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ORIGIN AND EARLY DEVELOPMENT OF THE CANINE CIRCUMANAL GLANDS

by

GODWIN NWACHUKWU ISITOR

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D. E. Wiseman

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INTRODUCTION

The circumanal glands (CGs) are the hepatoid glandular fractions, found in the skin around the anal orifice of the dog; they occupy a circular zone, next to that of the muco-cutaneous sebaceous glands around the anal orifice. Glands, comparable to CGs, have been observed in the skin at the prepuce, dorsal and ventral parts of the tail, loin, and the groin.[23] The anal sac glands should not be confused with the CGs, because they are sweat glands (in dogs) which empty into the Lumina of the paired anal sacs, and are located lateral to the anus[5] (Fig 65).

Since the first description of the CG,[35] there are three major reasons for an increased interest in them. First, the gland is found only in the dog,[1] where it is a good source of neoplasm,[1,7,8,10,15,17,18,24,27,36,37,38] and ranks third in canine neoplasia, the mastocytoma and the mammary neoplasia ranking first and second respectively.[6,11,12,13,14,31] Second, the function of the CG remains unknown despite various attempts at a morphological evaluation. However, the greater development of the CG in the males than in the females,[3,30] and the failure, by some earlier workers, to observe patent excretory ducts in the matured CG[3,30] suggests a testosterone dependent endocrine function. Finally, at present, there are varying opinions about the early development of the CGs; some workers claim that they do not appear until about two weeks post-partum,[23]

others claim that they observed the gland at birth.[3,7,8,9,10,29] Most believers of this later school of thought, however, fail to give a detailed description of their early morphological observations on the CG,[3] and some have confused the CG with the sebaceous gland.[35]

The objectives of this present work are: to carry out a detailed study on the origin of the CG in relation to the surrounding sebaceous glands, and to study the early developmental stages of the CG, using the techniques of light and scanning electron microscopy. It is hoped that the fulfillment of these objectives, when combined with earlier studies on the matured CG, will form a sound basis for future functional evaluation of the canine CG.

LITERATURE REVIEW

Siedamgrotzky[35] was the first to describe the CGs as glands with compact polyhedral glandular cells lining their long ducts. He concluded that they were modified sebaceous glands. Siedamgrotzky[36] also concluded, from his study on perianal skin of young dogs, that the CGs originate from sebaceous glands and gradually undergo modification as the animal grows until puberty when they begin secretion. He pointed out for the first time that the CGs are sources of adenoma.

Mladenowitsch's[26] description of the CGs was identical to that of Siedamgrotzky, although he corrected Siedamgrotzky's description of their ducts by stating that the duct lining cells lacked myoepithelial cells, and their lumen was filled with fat.

Schaffer[32,33,34] reported on a system of intracellular and inter-cellular canaliculi in the CGs, reminiscent to those of bile canaliculi of the liver. He also described presecretory granules in the cytoplasm of their glandular cells, which he interpreted as precursors of the serous materials which were secreted through the canaliculi.

According to McClelland,[25] there are two phases of CG secretion; an active phase resembling that of sebaceous and apocrine sweat glands of the entire skin of the dog, and a resting phase in which the CGs appear in groups of "cuboidal

and spindle-shaped epithelial cells, with nest of sebaceous glands" but without Lumina.

Jones[21] stated that the CGs were modified sebaceous glands, although he described them as tubular glands with double rows of large polyhedral lining cells which usually occluded the lumen.

Parks[30] described the well developed CG as a "bipartite structure consisting of a normal fat-secreting sebaceous component proximally and a non-sebaceous protein elaborating component distally." He also described the CG duct as originating from the hair follicle and branching immediately into the two typical lobes of a CG which were located at either sides of the hair follicle; each branch of the duct divides further into intralobular and terminal ducts which connected the glandular alveoli (about three alveoli per terminal duct). He stressed that the proximal portions of the ducts were patent, and served as a conduit for the secretory products of the superficial sebaceous components of the CGs. By contrast, the intralobular and terminal portions of the duct were non-patent, apart from the presence of few "non-sebaceous cyst" which contained "one or two central cells degenerate" and surrounded by "concentrically arranged fusiform or squamous cells," so that the entire structure resembled a thymic corpuscle. He associated the "non-sebaceous cyst" with partially successful attempts at duct lumenization in the absence of sebaceous transformation. He also described a second type

of cyst within the superficial portions of the CG in males, which he called "sebaceous-cyst", and associated them with sebaceous transformation of superficial CG alveoli which had no place to empty their sebaceous products. He further described a third type of Cyst - "intra-lobular cysts" in the non-sebaceous lobules of old female dogs, and interpreted them as end products of cellular degeneration and liquefaction rather than an active merocrine secretion. He concluded that the CG was an "abortive attempt to form a sebaceous gland" rather than being a functional secretory entity, since each CG had superficial sebaceous component which originated from the non-sebaceous or hepatoid component.

Baker[3] observed that the CGs were already present as "buds about hair follicles" at birth, and that their cells at that stage had non-proteinaceous, non-lipid granules in their cytoplasm, and approximated the adult appearance. He failed to observe any system of ducts connecting gland acini to the hair follicles. He suggested on this basis of failure to observe excretory ducts of the CG, and on observation of an extensive vascular network within the CG, that the glands are endocrine glands which initially are under the influence of pars distalis, until after puberty when they become influenced by the gonads. He agreed with Parks that the CGs are bipartite, with major hepatoid element and a minor sebaceous element. However, he disagreed with Park's rigid separation of the sebaceous and the hepatoid elements into proximal and distal portions of

the glands respectively; suggesting that whenever the sebaceous parts were present, they were being surrounded by the hepatoid elements.

Gerisch and Neurand[16] observed a system of secretory canaliculi within CGs during certain developmental periods, which were similar to those described previously by Schaffer.[32,33,34] They interpreted the secretion pattern of those canaliculi as involving the transfer of their products into the blood capillaries which were very abundant in the CGs. They, however, supported Baker's suggestion that the CGs can be assigned an endocrine function, at certain stages of development, on the basis of the involvement of the blood vascular system in the secretion pattern. They also associated cystic CG formation with seasonal regression changes, and stated that the products of the cyst were usually retained without being transferred to the surface of the skin.

Maita and Ishida[23] studied the structure, development and effect of testosterone propionate on the CGs, with the techniques of light and transmission electron microscopy. They failed to observe the gland in pups less than 14 days old. They observed excretory ducts (partially solid in pups less than 20 days old, completely closed or solid in older dogs) which connected the CGs to the hair follicles, in dogs from 14 days to 9 months of age. In dogs older than 9 months, they did not observe any duct between the CGs and the hair follicles. They described the CGs of adult dogs as developing

"flourishingly around semiclosed adult-like structures which fused with each other forming a 'tortoise-shell' lobule without connection to hair follicles." They also described two types of glandular cells - light and dark cells, with the aid of TEM; both types were devoid of secretory granules. They also observed glandular tissue which is comparable to CGs, in the skin at the loin, prepuce, groin, dorsal and ventral parts of the tail. They suggested that the CGs might secrete "some attractive substance in the breeding season" on the basis of testosterone propionate induced proliferation of the glandular tissues of the CG in 60-day-old pups.

MATERIALS AND METHODS

A total of 4 fetuses and 14 dogs of various breeds were utilized in the study. The animals were arranged in 9 groups. Groups 1 through 7 each consisted of a male and female animal of similar age and breed. Groups 1 and 2 were the fetal groups, consisting of fetuses, 32 days of age and 38 days of age respectively. The remaining Groups 3 through 9 consisted of identically aged dogs; these ages were 3, 11, 14, 20, 60 and 152 days old respectively.

Collection of circumanal tissue from each animal was preceded by euthanasia of the pregnant bitches (fetal specimens) and the dogs with T-61[a] Euthanasia Solution. The fetal circumanal specimens were collected after surgical removal of the fetuses from the euthanised pregnant bitches, and consisted of the entire hind quarter of the fetus with the intact skin, tail and hind limbs for proper orientation. Circumanal tissue of a few centimeters in length, was removed from the dogs, beginning from the mucocutaneous junction and extending away from the anal orifice. The tissues were collected with sharp razor blades, after thorough clipping of the hairs of the skin around the area, and rinsing the area with a swab that had been soaked in physiological saline solution. Most of the specimens

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[a]National Laboratories Corporation, Somerville, New Jersey 08876.

included a portion of the anal sacs, which aided in proper orientation. Tissue were fixed in Bouins Fixative, in 10% buffered neutral formalin (BNF) or in 3% glutaraldehyde in 1.0 M sodium phosphate buffer of pH 7.4 (Kessel and Slih, 1974).

The majority of the tissue was processed for light microscopy study immediately after collection. However, tissue from the 1, 20 and 152 days old specimens was divided into two portions; one portion for light microscopy study, and the other for scanning electron microscopy study.

Light Microscopy Study: The specimens for light microscopy study, apart from the fetal specimens, were further subdivided into three portions for corresponding Mallory Triple, Periodic Acid Shiff (PAS), and Oil Red O stainings. Only the Mallory Triple staining technique was used on the fetal specimens.

a. Mallory Triple and PAS staining techniques: Both staining techniques were used for observations on the origin and early morphological characteristics of the CGs. The specimen preparation, after collection, for both staining techniques were identical and involved fixation, dehydration, embedding, sectioning, mounting and staining.

The specimens were placed in the Bouins fixative for a minimum period of 48 hours in order to permit adequate penetration of the fixative, before the next process of dehydration.

Dehydration of the fixed specimen, carried out in a Tri-3-matic[b] Automatic Tissue processor, involved immersion of the specimen in graded concentrations of ethanols [70%, 80%, 85%, 90% (x2)] for one hour each, and infiltration with Paraplast[c] (M.P. 60 degrees C).

The tissue was oriented in embedding boats according to the types of sections desired (vertical or horizontal), and embedded in Paraplast (M.P. 60 degrees C). The blocks were hardened in water at room temperature,[20] and cooled in the deep freezer for a period of about 5 minutes.

Tissue was softened prior to sectioning (Gier, Personal Communication) (See Appendix I). Thin serial sections (8 µm thick) were prepared from the embedded tissue with the Spencer Microtome (model 820); mounted on slides with albumin fix; dried in the oven at 60 degrees C for an hour; stained sections were covered with cover slips, labelled and viewed with an Olympus light Microscope. Photomicrographs were taken on a Zeiss RA Research Microscope with CSmatic Camera attachment.

b. Oil Red O staining technique: This staining technique was used for distinguishing the CGs and the neighbouring sebaceous glands. Specimens were frozen prior to the staining procedure.

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[b]Lipshaw Manufacturing Corporation, Detroit, Michigan 48210.

[c]Curtis Scientific Co.

The formalin fixed specimens were frozen in 2-methyl-butane liquid and cooled to the temperature of liquid nitrogen. The frozen specimens were stored at -85 degrees C until sectioning which was done with Slee HR 10 Cryostat Microtome, at -19 degrees C. Thick sections (12 um thick) were collected on coverslips which have been previously immersed in subbing solution and allowed to dry overnight in a dust-free environment.[20]

The Oil Red O staining procedure for the frozen sections was done according to the standard method,[20] although some steps were slightly modified. Below is a summary of steps, describing the solutions, and the times used to provide for best results.

The frozen sections on the coverslips were immersed in staining dishes containing:

a. 100% propylene glycol for 10 minutes.
b. 0.5% solution of Oil Red O for 35 to 40 minutes.
c. 85% propylene glycol for 6 to 10 minutes.
d. Distilled water for 5 minutes.
e. Harris Hematoxylin (undiluted stock solution) for three minutes.
f. Distilled water - a quick rinse; sections were not allowed to stand.
g. 1% lithium carbonate for 15 seconds (until section turned blue).
h. Distilled water.

The stained sections on the coverslips were mounted on the slides with warm glycerin jelly; the edges of the coverslips were sealed to the slide with melted paraffin. Viewing and photomicrography of the sections were identical to those of PAS and Mallory Triple stained sections.

Scanning Electron Microscopy Study: The glutaraldehyde fixed specimens were processed for scanning electron microscopy (SEM) as follows:

Each specimen was fixed in the primary fixative of glutaraldehyde for a minimum period of 48 hours, for maximum penetration of the fixative; dehydrated in graded concentrations of ethanols (30%, 60%, 70%, 80%, 90% and 100%), for a period of 30 minutes; critical point dried with DCP-1 Critical Point Dryer[d]; and gold-palladium coated with KSE-2A-M Vacuum Evaporator[e] (see Appendix 2).

The coated specimens were viewed under at ETEC Autoscan[f] at accelerating voltages of 10 or 20 KV, and working distance of 8 to 26 mm. Photomicrographs were taken.

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[d]Denton Vacuum, Inc. Cherry Hill, New Jersey

[e]Kinney Vacuum Company, Boston, Mass. 02130

[f]ETEC Corporation, Hayward, California 94545

RESULTS

Scanning Electron Microscopy Observation:

In general, the typical elongated shape of the circumanal glands (CG) and their more compact nature (solid acini) formed firm basis for distinguishing them from the mucocutaneous sebaceous glands, which were made up of hollow acini. A brief description of observations corresponding to various ages are as follows:

The specimens from the one-day-old Beagle-cross dogs contained small developing CGs, which were closely located near the mucocutaneous junction. These glands (Fig 1 and 2) which were connected by extremely short main ducts, were elongated and directed posteriorly towards the inner dermal region; the entire gland lacked division into acini, as shown by the absence of basement membranes within the gland. Both vacuolated and non-vacuolated cells consisted mainly of single large vacuoles of about 7 μm mean diameter. Apart from the connecting tissue network of surrounding dermis which probably formed the gland capsule, the glandular interior lacked connective tissue.

The 20-day-old Black and White Poodle cross specimens showed more matured CGs; the glands had elongated from both proximal and distal ends, thus their main ducts had occupied a more or less central position (Fig 4). Partitioning into

spherical acini was evidenced by the presence of distinct basement membranes (Fig 6) which limited each acinus, and by the connective tissue network (probably collagen and reticular fibers) that formed a meshwork at the interacinar spaces (Fig 5 and 7). Each typical acinus consisted of mainly non-vacuolated cells, of about 20 um mean diameter; the intercellular spaces contained few networks of connective fibers (probably reticular fibers). Vacuolated cells were scarcely observed within the entire acini.

The CGs of the 152-day-old German shepherd cross (Fig 8 and 9) were fully matured, typically elongated and multiacinar; their cells contained vacuoles which, unlike the one-day-old glands, were smaller sized vacuoles, and there were many per cell (Fig 11). The acinar basal cells rested on thick basement membrane; adjacent basement membranes were connected by meshwork of connective tissue. The observed duct (Fig 10), which was probably the interacinar duct, consisted of vacuolated inner cells. Sebaceous glands (Fig 3) were located close to the mucocutaneous junction, and they consisted of hollow acini, about 100 µm mean diameter. Remnants of disquamated cells were clearly seen within the hollow sebaceous acini.

Light Microscopy Observation:

32-day-old Male and Female Cocker Spaniel Cross Fetuses. The skin of the entire fetal body, including that of the anal

region was entirely free of any glands, hair and other appendages; the epidermis showed more signs of differentiation than did the dermis.

The epidermal layer was thicker at the future circumanal region than elsewhere, and it consisted of four major layers: an inner stratum basale, made up of a single cuboidal cell layer and resting on a distinct basement membrane; a middle stratum spinosum of two polyhedral cell layers; and an outermost stratum granulosum layer of flattened cells. No layer of cornified cells was observed in the epidermis. An anal membrane which was a continuation of the spinous and granular layers of the epidermis, was observed covering the anal orifice (Fig 12). Few hair follicle primordia were observed in the dorsal skin of the thoracolumbar region only; they consisted of rounded masses of epidermal cells invaginating into the underlying dermal mesenchyme.

The dermis at the circumanal area was remarkable in that greater condensation of the mesenchyme tissue, and blood capillaries, were present when compared to the rest area of the skin (Fig 12). This dermal mesenchymal condensation was located at a mean radius of 60 μm from the anal orifice, and extended 280 μm mean distance at the circumanal region; the mean depth into the body, from the surface of the skin, was 430 μm.

38-day-old Male and Female Beagle Fetuses. The skin at the circumanal region and elsewhere was devoid of glands and

appendages; however, hair follicle primordia were sparsely distributed all over the entire skin (Fig 13).

The epidermis consisted of an outer layer of flat granular cells (stratum granulosum), a middle three-cell layer of polyhedral, densely packed cells (stratum spinosum), and an inner cuboidal cells layer (stratum basale), resting on a well defined basement membrane. The hair follicle primordia were button-shaped accumulations of cells of the stratum basale, which bulged into the underlying dermal mesenchyme tissue. The anal membrane, which was very pronounced in the 32-day-old fetus, had started disquamating; however, its original position was marked by the remnants of clumped cells of stratum spinosum.

The dermis was like those of the 32-day-old fetuses; however, greater differentiation was evidenced by dense accumulation of mesenchyme tissues at the future circumanal glandular zone, by the presence of more mitotic figures, and by the development of more blood islands.

One-Day-Old Male and Female Terrier Cross Dogs:

Mallory Triple Stain Observation. CGs, resembling mucocutaneous sebaceous glands, were observed. The typical elongated shape of the CGs (compared to the flask-shaped mucocutaneous sebaceous glands), in addition to their cellular make up and dermal locations, allowed identification of gland types (Fig 14 and 17). The mucocutaneous sebaceous glands (their typical branching nature rendered them different

from sebaceous glands found elsewhere) were located 1.6 mm mean distance from the mucocutaneous junction; their depth extended an average of 276 µm into the reticular dermis. The CGs occupied a circular zone about 2.2 mm wide next to the mucocutaneous sebaceous glands. Larger glands about 220 µm and 52.8 µm mean length and width respectively, were located nearer the mucocutaneous sebaceous glands than the smaller glands.

Their dimensions decreased with increasing distance from the mucocutaneous junction; they extended a maximum depth of 377 µm within the reticular dermis. The typical elongated shape of these young circumanal glands was narrowed at their superficial ends, as they formed a direct continuation of the main duct. In general, two CGs were located at positions medial and lateral to the hair follicle of origin.

The main duct had a mean diameter of 14.2 µm; it was lined by a layer of low cuboidal cells and was filled with cells. The duct bulged out of the hair follicle external root sheath, and continued directly as the proximal portion of the CG (Fig 15); its limit being indicated by the first layer of linear clumps of few cells across the gland. The inner cells main duct were entirely vacuolated (mostly small vacuoles), sebaceous-like and polyhedral, and were clearly demarcated by the layer of linearly clumped cells. There were no extensions of the main duct into the gland beyond the layer of clumped cells.

There were no acini within the circumanal glands, although some clumping of cells (inner cell type) was observed across the gland, almost parallel to those at the limit of the main duct. These cell clumps almost partitioned each gland into 4 to 6 acini. The entire gland was like an elongated bag, filled with vacuolated and non-vacuolated polyhedral inner cells and lined by low cuboidal basal cells (Fig 16); the vacuolated inner cells were more abundant at the proximal part of the gland. The cuboidal basal cells resembled the external root sheath cells of the hair follicle, they had a fusiform nucleus, 8.0 μm mean length, per cell; the single nucleus was heterochromatic and contained two to three nucleoli. Both vacuolated and non-vacuolated inner cells had indistinct limits. The mean cell diameter of both cell types was 16 μm, and their single spherical nucleus had two to three nucleoli, one of the nucleoli was bigger than the rest, and was connected to slender radiating strands of chromatin in an eccentric position. The non-vacuolated cells had fine purplish cytoplasmic granules. Vacuoles of the vacuolated cells ranged in size and number from a few large singles to many of small size.

PAS Stain Observation. The CGs, sebaceous and sweat glands were PAS negative (Fig 18).

Within the dermis, stratum spinosum cells contained many PAS positive granules, while only a few granules were observed in the stratum granulosum cells; the stratum basale was completely negative.

Apart from the PAS positive reaction of the abundant collagen fibers of dense irregular connective tissue in the dermis, only the internal root sheath cells of the hair follicle demonstrated a significant amount of PAS positive granules (Fig 19). The external root sheath cells contained small amount of such granules. In general, the PAS positive reaction of hair follicular cells was confined beneath the level of the origin of the CG main ducts (Fig 19).

Oil Red O Stain Observation. The staining reaction of the sebaceous glands and CGs to Oil Red O was identical (Fig 20 and 21). However, most of the sebaceous inner cells (including those of their duct) were loaded with liquid droplets, so that their pyknotic nuclei were hardly discernable (Fig 20); their cellular outline was very distorted. Comparatively, such lipid loaded, distorted cells were contined to the inner cells of the main duct of the circumanal glands.

All the Oil Red O positive inner cells of the CGs, which were more abundant at the proximal portion of the gland, corresponded with the vacuolated cells under the Mallory Triple staining technique. Their cytoplasm contained lipid droplets ranging from a few large droplets to many small droplets which almost formed ring-like accumulations around their nuclei (Fig 21).

Most basal cells of the sebaceous and CG acini, cell layer lining the ducts and the external root sheath cells of the

corresponding hair follicles reacted negatively to the Oil Red O stain. Most of the hair follicle internal root sheath cells were very positive.

Three-Day-Old Male and Female Mongrels:

Mallory Triple Stain Observation. CG at three days (Fig 22) have elongated at both the superficial and deep ends; as a result, their main ducts, which joined directly to the superficial ends of the glands in the one-day old, were located near the upper third of the glands. Due to their typical branching nature, and abundance of many vacuolated inner cells within the gland, the mucocutaneous sebaceous glands (300 µm and 16 µm, mean length and width respectively) were quite distinct from the CGs; they occupied a circular zone with a 2.1 mm mean radius from the mucocutaneous junction of the anal orifice, and a depth of 0.53 mm mean length from the surface of the skin into the dermis. The CG occupied a circular zone peripheral to the mucocutaneous sebaceous glands. This zone extended 2.9 mm (mean) from the zone of mucocutaneous sebaceous gland. The CGs were located at a 0.69 mm mean dermal depth from the skin surface; they had a mean length and width of 360 µm and 120 µm respectively.

The obliquely directed main duct connected each CG at their upper third to their hair follicle of origin (Fig 23). The duct which had elongated (40 µm and 44 µm, mean length and width respectively), when compared to those of day-one, was

solid and was filled with polyhedral, vacuolated inner cells of indistinct cellular limits, and lined by low cuboidal cells. The lining cells of the main duct had distinct cellular limits, and a spherical nucleus which almost filled the entire cell; each nucleus contained 3 to 4 nucleoli. The inner cells of the main duct had many vacuoles (like those of the sebaceous gland inner cells); some had a normal spherical nucleus (one per cell) of 16.8 μm mean diameter, with 2 to 4 nucleoli per nucleus, other cells showed many cytoplasmic vacuoles and a pyknotic nucleus. The main duct within the gland was not observed to extend beyond the first clump of cells demarcating the gland acinus (Fig 23).

Acinar organization of the CG was more advanced than in the one-day-old, as evidenced by more demarcating clumps of flattened cells which had almost divided the gland into 8 or 9 oblong acini of 69 μm and 39.5 μm mean length and width respectively (Fig 22). The basal cells of each organizing acinus (Fig 23) were squamous, and nearly filled with a single elongated nucleus; the nuclei had a mean length of 8.7 μm. The acinar inner cells completely filled the acinus, and were of both vacuolated and non-vacuolated types (Fig 23); the vacuolated type were fewer in number than in the one-day-old, and were most abundant in acini nearest to the main duct. These vacuolated inner cells had larger vacuoles in their cytoplasm than did the inner cells of the mucocutaneous sebaceous glands. The non-vacuolated inner cells were very

abundant in acini more distant from the main duct; their cytoplasm contained many fine purplish granules. Both vacuolated and non-vacuolated inner cells were polyhedral in shape, had similar mean cell diameters of 20.7 μm, and had a similar single spherical nucleus of 6.8 μm mean diameter, with two to three nucleoli and fairly coarse chromatin. One of the two or three nucleoli was eccentrically located, and was connected to radiating strands of chromatin network.

Connective tissue and capillary networks were not noticeably evident within the inter-acinar spaces of the circumanal gland, although evidence of blood vessel invasion was shown by the presence of a few capillaries at the peripheral limits.

PAS Stain Observation. As observed in one-day-old puppies, the CG, sebaceous and sweat glands were negative for PAS granules.

In the epidermis, only the stratum spinosum cells contained a few PAS positive granules; in the dermis, apart from the abundant PAS positive collagen fibers, the hair follicular cells, from below the level of the branching-off of the circumanal and sebaceous main ducts to the level of the hair papillae (Fig 24), contained many PAS positive granules. Above the level of the circumanal and sebaceous main ducts, only very few PAS positive granules were observed within some internal root sheath cells of the hair follicles.

Oil Red O Stain Observation. The inner cells of the mucocutaneous sebaceous gland were loaded with many lipid droplets of varying sizes which corresponded in size with the observed vacuoles using the Mallory Triple Stain; their inner duct cells contained similar lipid droplets; some of their nuclei were pyknotic, or completely displaced by the lipid droplets.

The CG had fewer lipid containing inner cells than those of the one-day-old, and they were mainly located in acini nearest to the main duct which also was completely filled with lipid contained inner cells. The sizes of the lipid droplets in the cytoplasm of these acinar inner cells corresponded with those of the vacuoles which were observed in vacuolated inner cells with the Mallory Triple Stain.

11-Day-Old Male and Female German Shepherd Cross Dogs:

Mallory Triple Stain Observation. The CGs were typically paired around each hair follicle of origin; with both medial and lateral glands forming a pair (Fig 25). When seen in a good longitudinal or oblique section of the skin, they resembled a miniature left and right mammalian lung. The glands were located a few millimeters from the mucocutaneous junction of the anal orifice, and extended a short distance peripherally (this extent was not measured); each typical gland averaged 570 μm in length and between 170 to 190 μm in width.

Each gland was connected to the hair follicle by a solid main duct, at a central position of the gland. These main

ducts averaged 57 μm in length and 42 μm in diameter; their lining cells appeared the same as those of the external root sheath of the hair follice, to which they were connected. Most inner cells of the main duct, tended to be vacuolated in a manner similar to that of sebaceous cells. No extensions of the main duct into the glandular tissue were observed.

The dense irregular connective tissue of the dermis served as an organ capsule which enclosed the solid spherical acini. Some CGs had a few sebaceous acini, which were located at the superficial end of the gland; about 12 to 22 acini were observed in typical glands in a good longitudinal section. In general, the acinar organization was by far more advanced than that of three-day-olds, as evidenced by a more compact and distinctly demarcated nature (Fig 26); each acinus averaged 60 to 74 μm in diameter. Squamous basal cells, with an indictinct cellular limit formed the limit of each acinus; they were similar to the lining cells of the main duct from which they originated; their elongated heterochromatic nuclei averaged 7.9 μm in width, and appeared to nearly fill the entire cell. Each basal cell nucleus contained one or two dense nucleoli, of about 1.6 μm in diameter. The acinar inner cells were spherical to polyhedral in shape, with indistinct cellular limits. Towards the center of the acinus, non-vacuolated inner cells (11.9 μm mean diameter) were frequently seen; their cytoplasm was dense with purplish granules (Fig 27). The vacuolated inner cells were larger cells; some had typical large vacuoles

almost coalescing. Both non-vacuolated and vacuolated inner cells had spherically shaped single nucleus (4.7 to 7.9 μm in diameter) with one or two nucleoli of 3.2 mean diameter. The nucleoplasm of non-vacuolated inner cells had spoke-like chromatin threads attached to an eccentric dense nucleolus.

The interacinar spaces contained narrow strips of connective tissue, and capillary networks.

PAS Stain Observation. Apart from the connective tissues of the interacinar basement membrane, the CG, sebaceous and sweat glands were PAS negative.

The entire epidermal strata were also PAS negative. Within the dermis, the collagen fibers of the dense irregular connective tissue and, in particular, the hair follicle cells below the level of the CG main ducts, were PAS positive. The internal root sheath cells contained more PAS granules than did the external root sheath cells. The external root sheath cells above the level of the main ducts of the CGs were PAS negative, while only a few PAS granules were observed within internal root sheath cells of the corresponding region.

Oil Red O Stain Observation. The sebaceous glands were positively stained for lipid. Their inner cells were completely filled with reddish droplets. The typical droplets which were spherical in shape varied in size, with the smaller droplets predominating and the larger droplets completely distorting the cellular outline. The cellular outlines with typical pyknotic wavy dense nuclei were quite discernible.

A few cells of the CG acini were also positive for lipid. These positively stained cells were invariably the vacuolated inner cell type. Unlike the sebaceous cells, these lipid containing inner cells of the circumanal gland had indistinct cellular limits, but with a normal nuclear pattern. Most of the inner cells of the ducts, and the internal root sheath cells of the hair follicle, contained lipid droplets. The lipid droplets in the CG varied in size; some very large lipid droplets may have resulted from rupture of vacuoles and subsequent seepage and accumulation of contained lipid.

14-Day-Old Male and Female German Shepherd Cross Dogs:

Mallory Triple Stain Observation. The CGs at this stage of development, were basically the same as those of 11-day-old pups except for a more distinct acinar organization and extensions of the main duct into the glands in the 14-day-old. The immediate periphery of the anal orifice, which also included the mucocutaneous junction, was devoid of CGs but mucocutaneous sebaceous glands were present. The next zone approximately 1.2 mm from the anal orifice or mucocutaneous junction and extending for about 2.35 mm, contained the CGs; from this point on only normal sebaceous glands without CGs were found.

The main duct extension consisted of solid inter-acinar and intra-acinar ducts (Fig 28 and 29). The inter-acinar duct, which averaged 20 µm in diameter, was a direct continuation of the main duct which was also solid and transversed through the middle portion of the gland in a length-wise

fashion. The lining cells of the duct resembled those of the acinar basal cells; its inner cells also resembled those of the acinus. The continuity of the inter-acinar ducts was periodically interrupted by the branching off of the intra-acinar duct. The intra-acinar ducts were immediate branches off the inter-acinar ducts, they were short and roughly of the same diameter as the inter-acinar ducts, if they were connected to a nearby acinus. If, however, they were connected to a more distant acinus they were narrower. The narrow intra-acinar duct had an average diameter of about 12 um. There was one intra-acinar duct per acinus; its lining and inner cells resembled those of the inter-acinar ducts. Other than being more vacuolated, the inner cells of the intra-acinar duct also resembled those of the glandular acinus.

The typical acinus at this stage of development was better defined and organized than those of 11-day-old pups (Fig. 30); unlike the acinus of the 11-day-old, it had fewer vacuolated inner cell type. Their non-vacuolated inner cells had more conspicuous cytoplasmic purplish granules. The nuclei of the acinar inner cell, in general, were condensed and averaged 7.9 μm in diameter. The characteristic spoke-like chromatin pattern of the 11-day acinar inner cell nuclei was less distinct in these inner cells. Each nucleus had two or three dense nucleoli. The basal cell of the acinus were very similar to those observed in 11-day-old, i.e. squamous to low cuboidal; each basal cell contained a single elongated

nucleus. The inter-acinar spaces contained blood capillaries as well as connective tissue fibers.

PAS Stain Observation. The CGs, sebaceous and sweat glands, apart from their inter-acinar basement membrane connective tissue, were significantly PAS negative.

As in 11-day-old, all the cells of the epidermal strata were also negative for PAS.

The positive-reacting dermal elements were: collagen fibers of dense irregular connective tissue, and granules in hair follicle cells of internal root sheath cells, most particular, below the level of the branching of the CG main ducts (Fig 33).

Oil Red O Stain Observation. As in 11-day-old puppies, the inner cells of the sebaceous glands were completely filled with lipid droplets (Fig 32). The inner cells of the duct and the internal root sheath cells to which they were connected were also completely filled with lipid droplets.

The total number of CGs cells stained positive for lipid decreased in number with respect to 11-day-old puppies. The positive cells were limited mostly to the inner cells of the duct, and to a few inner cells of the acini which were closer to the main duct (Fig 31). Those acini more distant from the main duct of the gland were virtually devoid of lipid cells. The few vacuolated inner cells of the acinus closest to the main duct in the Mallory Triple Stain, were the lipid cells.

Many small droplets of lipid or a few large droplets were observed in these lipid cells. Almost all the basal cells, including the external root sheath cells and the lining cells of the ducts, were negative for lipids.

20-Day-Old Male and Female Black and White Poodle Cross Dogs:

Mallory Triple Stain Observation. The numbers of CGs observed in the male and female were generally less than those in the 14-day-old German Shepherd pups, although greater development was evidenced in the CGs of the 20-day-old Black and White Poodle pups by increased size and greater extension of their main ducts. The CGs varied in size; the smaller ones being located on both the medial and lateral peripheries of the entire CG zone (Fig 34). The gland length ranged from 480 to 720 µm; mean width at the widest points (which was near the deep ends) was 320 µm. In general, they were typically located medial and lateral to the hair follicle of origin; two glands per hair follicle. Each gland was typically elongated, narrowed at the superficial ends, but widened at their deep ends. The glands occupied a circular zone, next to that of the mucocutaneous sebaceous glands. The mucocutaneous sebaceous glands, which were typically branched and located at the immediate periphery of the anal orifice, were shorter in length (286 µm mean length) than the CGs, although their mean width (346 µm) approximated that of the CG. They consisted of vacuolated glandular cells. In between the zone of the mucocutaneous sebaceous glands and the CGs were glands which were

transitional between both glandular types (Fig 35); these glands had typical sebaceous acini at their superficial ends, while their deeper acini were hepatoid, resembling the CGs.

Two types of solid main ducts (short and long), which connected each pair of CGs to a hair follicle of origin, were observed (Fig 36). The short main duct was often connected with the lateral CG, while the medial CG was usually connected to the hair follicle by the long main duct. However, either the lateral or the medial CGs could be associated with either the short or long main duct. The short main ducts had a mean length and width of 100 µm and 46 µm respectively. They extended in a transverse direction from the hair follicles into the inter-acinar ducts of the glands which, in turn, extended through the entire length of the gland and eventually branched into short narrow intra-acinar ducts which connected with the solid glandular acini (Fig 37). The long main ducts were of 255 µm and 45 µm mean length and width respectively. They connected the hair follicles with inter-acinar ducts of the CGs, near their deep ends (CGs). In general, the short main ducts entered the CG at a more or less central position of the gland, while the long main ducts entered the gland near their deep ends. The CGs which were connected to the short main ducts were more superficial in location (1.1 mm mean dermal depth from the skin surface), while those connected to the long main duct were located deeper in the dermis (1.4 mm mean dermal depth from the skin surface). All the ducts of the CG - main, inter-acinar and intra-acinar, were solid.

The lining cells were low cuboidal, with a high nuclear to cytoplasm ratio, and their cell membranes were indistinct. The duct inner cells were predominantly non-vacuolated, polyhedral cells and contained fine purplish granules (Fig 38). Cells with large vacuoles, and fibrocytes with collagen fibers were observed in the ducts (Fig 38).

The acini of the CGs were spherical to oblong in shape. Their number (20 to 31 acini per CG) varied directly with the size of the gland. The acini ranged from 72 to 128 µm in diameter. Each typical acinus was lined by cuboidal basal cells, polyhedral inner cells filled the lumen. The basal cell nuclei were elongated to spherical, heterochromatic and filled the entire cell. They had a mean diameter of 5.7 µm and an indistinct cell membrane. The inner cells had indistinct cell membranes, were filled with purplish granules (.08 µm mean diameter), had a mean cell diameter of 20.4 µm and a mean nuclear diameter of 8.3 µm. The nuclei of the inner cells contained two to three nucleoli and were more euchromatic than those of the 14-day-old. Vacuolated inner cells were not observed in the acini. In general, the glandular acini of the 20-day-old CGs were more organized and hepatoid than those of the 14-day-old.

The inter-acinar spaces (5 µm mean width) contained blood capillaries and collagen fibers with fibrocytes oriented in the direction of the fibers (Fig 39).

PAS Stain Observation. The sebaceous, sweat and circum-anal glands were PAS negative, except for collagen fibers of basement membranes in the inter-acinar spaces which were PAS positive. The lobulation of the CG into solid compact acini was distinct.

The hair follicular cells above the level where the main ducts originated were PAS negative. Below the level of the main ducts many PAS positive granules were observed in the hair follicle cells. The collagen fibers of the dense irregular connective tissue in the dermis were PAS positive (Fig 40).

Oil Red O Stain Observation. The inner cells of the sebaceous glands, and those of the superficially located sebaceous acini of the transitional CGs (located between the zone of the mucocutaneous sebaceous glands and the CGs) were Oil Red O positive (Fig 41). The positive cells were laden with lipid droplets which corresponded in size with vacuoles of similar cells seen in the Mallory Triple Stain.

The CGs were devoid of lipid cells, except for a few cells located in the ducts which contained large lipid droplets and also corresponded in size with the large vacuoles observed in similar cells stained with Mallory Triple Stain. In general, the glandular cells of the CGs were non-lipid and hepatoid. Their ducts contained fewer lipid cells than those of the 14-day-old (Fig 42).

60-Day-Old Female German Shepherd Cross Dog:

Mallory Triple Stain Observation. The three major glands of the circumanal skin - sweat, sebaceous and CGs, were well developed. The mucocutaneous sebaceous glands were typically branched (Fig 45 and 46); each branch emptied into the hair follicle by a main duct which, unlike that of the CG, was patent. They occupied a circular zone, of a mean distance of 8.6 mm from the anal orifice, which included the mucocutaneous junction. Their sizes were 0.8 mm and 0.7 mm mean length and width respectively. The CGs occupied a circular zone, next to that of the mucocutaneous sebaceous glands, which extended a mean distance of 1.4 cm away from the last mucocutaneous sebaceous glands; the mean dermal depth of the CGs within the zone was 2.0 mm from the surface of the skin. The CGs varied in sizes (1.2 to 2 mm length and 0.4 to 0.6 mm width at their widest points); the larger glands were located within the zone. The typical elongated shape of the CGs, with a narrow superficial end, was observed (Fig 43). Development of smooth and skeletal muscle bundles within the area limited the expansion of some glands, resulting in a very narrowed middle portion of the gland. However, an increased width of such gland, in the regions beyond the zone of contact with the muscle bundle, tended to compensate for the narrowness. The zone between the mucocutaneous sebaceous glands and the CGs contained glands which had sebaceous acini at their superficial portions, and hepatoid acini at their deep portions.

The hepatoid acini of such transitional CGs were identical to those of typical CG acinus, and they were larger than the sebaceous components which resembled typical mucocutaneous sebaceous glands. However, a few transitional CGs with a greater sebaceous component (2/3 of the entire gland) were present.

The increased width of the CGs due to growth of the acinus towards the follicle allowed the acinus to completely envelope the main ducts with the result that the main ducts were located entirely within the gland (Fig 43). The ducts were difficult to observe within the glandular tissue. The CGs were almost in contact with their hair follicles of origin, except for a thin layer of connective tissue which separated both structures (Fig 43). In general, the CGs appeared ductless and surrounded their hair follicles of origin. Some lateral and medial CGs also appeared to have fused with each other.

The typical acinus varied in size. There were about 32 to 81 acini per CG; the acini ranged from 84 mm to 140 mm in diameter. The larger acini were located towards the deep portion of the gland. Their shape, which was dependent on the pressure of the surrounding acini, varied from an incomplete sphere to angular, to oblong. Each acinus was lined by low cuboidal basal cells, and filled with polyhedral hepatoid inner cells (Fig 44). The basal cells had indistinct cell membranes, high nuclear to cytoplasm ratio, and rested on the basement membrane located within the inter-acinar spaces. The basal cell nucleus was spherical, 6.9 µm mean diameter,

and contained one or two nucleoli. The inner cells which had a diameter of 16.6 μm mean, were filled with purplish granules, and adhered tightly to one another, resulting in total occlusion of the interior of the acinus. The spherical nucleus of each inner cell measured 7.6 μm mean diameter, and had one or two nucleoli. The acini of the CGs were entirely devoid of vacuolated inner cells.

Inter-acinar spaces contained basement membranes composed of collagen fibers, fibrocytes and blood capillaries (Fig 44). The basement membranes were thicker and more developed than those of the 20-day-old, and completely separated the adjacent acini from one another.

PAS Stain Observation. The sweat, sebaceous and circumanal glands were PAS negative, except the basement membranes within their glandular tissues. The distinct separation of the circumanal glandular tissue into acini was very remarkable with the PAS stain.

The collagen fibers of the dermis were PAS positive, as were granules of those hair follicle cells, located below the level of the CGs. A few PAS granules were observed within the internal root sheath cells of the hair follicles, above the levels of the CGs, but the external root sheath cells similarly located above the CG levels were completely devoid of PAS granules.

Oil Red O Stain Observation. The sebaceous glands were laden with lipid cells, as evidenced by the presence of many small reddish droplets within their cytoplasm (Fig 47). The nuclei of the lipid cells were often times pyknotic.

The CGs were completely Oil Red O negative, apart from the sebaceous acini of the transitional CGs.

152-Day-Old Female German Shepherd Cross Dogs:

Mallory Triple Stain Observation. The two female dogs of this group, which were litter mates of the 11-, 14-, and 60-day-old dogs, had mucocutaneous sebaceous glands and CGs that were identical in sizes with similar glands observed in the 60-day-old dogs. The mucocutaneous sebaceous glands measured 0.5 mm and 0.3 mm mean length and width respectively, and they occupied a region which extended a mean distance of 3.3 mm from the periphery of the anal orifice. They were quite distinct and separate from the CGs which were located within the zone next to that of the mucocutaneous sebaceous glands, in an irregular circular manner. The CGs varied in size (0.6 to 2.0 mm length and 0.2 to 0.6 mm width), with the smaller glands being located closer to the mucocutaneous sebaceous glands, and at the medial periphery of the zone. Glands (Fig 55), which were transitional between the mucocutaneous sebaceous glands and the CGs, were observed between both glandular zones. They had elongated hepatoid acini at their superficial portions. The hepatoid component of some of the transitional glands were separate

from their corresponding sebaceous acini. The transitional glands were not observed in other locations apart from the one described above.

The ducts of the CGs, like those of the 60-day-old, were generally not discernible. However, a few ducts with cysts were observed in some of the glands (Fig 49, 50, 51 and 52). The cysts were cavitations within the duct remnants. The concentric layers of spindle shaped cells and connective tissue fibers of the duct walls were identical respectively with the fibrocytes and collagen fibers previously described in the ducts of the 20-day-old CGs. They were circular, contained solid reddish substances in their lumen and ranged from 100 to 160 µm in diameter. From two to three cysts were observed within both the incorporated main duct, and the inter-acinar ducts of some CGs; a few cysts of the main duct opened directly into the hair follicle of origin. Some CGs had only one cyst within their duct remnants, while most of the smaller CGs, which were located close to the mucocutaneous sebaceous glands, were devoid of cysts.

The CG acini ranged from 40 to 62 in number per gland, and the smaller glands had fewer acini. They were oblong to angular in shape, of variable diameter (80 to 150 µm), and were filled with glandular polyhedral inner cells and lined by a layer of low cuboidal to squamous basal cells (Fig 54). The acini of the smaller CGs were closer to one another than those in the larger glands. The acinar basal cells were nearly filled with a fusiform or spherical nucleus with a

7.0 μm mean length; each nucleus contained one or two dense nucleoli. The inner cells measured 13 μm mean diameter, contained a spherical nucleus of 7.0 μm mean diameter, had one or two dense nucleoli, and were filled with purplish to reddish granules of 0.08 μm mean diameter, which formed dense aggregates around the nucleus. Some of the inner cells, located towards the center of some acini, had indistinct cell membranes and nuclei that were undergoing pyknosis (Fig 54). The cytoplasmic granules within these abnormal inner cells were more faint and reddish, and some signs of cellular breakdown were also evident by shrinkage of some of the cells.

The inter-acinar spaces ranged from 20 to 60 μm in width; those of the larger CGs were wider and they contained blood capillaries, arterioles and venules, connective tissue fibers and fibrocytes. The greater width of the inter-acinar spaces in the larger CGs rendered each acinus in the gland almost a separate glandular unit. However, some of the acini still maintained contact with one another through the remnants of the ducts.

PAS Stain Observation. The tissues appeared to be identical to those of 60-day-old dogs. The sweat, sebaceous, and circumanal glands were PAS negative, apart from their inter-acinar connective tissue fibers. PAS granules were also observed within the hair follicle cells, beneath the level of origin of the CG main ducts.

Oil Red O Stain Observation. The glandular cells of the sebaceous glands and the sebaceous acini of the transitional glands, contained reddish lipid droplets, similar to those described in previous specimens (Fig 56).

The CGs were totally negative for lipids; there were no lipid droplets, observed within any portion of the glands. The cysts and their products were also Oil Red O negative (Fig 57).

DISCUSSION

To more easily describe and clarify the sequential development of the CGs, composite diagrams of the developmental stages were made from micrographs. These diagrams will be referred to in the discussion.

The light and scanning electron microscopy data provide evidence to support the hypothesis that the CGs are present at birth, and that they are unique and distinct from the sebaceous glands which are also present at birth. Although the CGs, at birth, contain some lipid cells which render them similar to the sebaceous glands, their typical elongated shape and the absence of acini within the gland, (as evidenced by inability to observe basement membrane within the gland with the aid of SEM), distinguish them from the neighbouring mucocutaneous sebaceous glands which are usually typically branched, possess acini, and are almost oval in shape. The lipid cells of the CGs, at birth, are concentrated mostly in the main duct and the superficial portion of the gland, while the sebaceous glands are completely filled with lipid cells. In addition, the position occupied by these CGs within the skin, relative to the mucocutaneous sebaceous glands, is identical to that observed in the older specimens. This position is always a circular zone, around the anal orifice, and is sandwiched by a zone of mucocutaneous sebaceous glands medially and ordinary

sebaceous glands laterally. The width of the zones vary with size, age and breed of the animal. In general, the CGs of older and larger dogs occupy a wider zone. The observation of this study that CGs are present at birth agrees with similar findings by Baker[3], and with the descriptions by Cotchin[7,8,9,10] and Nielsen.[29] Our observation, however, disagrees with those of Maita and Ishida[23] who failed to observe the glands in dogs less than 14 days of age. This disagreement might be attributable to the selection of routine H & E stains for their study. We found during our preliminary studies that H & E failed to distinguish the CGs from the mucocutaneous sebaceous glands. By using a modified Mallory Triple Stain (Gier, personal communication), we were able to readily distinguish the difference between both glands. The peculiar reddish colour of the lipid droplets, with Oil Red O Stain, also rendered differentiation between both glands possible.

The CGs originate from the external root sheath cells of the hair follicle as evidenced by both the light microscopy and SEM data. In the 1-day-old, pairs of CGs are seen directly connected to their hair follicle of origin, by very short main ducts which continue into the superficial portion of the glands (Fig 59). Involvement of only the external root sheath cells in the formation of the entire glandular tissue is indicated in all the specimens by inability to observe PAS granules in the CGs and external root sheath cells above the point of

origin of the glands. PAS granules, however, were observed in both the external and internal root sheath cells below the level of the origin of the CG main ducts; but above this level, the few granules observed were mostly confined to the internal root sheath cells. Since the CGs in all the specimens were significantly PAS negative, they may have originated from only the external root sheath cells of the hair follicle, which were also significantly PAS negative above the level of the origin of the main duct. The finding agrees with the statement by Maita and Ishida,[23] that the CGs originated from the hair follicle external root sheath cells, although it was not based on similar reasoning or data.

A definite period of rapid CG development is evident during that period which extends roughly from birth to about 60 days post partum. These results are summarized in the diagrams (Fig 59, 60, 61, 62 and 63) and graphs (Fig 64). The slight deviation of the curve of the mean gland length could be due to breed differences. During the period of rapid CG development, the glands increase remarkably at both the superficial and the deep ends, resulting in a shift of the main duct from the superficial end to a more or less central position on the glands. The main duct then extends into, and through the entire length of the gland which is being demarcated into solid acini, each inter-acinar duct divides into smaller intra-acinar ducts that supply each developing acinus. All of the ducts, and acini are solid, during the period of rapid development. There is also a rapid decrease of the number of lipid

cells in the CGs during this period, while similar cells persist and multiply in the surrounding sebaceous glands. The CGs of the 60-, and 152-day-old dogs were completely negative for lipids. The observation of CG ducts agrees with those of Parks,[30] but, disagrees with his description of the branching of a single glandular duct, immediately after originating from the hair follicle, into the two glands at either sides of the hair follicle. Each gland on either side of a hair follicle, which Parks[30] regards as two major lobes of the CG, has been shown, in this study, to be a separate gland, with its separate main duct originating directly from the hair follicle. The fact that we were able to identify ducts between the CGs and the hair follicles which Baker[3] did not, may be a consequence of our decision to serialy section our tissue and thus observe the entire glandular structure. By doing so, we have demonstrated that the CG ducts are so distinct that their presence cannot be disputed. Maita and Ishida[23] also observed ducts of the glands in dogs less than 9 months of age, although they stated that those ducts were partially solid. In this study, the ducts were observed to be filled with lipid cells at early stages, while towards the end of the rapid developmental period, the lipid cells become gradually replaced by non-vacuolated inner cells, fibrocytes, and collagen fibers. On the second growth phase (day 60 to 152) which is one of limited cellular multiplication and growth, cysts appear within the ducts. Note (Fig 64) the decrease in the mean inner cell diameter and the

glandular length during this period; this decrease is due mainly to formation of new smaller CGs from the mucocutaneous sebaceous glands. An explanation of the process of post partum CG formation will follow later. The cysts, which were observed in 152-day-old dogs, could be initial steps toward lumenization of the ducts for conduit of the glandular products. Parks[30] called those cysts "non-sebaceous cysts", and designated them as unsuccessful attempts at lumenization. Maita and Ishida[23] also observed similar cysts, although they did not assign a function to them. Further study is recommended in order to determine the role of these cysts which appear, from our observation, to be initial steps towards definite lumenization. There was no observation of system of canaliculi within the CGs in the present work, as was reported by Schaffer,[32,33,34] and later by Gerisch and Neurand.[16]

Evidence is also presented from light microscopy analysis to show that during the Period of concentration, new CGs develop from the deeper acini of the sebaceous glands on either sides of the CG zone, in particular, from the mucocutaneous sebaceous glands. The distribution of the CGs, during the Period of Concentration in which the larger glands are located mainly within the middle of the zone and occasionally including the lateral portion of the zone too, suggests that the smaller glands are added mainly into the zone from the mucocutaneous sebaceous glands which are close to the medial portion of the zone. In 60- and 152-day-old dogs, glands which are transitional,

with superficial sebaceous acini and deep hepatoid or CG acini, were most frequently observed between the zones of CGs and mucocutaneous sebaceous glands. The decrease in width of mucocutaneous sebaceous glandular zone in 152-day-old dogs, compared to those of the 60-day-old, also indicates transformation of some of the sebaceous glands into CGs. The older, larger CGs had acini that were widely separated by well developed inter-acinar tissue of collagen fibers and blood vessels, and their duct remnants had cysts. In comparison, the younger CGs were more compact, and lacked cysts. The transitional glands were rarely observed in other parts of the skin, apart from the one indicated above. The statement by Parks,[30] that the typical CG is bipartite with superficial sebaceous and hepatoid components, is highly contradicted by the result of our work. Only the glands we describe as transitional glands can match those of Parks' description. From birth, the typical CG is hepatoid and quite distinct from the sebaceous glands. Parks[30] concluded that the CGs are glands which have failed to develop into sebaceous glands; therefore, implying that the CGs have no particular function. Our result suggests that the reverse is the case. The CGs are unique from birth, and as the animal grows, new CGs originate from the neighbouring sebaceous glands. Acquisition of ducts by these glands and later cyst development of the ducts, coupled with good vascularization, and production of granules within the glandular (inner) cells, would strongly suggest that

these glands are quite functional. Baker,[3] and Maita and Ishida[23] suggested that an endocrine-like function is possible for the CGs. This hypothesis is supported by our observation that the glandular ducts are incomplete or cystic; this would argue strongly against any exocrine type activity. In addition, the presence of an extensive capillary bed around the CGs of adult dogs is strongly suggestive of an endocrine-like function. What the function may be remains to be established.

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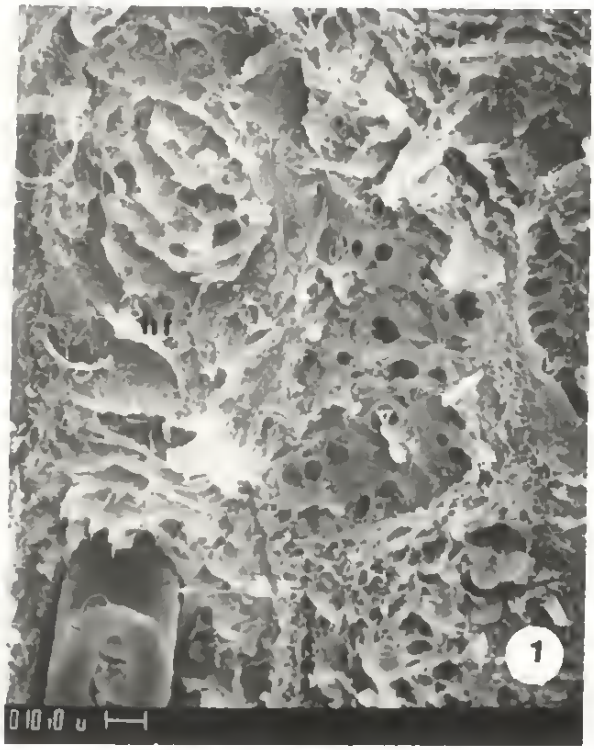
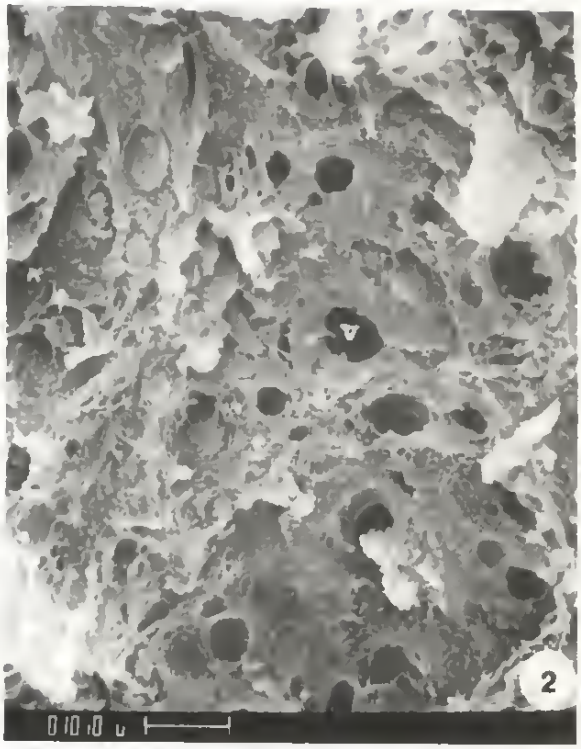
EXPLANATION OF FIGURES

Fig. 1. Scanning Electron micrograph of young circumanal gland from 1-day-old Beagle Cross dog, attached to the hair follicle (hf) of origin. The gland lacks basement membrane, and contains many inner cells with vacuoles (v). (20 KV; W.D. = 24 mm; X110).

Fig. 2. Higher magnification of Fig. 1 CG, showing the inner cells with vacuole (v). (SEM; 20 KV: W.D. = 17 mm; X500).

Fig. 3. Scanning Electron micrograph of sebaceous gland from 152-day-old German Shepherd Cross dogs, with hollow acini (ha) and desquamated cell remnants. (10 KV; W.D. = 26 mm; X100).


hf
1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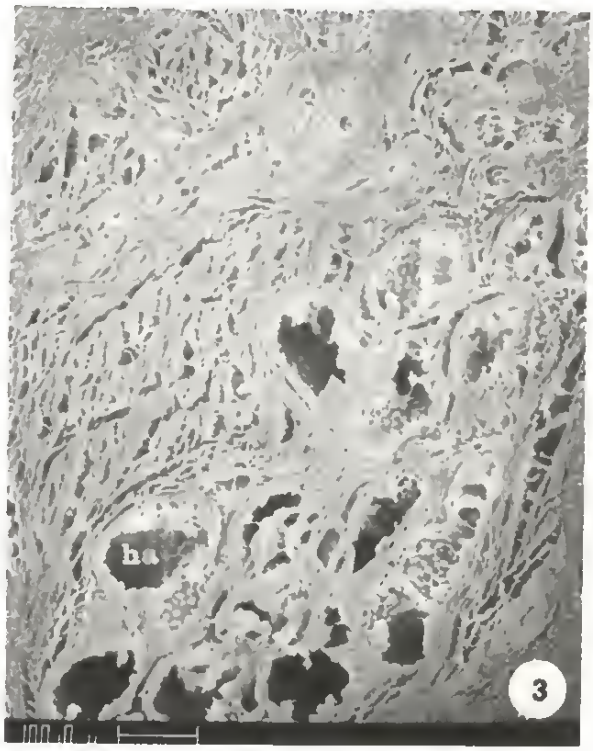

ba
3

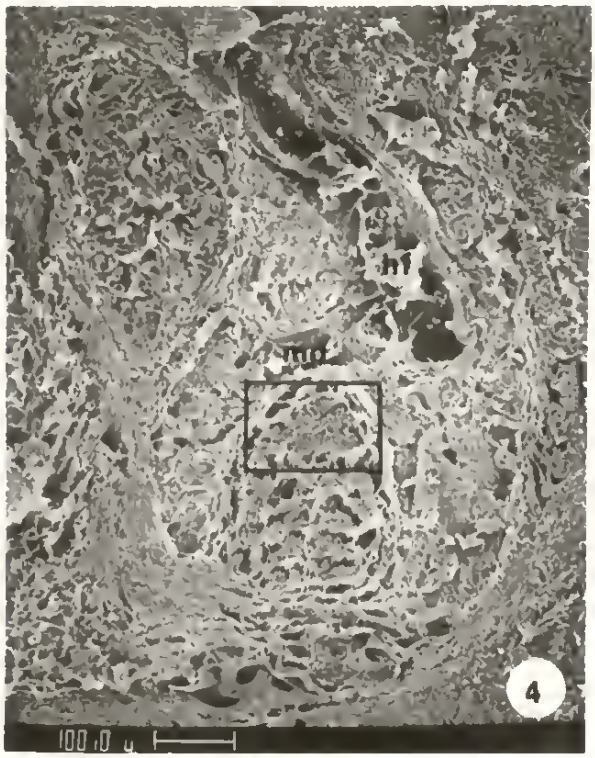
EXPLANATION OF FIGURES

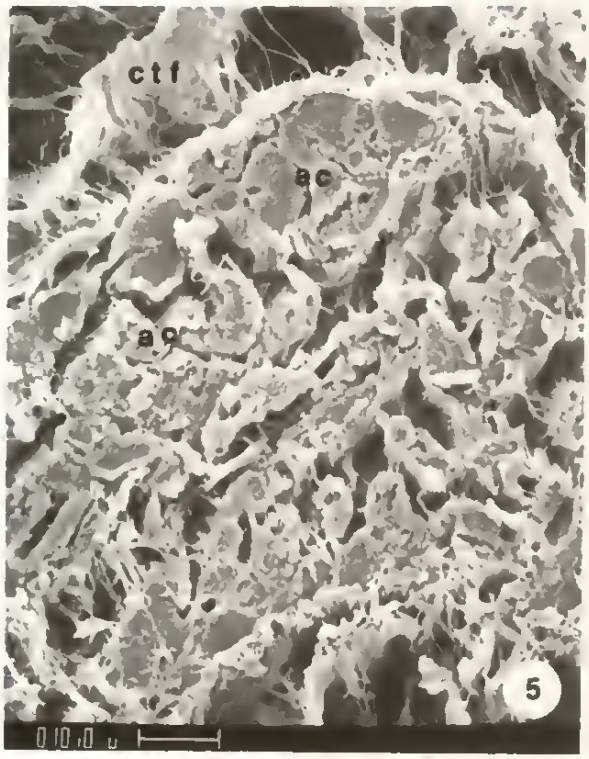
Fig. 4. Scanning Electron micrograph of circumanal gland from 20-day-old Black and White Poodle Cross dog. The gland is attached to a hair follicle (hf) of origin by the main duct (md). (10 KV; W.D. = 25 mm; X100).

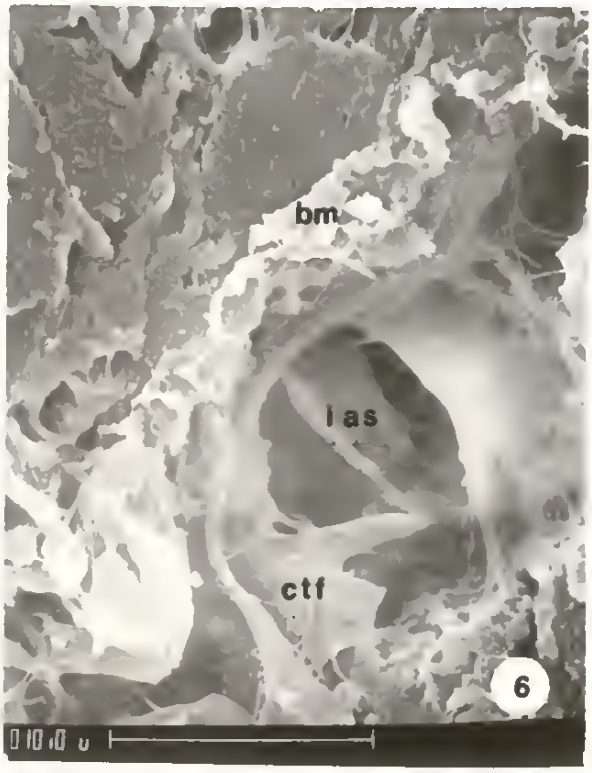
Fig. 5. Magnified group of acini (ac) from the area outlined in Fig. 4, showing connective tissue fiber support (ctf). (10 KV; W.D. = 15 mm; X1000).

Fig. 6. High magnification of an area outlined in Fig. 5. The acinar cells are shown resting on the basement membrane (bm); the inter-acinar space (ias) contains connective tissue fibers (ctf). (20 KV; W.D. = 10 mm; X3400).

Fig. 7. A few inner cells of the 20-day-old CG are magnified to show connective tissue fibers (ctf) in the inter-cellular space (ics). (20 KV; W.D. = 10 mm; X3400).


hf
mpf
4


ctf
ac
ac
5


bm
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ctf
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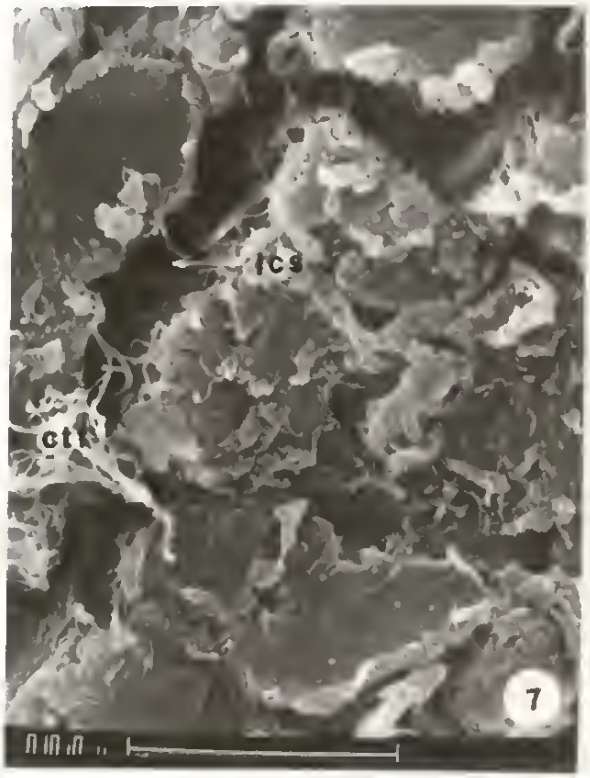

fcs
ctf
7

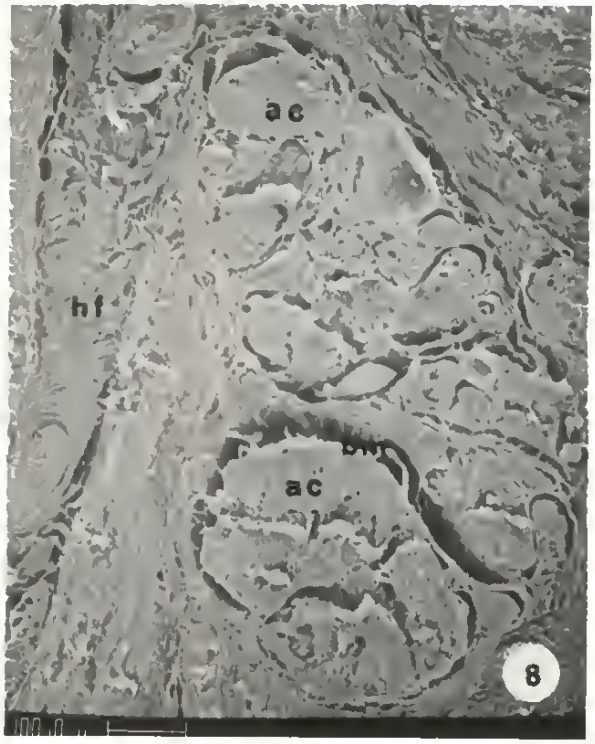
EXPLANATION OF FIGURES

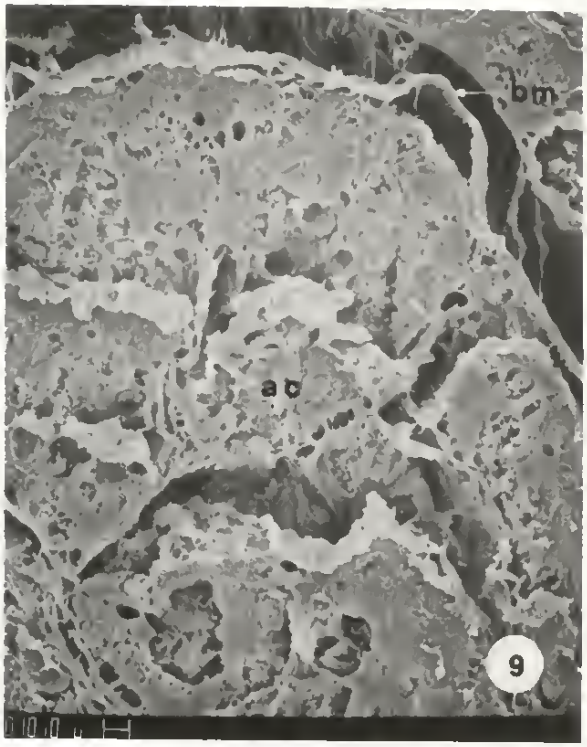
Fig. 8. Scanning Electron micrograph of very matured circumanal gland from 152-day-old German Shepherd Cross dog, containing many acini (ac), and very close to the hair follicle (hf) of origin. (10 KV; W.D. = 24 mm; X100).

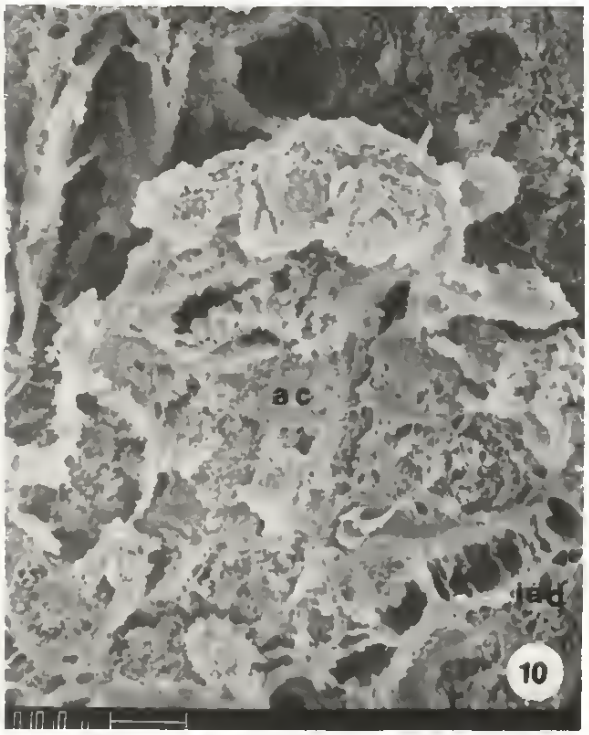
Fig. 9. Higher magnification of group of acini (ac) in the 152-day-old CG. Cysts are present towards the lower acini. (10 KV; W.D. = 24 mm; X300).

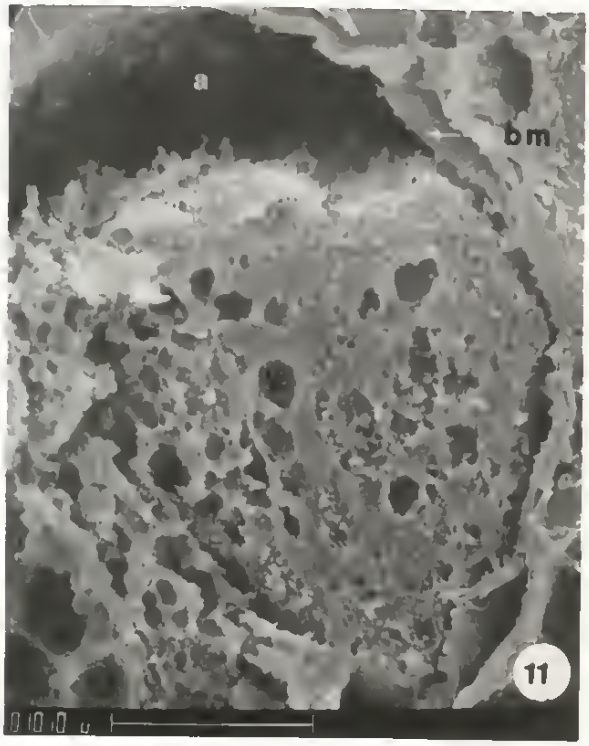
Fig. 10. A single acinus (ac) of the 152-day-old CG is magnified. The inter-acinar duct (iad) is shown close to the acinus. (10 KV; W.D. = 24 mm; X1000).

Fig. 11. A cell in the acinus of the 152-day-old CG is magnified. The cell rests on the basement membrane (bm), and contains many small vacuoles (v), which probably may represent early signs of cysts formation. The space between the cell and the basement membrane is an artifact (a). (20 KV; W.D. = 11 mm; X2600).


ac
hf
ac
8


bm
ac
9

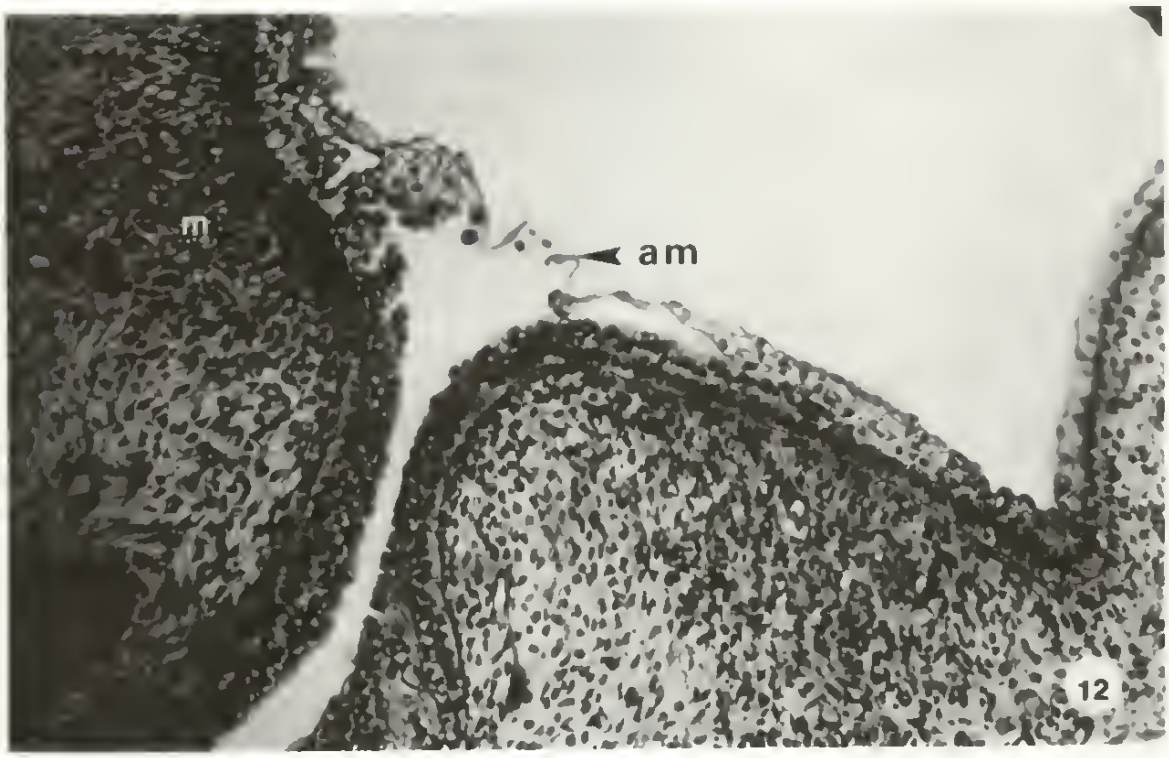

ac
10

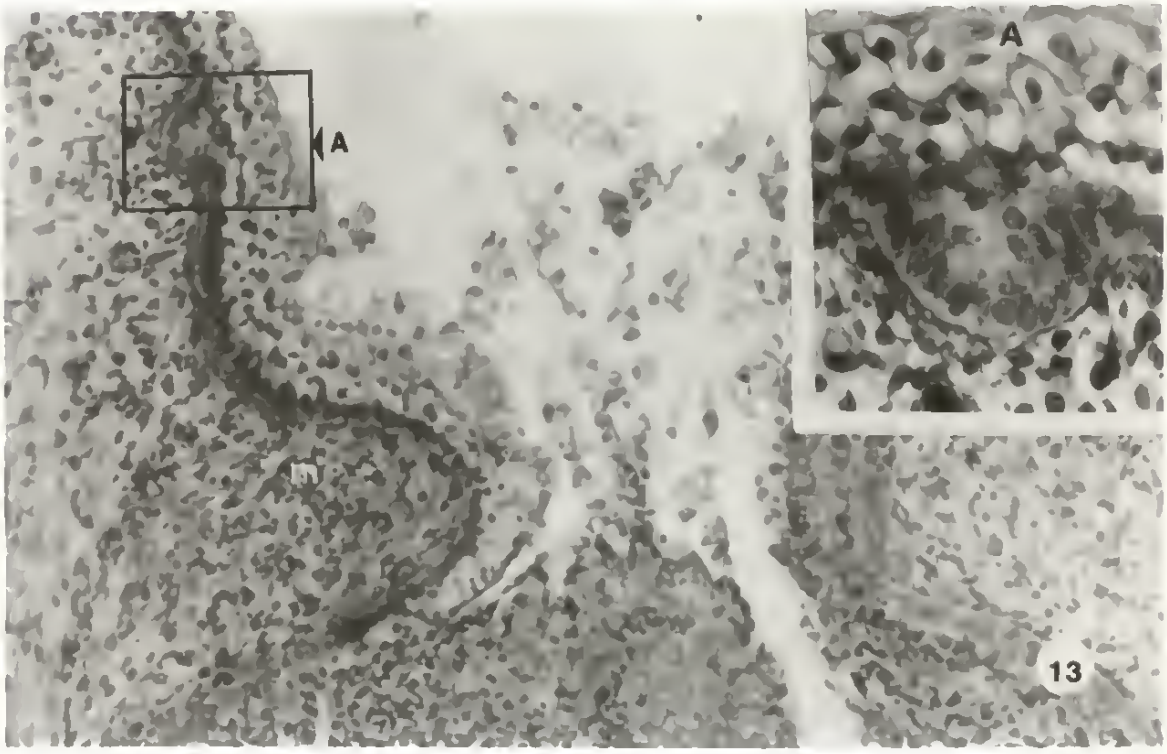

a
bm
11

EXPLANATION OF FIGURES

Fig. 12. Anal region of a 32-day-old Cocker Spaniel Cross fetus. There are no glands within the developing skin, but there are condensations of mesenchyme tissue (m) in the fiture circumanal glandular tone of the dermis. The anal membrane (am) still covers the anal orifice. (Mallory Triple Stain; X200).

Fig. 13. Anal region of a 38-day-old Beagle fetus. The anal membrane is desquamating; the hair follicle primordium (A) is represented by invagination of some stratum basale cells into the underlying mesenchyme tissue (m) which is more in the future circumanal glandular zone. (Mallory Triple Stain; X240).


m
am
12

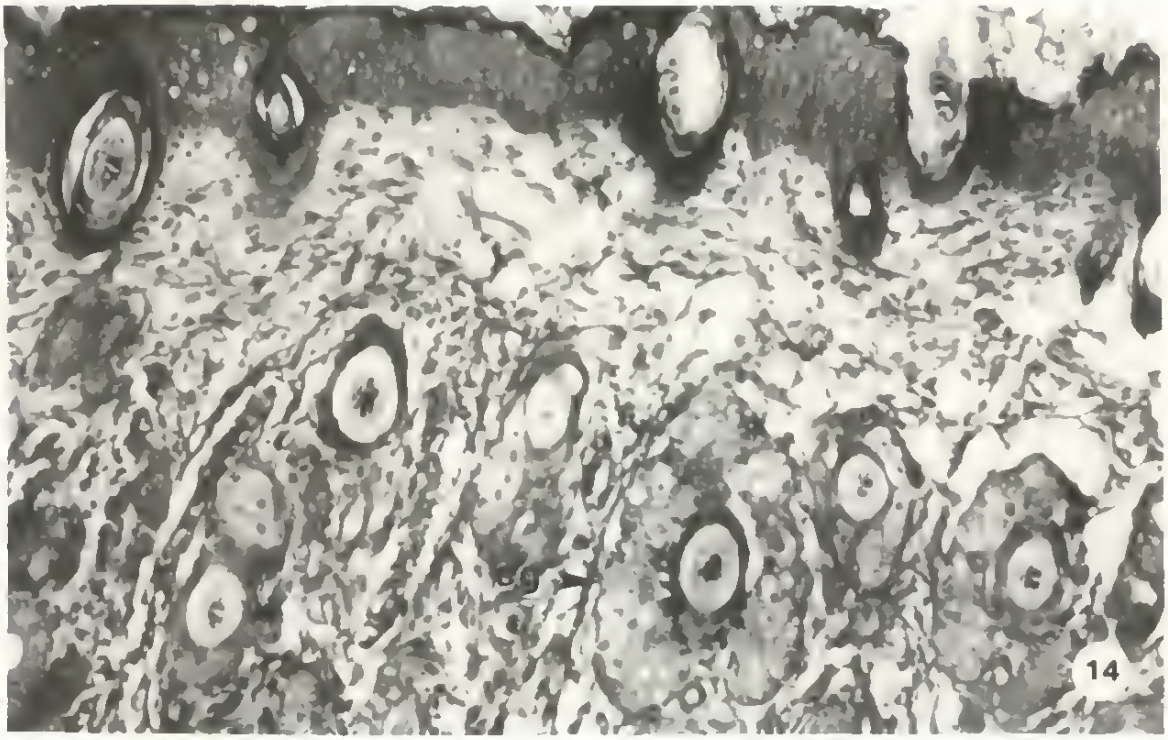

A
A
m
13

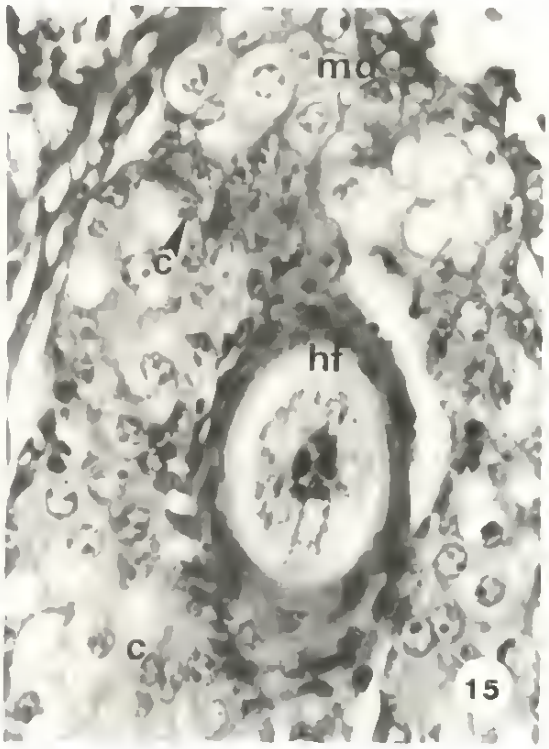
EXPLANATION OF FIGURES

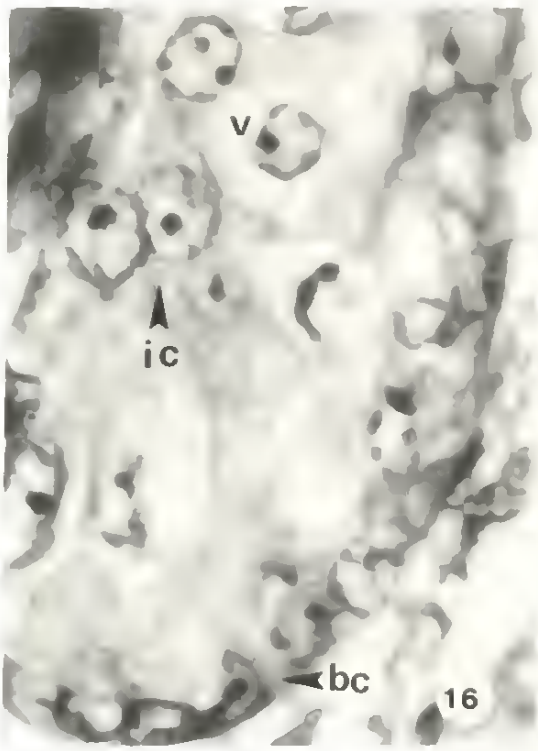
Fig. 14. Circumanal glandular zone of 1-day-old Terrier Cross dog. The circumanal glands (cg) are typically elongated. (Mallory Triple Stain; X200).

Fig. 15. Higher magnification of two CGs of 1-day-old Terrier Cross dog. The short main ducts (md) connect the superficial ends of the glands to the hair follicle (hf) of origin. Acinar organization is just beginning, as indicated by the presence of linear clumps of cells (c) across the gland. (Mallory Triple Stain; X480).

Fig. 16. Higher magnification of the deep portion of one of the CGs in Fig. 15. The gland is lined by low cuboidal basal cells (bc), and filled with inner cells (ic). Some of the inner cells contain vacuoles (v) while others are non-vacuolated and contain fine purplish granules. (Mallory Triple Stain; X1440).


14


mc
c
hf
c
15

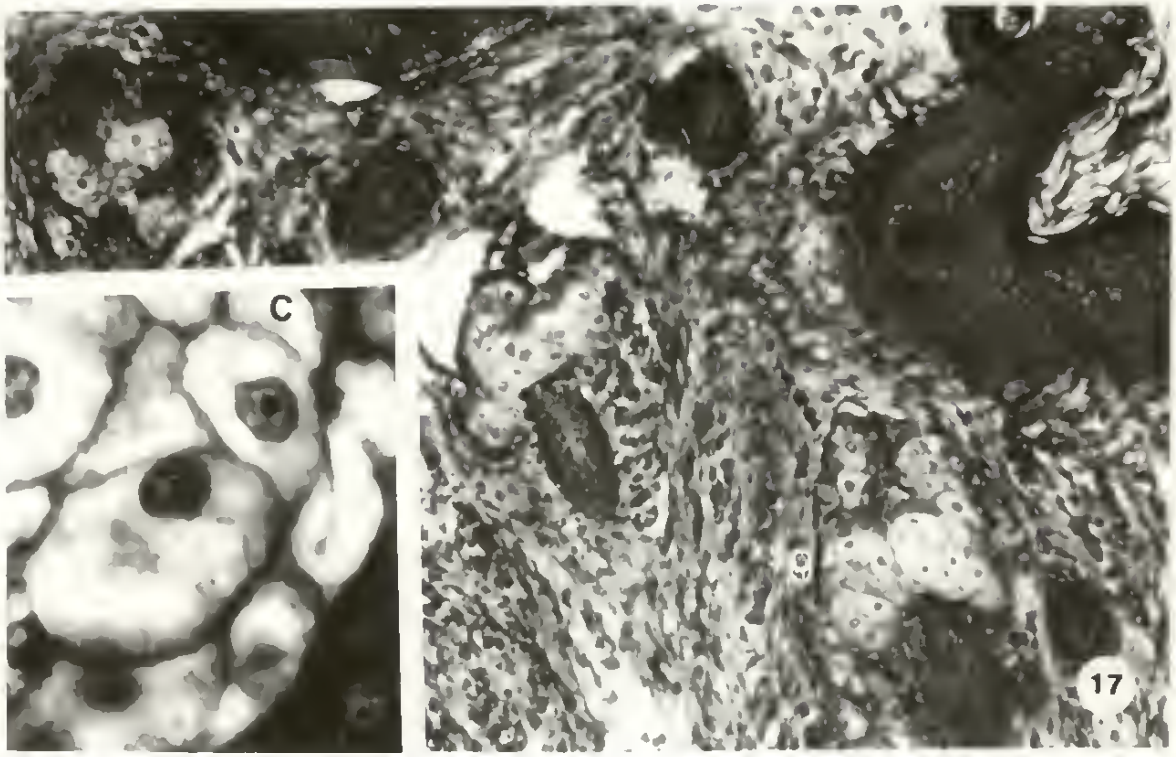

v
ic
bc
16

EXPLANATION OF FIGURES

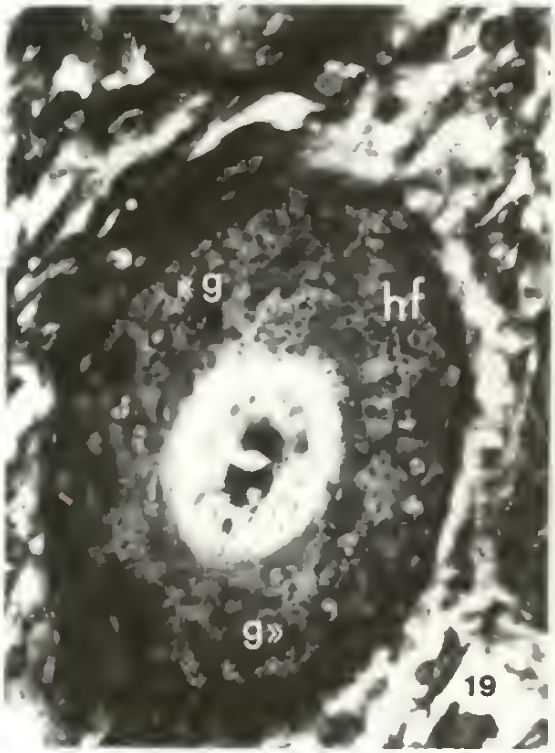
Fig. 17. Mucocutaneous sebaceous glandular zone of the 1-day-old Terrier Cross dog. The mucocutaneous sebaceous gland (sg) are flask-shaped, and are located very close to the anal orifice. C is a higher magnification of some sebaceous inner cells, showing their typical vacuolated nature. (PAS; X200).

Fig. 18. A circumanal gland from 1-day-old Terrier Cross dog. The entire gland which is connected by means of the main duct (md) to a hair follicle (hf) of origin and the hair follicle cells above the level of the main duct are PAS negative. (PAS; X640).

Fig. 19. An oblique section of a hair follicle (hf) beneath the level of origin of the CG main duct, in the 1-day-old Terrier Cross dog. The hair follicle cells are laden with PAS granules (g), especially the internal root sheath cells. (PAS; X1200).


c
17

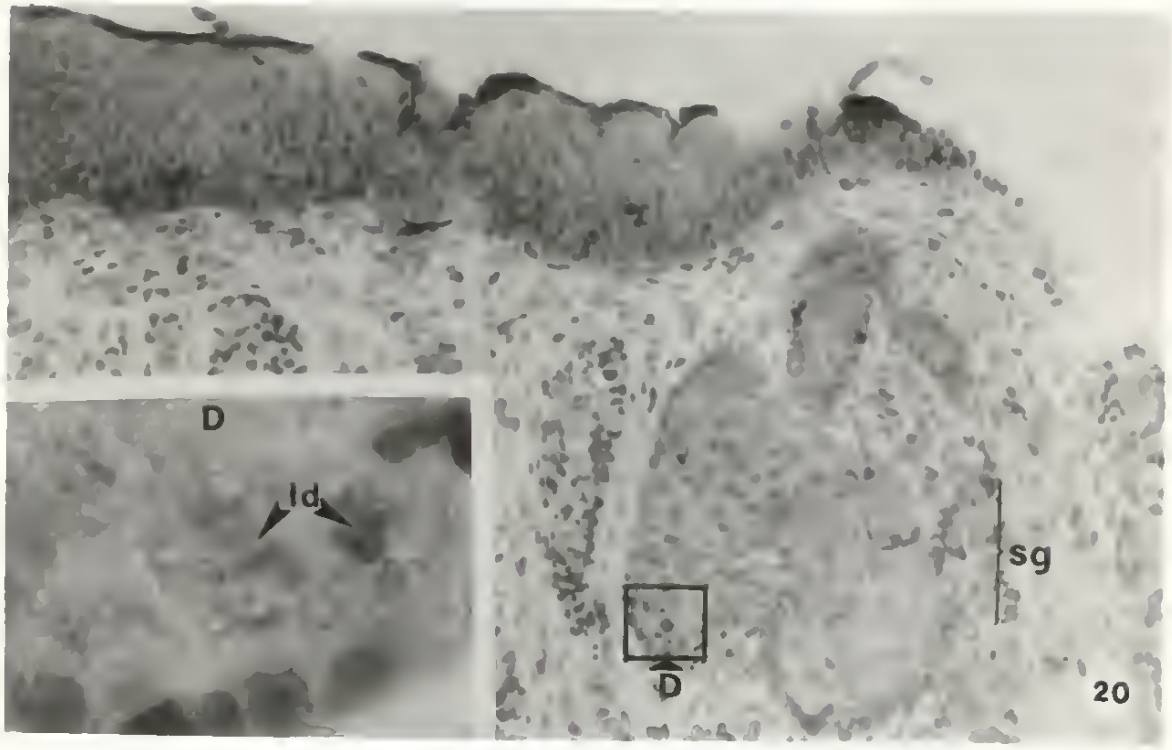

md
hf
18

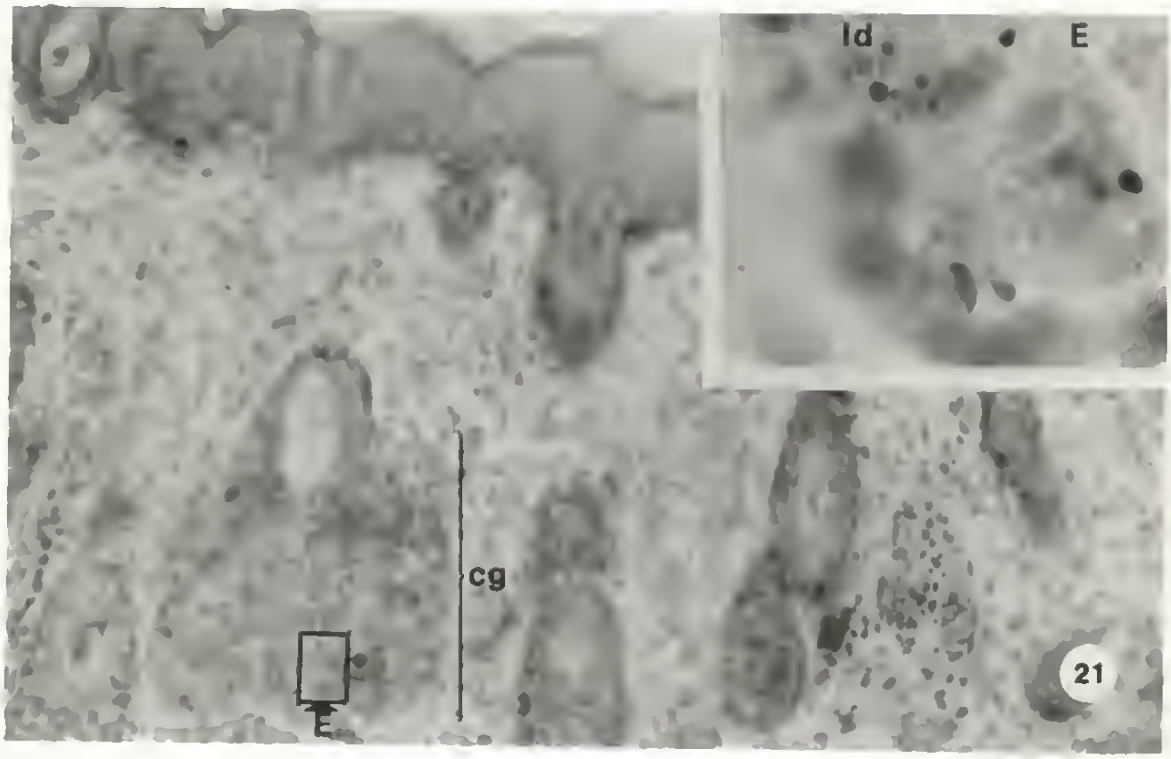

g
hf
g»
19

EXPLANATION OF FIGURES

Fig. 20. Oil Red O stained section of 1-day-old Terrier Cross dog, showing the mucocutaneous sebaceous gland (sg). D is a magnified portion of the gland, showing the presence of many lipid droplets (ld) within the sebaceous inner cells. Their nuclei are covered by the lipid droplets. (Oil Red O, X200).

Fig. 21. Oil Red O stained section of the 1-day-old Terrier Cross specimen, showing the circumanal glands (cg). E is a magnified portion of the gland, showing lipid droplets (ld) in some inner cells. The lipid droplets are fewer in number than those of the sebaceous gland shown in Fig. 20. (Oil Red O, X200).


D
ld
sg
D
20

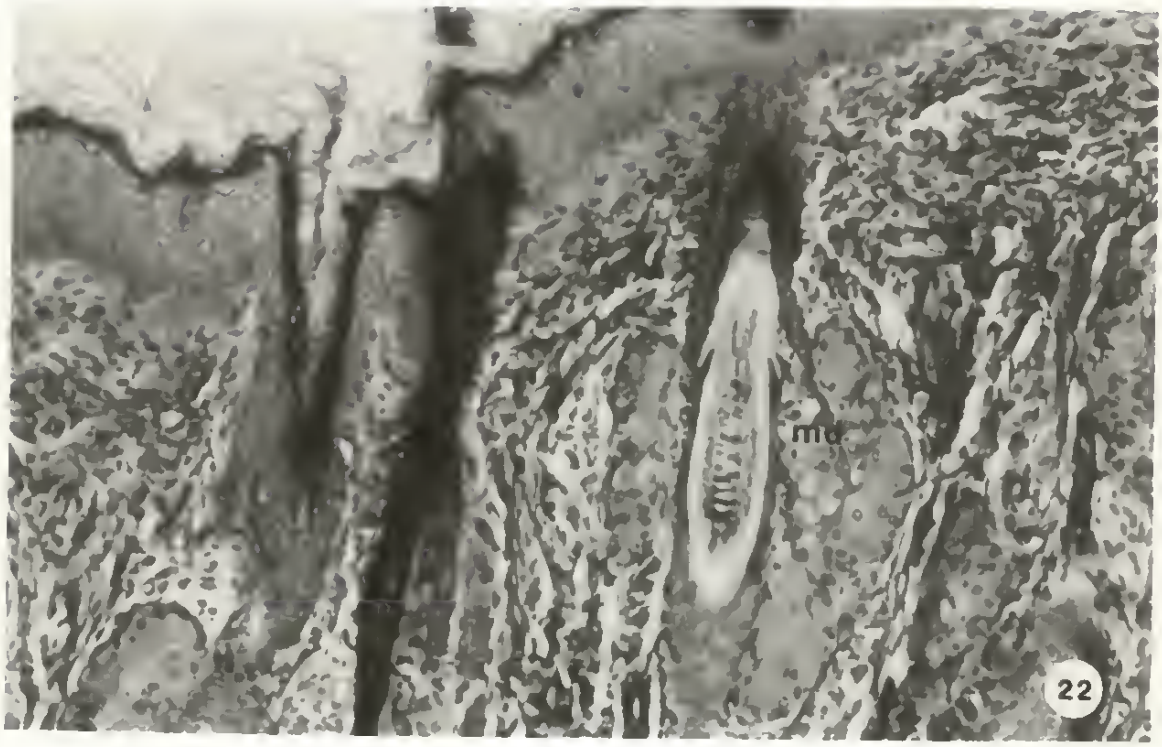

ld
E
cg
E
21

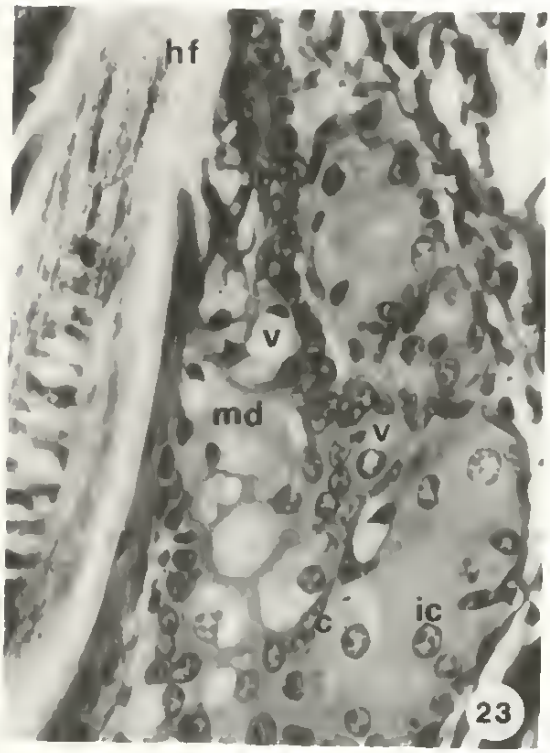
EXPLANATION OF FIGURES

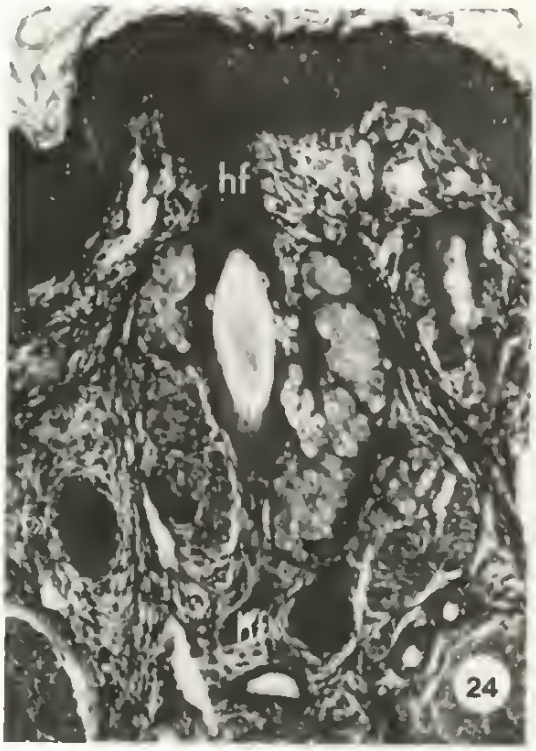
Fig. 22. A section through the circumanal glandular zone of a 3-day-old Mongrel. The main duct (md) of the circumanal gland is longer than that of 1-day-old dog, and connects the gland at an upper third position to the hair follicle. (Mallory Triple Stain; X160).

Fig. 23. Magnification of the CG in Fig. 22. The main duct (md) connects the gland to the hair follicle (hf) of origin, and it is filled with cells, some of which contain vacuoles (v). The limit of the main duct is represented by clumps of cells (C) which are also found within the gland, where they are demarcating the entire gland into several acini. Some inner cells of the gland contain vacuoles (v). (Mallory Triple Stain; X544).

Fig. 24. A section of the 3-day-old Mongrel specimen. The hair follicles (hf) below the level of the CGs, are filled with dark PAS granules. The CGs and the hair follicle cells above the level of the origin of the CG main ducts are PAS negative, apart from few internal root sheath cells of the hair follicle. (PAS; X160).


md
22


hf
v
md
v
c
ic
23


hf
24

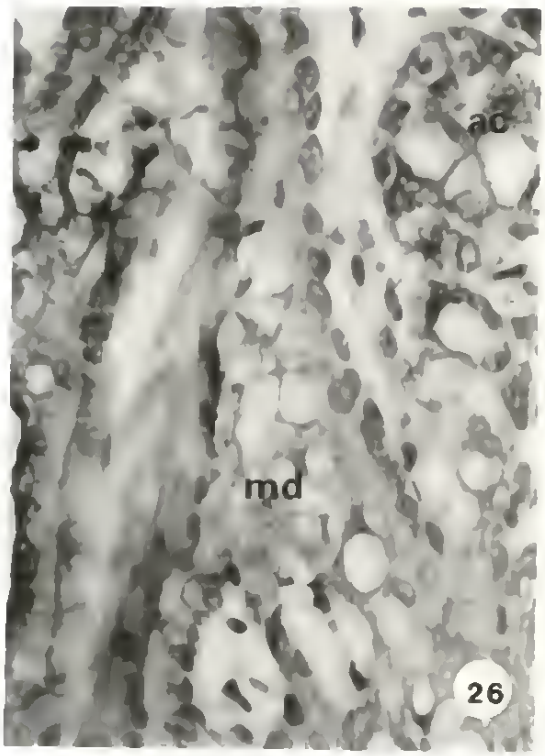
EXPLANATION OF FIGURES

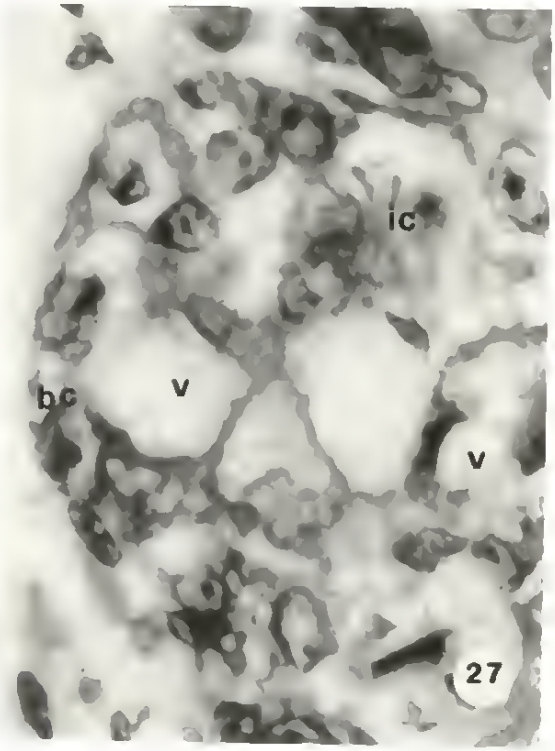
Fig. 25. A section of 11-day-old German Shepherd Cross dog, showing circumanal glands (cg). (Mallory Triple Stain; X133).

Fig. 26. Magnification of Fig. 25 CG, showing solid main duct (md), and better organization of the gland acinus (ac). (Mallory Triple Stain; X640).

Fig. 27. Magnified acinus of the 11-day-old circumanal gland. Low cuboidal or squamous basal cells (bc) line the acinus which is filled with polyhedral inner cells (ic), some of which contain vacuoles (v), while others contain fine purplish granules. (Mallory Triple Stain; X1467).


cg
25


ac
md
26


ic
bc
v
v
27

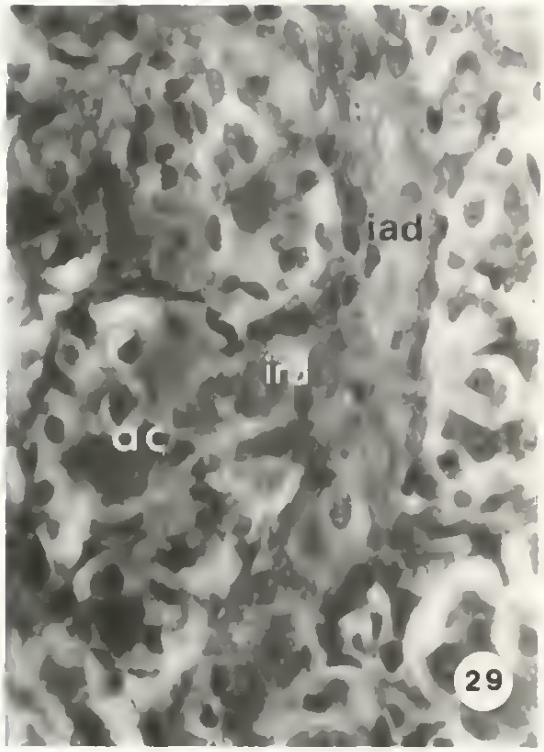
EXPLANATION OF FIGURES

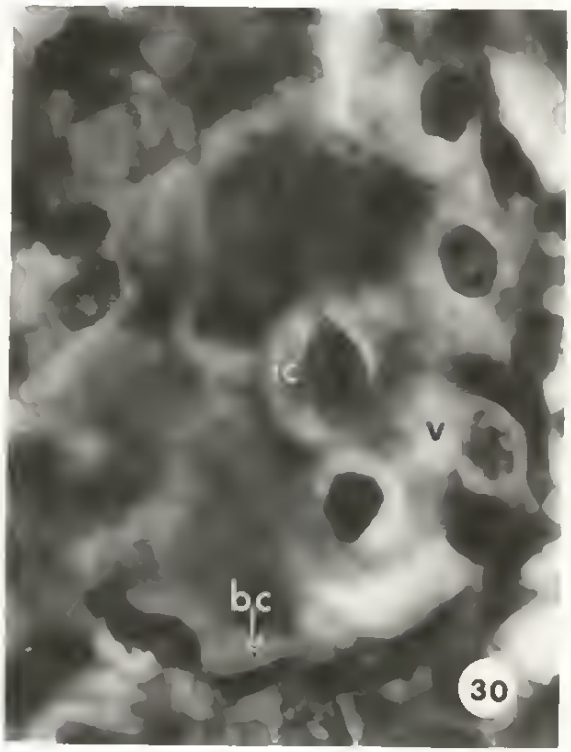
Fig. 28. A circumanal gland of a 14-day-old German Shepherd Cross dog, showing the inter-acinar duct (iad) and its extensions. (Mallory Triple Stain; X250).

Fig. 29. Higher magnification of a portion of Fig. 28 circumanal gland. The inter-acinar duct (iad) branches into shorter inter-acinar ducts (ird) which terminates in acini (ac). All the ducts and the acini are filled with cells. (Mallory Triple Stain; X480).

Fig. 30. A magnified acinus of the 14-day-old circumanal gland. The acinus is better defined than the 11-day-old, and the inner cells (ic) with vacuoles (v) are reduced. Most of the inner cells are filled with fine purplish granules. The basal cells (bc) which are low cuboidal or squamous line the acinus. (Mallory Triple Stain; X1600).


iad
28


iad
ac
29

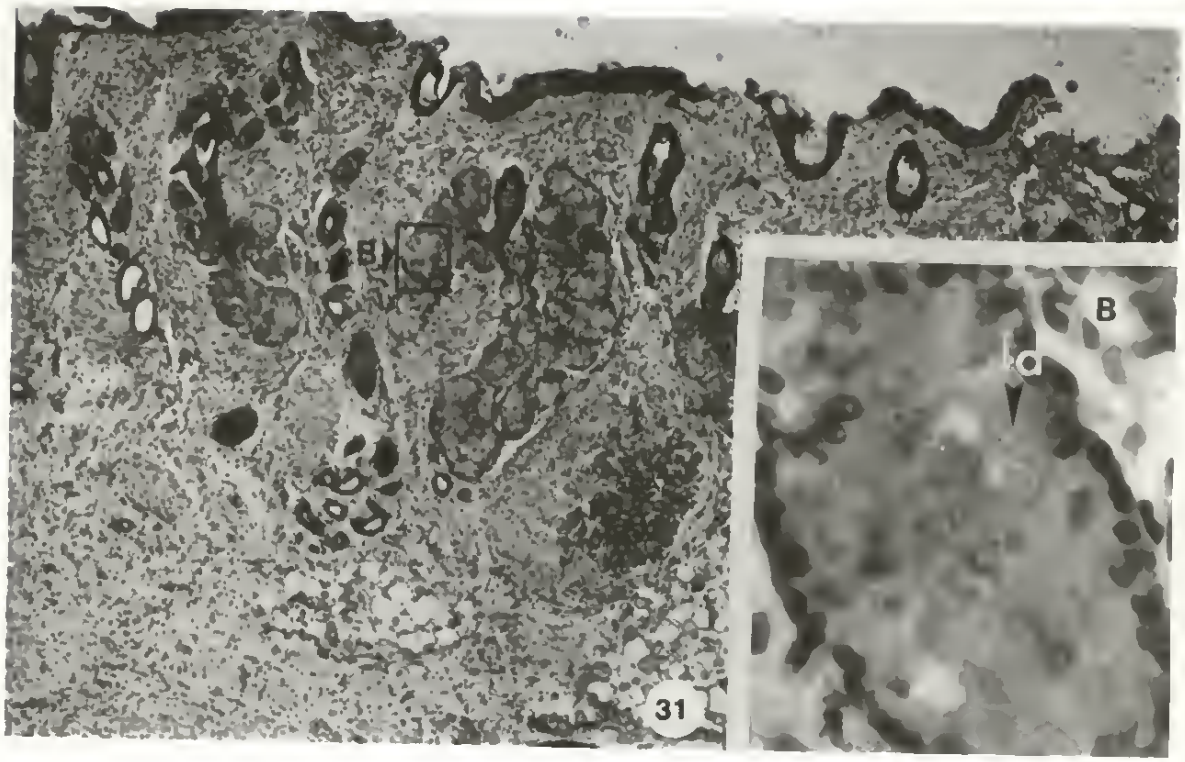

ic
v
bc
30

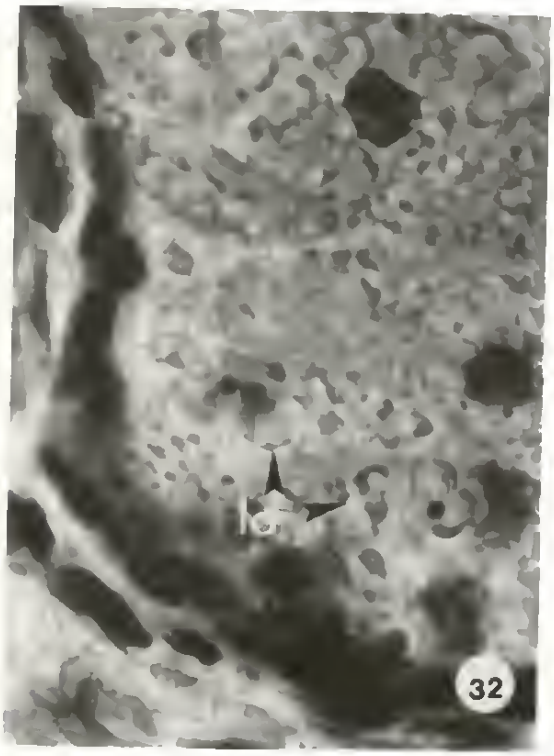
EXPLANATION OF FIGURES

Fig. 31. A section through the circumanal gland of a 14-day-old German Shepherd Cross dog, stained for lipids. B is a magnified acinus, close to the main duct of the gland, and showing lipid droplets (ld) within some inner cells. (Oil Red O Stain, X53).

Fig. 32. A portion of a mucocutaneous sebaceous gland from the 14-day-old dog, showing inner cells which are laden with lipid droplets (ld). The cellular outlines are completely distorted, and their nuclei are pyknotic, compared with the CG inner cells in Fig. 31 which have fewer lipid droplets in some inner cells only. (Oil Red O Stain; X1200).

Fig. 33. A section through the CG zone of the 14-day-old dog, showing hair follicle cells (hf) which are filled with PAS granules below the level of origin of the CG main duct (md). Few PAS granules are present mainly in the internal root sheath cells of the hair follicle, above the level of the origin of the main duct (md). The CGs are PAS negative. (PAS; X140).


B
C
31
B
C


C
32

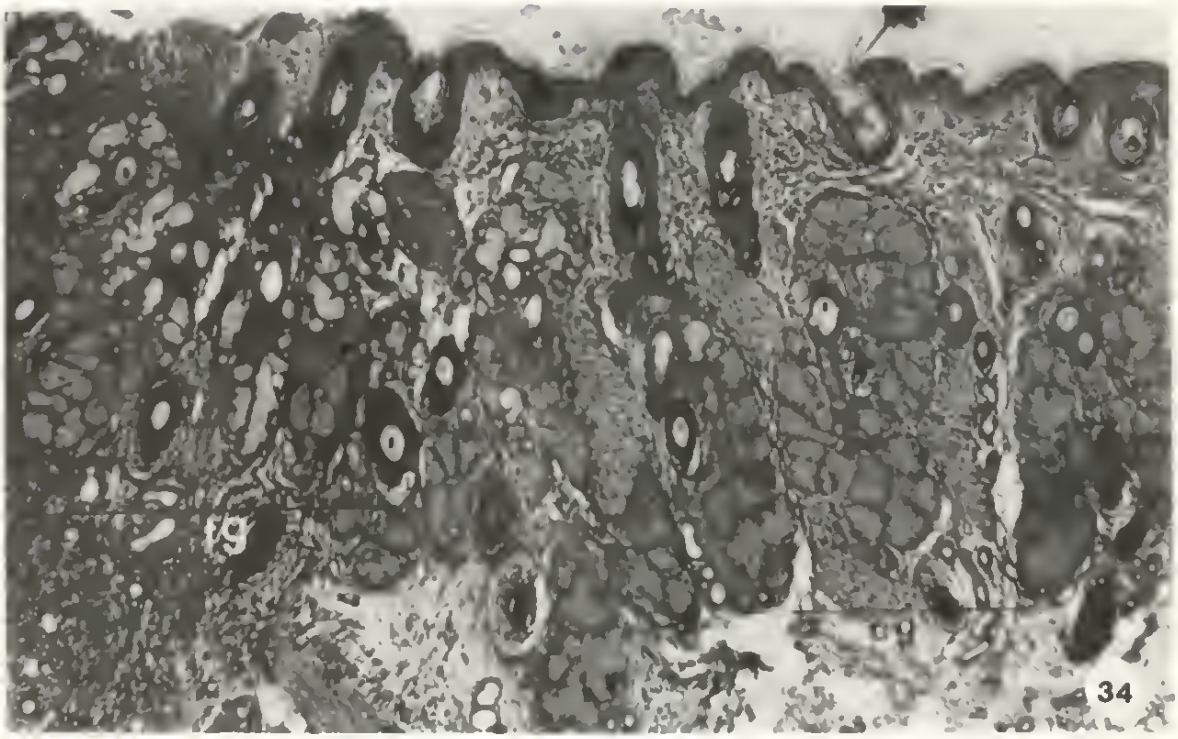

33

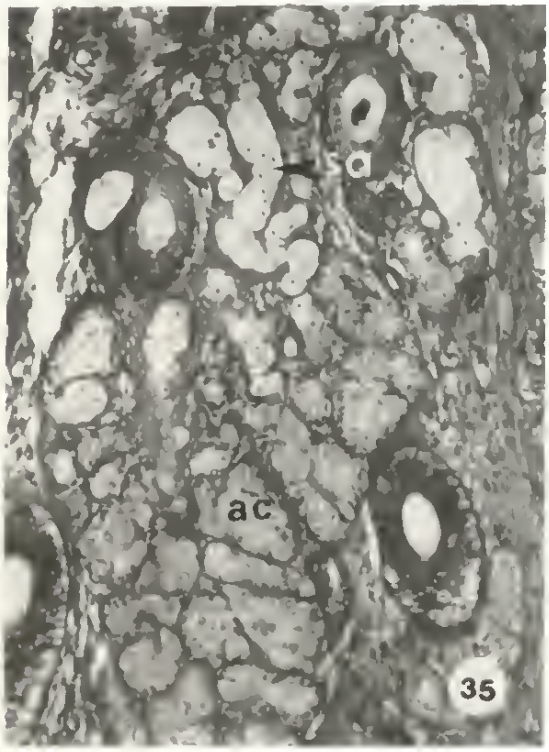
EXPLANATION OF FIGURES

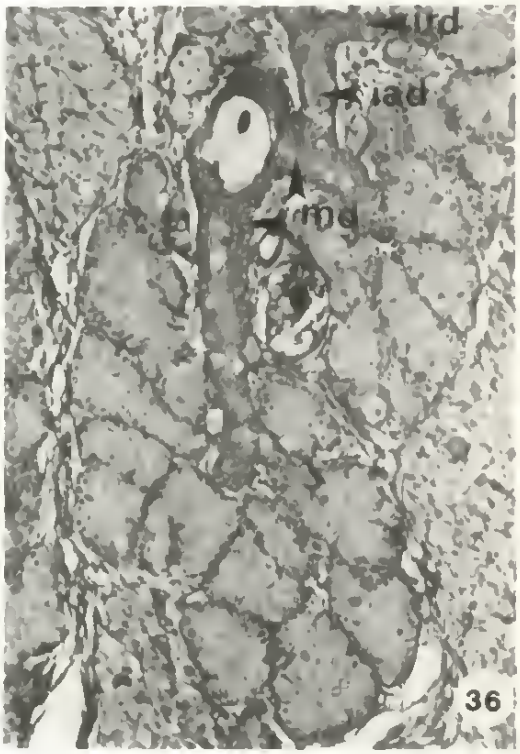
Fig. 34. A section through the circumanal glandular zone of a 20-day-old Black and White Poodle Cross dog. The transitional glands (tg) are located in between the CG zone and the muco-cutaneous sebaceous glands (not sbown). The circumanal glands (cg) are smaller at both medial and lateral periphery of the zone. (Mallory Triple Stain; X40).

Fig. 35. Higher magnification of some of the transitional glands in Fig. 34, showing the superficial sebaceous acini (sa) and the deep circumanal acini (ac). (Mallory Triple Stain; X120).

Fig. 36. Two circumanal glands of the 20-day-old dog. One of the main ducts (md) is longer than the other. The short main ducts (md) of the lateral gland branch into the inter-acinar duct (iad) which in turn branches into smaller intra-acinar duct (ird). (Mallory Triple Stain; X100).


fg
34


a
ac
35


ud
iad
md
36

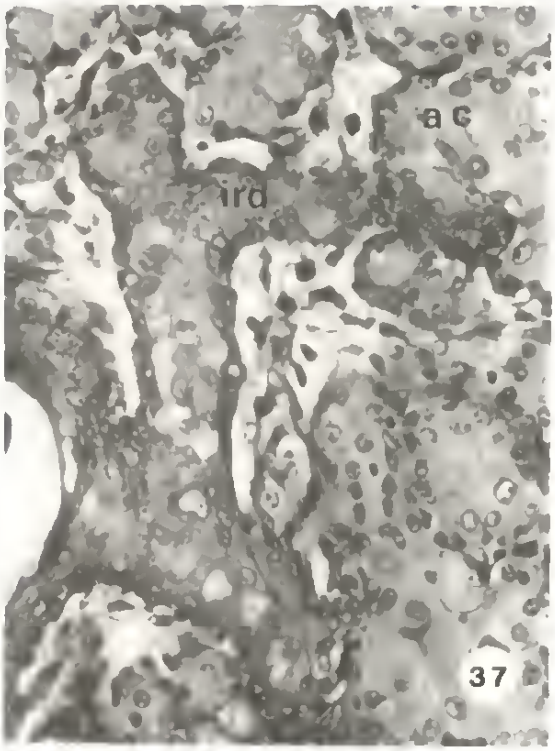
EXPLANATION OF FIGURES

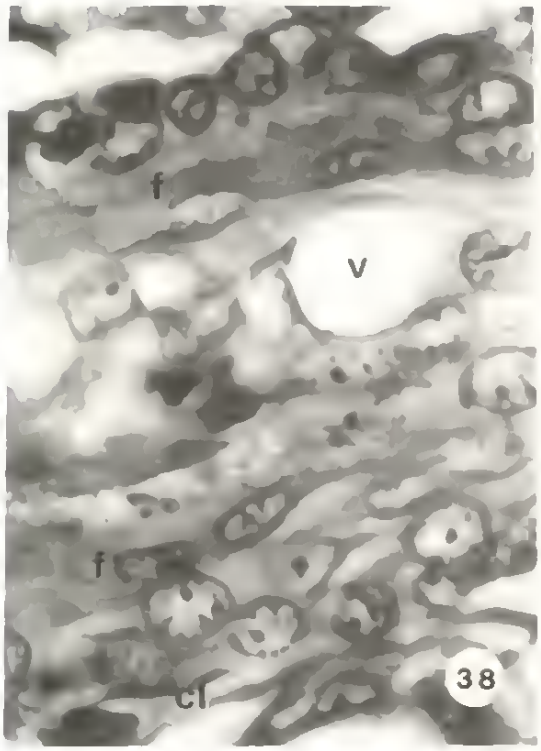
Fig. 37. A higher magnification of the lateral gland shown in Fig. 36, showing the intra-acinar duct (ird) which terminates in an acinus (ac). (Mallory Triple Stain; X300).

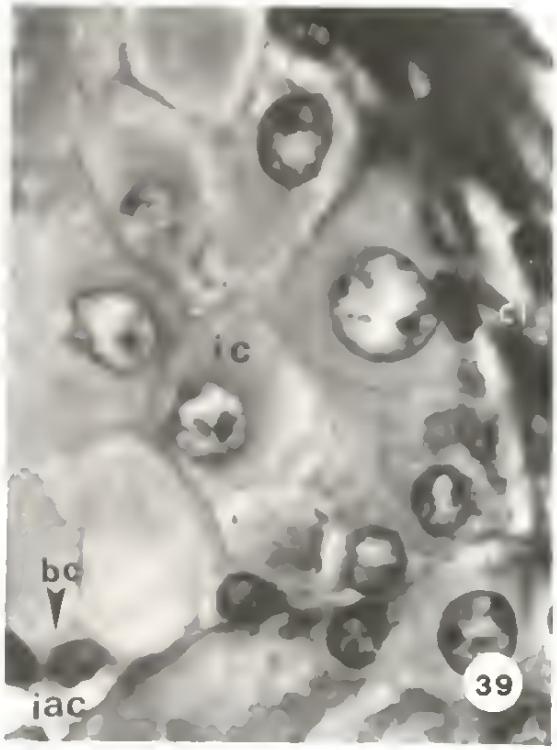
Fig. 38. A longitudinal section through the inter-acinar duct of the 20-day-old CG, showing the typical solid nature of the ducts. The duct is filled with fibrocytes (f), collagen fibers (cl), and cells with large vacuoles (v). (Mallory Triple Stain; X880).

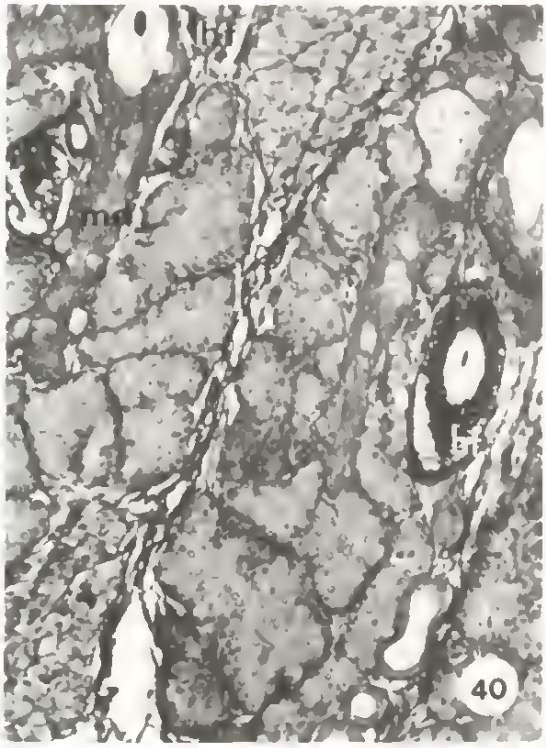
Fig. 39. A magnified portion of two acini of the 20-day-old CG. Each acinus is lined by low cuboidal or squamous basal cells (bc), and contains only inner cells (ic) which are filled with purplish fine granules. Collagen fibers (cl) are abundant in the inter-acinar spaces. (Mallory Triple Stain; X1500).

Fig. 40. A section of the 20-day-old specimen stained with PAS stain. The CGs are PAS negative; the hair follicle cells below the level of origin of the CG main ducts contain dark PAS granules. (PAS; X120).


ac
ird
37


f
v
f
cl
38

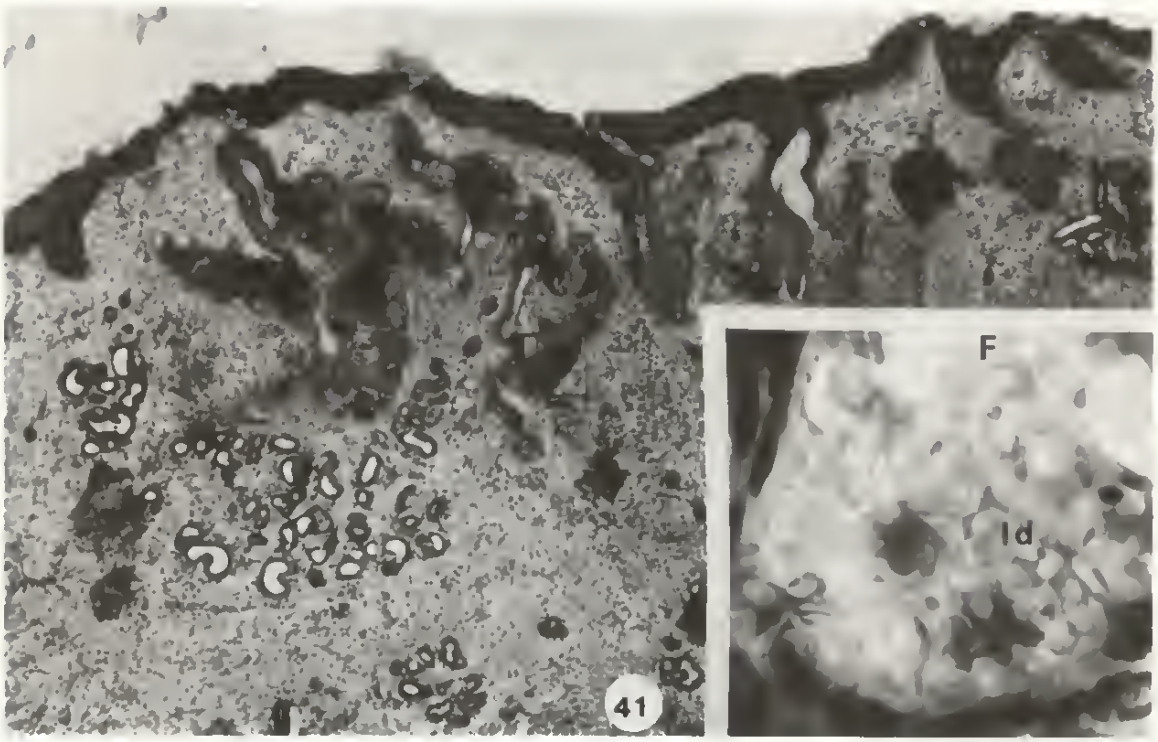

ic
cl
bc
iac
39

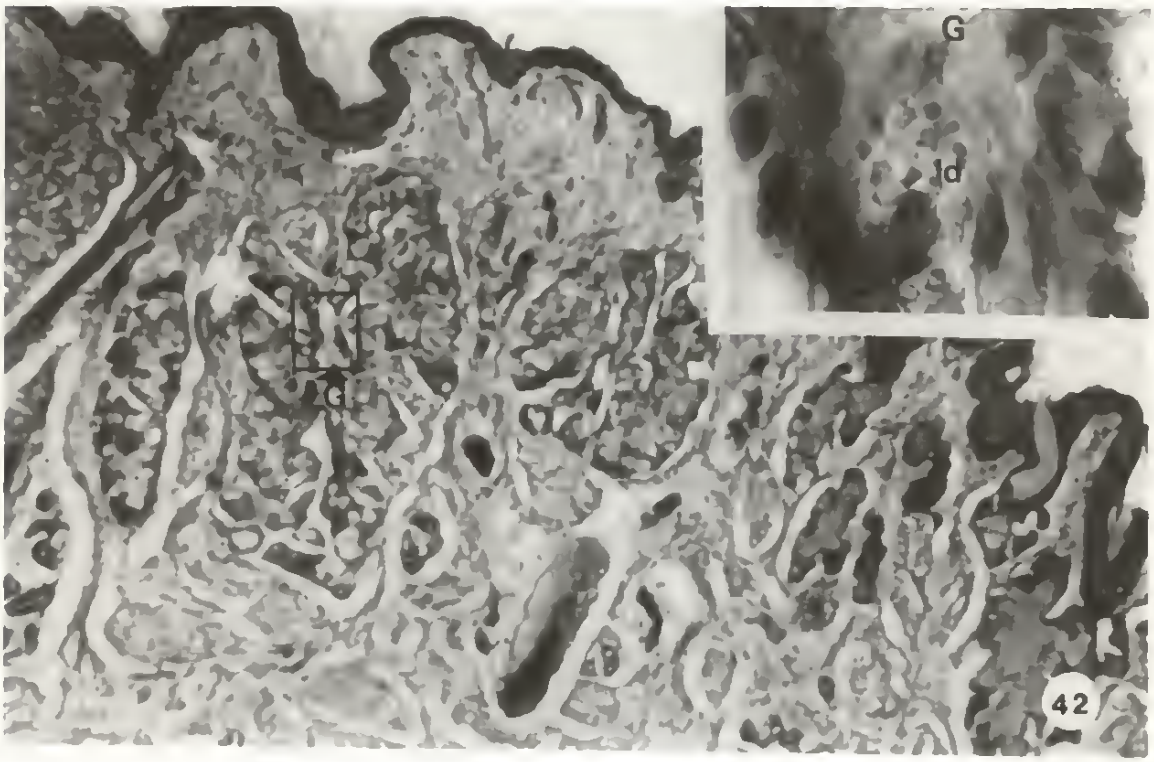

bf
mc
bf
40

EXPLANATION OF FIGURES

Fig. 41. A section through the 20-day-old mucocutaneous sebaceous glandular zone, stained for lipid. The darkness of the gland is due to the filling of their inner cells with lipid droplets. F is a portion of one of the glands, magnified to show the inner cells which are heavily loaded with lipid droplets (ld). (Oil Red O; X60).

Fig. 42. A section through the 20-day-old CG zone, similarly stained for lipids. The CGs were negative for lipids except for few droplets within their ducts. G is a magnified portion of the inter-acinar duct, showing few lipid droplets (ld) within some inner cells. (Oil Red O Stain; X45).


F
ld
41


G
ld
42

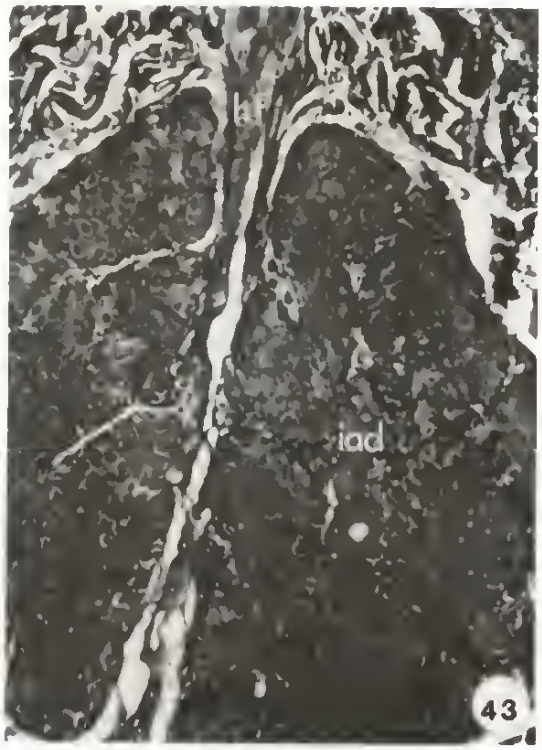
EXPLANATION OF FIGURES

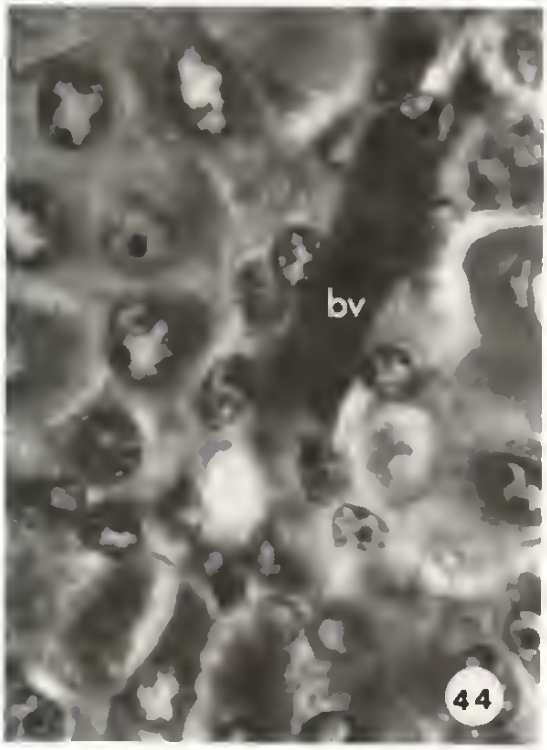
Fig. 43. A longitudinal section of 60-day-old German Shepherd Cross dog. The typical elongated shape of the CG, with wider deep end and narrow superficial portion, is shown. The CGs are very close to their hair follicle of origin (hf). Most of their main ducts have been incorporated into the gland; some portions of the inter-acinar duct (iad) are shown. (Mallory Triple Stain; X120).

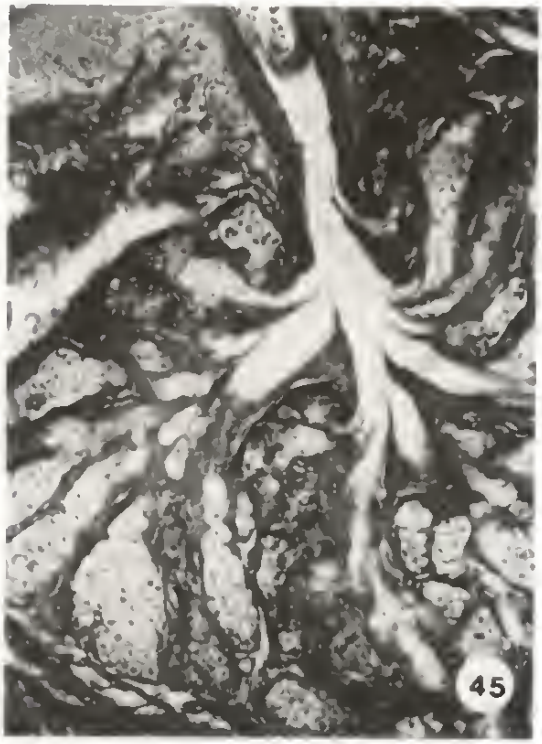
Fig. 44. Few magnified acini of the 60-day-old CG. Each acinus is lined by low cuboidal cells, and filled with hepatoid polyhedral inner cells. A blood vessel (bv) is present in the inter-acinar spaces. (Mallory Triple Stain; X1600).

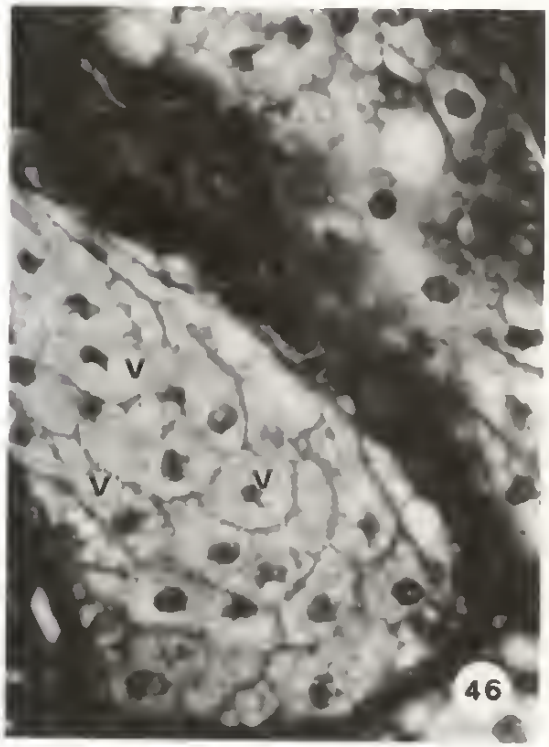
Fig. 45. A longitudinal section through a mucocutaneous sebaceous gland of the 60-day-old dog, showing the typical branched nature of the gland. (Mallory Triple Stain; X120).

Fig. 46. A magnified sebaceous acinus of the gland shown in Fig. 45, showing the inner cells with many vacuoles (v). (Mallory Triple Stain; X480).


iad
43


bv
44

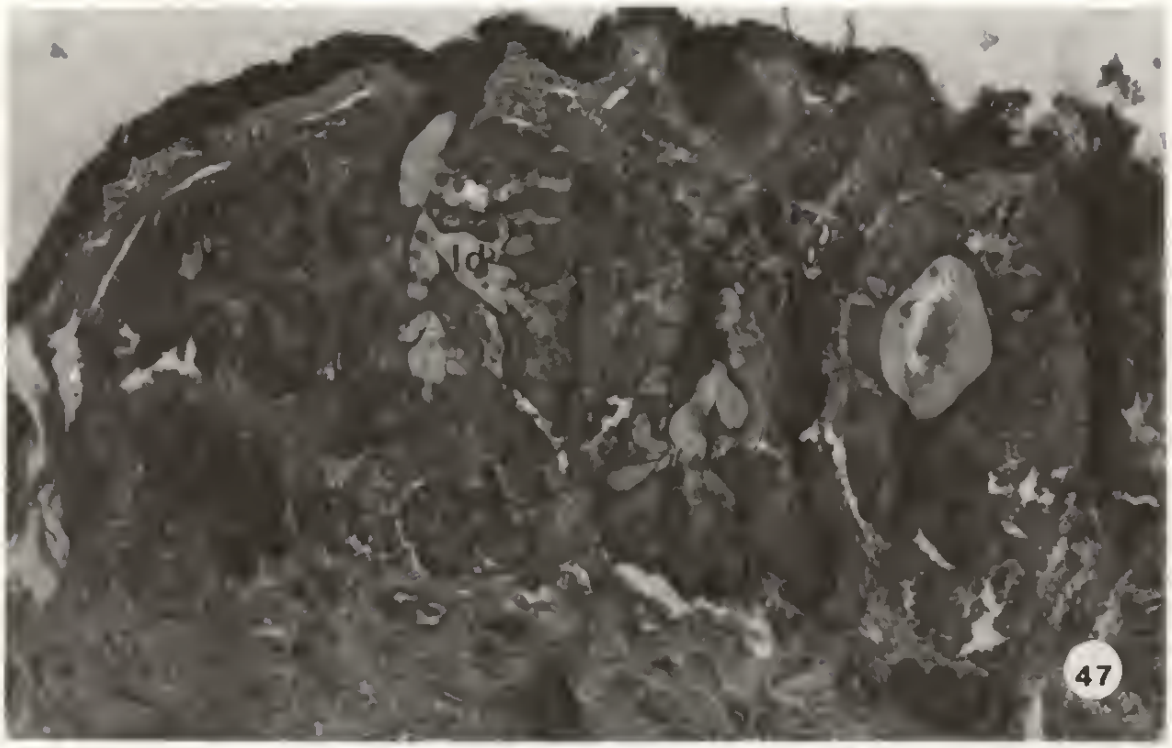

45

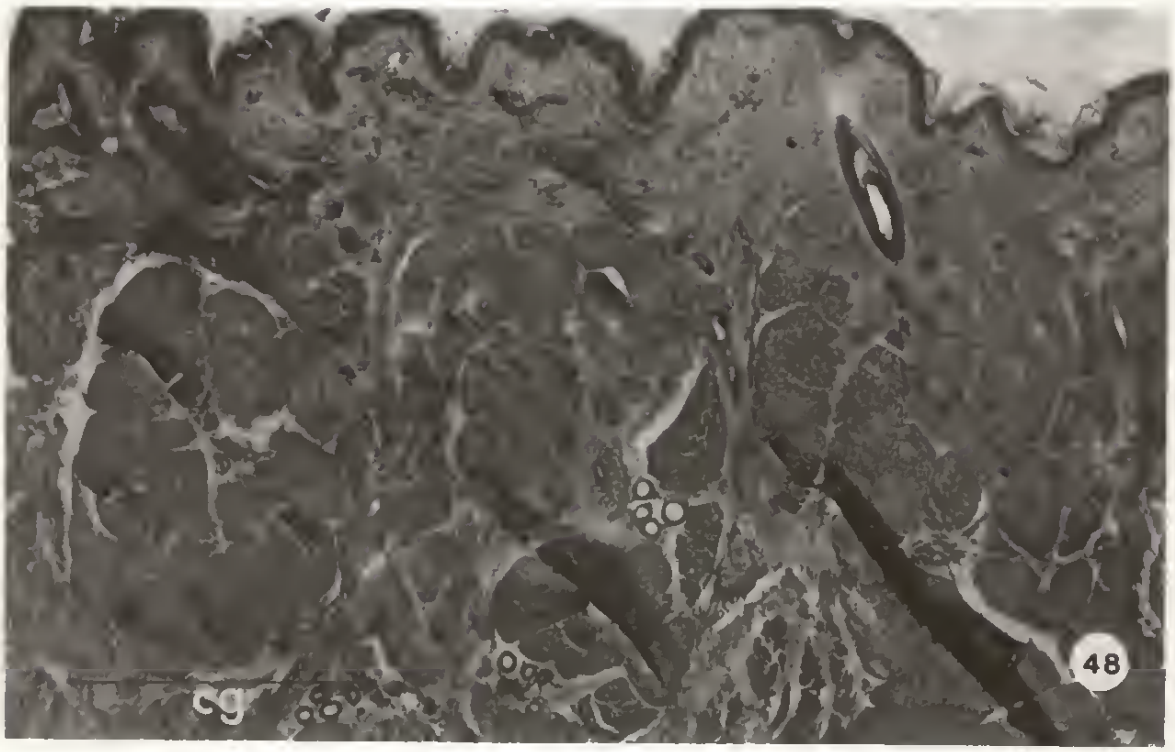

v
v
v
46

EXPLANATION OF FIGURES

Fig. 47. A section through the 60-day-old mucocutaneous glandular zone, stained for lipids. The sebaceous inner cells as well as their ducts are filled with lipid droplets (ld). (Oil Red O Stain; X100).

Fig. 48. A section through the 60-day-old circumanal glandular zone, similarly stained for lipids. The circumanal glands (cg) are completely negative for lipids, as shown by comparison of Fig. 47 and 48. (Oil Red O Stain; X40).


ld
47


cg
48

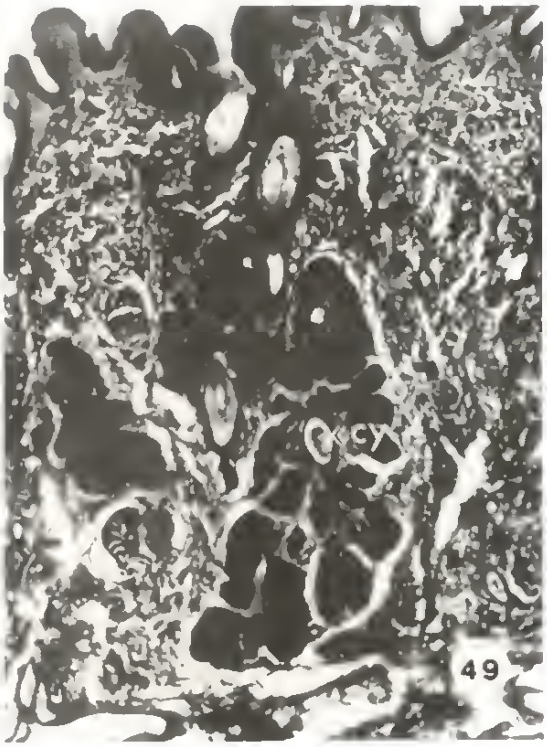
EXPLANATION OF FIGURES

Fig. 49. 152-day-old German Shepherd Cross CG, showing their typical elongated nature and presence of cyst (cy) within their interacinar duct. (Mallory Triple Stain; X30).

Fig. 50. A magnified portion of the CG in Fig. 49, showing the cyst (cy) within the interacinar duct (iad). The wall of the cyst consist of concentric layers of spindle cells, and the lumen contains solid reddish substance. (Mallory Triple Stain; X200).

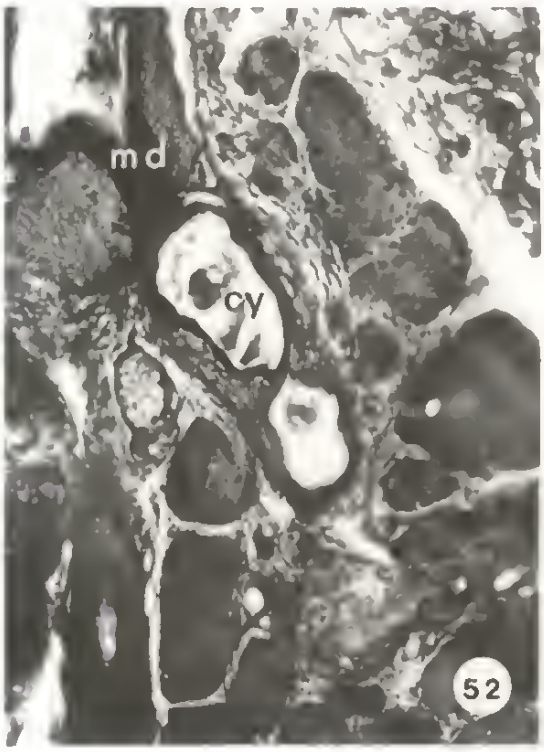
Fig. 51. Another section through the same CG shown in Fig. 49, showing two more cysts (cy) in the main duct. (Mallory Triple Stain; X30).

Fig. 52. Magnified portion of the CG in Fig. 51, showing the cysts (cy), within the main duct (md). The concentric spindle-shaped cells of the cysts wall is well shown. (Mallory Triple Stain; X107).


cv
49


cv
iad
50


cv
51

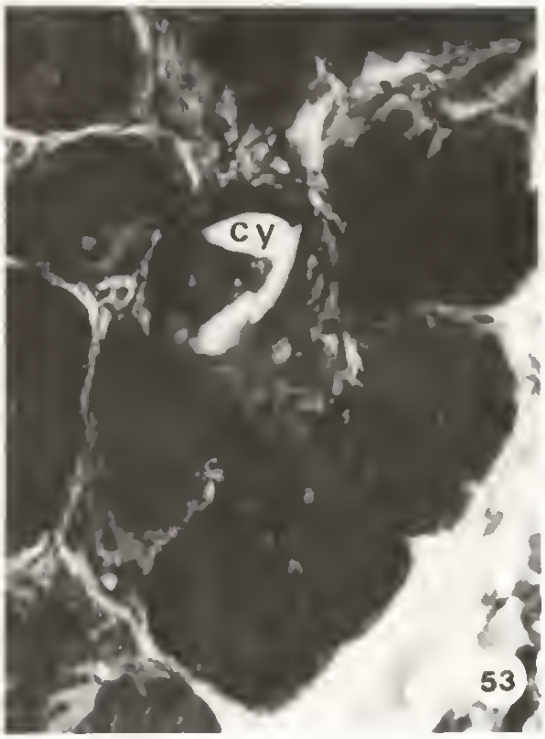

md
cv
52

EXPLANATION OF FIGURES

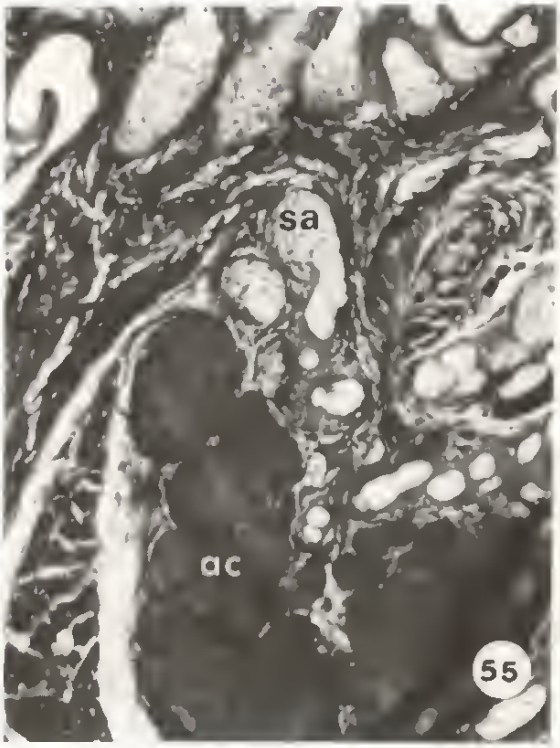
Fig. 53. A longitudinal section through the deep portion of a 153-day-old CG, showing a cyst (cy), probably within the inter-acinar duct. (Mallory Triple Stain; X300).

Fig. 54. A magnified portion of an acinus of the 152-day-old CG, showing the squamous basal cell (bc) layer, and the polyhedral inner cells (ic). The granules in the inner cells formed ring-like aggregates around the nucleus. Some of the inner cells (arrow) have pyknotic nucleus and faint cytoplasmic granules. (Mallory Triple Stain; X1600).

Fig. 55. A longitudinal section through a transitional gland of the 152-day-old dog, showing the superficial sebaceous acini (sa) and the deep circumanal acini. (Mallory Triple Stain; X120).


cy
53

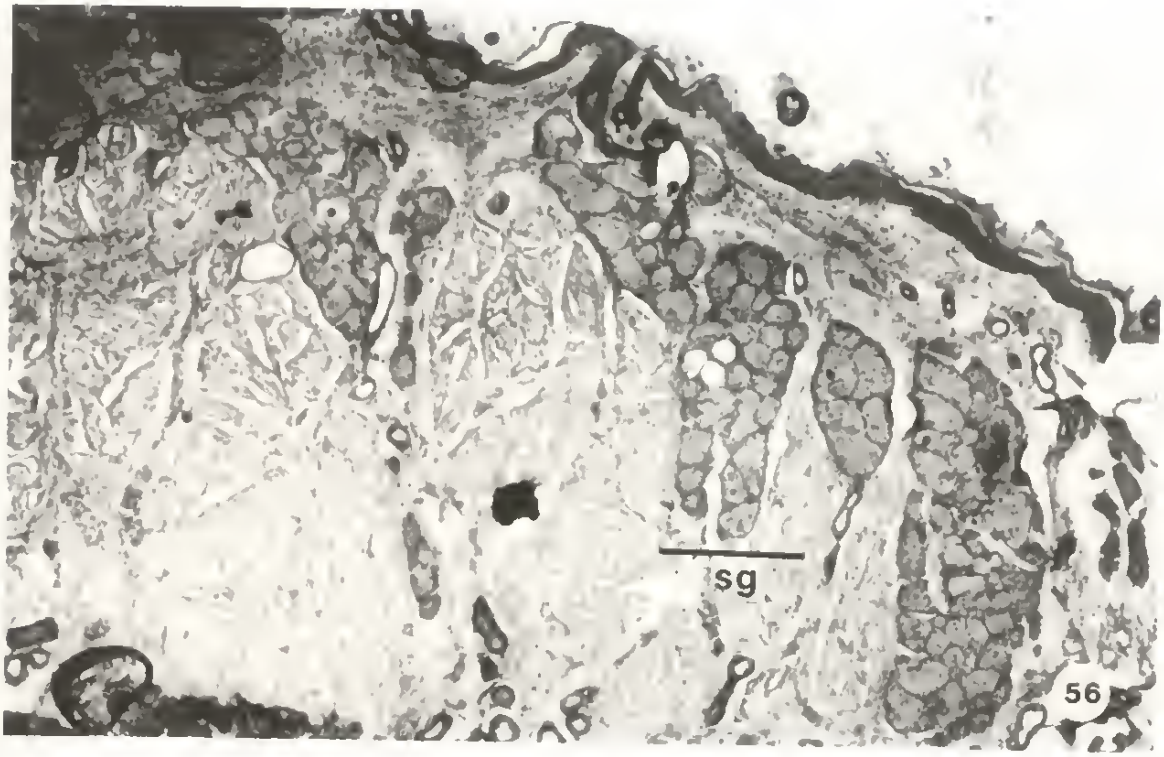

ic
bc
54

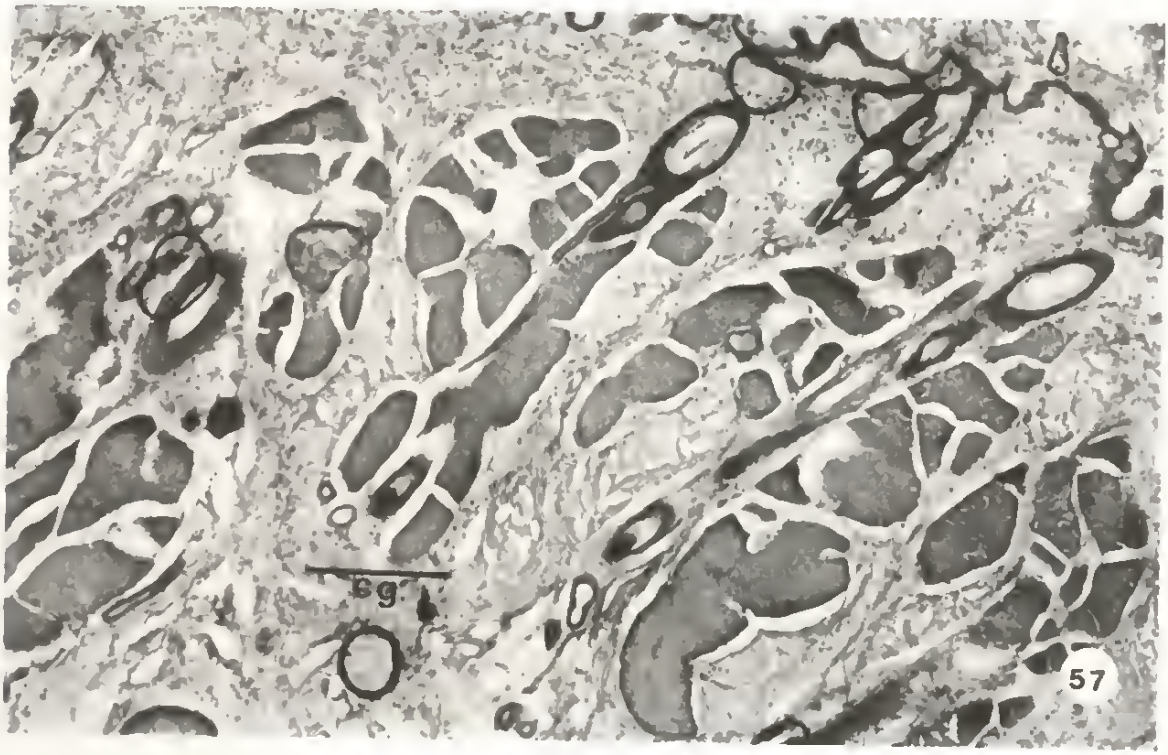

sa
ac
55

EXPLANATION OF FIGURES

Fig. 56. A section of 152-day-old mucocutaneous sebaceous glandular zone, stained for lipids. The sebaceous glands (sg) are filled with lipid droplets, as those of the 60-day-old. (Oil Red O Stain; X40).

Fig. 57. A section through the circumanal glandular zone of the 152-day-old dog, similarly stained for lipids. The circumanal glands (cg) are completely negative for lipids, as shown by comparing Figs. 56 and 57. (Oil Red O Stain; X40).

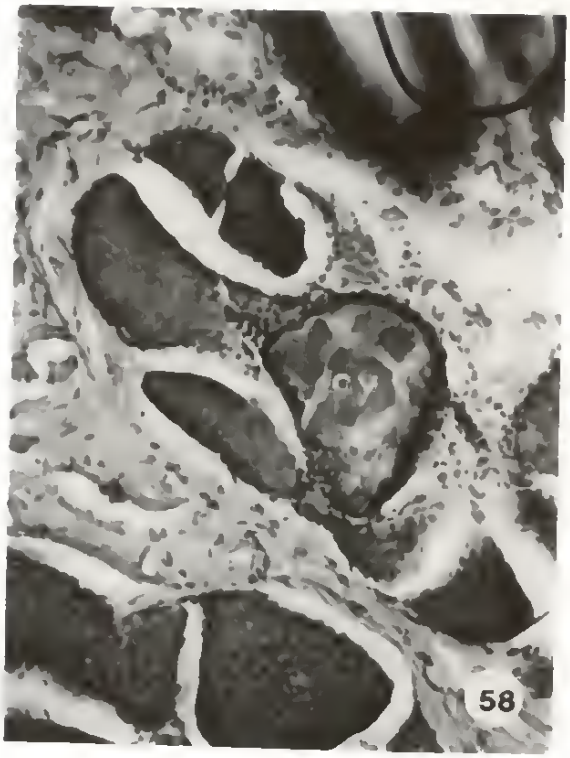

sg
56

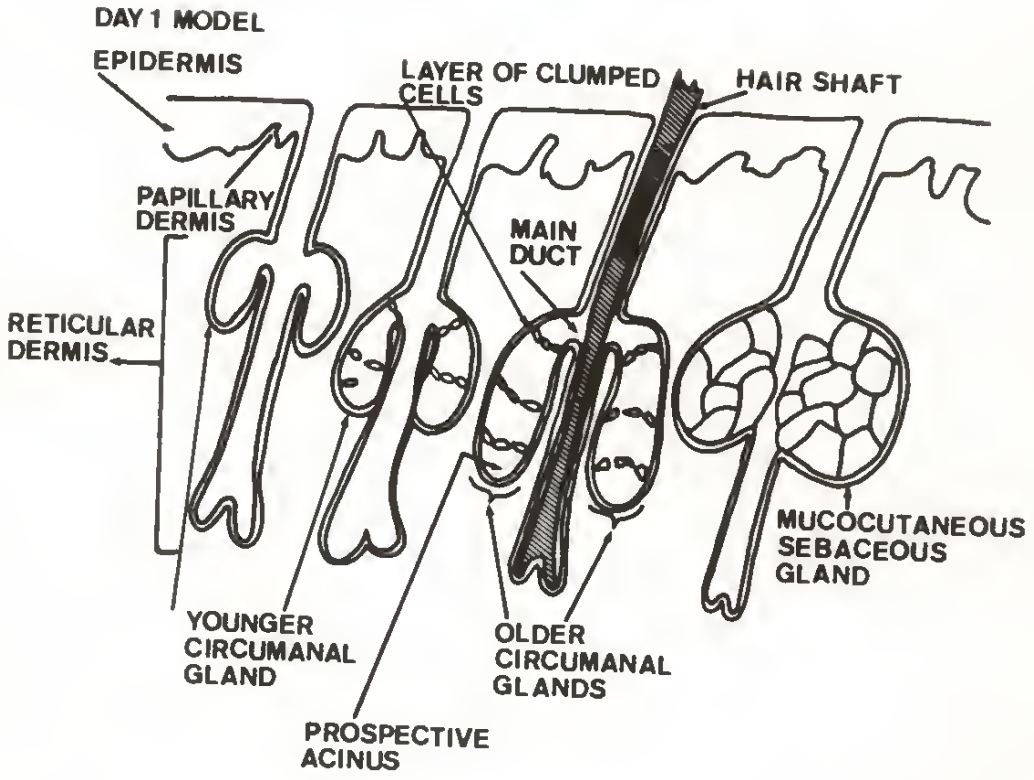

sg
57

EXPLANATION OF FIGURES

Fig. 58. Magnified portion of one of the CGs in Fig. 57, showing a cyst (cy) which is also completely negative for lipids. (Oil Red O Stain; X150).

Fig. 59. A diagram representing the developmental stage of sebaceous gland and CGs in one-day-old dog. The CGs lack acini.

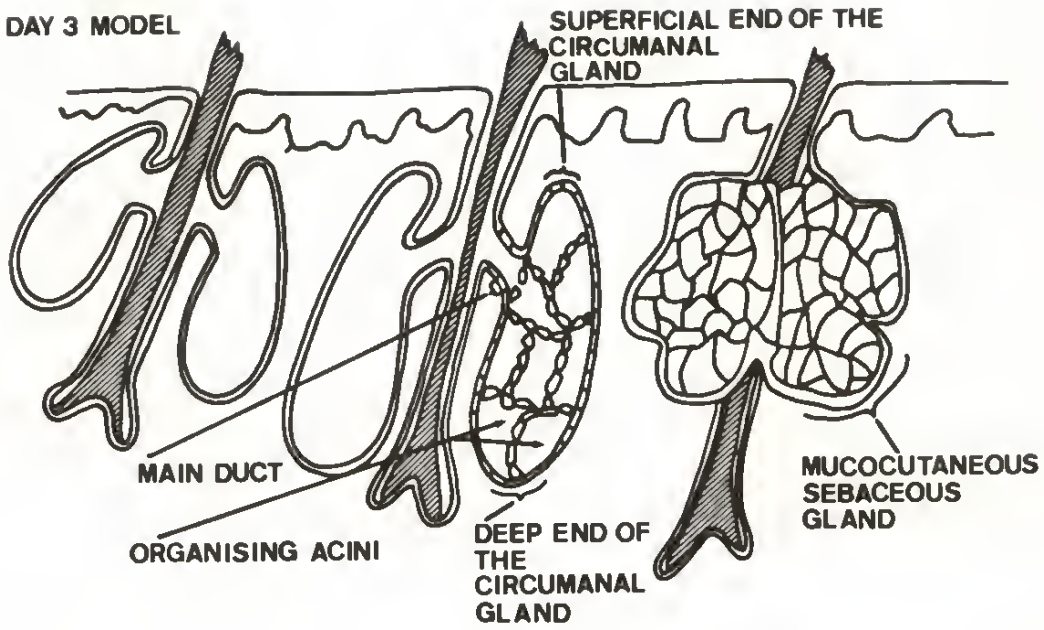

58


DAY 1 MODEL
EPIDERMIS
LAYER OF CLUMPED CELLS
HAIR SHAFT
PAPILLARY DERMIS
MAIN DUCT
RETICULAR DERMIS
MUCOCUTANEOUS SEBACEOUS GLAND
YOUNGER CIRCUMANAL GLAND
OLDER CIRCUMANAL GLANDS
PROSPECTIVE ACINUS

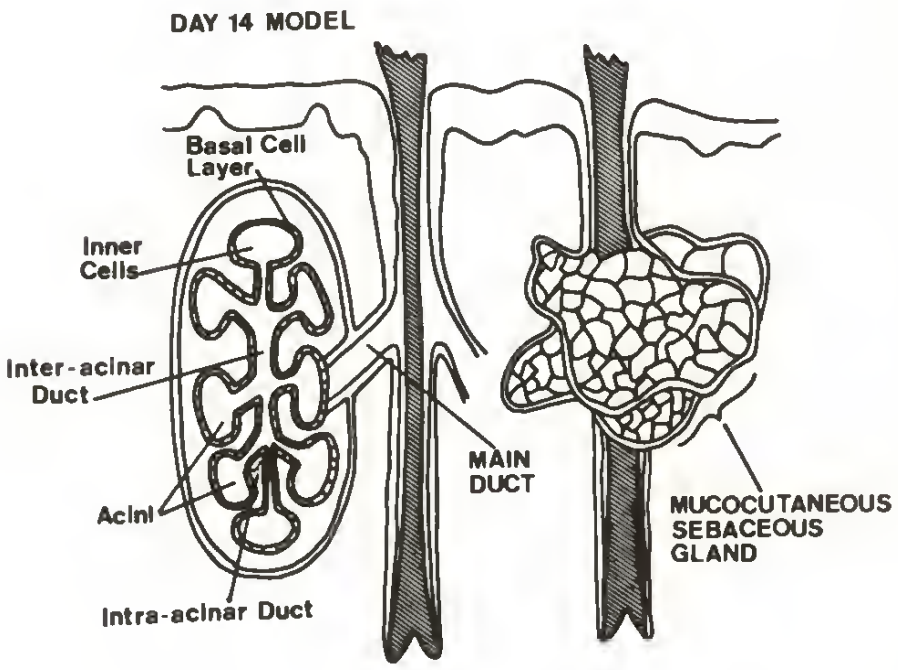
EXPLANATION OF FIGURES

Fig. 60. A diagram of the developmental stage of glands in three-day-old dogs. The elongation of the CGs has resulted in repositioning of the main duct in an upper third portion. Acinar organization is in progress within the glands.

Fig. 61. Diagram of the glands in 14-day-old dogs. The main ducts have extended within the gland, giving off several branches. Acinar organization is quite distinct.


DAY 3 MODEL
SUPERFICIAL END OF THE CIRCUMANAL GLAND
MAIN DUCT
ORGANISING ACINI
DEEP END OF THE CIRCUMANAL GLAND
MUCOCUTANEOUS SEBACEOUS GLAND

60

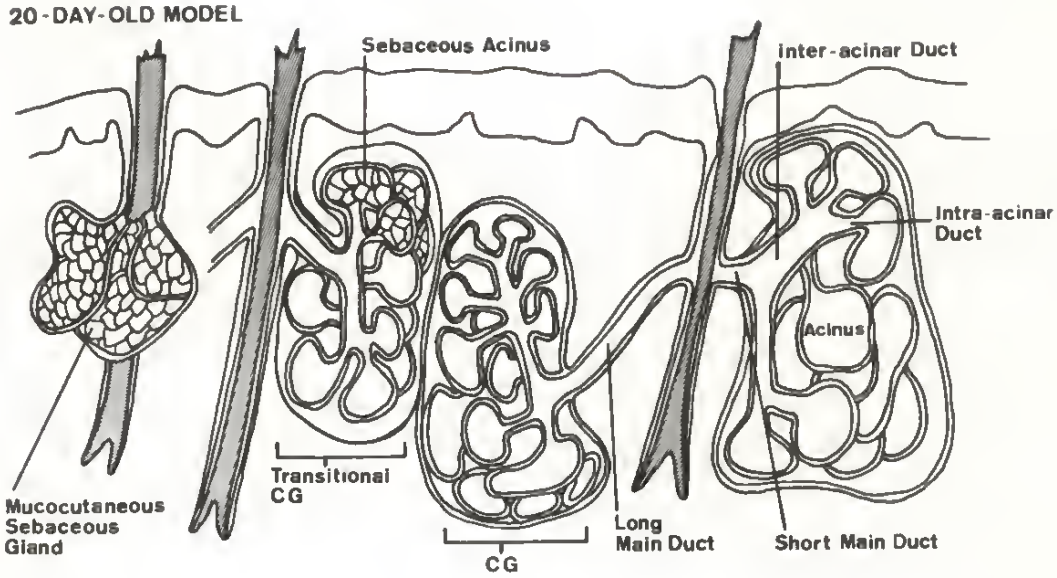

DAY 14 MODEL
Basal Cell Layer
Inner Cells
Inter-acinar Duct
Acini
Intra-acinar Duct
MAIN DUCT
MUCOCUTANEOUS SEBACEOUS GLAND

61

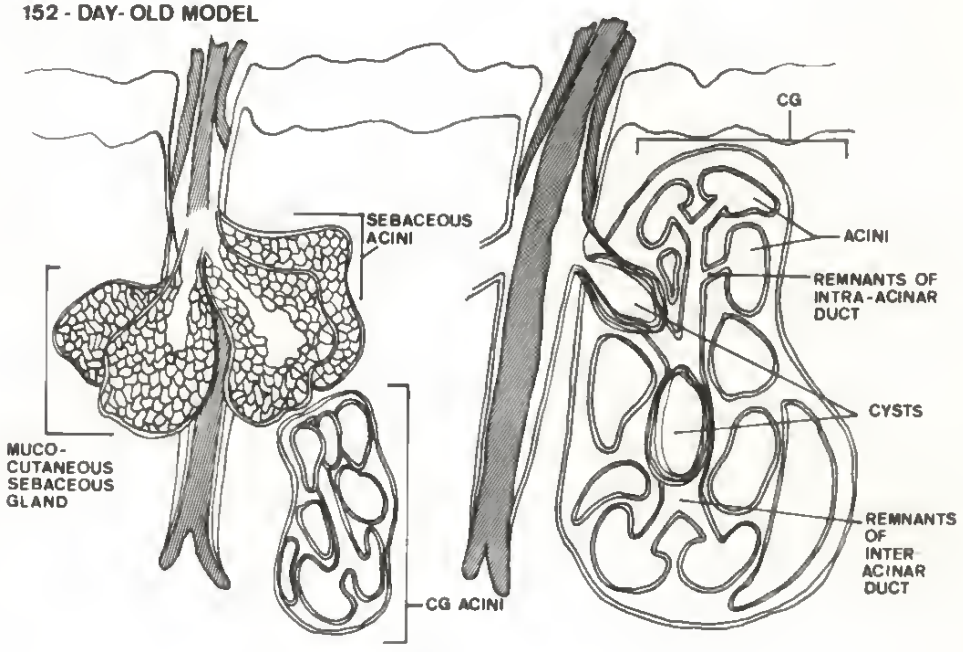
EXPLANATION OF FIGURES

Fig. 62. Diagram of glands in 20-day-old dogs. Formation of new CGs from mucocutaneous sebaceous glands is beginning. Elongation of the main ducts, and greater growth of the CGs are evident.

Fig. 63. Diagram of glands in 152-day-old dogs, showing development of cysts within their ducts.


20-DAY-OLD MODEL
Sebaceous Acinus
Inter-acinar Duct
Intra-acinar Duct
Acinus
Transitional CG
Mucocutaneous Sebaceous Gland
CG
Long Main Duct
Short Main Duct

62

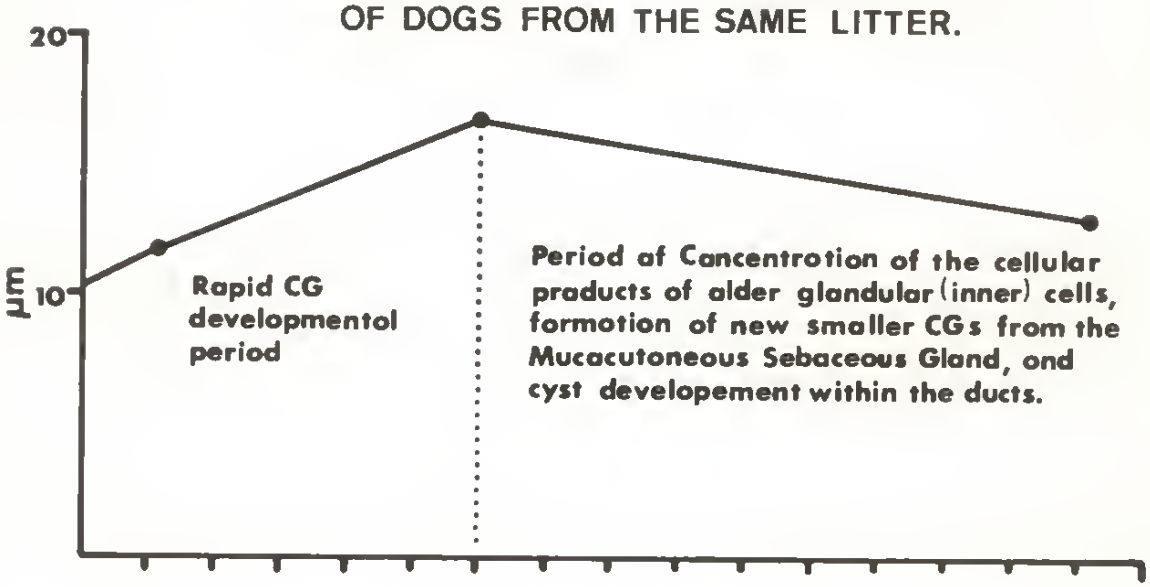

152 - DAY- OLD MODEL
CG
SEBACEOUS ACINI
ACINI
REMNANTS OF INTRA-ACINAR DUCT
CYSTS
MUCO-CUTANEOUS SEBACEOUS GLAND
REMNANTS OF INTER-ACINAR DUCT
CG ACINI

63

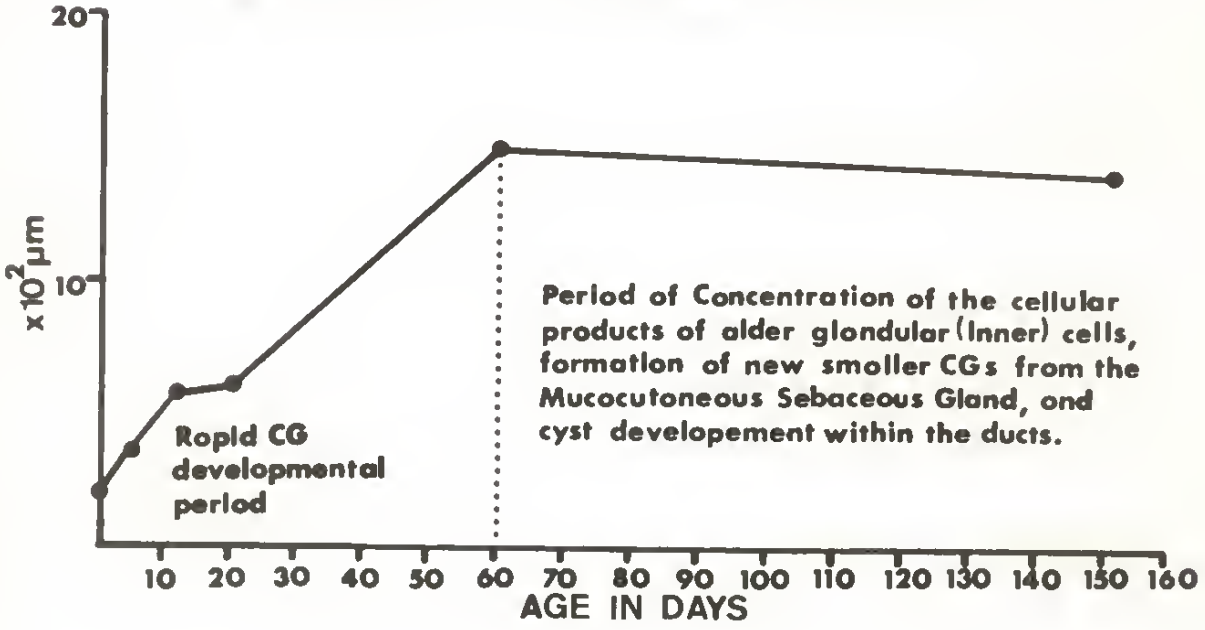
EXPLANATION OF FIGURES

Fig. 64. Graphical representation of the development of the circumanal glands from birth to 152 days post partum.

MEAN DIAMETER OF CG INNER CELLS
OF DOGS FROM THE SAME LITTER.



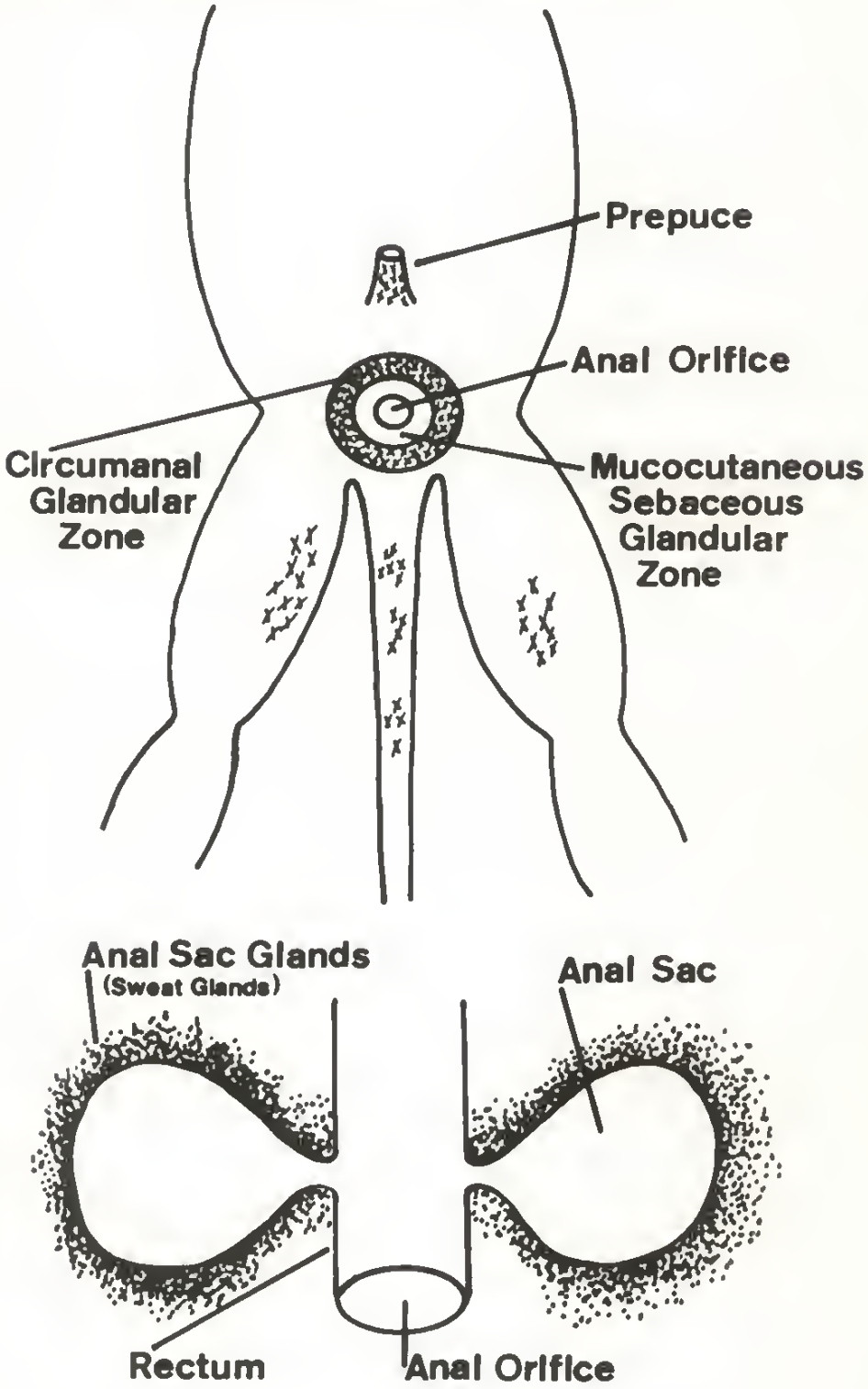
MEAN LENGTH OF CGs FROM ALL THE DOGS



EXPLANATION OF FIGURES

Fig. 65. Diagram of the distribution of anal glands, based on the data in the literature and the present study.

DISTRIBUTION OF ANAL GLANDS



APPENDICES

APPENDIX 1

Details on Tissue Softening Process Prior to Sectioning: The surface of the specimen was exposed in the paraffin block by the removal of few paraffin sections with the microtome. The block was immersed in 20% ethanol, with the exposed specimen surface resting on a filter paper which was placed on the bottom of the ethanol container. A minimum period of 36 hours was used for the softening process, in order to permit adequate diffusion of the ethanol into the specimen. In general, the longer the softening period, the better the result.

Sectioning of the softened skin specimen yielded smooth ribbons without damage to the glands. Comparatively, the non-softened specimens, which were used during the preliminary study, yielded torn ribbons during sectioning; glands contained in those sections were generally distorted in shape.

APPENDIX 2

Details on Method of Drying and Coating of the Specimens used for SEM: The specimens were critical point dried according to the methods of Boyde and Wood[4]; Horridge and Tamm.[19] This method was applied for overcoming the surface

tension damage to the specimen at liquid/gas boudary during drying. It involved the transfer of the specimen in 100% ethanol into the drying chamber of DCP-1 Critical Point Drier, which was immediately sealed off and saturated with liquid CO_2 for a period of 4 minutes. The saturation period was followed by a 4 minute period of escape of gaseous CO_2 from the drying chamber, by opening of the outlet valve. Since there was no change of the volume of the CO_2, as it moved from the liquid to gaseous phase, the surface tension damage was eliminated. The phase of saturation and gas escape was repeated five times, or until the specimen was completely dried as was indicated by the sublimation of the CO_2 flakes without leaving any drop of liquid on a dark background.

The specimen was gold-palladium coated from an evaporation source of 22.5 cm. length of 60% gold and 40% palladium, wrapped around a tungsten filament, and 3 mm carbon electrodes, in order to ensure the conductivity of the specimen surface and adequate secondary electron emission from such surface. KSE-2A-M[g] Vacuum Evaporator was used for the purpose. The specimens, about 10 cm. from the coating sources of the carbon and metal were rotated and tilted in perpendicular directions, as both the mechanical and diffusion pumps created 1×10^{-4} torr vacuum pressure in the evaporator during the operation. Thus, a thin layer

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[g]Kinney Vacuum Company, Boston, Mass. 02130

of carbon evaporated onto the specimen; the specimen being finally coated with 10 to 30 nm. layer of gold and palladium. The perpendicular tilting and rotation of the specimens during the process ensured uniform and continuous coating.[2]

ORIGIN AND EARLY DEVELOPMENT OF THE CANINE CIRCUMANAL GLANDS

by

GODWIN NWACHUKWU ISITOR

D.V.M., Ahmadu Bello University, 1975

AN ABSTRACT OF A MASTER'S THESIS

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Department of Anatomy and Physiology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1978

ABSTRACT

A detailed light and scanning electron microscopy study on the origin and early development of the canine circumanal glands (CGs), with respect to the surrounding sebaceous glands, is presented.

The data presents evidence that the CGs are present at birth, and quite distinct from the sebaceous glands which are also present at birth. The CGs originate from the external root sheath cells of the hair follicles, as evidenced by the distribution of PAS granules within the hair follicle cells and the CG cells. An approximate 60 days period of rapid CG development occurs, immediately after birth, during which there are increases in glandular size, organization of the gland into solid acini, and extension and branching of the solid main ducts into the gland. The rapid developmental period is followed by a period of negligible growth of the CGs which were present at birth, but in which occurs differentiation of new CGs from the mucocutaneous sebaceous glands, and development of cysts within the course of the solid ducts. An endocrine-like function is suggested for the CGs, on the basis of observation of good vascular network, and incomplete ducts with cysts in the gland.