THE ANAL SACS OF THE DOMESTIC CAT, *Felis domesticus*

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THE ANAL SACS OF THE DOMESTIC CAT, *Felis domesticus*

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INTRODUCTION

Anal sacs (*Sinus paranales*) are small, paired organs, the walls of which contain glands. These sacs are located lateral to the anal canal and connect to it by means of short ducts. The presence of these sacs is characteristic of many species of the Order Carnivora (Class Mammalia).

Investigation of these small organs seemed justified because little regarding the anal sacs of cats has been published since an article by Otto Krolling appeared in 1926. The study for the following report was undertaken in order to gain a better understanding of the gross and microscopic anatomy of the sacs and of some of the chemical properties as revealed by simple histochemical techniques. Some of the data presented here are in extension, clarification and corroboration of the fine work published by Dr. Krolling.

LITERATURE REVIEW

General Summary of the Literature

In 1926, an article written by Otto Krolling (1926) about the anal sacs of the domestic cat appeared in a German anatomy journal. In this article, the microscopic anatomy of the anal sacs of the adult animal and of the sacs of embryos of different ages was described. Remarks related to the function of the organ and speculations regarding phylogeny also were made. Another review of the subject appeared in Schaffer's work (1940) on the skin glands of mammals, with special emphasis on the glands of the anal region of the dog, including those of the anal sacs.

Descriptions of anal sacs in text books of anatomy of the domestic animals have been brief (Ellenberger, 1911; Ellenberger and Baum, 1943; Martin, 1923; Reighard and Jennings, 1940; Trautmann and Fiebiger, 1952). Mention of the anal sacs of cats, dogs, and weasels was included in an English summary of a Japanese article (Katsuna, 1959) about the comparative histology of glands of the perineum of female mammals.

Descriptions of the anal sacs of other carnivores, especially dogs, have been published. Titkemeyer (1958) clarified some of the confusing terminology used in relation to the glands and other structures of the perineal region of the dog. Diseases and treatment of anal sacs of dogs were discussed by Baker (1962). An extensive

histochemical study of the anal sacs of dogs was made by Montagna and Parks (1948). Discussions of anal sacs appear in reports of studies on other animals, such as the mink (Kainer, 1954), tiger (Hashimoto, et al., 1963) and the skunk (Mephitis mephitis) (Cuyler, 1924 and Schwartz and Schwartz, 1959).

Function of glands, similar to anal sac glands, found in the armadillo (Dasypus novemcinctus, Order Edentata) was investigated by Haynes and Enders (1961), and functional aspects of glands, including anal sac glands, which produce odorous substances in mammals were discussed by Parkes (1960). Cuyler (1924) and Schwartz and Schwartz (1959) discussed the functional aspects of the anal sacs of skunks.

Gross Anatomy

The gross anatomy of the anal sacs of various members of the Order Carnivora has been discussed by various authors, but usually briefly. According to Reighard and Jennings (1940), the anal sacs of the cat are approximately one centimeter in diameter and open into the anus at a point one or two millimeters from the caudal boundary of the anus.

The anal sacs of dogs are reported to be lateral and slightly ventral to the anus between the M. sphincter ani externus and M. sphincter ani internus (Martin, 1923 and Titkemeyer, 1958). Ellenberger and Baum (1943) stated that the anal sacs are the size of peas in young dogs, and as large as walnuts in adults. They further stated that the duct of each sac opens into the Zona cutanea

by way of an orifice the size of a pinhead. Montagna and Parks (1948) reported that the anal sacs of the dog are the size of a pea or hazel nut, are located between the internal and external anal sphincter muscles, and open "ventrally on the lateral margin of the anus". Baker (1962) reported the sizes of the anal sacs in the dog to be between seven and twenty millimeters and said the sac ducts open at the ano-rectal junction. Schaffer (1940) described the anal sacs as egg-shaped and reported that they measure nine by seven millimeters.

Hashimoto, et al. (1963) found the anal sacs of the tiger positioned similarly to those of the dog and cat and that they are three by two and one-half centimeters in size. He reported each sac duct opens into a nipple located in the cutaneous zone adjacent to the ano-cutaneous line. Cuyler (1924) and Schwartz and Schwartz (1959) wrote that the orifices of the anal sac ducts of the skunk (Family Mustelidae) also are located on nipples in the anal wall and that the nipples are projected to the outside when the tail is raised in readiness for discharge of the musk. Cuyler (1924) noted that the skunk sacs are egg-shaped.

Kainer (1954) described the sacs of the mink, another mustelid, on each side of the cutaneous zone of the anus, and connected by means of a short duct to an opening near the ano-cutaneous line. He wrote that part of each sac is located between the internal and external anal sphincters, that the external sphincter is thin at the dorso-lateral surface of the sac, and the fundus of the sac extends beyond the anterior border of the thin part of this muscle into the

fat-filled ischio-rectal fossa. He reported a lobulated, gland-like structure surrounding the neck of each sac.

According to Titkemeyer (1958) the anal sac of the dog receives its blood supply from the caudal hemorrhoidal artery and is innervated by the internal pudendal nerve.

Microscopic Anatomy and Histochemistry

The most comprehensive discussion of the microscopic anatomy of the anal sacs of the cat was presented by Krolling (1926). This work will be referred to later, in the discussion of the microscopic aspects of these organs. Montagna and Parks (1926) in their report on the histo-chemical aspects of the anal sacs of dogs, stated that these organs are situated between the external and internal anal sphincter muscles. The sacs and ducts were said to be lined with keratinized, stratified squamous epithelium, surrounded by glands embedded in a connective tissue stroma rich in diffuse lymphatic tissue with occasional lymph nodules. They reported apocrine, sudoriparous tubules with myoepithelial cells and basement membranes, surrounding the fundus of the sac and opening into it, and large sebaceous acini in the walls of the sac ducts. They also reported that the apocrine glands secrete serous fluid and the sebaceous glands secrete most of the lipid portion of the anal sac products.

Ellenberger (1911) stated that the wall of the anal sac of the dog contains a type of sweat gland which produces a fatty secretion, and that the wall of the sac duct contains sebaceous

glands. In cats, he described branched alveolar sebaceous glands, as well as branched tubular sweat glands, in the sac wall. He further reported lymph nodules in the lamina propria of the anal sacs, and peculiar cells "not dissimilar to neuro-epithelial cells" in the epithelium of the sac wall. Contrary to other accounts, Trautmann and Fiebiger (1952) believed there are both tubular and alveolar glands in the excretory ducts of both dog and cat. The presence of leucocytes in the apocrine tubules in the dog was noted by Schaffer (1940) and in the cat by Krolling (1926).

Hashimoto, et al. (1963) found that the anal sacs of a tiger also possess a keratinized lining of stratified squamous epithelium and a thick muscular band of irregularly arranged skeletal muscle bundles. They found the connective tissue stroma contains many highly convoluted, branched tubules connected to a collecting duct opening into the sac lumen, and that the tubules possess myoepithelial cells and basement membranes. The presence of a few alveolar glands composed of polygonal cells which stained strongly with Sudan IV also was reported.

Tagawa (cited by Hashimoto, et al., 1963) reported that the anal sacs of the skunk have only apocrine glands which are arranged mainly around the equatorial zone of the sac.

Function

Various statements have been made regarding the function of anal sacs. Krolling (1926) reported that the activity of the glands is related directly to the breeding condition of the animal.

According to his results, the apocrine and sebaceous glands reach their maximum activity during mating seasons, and that the sebaceous glands are inactive at times other than the mating seasons. He found no difference in secretion intensity between the sexes. He concluded that the function of the sac glands is to provide a secretion, which by virtue of its odor, would help to bring the male and female together during the mating seasons. Schaffer (1940) concluded a similar function and added that at times other than the heat period, the odor of the sac secretion served for identification.

Gerstenberger (cited by Krolling, 1926) reported a correlation between anal sac secretion of the dog and the reproductive cycle, as did Schaffer (1940). Baker (1962) believed the biological significance of the dog anal sacs is unknown, but listed three theories related to their function. In addition, he reported that the dog may discharge anal sac fluid when frightened. He discredited the theory that the secretion provides a lubricant to aid defecation, on the basis of the distal location of the sacs. The other theories cited were that the secretion provides a characteristic smell, and that it might be used for territory marking. Parkes (1960) supported these last two theories by asserting that they apply to odorous secretions in general from glands of mammals. He further stated that such secretions provide an identifying odor to attract the male to the estrous female. George A. Petrides (personal communication) stated that tom cats, captured in traps set for wild fur-bearing animals, released a marked odor, subsequent to handling, which he believed to originate from anal sac secretions.

Hashimoto, et al. (1963) believed the biological and physiological significance of the anal sacs of the dog and cat is obscure. Montagna and Parks (1948) in their report on the histochemistry of the anal sacs of the dog, advanced no theories or conclusions regarding function.

Haynes and Enders (1961) reported glandular structures of the armadillo, Dasypus novemcinctus, to be active all year. The description of these organs was similar to that of anal sacs. They suggested that the secretion might serve in marking trails or as a defense mechanism. The only relation to the reproductive cycle found was an increase in glycogen in the apocrine glands of the sac during induced ovulation.

Schwartz and Schwartz (1959) reported that a "strong odor" is given off by the musk, or anal sac, glands of the mink, especially during the breeding season, but also during any period of intense excitement. They reported that musking by the skunk occurs mostly in self-defense and that only rarely is the anal sac secretion released when skunks play, engage in fights with other animals, or when the females are in heat.

MATERIALS AND METHODS

Gross Anatomy

Of a total of 57 cats used in this study, 17 adult female and 14 adult male embalmed house cats were chosen for gross dissection and measurements. Dissection and measurements were also performed on 4 adult female, 1 immature male and 2 adult male cats before fixation. The following measurements were made: internal lengths of the sac lumens of embalmed specimens, dimensions of the sacs of the unembalmed specimens, both including and excluding the external anal sphincter muscle covering the sacs, and dimensions of the sebaceous gland complexes. A caliper was used to make the measurements. The measurements were correlated with sex and size differences. The arterial supply to the sacs was noted in embalmed specimens which had been injected with colored latex. General gross observations were made on all animals dissected.

Microscopic Anatomy and Histochemistry

Anal sacs of 5 adult cats (2 males and 3 females) and 10 immature, unembalmed cats (8 males and 2 females) were selected to be fixed, embedded in paraffin blocks, sectioned and stained. In addition, 4 embalmed cats (1 male and 3 females) were handled similarly. Some cellular and tissue distortion was present in the sections made of the sacs taken from the embalmed cats; nevertheless,

they were useful in investigation of general structural characteristics.

With the exception of one sac which was fixed in 10% alcohol-formalin for preservation of glycogen, all the anal sac specimens were fixed in 10% formalin. The specimens were stored in 70% alcohol until further processing.

Most of the specimens were dehydrated and cleared in four changes of dioxane. Three specimens were dehydrated in a graded series of alcohol and cleared in cedar oil and xylene in an unsuccessful attempt to obtain a tissue specimen which could be more easily sectioned. The specimens were infiltrated with paraffin in a vacuum oven prior to embedding in paraffin. During infiltration, the oven mechanism was operated so that 15 minute periods of atmospheric pressure alternated with 15 minute periods of partial vacuum. One specimen was double-embedded in celloidion and paraffin. Sections were cut at 6-7 microns on a rotary microtome.

The tissue sections tended to fragment when floated in a water bath, so were placed directly on a glass slide to avoid fragmentation. A few drops of water, in which a small amount of gelatin had been dissolved, were placed on the slide to aid in flattening the section, and the slide was drained and dried. The slides were coated with albumin adhesive until it was ascertained that this precaution was unnecessary. The sections were subsequently stained and mounted. One formalin-fixed specimen was sectioned on a sliding, freezing microtome prior to staining for evidence of lipids.

The tissues were found to be especially difficult to prepare for sectioning and several variations in schedules of the dehydration

and embedding procedures were employed. No completely satisfactory method was devised since the reasons for the difficulties were not found.

Several different stains were used to investigate various aspects of the chemical and structural nature of the anal sacs. General tissue staining was achieved with a modified Harris' hematoxylin-eosin stain (Malewitz and Smith, 1955). Crossmon's modification of Mallory's triple stain (Crossmon, 1937) was used as a general stain and to differentiate collagenous connective tissue. Reticular connective tissue was demonstrated, using a Lillie modification of Gomori's silver technique (Lillie, 1954) and by a modification based on a combination of techniques of Gridley (1957) and Lillie.

The presence of acid mucopolysaccharides was tested by Gridley's (1957) Alcian blue method. P.A.S. techniques of Lillie (1954) and Gridley were used to test for the presence of carbohydrates with 1,2 glycol groups; a combination Alcian blue-P.A.S. technique of McManus and Mowry (1960) also was used. Gridley's Oil Red O procedure was used on frozen sections to determine the presence of lipids.

Various structures were measured with an ocular micrometer. Measurements and means were recorded (Table 3). Due to irregularities in the shapes of the structural components of the anal sacs, measurements which could be considered representative were limited. In addition, special care was taken in selection of the various measurements and in interpreting those measurements. Because of

the irregular shapes of the structures in the sac, and the aberrations which naturally result from sectioning tissue, some of the figures presented may be regarded as approximations. Large variations (Table 3) made statistical analyses impractical.

RESULTS AND DISCUSSION

Terminology

The anus usually is considered to be divisable into three zones, viz.: the Zona columnaris, the Zona intermedius and the Zona cutanea. The anus is lined by stratified squamous epithelium. The Zona columnaris is the most cranial portion and joins the rectal mucosa at the Linea ano-rectalis. It is characterized by longitudinal folds, the Columnae ani, and is found only in man, dog, cat and swine (Ellenberger and Baum, 1943). The Z. intermedia is hairless and glandless and joins the Z. cutanea at the Linea ano-cutanea. The Z. cutanea is the distal link to the outer skin of the perineum and has dermal papillae, hairs and associated sebaceous and sweat glands and, in the dog and cat, circumanal glands (Ellenberger and Baum). The above classification was made by Mladenowitsch (cited by Ellenberger and Baum) and both the Latin and Anglicized versions of the terms are used in the following report.

Gross Aspects

The two anal sacs of the cat were situated laterally and somewhat ventrally on opposite sides of the anus (Plate I). Their caudal walls were approximately one centimeter from the perianal skin. The short ducts opened at the level of the ano-cutaneous

line (designated as that line cranial to which hair was not discernable). The ducts, at their openings into the anus, appeared as small, wrinkled tubules. Thin layers of the external anal sphincter muscle, which also encircled the anus, covered the sacs and appeared as a common sheath surrounding both sacs and the portion of the anus between the sacs. The sacs, with their muscular covering, were embedded in a pad of fat. On removal of the muscle, a capsule of connective tissue was seen in which were embedded four to nine yellow, spherical bodies (an average of 5.8 on the basis of six observations). These yellow bodies were later found to be sebaceous gland complexes (so designated to distinguish them from the sebaceous glands typically associated with skin), which measured up to 3 to 4 mm. at their longest axes (Table 2). The linings of the sacs of fresh specimens were smooth and white. The lumens of the sacs of the embalmed cats were kidney-shaped or bean-shaped, but the shapes of the lumens of the fresh specimens could not be observed due to their tendency to collapse when opened.

Each sebaceous gland complex opened by a single duct into the lumen of the anal sac; the tiny openings of these small ducts were visible grossly on examination of the interior of the sac. Various amounts of yellowish or grayish-white liquid, slightly more viscid than water, usually could be expressed from the unembalmed specimens. This secretion had a characteristic, pungent, "cat-like" odor.

Dissection of embalmed specimens showed the anal sacs to be supplied by branches of the middle hemorrhoidal artery, the principal vessels passing over the muscular covering at the ventro-medial

aspect of each sac. The middle hemorrhoidal nerve, a branch of the pudendal, was reported by Reighard and Jennings (1940) to innervate the muscles and other structures about the caudal end of the gastro-intestinal tract, so was assumed to innervate the anal sacs also. The innervation might be bilateral since the external anal sphincter muscle receives bilateral innervation from the pudendal nerves (Bishop, 1959). However, identification of the nerve supply was not made in the present study.

The measurements made on adult, embalmed cats are recorded and summarized in Table 1. In order to relate the size of the sac lumen to the size of the animal, the length of the sac per millimeter of body length and per millimeter of hind foot length was determined. Body length was measured from the base of the tail to the tip of the nose, and the hind foot was measured from the end of the calcaneous bone to the tip of the longest toe.

The average size of the female sac lumens was greater than in the male. Moreover, the sizes of the anal sac lumens of the females as related to body length and to hind foot length were, on an average, larger than those of the males (Table 1). The differences between the males and females in the sac-body length ratios and the sac-hind foot length ratios were found to be significant using a two-tailed test at the 5 per cent level.

Measurements made of unembalmed cats are recorded and summarized in Table 2. Statistical analyses were not attempted because of the small size of the sample, but comparison does not show a marked or surprising deviation from the figures for the embalmed specimens (Table 1).

Table 1. Body lengths, hind foot lengths and dimensions of gross aspects of anal sacs of embalmed cats

	Body length	Hind foot length	Sac lumen length	Sac length to body length ratio	Sac length to hind foot length ratio
Females	480*	106	10.1	.0210	.0953
	468	104	10.4	.0222	.1000
	457	111	9.5	.0208	.0856
	455	110	10.1	.0222	.0918
	445	104	7.5	.0169	.0721
	444	109	7.1	.0160	.0651
	430	104	8.5	.0198	.0817
	429	99	10.2	.0238	.1030
Range	429-480	99-109	7.1-10.4	.0160-.0238	.0651-.1030
Mean	451	106	9.18	.0203	.0868
S.d.**			1.3	.0027	.0133
Males	526	115	8.2	.0156	.0713
	525	123	10.1	.0192	.0821
	510	116	10.6	.0208	.0914
	505	120	10.5	.0208	.0875
	502	113	6.7	.0134	.0593
	475	115	6.4	.0135	.0557
	475	119	7.8	.0164	.0656
	474	115	6.6	.0139	.0574
	473	113	6.7	.0142	.0593
	463	113	10.1	.0218	.0894
	459	107	7.0	.0153	.0654
	455	108	9.7	.0213	.0898
	446	109	7.7	.0173	.0706
Range	446-526	109-123	6.6-10.6	.0134-.0218	.0557-.0914
Mean	484	114	8.32	.0172	.0727
S.d.**			1.3	.0032	.0136

* All measurements in millimeters
** Standard deviation

Table 2. Body lengths, hind foot lengths and dimensions of gross aspects of anal sacs of fresh cats

	Body length	Hind foot length	Sac length including muscle	Sac length without muscle	Number of sebaceous complexes	Length of largest sebaceous complex
Mature females	424*	117	11.3	---	---	---
	432**	114	10.1	---	---	---
			12.3	---	---	---
	477	121	8.0	6.7	5	3.3
	481**	121	10.5	7.3	5	3.8
			11.1	9.0	4	3.7
Mean	454	118	10.5	7.7	4.7	3.6
Immature male	281**	83	8.4	---	---	---
			7.7	---	---	---
Mean			8.1			
Mature males	430	123	10.5	---	9	4.0
	---	117**	8.8	5.8	6	3.2
			9.5	6.1	6	
Mean		120	9.6	6.0	7	3.6

* All measurements in millimeters

** Measurements of both anal sacs of this specimen included

Microscopic Aspects

General Appearance. Microscopic sections of anal sacs of cats cut on a plane transverse to the anus (Plate II) showed the walls to be composed of white fibrous connective tissue in which were embedded two types of glands, viz.: alveolar sebaceous glands clustered together in large, ovoid complexes, and coiled, tubular apocrine glands structurally similar to other apocrine glands of mammalian skin. The glands were arranged densely in the sac wall with apocrine glands interspersed between sebaceous complexes. The sacs were lined with keratinized, modified stratified squamous epithelium. The walls of the sac excretory ducts also consisted of white fibrous connective tissue, and were lined with keratinized, stratified squamous epithelium. The duct epithelium was continuous with that of the sacs, but consisted of more layers and was more highly keratinized than the epithelium of the sac. The ducts connected the sacs to the anus and opened at the Linea ano-cutanea. A band of skeletal muscle fasciculi (M. sphincter ani externus) was found between the external sphincter muscle surrounding the sac, and the anus, but extended only to the level of the ano-cutaneous line, and did not appear related to the anal sacs or ducts.

Anal Sac and Duct. The anal sac was lined by keratinized, modified stratified squamous epithelium which did not form rete pegs (Plates IX and X). The basal layer of epithelial cells consisted of regularly arranged, columnar or cuboidal cells resting on a flat basement membrane. The nuclei of the basal layer contained dense

chromatin, and were situated at approximately the same level, i.e., at the same distance from the basement membrane. These basal cells usually were overlaid with one, but sometimes two, layers of irregularly arranged polygonal cells, with a final layer of squamous cells, although often only the basal layer of columnar cells was evident. The intermediately placed cells were larger and had nuclei with less dense chromatin than the basal cells. An area of keratinization separated these epithelial cells from the sac lumen. The epithelial height varied considerably, but averaged about 11 microns high at the lowest points. (The true, overall average was not determined due to the probability for error caused by tangential sectioning.) The epithelial lining rested on a bed of connective tissue which was composed mainly of collagenous tissue containing blood vessels, nerves, and, in one specimen, two small Pacinian corpuscles were observed (Plate III). Often, the area of connective tissue was infiltrated with leucocytes. Very little elastic tissue---less than that observed in the dermis of the anus---was demonstrated. Argyrophilic reticular tissue was present, mostly concentrated directly under the epithelium in the form of fibers running parallel to the basement membrane (Plate IV).

The anal sac duct (Plate II) was lined by stratified epithelium similar to that observed in the sac lining, but had more cell layers between the basal layer and the lumen, and had a thicker area of keratinization. The epithelium was usually two to four layers in thickness. (At the areas where the epithelium was lowest, the average height was about 19 microns.) By observing a series of cross

sections of the anus it was determined that each anal sac duct opened at the ano-cutaneous line, so that the caudal part of the orifice was continuous with the cutaneous zone, and the cranial part with the intermediate zone. Plate II is a photograph of a section cut at an area where the duct opens into the intermediate zone. The duct lumens, measured at the widest points, averaged about 1 mm. in diameter and the lengths, measured between the anus and the anal sac, averaged about 4 mm. (Table 3). The connective tissue surrounding the duct, similar to that around the sac, was mainly collagenous with argyrophilic reticular fibers concentrated under the epithelium. The elastic fibers were more abundant than in the sac wall and appeared to be of the same concentration as that seen in the dermis of the anus.

Small fasciculi composed of thin skeletal muscle fibers surrounded the anal sac (Plates II and V). As noted grossly, this band of muscle also encircled the anus. As seen microscopically, parts of this muscle layer extended into the area between the sacs and the anus. Smooth muscle of the internal anal sphincter muscle was observed between the skeletal muscle and the anus cranial to the level of the duct opening (i.e., at the ano-cutaneous line), but did not appear related to the sacs or ducts. Skeletal muscle also was arranged circularly around the sac duct, external to the connective tissue sheath of the duct (Plate II). The skeletal muscle was supplied with numerous nerves and blood vessels.

Sebaceous Gland Complexes. The sebaceous gland complexes, the gross appearance of which was described earlier, were seen to be dispersed in the connective tissue of the wall of the anal sac at fairly regular intervals. Cross sections of the anal sacs showed two

or three sebaceous gland complexes in each cross section, giving a general idea of the distribution of the complexes in the sac wall.

The connective tissue surrounding a complex was similar to, and continuous with, that surrounding the epithelial lining of the anal sac and that in which the apocrine glands were embedded. The thickness of the connective tissue layer between the sebaceous complexes and the surrounding skeletal muscle varied between 12 and 174 microns (Table 3). This was considered as an outer capsule enclosing the anal sac and the anal sac glands. The connective tissue surrounding the sebaceous complex was continuous with the sparse stroma around the individual alveoli of the sebaceous complex itself. Often lymphocyte infiltration was noted both in the stroma of the glandular complex, as well as in other parts of the anal sac wall. Several of the sebaceous complexes appeared to be composed of indistinct lobules of sebaceous gland units; the lobules were separated from each other by small amounts of collagenous and reticular connective tissue. Blood vessels and occasional apocrine ducts were embedded in the connective tissue (Plate IX).

The typical individual sebaceous gland unit, or alveolus, was similar to sebaceous glands associated with hair follicles of the skin in that it was composed of central polygonal or spherical, vacuolated, sebaceous cells enclosed by smaller, flatter cells forming a capsule-like arrangement around the central cells (Plates VI and IX). However, the sebaceous cells of the alveoli of the anal sac glands were generally smaller and usually stained more darkly than those of the sebaceous glands of the dermis of the cutaneous zone of

the anus. Measurements of the lengths of the sebaceous alveoli averaged about 85 microns (Table 3). Each individual alveolus was surrounded by a thin, and sometimes indistinct, framework of collagenous and reticular connective tissue (Plate IV).

Krolling (1926) stated that the sebaceous excretory ducts were tubules consisting of one to two layers of epithelium. The present investigation indicated that the beginning of an excretory duct was formed by degeneration of sebaceous cells located in the center of an alveolus (Plate VI). The lumens formed by these areas of disintegration led into epithelial-lined ducts similar to those described by Krolling. The ducts, scattered throughout the sebaceous gland complex, converged and joined to form larger ducts. The final union of the ducts formed a large cistern which emptied into the lumen of the anal sac (Plate IV). The ducts were supported by a stroma of collagenous and reticular connective tissue. Measurements of the lumen diameters of the largest ducts, excluding the cisterns, of the sebaceous complexes varied between 23 and 160 microns with an average of 2-3 cell layers composing the epithelial linings.

The mouths of the cisterns, as noted earlier, were visible grossly as they opened into the sac lumen. The cistern lumens at the widest diameters varied between 117 and 560 microns (Table 3). The epithelial linings of the ducts, cistern and anal sac were continuous. At the beginnings of the sebaceous gland duct systems, the lining cells were greatly flattened, only one layer thick, and not keratinized. Progressing toward the cistern, the lining cells became more cuboidal and the layers more numerous, and abundant keratinization was visible. Where the diameters of the cisterns were

widest, the average height of the epithelium was about 20 microns with three cell layers.

Apocrine Glands. The apocrine glands were embedded in the connective tissue of the anal sac wall (Plates II and XII) between and surrounding the sebaceous complexes, except at the outer-most limits of the sac wall. Krolling (1926) stated that there were up to 30 apocrine glands present in one anal sac. In the present study attempts to count the apocrine glands were not undertaken, because it seemed impossible to distinguish one gland from the adjacent glands by examination of microscopic cross sections. The connective tissue in which the glands were embedded was similar to other connective tissue stroma described earlier. It was composed mainly of collagenous tissue with little elastic tissue, contained blood vessels and nerves, and frequently was infiltrated by diffuse lymphatic tissue, and occasionally organized nodules. There was considerable argyrophilic, reticular tissue present around the tubules (more than that observed around the sebaceous alveoli), so that the silver stain clearly outlined these glands (Plate XIII). The tubules of the glands appeared tortuous and coiled as evidenced by the large numbers of cross sections of tubules seen in one area (Plate II). The most extensive glands, measured from the sac lumen to the distal limit of the gland, were about 2 mm. in depth (Table 3). The outer, or distal, limit corresponded approximately with the outer limits of the sebaceous complexes. In places, the tubules appeared to branch, or formed diverticuli similar to the apocrine glands of human skin described by Montagna (1962). The tubules were lined by simple columnar or cuboidal

epithelial cells which varied in height and structure. Many of the luminal borders of the cells appeared to form cytoplasmic extensions into the lumens, whereas some were smooth (Plates XII and XI). The tubules were surrounded by a thick-appearing basement membrane and myoepithelial cells were discernible between the basement membrane and the epithelial cells (Plate XI). Based on the shapes of the nuclei, these myoepithelial cells appeared to be arranged in the manner described by Goldstein (1961) for myoepithelial cells of apocrine glands of the human ear, i.e., as elongated cells with their long axes parallel to the long axis of the tubule. Tubules of all specimens examined contained leucocytes in various concentrations and many tubules contained small globules of unidentified material (Plate XI).

Diameters of the lumens of the apocrine glands and heights of epithelial cells varied widely (Plates XI and XII and Table 3). Measurements of the largest lumens ranged up to 488 microns and averaged about 100 microns (Table 3). Lumens, other than the largest ones just mentioned, and which appeared to be fairly representative of a random selection, ranged from 4 microns to 30 microns and had an average diameter of about 12 microns. The lumens of some tubules appeared completely obliterated, due to the small tubule cross sections and high epithelium (Plate XI).

Excretory ducts were difficult to identify, but tubules located in the inter-lobular connective tissue (Plate IX) of the sebaceous complexes were so designated on the basis of (1) their position, i.e., they were not situated within the sebaceous gland tissue as sebaceous

excretory ducts, and (2) because of the presence of leucocytes in the lumens which was characteristic of the apocrine tubules. The excretory ducts were lined by cuboidal epithelium of one or two layers.

Krolling (1926) stated that there was one apocrine gland in the anal sac which embryologically developed independently of any hair follicle and in the post-natal animal opened by means of a duct directly into the sac lumen (Plate IX). He contrasted this with the other apocrine glands which developed, along with the sebaceous glands, as outgrowths of hair follicle primordia. The hair follicles later degenerated and were not demonstrable in adult cats. These apocrine glands were reported to open into the cisterns previously described for the sebaceous complexes. In the present study, the openings of the individual apocrine glands into the sac lumen were observed only infrequently. In one specimen, a situation similar to that described by Krolling appeared to occur, in which a duct lined by several layers of epithelium seemed to open directly into the anal sac and was not related to a sebaceous complex (Plate X). In other cases, ducts appeared to pass between the sebaceous lobules, which was described by Krolling as the route of exit for some of the apocrine glands associated with the sebaceous glands, but they were seen to empty directly into the anal sac lumen, not into the sebaceous complex cistern (Plate IX). In many specimens apocrine ducts were found in sebaceous complex stroma, but in no cases could it be ascertained that the ducts emptied into the sebaceous cisterns. The morphological differences pointed out by Krolling,

viz.: the wide variation seen in lumen diameters and epithelial heights present in the "freely-opening" gland, in contrast to the uniformity of the other apocrine glands, was not observed in the present study. Wide variations were noted, but did not appear confined to one particular apocrine gland.

Table 3. Body lengths and dimensions of microscopic parts of anal sacs of cats

		Body length (in millimeters)	Anal sac (in microns)			Sebaceous complex (in microns)				Apocrine glands (in microns)			
			Width of surrounding muscle	Widest diameter of duct	Duct length	Areas of cell disintegration	Diameter of largest duct	Length of selected alveolus	Widest diameter of cistern	Diameter of widest tubule	Epithelial ht. of widest tubule	Diameter of selected tubule	Epithelial ht. of selected tubule
Immature ♂		246	400	1359	2567	3.5	25.6	104	423	112	11.0	28.4	7.6
		280	---	---	---	8.0	73.1	123	408	58	13.9	29.6	13.9
		285	642	---	---	5.5	106.7	129	275	84	15.7	13.3	14.5
		285	529	876	---	---	---	64	---	85	5.8	31.3	4.6
		291	---	1208	---	4.0	66.7	125	321	41	17.4	18.6	15.1
		320	831	1661	4304	2.0	23.2	67	434	163	12.2	22.1	8.1
		321	189	1133	3926	8.0	70.8	46	281	143	8.7	31.9	20.3
		328	1284	1208	4228	9.0	157.7	58	306	204	9.3	23.2	23.2
	X	295	646	1241	3756	5.7	74.8	89	350	111	11.7	24.8	13.4
Mature ♂		357	529	921	---	9.0	141.5	67	---	97	12.8	31.3	12.2
		430	---	---	---	9.0	123.4	104	117	---	---	---	---
		---	1076	876	4530	---	56.3	77	---	78	7.3	15.1	6.1
		481	793	---	---	3.5	87.0	99	---	275	23.2	5.8	8.1
	X	423	729	899	4530	7.2	102.1	87	117	150	14.4	17.4	8.8
Immat. ♀		296	302	1163	3473	3.4	56.9	80	408	32	9.0	10.2	10.8
		328	604	770	---	6.5	68.6	92	559	32	11.6	16.3	12.8
	X	312	453	967	3473	5.1	63.3	86	483	32	10.3	13.3	11.8
Mature ♀		395	---	1306	3737	12.0	83.5	133	---	41	9.3	14.5	9.3
		442	---	568	3775	---	87.0	---	---	124	10.2	16.9	10.2
		470	1208	---	---	---	91.3	48	287	48	16.2	9.3	9.3
		---	529	559	---	7.5	112.2	61	510	23	20.3	4.1	9.9
		480	1133	483	---	6.5	17.4	---	---	116	3.5	26.1	6.4
	X	447	957	729	3756	8.7	78.3	81	399	70	11.9	14.2	9.0

Histochemistry

Staining with Oil Red O (Plate VII) showed lipid distribution in the sebaceous complexes similar to that described by Krolling (1926) for the anal sac, and by Montagna (1962) for human skin. The contents of sebaceous gland ducts showed a strong reaction. The peripheral cells of the individual alveolus reacted little, or not at all. The central cells often gave a positive reaction, although not as intensely as the contents of the lumens of the ducts. Reaction to this stain did not occur elsewhere.

Glycogen precipitation, as demonstrated by the Best's carmine technique, was seen to have a scattered distribution in the sebaceous cells, and was more constantly and regularly distributed in the epithelial cells of the sebaceous complex ducts, the epithelium of the anal sac lining and of the sac duct lining (Plate VIII). The apocrine glands seemingly lacked glycogen.

An Alcian blue staining procedure was used to indicate the presence of acid mucopolysaccharides. The luminal edges of the apocrine glandular cells were stained slightly to moderately with Alcian blue and the cytoplasm between the nuclei and the lumens occasionally stained lightly. In some specimens the contents of the lumens were stained. None of the anal sac tissue reacted to the Alcian blue as intensely as did the goblet cell mucus of the rectal mucosa. The sebaceous glands did not stain with Alcian blue.

The P.A.S. technique demonstrated polysaccharide distribution similar to the glycogen distribution shown by the Best's carmine

technique, although the reactions were weak. This may have been because most of the specimens were fixed in 10% formalin, a procedure reported to have a tendency to dissolve glycogen (Lillie, 1954). In addition, some of the specimens exhibited droplets of P.A.S. reactive material at the basal portions of the apocrine tubules. Finely granular deposits of P.A.S.-reactive material also were found at the basal portions of other apocrine tubules. The droplets may have been associated with myoepithelial cells, whereas the finer deposits appeared to be in the glandular epithelial cells. The basement membranes of the apocrine tubules reacted mildly with the P.A.S. technique.

No conclusions were drawn relating the chemical nature of the glands to the age or sex of the animals. The apocrine secretion was believed to be mainly serous, instead of mucous, since the reaction to Alcian blue was weak and inconsistent. The sebaceous contribution was considered lipid in nature on the basis of the results with Oil Red O stain. The remainder of the anal sac contents appeared to be composed of cellular debris. Similar results were reported by Montagna and Parks (1948) for the anal sacs of dogs.

SUMMARY AND CONCLUSIONS

Fifty-seven anal sacs were selected for study of gross, microscopic and histo-chemical characteristics. Gross measurements were made of entire sacs and microscopic measurements were made of sac components as seen in cross sections of the organ.

These organs are paired, hollow, ovoid or spherical structures about one centimeter in length, situated lateral to and on opposite sides of the anus. Each sac was connected to the anal canal by a narrow duct about three and one-half millimeters long which opened at the level of the ano-cutaneous line. The sacs and anus, surrounded by the external anal sphincter muscle, were embedded in fat.

The walls of the sacs were composed of white fibrous connective tissue in which were embedded up to nine ovoid complexes of sebaceous glands measuring up to four millimeters in length. Coiled tubular apocrine glands were present in the connective tissue between the sebaceous complexes. The sacs were lined with keratinized, modified stratified squamous epithelium usually of two layers. Blood vessels and nerves were observed in the connective tissue of the sac and in the surrounding skeletal muscle. Leucocyte infiltration in the connective tissue was common. The sac ducts also contained white fibrous connective tissue, surrounded by skeletal muscle, and lined by keratinized, stratified squamous epithelium. No glands were observed in the walls of the ducts.

The sebaceous gland complexes consisted of masses of sebaceous alveoli separated by thin layers of connective tissue. These glands were believed to be holocrine in nature, in that degeneration of the sebaceous cells in the centers of the alveoli was thought to be responsible for the formation of the beginnings of the sebaceous duct systems. Each complex contained, in addition, a series of epithelial-lined ducts terminating in a common cistern which opened into the lumen of the anal sac. The apocrine glands were branched tubules lined by simple cuboidal to columnar epithelium. Myoepithelial cells were between the epithelium and a basement membrane. The tubules appeared to be arranged as dense masses of coils. In some cases the excretory ducts of the apocrine glands were embedded in the connective tissue of the sebaceous complexes. The excretory duct orifices were located near the sebaceous cistern openings, as well as at points between the sebaceous complexes. The tubules contained leukocytes and cellular debris.

The excretory products of the anal sac consisted of a mixture of fatty and serous materials and cellular debris. The secretion from the sebaceous alveoli consisted of lipid material, whereas the apocrine gland secretion was believed to be serous. Also, keratinized cells sloughed off from the sac lining and cellular debris from both types of glands were part of the sac contents.

The present study produced no evidence which could be used in support of theories related to function. Both apocrine and sebaceous glands appeared active in immature and mature animals of both sexes, and at various times of the year.

Perhaps investigation of the effects of hormones, breeding condition, and removal of the sacs, on cats of known age would reveal some facts about the function of the anal sacs as related to the physiology and behavior of the animals.

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Plate I

Anal sacs exposed by dissection, ventral view. Fresh specimen. About 2.2X.

1. Anal sacs
2. Pubic symphysis
3. Urethra
4. Obturator foramen
5. Ventral surface of tail

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Plate II

Anal sac with duct opening into anal canal near Linea ano-cutanea, cross section. Harris' hematoxylin and eosin stain. 25X.

1. Sac lumen
2. Keratinized material in lumen of sac duct
3. Linea ano-cutanea
4. Apocrine glands
5. Sebaceous complex
6. Skeletal muscle
7. Circumanal glands

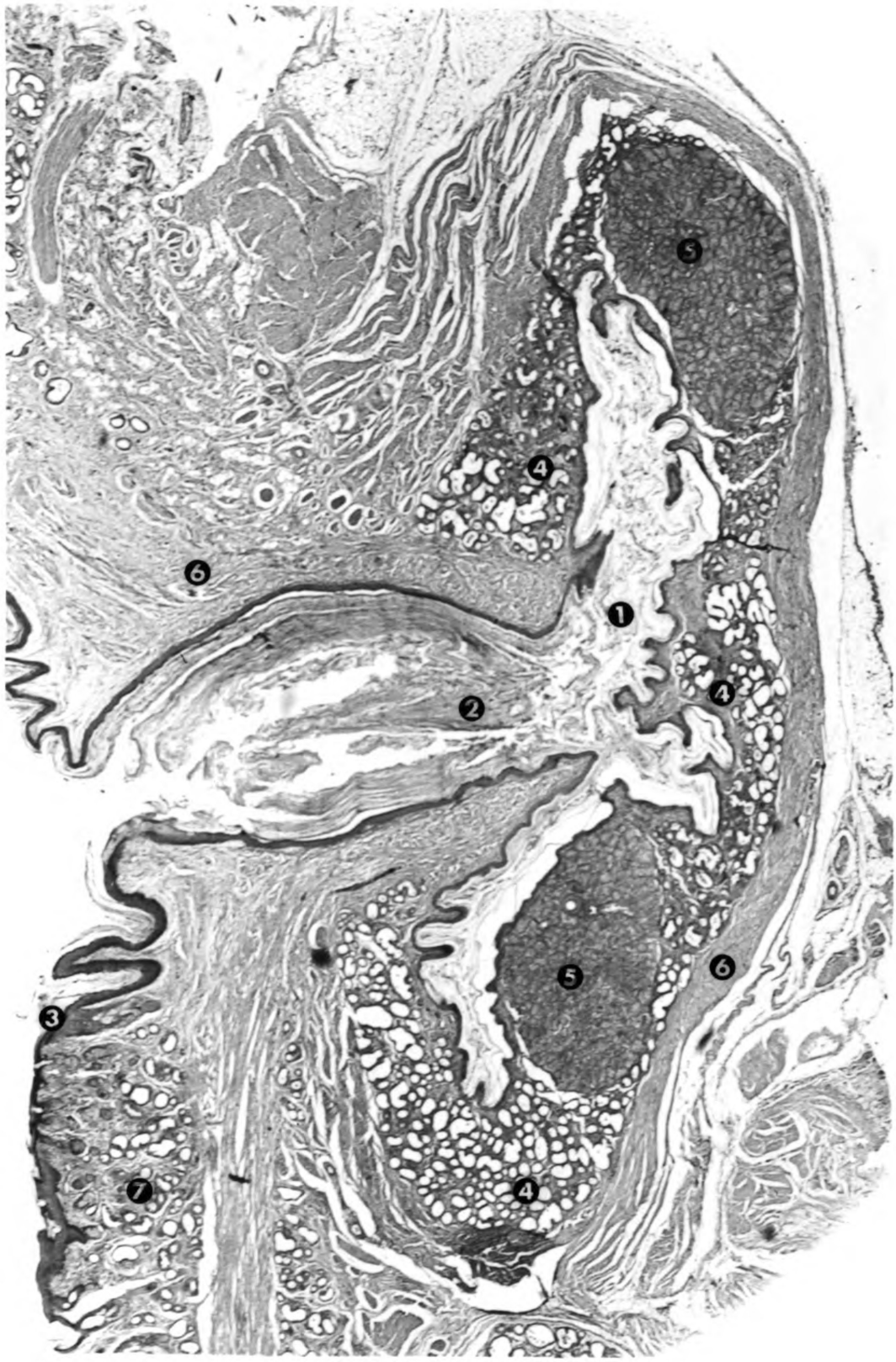
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Plate III

Pacinian corpuscle in connective tissue of sac wall. Harris' hematoxylin and eosin. 1430X.

1. Pacinian corpuscle
2. Keratinized epithelium of sac lining

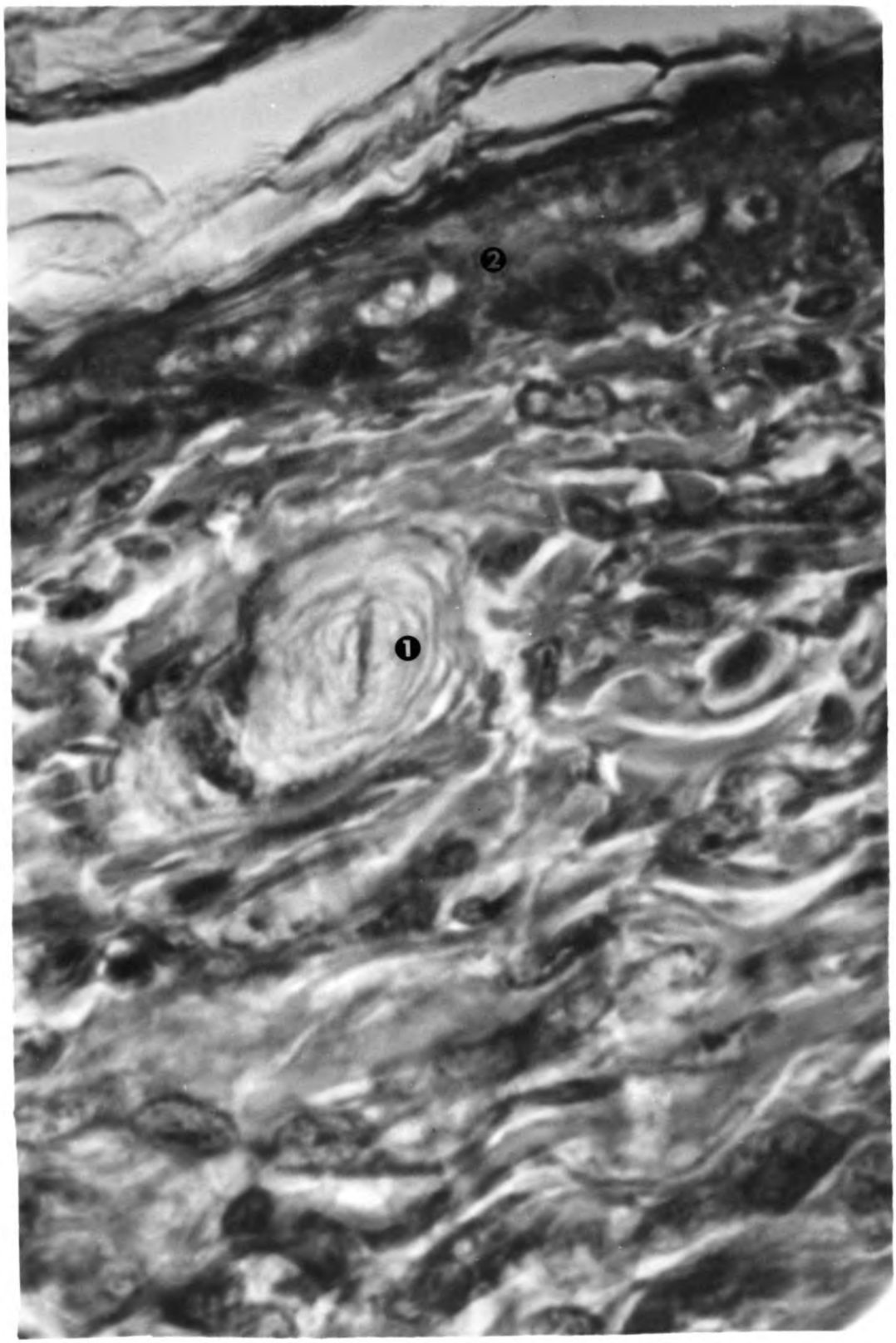
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Plate IV

Reticular tissue appearing as black fibers surrounding sebaceous alveoli and in the connective tissue. Silver technique and Nuclear Fast Red stain. 240X.

1. Sebaceous alveoli
2. Sebaceous complex cistern
3. Epithelium of sac lining

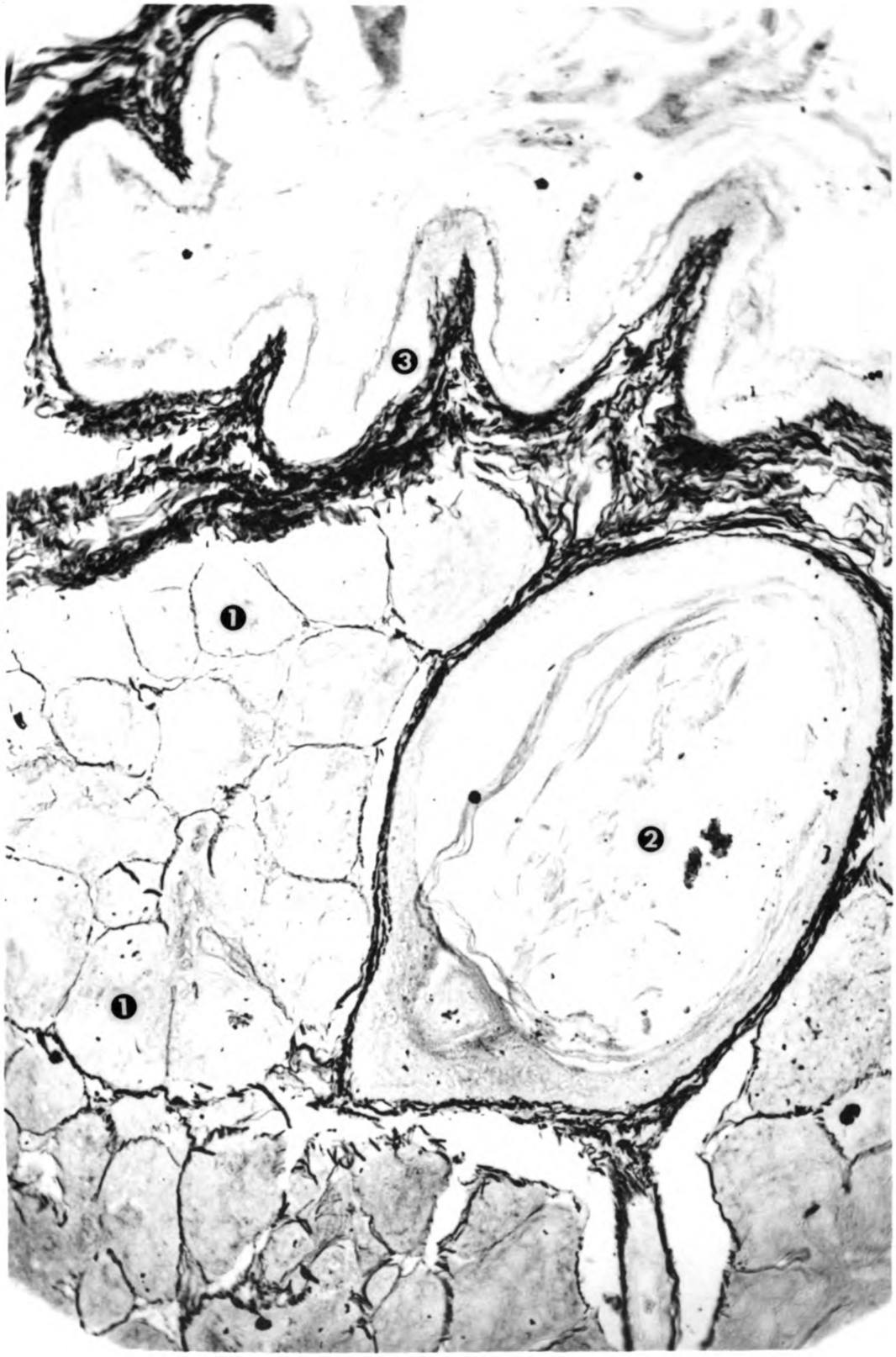
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Plate V

Sebaceous gland complex, longitudinal section. Mallory's trichrome stain. 105X.

1. Sebaceous alveoli
2. Beginnings of sebaceous ducts
3. Sebaceous ducts
4. Anal sac lumen
5. Apocrine tubules
6. Skeletal muscle
7. Connective tissue

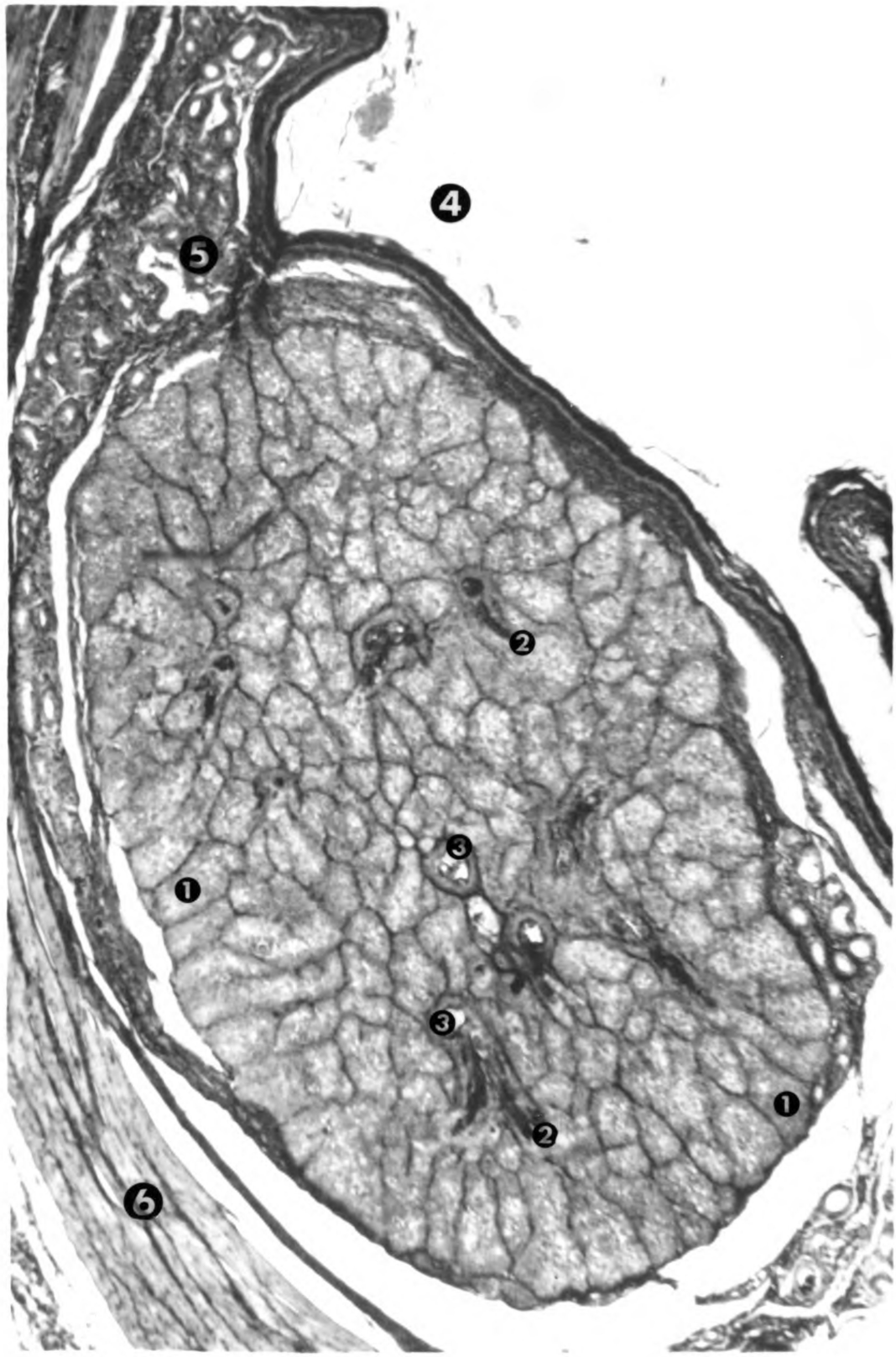
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Plate VI

Portion of sebaceous gland complex showing cellular disintegration leading to duct formation. Mallory's trichrome stain. 519X.

1. Areas of cellular disintegration
2. Sebaceous alveoli
3. Sebaceous complex ducts

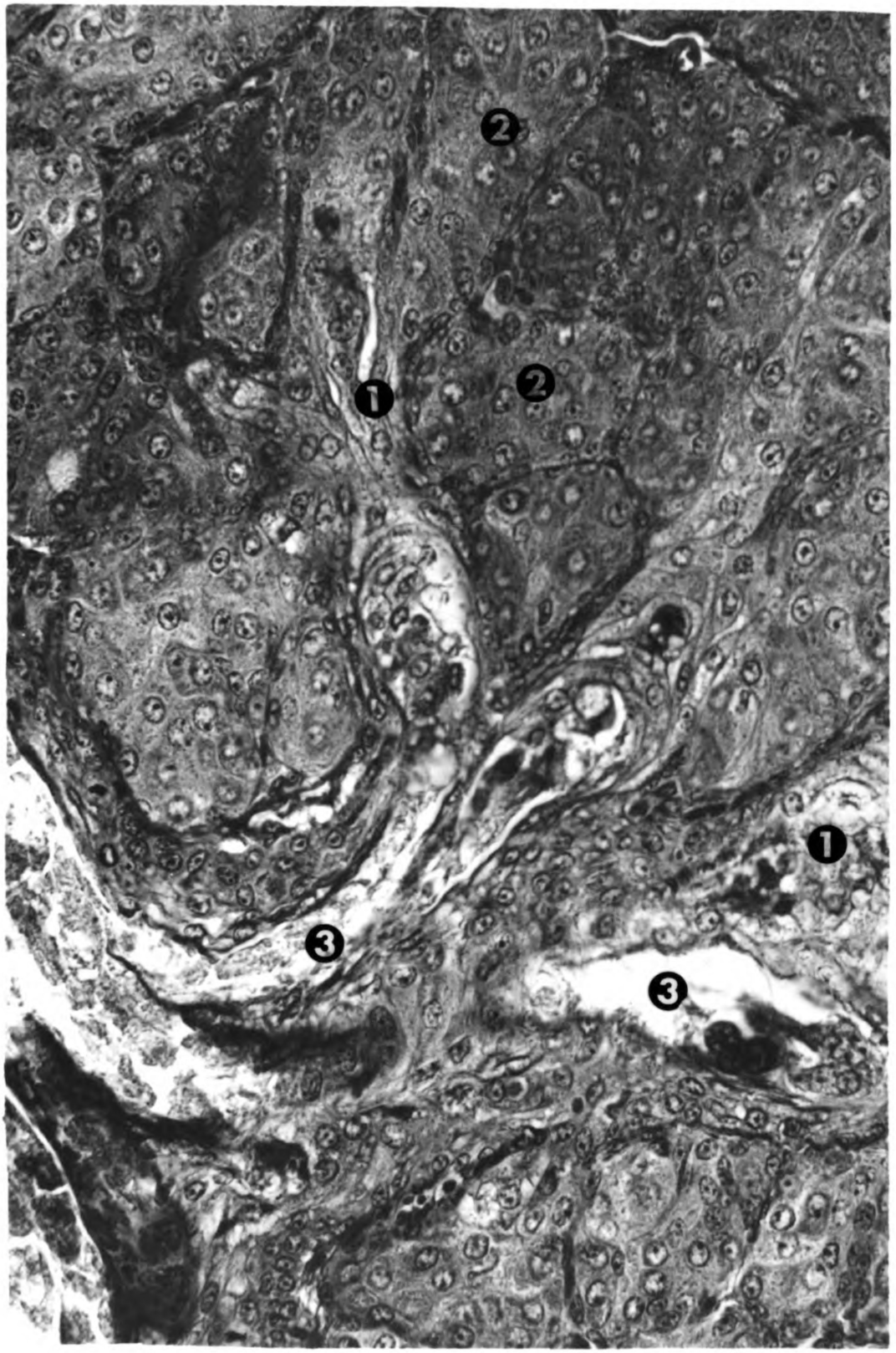
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Plate VII

Portion of sebaceous gland complex showing fat distribution.

Oil Red O stain. 450X.

1. Sebaceous ducts containing fat

2. Sebaceous alveoli containing fat

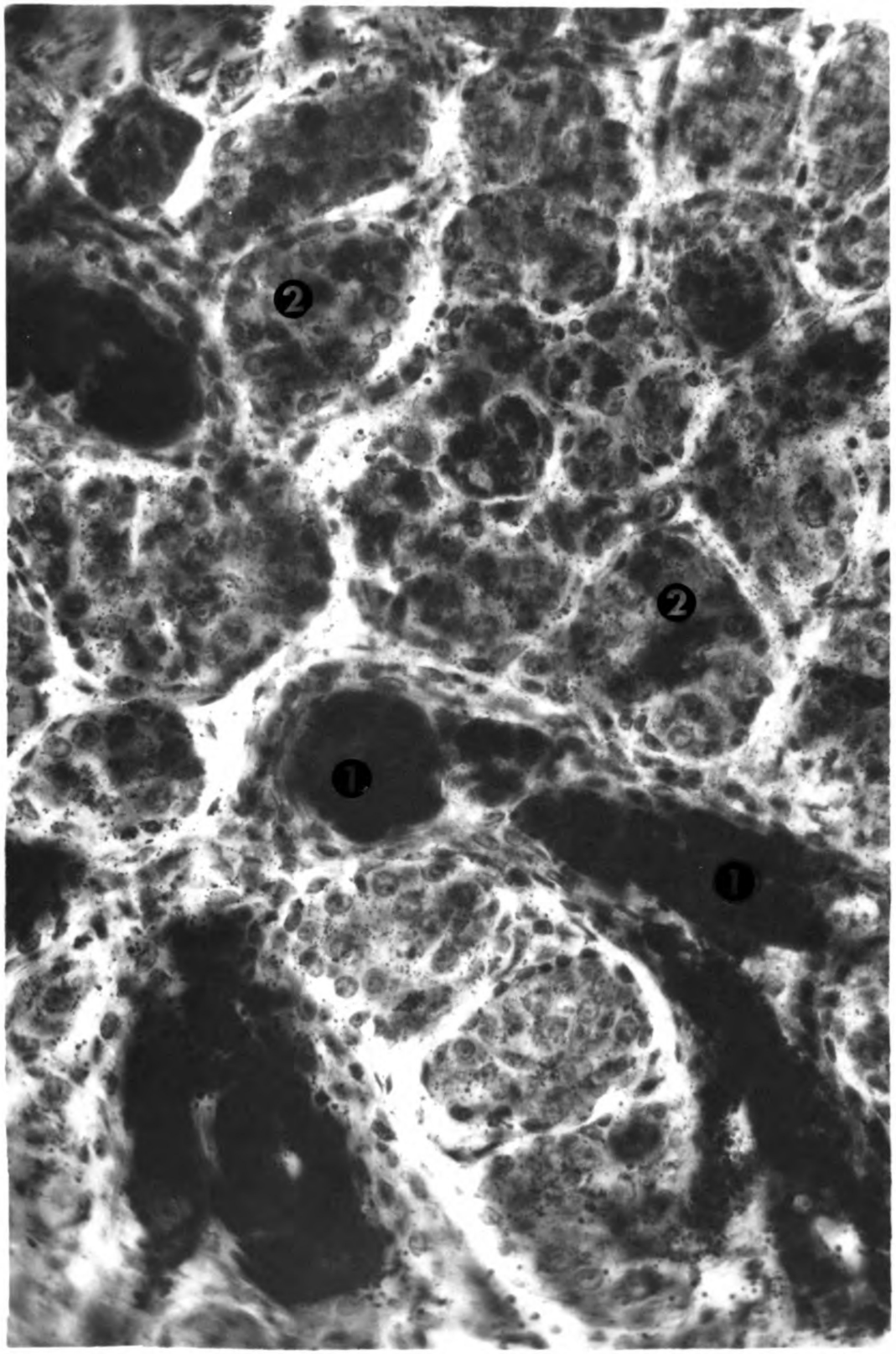
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Plate VIII

Portion of sebaceous gland complex showing glycogen distribution. Best's carmine stain. 525X.

1. Glycogen deposits in sebaceous alveoli
2. Glycogen deposits in sebaceous ducts

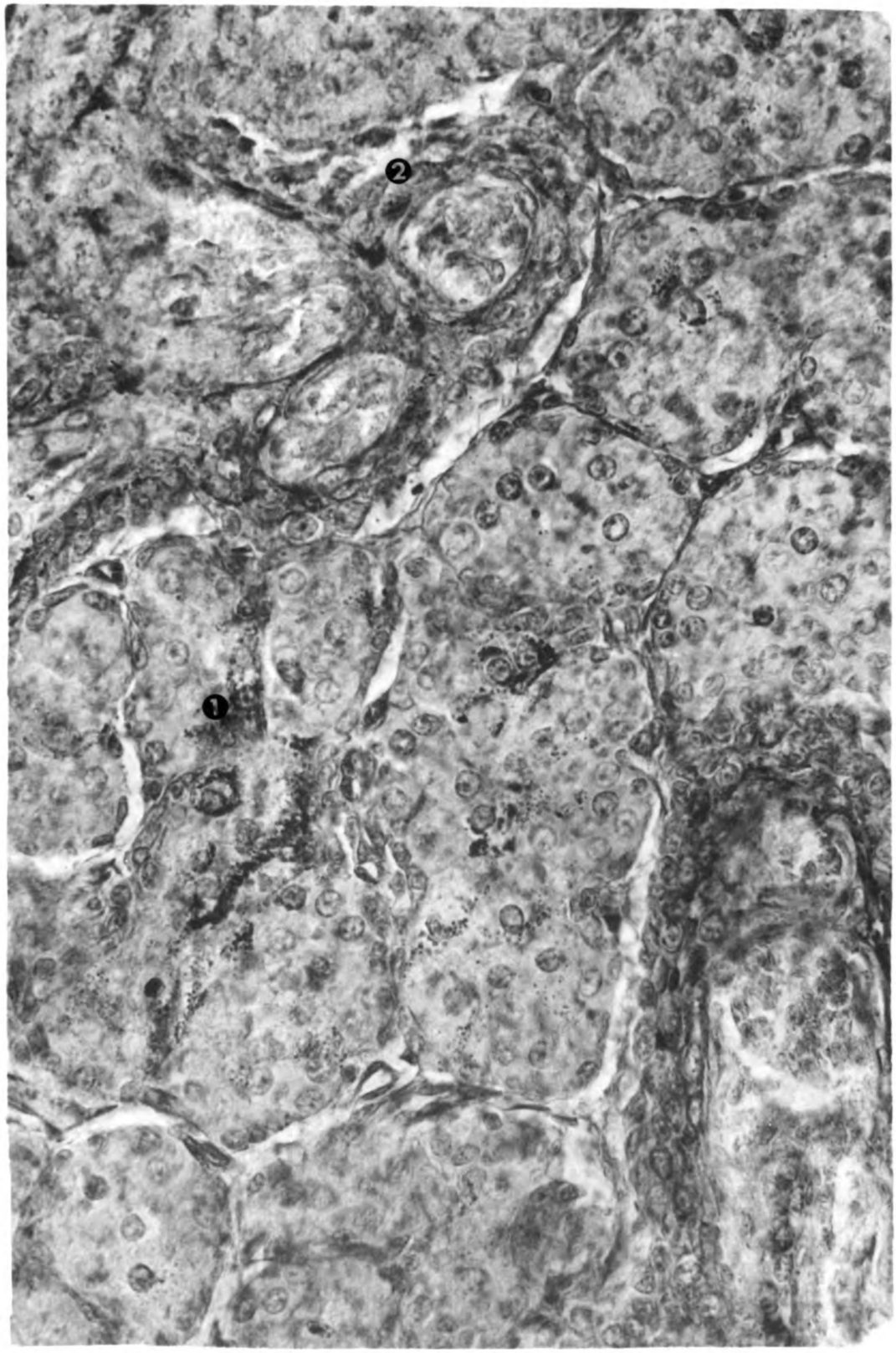
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Plate IX

Apocrine duct opening into sac lumen near sebaceous gland complex. Mallory's trichrome stain. 344X.

1. Apocrine gland duct containing leucocytes opening into sac lumen
2. Sac lumen
3. Epithelium of sac lumen
4. Sebaceous alveoli
5. Apocrine gland duct containing leucocytes passing through connective tissue of sebaceous gland complex

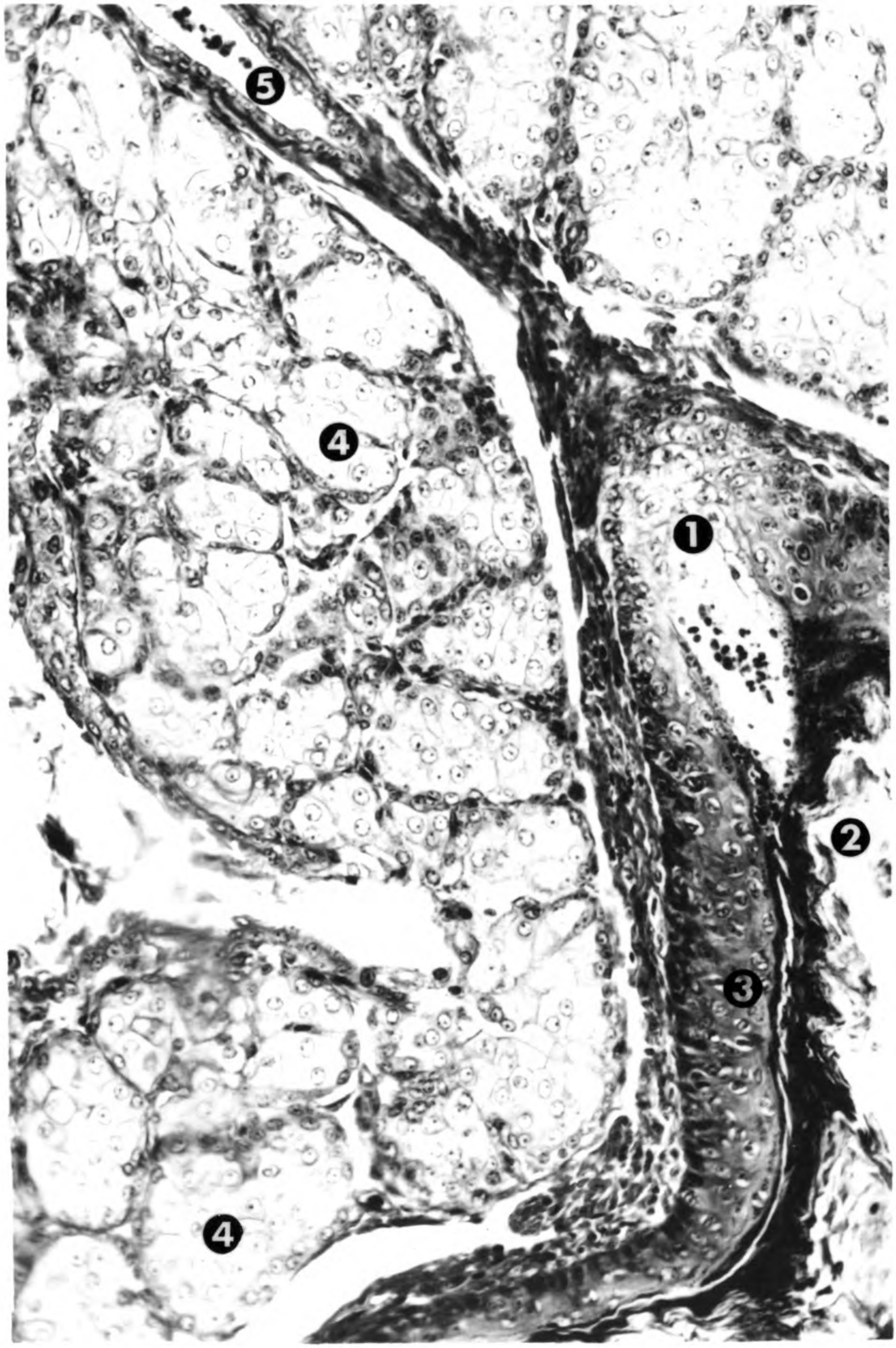
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Plate X

Apocrine gland duct opening into sac lumen. Mallory's trichrome stain. 335X.

1. Apocrine gland duct
2. Apocrine tubules
3. Connective tissue
4. Epithelium of sac lining
5. Keratinized material in lumen of sac

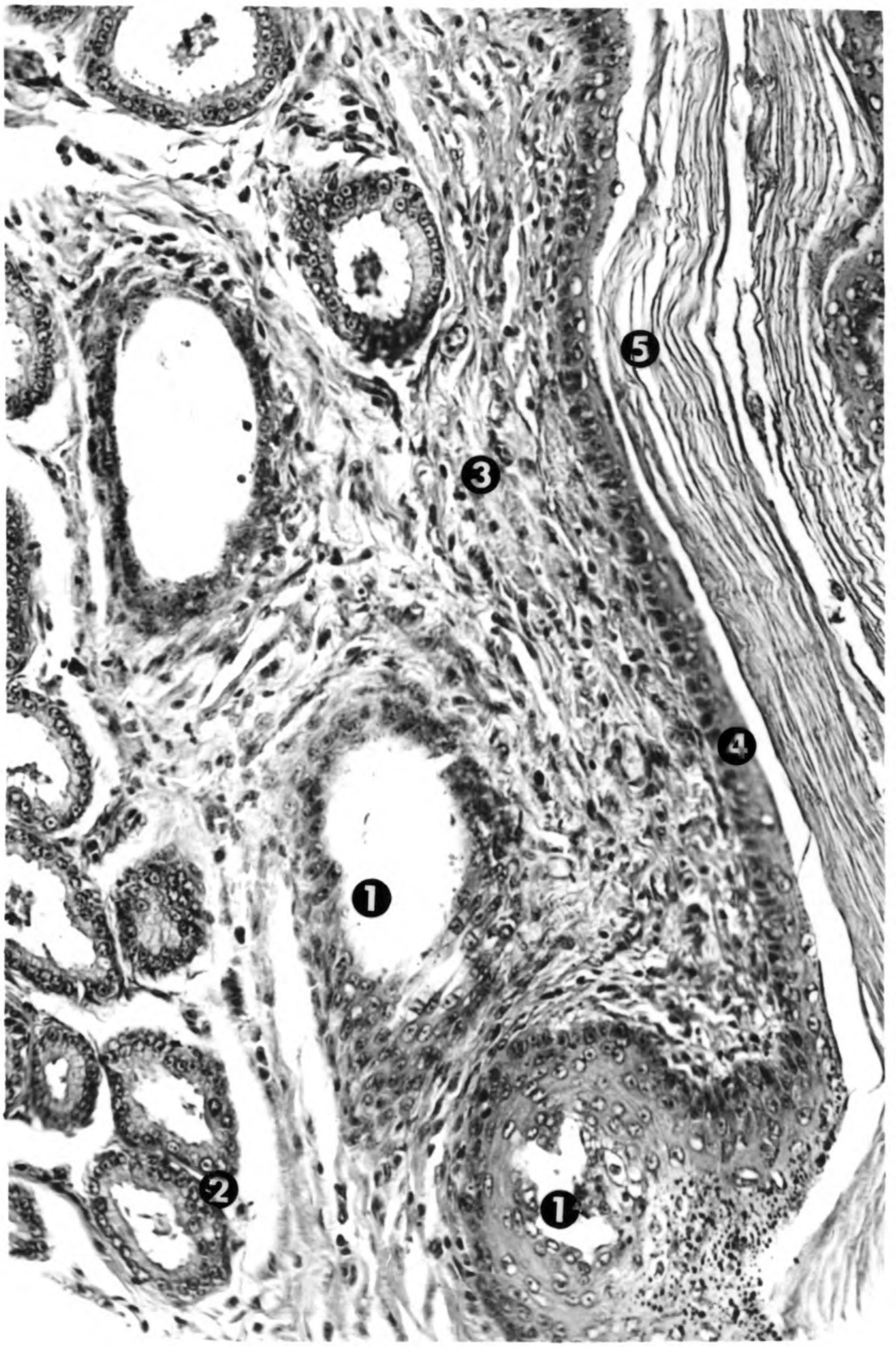
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Plate XI

Apocrine gland tubules. Harris' hematoxylin and eosin . 1325X.

1. Glandular epithelium
2. Nuclei of myoepithelial cells
3. Leucocytes

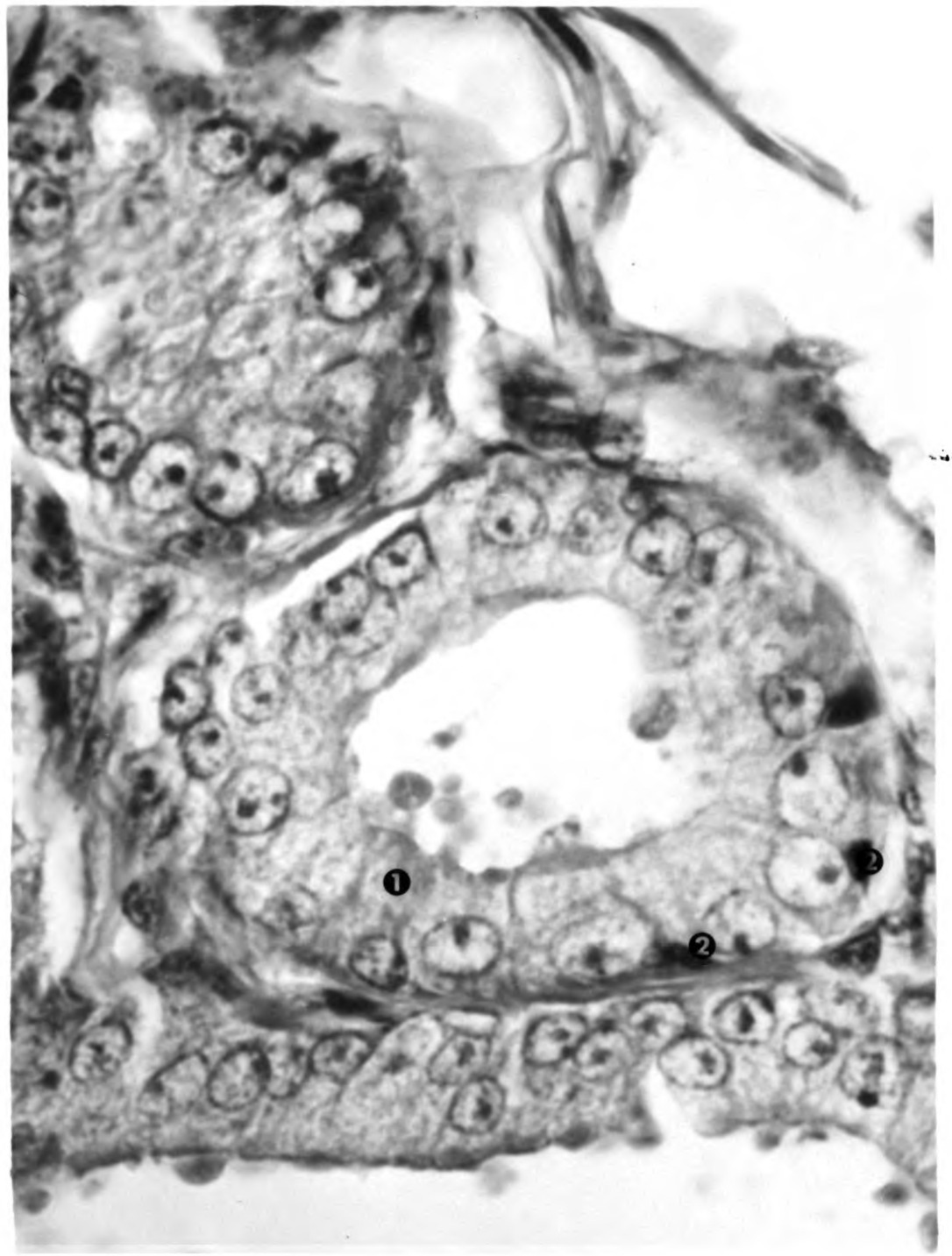
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Plate XII

Apocrine gland tubules. Harris' hematoxylin and eosin. 440X.

1. Apocrine tubules containing leucocytes
2. Portion of sebaceous complex
3. Skeletal muscle

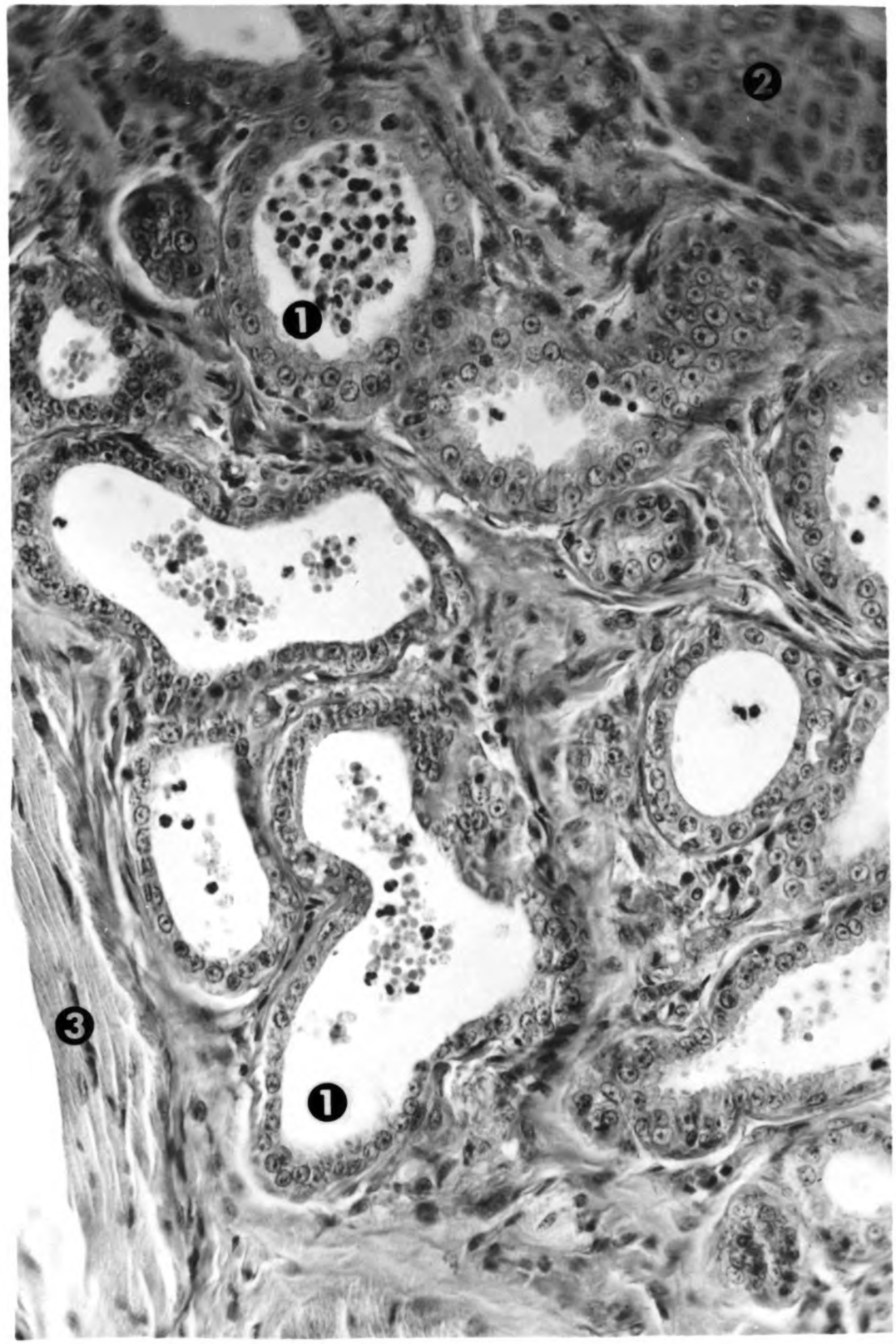
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Plate XIII

Reticular tissue seen as black fibers surrounding apocrine tubules and as irregularly arranged fibers in the connective tissue stroma between the apocrine tubules. Silver technique and Nuclear Fast Red stain. 245X.