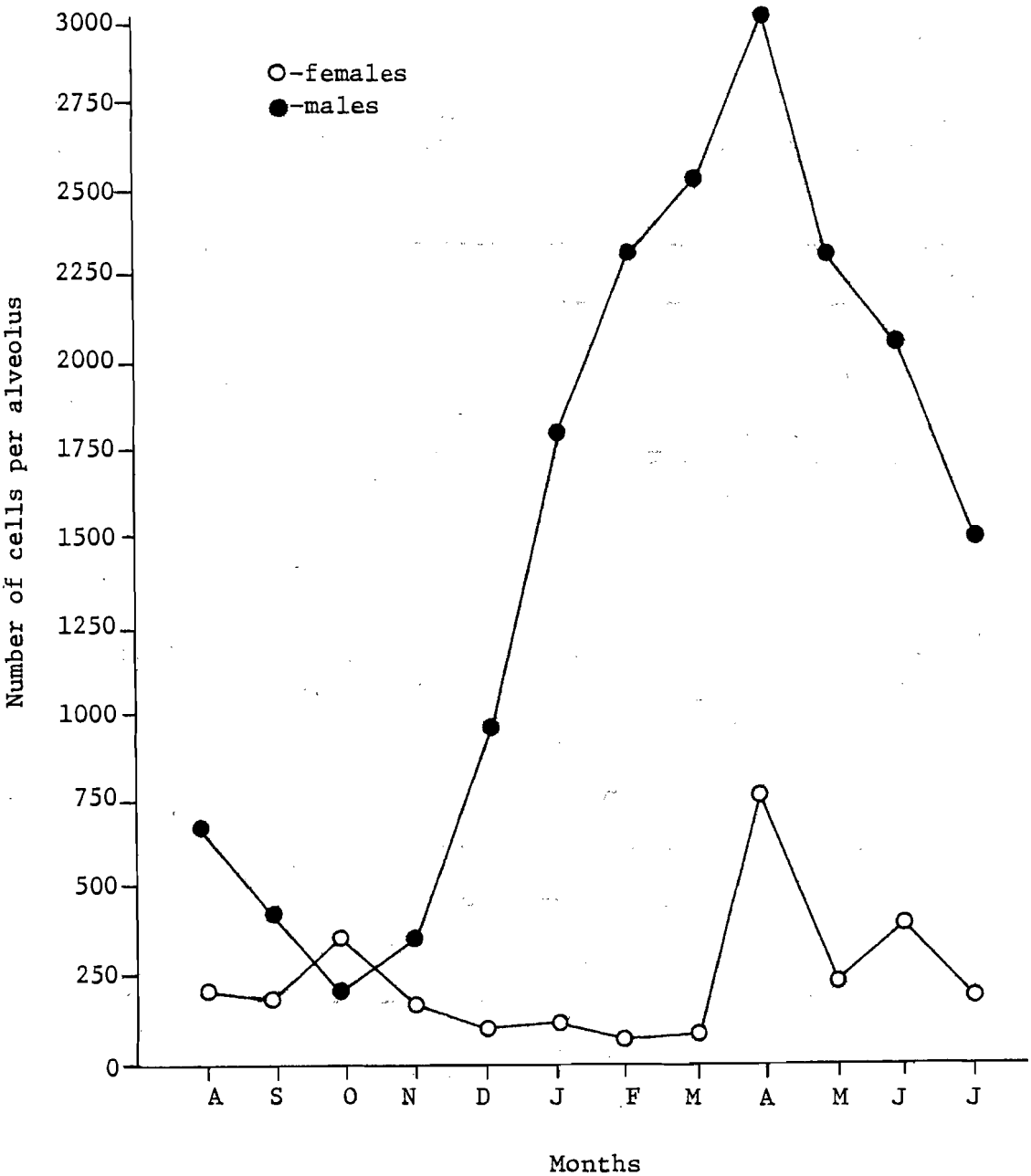

Figure 10. Monthly changes in number of cells per alveolus in the midventral gland for adult N. floridana from August, 1972 through July, 1973.

Table III. Significant (+) and non-significant (0) differences at 0.05 level in various measurements of the midventral gland between male and female Neotoma floridana. MGA, midventral gland area; AAS, average alveolar size; ACS, average cell size; C/A, number of cells per alveolus; ST, skin thickness; ET, epidermis thickness; and ACL, number of acidophilic cell layers in the alveoli.

	MGA	AAS	ACS	C/A	ST	ET	ACL
ADULTS	+	+	+	+	+	+	+
SUBADULTS	+	+	+	+	0	+	0

Table IV. Significant (+) and non-significant (0) differences between sexes of adult N. floridana in measurements of sebaceous glands and skin lateral to the midventral gland. Legend same as above.

	AAS	ACS	C/A	ST	ET	ACL
ADULTS	+	0	0	0	0	0

Table V. Average skin thickness (ST), epidermis thickness (ET), and number of acidophilic cell layers (ACL) in alveoli for N. floridana.

	ADULT		SUBADULT		JUVENILE	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
ST (mm)	0.98	0.77	0.83	0.60	0.40	0.47
ET (μ)	28.3	9.3	23.3	8.8	8.0	7.0
ACL	7.9	1.5	1.7	1.3	0.0	0.0

Table VI. Measurements on sebaceous glands and skin lateral to the midventral gland in adult N. floridana. AAS, average alveolar size; ACS, average cell size; C/A, number of cells per alveolus; ST, skin thickness; ET, epidermis thickness; and ACL, acidophilic cell layers in alveoli.

	MALE	FEMALE
AAS (μ)	74.5	51.0
ACS (μ)	17.1	15.3
C/A	86	43
ST (mm)	.70	.60
ET (μ)	10	11
ACL	1.0	0.8

significant differences (0.05 level) between skin thickness, epidermis thickness, or layers of acidophilic cells in the alveoli. However, alveolar diameters in the ventral gland of adult males were significantly larger than those in the lateral skin of adult females.

In no female did the gland units occupy more than one-third of the ventral dermis thickness. Generally, there was only one sebaceous gland, or occasionally two, associated with a follicle; none was observed to be miltilobulated.

Subadult females captured in the spring and summer had sebum globules on the hairs in the midventral region. The glandular area was only about one-third of that found in adult females of the same months (Table II). No subadult female was found with stained hairs on the venter. Glandular alveoli averaged 73.6 microns in diameter (Table VII), which is significantly smaller than adult female alveoli ($P<0.05$). The glandular units were not as abundant in subadults as in adult females (Fig. 11).

No juvenile was found with an active abdominal gland. The midventral sebaceous glands could not be differentiated from unspecialized glands lateral to the abdomen in either size or number (Fig. 12). No difference was noted between the sexes, and epidermal and dermal thicknesses were not different than those of lateral skin.

Males

Adult males exhibited a distinct seasonal cycle in which hairs on the venter were stained from the abdominal gland exudate (Fig. 9 and Table VIII). The intensity and darkness of the stain varied from one individual to another. During seasons when ventral staining was present,

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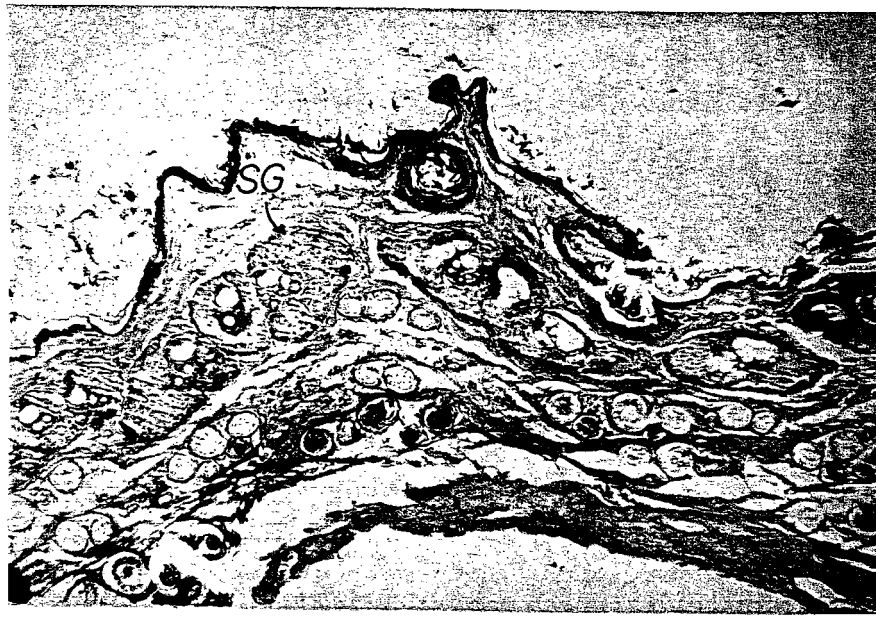

Fig. 11. Transverse section of the midventral gland in a subadult female (60x). SG, sebaceous gland.

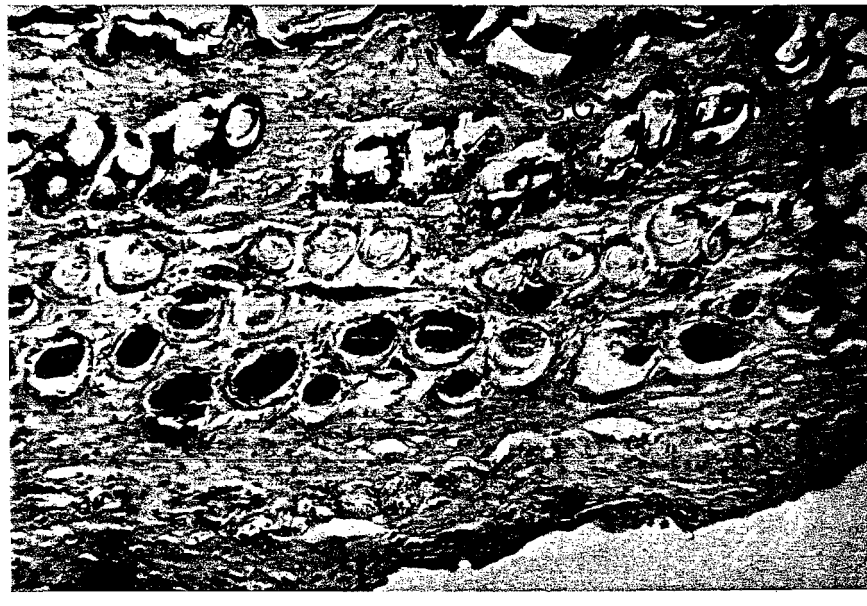

Fig. 12. Transverse section through the midventral skin in a juvenile female (150x). SG, sebaceous gland.

Table VII. Average alveolar size (AAS), average cell size (ACS), and numbers of cells per alveolus (C/A) in the mid-ventral gland of subadult and juvenile N. floridana. Alveolar and cell measurements are in microns.

	SUBADULTS		
	AAS	ACS	C/A
MALE	192.5	17.6	1323
FEMALE	73.6	15.1	122

	JUVENILES		
	AAS	ACS	C/A
MALE	75.1	15.2	123
FEMALE	59.8	12.1	121

Table VIII. Monthly percentages of presence of the midventral stain in 103 specimens of *N. floridana*. Numbers in parentheses indicate sample size.

	ADULTS		SUBADULTS		JUVENILES	
MONTH	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
JAN	100 (2)	0 (4)				
FEB	100 (1)	0 (2)				
MAR	100 (2)	0 (2)				
APR	100 (1)	0 (1)				
MAY	100 (2)	0 (5)	100 (1)		0 (2)	0 (3)
JUN	100 (3)	20 (5)	100 (3)	0 (2)	0 (1)	0 (1)
JUL	75 (4)	0 (3)	100 (1)	0 (1)		
AUG	25 (4)	0 (2)	0 (1)	0 (2)	0 (2)	0 (1)
SEP	33 (3)	0 (7)				
OCT	33 (3)	0 (7)				
NOV	25 (4)	0 (1)				
DEC	100 (5)	11 (9)				

there were few sebum globules at the bases of hairs along the gland region. The globules were observed in greatest numbers along the lateral edges of the abdominal region and in the mid-pectoral region. A gradual loss of hair on the ventral gland was also associated with the presence of stain, which became greatest in the times of largest glandular proliferation and activity.

Thirty-three per cent of the adult males captured during September through November showed ventral staining. In these individuals, the stain was light brown. No specimen examined had an area devoid of hair in the glandular region at that time. The midventral gland area averaged 763 mm^2 (Table II and Fig. 6), which was the most reduced size of the year.

During the fall, the alveolar size in the midventral gland reached its lowest point of the year (Fig. 13), but was still significantly larger than the alveolar size of females at any time of the year. The number of cells per alveolus was also greatly reduced, although the cell size remained about the same (Table I). The reduced size of the male ventral gland is shown in Figure 13.

All adult males captured in the winter had midventral stains. This staining became increasingly greater in intensity with time, as shown in the following examples. A 18 December specimen, with abdominal testes, had a sparse scattering of sebum globules across the entire venter (30-40 mm wide). However, most of the exudate was at the subpectoral location, and from there tapered to a narrow strip (1 mm) on the stomach. The hair over the venter was coarser than hair elsewhere on the body. A midventral staining of the hair (light brown) was present over the stomach region, which was posterior to the area of greatest number of sebum

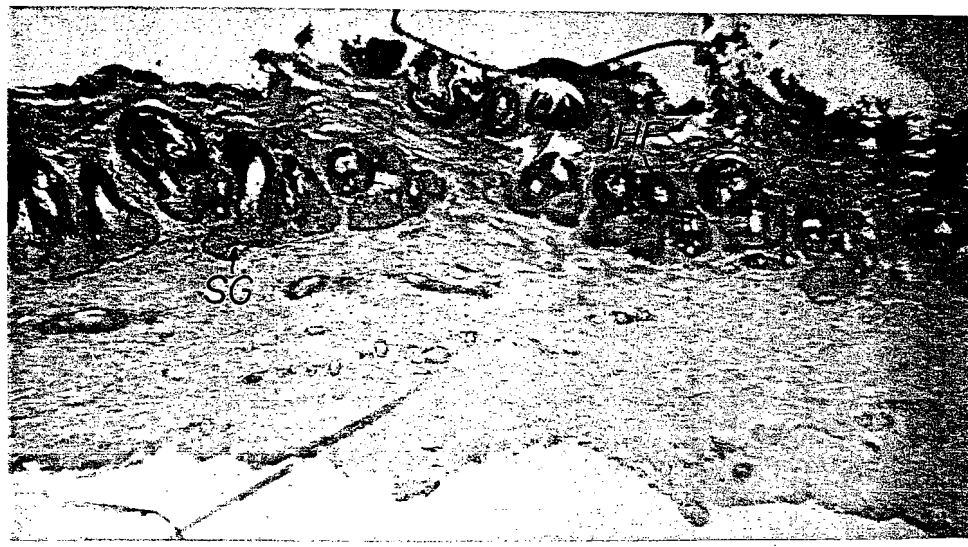

Fig. 13. Transverse section of an adult male inactive ventral gland (40x). HG, hair follicle; SG, sebaceous gland.

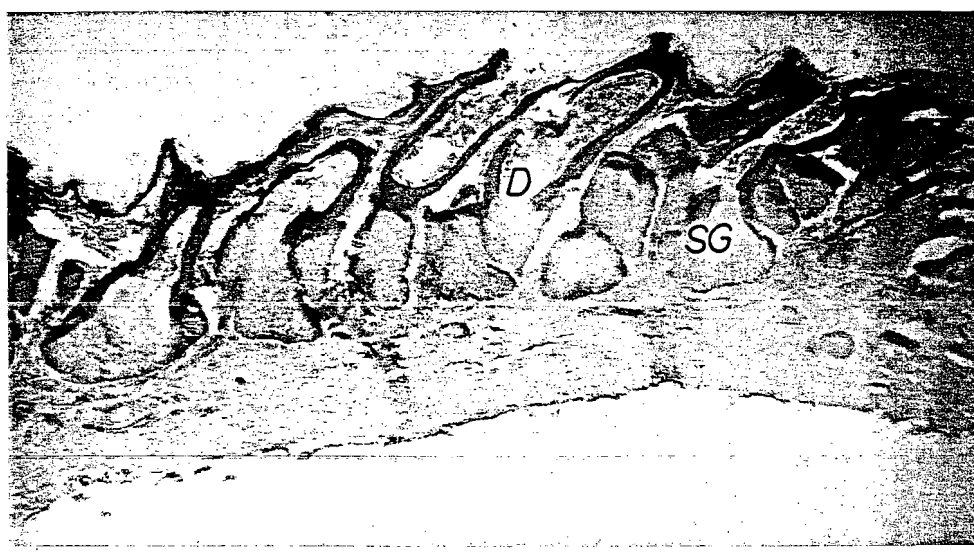

Fig. 14. Transverse section of an adult male active ventral gland (40x). Note absence of hair follicles. D, duct; SG, sebaceous gland.

globules (subpectoral). This stained region was 58 by 24 mm.

A 19 January male, with scrotal testes, had virtually no sebum globules present, except in a 3 mm^2 area on the pectoral region. The majority of the ventral hair was discolored, with the midline of the abdomen being darkest. A yellowish tint bordered the dark brown stain. The area of discoloration extended from a point between the forelimbs to the testicular region, tapering at both ends. The dark stain (45 x 18 mm) was heaviest on the stomach area about 25 mm anterior to the penis. In the region of heaviest staining, there were several places where the hairs were matted together with the exudate.

A male captured 26 February with scrotal testes had stained hair on a large portion of the venter. The darkest stain was again over the stomach region and measured approximately 50 mm long by 25 mm wide. Progressing outward from the dark stain, the discoloration gradually changed to a medium brown, a yellowish-brown, and, along the outer periphery of the stain, a subtle yellow.

The midventral gland area increased in winter males to an average of 903 mm^2. Table I shows that the alveolar diameters in the abdominal gland increased in size each month, in addition to an increase in the number of cells per alveolus. Glandular proliferation took place at its greatest rate during these months, especially in January.

Adult males captured in the spring exhibited the maximum gland area, 1324 mm^2 (Table II), and the maximum alveolar size, 299 microns (Table I), of the year. Some of the sebaceous glands became multilobulated while others simply enlarged and remained as a single entity (Fig. 14). All specimens during this time period had midventral staining similar to those described for January and February. However, it was during these months when hair

loss began to occur on the ventral gland. A 18 March male had an area, directly down the midline of the venter, that was not bare, but hairs were sparse and coarse to the touch. A 16 April male showed an area 20 x 5 mm directly over the xiphoid region that contained no hair, and a 4 May specimen had a hairless area measuring 24 x 5 mm.

Sixty-four per cent of the summer males had ventral staining (Fig. 9). The staining was not as intense as in previous months and was yellow-brown. The areas which were devoid of hair on the venter appeared to enlarge and then began to diminish. A bare area 65 x 9 mm was found on a 8 June specimen (Fig. 2), whereas a 26 June male had a 45 x 5 mm area devoid of hairs. The bare areas were rough and scaly. No August males were found with hair loss on the venter.

Table I shows a steady decrease in the alveolar size and number of cells in the alveoli of the midventral gland from June through August. The size of alveolar cells did not change appreciably from other times of the year. The reduction in gland activity was accompanied by a reduction in the ventral gland area (Fig. 6).

Subadult males captured in May through July (with abdominal testes) all had midventral staining (Table VIII). The stain was yellowish-brown and darkest on the stomach area, but was never as intense as in adult males. The average size of the stain was 45 mm long by 7 mm wide. A yellowish discoloration surrounded the darker region. Fairly heavy sebum production was evidenced by a large number of sebum globules on hairs throughout the glandular area. No hair loss on the venter was observed in subadult males and the ventral hair was soft, not coarse as in adult males, at that time.

The midventral gland area of subadult males was not as large as in

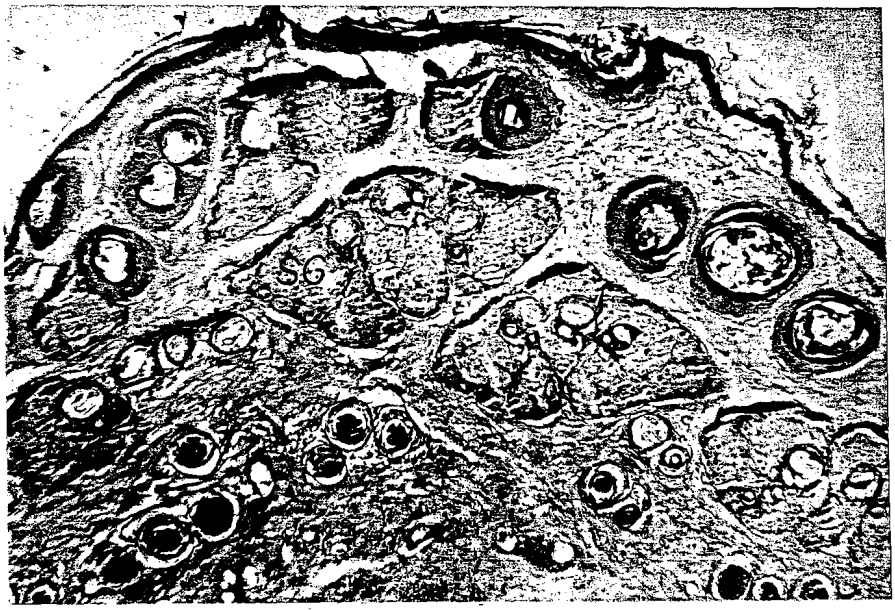

Fig. 15. Transverse section of the ventral gland in a subadult male (100x). HG, hair follicle; SG, sebaceous gland.

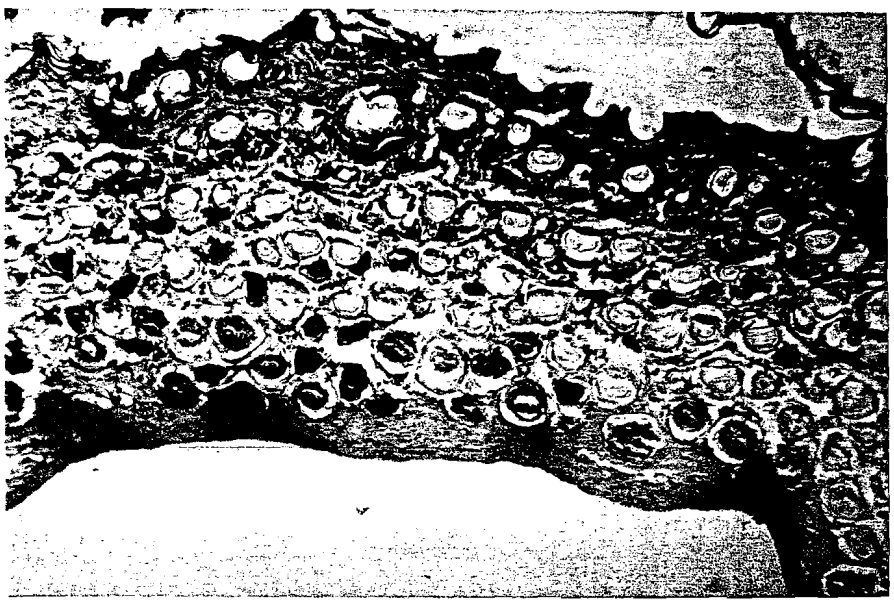

Fig. 16. Transverse section of the ventral skin in a juvenile male (150x). No sebaceous glands can be distinguished in the photograph.

adult males, and was approximately equal to that of adult females (Table II). However, alveoli of subadult males were greatly enlarged, averaging 192.5 microns in diameter (Table VII and Fig. 15). This surpassed any adult female of equivalent gland area. The number of cells per alveolus was comparable to that of adult males, while the alveolar cell diameters were more like those of adult females (Table VII).

No juvenile male examined had evidence of glandular proliferation. There were no sebum globules found along the gland region. Histological sections revealed the gland primordia to be indistinguishable from unspecialized sebaceous glands of the lateral skin (Fig. 16).

The portion of skin containing the ventral gland was significantly thicker in adult males than in females (Table III). Epidermal thickness, due to an increased number of cell layers, was also greater for males. In the ventral gland alveoli, adult males had a significantly greater number of acidophilic cell layers than females. Subadult males had a greater total skin thickness than either subadult or adult females. These thicknesses approached those of adult males (Table V). However, subadult males did not have as many acidophilic cell layers as adult males. When compared with sebaceous glands in the skin lateral to the abdominal gland (Table VI), both adult and subadult males had larger measurements than females in all cases. Among the lateral sebaceous glands alone (Fig. 17), the males had significantly larger alveolar diameters than females (Table IV).

Gland Development

Figure 18 shows that ventral gland proliferation began at approximately 45 to 55 days of age in males, and from 70 to 80 days in females. Not

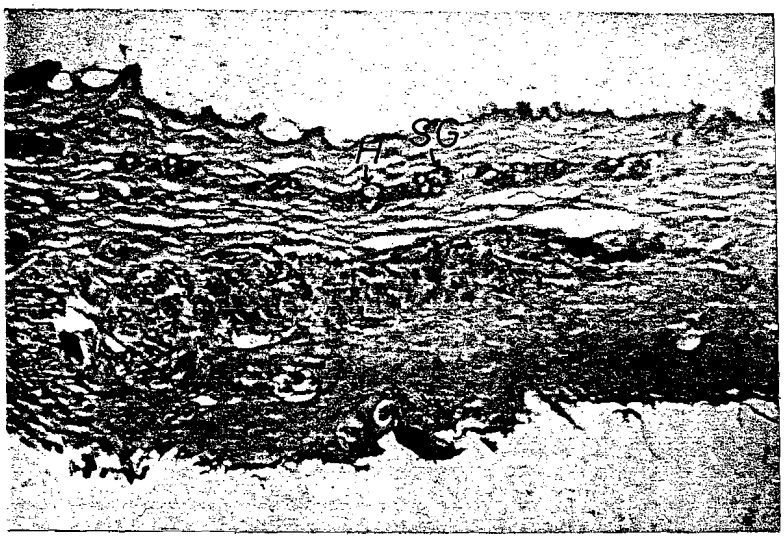

Fig. 17. A shows a transverse section of skin lateral to the ventral gland in an adult female (40x). B shows a sebaceous gland (SG) and a hair follicle (H) at higher magnification (400x).

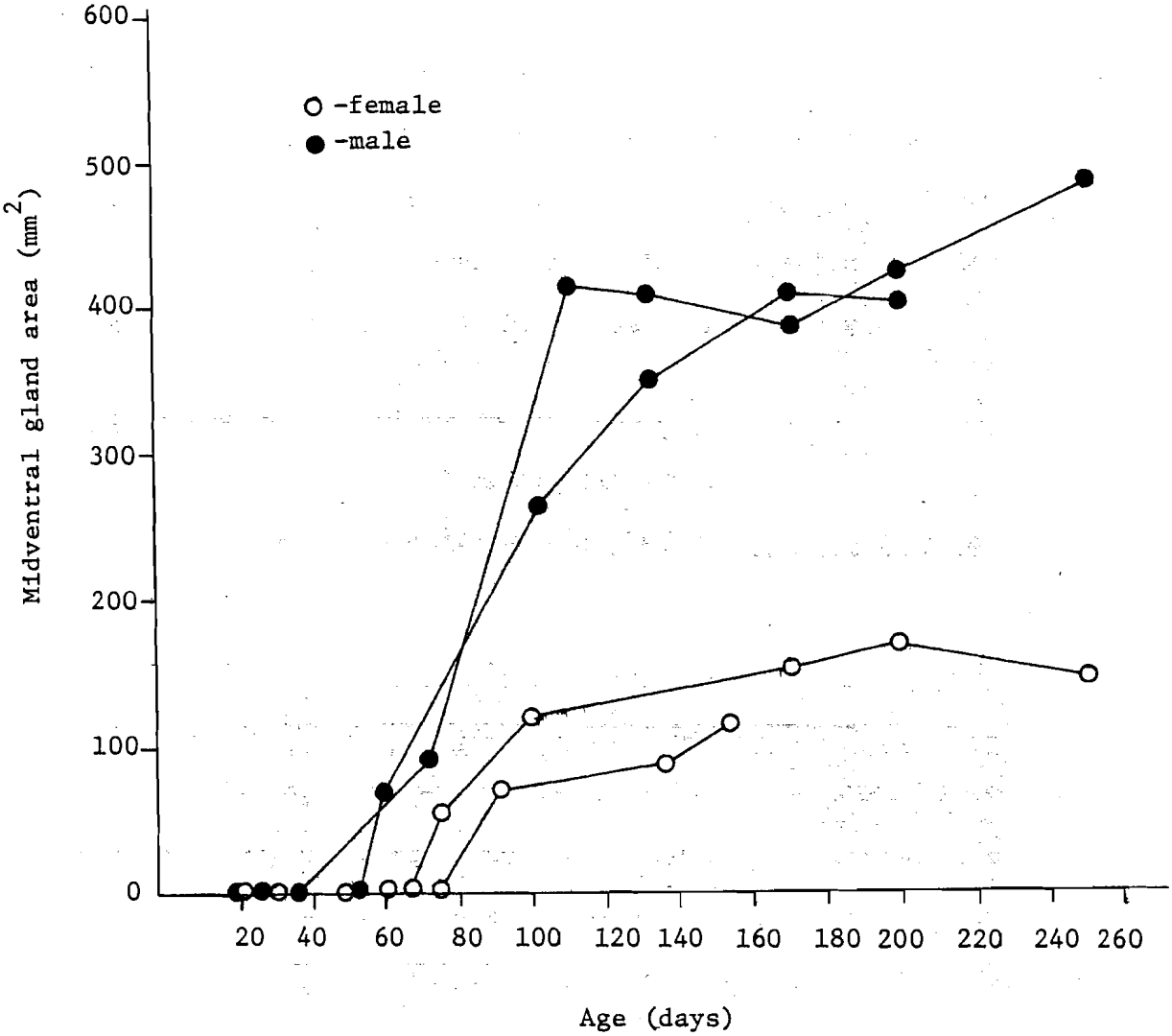

Figure 18. Development of the midventral gland in two male and two female N. floridana.

some instances was continuous with the surface epithelium. The majority of ducts, however, were joined to the hair follicles, in which case the duct epithelium was continuous with the outer layer of follicular epithelium. The ducts were composed of many layers of cells which were gradually reduced in number until a single layer of cells became the basal layer surrounding the gland alveolus. The cells of the basal layer were flattened and somewhat overlapping, and contained small flattened nuclei. No visible lipid droplets were discernible in this basal layer.

Next to the basal layer, were two or three layers of cuboidal cells, which represented the immature sebaceous cells. These contained few, if any, lipid droplets and were smaller in size than the majority of alveolar cells. Large, polyhedral-shaped cells constituted the bulk of the alveolus. These cells had large, round or ellipsoid nuclei, with many chromatin granules and a prominent nucleolus present. The cell cytoplasm was filled with a multitude of lipid droplets. Various structures of the sebaceous alveoli are shown in Figure 20.

Toward the neck of the alveolus, there were several layers of cells which were in the process of breaking down to form sebum. Close examination revealed that the nuclear membrane probably dissolved first, resulting in a release of the nuclear contents into the cytoplasm. It was at this point that the cells changed from basophilic, as exhibited by the majority of alveolar cells, to acidophilic. This view was supported by the following observations. Many cells in this borderline region were observed to have irregularly shaped nuclei (Fig. 21), thus indicating a recent rupture or disintegration of the nuclear membrane. Directly surrounding these nuclei, the cytoplasm was acidophilic (red), while the remainder of the

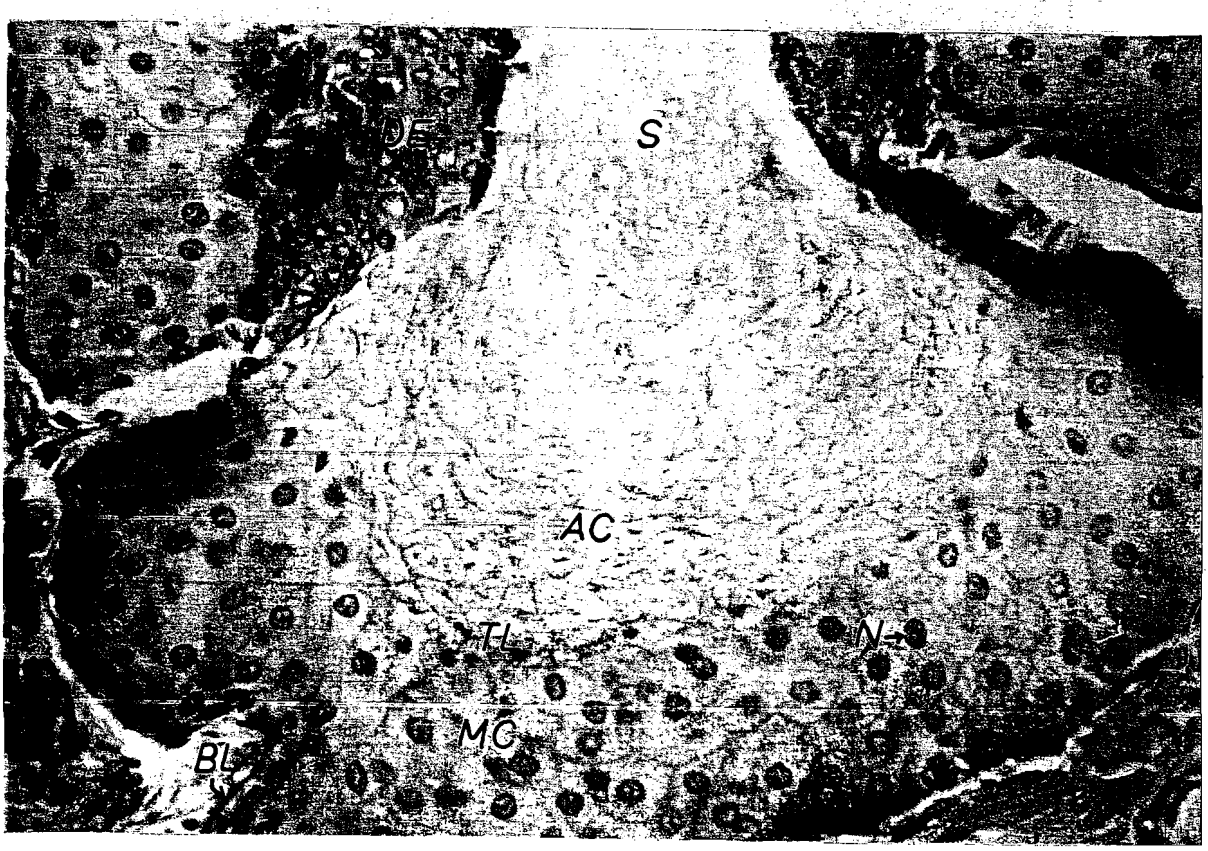

Fig. 20. Transverse section (400x) through a midventral sebaceous alveolus indicating various structures mentioned in the text. Also shown are zones of cellular development. AC, acidophilic cells (Zone 4); BL, basal cell layer (Zone 1); DE, duct epithelium; IC, immature cells (Zone 2); MC, mature cells (Zone 3); N, nucleus; S, sebum (Zone 4); TL, transition layer between basophilic and acidophilic stained cells.

peripheral cytoplasm was basophilic (blue). Cells farther along in degeneration were always completely acidophilic. In the following stage, the cell membrane ruptured, and the cellular constituents were dumped into the duct to form the sebum.

The number of acidophilic cell layers in the alveoli varied with sex, age, and season. During the breeding season adult males exhibited the largest number of these cell layers with acidophilic cells occupying up to two-thirds of the alveolus at that time. The number decreased when the glandular activity decreased during the fall. Subadult and juveniles of both sexes, as well as adult females, generally had from one to four acidophilic cell layers, which did not change in number at different times of the year.

Sebum in the ducts was always acidophilic. There was no sebum pillar that extended through the duct to the surface. The sebum remained as an amorphous oily material in the duct until it was pushed to the surface by pressure of new sebum being formed below. The sebum was in largest quantities in the ducts of adult males during the breeding season.

Stroma

Each sebaceous gland was surrounded by a thin connective tissue capsule which was continuous with the rest of the stroma. When the gland was partially lobed or divided into separate alveoli, an invagination of elastic connective tissue was seen to permeate the interlobular septa. Collagenous fibers made up the majority of the stroma which contained numerous fibroblasts. Elastic fibers were present and found scattered throughout the collagenous fibers. Fibroblasts were the most numerous cells in the stroma with histiocytes, lymphocytes, and leucocytes also

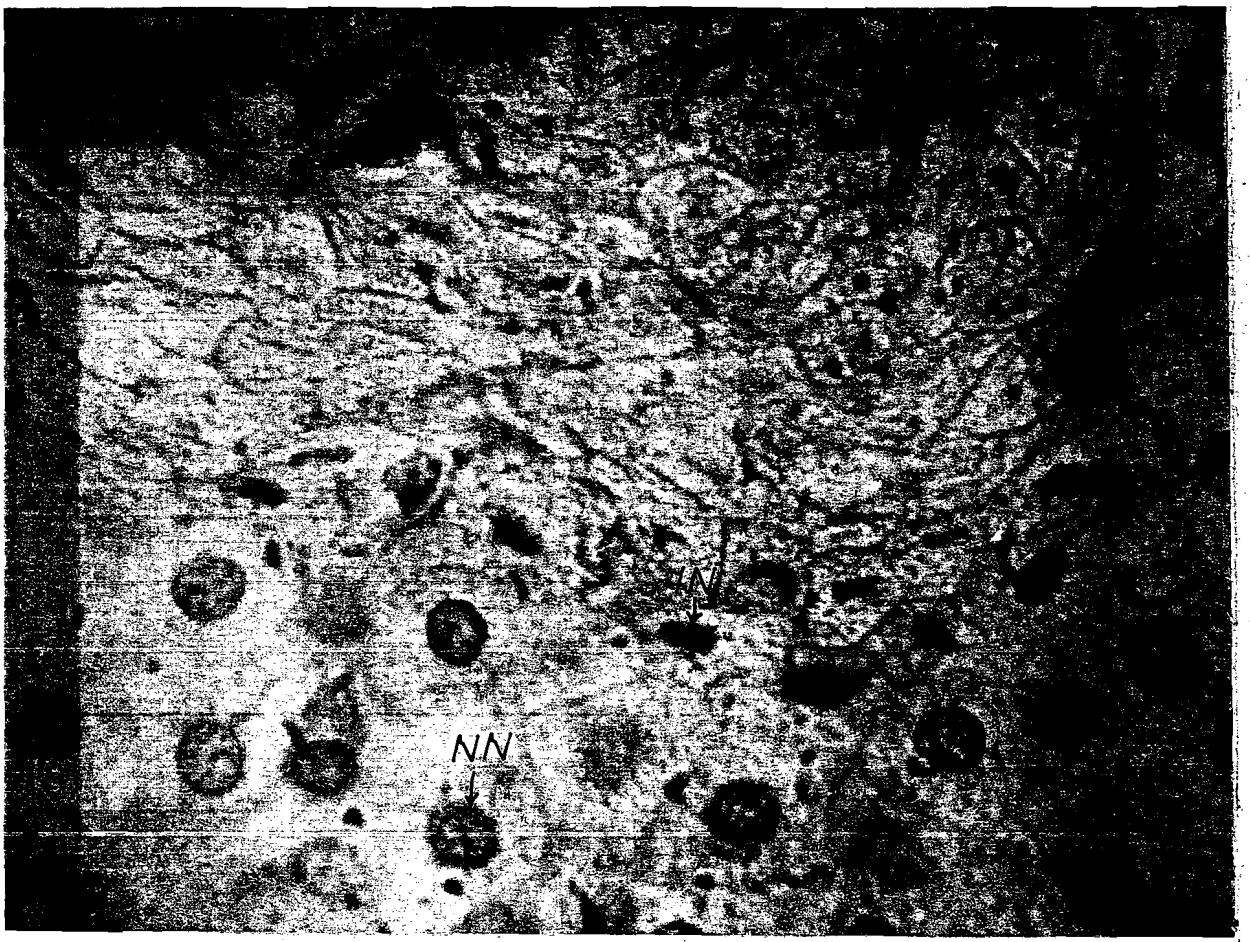

Fig. 21. Photomicrograph of irregularly shaped nuclei (IN) in borderline (transition) zone of sebaceous alveoli (400x). Also shown are the normal shaped nuclei (NN) of cells in Zone 3.

present. Mast cells, stained with Neutral Red, were present in the upper portion of the dermis. Venules, arterioles, and capillaries were abundant in the dermis, and were found most frequently in the upper one-half. Few arrector pili muscles were observed in the ventral gland area.

Testicular Weight - Gland Area Relationship

A direct correlation exists between wet testes weight (grams) and ventral gland area (mm^2); $r = 0.95$, $P < 0.005$ (Fig. 22). A partial correlation, to rule out the effects of body weight, revealed a smaller, but still significant, correlation between testes weight and gland area ($r = 0.83$; $P < 0.005$). There was a marked tendency for males with scrotal testes to have a larger glandular area and more intense staining of ventral hair than those males with abdominal testes. Only one captured male (December) with abdominal testes had a large gland area and ventral staining.

Influences of Gonadal Hormones

The juvenile female given injections of testosterone propionate showed a massive enlargement of the ventral gland tissue, both in number of glands and gland size, compared to the control female (Figs. 23 and 24). A large number of acidophilic cells was present in the alveoli of the injected female. External appearance of the ventral gland remained unchanged until day 12, at which time the experimental female began to develop a few sebum globules at the hair bases along the midline of the venter. By day 14, when the female was sacrificed, the glandular exudate was found in an area 9 mm long by 3 mm wide immediately posterior to the xiphoid cartilage. The control female, on day 14, showed no signs of ventral glandular activity.

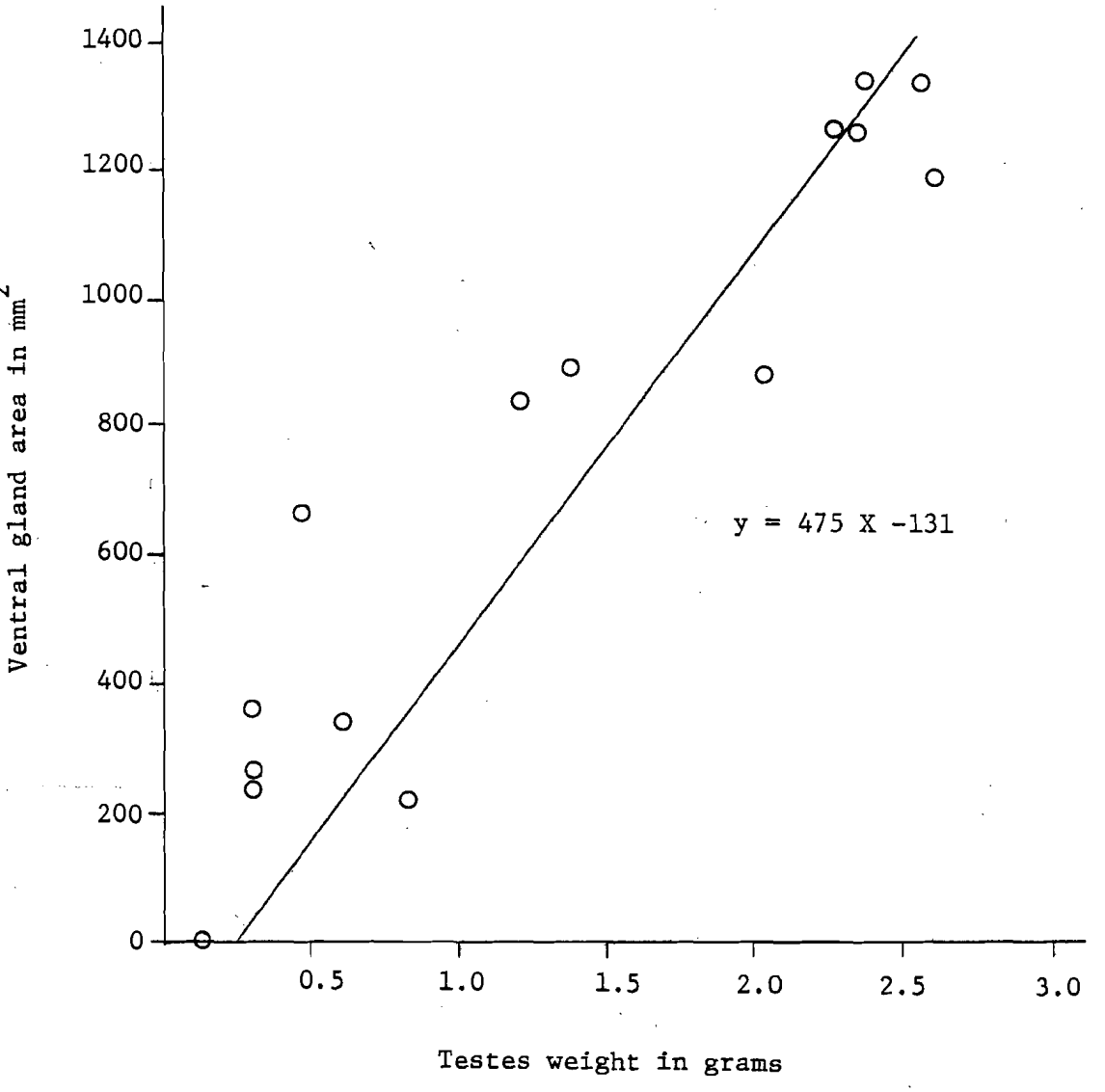

Figure 22. Relationship of wet testes weight to ventral gland area in 15 male N. floridana.

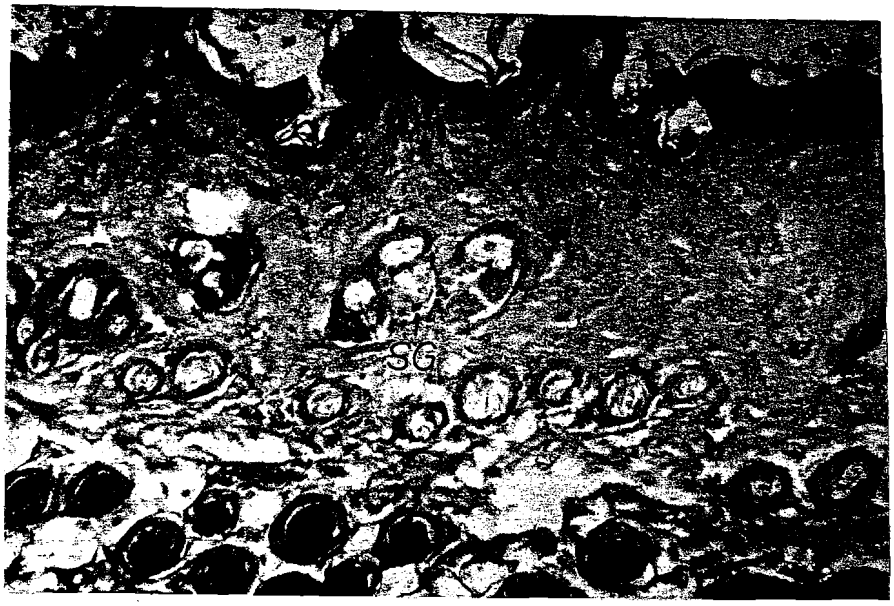

Fig. 23. Transverse section through ventral skin of control juvenile female not receiving testosterone propionate injections (60x). SG, sebaceous gland.

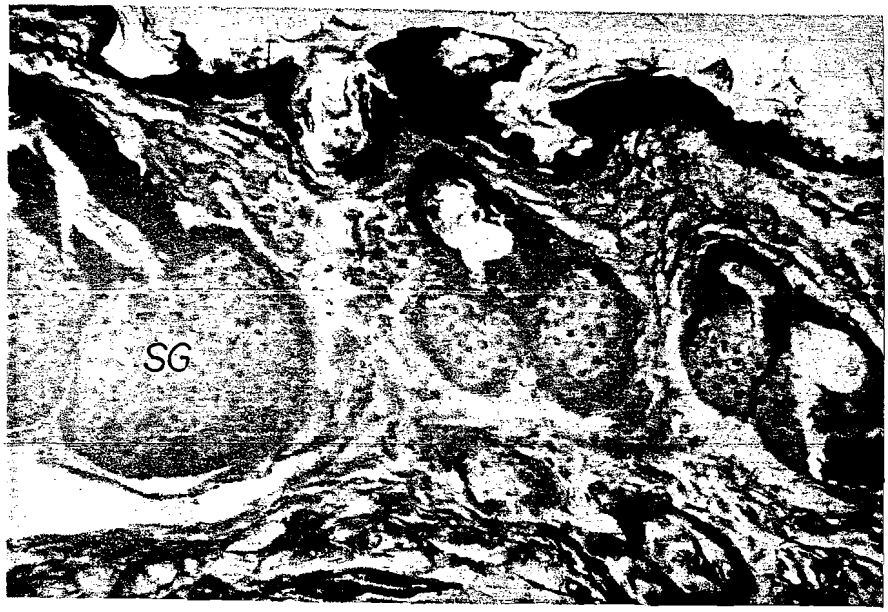

Fig. 24. Transverse section of ventral gland of juvenile female which received testosterone propionate injections (60x). SG, sebaceous gland.

only do male glands begin to develop at an earlier age, but they also proliferate at a much faster rate than in females. There was an abrupt increase of glandular growth in young males 30-40 days after initial gland development, and then the proliferation leveled off. Young females attained a near maximum gland area about one month after gland growth began. All of these individuals were raised in the laboratory, so perhaps the laboratory light cycle (9 L - 15 D) affected the glandular growth in a way that may not occur in the natural environment.

Histology

General Description

The specialized midventral gland in Neotoma floridana is composed of many enlarged, holocrine sebaceous glands. The sebaceous gland units (alveoli) vary in size, being largest directly along the midline of the venter and becoming diminishingly smaller toward the periphery of the gland. Also, the gland units are of greatest size directly below the xiphoid region of the breastbone, and again decrease in size toward the pectoral region and lower abdominal region.

Transverse sections of the skin through the glandular region revealed an abundance of papillae-like projections (Fig. 14), whereas skin lateral to the ventral gland has a smoother appearance (Fig. 17). These folds of skin projected posteriorly as did the hair shafts. Thickness of the midventral skin ranged from 0.6 to 1.0 mm as contrasted to the 0.4 to 0.6 mm thickness of lateral skin. Most of the increase in midventral skin thickness was in the dermis. However, there were additional layers which added thickness to the epidermis as well.

The alveoli are usually associated with a hair follicle, where a short

duct joins the gland directly to a follicle. From the follicle, the sebum is carried to the external surface of the skin. Some follicles appear rudimentary, with no hair shaft present, and perhaps function solely as export canals for the sebaceous glands. Other sebaceous glands, especially during their active season, were not associated with hair follicles, and the sebum duct opened directly to the skin surface. In most instances, there were only one or two glands per follicle, and these were not lobulated. However, in active male glands, the gland units enlarged and in some cases, became partially lobed into two or three portions. At such times, it became difficult to distinguish exactly the number of alveoli associated with any one follicle.

The glandular alveoli occured at a consistent depth in the dermis of the midventral skin. In the active state of glandular secretion, the alveoli occupied approximately one-third to one-half of the dermis area (Fig. 14). Inactive glands were somewhat spherical in shape, while active glands assumed a variety of shapes ranging from bell-shaped to elongated allipses.

Cross sections of the hairs in the gland region revealed a modified form of hair shaft that was flattened and curved (Fig. 19). These hairs, as well as those from other parts of the body had a segmented appearance with ringed protrusions along the entire shaft. Sebum was often found clinging to these protrusions of the hair shaft in the ventral gland area. No hairs were found on other parts of the body that were flattened and curved as were the midventral hairs. Lateral hairs were cylindrical in shape.

Parenchyma

Gland ducts were lined with stratified squamous epithelium, which in

Fig. 19. Cross section of the modified hairs (MH) in the ventral gland region showing the peculiar trough-like shape (400x). F, follicle.

Castrated males showed a direct dependence upon testosterone for ventral gland maintenance and development (Fig. 25). Twenty days after castration, the gland area had been reduced to about one-third its initial size. The reduction in gland size was slow until day 14. A rapid reduction in gland size occurred during the next six days. Testosterone propionate injections started on day 15, quickly restored the gland area within four days, and resulted, after seven days, in a ventral gland area that was approximately 400 mm^2 larger than at the beginning of the experiment.

Ovariectomized females showed no noticeable difference in ventral gland area. The abdominal glands initially averaged 239.6 mm^2; at the end of the experiment the gland areas averaged 236 mm^2.

Histochemistry

General

Although hematoxylin and eosin are not generally utilized as histochemical stains, their use revealed that the alveoli of the ventral gland possessed distinct areas of acidophilic and basophilic staining cells, which has been described in the earlier section on histology. Negative results were obtained with ninhydrin for presence of alpha-amino acid groups; no positive reaction was observed in any portion of the gland alveolus or in the sebum. Untreated frozen sections examined for natural color revealed that the most exposed sebum in the alveolus neck was yellowish-brown, while the remainder of the alveolus was faint yellow.

Lipids

Staining with Sudan Black B revealed lipids to be present in all

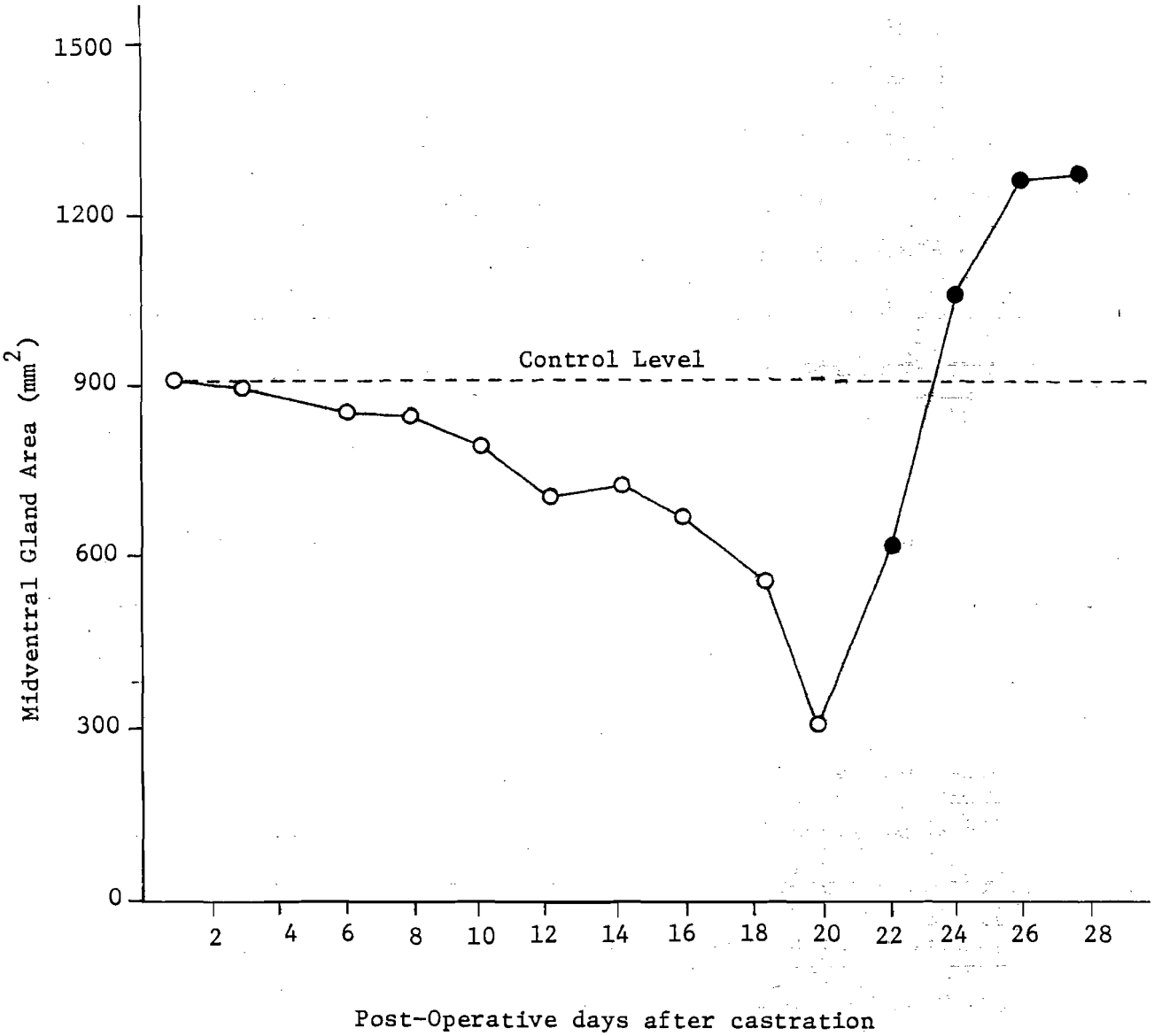

Figure 25. Relationship between midventral glandular area and testicular hormones. Open circles represent glandular area of castrated individuals; solid circles represent glandular area of castrated individuals receiving testosterone propionate injections.

cells of the alveolus and in the sebum (Fig. 26). The basal layers of cells were extremely dark, with many fine granules. Zone 3 (Fig. 20) was not as heavily stained as Zones 1 and 2, but also showed an abundance of cytoplasmic granules. Individual cells contained more granules around their periphery, and possibly indicated a central, internal vesicl in each cell. However, no such vesicle was ever positively identified. The sebum, and the most ental cells stained intensely blue-black. Those sections that were extracted with xylene:glacial acetic acid stained darkly in Zones 1, 2, and 4 (Fig. 27).

Oil Red O stained the glands in much the same manner as Sudan Black B. Zones 1 and 2 appeared darker due to the more numerous granules which stained dark orange-red; some were darker than others. In Zone 3, the granules were less numerous, somewhat larger, and stained pale orange. Zone 4 stained bright orange with no granules present. A few cells along the border line of Zones 3 and 4 contained smaller nuclei (half size) and stained bright orange, which contrasted with those cells found in Zone 3. This may represent the transition of one chemical structure to another.

With Nile Blue A, the alveoli of the ventral gland stained in a manner similar to other stains. Zones 1, 2, and 3 were red and indicated the presence of neutral lipids such as triglycerides. Zone 4 stained blu revealing the presence of acidic lipids such as fatty acids and phospholipids. This further verifies the results obtained with hematoxylin and eosin stains. No vesicles with lipid caps were apparent in any of the ce The lipid droplets all stained with the same intensity in any given zone.

Phospholipids

The presence of phospholipids in the glandular alveoli appeared to be restricted primarily to the periphery of the alveolus, particularly in

Fig. 26. Transverse section of ventral gland stained with Sudan Black B (100x). HF, hair follicle; SG, sebaceous gland.

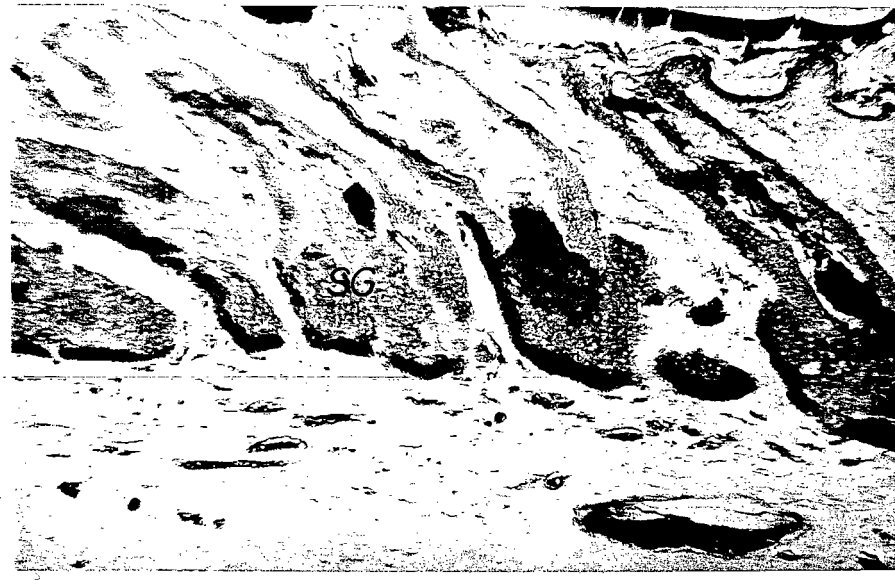

Fig. 27. Transverse section of ventral gland extracted with xylene: glacial acetic acid and stained with Sudan Black B (100x). SG, sebaceous gland.

Zones 1 and 2 (Fig. 28). The sebum also stained positive for phospholipids. Similar results were obtained when xylene-extracted tissue was stained with Sudan Black B which indicated that the phospholipids were not removed by extraction as were the lipids. Granules of approximately the same size as those found with lipid stains were found in the cytoplasm of Zones 1 and 2. Minute cytoplasmic granules in Zone 3 also stained to a lesser degree.

Fluorescence

The brightest fluorescence, in both paraffin and frozen sections, was obtained when the combination of a Schott BG 12 emission filter and a 510 eyepiece filter were used. The sebum and cells in the process of breaking down fluoresced bright yellow-green (Fig. 29). There was also a peripheral ring of bright yellow-green fluorescence corresponding to Zones 1 and 2. There were some free portions of sebum in the duct which did not fluoresce. The remainder of the glandular cells (Zone 3), showed dull yellow-green fluorescence. The epithelium of the ducts did not fluoresce. The stroma surrounding the glands had darker gray-green coloration.

External examination of the glandular region with a shortwave UV light revealed a soft yellowish glow from the sebum-covered ventral gland hairs. Adult males that had lost hair over the glandular area did not show fluorescence from the bare skin. In adult and subadult males, during the breeding season when the venter became discolored, a large portion of the stained region fluoresced. In one male that had considerable staining of the venter, the entire scrotum was stained and fluoresced.

Female venters did not fluoresce unless the hair was shaved, thus exposing the glandular area. A similar situation was found in subadult females which had active glands. No juvenile venters fluoresced whether

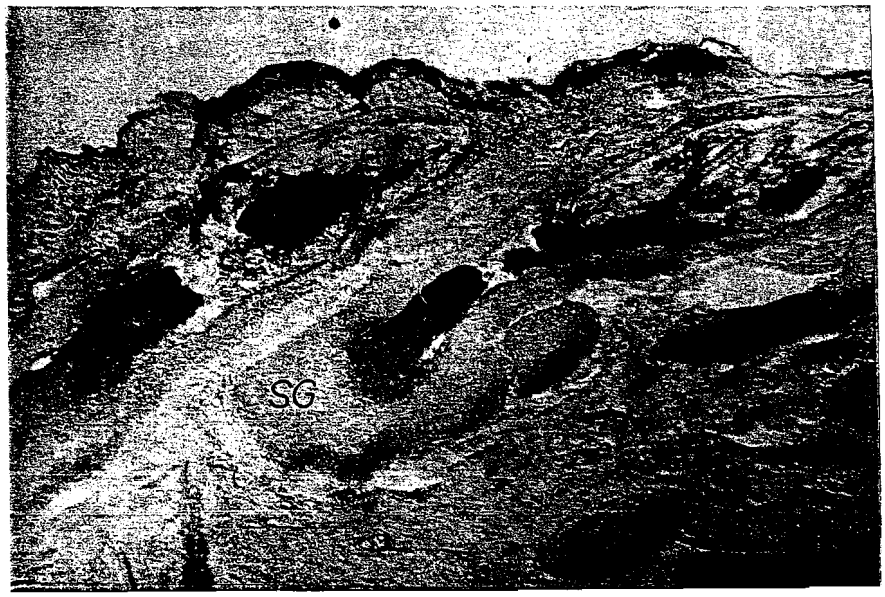

Fig. 28. Transverse section of midventral gland stained for phospholipids (100x). SG, sebaceous gland.

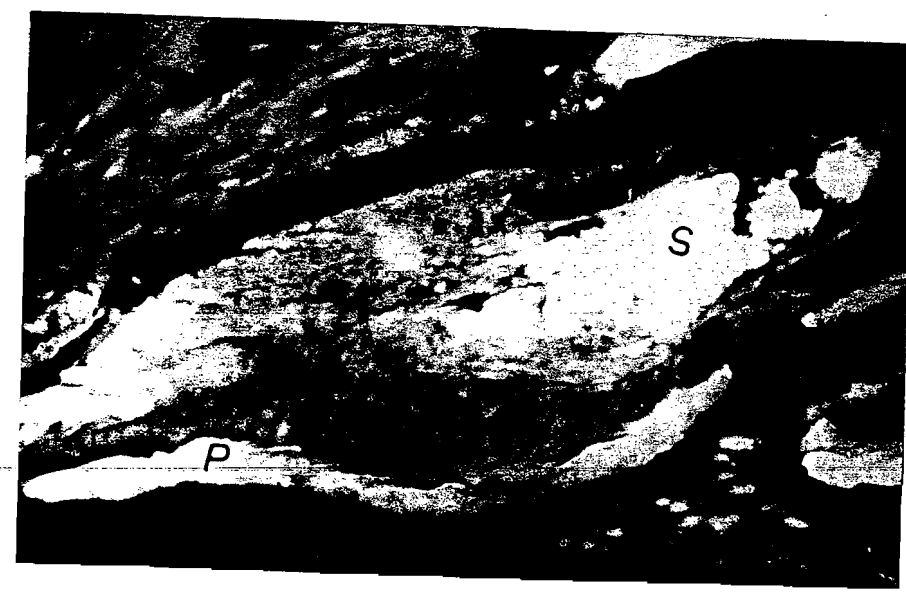

Fig. 29. Section of midventral gland showing areas of fluorescence (200x). P, peripheral layers of alveolus; S, sebum.

they were shaved or unshaved. The soles of the feet were found to fluoresce in a few individuals. However, this could be attributed to factors other than glandular secretion. These factors are discussed later in the paper.

DISCUSSION

Scent Marking

Scent marking can be used for many purposes, but probably its most important function is to foster familiarity between the occupant and its home (Ewer, 1968). In moving around the home range, the individual invariably comes in contact with a variety of foreign odors, as well as its own scent. Reassurance, by smelling its own scent, probably relieves anxiety and timidness in the animal. In territorial animals, the scent becomes an important means of reassurance for the occupant of the territory and a warning that tends to inhibit intruders that venture into the territory.

In wood rats, maintenance of the territory may be due, in part, to the specialized ventral gland. Fecal droppings and urine are also possible territorial markers. Wood rats possess a circular anal gland (Howell, 1926) that probably functions to deposit scent on fecal material. Linsdale and Tevis (1951) reported that individuals of Neotoma fuscipes, released at their houses, often smelled a pile of fecal pellets before entering. They cited an example of one male which, when released at its home, entered, came back out, crawled to the top of the house, and repeatedly rubbed its anal region over sticks, as if depositing scent.

Apparently, N.f. osagensis is more territorial than other subspecies of the genus Neotoma which have been described in the literature. English (1923) kept one male and two female N. fuscipes in a cage for five months, during which time they "lived together harmoniously, sleeping side by side in the same compartment." Other studies have shown that N. f. floridana seems to be mild tempered, and group living may occur without

discord (Pearson, 1952; Hamilton, 1953). Individuals of both sexes of N. f. osagensis are extremely territorial, and will fight to the death or exhaustion if placed together in an enclosed cage (Spencer, pers. comm.).

There is usually only one individual N. f. osagensis living in a house at one time, except during the breeding season when juveniles are present. The juveniles are quickly forced out of the house at about the age of 60 to 85 days to search for houses of their own (Hamilton, 1953; Knoch, 1968; and Spencer, pers. comm.). At this time ventral gland proliferation begins in young wood rats (Fig. 18). Therefore, it is possible that the gland may have a territorial and survival significance, since young wood rats develop the gland at the same time at which they must find and defend a house of their own. If they were forced out of every house that they occupied because of inability to mark their territory, exposure to predation would be greater, and thus, survival value would be placed on development of the ventral gland at an early stage in life. Also, sub-adult males exhibit ventral staining (active gland), but are sexually immature, thus possibly indicating the scent function is for territoriality.

Poole (1940) and Spencer (1968) have both described Neotoma floridana males as having extensively rubbed their venters over objects during the breeding season. This obviously deals with some kind of scent marking, as it correlates with the time of greatest gland activity (Fig. 6), but the specific use is somewhat vague. One possible interpretation is that the male is applying scent around the immediate vicinity of his house in order to keep other males away during the breeding season. This hypothesis loses credance when one considers that due to the positioning and spacing of the rat houses there are probably no females within his marking distance. Thus it would serve no useful purpose in defending a

Another interpretation is that the male is applying scent around his house so that a female, in searching for a mate, can recognize it as a male's house and enter for mating. This would save energy and involve less exposure for the rats compared to a situation in which the rats must move from house to house in a hit-and-miss fashion searching for a mate. Moore (1965) describes mate-searching behavior directed by olfactory stimuli in Peromyscus maniculatus. That the female wood rat goes in search of the male is suggested by the fact that, in courtship behavior, the female is the one that initiates the series of events leading to coitus (Spencer, pers. comm.). This latter hypothesis is more probable than the former in which the male applies scent for purposes of keeping other males away.

Movement studies have been conducted on a number of wood rat species, and in most of them males moved greater distances than females (Linsdale and Tevis, 1951; Raun, 1966; Stones and Hayward, 1968). Among the studies on N. f. osagensis, Rainey (1956) and Goertz (1970) found males to have greater home ranges while Johnson (1967) and Wiley (1971) found females to have greater home ranges. This discrepancy may be due to different types of habitat and trapping techniques involved in the various studies. It is important to note that females do get out and move around and are not restricted to the immediate area around their houses. Thus, females could conceivably search for a mate, at least in N. f. osagensis.

The question arises why female wood rats, which also possess the gland, do not have stained venters if they scent-mark in the same manner as males? The fact that females do not have ventral discoloration shows that they do not rub their venters as males do, or at least not with the frequency of males. From a physical viewpoint, if females rubbed

their venters, the sebum globules at the bases of the hairs would be mashed and spread out, and would obviously stain the hair that was rubbed upon it. Also, the hair would become coarse and hair over the gland would be lost. None of this occurs in the majority of females. Therefore, females probably do not use the ventral gland as a territorial marker. Fecal droppings and urine stations may provide the necessary olfactory signposts for establishment of a territory around their homes.

It is concluded that neither male nor female N. f. osagensis utilize the ventral gland for the purposes of territorial marking. A territory is defined as an area claimed and defended by one animal against others of the same species (Smith, 1966). Such territoriality occurs in N. floridana throughout the year. However, the ventral gland is only active during the breeding season, so it could not be used to maintain a territory throughout the year. The male glandular scent probably has the dual purpose of attracting females and also deterring males, which results in a place for courtship and mating without interference from other males. This would not fit the definition of a territorial marker since the scent is acting as an attractant for females. This is not the same situation as in birds where only the male establishes a territory during the breeding season, because the female bird is always present in the territory and the male actively defends his mate from conspecific males.

When a male and female wood rat meet for mating purposes, there is a preliminary sparring event where both individuals are in an upright position facing one another (Spencer, 1968). In this posture, it becomes evident that the ventral gland is in the best position where both individuals could smell the other's glandular pheromone, since it is directly below the other's nose. Thus, the gland may play a role in recognition of the

sexes in order to carry out a successful mating. The information relayed at this point may result from presence of a species specific pheromone so that the partners must be of the same species before mating behavior could proceed. A close similarity in such a reproductive isolating pheromone might lead to failure of species differentiation, and result in hybridization between sympatric species, such as occurs between N. floridana and N. micropus (Spencer, 1968).

Another possible use of a ventral gland pheromone may be as a recognition odor for litter newborns to recognize their mother. Since wood rats are blind at birth, visual cues cannot be used, leaving odors as the most important means of a relationship between the rats and the environment. This supposition also fits the present data, in that the female gland area is largest during the time of year when she is lactating and nursing young. In this case, the female would not need to rub her venter, as the odor would only need to be present on the body.

Observations of the behavioral habits of wood rats reveal that they are an extremely cautious, nocturnal creature. It is safe to assume that greater survival value would be placed on those factors that enable the rats to escape predation more efficiently. This may be accomplished by the rats marking their major pathways around their houses with an odor so that they can quickly return to the house in the shortest amount of time if chased by a predator in the dark. This is a possible explanation for subadult glandular development, but it can not explain the seasonal variations in the gland area exhibited by adults. It is most probable that the odors along the pathways are placed there in the form of feces and urine.

Of course, one possible explanation for the presence of the ventral

gland is its role in establishing individuality between members of the rat colony. Each individual's pheromone production probably varies slightly in chemical composition. This would aid in identification of the individual. The importance of maintaining individuality would be to recognize one's own house from an adjacent house, and thus reduce agonistic encounters.

The mammalian olfactory system is extremely complex and has the capability of separating and recognizing an almost limitless number of odors. It is doubtful that there is a single function of the ventral gland pheromones in wood rats. The gland probably yields a number of important identification cues for distinguishing sexes, individuals, pathways, and others mentioned above. The uses of the specialized scent gland can only be determined by further behavioral studies and use of a bioassay system with isolated gland pheromones.

An interesting observation in this study was that, in some wood rats, the soles of the feet fluoresced externally. If this is the same fluorescing material as produced by the ventral gland, then it has marked implications. The feet could be used as an additional device for spreading the scent from the ventral gland. By simply rubbing the paws over the glandular region, in the course of normal movements the scent would be spread around the rat's territory. There has been no behavioral evidence that this does occur, however, future studies should include attempts to ascertain the possibility of this function.

Hormonal Control

From the results obtained, it may be concluded that the development and maintenance of the ventral gland in Neotoma floridana is under the control of androgens. This is in agreement with work done on gerbils

(Glenn and Gray, 1965) and Peromyscus maniculatus (Blum, et. al., 1971; Doty and Kart, 1972). However, Doty and Kart (1972) reported that continued injections of high doses of testosterone did not increase the glandular area beyond that of controls. It was found in the present study that continued injections increased the glandular area past the control level by a factor of one-third, after which there was no further increase. This higher level is comparable to the gland area during the active breeding season in the month of April (Table II). The peak gland area obtained by the injections most likely represents the maximum gland size obtainable in the natural environment.

Since the juvenile female responded to testosterone injections by increased glandular development, it was shown that gland development in young wood rats is probably initiated by the presence of androgens. This is further evidenced by the high degree of correlation which exists between ventral gland area and testicular size in male wood rats (Fig. 22). This study revealed a much higher correlation between gland area and testes size than found in Peromyscus (Blum, et. al., 1971; Doty and Kart, 1972). Even though in subadults the testes were not in an active state, the ventral gland began development, indicating an early initiation of testosterone production by the testes. There must be a period of growth in early subadults, during which the androgen levels are rising and alveolar size increasing. Not until the hormone levels have peaked, does the ventral gland begin active secretion.

The few females that exhibited ventral staining and large gland proliferation were probably old females. At increased age, as in other mammals, the ovaries stop producing female hormones, and their inhibitory effects over the androgen levels in the blood become reduced. When this

happens, the androgens have the capability of stimulating ventral gland growth. It is not unlikely that old females might exhibit gland sizes comparable to those of adult males, although none were found in this study. When the ventral glands of males become inactive, they are still much larger than the glands of females. This is due to the high level of androgens which is present in males even during the non-breeding season. The large number of blood vessels found surrounding the glands may help to increase the amount of hormone reaching the glands.

Ebling (1948) found that continued injections of estrogens resulted in atrophy of the sebaceous glands in laboratory rats. Doty and Kart (1972) observed that either one or both of progesterone and estrogen somehow inhibit sebaceous gland growth. No significant change in the ventral gland size was found in gerbils after estrogen - progesterone injections (Glenn and Gray, 1965). In the present study, no difference was noted in gland size of adult female wood rats after ovariectomy. If an inhibitory effect were present with estrogen, the gland size should have increased after ovariectomy, but they did not. As pointed out by Doty and Kart (1972), there is still an apparent confusion as to the exact nature of sebaceous gland response to female sex hormones, and further research needs to be conducted in this area.

Since wood rats are seasonal breeders, it follows that during the breeding season there would be an increased androgen titer in the blood. This would lead to an increase in glandular activity due to the gland dependence upon androgen. The present data confirm this point of view. Similarly, females during the breeding season should have an increased ovarian hormone titer, which would lead to suppression of the ventral gland. However, the opposite effect was found, and remains unexplained

at the present. Possibly another hormone in females acts in conjunction with androgens during the breeding season and is responsible for the increase in glandular proliferation.

Histology and Histochemistry

The histological structure of the ventral gland in Neotoma floridana is quite similar in many respects to descriptions given for other rodents (Quay, 1953, 1954, 1968; Glenn and Gray, 1965; Doty and Kart, 1972). The rodent which showed greatest similarity in gland structure to Neotoma was Peromyscus maniculatus (Peromyscus belongs to the same family as Neotoma) whereas Meriones and Dipodomys, with greater structural differences in the glands, belong to different families.

Dipodomys exhibits sebum pillars which extend beyond the skin surface, while Neotoma has no such pillars. The alveolar size in Dipodomys occupied much more of the dermal space than did the maximum alveolar size in Neotoma. There was similarity in all species in that the alveolar size was greatest in males during the breeding season. Quay (1954) reported that the peripheral alveolar cells in Dipodomys contained several small vesicles which fused to form one large vesicle as the cell was moved inward in the alveolus. The present study found no such vesicles to be present in Neotoma. The reduction in number of arrector pili muscles in the glandular region is in agreement with studies on Rattus exulans (Quay and Tomich, 1963) and Dipodomys (Quay, 1954). The glandular alveoli in Neotoma were not as multilobular as those described in Peromyscus (Doty and Kart, 1972).

The portion of skin with the largest number of sebaceous glands was found to be in the subpectoral region. This would seem reasonable when the mode of scent marking is considered. The male wood rat drags its

venter over objects to leave scent on them. From the rats' profile it can be seen that the lowest portion of the wood rat venter is the sub-pectoral area. This region would be in contact with the substrate most often. Also, beneath this region lies the xiphoid cartilage of the breast bone which allows the wood rat to apply considerable pressure to the substrate and more efficiently rub off glandular exudate than would be accomplished by rubbing with the softer body parts of the abdomen.

In adult males during the breeding season, many of the glandular ducts opened directly to the skin surface. This may be the most efficient means of exposing the sebum to the outside for purposes of depositing it. Thus, hair loss on the venter may be not only due to excessive rubbing, but also to a physiological adaptation where hair is shed from the venter to improve the scent marking capabilities of the rat. Those glands that were associated with hair follicles may be aided in sebum transfer by the trough-like modified hairs found in this region. The protrusions on the hair shafts also provide a means of holding the exudate until it is appropriately rubbed in the scent marking process.

Implications of the histochemical properties of the ventral gland are not clearly understood. Adult male Neotoma floridana exhibited a much larger acidophilic cell region than any of the other species. This acidophilic region probably is due to the lysis of unsaturated glycerides which releases fatty acids. There was a distinct change in chemical properties as the cells moved inward in the alveolus. Phospholipids were most abundant around the periphery and in the sebum, but in the middle zone something may alter the phospholipid structure, for it does not stain.

It is interesting to note that the fluorescence studies also revealed

the same segregation of Zones 1, 2, and 4 from Zone 3, as did the phospholipid stains. However, since phospholipids generally do not fluoresce, there is apparently no connection between the two results. Since many chemicals will fluoresce in a given range of light wavelengths, it is possible that the fluorescent compounds in Zones 1 and 2 are completely different from those found in Zone 4. These fluorescing compounds might be a number of substances such as flavins, lipid vitamins, or some triglycerides.

Where the actual pheromone production takes place is unknown. One possibility is that when the alveolar cell nucleus disintegrates the nuclear components are broken down and used for the final pheromone structure. This would account for the increase in numbers of acidophilic staining cells in the alveoli during the breeding season. Quay (pers. comm.) suggested that the pheromone possibly might not be activated until oxidation of the sebum at the skin surface. Further experiments need to be performed to test these possibilities.

SUMMARY

1. A study of the specialized midventral gland in Neotoma floridana osagensis was conducted from August, 1972, through July, 1973. Because of lack of previous studies conducted on ventral glands in the genus Neotoma, this study was initiated to establish the various aspects of the gland structure and function.

2. Comparisons were made of the gland size between sexes, ages, and seasons of the year. Histological and histochemical properties were described for the specialized gland. The effects of gonadal hormones on the ventral gland maintenance were studied on both males and females.

3. The gland size was much larger in adult males than in adult females. In both sexes, the gland enlarged during the breeding season (March - September). Males, during the breeding season, had a copious dark brown stain on the ventral hairs from the gland exudate. Hair loss on the gland region occurred in males, due to excessive rubbing of the venter over objects. Female glands were never as active as those in males.

4. Subadult males began development of the gland at about the age of 50 days whereas subadult females began gland development at about the age of 75 days. Glandular alveoli of subadult males surpassed the size of alveoli in adult females. Midventral staining occurred in subadult males, but with only moderate intensity.

5. No juveniles were found with active ventral glands. The gland primordia were indistinguishable from sebaceous glands in skin lateral to the abdomen.

6. A direct correlation was found between wet testes weight and

size of the ventral gland area in males, emphasizing the dependence of the ventral gland on androgens.

7. It was found that the specialized ventral gland is under control of androgens in both males and females. Female ovarian hormones had no visible effect upon the gland area.

8. Histochemical properties of the glandular area revealed both similarities and dissimilarities between the scent gland of Neotoma and those of other rodents.

9. It was concluded that the ventral gland probably plays no role in territorial marking in either sex. Its most probable function is that, during the breeding season, the males mark their houses with the glandular exudate which acts as a sex recognition agent when females go in search of mates. The pheromones produced from the ventral gland probably have more complicated meanings than expected, e.g. individual discrimination, interspecific reproductive isolation, mother - litter relationships, and others.

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