HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF GROWTH CHANGES OF CANINE SKIN

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Anatomy

Iowa State College

1955

1495785

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I. INTRODUCTION

The control and treatment of canine skin disorders has been a perplexing problem for many years, for both the dog owner and the veterinarian. The methods used to combat cutaneous involvements of the dog have not been entirely satisfactory because of an incomplete knowledge of the physiological and pathological factors involved. A vast amount of research has been done on the anatomy, physiology, and pathology of human skin. With a basic understanding of the structure and function of the skin, human dermatologists are able to attack skin problems on a scientific level. Unfortunately, very little of the information and experience developed in human dermatology is directly applicable to veterinary medicine because of differences between the skin of man and animals. Due to a lack of information concerning the normal histological structure of the skin of the dog, physiological and pathological studies of canine skin have been retarded.

A review of the literature reveals that histological work done on the skin of the dog, in the past, was largely confined to morphological studies. The bulk of these studies have been done on random dogs with very little consideration of their ages.

The histochemical study of the anal sac of the dog by

Montagna and Parks (1948) and the cytochemical study of the circumanal glands of the dog by Parks (1950) are the only investigations, known to the author, into the histochemical or cytochemical nature of canine skin.

This study, therefore, is an attempt to determine the changes that take place in canine skin between birth and 28 weeks of age, utilizing histological and histochemical methods.

II. REVIEW OF LITERATURE

A. General

Literature concerning the microscopic anatomy of the
integumentary system of the dog is quite limited. The first
recorded research on the histology of canine skin was done
by the German worker, Gurlt (1835), in which he did a com-
parative study of the skin of domestic animals and man.
During the century following Gurlt's work, other investiga-
tions into the structure of the dog's skin were reported
in the German language. These were summarized very well
by Claushen (1933). Ellenberger (1906) presented a good
textbook summary of the work up to that date. Trautmann
and Fiebiger (1949) also gave a textbook discussion of
canine skin. Recent work has been reported by the follow-
ing workers: Muto (1925), Gair (1928), Fuchs (1934), Speed
(1941), Varicak (1941), Hildebrand (1952), Nielsen (1953),
Webb and Calhoun (1954), Epling (1953), Hansen et al. (1950),
and Hansen et al. (1954).

B. Epidermis

Hansen and co-workers (1950), in studies of the skin of
dogs on normal and fat deficient diets, described

histological sections taken from the dorsal surface of the
thigh and stained with hematoxylin and eosin. They found
the normal epidermis to consist of two to four cell layers,
and that the nuclei of the cells were oriented parallel to
the surface. They observed no epidermal pegs, and that
the absence of intercellular bridges caused the cytoplasm
to appear syncytial. The stratum corneum was found to be
thin and without nuclei. In their material, neither the
stratum granulosum nor stratum lucidum was evident. In
biopsies from the dorsal aspect of the thigh and from the
ventral surface of the chest, fixed in Bouin's solution and
stained with hematoxylin and eosin, Hansen et al. (1954)
described the epidermis as being two to three cells in thick-
ness. The outer layer was flattened to a granulosum type
containing a few basophil granules. Above this layer, they
described a thin lace-like stratum disjunctum of kera-
tinized material, with hardly a trace of stratum lucidum.
They found the basal layer of the stratum germinativum
to be low columnar to cuboidal with little mitosis.

Webb and Calhoun (1954) described four distinct epi-
dermal layers: stratum germinativum, stratum granulosum,
stratum lucidum, and stratum corneum. According to their
measurements, the epidermis varied from 26.5 microns in the
sternal region to 1685.4 microns in the digital pad. They
found the stratum germinativum to vary from two cell rows

in thin skin to 35 rows in the nasal septum. The stratum granulosum, varying from one row of cells in thin skin to 15 rows in the digital pad, was generally present. According to their findings, the stratum lucidum was best developed in the digital pads, and was absent in most of the thin epidermis. They found that the stratum corneum varied from 5.3 microns in the lip to 1526.4 microns in the digital pad.

Epling (1953) described the epidermis of the carpal, metacarpal, metatarsal, and digital pads as consisting of three layers: stratum germinativum, stratum granulosum, and stratum corneum. In describing the thin skin, he reported that all three layers of the epidermis are decidedly reduced in thickness, and the stratum granulosum is sometimes absent altogether.

Varicak (1941) compared the epidermal layers of the skin of the domestic animals with those of man. He found that the epidermis of the dog resembles that of man.

Webb and Calhoun (1954), Epling (1953), and Hansen et al. (1954) made comments on the junction of the epidermis and dermis. Epling mentioned a network of reticular fibers existing between the stratum germinativum and the dermis, which is commonly termed the basement membrane. Hansen described the lower surface of the epidermis as being quite smooth and resting on a rather homogeneous collagenic layer of dermis containing a few fibroblasts, capillaries, and

nerve fibers.

Mitosis in the epidermis was discussed by several authors. Hansen et al. (1954) stated that the basal layer of the stratum germinativum is low columnar to cuboidal with little mitosis. Epling (1953) reported that mitoses are most numerous in the deeper layer but also occur in other parts of the stratum germinativum. He also stated that pigment is observed as fine granules within the cells of the stratum germinativum and to a lesser degree in the remaining strata. Webb and Calhoun (1954) found that white or light colored skin was characterized by a few melanin granules in the cytoplasm of the basal layer of the stratum germinativum. The amount of pigment increased with darkness of the skin. Black skin had melanin in all layers of the epidermis. Nielsen (1953) found the hairless, heavily-pigmented skin on the tip of the nose to be without sweat glands.

Trautmann and Fiebiger (1949) stated that the planum nasale of Carnivora is free from glands.

C. Dermis

Epling (1953) stated that the dermis of the hairy skin of the dog is composed of dense irregularly arranged elastic, collagenous, and reticular fibers, along with

fibroblasts and histiocytes. Hansen et al. (1950) found the dermis to consist of sharply demarcated and loosely woven collagen bundles and blood vessels.

Webb and Calhoun (1954) found that collagenous and elastic fibers were finer in the superficial or papillary zone of the dermis, and thicker in the deep or reticular layer. They noted numerous reticular fibers just beneath the basement membrane and around hair follicles. Sisson and Grossman (1953) stated that the corium of the foot pads have large papillae and contain sweat glands and lamellar corpuscles.

D. Hair and Hair Follicles

A comprehensive study of the hair of the costal region of the dog was done by Gair (1931). He observed hair length, thickness, type, and number of hairs per square millimeter, and classified the hair coats of dogs into three types: normal, short, and long.

The hair follicle of the dog has been discussed by various authors. Sisson and Grossman (1953) stated that the hairs in the dog's skin are arranged in groups of three to a large extent. According to Hansen et al. (1950), the hair follicles from the skin of the dorsal surface of the thigh have an epidermal layer of two to three cells. Epling

(1953) stated that the hair follicle is essentially an epithelial invagination of the skin, and that it is common to find that many hairs may share the upper portion of a follicle, and more deeply, branch away from one another to form individual follicles. As many as 13 hairs may share one hair follicle. Claushen (1933) found that the hairs occur in groups, and each group consists usually of only one main hair and three to nine accessory hairs, all of which arise out of a common hair bed. Fuchs (1934) reported that on the head and limbs, the hairs lie in contiguous groups, each with a principal hair and associated accessory hairs. Hildebrand (1952, p. 421) gives the following monograph on the hair follicles of canids by de Meijere (1894):

> On the well-furred parts of the bodies of
> many mammals, each hair follicle becomes divided
> and produces a bundle of hairs. In canids, the
> bundles may be isolated, but are usually in groups
> of two to four or more; three-bundle groups are
> by far the most frequent. The groups are oriented
> into somewhat irregular rows. The typical bundle
> consists of several wool or under hairs and a
> single longer and stiffer guard or over-hair. The
> guard-hair of the central bundle of a three-bundle
> group is coarser than that of the lateral bundles.

Ellenberger and Baum (1943) describe the arrangement of hairs in groups in the dog, and show illustrations of a main hair with a group of smaller hairs clustered around it. Also, they show an illustration of the surface contour of the skin with the bunches of hairs grouped in three's, emerging from under folds of the surface.

E. Sweat Glands

Gurlt (1835) was the first to record research into sweat glands of horses, cattle, pigs, and dogs. In the dog, he found that in all hairy parts, the sweat glands are small and elongated, not very curved, and not easily found.

The work of Claushen (1933) contains a comprehensive review of the German literature and includes the observation that sweat glands are present in all parts of the skin. Their ducts open into the hair follicle above the sebaceous gland, 300 to 500 microns under the epidermis. Fuchs (1934) found that the sweat glands are most numerous on the distal end of the limbs, next on the lips, then the hairy part of the nose, and about as many on the proximal ends of the limbs.

Speed (1950) gives a very good survey of the literature in English and gives histological evidence for the following conclusions, page 256:

> Sweat glands in the dog are numerous. They occur in a simple uncoiled form on the hairy parts of the body. In the pads of the feet, they are coiled. With the exception of those in the pads of the feet, all sweat glands in the dog open into hair follicles and have the appearance of buds or branches of the hair follicle. Contrary to Hirt's findings, the opening of the sweat gland into the hair follicle is separate from that of the sebaceous gland, and nearer the surface of the skin.

Aoki and Wada (1951) suggest that the sweat glands in the hairy skin of the dog do not participate actively in the central thermo-regulatory mechanism but that they sub-serve chiefly for the protection of the skin from excessive rise of temperature.

Trautmann and Fiebiger (1949) stated that the apocrine glands of the dog are serpentine small tubular glands, that are better developed in some regions, such as the back, mouth, and anus. Nielsen (1953) classified the sweat glands of the dog into merocrine and apocrine, according to the classification of Schaffer (1924). Nielsen stated that the apocrine glands of the dog are large loosely-coiled sweat glands, distributed over the entire skin as an appendix to the hair follicles. The small densely-coiled merocrine sweat glands are present only in the thick fatty fibrous tissue under the foot pads. He included a description of the secretion and resting cycle of the apocrine glands with photomicrographs.

F. Sebaceous Glands

In writing on the skin of the dog, Sisson and Grossman (1953) stated that sebaceous glands are best developed in short and rough haired breeds; and that they are largest and most numerous at the lips, anus, dorsal surface of the

trunk, and sternal region. Fuchs (1934) reported that
sebaceous glands occur in elongated and alveolar forms and
of varying sizes, the largest being on the head, the lip,
and the hairy part of the nose. Claushen (1933) found seba-
ceous glands present in all parts of the skin that he examined.
Trautmann and Fiebiger (1949) stated that, in the dog, seba-
ceous glands are often club-shaped and coiled, or even
branched. Webb and Calhoun (1954) reported that the seba-
ceous glands of the dog were generally larger than those of
the human species. Nielsen (1953) stated that glands with
a sebaceous holocrine type of secretion are distributed
over the entire integument in connection with the hairs.
Morphologically, they do not differ from the sebaceous
glands in the skin of other mammalian species. Epling
(1953) described the histological structure of sebaceous
glands of the dog. Hansen et al. (1954) stated that at the
level of the stratum reticularis, sebaceous glands are
bunched on the sides and lower slope of the follicle. They
show a complete gradation from germinative to degenerative
cells, with little sign of a lumen in any gland. According
to Schaffer (1930), all sebaceous glands are developmentally
related to hair follicles.

G. Arrector Pili Muscle

Very little has been written about the arrector pili muscle of the dog. Claushen (1933) reported that arrector pili muscles are strongly developed on the back and often absent in the remaining parts of the body. Epling (1953) agreed that the arrector pili muscles are best developed in the dermis of the dorsal aspect of the body. He also stated that each muscle arises in the dermis by several ends, runs a short distance as a compact bundle, then divides into smaller branches which serve the individual hairs of a group.

III.   METHODS OF PROCEDURE

A.   Obtaining Skin Samples

The specimen material was secured from 13 dogs, which
were whelped and raised in the dog colony of the Veterinary
Anatomy Department, Iowa State College.   At the time of
whelping, the birth dates were carefully recorded so that
the age of the dogs would be definitely known.   After wean-
ing, the dogs were fed dry commercial dog food[1] in self
feeders.

Half of the protein of the commercial dog food was
from animal sources, one-third from special vegetable sources,
and one-sixth from cereals.   The food provided 1500-1600
calories per pound.   The dogs were allowed all the water
they wanted by means of automatic waterers.   Intestinal
parasites were controlled by treating the bitches with a
commercial anthelmintic prior to parturition, and cleaning
the dog runs daily.   Protection against canine distemper
and infectious hepatitis was afforded by means of a double
fence with a five-foot alleyway around the dog runs to

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[1]The information concerning source and caloric value
of the feed was obtained from a report of the Gaines Research
and Development Laboratory, October 2, 1953.

prevent exposure by stray dogs. There was no evidence of excessive parasitism or nutritional deficiency observed during the feeding period or at the time the material was collected. The sex, breed characteristics, and age, at the time the skin samples were collected, were as follows:

Birth	- Coon hound	Female
1 week	- Coon hound	Female
2 weeks	- Coon hound	Female
3 weeks	- Coon hound	Female
4 weeks	- Collie cross	Male
6 weeks	- Collie cross	Female
8 weeks	- Collie cross	Female
10 weeks	- Coon hound	Female
12 weeks	- Coon hound	Female
16 weeks	- Coon hound	Male
20 weeks	- Coon hound	Male
24 weeks	- Cocker cross	Female
28 weeks	- Cocker cross	Female

At the specific ages the dogs were destroyed by electrocution and the specimen material was immediately collected from the body areas shown on Fig. 1. The strips of skin were cut parallel with the direction of the slant of the hair, whenever possible. Two samples of skin were collected from each area. One, 1/2 centimeters by 2 centimeters, was fixed in formalin; the other, 2-3 millimeters by 2 centi-

meters, was fixed in chilled alcohol.

B. Histological and Histochemical Methods

1. Histological techniques

For a general fixative, a 10 per cent solution of neutral formalin (equal to 4 per cent of formaldehyde) was used. The skin samples were allowed to remain in the formalin fixative for 24 to 48 hours. After the fixation period, the hair was trimmed as short as possible. The edges of the samples were trimmed square with a razor blade and the samples were stored in 70 per cent ethyl alcohol. Dehydration and embedding were accomplished through butyl alcohol and Altmann's mixture, using the technique of Johnson et al. (1943) for muscle, which was modified by reducing the time in each paraffin mixture to 30-60 minutes, making a total of 2-4 hours in the paraffin oven. It was found that the skin samples became too hard if they were allowed in the oven more than 3-4 hours.

Sections of the paraffin blocks, 6-10 microns in thickness, were routinely stained with Harris' alum hematoxylin, Mallory (1938, p. 72), and precipitated eosin Y technique according to Pearson (1942). For the staining of specific structures, the following three staining techniques were

used in succession: Van Gieson's acid fuchsin and picric
acid stain for collagenous fibers (Guyer, 1936, p. 250);
Weigert's stain for elastic fibers (Mallory, 1938, pp. 168-
169); and Heidenhain's iron hematoxylin for nuclear detail
(Mallory, 1938, pp. 74-75). For the demonstration of reti-
cular fibers, the rapid method of silver impregnation of
reticulum according to Foot and Menard (1927) was used.

2. Glycogen technique

For the demonstration of glycogen, the tissue was fixed
in 80 per cent alcohol at zero degrees Centigrade, cleared
in cedar oil, and embedded in paraffin. The sections were
cut at 6-10 microns and stained with the Feulgen-Bauer
technique (Bensley, 1938, p. 105). Control sections were
stained simultaneously from which the glycogen had been
digested in saliva or diastase.

3. Phosphatase techniques

For the histochemical demonstration of alkaline and
acid phosphatase, the methods of Gomori (1952) were followed.
The calcium phosphate method was used for the demonstration
of alkaline phosphatase, and the lead method was used for
the demonstration of acid phosphatase. Dempsey and Deane

(1946) found that chilled alcohol was satisfactory for the fixation and demonstration of both alkaline and acid phosphatase.

The skin samples were cut at 2 millimeters in thickness and fixed in cold 80 per cent alcohol (zero degrees Centigrade) for 24 hours. They were then changed every 24 hours to 90 per cent, 95 per cent, and finally absolute alcohol.

The tissues were cleared in cedar oil for 24 hours and then placed in three changes of benzene of 1 hour each. Infiltration was accomplished by means of three changes of low melting point paraffin. The tissues were not allowed in the paraffin oven for more than 3 hours. Altmann's mixture was used as the final embedding medium. The paraffin blocks were trimmed and sectioned from 6-10 microns in thickness, and mounted on glass slides by means of Mayer's albumin-glycerine mixture.

After deparaffinization, the sections were incubated in calcium solutions, containing sodium glycerophosphate and either lead or calcium ions. The solutions were buffered with acetate or sodium barbital, depending on the pH desired. All solutions were examined immediately prior to incubation by means of a Beckman pH meter and were adjusted to Ph 9.4 for alkaline phosphatase and pH 5.0 for acid phosphatase. The sections were incubated for 6, 12,

and 24 hours. Control sections were prepared by omitting
the substrate in the incubating solutions, in order to reveal
any possibilities of preformed insoluble native phosphate.
The sites of phosphatase activity were made visible micro-
scopically by converting the precipitated phosphate into
visible brown sulfides as described by Gomori (1939).

4. Microscopic techniques

Observations of the stained sections were made by means
of a Bausch and Lomb binocular research microscope. The
measurements were made with a Bausch and Lomb eyepiece
micrometer.

Some observations of the surface contour of fresh skin
and the arrangement of the hairs and hair follicles, to
confirm the microscopic observations made from the sections,
were made by means of an American Optical Company binocular
dissecting microscope at 15X magnification.

IV. OBSERVATIONS

A. Epidermis

In the epidermis of the foot pads, five layers of epithelium were observed: stratum cylindricum, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The stratum cylindricum and stratum spinosum are frequently referred to collectively as the stratum germinativum.

The stratum cylindricum was made up of a single layer of basal cells resting on the connective tissue of the dermis. The cells of this layer had little cytoplasm and dark staining nuclei which occasionally showed mitotic activity.

The stratum spinosum was observed to be composed of diamond or dome shaped cells containing more cytoplasm and lighter staining nuclei than the stratum cylindricum. In the foot pads and planum nasale, cell outlines and intercellular bridges were observed. In the hairy skin, the stratum spinosum cell outlines were more visible after alcohol fixation than following formalin.

The stratum granulosum was made up of flattened cells which contained basophilic keratohyalin granules in their cytoplasm. The stratum granulosum was the thickest in the foot pad epidermis. In the thin hairy skin, the stratum

granulosum was one to three cells in thickness; whereas, in the foot pad, it was four to five cell layers thick.

The stratum lucidum was consistently observed only in the foot pads as a shiny acidophilic layer of homogenous substance called eleidin by Maximow and Bloom (1952). This layer was lacking entirely in the planum nasale and was inconstant in the thin hairy skin, being observed only in the thicker regions.

The stratum corneum was observed to consist of a layer of flattened, filamentous, keratinized, non-nucleated material. The stratum corneum of the planum nasale was very thin in comparison to the thickness of the epidermis. The cells of the corneum of the nasal region contained nuclei. The stratum corneum made up from one-third to one-half of the total thickness of the epidermis in all areas studied, with the exception of the planum nasale.

The measurements of the epidermis include the thickness of all of the layers present including the stratum corneum. The counts of the number of layers of cells in the epidermis do not include the stratum corneum or stratum lucidum because of their non-cellular nature.

The areas of non-hairy thick epidermis, like the planum nasale and foot pads, increased in thickness with an increase in age.

Table 1 shows the measurements that were made, of all

Table 1. Thickness of epidermis in various body areas at various ages
(measured in microns with an eyepiece micrometer)

		Age in weeks											
	Birth	1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	37	30	28	25	32	32	32	36	32	38	31	39	39
Front foot pad	225	520	360	472	750	652	877	900	870	990	950	1620	1800
Sacral	36	36	32	35	32	40	36	34	33	40	38	40	42
Hind foot pad	210	468	500	495	702	720	895	990	900	900	1061	1790	1800
Paraumbilical	35	35	40	46	35	30	42	35	33	55	32	32	33
Planum nasale	194	234	300	220	225	270	450	396	405	540	620	640	630
Margin of teat	35	52	51	50	40	34	46	43	35	56	40	25	60
Hairy eyelid	37	25	45	43	32	28	36	40	40	30	26	28	23
Eyelid margin	59	68	60	75	70	62	80	81	56	60	72	69	84
Conjunctiva	48	43	45	53	47	47	60	49	46	48	55	59	45
Fold of flank	46	49	46	47	43	49	41	49	45	50	42	40	36
Ear flap	34	23	61	32	37	40	28	41	38	45	42	26	20

layers, of the various areas at different ages. The epidermis of the foot pads increased from 225 microns at birth to 1800 microns at 28 weeks. The epidermis of the planum nasale increased from 194 microns at birth to 630 microns at 28 weeks.

Table 1 also shows that the regions of hairy skin, like interscapular, sacral, paraumbilical, margin of teat, hairy eyelid, fold of flank, and ear flap, remained quite constant, 25 to 50 microns, during the 28 weeks studied. The non-hairy margin of the eyelid was thicker, measuring 56-84 microns in thickness. The conjunctiva was found to be thicker than the epidermis of the hairy areas, 43-60 microns.

Fig. 2 and Fig. 3 show a comparison of the thickness of epidermis of the planum nasale at birth and 28 weeks. Both photomicrographs were taken at the same magnification.

Figs. 5, 7, and 9 show photomicrographs of the epidermis of the foot pad from birth, 8 weeks, and 28 weeks, respectively. They were taken at the same magnification. The increase in thickness is readily observed.

Figs. 4, 6, and 8 show photomicrographs of the epidermis of the hairy skin at birth, 8 weeks, and 28 weeks, respectively, taken at the same magnification. The similarity in thickness of the epidermis at these three ages is easily seen.

Table 2 shows the average number of cell layers counted

Table 2. Thickness of epidermis in various body areas at various ages (values represent the average number of cell layers, not including the stratum lucidum or stratum corneum)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	3	5	4	3	6	4	5	4	5	5	5	5	5
Front foot pad	9	18	18	16	12	14	16	15	18	18	17	18	20
Sacral	4	3	5	4	5	4	5	4	5	4	5	5	5
Hind foot pad	12	18	15	17	16	15	17	16	20	18	15	16	20
Paraumbilical	4	8	6	6	5	4	5	4	5	6	7	4	5
Planum nasale	10	14	19	25	15	20	18	18	21	21	25	23	19
Margin of teat	4	8	6	6	5	4	5	5	4	5	5	5	5
Hairy eyelid	6	6	5	5	4	5	5	4	6	4	7	5	5
Eyelid margin	9	10	9	10	9	9	11	9	11	10	11	10	10
Conjunctiva	8	7	8	10	7	9	8	6	8	6	8	7	6
Fold of flank	5	6	4	6	4	6	5	5	6	5	7	5	4
Ear flap	6	5	6	4	5	5	6	5	5	6	6	4	5

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in the stratum cylindricum, spinosum, and granulosum in
the various body areas at different ages. There was very
little fluctuation in the number of cell layers of the
epidermis of the hairy skin. The areas of thick skin, like
the foot pads and planum nasale, increased slightly in the
number of cell layers during the 28 weeks studied. It was
observed that most of the increase in thickness in the foot
pads was a result of the increased undulation of the basal
layer of epidermal cells to form epidermal pegs and an
increase in thickness of the stratum corneum, rather than
an increase in number of cell layers.

Table 3 gives the distribution and location, according
to ages, in which epidermal papillae were observed. These
papilla-like structures were covered by a thickened epidermis
which was usually 6-12 cell layers thick, about twice as
thick as the surrounding epidermis. The dermis under this
area was thickened, more dense and filled or extended up
under the thickened epidermis to form a papilla-like struc-
ture. Fig. 10 shows a picture of an epidermal papilla which
was taken through a dissecting microscope at 15X magnifica-
tion. Figs. 11, 12, and 13 show photomicrographs of sections
of epidermal papillae.

Many clear cells were observed in the stratum cylindri-
cum. The cytoplasm of the cells appeared to be completely
transparent and the nuclei were extremely basophilic. These

Table 3. Epidermal papillae were observed in skin sections taken from various body areas at various ages as indicated below

	Birth	Age in weeks											
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	Pap*	Pap	–	–	Pap	–	Pap	–	–	–	–	–	Pap
Front foot pad	–	–	–	–	–	–	–	–	–	–	–	–	–
Sacral	–	–	–	–	Pap	–	–	–	–	–	–	Pap	–
Hind foot pad	–	–	–	–	–	–	–	–	–	–	–	–	–
Paraumbilical	Pap	–	–	–	–	–	–	–	Pap	Pap	–	–	–
Planum nasale	–	–	–	–	–	–	–	–	–	–	–	–	–
Margin of teat	Pap	–	–	–	–	–	–	Pap	–	–	–	–	–
Eyelid	–	–	–	–	–	–	–	–	–	–	–	–	–
Fold of flank	Pap	–	–	–	–	–	–	–	–	–	–	–	–
Ear flap	–	–	–	–	–	–	–	–	–	–	–	–	–

*The abbreviation Pap indicates the presence of papillae

clear cells were more numerous in the stratum cylindricum of the thick skin of the planum nasale and foot pad than in the hairy skin, as can be seen by comparing Fig. 14 and Fig. 15. Many of these clear cells showed evidence of mitotic activity as illustrated in Fig. 16 and Fig. 17, which show cells in telophase. Because the nuclear material of the clear cells stained so intensely basophilic and was observed in various stages of mitosis, it was assumed that all clear cells with the dark basophilic nuclei represented mitotic cells. Table 4 gives the counts of mitotic figures that were made on this basis, of various areas at different ages. The columns of the table were totaled both vertically and horizontally. Vertically there was no apparent correlation between age and number of mitotic figures per section. Horizontally it appeared that the number of mitotic figures was greater in the foot pads and planum nasale than in the thin hairy skin.

Table 5 shows the distribution of glycogen in the epidermis of various body areas at various ages. In the hairy skin, very little glycogen was observed. Only a few very fine granules were observed around the openings of hair follicles. In the thick skin of the foot pads and planum nasale, glycogen granules were observed in the cytoplasm of the spinous cells, only in the very young material.

Fig. 18 shows a section of the fused eyelids of a

Table 4. Mitotic figures in skin sections from various body areas at various ages (values represent the number of mitotic figures counted in one section)

						Age in weeks								
	Birth	1	2	3	4	6	8	10	12	16	20	24	28	Total
Interscapular	1	17	4	8	11	0	11	4	6	2	6	13	5	88
Front foot pad	0	10	5	95	0	5	33	8	3	8	16	27	5	215
Sacral	4	1	2	10	0	0	10	0	3	1	5	5	5	46
Hind foot pad	4	15	15	15	11	4	37	10	17	0	0	30	12	170
Paraumbilical	1	2	4	5	1	0	8	10	12	18	7	0	3	71
Planum nasale	2	15	10	0	7	15	13	3	8	14	8	23	5	123
Margin of teat	5	10	1	5	1	0	14	3	0	11	8	8	6	72
Eyelid	0	0	5	0	0	0	6	4	2	0	4	2	0	23
Fold of flank	5	10	0	4	0	6	8	4	0	3	2	1	0	43
Ear flap	0	0	0	2	0	3	7	8	0	2	4	4	0	30
Total	22	80	46	144	31	33	147	54	51	59	60	113	41	

Table 5. Glycogen content of epidermis in various body areas at various ages (values indicate degree of reaction to Feulgen-Bauer technique*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	0	5	0	0	5	0	0	0	0	0	0	0	0
Front foot pad	10	0	0	0	0	0	0	0	0	0	0	0	0
Sacral	5	0	0	0	0	0	0	0	0	0	0	10	0
Hind foot pad	30	5	0	0	0	0	0	0	0	0	0	0	0
Paraumbilical	5	5	0	0	5	0	5	0	0	0	0	0	0
Planum nasale	20	20	5	30	5	0	0	0	0	0	20	0	0
Margin of teat	5	0	0	0	0	0	0	0	5	0	0	0	0
Hairy eyelid	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	5	10	30	30	30	20	20	20	20	10	20	20	20
Fold of flank	0	0	0	0	0	0	0	0	0	0	0	0	0
Ear flap	5	5	5	0	0	0	0	0	0	0	0	0	0
Hairy lip	0	0	0	0	0	a							
Buccal mucosa	40	30	20	20		a							
Hairy vulva	5	5	5	5	0	a							
Mucosa of vulva	40	40	20	30	40	a							

* 5 - very slight
10 - slight
20 - moderate
30 - intense
40 - very intense

[a]Material was not collected from the lip and vulva above the age of 4 weeks.

28

day old puppy with glycogen granules in the cytoplasm of
the epidermal cells at the margin of fusion. Fig. 19 shows
a section of mucous membrane from the vulva in which the
cytoplasm of the cells contain large quantities of glycogen.
The mucous membrane of the conjunctiva, lip, vulva, and
prepuce all contained large quantities of glycogen as shown
in Table 5.

Table 6 shows the distribution of alkaline phosphatase
in the epidermis of various body areas at various ages.
In the hairy skin, alkaline phosphatase was found to be
present, in very small quantities, only in the nucleoli of
the spinous cells of the stratum spinosum and the kerato-
hyaline granules of the stratum granulosum. The epidermis
of the thick skin, like the foot pads and planum nasale,
showed a moderate to very intense reaction for alkaline
phsophatase in the nuclei and cytoplasm of the cells at the
depths of the epidermal pegs, which extended deep into the
dermis. This may be seen in Fig. 21. The nuclei and cyto-
plasm of the cells of the mucous membranes showed the strong-
est reaction. The mucosa of the vulva and lip reacted to
a degree of very intense, as seen in Fig. 20.

In heavily pigmented parts of the skin, many of the
basal cells contained a large amount of pigment. These
probably represent melanoblasts. The ordinary epidermal
cells also contained melanin granules on the distal side of

Table 6. Distribution of alkaline phosphatase in epidermis of various body areas at various ages (values indicate degree of reaction to the Gomori technique*)

| | Birth | Age in weeks |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	0	5	5	5	0	0	0	5	5	0	0	5	0
Front foot pad	20	20	20	30	30	20	30	30	30	20	30	40	20
Sacral	0	0	5	5	0	0	0	0	0	5	0	5	0
Hind foot pad	20	20	30	30	30	20	30	30	20	20	40	40	30
Paraumbilical	0	5	5	5	5	0	0	20	20	10	5	10	0
Planum nasale	40	20	20	40	30	20	20	30	20	20	40	40	20
Margin of teat	0	5	5	5	0	0	0	5	0	0	0	5	0
Hairy eyelid	0	0	5	0	0	0	0	0	0	0	10	0	0
Conjunctiva	20	20	30	20	30	30	30	30	20	30	30	30	30
Fold of flank	0	5	5	5	0	5	0	5	0	0	5	10	0
Ear flap	0	5	5	5	0	5	0	5	5	0	0	10	0
Hairy lip	5	0	5	0	0	[a]							
Buccal mucosa	40	40	40	40	40	[a]							
Hairy vulva	5	0	10	5	0	[a]							
Mucosa of vulva	40	40	40	40	40	[a]							

* 5 – very slight
  10 – slight
  20 – moderate
  30 – intense
  40 – very intense

[a]Material was not collected from the lip and vulva above the age of 4 weeks.

the nucleus in varying amounts, depending on the degree of
pigmentation of the area. Fig. 22 shows a pigment cell
which has dendritic processes. This cell type is suggestive
of those described by Billingham (1948) after dopa reaction.

Fibroblasts were observed between the cells of the
basal layer of epidermis as illustrated in Fig. 23. These
fibroblasts were more visible in the formalin fixed tissue
than in that fixed in 80 per cent alcohol.

Fig. 24 shows the difference between the dermal-
epidermal junction of the mucous membrane and the hairy
portion of the lip. These is no pegging of the epidermis
of the hairy part of canine skin. The basal layer of cells
forms a line parallel with the surface contour of the skin.
The surface of the hairy skin of the dog is normally thrown
up into folds, which form troughs or grooves, into the
depths of which the hair follicles invaginate. The mucous
membrane of the lip, as shown in Fig. 25, forms an undulat-
ing line at the dermal-epidermal junction. This is charac-
teristic of the non-hairy portions of canine skin, like the
planum nasale and foot pads.

B. Dermis

The dermis was thickest, at all ages, in the inter-
scapular and sacral regions. As seen in Table 7, it

Table 7. Thickness of dermis in the various body areas at various ages[a]
(measured in microns with an eyepiece micrometer)

	Birth	Age in weeks											
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	720	990	495	405	518	495	675	810	830	1080	1440	1620	1575
Front foot pad	270	473	405	440	315	450	585	585	495	900	900	900	810
Sacral	630	698	563	600	563	518	810	810	585	1350	1420	1440	1530
Hind foot pad	315	405	370	585	583	518	810	675	675	900	630	630	720
Paraumbilical	324	315	338	405	405	495	675	495	585	850	630	990	495
Planum nasale	270	225	243	315	350	540	608	653	540	675	630	900	840
Margin of teat	315	405	270	315	293	450	653	495	675	810	585	1080	810
Fold of flank	225	293	270	315	360	360	315	540	720	720	585	540	810

[a]The dermis of the eyelid, lip and ear were not clearly defined and could not be measured.

increased from approximately 700 microns at birth to 1500
microns in thickness at 28 weeks of age. The paraumbilical
region, margin of teat, fold of flank, foot pads, and planum
nasale were all quite similar. They increased from about
300 microns in thickness at birth to about 900 microns at
28 weeks of age.

In all areas studied, it was found that the collagenous
fiber bundles increased in size and number with an increase
in age (see Table 8). At birth, the collagenous fiber
bundles were found to average 3 to 4 microns in diameter.
With an increase in age, there was a gradual increase in
these bundles so that at 28 weeks of age they measured 19
to 20 microns in diameter. Concurrent with this increase
in the size of bundles, there was a corresponding decrease
in the size of the spaces between the fiber bundles.

With the increase in age, there was noted a definite
increase in the size and number of elastic fibers in the
dermis in all areas except the foot pads. In all areas,
other than the foot pads, the elastic fibers increased from
small branched filamentous fibers, less than one-half of
one micron in diameter to the thicker undulating fibers of
about 1.5 to 2 microns in diameter as shown on Table 9.
The elastic fibers were not very plentiful. This can easily
be observed in Fig. 27, which shows an area containing the
highest proportion of elastic fibers observed. In the

Table 8. Size of collagenous fibers in the dermis of the skin of various body areas at various ages (values represent the diameter of the collagenous bundles in microns)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	4	6	8	9	13	15	14	15	16	17	16	18	19
Front foot pad	2	3	7	9	14	13	15	14	16	18	16	17	20
Paraumbilical	3	4	5	7	9	8	11	13	14	16	17	20	19
Planum nasale	5	8	13	12	14	17	15	18	15	17	16	17	20

Table 9. Size of elastic fibers in the dermis of the skin of various body areas at various ages (values represent the diameter of the elastic fibers in microns)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	.2	.4	.5	.4	.7	.5	.8	1.2	1.4	1.6	1.8	1.9	2.1
Front foot pad	.3	.5	.6	.4	.3	.5	.7	.8	.0	.8	.0	.5	.3
Paraumbilical	.3	.2	.2	.6	1.0	1.2	.9	1.3	1.4	1.6	1.5	2.0	1.6
Planum nasale	.2	.2	.4	.6	.8	1.0	1.0	1.3	1.6	1.7	1.5	1.3	1.2

deeper portions of the foot pads, there are some thick bundles
of elastic fibers which extend down into the subcutaneous
tissue under the foot pad. These are made up of dense elas-
tic tissue mixed with reticular fibers. There were fine
elastic fibers observed extending from the end of the
arrector pili muscle to the basal layer of the epidermal
cells, as shown in Fig. 26.

In all areas and ages studied, reticular fibers were
observed in the sections prepared with Foot and Menard (1927)
rapid technique of silver impregnation. It was noted that
in the younger material, there were more reticular fibers
in the dermis than in the older ages. At birth, there were
many reticular fibers distributed throughout the dermal
ground substance. With an increase in age, there was a
gradual decrease in the number of reticular fibers. At
four weeks of age, there was only a reticular network
remaining under the basal layer of the epidermis and around
the hair follicles, glands, arrector pili muscles, and blood
vessels as shown on Table 10 and Figs. 28 and 29.

The reticular network was most concentrated under the
mucous membrane of the conjunctiva, lip, and vulva (Fig.
31). In all areas, the reticular fibers interdigitated be-
tween the basal cells of the epidermis and were apparently
continuous with the collagenous fibers of the dermis (Figs.

Table 10. Concentration of reticular fibers in the dermis of the skin of various body areas at various ages (values represent the concentration of reticular fibers as indicated below*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	40	40	30	20	10	10	10	10	10	10	10	10	10
Front foot pad	40	30	20	20	10	10	10	10	10	10	10	10	10
Paraumbilical	40	30	20	10	10	10	10	10	10	10	10	10	10
Planum nasale	30	30	10	10	10	10	10	10	10	10	10	10	10

*40 - Very many reticular fibers distributed throughout the dermal ground substance
30 - Many reticular fibers distributed throughout the dermal ground substance
20 - Few reticular fibers distributed throughout the dermal ground substance
10 - Very few reticular fibers in dermal ground substance mostly concentrated around the hair follicles, glands, arrector pili muscles, and under the epidermis

30, 32, 33, and 34).

In the skin studied from birth, there were numerous nuclei in the connective tissue of the dermis. As the age increased, the number of nuclei in a given area decreased, as can be seen on Table 11. The nuclei counted in one half the microscopic field on oil immersion decreased from 65 at birth to 30 at 28 weeks of age.

In the material prepared by the Gomori technique for alkaline phosphatase, reactions were noted in the nuclei of the fibroblasts of the dermis and in the endothelial cells of the blood vessels and capillaries of the dermis. Table 12 shows the distribution of the enzyme, alkaline phosphatase, in the dermis of the various body areas at the various ages. In the interscapular and the paraumbilical areas, alkaline phosphatase activity was observed only during the first two weeks of age. In the skin from dogs older than this, no activity was observed. In the thick skin, like the foot pad and the planum nasale, alkaline phosphatase activity was observed for all ages, especially in the dermal papillae, in which the nuclei of fibroblasts and the capillary endothelium reacted quite consistently.

There was no glycogen observed in the dermis of any of the tissue studied with the exception of a few instances where it was observed in the walls of blood vessels in the form of very fine granules. These blood vessels were

Table 11. Comparative number of nuclei in the dermis of the skin of various body areas at various ages (values represent the number of nuclei counted in one-half of an oil immersion field)

	Birth						Age in weeks						
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	75	68	55	47	41	39	41	32	33	27	34	21	20
Front food pad	66	75	79	65	66	55	57	47	35	35	38	32	42
Paraumbilical	60	53	61	60	45	32	42	41	30	34	29	24	29
Planum nasale	69	75	72	58	60	59	45	50	37	47	53	34	35

Table 12. Distribution of alkaline phosphatase in the dermis of the skin of various body areas at various ages (values represent the concentration of alkaline phosphatase as indicated below*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	30	20	10	0	0	0	0	0	0	0	0	0	0
Front foot pad	30	30	30	30	30	20	30	30	20	20	20	40	20
Paraumbilical	30	20	30	10	0	0	0	0	0	10	0	0	0
Planum nasale	30	20	20	40	10	20	10	30	30	20	30	30	30

*40 - Very intense in fibroblast nuclei and capillary endothelium
 30 - Intense in fibroblast nuclei and capillary endothelium
 20 - Moderate amount of alkaline phosphatase in fibroblasts and endothelium
 10 - Slight amount of alkaline phosphatase in fibroblasts and endothelium

usually located in the deeper portion of the dermis.

Lamellar corpuscles were observed in the subcutaneous tissue of the foot pads just below the dermis. They were only found in the four and 12 week old specimen, Fig. 35 and Fig. 36.

C. Hair Follicles

The hair follicles were found to develop from simple follicles (each follicle containing a single hair) at birth to a compound follicle (each follicle containing as many as 7 to 10 hairs) at 28 weeks. Table 13 shows the results of hairs counted per follicle in several representative areas at various ages. It is obvious from this table and from a comparison of Figs. 37, 38, 39, 40 and 4, 6, 8 that the number of hairs per follicle gradually increased concurrently with an increase in age. The additional hairs developed in accessory follicles which arose as buds from the original single follicle. The multiple hairs in the follicle diverged from one another in the deeper layers of the dermis and branched out into separate follicles, where the hairs originated from individual hair bulbs.

In the skin of the margin of the ear, it was noted that immature developing hair follicles were present. These may be seen in Fig. 41 as long epidermal pegs extending down

Table 13. Number of hairs emerging from a single follicle orifice in the various body areas at various ages

						Age in weeks							
	Birth	1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	1	1	1-2	1-3	1-3	3-5	3-5	4-5	4-5	3-6	4-7	5-8	6-9
Paraumbilical	1	1-2	1-2	2	2-3	4	3-5	3-4	5	4-6	7-8	7-9	7-9
Margin of teat	1	1-2	1	2	1-3	3	5	2-5	3-4	4-6	7-8	7-9	7-9
Fold of flank	1	1	1-2	1-2	3	4	3-4	2-5	3-4	3-5	7	8-10	7-10

into the dermis. The hair matrix and dermal papillae have not yet developed in these follicles.

Tactile hairs were observed in the sections of the lip. The connective tissue portion of the follicle supporting these hairs was highly developed. A blood sinus, lined with endothelium, was observed between the outer and inner dermal sheaths. Because of this, these hairs are referred to as sinus hairs. They have been described by Trautmann and Fiebiger (1948). Fig. 42 shows a tangential section of a sinus hair.

Club hairs and resting hair follicles have been described by Rothman (1954) representing the inactive part of the human hair cycle. No evidence of club hairs or resting follicles was observed in any of the areas studied.

Table 14 and Figs. 43 and 55 show that large quantities of glycogen granules were present in the cytoplasm of the cells of the middle one third of the outer root sheath in all ages. No glycogen was present in the hair matrix cells and dermal papilla except during the first week of age. Small quantities of glycogen were also observed in the medulla of the hair shafts in most instances.

The highest concentration of alkaline phosphatase was observed in the dermal papilla of the hair bulb as shown on Table 15 and Fig. 44. No dermal papillae of hair follicles were observed which did not contain alkaline phosphatase.

Table 14. Distribution of glycogen in the hair follicles of the skin of the interscapular area at various ages (values represent the concentration of glycogen granules as described below*)

	Birth	Age in weeks											
		1	2	3	4	6	8	10	12	16	20	24	28
Upper one third	10	10	10	10	0	5	5	5	5	10	5	5	0
Middle one third	40	40	40	40	20	40	40	40	40	30	30	30	40
Lower one third	10	10	10	10	5	10	10	10	5	10	10	10	10
Hair matrix	5	5	0	0	0	0	0	0	0	0	0	0	0
Dermal papilla	5	5	0	0	0	0	0	0	0	0	0	0	0
Hair shaft medulla	5	5	5	0	0	0	5	5	10	5	5	5	5

* 5 - Very slight
  10 - Slight
  20 - Moderate
  30 - Intense
  40 - Very intense

Table 15. Distribution of alkaline phosphatase in the hair follicles of the skin of the interscapular area at various ages (values represent the concentration of alkaline phosphatase as shown below*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Upper one third[a]	5	5	5	5	0	0	0	0	0	0	5	5	0
Middle one third[a]	5	5	5	5	0	0	10	10	0	5	5	5	5
Lower one third[b]	10	10	10	10	0	5	5	5	0	5	10	10	10
Hair matrix[b]	20	20	20	20	10	20	20	20	10	10	20	20	30
Dermal papilla[c]	40	40	40	40	20	30	20	30	30	30	30	40	30

* 5 - Very slight
 10 - Slight
 20 - Moderate
 30 - Intense
 40 - Very intense

[a]Phosphatase located only in nucleoli
[b]Alkaline phosphatase located in nuclei
[c]Alkaline phosphatase located in both nucleus and cytoplasm

The nuclei of the hair matrix cells showed less evidence of alkaline phosphatase activity than the dermal papillae. The nucleoli of the middle and upper third of the root sheath showed a slight amount of alkaline phosphatase activity.

D. Sweat Glands

The heights of the secretory epithelium of the sweat glands of various body areas were measured with an eyepiece micrometer, Table 16. The merocrine glands of the foot pads had the lowest epithelium. There was considerable variation in the height of the glandular epithelium of the apocrine glands because of the variation in the stage of secretion. Fig. 48 shows that the actively secreting glandular epithelium is low cuboidal or flat in appearance. The presecretory stage is high columnar with apical knob-like processes of cytoplasm extending into the lumen of the tubule. The merocrine gland epithelium, of the foot pads, varied from 8 to 12 microns in height. The apocrine gland epithelium of the hairy skin varied from 10 to 25 microns in height. The greatest secretory activity was observed in the apocrine glands of the margin of the lip and vulva, and in the thin skin of the ventral part of the body. The measurement of the diameter of the glandular tubules varied

Table 16. Height of secretory epithelium of sweat glands of various body areas at various ages (values represent direct measurements with an eyepiece micrometer, expressed in microns)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	15	18	10	16	12	12	10	15	15	22	18	15	20
Front foot pad	10	10	12	8	9	10	10	10	10	12	10	12	11
Paraumbilical	15	25	18	10	12	15	10	12	14	10	12	15	16
Margin of teat	15	25	25	12	18	17	10	25	10	22	16	15	15
Fold of flank	10	10	19	15	15	12	25	25	8	18	15	12	15
Margin of lip	10	18	15	20	22	[a]							
Vulva or prepuce	18	23	18	20	20	[a]							

[a]Material was not collected from the lip and vulva above the age of 4 weeks

from 25 to 35 microns in the foot pad. In the apocrine glands, the diameter of the tubules varied from 30 to 90 microns in diameter. These measurements are shown on Table 17. The larger apocrine tubules appeared to be cyst-like dilatations. There was considerable variation in the diameter of the tubules because of the different stages of secretion. Figs. 45 and 46 show excretory ducts of apocrine glands emptying into a hair follicle above the level of the sebaceous glands.

Table 18 shows the comparative amounts of glycogen in the cytoplasm of the glandular epithelium in the various body areas for the various ages. Only traces of glycogen were observed in the apocrine glands near the junction of the secretory and excretory ducts. The merocrine glands of the foot pads showed the greatest concentration of glycogen, as shown in Fig. 49.

Table 19 shows the relative distribution of alkaline phosphatase in the sweat glands of the interscapular area and of the foot pad at various ages. The concentration of alkaline phosphatase was similar in all ages. The greatest concentration was observed in the myoepithelial cells, Fig. 50. The secretory cell nuclei, also, contained alkaline phosphatase to a lesser degree and the secretory cell cytoplasm showed no evidence of alkaline phosphatase activity. The reaction for alkaline phosphatase was greater in the

Table 17. Diameter of glandular tubules of sweat glands of various body areas at various ages (values represent measurements with an eyepiece micrometer, expressed in microns)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	50	50	40	45	33	40	29	38	33	70	60	65	55
Front foot pad	25	33	32	30	25	33	31	30	31	33	35	35	33
Paraumbilical	40	90	40	43	40	43	30	40	45	45	50	50	40
Margin of teat	45	57	50	30	40	45	32	72	35	55	49	53	42
Fold of flank	35	63	45	55	35	35	65	60	38	45	90	82	60
Lip margin	40	80	60	65	60	a							
Vulva or prepuce	70	70	65	70	75	a							

[a]Material was not collected from the lip and vulva above the age of 4 weeks

Table 18. Concentration of glycogen granules in the cytoplasm of the glandular epithelial cells of the sweat glands of the various body areas at various ages (values represent the concentration of glycogen as indicated below*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular	5	10	0	0	0	0	0	0	0	0	0	0	0
Front foot pad	20	20	30	20	0	0	5	5	5	0	0	0	0
Sacral	0	0	0	0	0	0	10	0	10	0	0	0	0
Hind foot pad	20	20	20	20	10	10	10	10	5	5	0	5	0
Lip	0	0	0	0	0	a							
Vulva	0	0	0	0	0	a							

* 5 - Very slight
 10 - Slight
 20 - Moderate
 30 - Intense

[a]Material was not collected from the lip and vulva above the age of 4 weeks

Table 19. Distribution of alkaline phosphatase in the sweat glands of interscapular area and foot pad at various ages (values represent the concentration of alkaline phosphatase as indicated below*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular													
Myoepithelial cells	30	30	30	40	20	30	20	30	30	30	30	20	20
Secretory cell nuclei	20	20	20	30	10	20	10	20	20	20	20	10	10
Secretory cytoplasm	0	0	0	0	0	0	0	0	0	0	0	0	0
Foot pad													
Myoepithelial cells	30	20	30	20	20	30	20	30	20	20	20	20	20
Secretory cell nuclei	20	10	20	10	30	30	30	20	20	20	20	30	30
Secretory cytoplasm	10	0	0	0	0	0	0	0	0	0	0	0	0

*10 - Slight
20 - Moderate
30 - Intense
40 - Very intense

apocrine glands than in the merocrine glands. The reaction
for both glycogen and alkaline phosphatase in the merocrine
glands was irregular or spotty. Fig. 51 shows the alkaline
phosphatase reaction in the merocrine gland tubules. Fig.
52 shows a picture of typical merocrine glands stained with
hematoxylin and eosin.

E.   Sebaceous Glands

Table 20 shows the size of sebaceous glands at the
various ages. The size of the sebaceous glands in the
interscapular region varies from 30 to 50 microns to 80 to
250 microns with a gradual increase occurring with the
increase in age. In the paraumbilical region, this correla-
tion with the increase in age was not apparent. The largest
sebaceous glands were observed at the muco-cutaneous junc-
tions of the lips, vulva, and eyelids.

Alkaline phosphatase was observed quite consistently
in the nuclei and cytoplasm of the peripheral cells of the
sebaceous glands as shown in Fig. 53. The central cells
reacted only in their nuclei, from a slight to a moderate
degree.

Without exception, there was no glycogen observed in
sebaceous glands in any of the tissue examined in this work.
Fig. 54 shows a picture of Meibomian gland of the eyelid,

Table 20. Comparative size of sebaceous glands in the dermis of the skin of various body areas at various ages (values represent measurements with eyepiece micrometer, expressed in microns)

	Birth						Age in weeks						
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular													
Largest	50	90	45	40	60	80	110	60	115	160	70	140	250
Smallest	30	50	35	35	20	25	70	30	50	90	70	40	80
Paraumbilical													
Largest	45	100	40	45	70	40	80	60	110	100	80	60	50
Smallest	40	40	35	40	18	20	40	30	78	38	60	45	33
Lip margin	270	310	180	180	270	[a]							
Vulva or prepuce	350	180	200	300	200	[a]							

[a]Material was not collected from the lip and vulva above the age of 4 weeks

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Table 21. Distribution of alkaline phosphatase in the sebaceous glands of the dermis of the skin of the sacral region at various ages (values represent degree of concentration of reaction as shown below*)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Nuclei of peripheral cells	30	20	30	10	30	30	30	30	20	30	40	40	30
Cytoplasm of peripheral cells	0	10	0	0	10	0	10	10	0	0	10	20	0
Nuclei of central cells	10	10	10	10	10	10	10	10	10	10	20	20	10
Cytoplasm of central cells	0	0	0	0	0	0	0	0	0	0	0	0	0

*10 - Slight
 20 - Moderate
 30 - Intense
 40 - Very intense

which is a large modified sebaceous gland.

F. Arrector Pili Muscles

The largest arrector pili muscles were observed in the sacral and interscapular regions. The arrector pili muscles of the skin of the ventral surfaces of the body were found with difficulty and were very small and indistinct, Table 22.

The arrector pili muscles showed a slight reaction for alkaline phosphatase, only in the nuclei, with no correlation with age as shown on Table 23. Glycogen was observed in the cytoplasm of the smooth muscle cells of the arrector pili muscles from all areas and in all ages. Fig. 55 and Fig. 43 show pictures of glycogen in the arrector pili muscles.

Table 22. Size of arrector pili muscles in the dermis of the skin of various body areas at various ages (values represent measurements taken with an eyepiece micrometer, expressed in microns)

| | Birth | Age in weeks | | | | | | | | | | | |
		1	2	3	4	6	8	10	12	16	20	24	28
Interscapular													
Largest	40	30	15	20	40	15	45	70	40	120	50	140	115
Smallest	10	10	9	11	18	9	19	38	15	44	31	42	62
Paraumbilical	-*	-	-	-	-	-	-	7	-	-	-	-	-

* - indicates that no arrector pili muscle was found in the section examined for the age and area

Table 23. Distribution of alkaline phosphatase and glycogen in the arrector
pili muscle of the dermis of the skin of the sacral region at
various ages (values represent degree of reaction as shown
below*)

| | | | | | | | Age in weeks | | | | | | |
	Birth	1	2	3	4	6	8	10	12	16	20	24	28
Glycogen	10	20	30	20	20	20	20	30	10	20	10	10	10
Alkaline phosphatase													
Nuclei	5	5	5	0	10	10	10	10	0	10	10	10	0
Cytoplasm	0	0	0	0	0	0	0	0	0	0	0	0	0

* 5 – Very slight
10 – Slight
20 – Moderate
30 – Intense

V. DISCUSSION

A. Epidermis

Clear cells in the epidermis have been reported in the
rat and man. Andrew and Andrew (1949) theorize that the
clear cells are lymphocytes, which migrate into the epi-
dermis, where they differentiate into epidermal cells.
Andrew and Andrew (1954) classified cells which were not
typical epidermal cells into three groups: clear cells,
lymphocytes, and intermediate cells. The clear cells
observed in this work on the skin of dogs did not conform
with their description of any one of the three groups. The
clear cells observed in this work on dog skin contained
nuclei which stained intensely basophilic and they were
observed in the process of cell division, especially telo-
phase. Since mitotic figures in the epidermis of rat and
human skin have been illustrated by Andrew and Andrew (1954)
and Rothman (1954) as cells with transparent cytoplasm and
dark staining nuclear material in various stages of cell
division, all of the clear cells observed in canine skin in
this work were regarded as representing mitotic figures.

The mitotic cells in this study were observed in the
stratum cylindricum, for the most part, rather than in the
stratum spinosum, as was reported for the human scalp by

Thuringer (1924). Thuringer and Cooper (1950) reported
that there was an increase in mitoses with advancing age
and the majority of mitotic figures were in the stratum
spinosum. Cooper and Schiff (1938) presented evidence that
mitotic rate is higher at night and lower in the day time
in human skin. In the dog skin examined in this study, the
rate of mitosis was higher in the thick skin of the planum
nasale and foot pad than in the thin skin. No attempt was
made to study any difference in mitotic rate at night or
in the day time and no correlation was evident between
mitotic activity and age.

Another theory on the clear cells was cited by Ham
(1953). He cited the work of Masson (1948, 1935) and
Becker (1942) who postulate that clear cells are of neural
crest origin and that, with the proper stimulation, they
manufacture melanin pigment.

Bullough and Eisa (1950) correlated the diurnal vari-
ation in mitotic activity with a corresponding variation in
tissue glycogen in the mouse. Bradfield (1951) postulated
that glycogen is necessary in the epidermal cells to provide
the energy for the synthesis of keratin. Glycogen has been
reported in the epidermis of man by Montagna et al. (1952) and
Scothorne and Scothorne (1953). Wislocki et al. (1951) re-
ported the occurrence of glycogen in the mucous membrane and
skin of the monkey and Tribby (1943) reported glycogen in the

vaginal epithelium of the guinea pig. Glycogen was observed
in the mucous membranes of the dogs studied in this work in
great quantities. Very small quantities of glycogen were
observed in the epidermis of the thin skin. These observa-
tions conform to the findings of Montagna et al. (1952) and
Tribby (1943) in primates. It was noted that the thick skin
of the planum nasale and the foot pads contained more glyco-
gen and alkaline phosphatase than the thin skin. This would
tend to indicate that the concentration of glycogen and alka-
line phosphatase is greatest in the areas of epidermis where
there is the greatest mitotic activity.

Very little alkaline phosphatase was found in the epi-
dermis of the thin skin of the dog skin studied. This agrees
with the findings of Bourne and MacKinnon (1943), who ob-
served slight alkaline phosphatase activity in the stratum
granulosum of guinea pig skin.

B. Dermis

The development of the dermis followed a definite
pattern. Coinciding with a decrease of reticular fibers,
there was an increase in the size of the bundles of col-
lagenous fibers, with a concurrent decrease in the number
of nuclei. This has been observed in other species.
Yuditskaya (1947) reported that the only fibrous protein

recovered from cattle embryo skin was reticulin. Gross
(1950) reported an overwhelming predominance of argyrophilic
reticulin in the newborn rat with a gradual transition to
typical collagen with aging. This evidence supports the
theories that reticulin is an early developmental stage of
collagen or that reticulin is basically collagen. Fig. 33
shows that the reticular fibers appear to be continuous with
collagenous bundles. This was observed by Odland (1950) in
human epidermis. The observation that alkaline phosphatase
was more plentiful in fibroblast nuclei in the younger skin
would tend to indicate that alkaline phosphatase is related
to collagen formation. Gold and Gould (1951) found this
to be true in healing wounds. Robertson et al. (1950) on
the contrary were unable to demonstrate alkaline phosphatase
in artificially produced areas of collagen formation.

The elastic fibers observed in this work were not as
plentiful as those described in the adult human skin by
Dick (1947). In the skin of children Dick reported few
elastic fibers. The observations of this work on the dog
agree with Dick's that elastic tissue of the dermis increases
with normal growth and development.

It was observed that the dermis increased in thickness
with an increase in age. This was accompanied by an increase
in the number and size of the collagenous bundles. Since
the reticular fibers and fibroblasts were most numerous

at birth and decreased in number with an increase in age,
it is logical to conclude that the reticular fibers are
developmentally related to collagenous fibers. The observa-
tion that collagenous fibers were continuous with reticular
fibers supports this conclusion.

C. Hair Follicles

Considerable information is available on the hair
cycle of rat, mouse, ferret, and human skin. Rothman (1954)
divided the hair cycle into three stages: (1) the anagen
stage, the phase of active proliferation; (2) the catagen
stage, a transitional phase, characterized by cessation of
active proliferation and by club hair formation; and (3)
the telogen (final) or resting stage. After this stage,
the hair cycle starts all over again. Chase and co-workers
(1953) described variations in the thickness of the epidermis
and corium which are directly related to the hair cycle in
the mouse. The development of the germ of the hair fol-
licle in rat embryos was reported by Fraser (1928). Butcher
(1934) found the hair cycle of the rat to be a 35-day cycle
with 17 days of growth and 17 days of rest. He found that
five days after birth, the first hair coat comes in and at
35 days after birth the second hair coat comes in. The
new hair does not push out the old, so two hairs remain in

the follicle. At 84 days after birth, he reported three hairs may be observed in a follicle. There are never more than three hairs in a follicle of rat skin.

Apparently the hair cycle of the dog skin is much different because of the compound nature of the follicle and the seasonal manner in which the dog sheds its hair coat. One could postulate that instead of the first hair becoming detached and a new hair bulb developing as occurs in the rat, that in the dog, the first hair remains attached and actively growing even after the second hair develops. The second hair develops as a bud or a branch from the main follicle, resulting in two hairs being present in the follicle. Then as the age increases and additional hairs are added, the follicle complex becomes larger until the condition of 28 weeks is reached, where there are 7 to 10 hairs embraced in a single hair follicle orifice.

Since no resting follicles were observed in the material studied, it is assumed that during the period from birth to 28 weeks of age the hair follicle of the dog does not make a complete cycle. Bissonnette (1935) reported that in the ferret the hair cycle is related to the anterior hypophysis and to light cycles. He found that the spring loss of winter coat in ferrets occurs five to six weeks after the beginning of estrous or two to five days before parturition. Hypophysectomy was followed by loss of some

hair but lead to suppression of the sexual cycle and hair cycle.

None of the dogs used in this study were observed to show any manifestations of estrous. If the hair cycle is related to the estrous cycle or sexual maturity of the dog, during shedding, one would expect to find the resting follicles in the skin after estrous. This would explain why no resting follicles were observed in this work. Additional research on the hair cycle of sexually mature dogs is necessary before definite conclusions can be made.

Histochemical studies of human skin have been made by Montagna, Chase, and Lobitz (1952), in which they found glycogen to be most abundant in the external sheath of active hair follicles. The only other regions of the follicle in which they found glycogen, were the medulla and the cuticle cells of the cortex. Usually, the internal sheath, cortex, and matrix cells of the hair bulb contained none. Johnson and Bevelander (1946) studied the distribution of alkaline phosphatase and glycogen in the developing hair follicle of the rat. They found both alkaline phosphatase and glycogen were present in the primitive hair bud. During the later stages of dif-

ferentiation and growth, they observed that the spatial
relations between phosphatase and glycogen correlated
well with the path of glucose entry into the tissues.
Johnson, Butcher, and Bevelander (1945) studied the dis-
tribution of alkaline phosphatase in the cyclic growth
of the rat hair follicle. They found the enzyme was
present to a very slight degree in the early epithelial
bud, becoming very abundant in the mesodermal papilla as
the follicle matured and elaborated the hair. With the
tapering off of the cellular activity the quiescent period
of the hair cycle was characterized by a negligible
amount of alkaline phosphatase. Bollinger and McDonald
(1949) investigated the glycogen in skin and hair of man,
sheep, rabbit, and phalanger. They observed granules
in the outer root sheath, which stained selectively with
periodic acid treatment and fuchsin sulphite. Hardy (1952)
studied the histochemistry of hair follicles in the mouse
and reported the presence of glycogen in the outer root
sheath and alkaline phosphatase in the dermal papilla
of the active hair follicle. The findings in this work
show that the distribution of glycogen and alkaline phos-
phatase in the dog hair follicle is similar to that re-
ported by Hardy (1952) in the mouse.

Alkaline phosphatase was present in the dermal papillae
of all hair follicles observed in this study. According
to Johnson et al. (1945) this indicates that these follicles
were in an active growing phase rather than a quiescent
phase.

D.   Sweat Glands

Montagna, Chase, and Lobitz (1952) reported that in
eccrine (merocrine) sweat glands of human skin, some secre-
tory cells are rich in glycogen while others are not.
Bunting, Wislocki, and Dempsey (1948) found that glycogen
was abundant in the secretory tubules of the eccrine glands,
but has not been found in apocrine glands.

Fisher and Glick (1947) reported alkaline phosphatase
activity in the endothelial cells and sweat glands of human
skin. They observed that the enzyme appeared to be concen-
trated at the cell walls and in the nuclei.

The observations made on dog skin that the merocrine
glands of the foot pads contain the greatest concentration
of glycogen and the apocrine glands contained only traces
agrees with the findings of Montagna, Chase, and Lobitz
(1952) and Bunting, Wislocki, and Dempsey (1948) in human
skin.

In dogs of all ages, alkaline phosphatase activity was observed in the nuclei of the secretory cells and the nuclei and myoepithelial cells of both merocrine and apocrine glands. Since phosphatase indicates cellular activity, this would lead to the conclusion that the sweat glands are actively secreting from the time of birth through 28 weeks of age.

E. Sebaceous Glands

Location of alkaline phosphatase in the sebaceous glands of the dog agrees with that reported by Montagna and Noback (1947) in the rat. The size of the sebaceous glands on the dorsal skin increased with age but the alkaline phosphatase activity remained constant for all ages.

F. Technique for Acid and Alkaline Phosphatase

The histochemical method for alkaline phosphatase, originally devised by Gomori (1939) and by Takamatsu (1939) has been widely employed in histology. The technique is based on the principle that if sections are incubated with glycerophosphate at an alkaline reaction in the presence of Ca ions, the phosphate ions liberated will be precipitated at the site of formation as insoluble Ca phosphate. The

latter is then transformed in a second step into black cobalt sulfide. With this method, the amount and distribution of phosphatase have been observed to change in a number of physiological and pathological states. The method seems to be of value since it provides an index by which the activity of the tissues may be measured. Danielli (1953), Martin and Jacoby (1949), and Gomori (1951) have made critical studies of the phosphatase technique. These workers suggest that nuclear staining in areas of high activity is an artefact due to the secondary adsorption of Ca phosphate. The evidence for this is that the Azo-dye methods, for the demonstration of alkaline phosphatase, do not show any alkaline phosphatase in cell nuclei. Martin and Jacoby (1949) make the conclusion that a positive result shows the presence of the enzyme, but a negative result does not necessarily demonstrate its absence. They also felt that a positive reaction indicates the presence of enzymes, but that it does not necessarily give its precise location and may even mask histochemically inherently negative structures.

The original histochemical method for acid phosphatase utilizes the hydrolysis of glycerophosphate at pH 5 in the presence of lead ions. Fixation in cold acetone or alcohol have been used (Dempsey and Deane, 1946). It is agreed by most authors that the results of this technique are not

nearly so satisfactory or consistent as those of the method for alkaline phosphatase.

It is recognized that the technique for the demonstration of acid and alkaline phosphatase have definite limitations and this causes considerable doubt as to the value of conclusions that can be drawn from results obtained from these histochemical methods.

The failure to demonstrate the presence of acid phosphatase in this work by no means is enough evidence to conclude that there is no acid phosphatase in canine skin. The presence of alkaline phosphatase in the nuclei of many areas reported in this work may be the results of diffusion artefacts. It is difficult or impossible to come to any conclusions in regard to the significance of these results. The findings here presented on the histochemistry of canine skin compare favorably with results reported on human skin using the same technique. More research should be done on the histochemistry of canine skin so that a more definite insight may be developed into the nature of the intermediary metabolism taking place at cell membranes and boundaries.

VI. SUMMARY

1. Skin samples from 10 or 12 different areas from 13
   normal dogs, ranging in age from birth to 28 weeks,
   were studied microscopically. A total of 140 skin
   areas were studied.

2. Observations have been made on the morphology, location
   of glycogen, and distribution of alkaline phosphatase in
   normal canine skin at various ages.

3. No acid phosphatase was observed in any part of the skin
   at any age.

4. From birth to 28 weeks alkaline phosphatase was observed
   in greatest quantities in the connective tissue hair
   papillae, nuclei, and cytoplasm of cells of the mucous
   membranes, peripheral cells of sebaceous glands, myo-
   epithelial cells of sweat glands, nuclei, and cytoplasm
   of the cells at the depths of epidermal pegs of foot
   pads and planum nasale, fibroblasts and capillary endo-
   thelial cells of the dermal papillae of foot pads and
   planum nasale, nuclei of the hair matrix cells, and
   nuclei of secretory cells of the sweat glands.

5. At all ages alkaline phosphatase was found to be present
   in lesser amounts in the nuclei of the smooth muscle
   cells of arrector pili muscles, nuclei of the central
   sebaceous gland cells, nucleoli of spinosum cells and

keratohyalin granules of the epidermis of the hairy skin.

6. The fibroblasts and capillary endothelial cells of the dermis of the hairy skin contained alkaline phosphatase only during the first three weeks.

7. Glycogen was observed in greatest quantities in the cytoplasm of the epithelial cells of the mucous membranes and middle third of the outer root sheath in all ages studied.

8. The next greatest quantity of glycogen was observed in the cytoplasm of the secretory cells of the merocrine glands of the foot pads and arrector pili smooth muscle cells.

9. During the first three weeks glycogen was present in the foot pad and planum nasale spinosum cells. A few very fine glycogen granules were observed around the hair follicle orifices in the hairy skin epidermis in all ages.

10. No glycogen was observed in the sebaceous glands or connective tissue of the dermis. A trace of glycogen was observed in the secretory cells of the apocrine glands and hair matrix and hair papillae in very young material.

11. The morphology and thickness of the epidermis of the hairy skin was observed to change very little, whereas

the thickness of the epidermis of the foot pads and planum nasale increase steadily with an increase in age.

12. A greater number of mitotic clear cells were observed in the epidermis of the foot pads and planum nasale than in the epidermis of the hairy skin. No correlation was apparent between age and number of mitotic figures in the epidermis per section.

13. Epidermal pegs were not observed in the hairy skin, but they were found to be very extensive in the mucous membranes and foot pads and planum nasale.

14. Thickened papilla-like areas of epidermis were observed in various parts of the skin at all ages. These were called epidermal papillae.

15. With an increase in age, the dermis was observed to increase in thickness. This was accompanied by an increase in the number and size of the elastic fibers and collagenous bundles.

16. Reticular fibers and fibroblasts were observed to be most numerous at birth and decreased in number with an increase in age.

17. Reticular fibers were observed interdigitating between the basal cells of the epidermis and appeared to be continuous with collagenous fibers of the dermis. Fibroblasts were also observed between the basal cells

of the epidermis.

18. The hair follicles were observed to develop from a simple form (each follicle embracing a single hair) at birth to a compound form (each follicle embracing 7-10 hairs) at 28 weeks of age. The additional hairs developed from buds off the original follicle which separated deeply into auxiliary follicles which united superficially with the main follicle.

19. Immature, developing hair follicles were observed on the margin of the ear, during the first week.

20. No resting follicles were observed in any of the skin areas examined.

21. Histological evidence of secretory activity was observed in merocrine and apocrine glands of areas studied at all ages.

22. The excretory ducts of the apocrine glands were observed emptying into the hair follicles above the level of the sebaceous gland.

23. It was observed that sebaceous glands in the inter-scapular area tended to become larger with an increase in age. The largest sebaceous glands were observed around the mucocutaneous junctions.

24. It was observed that the arrector pili muscles of the interscapular area tended to increase in width with

an increase in age. The largest arrector pili muscles
were observed in skin of the dorsal part of the body.

VII. CONCLUSIONS

1. Alkaline phosphatase is present in canine skin in areas of greatest cellular activity.

2. Alkaline phosphatase and glycogen are located in areas of canine skin comparable with those reported in human and rat skin.

3. Clear cells are present in the stratum cylindricum of the epidermis of canine skin and probably represent mitotic figures.

4. The rate of mitosis is greater in the epidermis of the foot pads and planum nasale than in the hairy skin.

5. Epidermal papillae are normally present in the epidermis of the hairy skin of the dog.

6. There is no developmental increase in the thickness of the epidermis in the hairy skin of the dog between birth and 28 weeks of age.

7. There is a normal increase in the thickness of the epidermis of the foot pads and planum nasale and the dermis of all body areas between birth and 28 weeks of age.

8. The hair follicle of the dogs studied developed from a simple follicle at birth to a compound follicle at maturity.

9. One entire hair cycle was not completed during the first six months of the life of the dogs studied.

10. Additional research should be done on the hair follicle of the dog before definite conclusions may be made concerning the hair cycle.

11. At the time of birth the sweat glands of canine skin are well developed and functional.

12. Reticular fibers are closely related to collagenous fibers, developmentally and morphologically.

13. At the time of birth the sebaceous glands of canine skin are well developed and functional.

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IX. ACKNOWLEDGMENTS

X. PHOTOGRAPHS

Fig. 1. Areas from which skin was studied

        Fig. 1a. Lateral view
        Fig. 1b. Ventral view

1. Interscapular area (dorsal shoulder region)
2. Front foot pad (metacarpal)
3. Sacral area (at base of tail)
4. Rear foot pad (metatarsal)
5. Paraumbilical area (ventral abdomen)
6. Tip of nose (planum nasale)
7. Base of teat (hairy margin)
8. Eyelid (upper)
9. Inside fold of flank (inguinal region)
10. External ear flap (pinna)
11. Margin of lip (upper)
12. Margin of vulva or prepuce
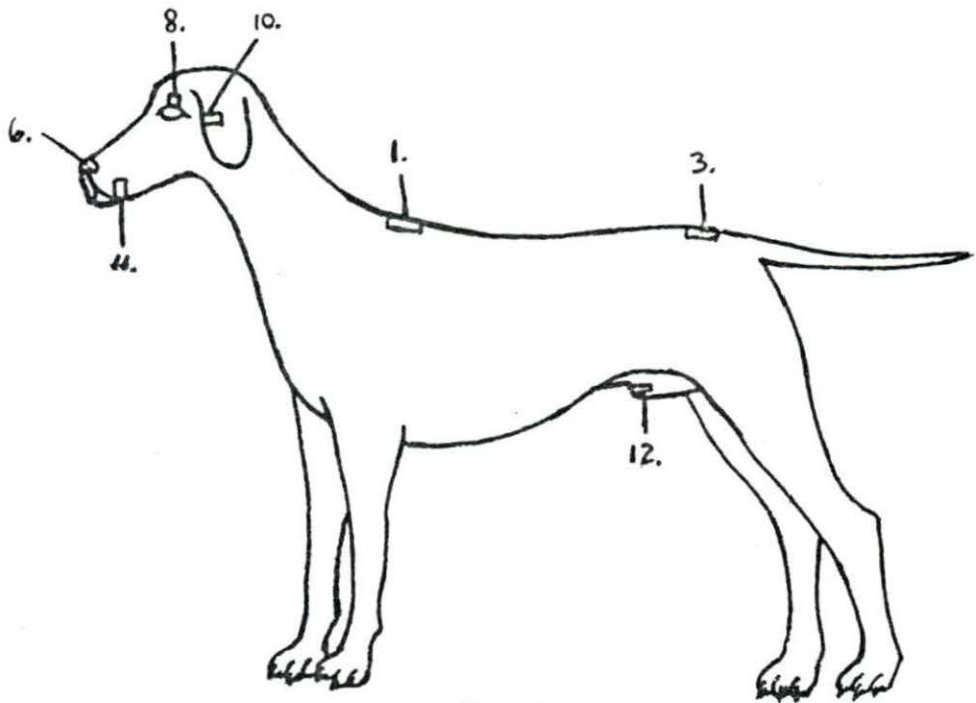
Fig. 1a
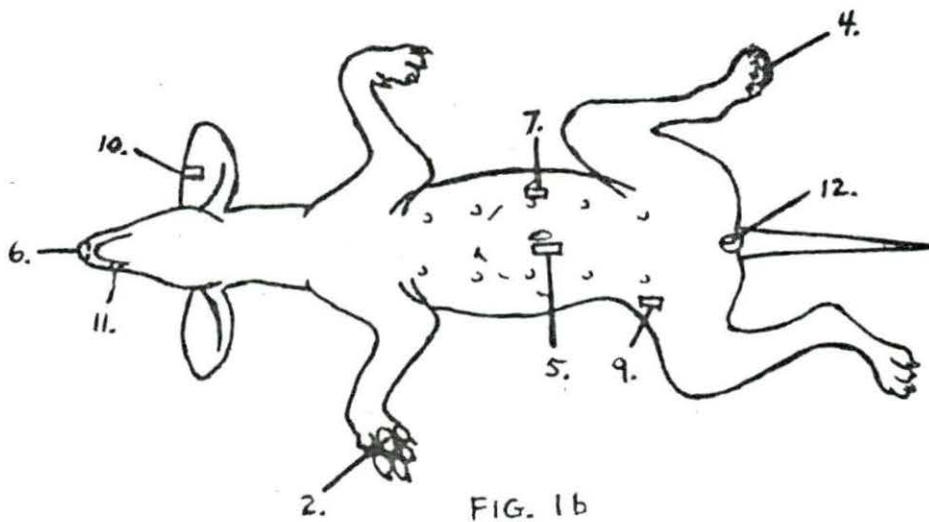
Fig. 1b

FIG. 1

Fig. 2. Nasal skin at birth - Section of skin from the
planum nasale of a dog shortly after birth,
fixed in formalin and stained with Harris' hema-
toxylin and eosin Y, 90X magnification; note
the undulation of the basal layer of epithelial
cells and the thickness of the epidermis as
compared with Fig. 3

    a. Stratum corneum
    b. Stratum spinosum
    c. Stratum cylindricum
    d. Dermis
    e. Undulation of basal layer to
        form epidermal pegs

Fig. 3. Nasal skin at 28 weeks - Section of skin from the
planum nasale of a dog 28 weeks of age, fixed in
10 per cent formalin and stained with Harris'
hematoxylin and eosin Y, 90X magnification; note
the thickness of the epidermis as compared to
Fig. 2, for contrast between the thickness of
the nasal epidermis of birth and 28 weeks of
age

    a. Nucleated stratum corneum
    b. Stratum spinosum
    c. Stratum cylindricum
    d. Dermis
    e. Epidermal peg
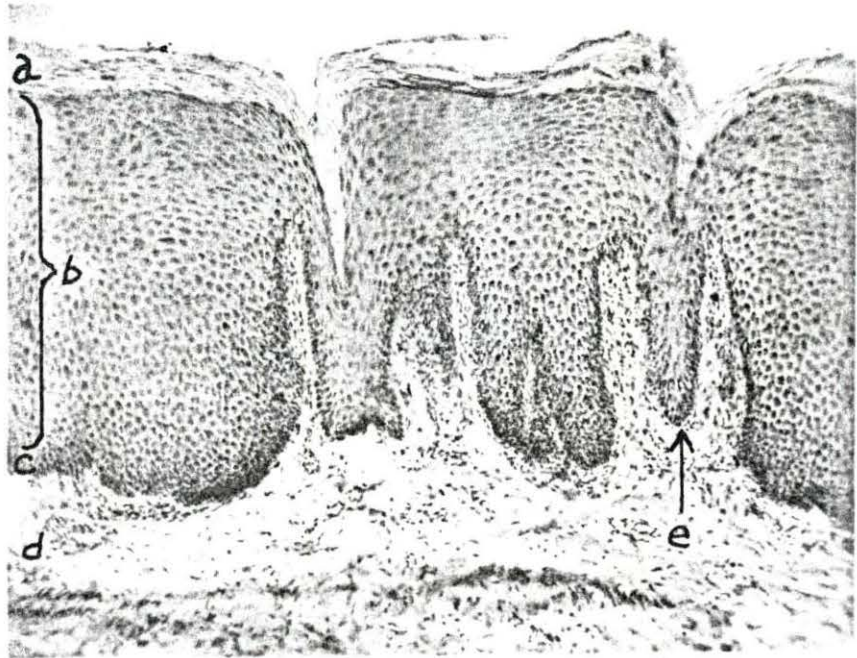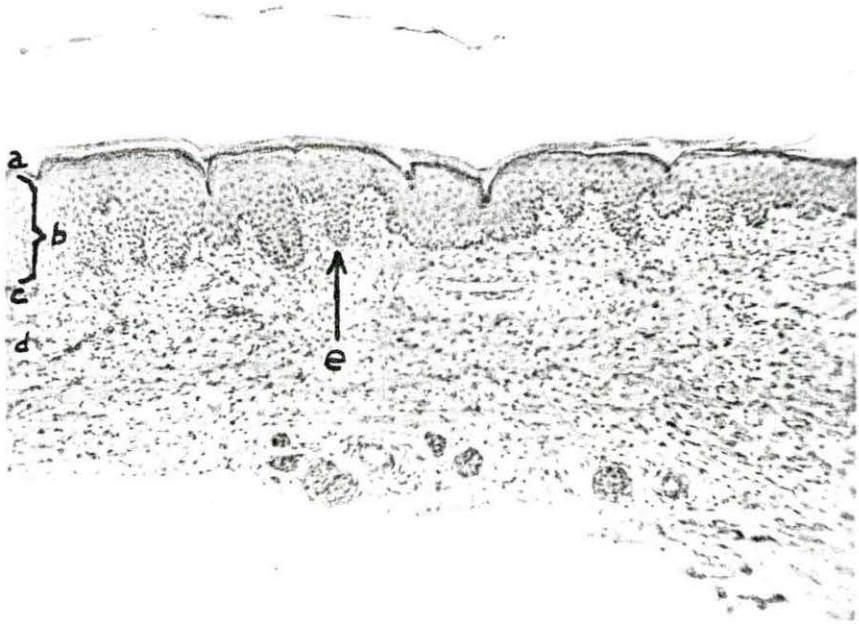
Fig. 4.   Hairy skin at birth - Section of heavily haired
          skin from the interscapular area taken from a
          dog shortly after birth, fixed in 80 per cent
          alcohol and stained with Harris' hematoxylin
          and eosin Y, 35X magnification

          a. Epidermis
          b. Dermis
          c. Subcutis
          d. Hair follicle containing one hair

Fig. 5.   Foot pad at birth - Section of skin from the
          front foot pad taken from a dog shortly after
          birth, fixed in 80 per cent alcohol and stained
          with Harris' hematoxylin and eosin Y, 35X
          magnification; sweat glands may be observed
          in the subcutaneous tissue

          a. Epidermis
          b. Dermis
          c. Subcutis
          d. Merocrine glands

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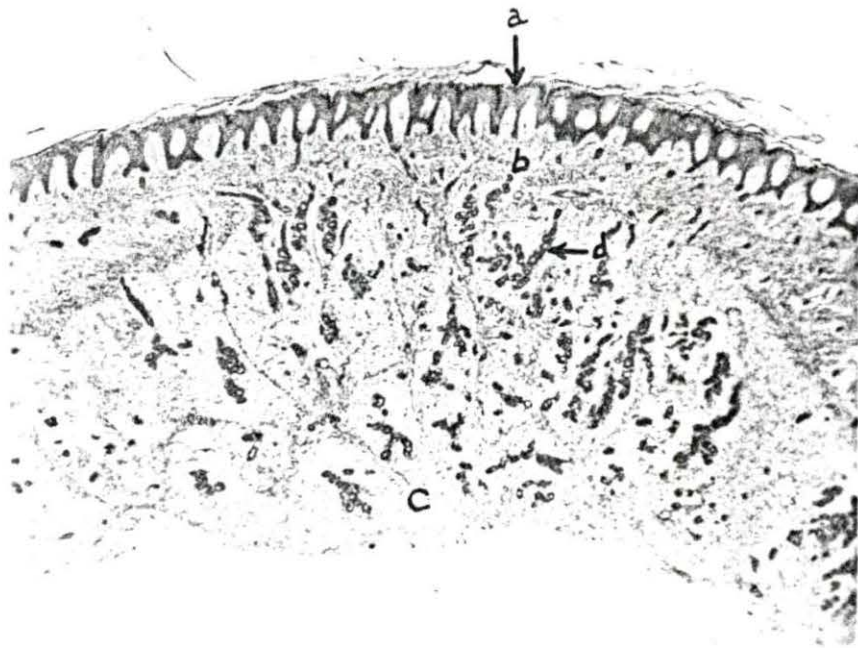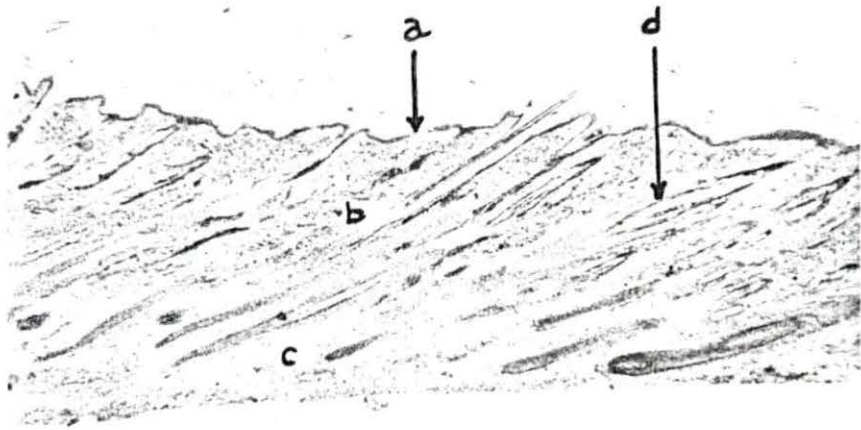
Fig. 6. Hairy skin at eight weeks - Section of heavily
         haired skin from the interscapular area of a
         dog eight weeks of age, fixed in 10 per cent
         formalin and stained with Harris' hematoxylin
         and eosin Y, 35X magnification

              a. Epidermis
              b. Dermis
              c. Subcutis
   d. Hair follicle containing three hairs

Fig. 7. Foot pad at eight weeks - Section of skin from
         the front foot pad of a dog eight weeks of age,
         fixed in 80 per cent alcohol and stained with
         Harris' hematoxylin and eosin Y, 35X magnifica-
         tion

              a. Stratum corneum and lucidum
              b. Stratum granulosum, spinosum and
                  cylindricum
              c. Dermal papilla
              d. Merocrine sweat gland
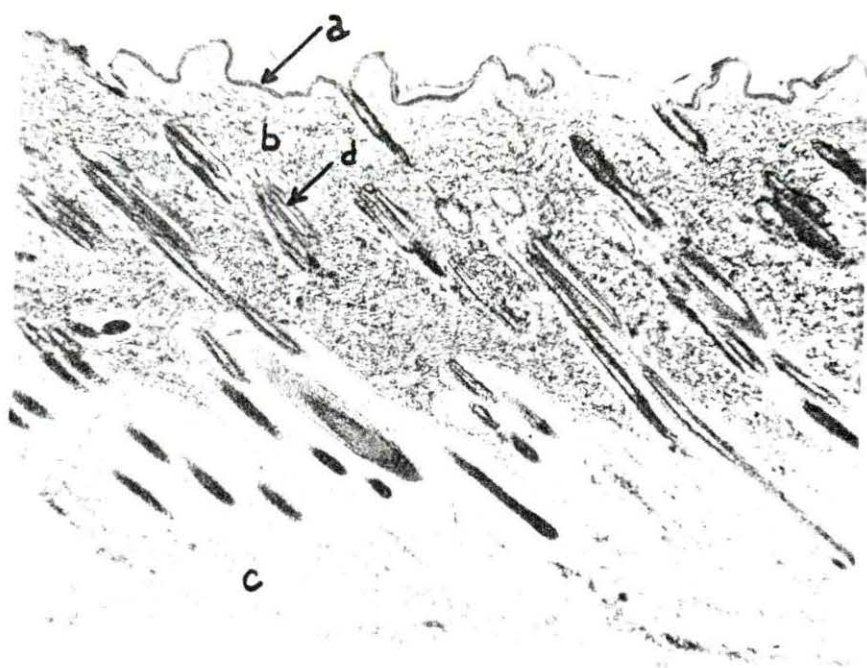
Fig. 8. Hairy skin at 28 weeks - Section of heavily haired
        skin from the interscapular area of a dog 28 weeks
        of age, fixed in 80 per cent alcohol and stained
        with Harris' hematoxylin and eosin Y, 35X magnifica-
        tion; notice that eight to ten hairs converge at
        the external follicle orifice

              a. Epidermis
              b. Dermis
              c. Subcutis
              d. Apocrine gland
              e. Sebaceous gland
              f. Follicle containing ten hairs

Fig. 9. Foot pad at 28 weeks - Section of skin from the
        front foot pad of a dog 28 weeks of age, fixed
        in 80 per cent alcohol and stained with Harris'
        hematoxylin and eosin Y, 35X magnification

              a. Stratum corneum
              b. Stratum lucidum
              c. Stratum granulosum
              d. Stratum spinosum
              e. Stratum cylindricum
              f. Dermis
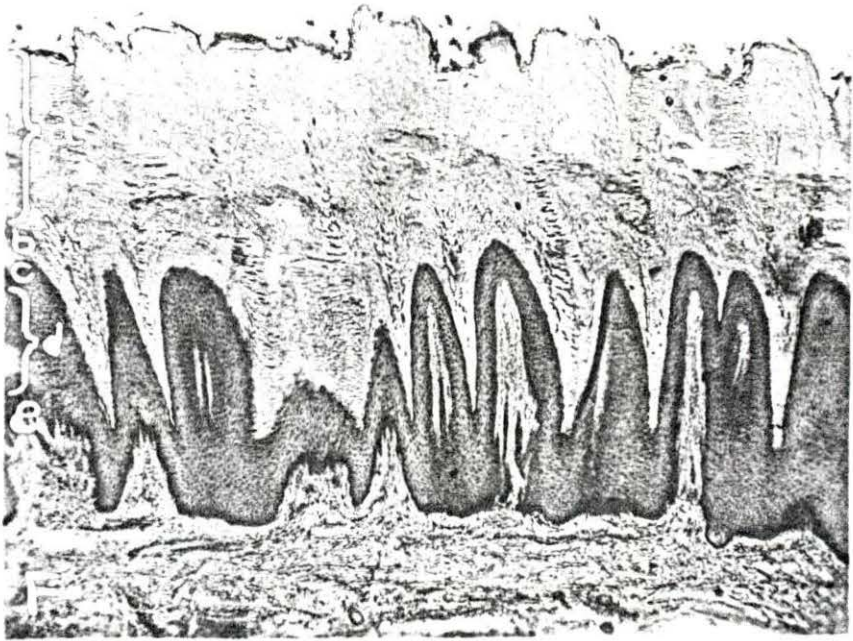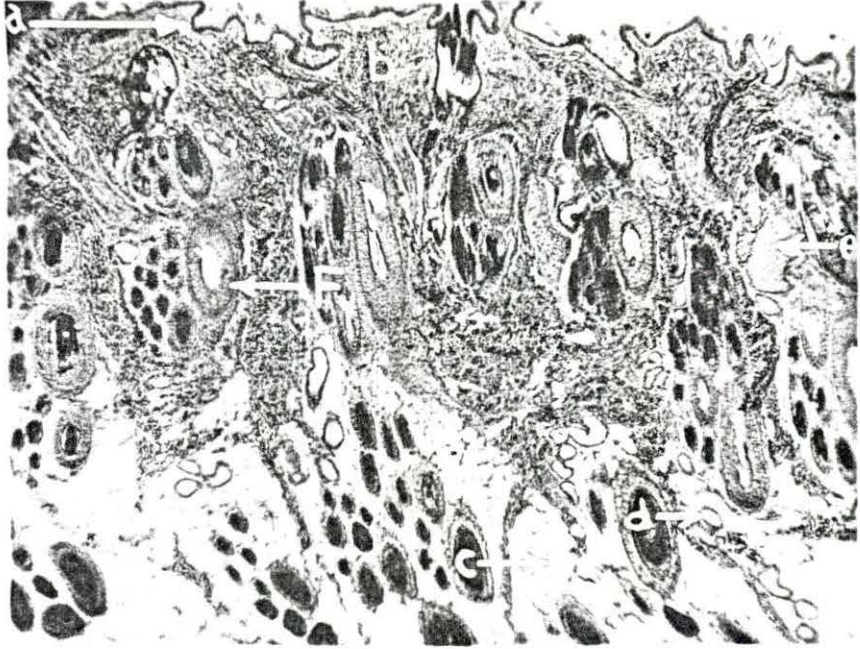
Fig. 10. Epidermal papilla - Picture of an epidermal
papilla taken at 18X magnification from a
mature dog

a. Epidermal papilla
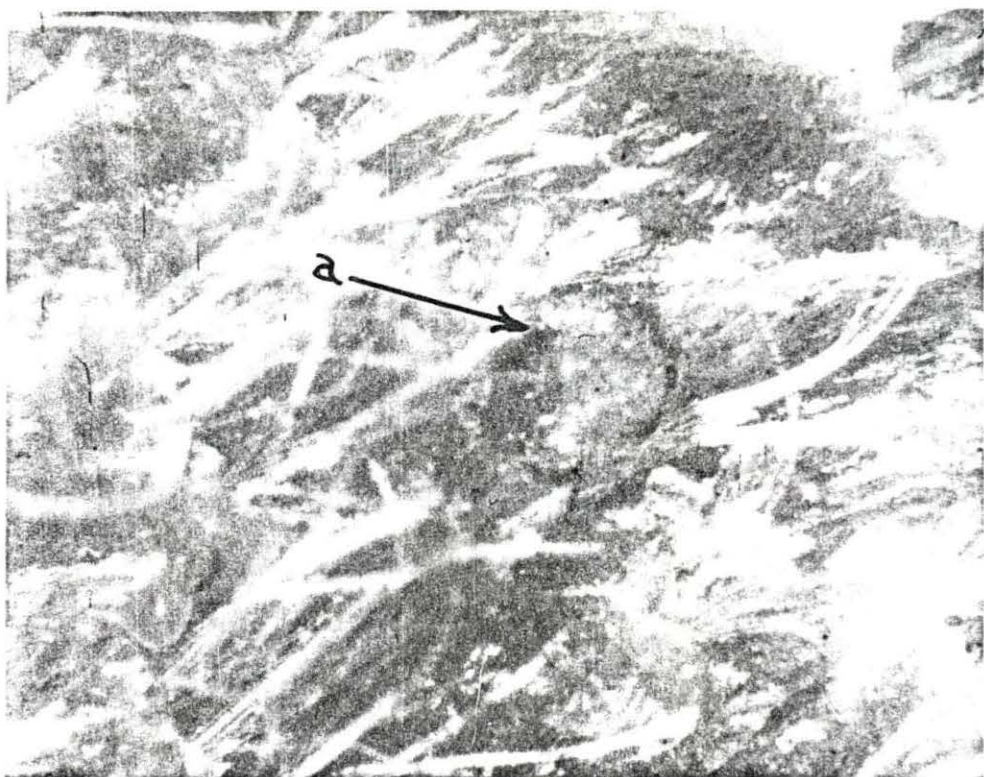
Fig. 11. Epidermal papilla - Section of skin from the
         interscapular region of a dog at the time of
         birth, fixed in 80 per cent alcohol and stained
         with Harris' hematoxylin and eosin Y, 470X
         magnification; it may be observed that the
         epidermis is thickened into a papilla-like
         formation in the center of the picture, the
         underlying dermis forms a core-like structure
         under this epidermal thickening

         a. Epidermis of epidermal papilla
         b. Dermis
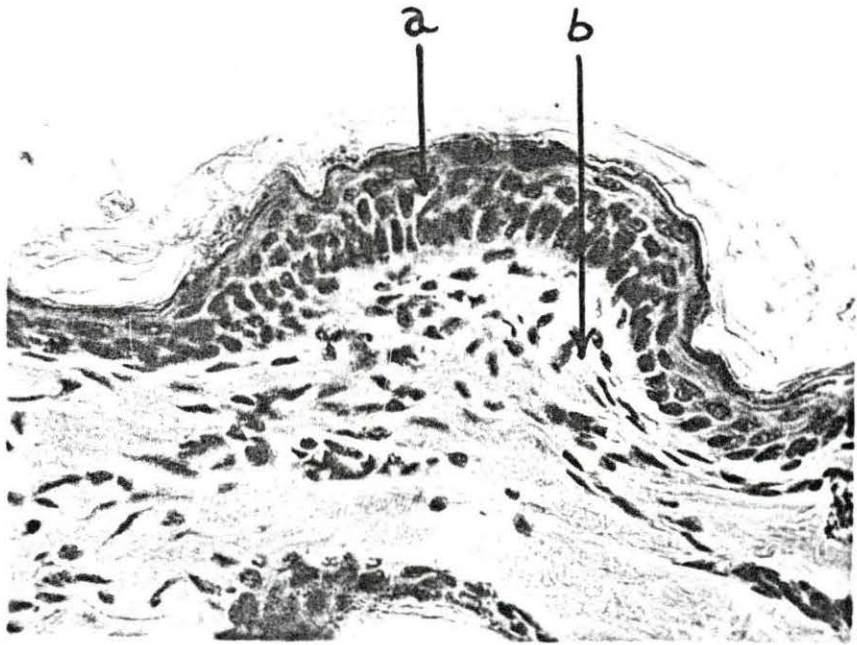
Fig. 12.   Epidermal papilla - Section of skin from the
           interscapular area of a dog 28 weeks of age,
           fixed in 80 per cent alcohol and stained with
           Harris' hematoxylin and eosin Y, 90X magnifica-
           tion; a typical group of three compound follicles
           containing seven to ten hairs each may be observed;
           an epidermal papilla is observable in the upper
           center region of the picture

                   a.   Epidermal papilla
                   b.   Group of three compound follicles

Fig. 13.   Epidermal papilla - Section of skin from the
           interscapular region of a dog 28 weeks of age,
           fixed in 80 per cent alcohol and stained with
           Heidenhain's hematoxylin and Weigert and Van
           Gieson's connective tissue stain, 180X magnifica-
           tion; in the center of the picture a very well
           developed epidermal papilla may be observed;
           note the fine connective tissue substance that
           fills the papilla

                   a.   Epidermis of epidermal papilla
                   b.   Connective tissue of papilla
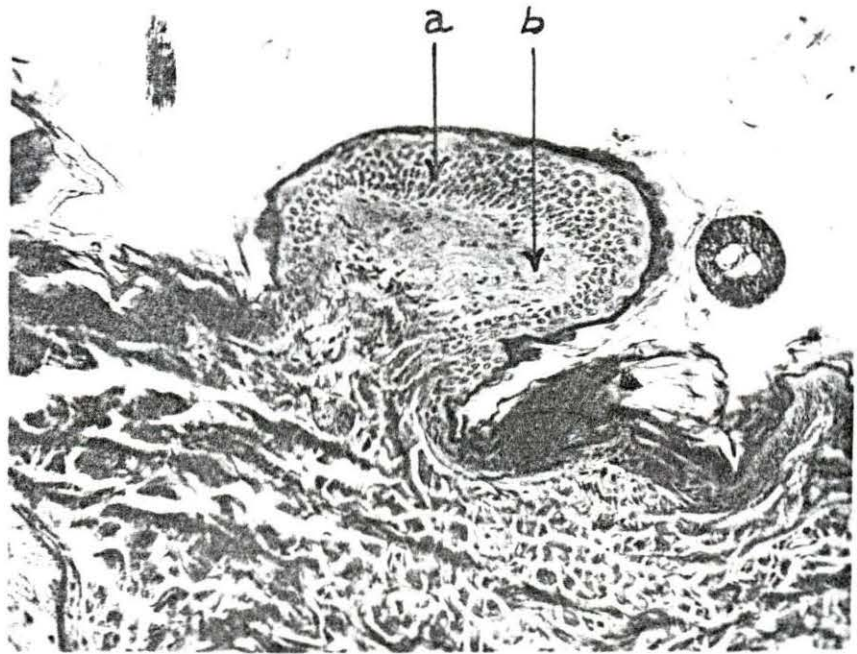
Fig. 14. Clear cells in foot pad epidermis - Section of
          skin from the front foot pad of a dog three
          weeks of age, fixed in 80 per cent alcohol and
          stained with Harris' hematoxylin and eosin Y,
          470X magnification; note the clear cells which
          are present in the basal layers of the epidermis;
          some dermal papillae are cut on a cross section
          and appear as islands of dermal tissue surrounded
          by epidermis; in the upper right corner there is
          a cross section of a sweat gland excretory duct

          a. Sweat gland excretory duct
          b. Cross section of dermal papilla
          c. Clear cells

Fig. 15. Clear cell in hairy skin - Section of skin from
          the sacral region of a dog one week of age, fixed
          in 80 per cent alcohol and stained with Harris'
          hematoxylin and alcoholic solution of precipitated
          eosin Y, 470X magnification; in the center of the
          picture there is a clear cell which is probably
          a mitotic cell in prophase or metaphase

          a. Epidermis
          b. Dermis
          c. Clear cell
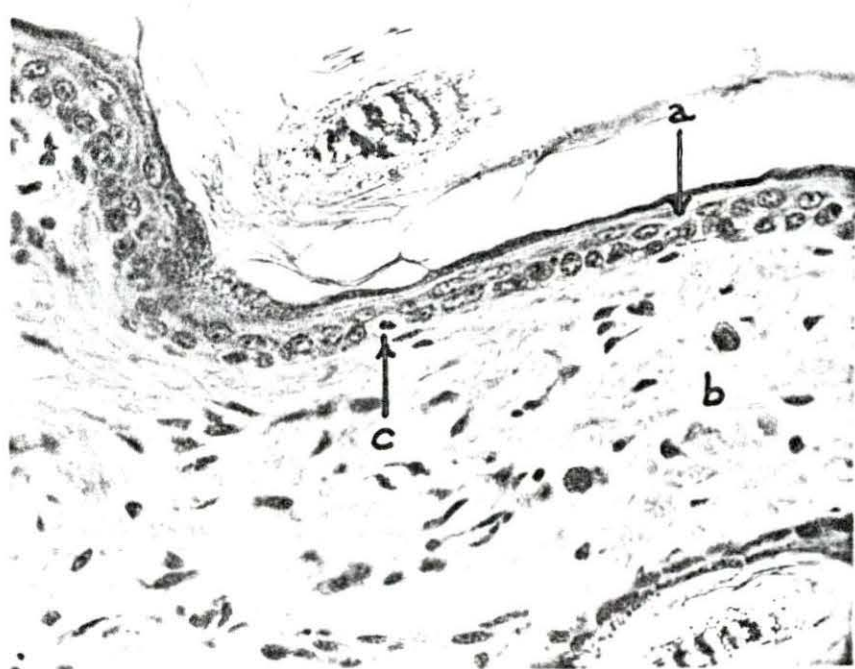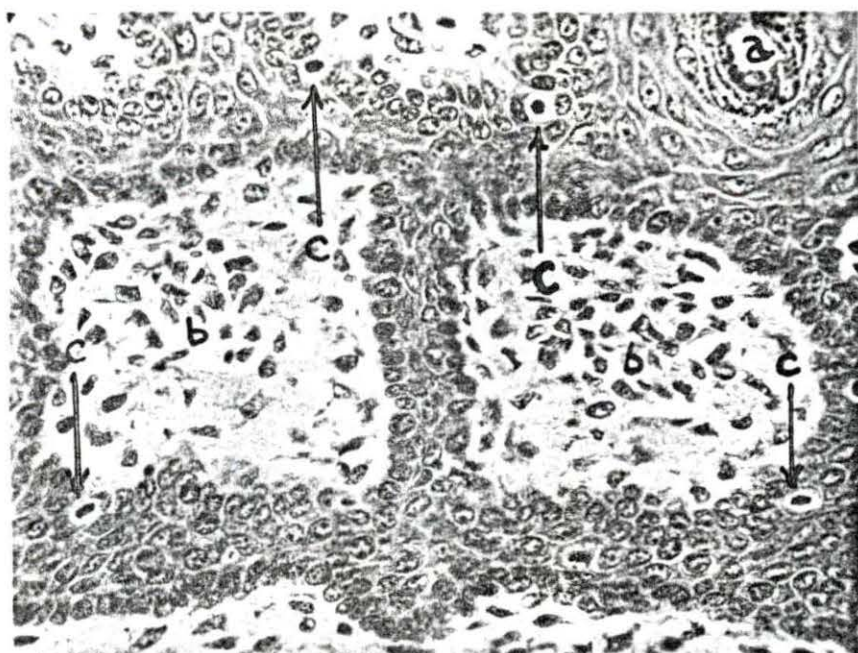
Fig. 16.   Mitotic clear cells - Section of skin from the
           paraumbilical region of a dog one week of age,
           fixed in 80 per cent alcohol and stained with
           Harris' hematoxylin and precipitated eosin Y
           solution, 470X magnification; several clear
           cells may be observed in various stages of
           mitosis; the stratum corneum has been separated
           from the stratum granulosum in sectioning

                  a.   Stratum corneum
                  b.   Stratum granulosum
                  c.   Clear cells in telophase

Fig. 17.   Clear cell in telophase - Section of skin from
           the sacral region of a dog one week of age,
           fixed in 80 per cent alcohol and stained with a
           combination of Heidenhain's iron hematoxylin,
           Van Giesen's acid fuchsin and picric acid stain,
           and Weigert's stain for elastic fibers, 470X
           magnification

                  a.   Clear cell in mitosis (telophase)
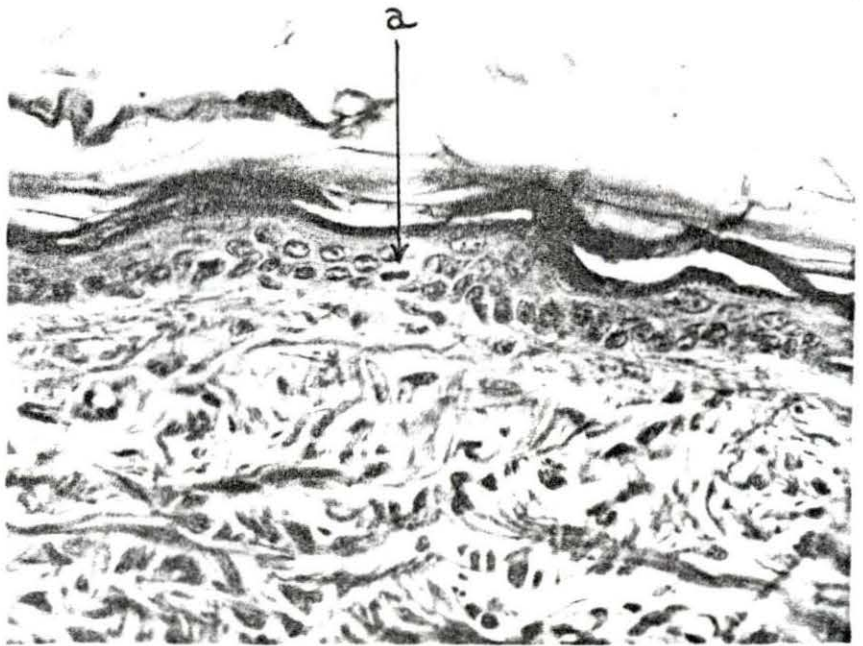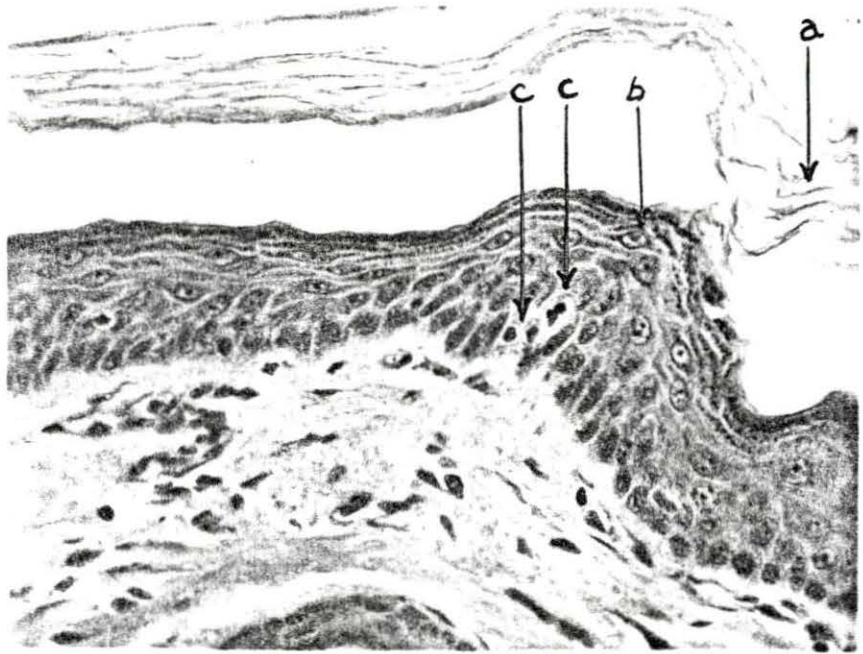
Fig. 18.   Glycogen in fused eyelids - Glycogen granules in
the epidermis of the fused eyelids of a one day
old puppy, fixed in 80 per cent alcohol and
stained with Feulgen-Bauer technique; no granules
appeared in control sections which were stained
simultaneously from which the glycogen had been
digested in diastase, 90X magnification; glycogen
granules are also present in the hair follicles

            a.   Fusion of eyelids
            b.   Conjunctiva
            c.   Hair follicle
            d.   Hairy skin
            e.   Glycogen granules

Fig. 19.   Glycogen in mucous membrane - Glycogen granules
in the epithelium of the mucous membrane of the
vulva, fixed in 80 per cent alcohol and stained
with Feulgen-Bauer technique, 470X magnification;
control sections were stained simultaneously
from which the glycogen had been digested in
diastase; the granules were not present in the
control; the tissue was collected from a dog
at birth

            a.   Epithelium of mucous membrane
            b.   Glycogen granule
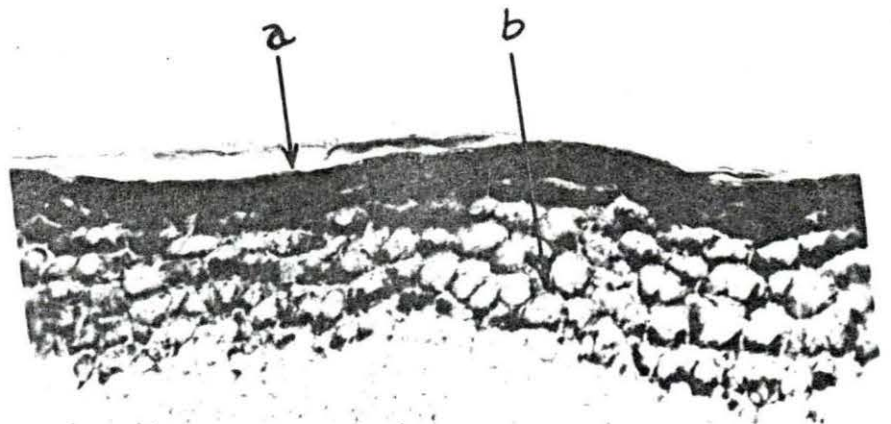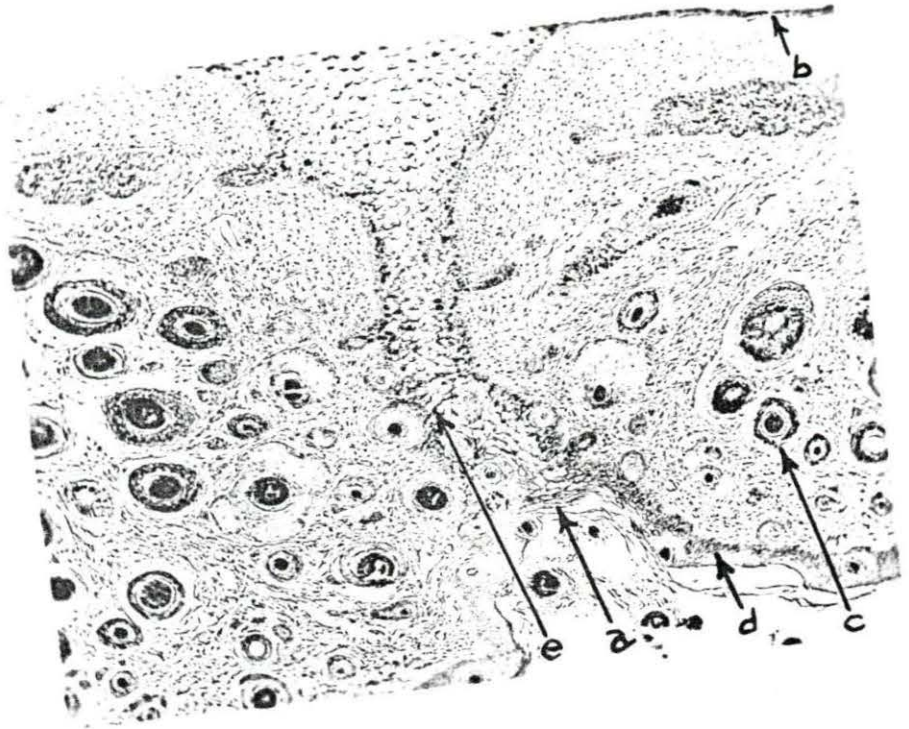
Fig. 20.   Alkaline phosphatase in mucous membrane - Section
          of the mucous membrane of the lip of a three weeks
          old dog, fixed in 80 per cent alcohol and prepared
          with the alkaline phosphatase technique of Gomori
          (three hours incubation), 180X magnification;
          evidence of the presence of the enzyme, alkaline
          phosphatase, may be observed in the superficial
          epithelial cells and the basal cells; the shading
          of the superficial cells was not present in the
          control, so it is assumed it indicates the presence
          of the enzyme; the capillary endothelial cells
          also show a positive response

                    a.   Superficial epithelial cells
                    b.   Basal cells of stratum cylindrum
                    c.   Capillary endothelium

Fig. 21.   Alkaline phosphatase in nasal epidermis - Section
          of nasal epidermis from a dog at birth, fixed in
          80 per cent alcohol and prepared with the alka-
          line phosphatase technique of Gomori (24 hours
          incubation), 180X magnification; evidence of the
          presence of the enzyme, alkaline phosphatase, may
          be observed in the cells of the basal layer of
          the epidermis at the depths of the epidermal pegs
          extending down into the dermis; the darkness
          in the superficial layers of the epidermis is
          probably caused by the presence of native
          phosphate because it was also present in the
          control section; the nuclei of the fibroblasts
          of the dermis and the endothelial cells of the
          capillaries also show a positive response for
          alkaline phosphatase

                    a.   Basal cells at the depths of epidermal
                         pegs
                    b.   Capillary endothelium
                    c.   Fibroblasts of dermis
                    d.   Superficial cells
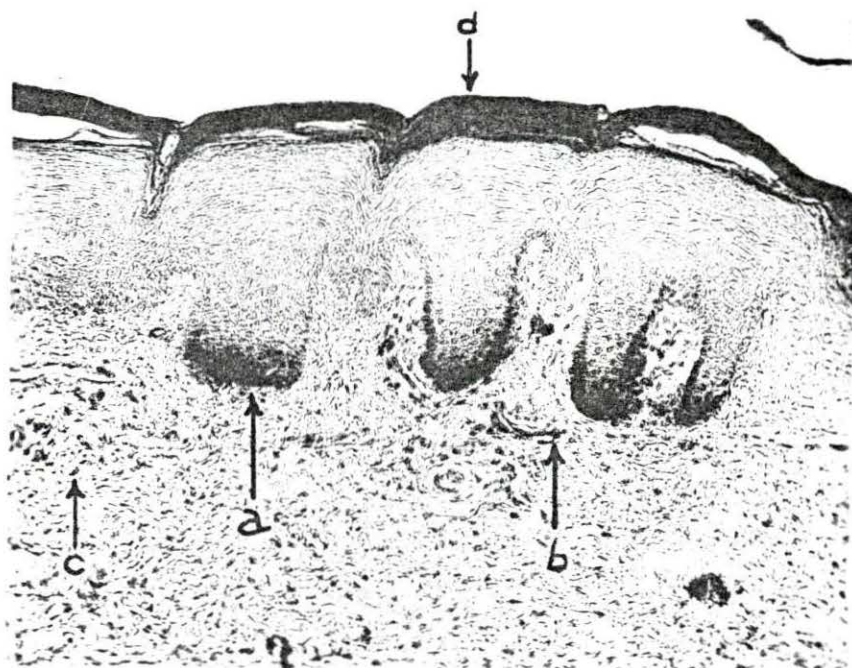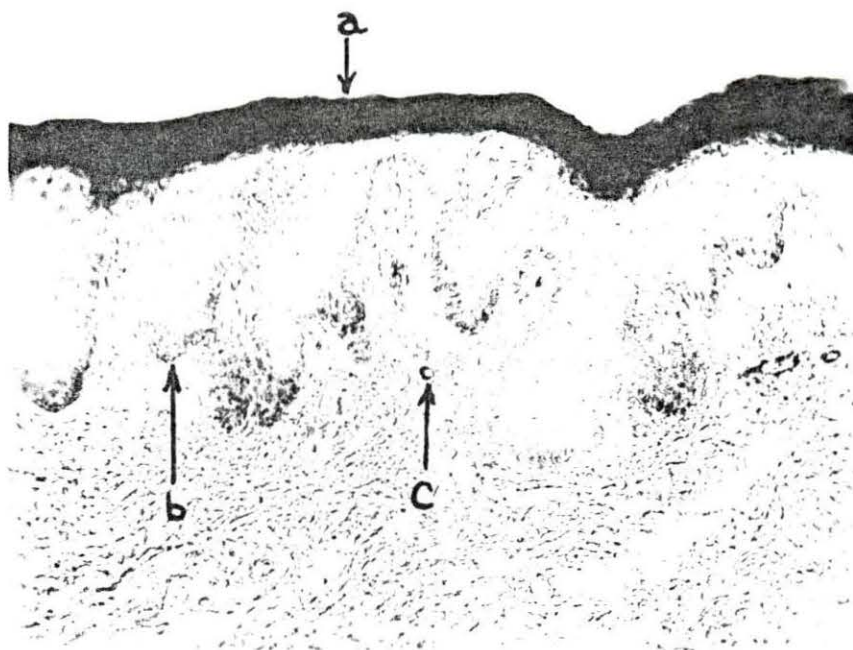
Fig. 22. Pigment cell with dendritic processes - Section
          of ear flap of dog taken the day of birth, fixed
          in 80 per cent alcohol and stained with Harris'
          hematoxylin and eosin Y, 470X magnification; note
          the melanoblasts in the basal layer of the epi-
          dermis; there is one near the center which shows
          dendritic processes

                    a. Stratum corneum
                    b. Stratum granulosum
                    c. Stratum spinosum
                    d. Stratum cylindricum
                    e. Melanoblast
                    f. Dermis

Fig. 23. Fibroblasts in epidermis - Section of skin from
          the interscapular region of a dog one week of
          age, fixed in 10 per cent formalin and stained
          with Harris' hematoxylin and precipitated eosin
          Y, 470X magnification; fibroblasts are observable
          between the cells of the stratum cylindricum or
          basal cells of the epidermis

                    a. Stratum corneum
                    b. Stratum granulosum (keratohyalin granules)
                    c. Stratum spinosum
                    d. Stratum cylindricum
                    e. Fibroblasts
                    f. Dermis
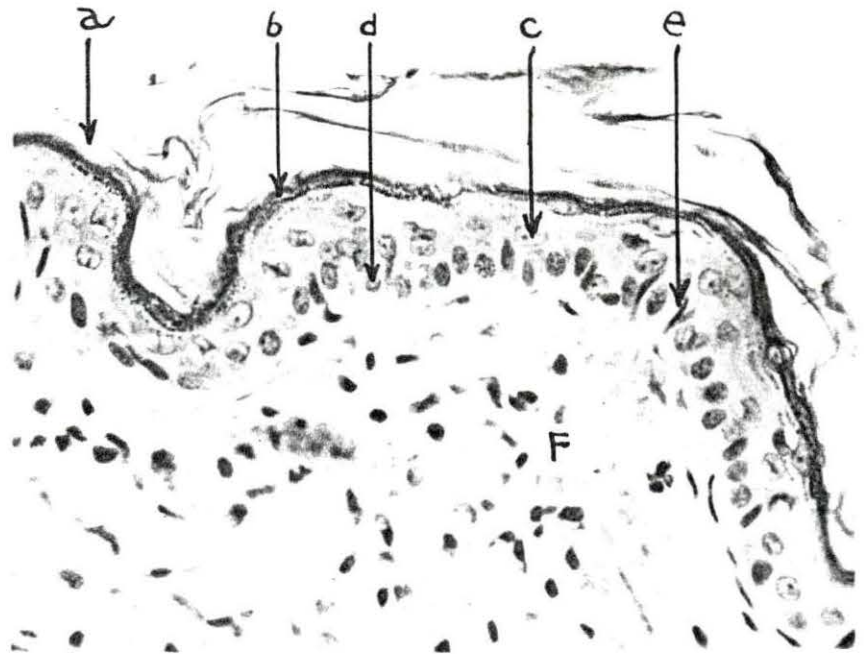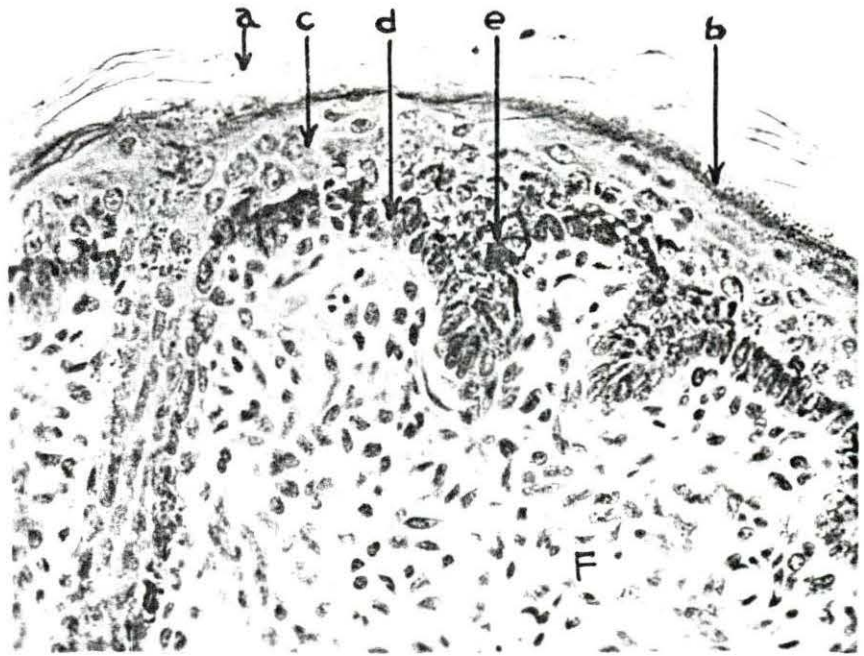
Fig. 24. Section of lip - Section of lip of one week old
          dog, fixed in 80 per cent alcohol and stained
          with Harris' hematoxylin and eosin Y, 15X
          magnification; note the undulation of the
          epidermal-dermal junction on the mucous membrane
          and non-hairy portion and the absence of epi-
          dermal pegging on the hairy part

          a. Mucous membrane
          b. Hairy skin epidermis
          c. Epidermal peg
          d. Area of Fig. 25
          e. Muscle

Fig. 25. Mucocutaneous junction of lip - Section of lip
          of one week old dog, fixed in 80 per cent alcohol
          and stained with Harris' hematoxylin and eosin Y,
          90X magnification; note the epidermal pegging of
          the non-hairy part of the lip; note also the size
          of the sebaceous and apocrine glands that are
          associated with the hair follicles near the
          mucocutaneous junction

          a. Epidermal peg
          b. Mucocutaneous junction
          c. Sebaceous gland
          d. Apocrine gland
          e. Hair follicle
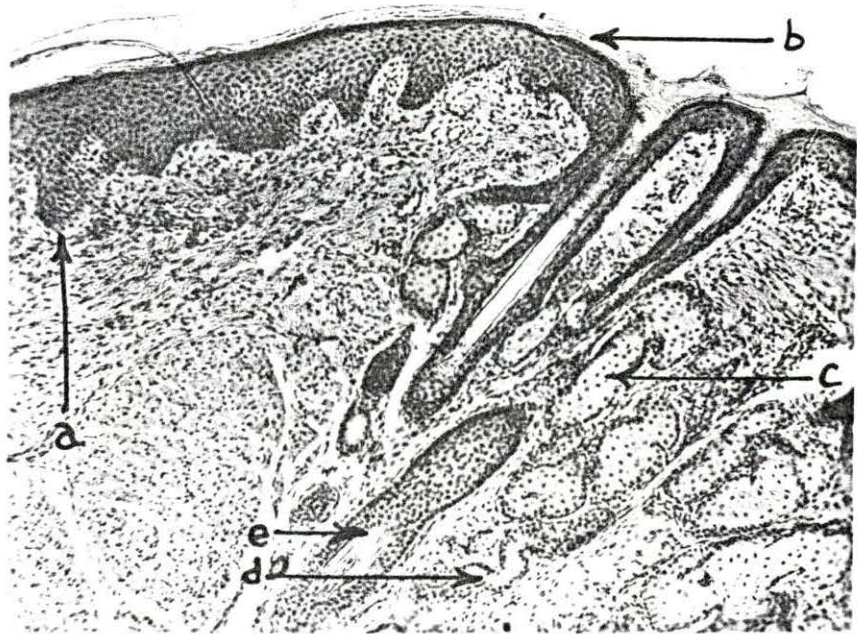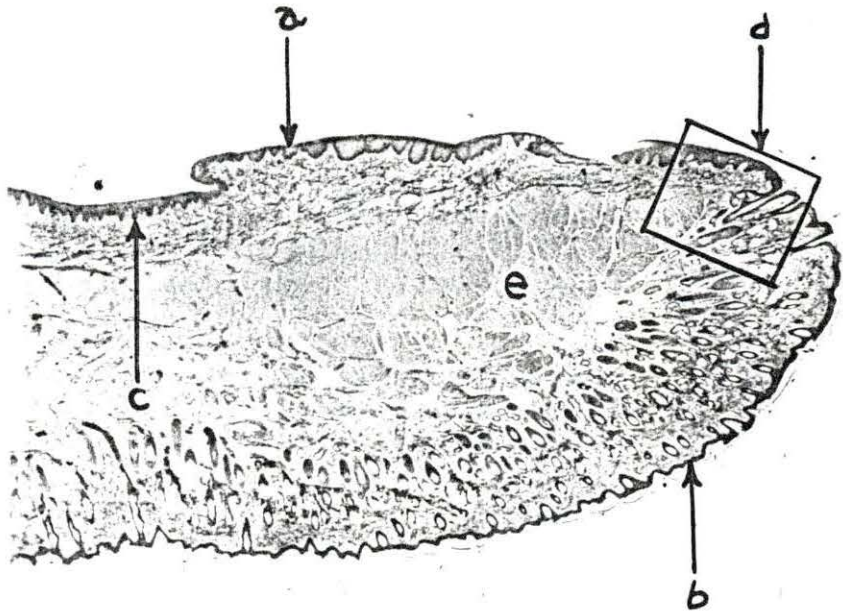
Fig. 26. Elastic fibers connected to arrector pili muscle -
         Section of skin from interscapular region of a
         dog 24 weeks of age, fixed in 80 per cent alcohol
         and stained with Weigert and Van Gieson's connec-
         tive tissue stain, 180X magnification; note the
         elastic fibers extending from the end of the
         arrector pili muscle toward the surface epi-
         thelium

              a. Arrector pili muscle
              b. Hair follicle
              c. Epidermis
              d. Elastic fiber
              e. Dermis

Fig. 27. Elastic fibers - Section of skin from the sacral
         region of a dog 28 weeks of age, fixed in 80 per
         cent alcohol and stained with Weigert and Van
         Gieson's connective tissue stain, 470X magnifica-
         tion; note the branching elastic fibers mixed in
         with the collagenous fibers in the dermis near a
         hair follicle

              a. Elastic fiber
              b. Collagenous fiber
              c. Hair follicle
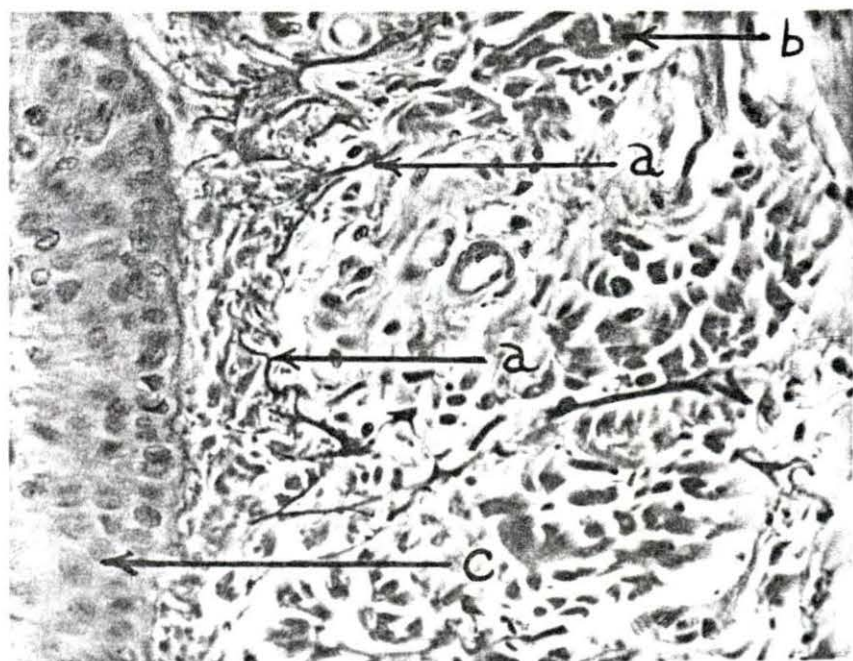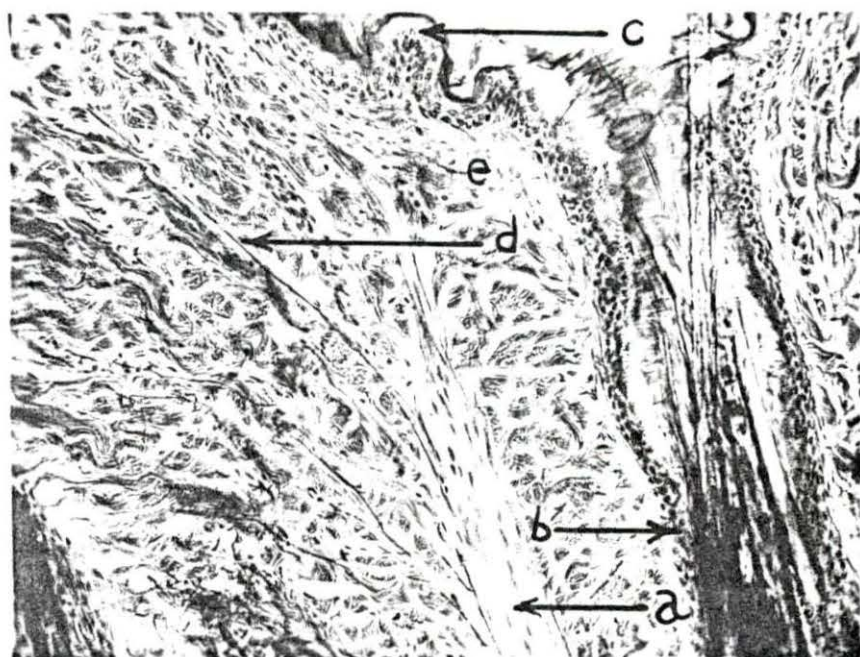
Fig. 28. Reticular network - Section of skin from the inter-
scapular region of a mature dog, fixed in 10 per
cent formalin and stained with Foot and Menard
rapid silver impregnation method, 90X magnifica-
tion; reticular fibers may be observed forming
a network around hair follicles, sebaceous glands,
arrector pili muscles, and apocrine glands;
collagenous fibers appear thicker and lighter

    a. Reticular fibers
    b. Collagenous fibers
    c. Sebaceous gland
    d. Hair follicle

Fig. 29. Reticular network - High power picture of the
same section as Fig. 28, 470X magnification;
reticular fibers may be seen around the hair
follicles, sebaceous glands, and arrector pili
muscle

    a. Reticular fibers
    b. Sebaceous gland
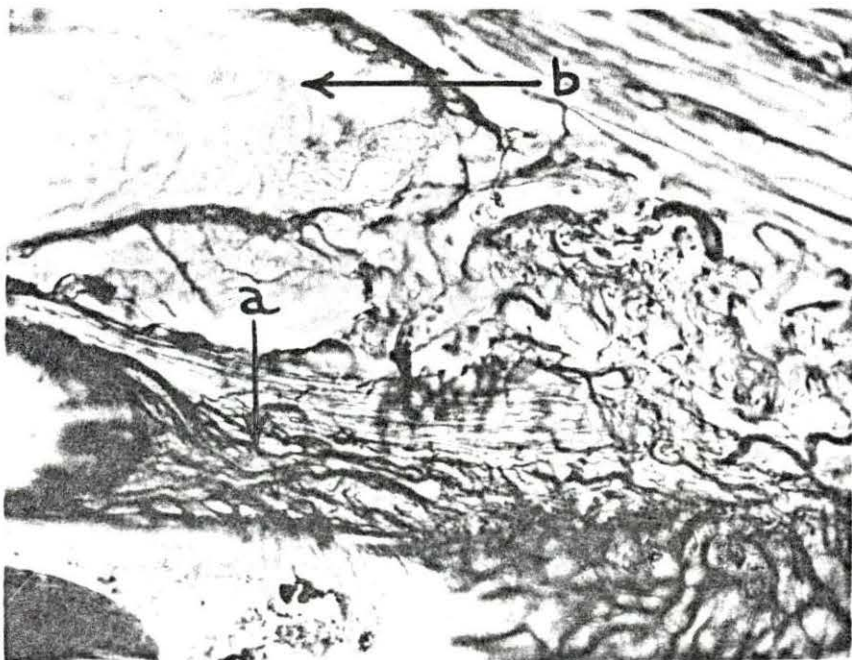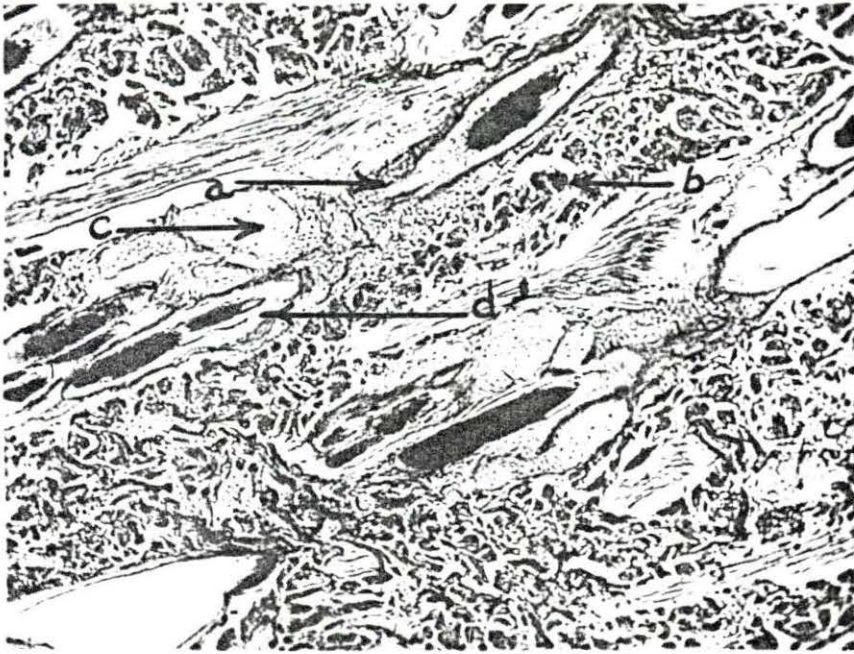
Fig. 30.   Reticular network - Section of paraumbilical skin
from a dog three weeks of age, fixed in 80 per
cent alcohol and prepared with Foot and Menard
rapid method of silver impregnation, 835X mag-
nification; evidence may be observed that the
so-called basement membrane is made up of a
network of fine reticular fibers which inter-
digitate between the basal cells of the epi-
dermis

    a. Epidermis
    b. Stratum cylindricum
    c. Reticular fibers

Fig. 31.   Reticular network - Section of the conjunctival
mucous membrane from the eyelid of a dog eight
weeks of age, fixed in 80 per cent alcohol and
prepared with Foot and Menard rapid method of
silver impregnation, 470X magnification; a net-
work of reticular fibers may be observed under
the epithelium which appear to be continuous
deeply with the thicker collagenous bundles

    a. Epithelium
    b. Reticular network
    c. Collagenous fibers
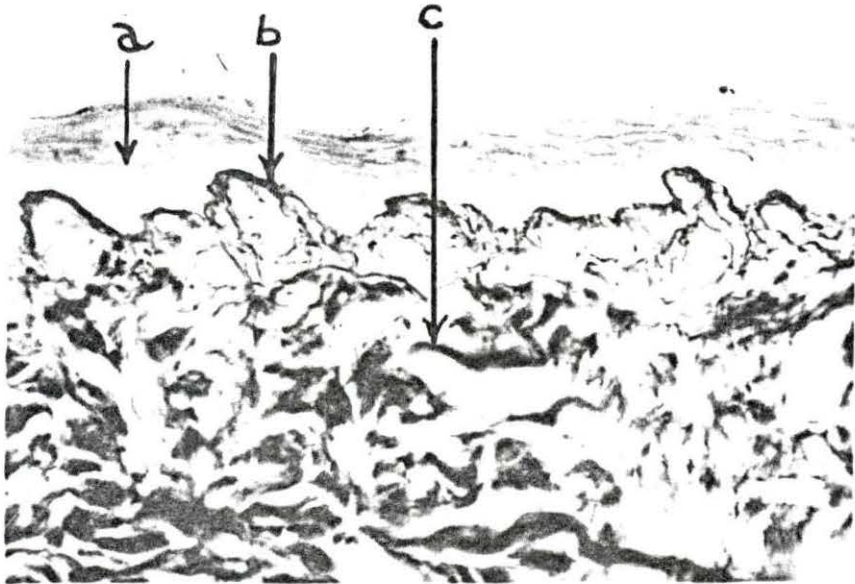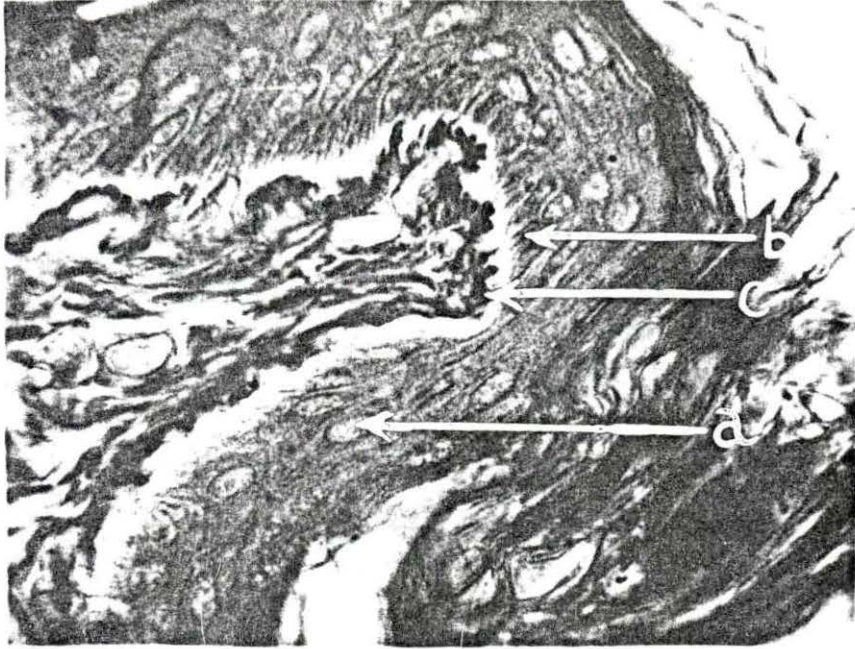
Fig. 32. Reticular fibers interdigitating with basal cells -
Section from the front foot pad of a dog eight
weeks of age, fixed in 10 per cent formalin and
prepared with the Foot and Menard rapid method
of silver impregnation, 835X magnification; fibro-
blasts may be observed between the basal cells
of the epidermis and the reticular fibers of
the dermis may be observed extending up between
the basal cells of the epidermis in the vicinity
of the fibroblasts

        a. Basal cell of stratum cylindricum
        b. Fibroblast
        c. Reticular fiber
        d. Spinous cell of stratum spinosum
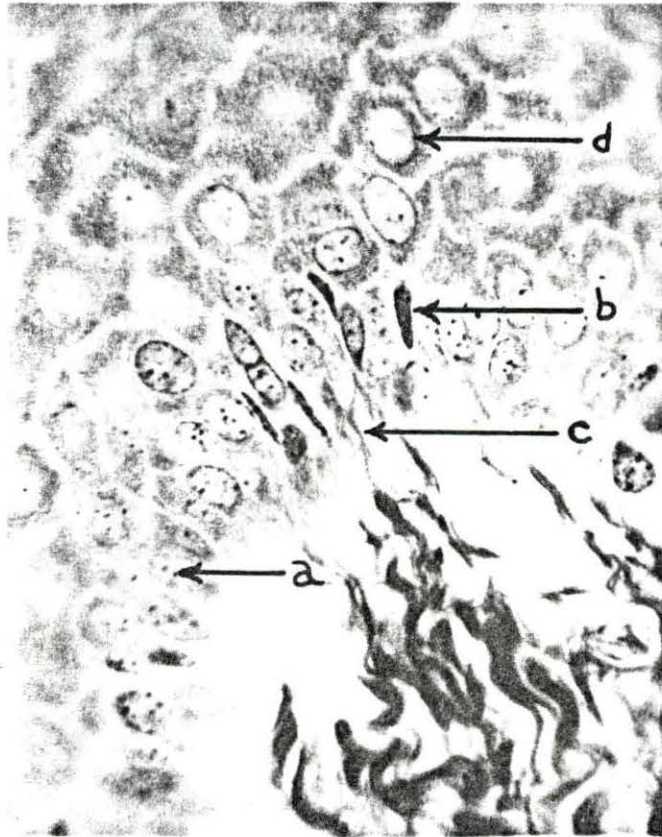
Fig. 33. Reticular and collagenous fibers - Section of
front foot pad from a two weeks old dog which
has been fixed in 80 per cent alcohol and treated
with the Foot and Menard rapid method of silver
impregnation, 470X magnification; the epithelial
cells are heavily pigmented; reticular fibers
may be observed directly under the epidermis
which form a network; these reticular fibers
appear to be continuous with the lighter stain-
ing collagenous fibers which are located deeper
in the dermis

           a.   Epidermal cells
           b.   Pigment granules
           c.   Reticular fibers
           d.   Collagenous fibers

Fig. 34. Reticular fibers interdigitating between basal
cells - Section of nasal epidermis from a dog
four weeks of age, fixed in 10 per cent formalin
and treated with the Foot and Menard rapid
method of silver impregnation (modified), 835X
magnification; reticular fibers may be observed
interdigitating between the basal cells of the
epidermis; intercellular bridges may be observed
in the upper right hand corner

           a.   Basal cell of stratum cylindricum
           b.   Reticular fiber
           c.   Spinous cell with intercellular bridges
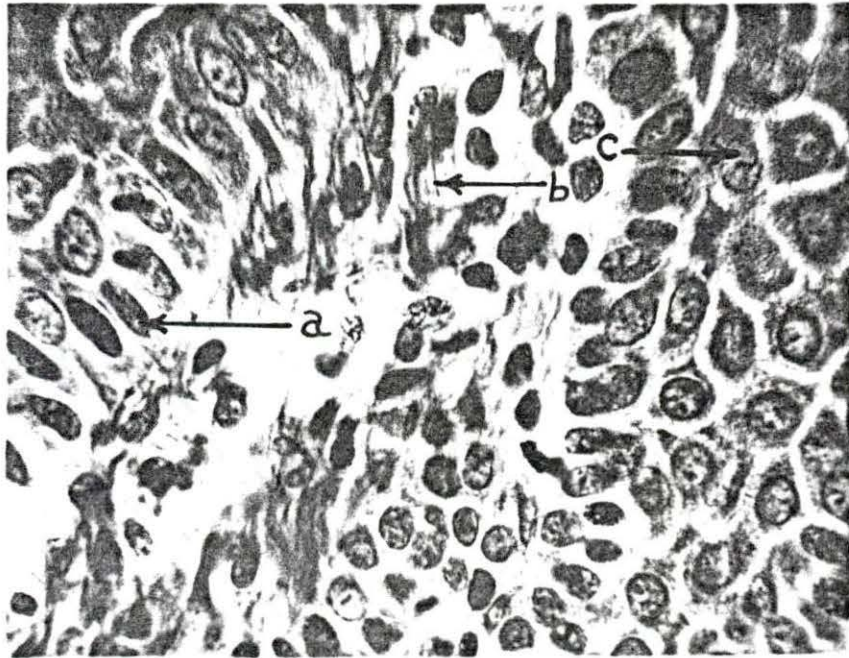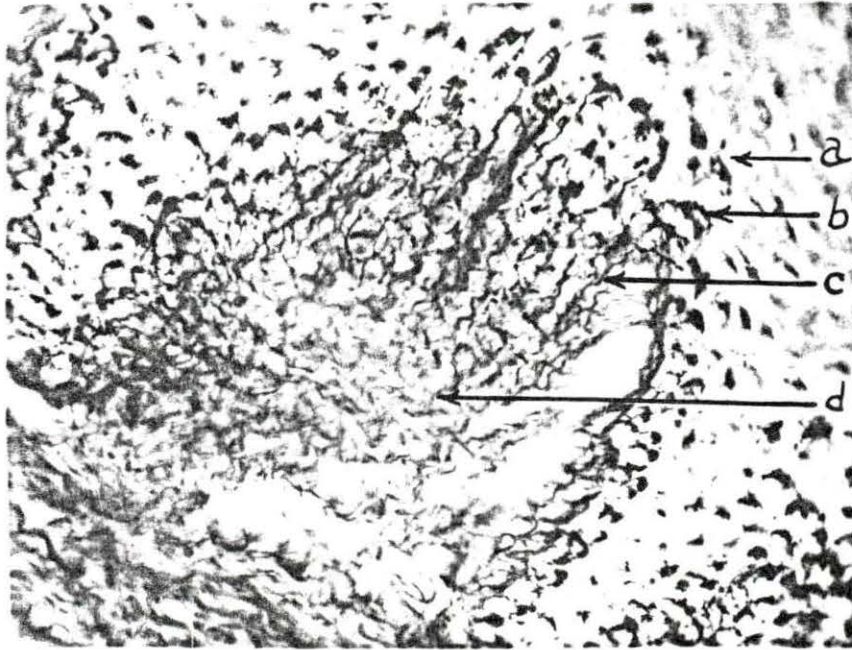
Fig. 35. Sensory lamellar corpuscle - Section of sub-
         cutaneous tissue of the front foot pad of a
         four week old dog, fixed in 80 per cent alcohol
         and prepared with the Feulgen-Bauer technique
         (this slide was a control), 180X magnification;
         note the lamellar corpuscle in the center of the
         picture

         a. Lamellar corpuscle

Fig. 36. Sensory lamellar corpuscle - Section of the sub-
         cutaneous tissue of the front foot pad of a
         12 week old dog, fixed in 80 per cent alcohol
         and prepared with the Gomori technique for
         alkaline phosphatase, incubation time 24 hours,
         470X magnification; note the lamellar corpuscle
         with alkaline phosphatase activity in the center

              a. Lamellar corpuscle
              b. Center of alkaline phosphatase activity
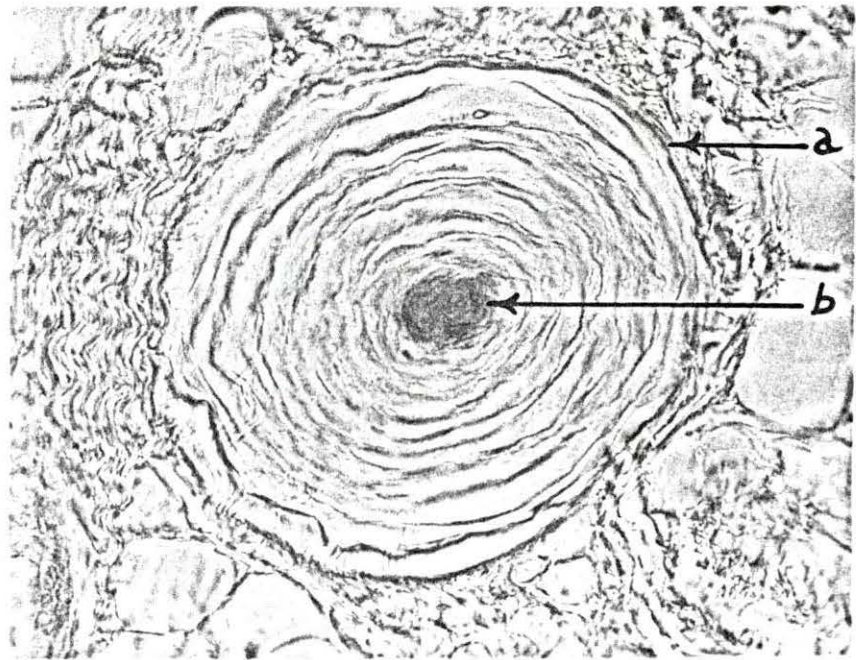
Fig. 37. Simple hair follicle at birth - Cross section of
         a hair and hair follicle from a dog shortly after
         birth, fixed in 80 per cent alcohol and stained
         with Harris' hematoxylin and eosin Y, 470X
         magnification; notice that this is a single hair
         follicle with a single hair, this is typical of
         the follicles of dog skin from dogs less than a
         week old; apocrine glands may also be observed
         on each side of the follicle

              a. Hair follicle
              b. Hair
              c. Apocrine gland

Fig. 38. Two or three hairs in a follicle at four weeks -
         Section of skin from the interscapular region of
         a dog four weeks of age, fixed in 80 per cent
         alcohol and stained with Harris' hematoxylin
         and precipitated eosin Y solution, 90X magnifica-
         tion; in the cross sections of the hair follicles, it may be observed that there are two
         hairs in each follicle superficially and more
         deeply they branch or separate into two indi-
         vidual hair bulbs

              a. Hair shaft
              b. Hair follicle containing two hairs
              c. Epidermis
              d. Dermis
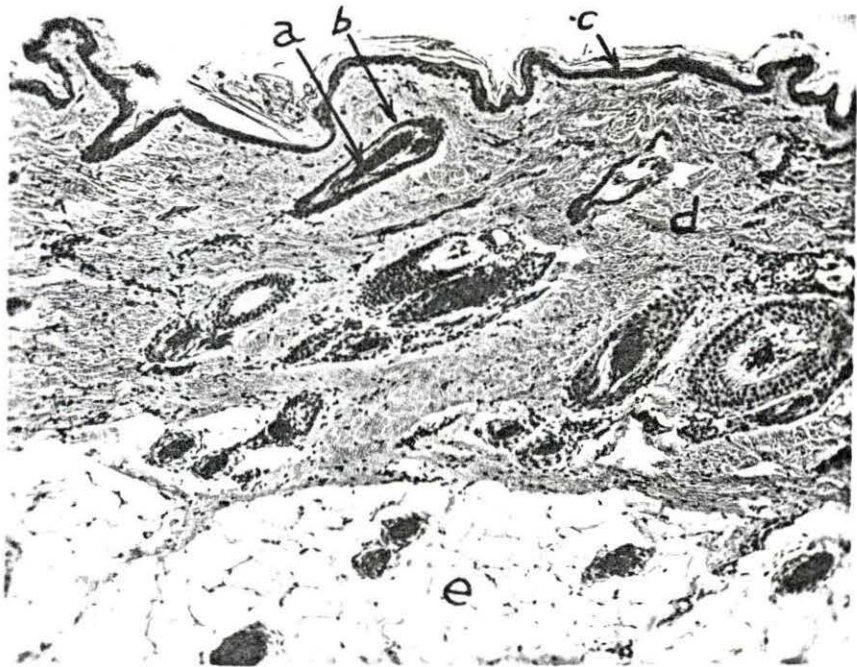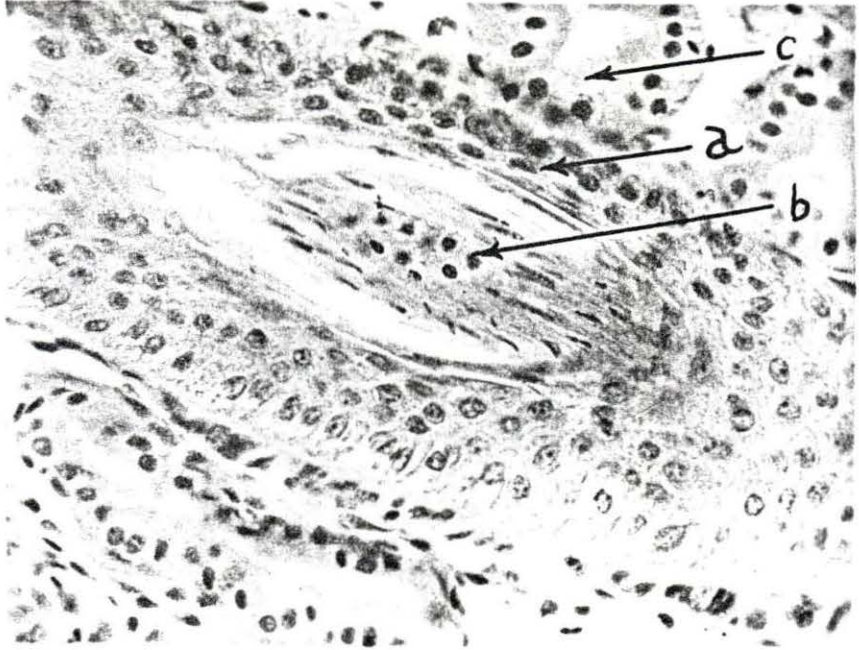   e. Subcutis

Fig. 39.   Four to six hairs in a follicle at ten weeks -
           Section of skin above the eye from a dog ten
           weeks of age, fixed in 80 per cent alcohol and
           stained with Harris' hematoxylin and alcoholic
           solution of precipitated eosin Y, 79X magnifica-
           tion; this section shows that the compound hair
           follicles of the dog's skin are for the most part
           arranged in groups of three; also it may be
           observed that there are from four to six hairs
           in each follicle

                a. A group of three follicles
                b. Five hairs in one follicle
                c. Sebaceous gland

Fig. 40.   Ten hairs in a follicle at 28 weeks - Section of
           skin from the flank region of a dog 28 weeks of
           age, fixed in 80 per cent alcohol and stained
           with Harris' hematoxylin and eosin Y, 180X
           magnification; the picture shows a cross section
           of a compound hair follicle which contains ten
           hairs; note the excretory duct of an apocrine
           gland on the lower side of the follicle

                a. Compound hair follicle
                b. Main hair
                c. Auxiliary hairs
                d. Apocrine gland duct
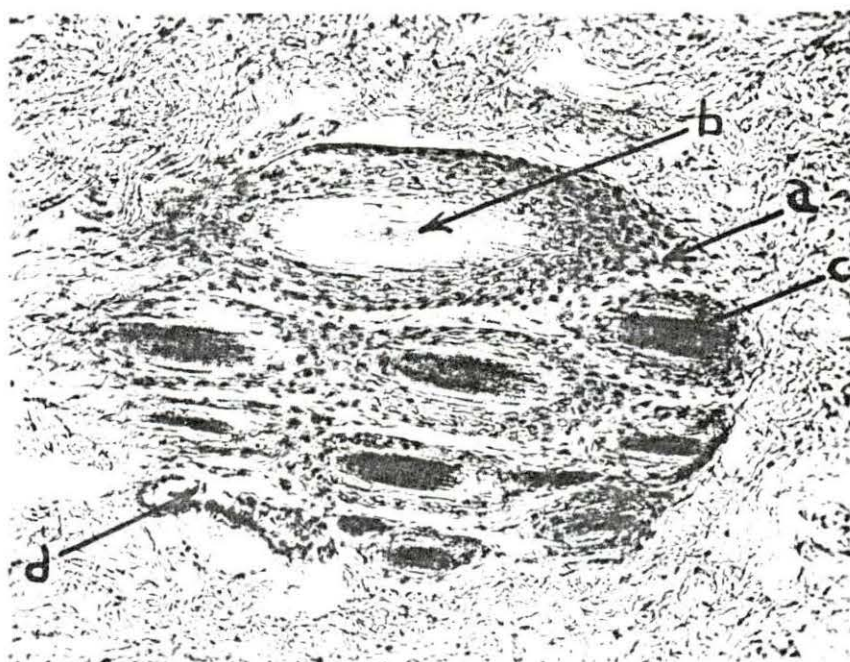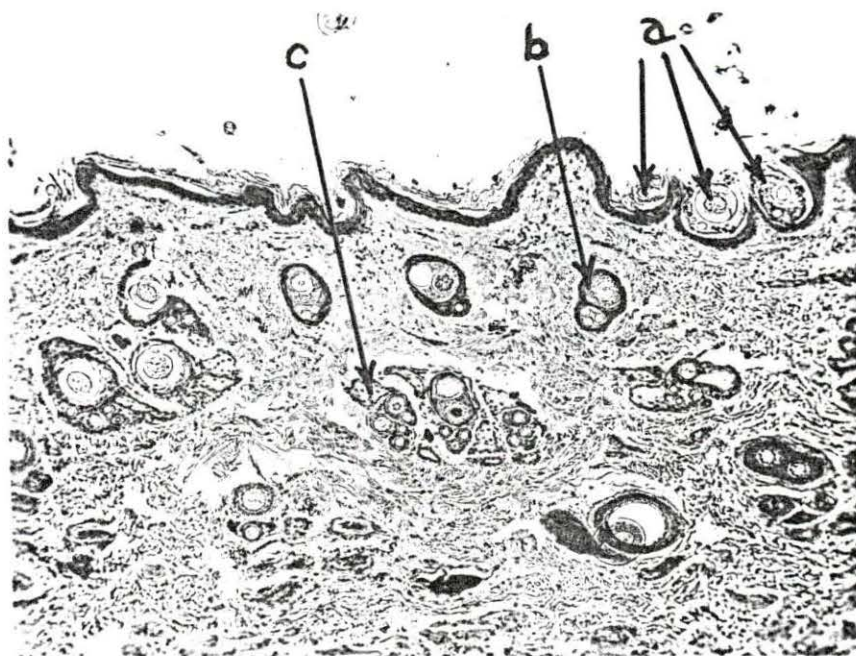
Fig. 41. Developing follicles - Section of earflap of
          dog collected shortly after birth, fixed in
          80 per cent alcohol and stained with Harris'
          hematoxylin and eosin Y, 180X magnification;
          note the developing hair follicles extending
          into the dermis

          a. Epidermis
          b. Developing follicle
          c. Mature follicle

Fig. 42. Sinus hair follicle - Section of lip of dog three
          weeks of age, fixed in 80 per cent alcohol and
          prepared with the Gomori technique for the
          demonstration of alkaline phosphatase, six hours
          incubation, 90X magnification; the picture shows
          a tangential section of a sinus hair follicle
          and a sebaceous gland duct opening into the
          hair follicle

          a. Hair follicle
          b. Sebaceous gland
          c. Blood sinus
          d. Connective tissue sheath of sinus
          e. Apocrine gland of adjacent follicle
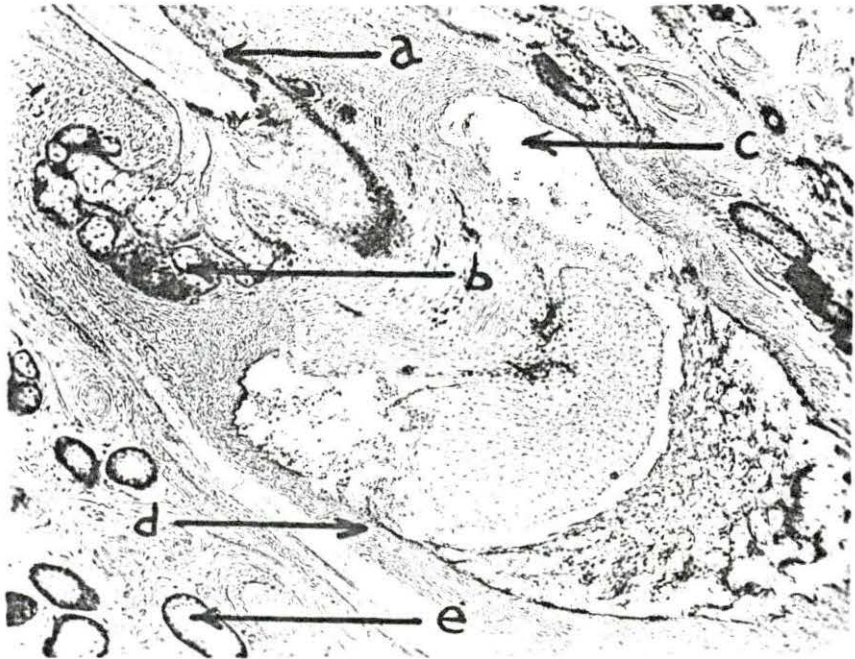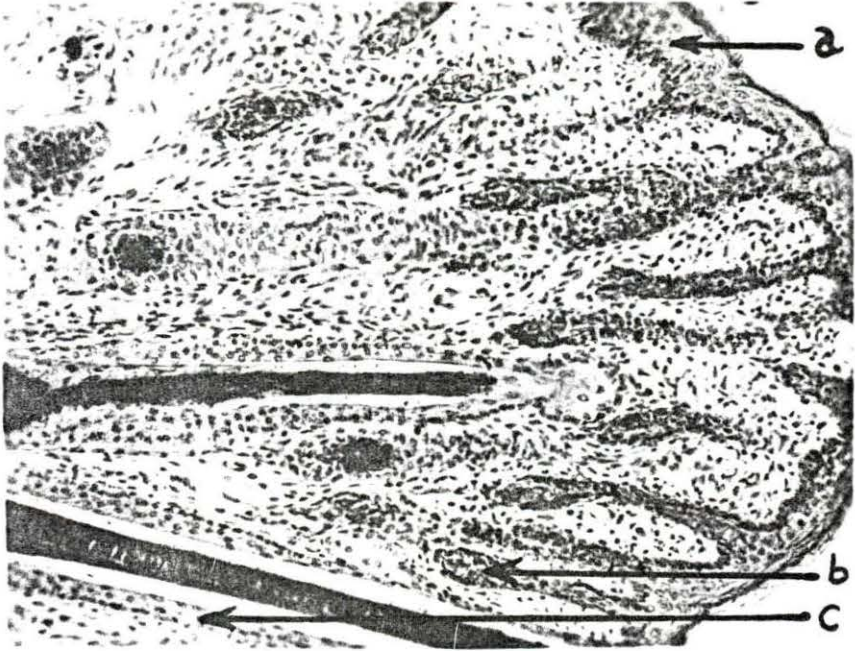
Fig. 43.   Glycogen in hair follicles - Section of skin from
           interscapular region of a dog one week of age,
           fixed in 80 per cent alcohol and prepared with
           the Fuelgen-Bauer technique for the demonstration
           of glycogen, 90X magnification; note the glycogen
           in the outer root sheaths of the hair follicles
           and in the arrector pili muscles

                a. Outer root sheat containing
                    glycogen granules
                b. Arrector pili muscles containing glycogen
                c. Epidermis

Fig. 44. Alkaline phosphatase in hair papilla and matrix -
          Section of skin from the interscapular area of a
          dog one week of age, fixed in 80 per cent alcohol
          and prepared with the Gomori technique for the
          demonstration of alkaline phosphatase, incubation
          six hours, 470X magnification; hair matrix and
          dermal papilla may be observed; note the intense
          reaction for alkaline phosphatase in the dermal
          papilla and the moderate reaction of the nuclei
          of the matrix cells

                a. Hair matrix
                b. Dermal papilla
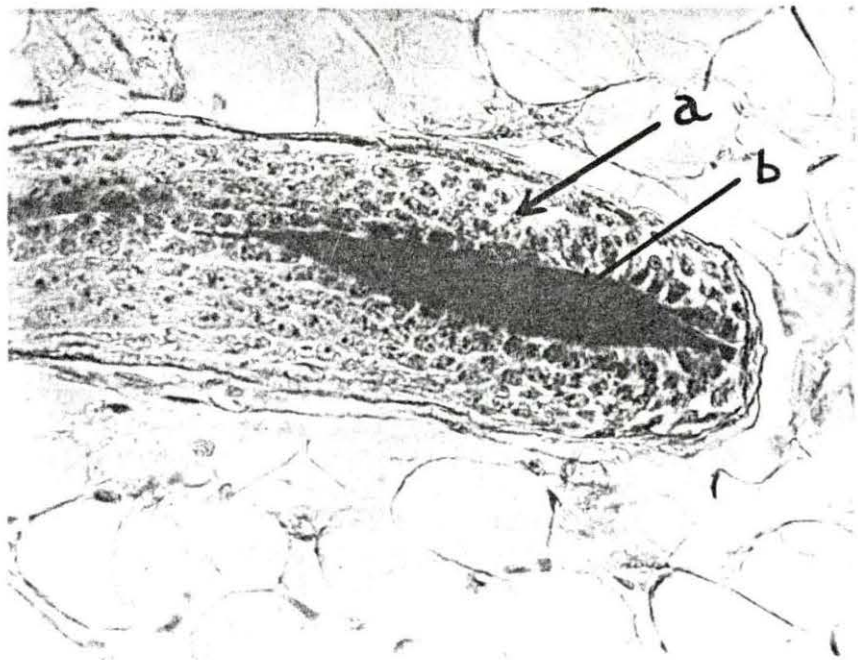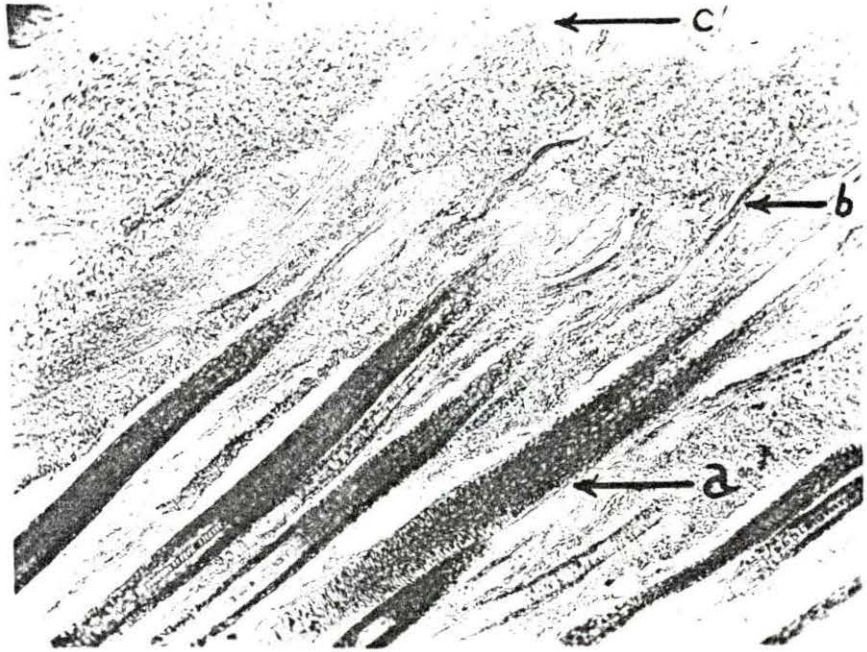
Fig. 45.   Sweat gland opening into hair follicle - Section
            of skin from the interscapular area of a dog one
            week old, fixed in 80 per cent alcohol and stained
            with Weigert and Van Gieson's connective tissue
            stain, 90X magnification; the picture shows the
            duct of an apocrine gland emptying into a hair
            follicle

            a. Hair follicle
            b. Secretory portion of apocrine gland
            c. Excretory duct of apocrine gland
            d. Sebaceous gland
            e. Point where gland opens into follicle

Fig. 46.   Sweat gland opening into hair follicle - Section
            of skin from the interscapular region of a dog
            one week of age, fixed in 80 per cent alcohol
            and prepared with the Gomori technique for the
            demonstration of alkaline phosphatase, 24 hours
            incubation, 90X magnification; note the apocrine
            gland tubule emptying into the hair follicle
            above the level of the sebaceous gland

            a. Hair follicle
            b. Secretory portion of apocrine gland
            c. Excretory duct of apocrine gland
            d. Sebaceous gland
            e. Arrector pili muscle
            f. Point where gland opens into follicle
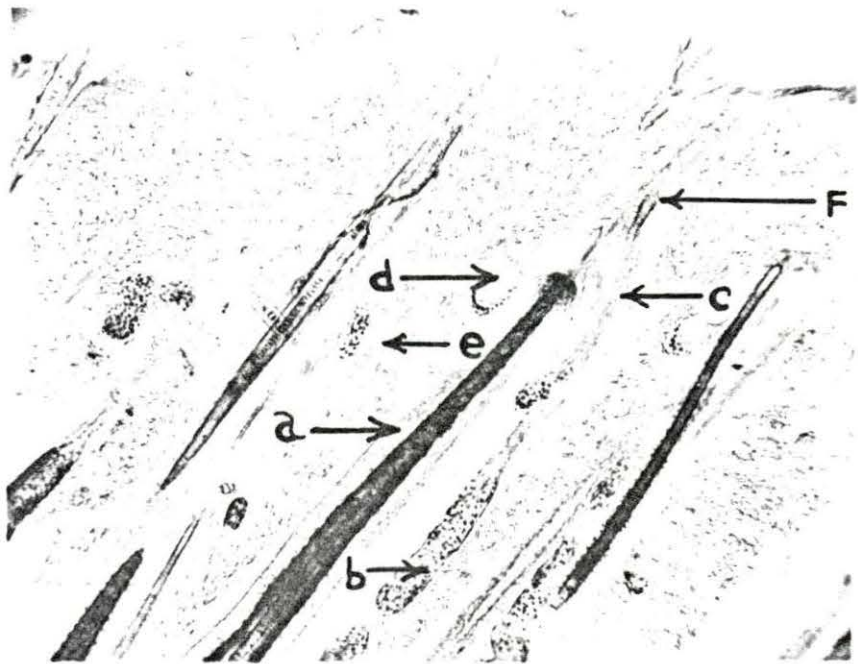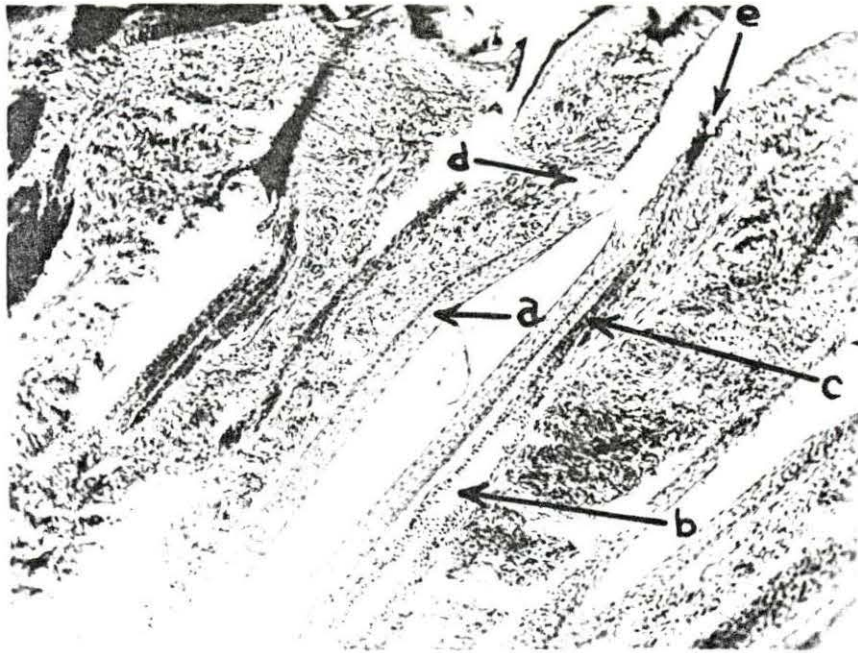
Fig. 47. Dilated apocrine tubules - Section of skin from
          the fold of the flank of a dog eight weeks of
          age, fixed in 80 per cent alcohol and stained
          with Harris' hematoxylin and eosin Y, 90X mag-
          nification; a series of cross sections of the
          secretory portion of an apocrine gland may be
          observed in the subcutaneous tissue; an excre-
          tory duct, smaller in diameter than the
          secretory tubule may be observed winding up
          toward the hair follicle and the surface

               a. Secretory portion of apocrine gland
               b. Excretory duct
               c. Hair follicle
               d. Epidermis
               e. Dermis
               f. Subcutis
               f. Sebaceous gland

Fig. 48. Active apocrine gland - Section of skin from the
          vulva of a dog three weeks of age, fixed in 80
          per cent alcohol and stained with Harris' hema-
          toxylin and eosin Y, 470X magnification; note
          the apical processes of the secretory cells of
          the apocrine tubule c.; tubule d. represents
          the post-secretory stage

               a. Apical process of apocrine cell
               b. Apocrine cell
               c. Pre-secretory tubule
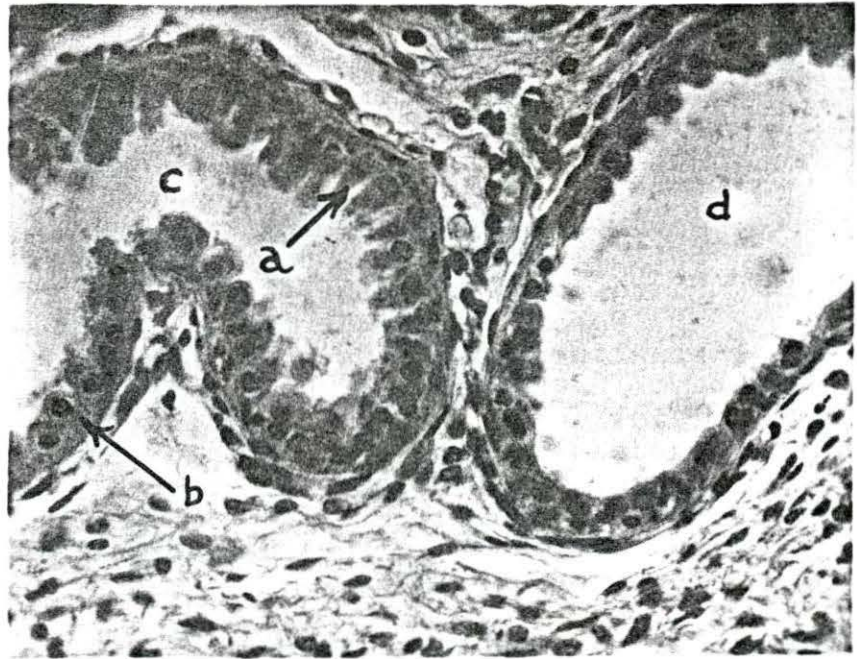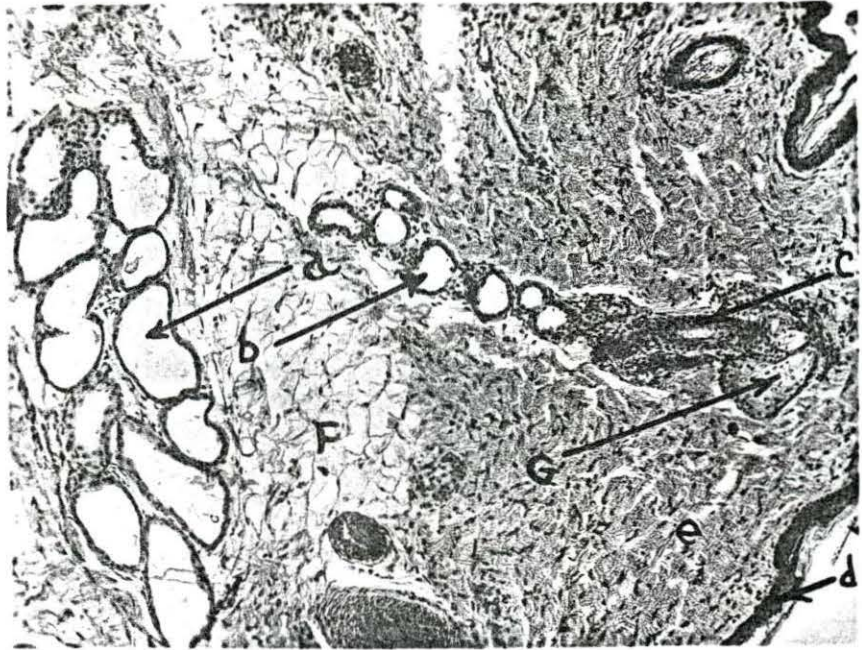   d. Post-secretory tubule

Fig. 49. Glycogen in merocrine gland – Section of front
         foot pad from dog two weeks of age, fixed in 80
         per cent alcohol and prepared with the Feulgen-
         Bauer technique for the demonstration of glycogen,
         470X magnification; note the glycogen granules in
         the cytoplasm of the merocrine gland cells

                 a. Merocrine gland tubule
                 b. Glycogen granules

Fig. 50. Apocrine glands showing myoepithelial cells –
         Section of lip of a dog three weeks of age,
         fixed in 80 per cent alcohol and prepared with
         the Gomori technique for the demonstration of
         alkaline phosphatase, six hours incubation,
         470X magnification; note the very intense
         reaction of the myoepithelial cells and their
         processes; note also that the nuclei of the
         secretory cells show evidence of alkaline
         phosphatase activity; the apical cytoplasmic
         projections into the lumen are characteristic
         of apocrine glands

                 a. Apocrine secretory cell
                 b. Fibroblast
                 c. Myoepithelial cell
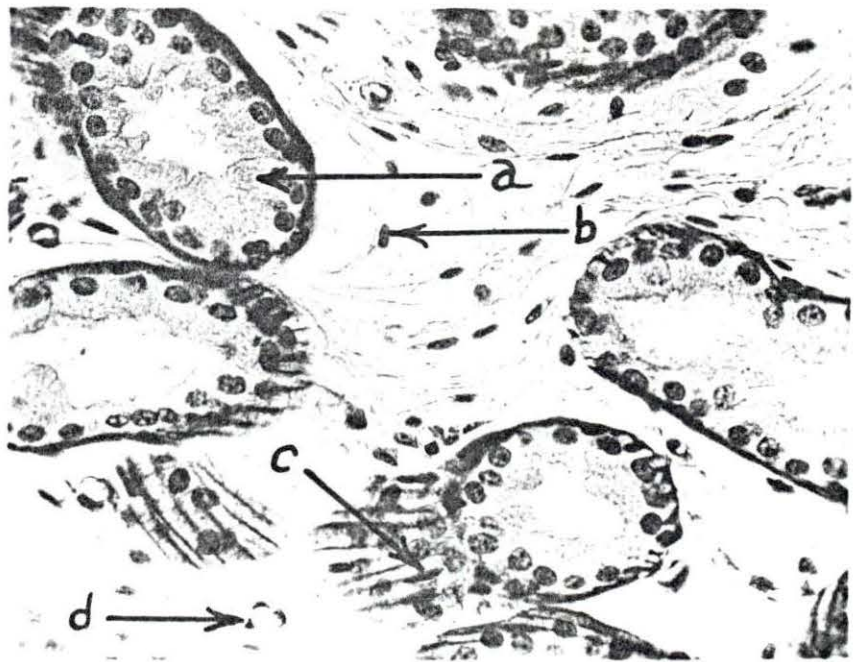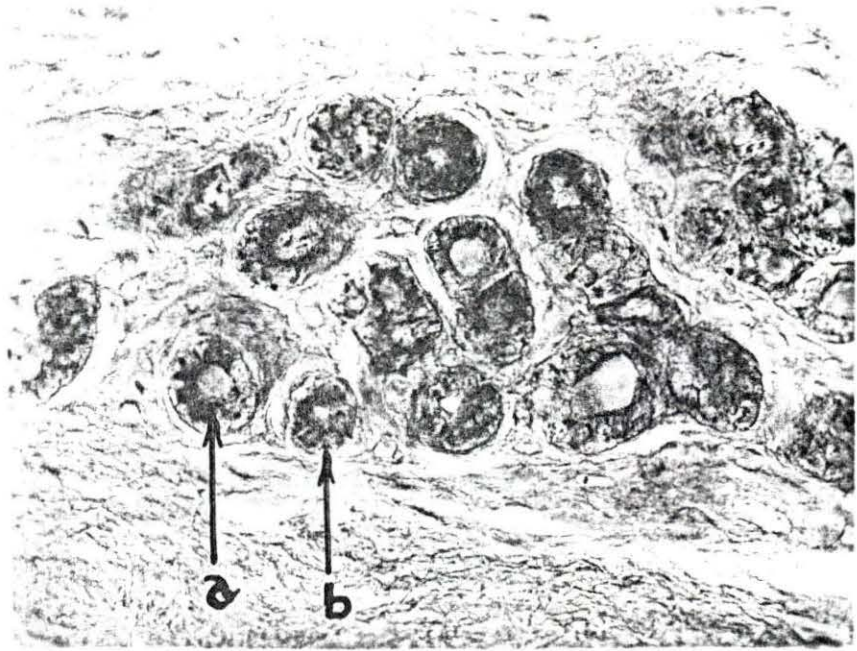   d. Capillary endothelium

Fig. 51. Phosphatase in merocrine glands - Section of subcutaneous tissue of the hind foot pad of a dog ten weeks of age, fixed in 80 per cent alcohol and prepared with the Gomori technique for the demonstration of alkaline phosphatase, 24 hours incubation, 470X magnification; note the reaction of the nuclei of the secretory cells and the fibroblast nuclei and the myo-epithelial cells

    a. Merocrine secretory cell
    b. Myoepithelial cell
    c. Fibroblast
    d. Fat cell

Fig. 52. Merocrine gland - Section of hind foot pad of a dog 12 weeks of age, fixed in 80 per cent alcohol and stained with Harris' hematoxylin and eosin Y, 470X magnification; the picture shows subcutaneous fat cells and cross sections of the merocrine secretory tubules; myoepithelial cells may also be observed

    a. Fat cell
    b. Merocrine secretory cell
    c. Myoepithelial cell
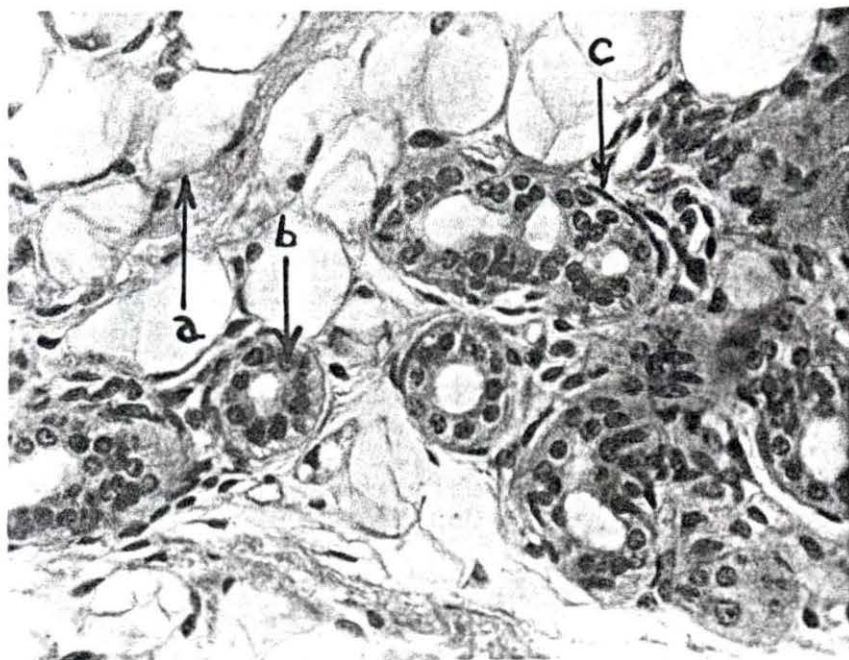
Fig. 53. Alkaline phosphatase activity in a sebaceous
          gland - Section of lip of dog three weeks of
          age, fixed in 80 per cent alcohol and prepared
          with the Gomori technique for the demonstration
          of alkaline phosphatase activity, six hours
          incubation, 470X magnification; note the very
          intense reaction of the peripheral sebaceous
          cells and the moderate to slight reaction of
          the nuclei of the central cells

              a. Peripheral sebaceous cells
              b. Central sebaceous cells
              c. Apocrine gland tubule
              d. Hair follicle

Fig. 54. Meibomian gland - Section of eyelid of a dog
          eight weeks old, fixed in 80 per cent alcohol
          and stained with hematoxylin and eosin Y, 90X
          magnification; note the large modified sebaceous
          gland at the margin of the lid

              a. Meibomian gland
              b. Epidermis
              c. Dermis
              d. Sebaceous gland and hair follicle
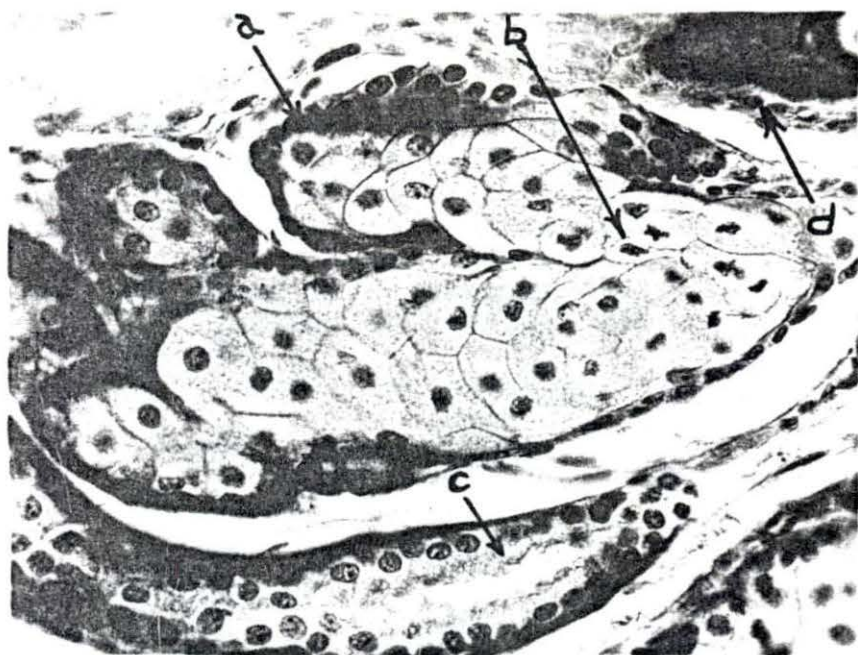   e. Conjunctiva

Fig. 55. Glycogen in arrector pili muscle - Section of
skin from the sacral region of an eight week
old dog, fixed in 80 per cent alcohol and pre-
pared with the Feulgen-Bauer technique for
glycogen, 90X magnification; note the glycogen
in the arrector pili muscle and outer root
sheath of hair follicles

        a. Epidermis
        b. Dermis
        c. Arrector pili muscle
        d. Hair follicle
        e. Glycogen granules
        f. Sebaceous gland