THE MICROSCOPIC ANATOMY OF THE INTEGUMENT OF HOLSTEIN CATTLE

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THE MICROSCOPIC ANATOMY OF THE INTEGUMENT OF HOLSTEIN CATTLE

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TABLE OF CONTENTS

<u>LIST OF TABLES</u>

LIST OF PLATES

INTRODUCTION

The skin of domestic animals has received less attention than human skin. As a result of better understanding of the functions of the skin and better diagnostic methods and modern treatment of specific skin diseases, the importance of veterinary dermatology has increased. To enrich this poorly investigated field of veterinary dermatology, the Anatomy Department of Michigan State University has taken the lead. Research has been conducted on the skin of mongrel dogs by Webb and Calhoun (1954), of Hereford and Aberdeen Angus beef cattle by Goldsberry and Calhoun (1959), of cats by Strickland and Calhoun (1960 and 1963), of newborn swine by Smith (1960), of rats by Holmes (1960), of fetal pigs by Fowler (1962), of mature hogs by Marcarian (1962) and of goats by Sar (1963).

Investigations on the cattle skin have been mainly carried out either on beef cattle or cattle in general. Very little specific histological work has been done on the skin of dairy cattle, especially of the Holstein breed. Since dairy cattle are the major source of milk, butter, cheese, cream, and a source of leather and meat as well, it is important to investigate the detailed microscopic structures of the integument of dairy cattle. This will be helpful for a proper diagnosis of skin diseases and their treatment. To fulfill this requirement, the present investigation of the microscopic anatomy of the integument of Holstein cattle, an important dairy breed of the U.S.A., has been undertaken.

REVIEW OF LITERATURE

A careful survey of the literature revealed that exhaustive investigations have been carried out on the histology, embryology, physiology, biochemistry, histochemistry and histopathology of human integument, but comparatively little work has been done on the skin of domestic animals especially of dairy cattle.

GENERAL FEATURES OF THE SKIN

Thickness

Ellenberger (1906), Sisson and Grossman (1953), and Trautmann and Fiebiger (1957) considered the skin of cattle as the thickest of all the domesticated animals. These authors, as well as Goldsberry and Calhoun (1959), stated that thickness of skin varies greatly according to breed, sex and body region. Dermal thickness was found greater in males than in females, but there was no significant difference in epidermal thickness. The thickest epidermis was found in the muzzle, head, neck, brisket and tail in both sexes while the thinnest was found in the axillary and ventral abdominal regions (Goldsberry and Calhoun, 1959). According to Bohm and Hlousek (1957), the thickest skin folds in red and white cattle were found on the larynx (15 mm), on the back and flanks (14 mm) and the thinnest on the olecranon, the metatarsus, the pastern joint area and the medial surface of the tibia (6 mm). According to

Sisson and Grossman (1953), the general thickness of cattle skin is about 3 to 4 mm, and the thickness of the skin of the tail root and the point of the hock is about 5 mm and of the brisket 6 to 7 mm. Dowling (1955b) studied thickness of the cattle skin. Tulloh (1961) described the skin and skinfold thickness in relation to the depth of subcutaneous fat in beef cattle. Patel and Anderson (1958) studied the variation of skin thickness in dairy cattle.

Pigmentation

According to Brody (1948) and others (cited by Yang, 1952), the problem of adaptability of different breeds of cattle to the tropics has drawn attention to the bovine skin pigmentation. Among European cattle breeds, Jersey cattle have the highest heat tolerance, presumably due to yellow cutaneous pigment (Bonsma and Pretorius (1943) cited by Yang (1952)). According to Yang (1952), Bonsma (1949) found dark pigmented skin with light colored hairs more resistant to the adverse effects of the long and short wave radiation of tropical countries. Spectrophotometric methods are generally applied to the analysis of human skin color (Rothman, 1954). Yang (1952) devised a method of assessing cutaneous pigmentation in bovine skin. Goldsberry and Calhoun (1959) modified Yang's method and analyzed the cutaneous pigmentation of Hereford and Aberdeen Angus cattle.

The coloration of the skin is mostly due to the pigment melanin which occurs as fine brown to black granules within and between cells of the stratum germinativum and occasionally in dermal cells. Melanin producing cells are called melanoblasts in embryos and melanocytes in adults. Dermal cells which contain, but do not manufacture melanin, are called chromatophores (Ham and Leeson, 1961). Previously melanocytes were commonly called melanoblasts (Rothman, 1954). Melanocytes are dendritic cells in the basal layer and appear as clear cells. Clear cells may be melanocytes or keratinocytes, but only melanocytes are positive for DOPA reaction (Clark et al., 1961). Melanoblasts originate from the neural crest of the embryo, migrate to the basal layer of the skin, mature to melanocytes and produce melanin (Arey, 1954). Melanocytes are the only melanin producing cells and the other cells which contain melanin are not true melanocytes since they obtain these pigment granules by their phagocytic action (Ham and Leeson, 1961). In the year 1948, Masson stated that pigment granules are distributed to adjacent cells via dendrites of melanocytes. These cells are known as 'cytocrine' (cited by Rothman, 1954). In the basal cells of the pigmented skin, these granules are arranged around the upper pole of the nucleus in such a manner that they appear as supranuclear caps (Hu and Cardell, 1962).

The synthesis of melanin in cells is not fully understood. Ham and Leeson (1961) stated that probably ultraviolet rays and catalysts break down tyrosine and form DOPA-like material which is acted upon by the DOPA oxidase of melanocytes and turned into melanin. According to Rothman

(1954) it is regarded as the oxidation product of tyrosine or an orthodihydroxyphenyl compound. On the basis of morphological, chemical and enzymatic observations, melanin granules have been regarded as kinds of mitochondria which differ from other mitochondria as they contain a melanogenic enzyme, tyrosinase, which oxidizes tyrosine to melanin. Melanin formation is also influenced by the following factors: Physical factors, viz., sunlight, ultraviolet rays, heat rays; nutritional factors such as trace metals--copper, iron, silver, gold, bismuth and arsenic; amino acids; vitamins--vitamin A, B-Complex and vitamin C; and hormonal--pituitary, thyroid, adrenal, and gonadal hormones. In the year 1948, Meirowsky and Behr observed melanin formation in the intranuclear vacuoles.

Keratinization

Keratinization or cornification is a process by which living epithelial cells are transformed into horny structures. By this process, the cytoplasmic proteins are changed into keratin, and the cytoplasm and nuclei of the cells degenerate (Rothman, 1954). According to Matoltsy (1962), it is a specific form of cell differentiation in which the active epithelial cells are packed with an insoluble and resistant horny material produced by various cytological and physiological processes.

Keratins are modified proteins which are found in keratinous structures such as the horny layer of skin, hairs, nails, hoofs, horns and claws (Rothman, 1954). Unna (1920,

1921) classified keratins into four groups, keratin A, B, C, and keratin--albumoses. According to Giroud *et al.* (1934), keratins are of two types, hard keratin and soft keratin. Hard Keratin has a high content of sulfur and cystine and does not desquamate, while soft keratin has a low content of sulfur and cystine and desquamates. Recently Matoltsy (1962) has described three kinds of keratin, amorphous, fibrous and mixed.

Keratohyaline granules, which occur in the cells of the stratum granulosum and stratum lucidum, are not keratin but a complex substance. According to Rothman (1954), they may be regarded as the decomposition products of protein. They are called keratohyalin granules because they are connected with keratin formation and they are hyaline in appearance. They do not contain SH group and -S-S- bond. Morphological and chemical properties of these granules were reported by Matoltsy and Matoltsy (1962). Rothman (1954) also described 'eleidin,' which occurs in the cells of the stratum granulosum and stratum lucidum, as a product of protein decomposition. It is called eleidin because it resembles fatty droplets (Gk. elaia--olive oil).

Many past and present workers have tried to understand the mechanism of keratinization, but it is still not fully understood. Giroud and Leblond (1951) described two forms of keratinization: soft keratinization and hard keratinization based on morphological appearance and chemical constitutions. In soft keratinization the epithelial cells transform into horny material passing through granular and glassy stages and contain small amounts of sulfur. The epidermis, medullary cell line of the hair, inner root sheath of hair follicles, corns and chestnuts are representatives of this form. In hard keratinization, the epithelial cells modify into horny material without passing into granular and glassy stages and also contain a great amount of sulfur. Its examples are cuticular and cortical cell lines of the hair, nail, horn, hoof and claw. In both types of keratinization, L-keratin containing tonofibrils plays a great role. Tonofibrils, which are usual features of cells of the Malpighian layer, were also observed in cells undergoing keratinization. They contain sulfhydryl groups. According to Matoltsy (1962), Birbeck and Mercer (1957) stated that besides tonofibrils, other cytoplasmic constituents also play a part in keratinization. Recently Matoltsy (1962) described three types of keratinization--keratinization resulting in formation of amorphous keratin, e.g., cuticular line of hair or wool; keratinization resulting in production of fibrous keratin, e.g., cortical cell line of hair, wool and quill; and keratinization resulting in manufacture of a mixture of both fibrils and granules (keratohyaline and trichohyaline granules), for example, mammalian epidermis, inner root sheath of hair follicle.

Matoltsy (1962) also stated that all keratinizing cells pass through three different stages--germinative, specialized and terminal horny stages. Each epithelial cell keratinizes individually. Cytoplasmic fibrils and keratohyalin granules play the principal role in the formation of cornified epithelial cells. Keratohyaline granules do not contain -SH group (Rothman, 1954, Matoltsy, 1962); whereas the cornified layer of epidermis contains disulfide groups which are supposed to be derived from sulfhydril groups of tonofibrils (Copenhaver and Johnson, 1958).

According to Rothman (1954), Claude (1859, 1878-79) observed that the soft keratogenous zone of the fetal cow hoof contained abundant glycogen which disappeared after keratinization. Rothman (1954) supposed the presence of glycogen was necessary for energy supply in keratinization. He pointed out that hormones and vitamins also influence keratinization. The administration of estrogen affects the epithelial activity in both sexes. It increases proliferation, keratinization and desquamation of cells. Vitamin A, like estrogen, also modifies keratinization. It inhibits the differentiation of stratified squamous epithelium and therefore acts as an antikeratinizing factor.

Aging of Skin

Little work has been done on the aging of skin of domestic animals. Klein (1959) worked on the chemical and microscopic changes of the aging human skin. He observed the flattening of the rete pegs and atrophy of the collagenous bundles in skin covered by clothes and senile degeneration of collagenous and elastic fibers in exposed skin. He also reported a decrease in cholesterol and an increase in calcium content of skin. According to him, greying of hairs is associated with cardiovascular disorders. Hafez *et al*. (1955) reported age differences in the histology of buffalo and cattle skin. He observed numerical differences of hair follicles per square centimeter in a five-month-old embryo, a calf at birth and adult buffaloes. There were no skin glands in 1 to 5-month-old fetuses. The sweat glands of the calf were smaller in size and fewer in number than those of the adults. In structure they were similar to those of adults. According to Rothman (1954), elastic tissue in skin is relatively more abundant in young animals than in adults. With advancing age, collagenous fibers become progressively matted and intertwined until dense bundles are formed in the adult. Andrew (1951) studied age changes in the skin of rats.

Functional Anatomy

General--The skin is a protective membrane against disease organisms, water and excessive light (Ham and Leeson, 1961). It regulates the body heat by radiation, convection, conduction and evaporation (Rothman, 1954).

Hair--Cattle hair furnishes protection against water, rain, cold and insects (Trumbower, 1904). The terminal tail hairs of cattle act as a swatter for biting insects. Eyebrows, eyelashes, hairs of the nostril, and external auditory canal hairs guard against the entrance of harmful substances like sweat and dust (Rothman, 1954). They help in heat regulation of the body by conduction, radiation and evaporation. They protect the body from extreme and sudden variations of temperature since they are a poor conductor of heat. Cold acts as a stimulus to the growth of hairs and therefore the coat becomes thicker in winter than in summer. Contraction of arrector pili muscles makes the hairs become erect and the coat thicker (Trumbower, 1904). Tactile hairs act as sensory apparatus. Hairs help in excretion of certain metals such as arsenic, iron, lime, lead, etc. (Rothman, 1954).

Sebaceous Glands--These holocrine glands secrete sebum, an oily semiliquid substance which solidifies upon exposure to air. The sebum makes the skin and hair smooth and pliable. It contains precursors of vitamin D (Trautman and Febiger, 1957; Kral, 1960; Dukes, 1955). The size and activity of sebaceous glands are proportionate to the amount of sexual hormones produced in the body. They are smaller in young animals and larger in matured animals. The production of sebum is continuous, but the amount is influenced by climate and environment (Kral, 1960). Rothman (1954) also reported that human sebaceous glands are influenced by age, sex, hormones and nutrition. According to Rothman (1954), Schaffer (1930) reported that sebaceous glands of mammals, like the apocrine coiled glands, excrete species and sex-specific odorous substances which play a role in sexual attraction and recognition of the animals of the particular species.

Sweat Glands--The skin may supplement the function of lungs and kidneys by excreting a considerable amount of water and small amounts of salts and urea (Trumbower, 1904). Cattle were found sweating when they were put to work in warm and humid conditions (Dowling, 1955a; Taneja, 1959a, b, and 1960). In 1955, Ferguson and Dowling observed moisture at the root of hair and concluded that sweat glands of cattle were probably functional. Findlay and Jenkinson (1960) reported that the lumina of sweat glands contained fluid-like material which probably was produced by the active secretion of the epithelium. Seasonal variations in skin secretions were observed by Tulloh (1962). Sweat glands of cattle help in regulation of body temperature (Knapp and Robinson, 1954; Ferguson and Dowling, 1955; Chowdhury and Sadhu, 1961). Temperature regulation by sweating is less effective in ruminants than in man (Dukes, 1955). Secretion of sweat is controlled by secretory nerve fibers of the sympathetic system. The secretory fibers of the sweat glands are cholinergic in cats and dogs and adrenergic in horses and sheep (Dukes, 1955). Sweat glands of cattle are adrenergic (Taneja, 1959a).

Vascular System--Cutaneous blood vessels are under control of the autonomic nervous system. They also play an important role in temperature regulation. Dilatation of arterioles and capillaries in a "flushed" skin helps in heat loss; whereas constriction of the arterioles in a blanched skin aids in conserving heat (Romer, 1962).

Blood Supply

Findlay and Yang (1948) described the capillary distribution in cattle skin. They divided the corium into five indistinct layers--the subepidermal, sebaceous gland, subsebaceous gland, sweat gland, and the subsweat gland levels. In the subepidermal level, fine capillaries form anastomoses and encircle hair follicles. In the sebaceous gland level, fine arterioles extend toward the epithelium. In the subsebaceous and sweat gland levels, groups of two or more hair follicles are surrounded by capillaries. In the subsweat gland level, few arterioles and venules are found. Goodall and Yang (1954) observed three plexuses of blood vessels in the skin of Ayrshire calves and embryos. The first lies below the corium, the second is situated between the sweat and sebaceous glands, and the third is a network of fine blood vessels extending from the second plexus to the dermal-epidermal junction. The sweat glands generally have a poor blood supply, while the hair follicles and papillae have a rich supply. Goodall (1955) observed arteriovenous anastomoses in the skin of the forehead, cheeks and ears of calves. Melczer (1926) and Eimer (1929) (cited by Hafez et al., 1955) found that lymphatics originated from intercellular spaces in the epidermis and passed through the dermis. According to Sisson and Grossman (1953), the arteries of the skin enter from the subcutis and form plexuses in the deep corium and under the dermal papillae. The deep plexuses supply vessels to fat and sweat glands;

while the papillary plexuses supply hair follicles and sebaceous glands. The veins also form two plexuses--one beneath the papillae and the other at the coriosubcutis junction. The lymph vessels form subpapillary and subcutaneous plexuses.

Nerve Supply

Since the skin is a sense organ, its nerves are mainly sensory which are peripheral branches of somatic ganglion cells. Efferent sympathetic nerve fibers supply the smooth muscle of the blood vessels, the arrectores pilorum and the secretory cells of the sweat glands (Copenhaver and Johnson, 1958). Kuntz and Hamilton (1938) described the afferent innervation of the skin of the cat. According to Weddell *et al*. (1955), Frey (1894-96) recognized four primary modalities: touch, cold, warmth and pain in the skin. He stated that each modality was associated with a morphologically specific nerve ending which lies beneath each respective sensory spot, viz. hairs and Meissner's corpuscles with touch, Krause's end bulb with cold, Ruffini corpuscles with warmth and free nerve terminals with pain sensibility. According to Rothman (1954), Saunders (1947) stated that Meissner's corpuscles, nerve terminals around the sheath of hair and Merkel's touch cells, mediate touch sensation; while pacinian corpuscles are assigned to pressure.

Epidermis of man contains free nerve endings (Copenhaver and Johnson, 1958). They are probably responsible for pain sensibility (Rothman, 1954). The epidermis around the nostril of moles, rats, cats, dogs and pigs contains intraepidermal nerve fibers (Cauna, 1959). Kadanoff (1924) observed three types of intraepidermal nerve fibers in the nasal skin of the cow. Intraepithelial nerve fibers are absent in the skin of the teat of the cow (Niggli-Stokar, 1961). Merkel's cells of mammals are found in the deepest layers of the epidermis (Trautmann and Fiebiger, 1957).

Dermis contains tactile corpuscles of Meissner in the papillae and Krause's end bulbs beneath the papillae (Copenhaver and Johnson, 1958). The connective tissue of the hoof and claws contains free nerve endings. The corium of the hoof contains the corpuscles of Pacini, Golgi-Mazzoni, and Ruffini (Trautmann and Fiebiger, 1957). Weddell *et al*. (1955) pointed out the presence of unencapsulated nerve endings in the dermis.

Hair follicles--As a rule human hair follicles contain at least one nerve fiber which enters the follicle below the opening of the sebaceous gland (Copenhaver and Johnson, 1958). According to Rothman (1954), Frey (1896) described two sets of nerve fibers in man: one at the level of the hair papilla and the other at the narrow part of the neck of the follicle. Stetson (1923) (cited by Rothman, 1954) stated that papillary nerve fibers are nonsensory, while fibers around the neck are sensory. Mammalian vibrissae are innervated by thick nerve bundles which enter the fibrous sheath obliquely and supply the epithelial root sheath (Dixon, 1961).

<u>Sebaceous glands</u>--The periglandular connective tissue of human sebaceous glands contains a ramifying bundle of nerve fibers. From these fibers, slender branches follow the intertubular septa, break up into very fine fibers and form a plexus on the basement membrane of the gland (Rothman, 1954). The secretory activity of sebaceous glands is controlled by the nervous system (Rothman and Felsher, 1946, cited by Rothman, 1954).

<u>Subcutis</u> contains larger nerve trunks. The Pacinian corpuscles are found in it. The finer branches from the large nerve trunks pass through the corium and form subpapillary plexuses of both medullated and nonmedullated nerve fibers (Copenhaver and Johnson, 1958). Pacinian corpuscles are present in the subcutis of the planum nasolabiale and near the coccygeal artery of long-tailed animals (Trautmann and Fiebiger, 1957). Moriconi and Renzoni (1955) observed the pacinian corpuscles in the muzzle of <u>Bos taurus</u>.

COMPONENTS OF THE SKIN

Epidermis

The skin or cutis consists of two layers, epithelial part (epidermis) and connective tissue part (dermis or corium). The epidermis is divided into four layers: the stratum germinativum or Malpighii, the stratum granulosum, the stratum lucidum and the stratum corneum (Trautmann and Fiebiger, 1957). The epidermis is nonvascular and presents openings of cutaneous glands and hair follicles (Sisson and Grossman, 1953). Epidermal thickness varies according to the exposure of the skin to physical influences (Yamane and Ono, 1936).

Stratum germinativum is further subdivided into two layers, the stratum cylindricum and the stratum spinosum or prickle cell layer (Trautmann and Fiebiger, 1957). The stratum cylindricum in cattle consists of a single layer of high columnar epithelial cells. They are tall in thick epidermis and low columnar in thin epidermis. Hereford cattle usually have taller basal cells than do Aberdeen Angus cattle. These cells rest on a thin basement membrane which replicates elevations and depressions of the corium. They have cytoplasmic processes which penetrate the basement membrane. These processes extend into the dermis where dermal fibers are fine and loose (Goldsberry and Calhoun, 1959). Mitotic activity is most prominent in this layer (Cowdry, 1944; Ham and Leeson, 1961). Mitotic frequency is greatest during the period of rest (Cooper and Franklin, 1940; Trautmann and Fiebiger, 1957). These cells may contain melanin granules. The stratum spinosum is composed of irregular polyhedral cells. These cells contain intracellular fibrils called 'tonofibrils' which converge towards the cell membrane and produce delicate spinelike processes and hence the name stratum spinosum is given. Under the light microscope, these processes appear as continuous cytoplasmic intercellular bridges. Electron microscopic studies reveal that there are no intercellular bridges, but the tonofibrils of adjacent cells converge towards the cell membrane and produce thickenings called desmosomes which give the appearance of intercellular bridges (Odland, 1958; Copenhaver and Johnson, 1958; Ham and Leeson, 1961). Intercellular bridges are more prominent where five or more rows of cells are present.

Stratum granulosum consists of one to five layers of rhomboidal cells just outside the stratum spinosum. These cells are arranged parallel to the surface (Ham and Leeson, 1961). In Aberdeen Angus and Hereford cattle, this layer is poorly developed and is generally represented only by occasional cells. Epidermis over the fetlock, which is fairly thick, contains one to two rows of granular cells, but the extremely thick muzzle epidermis contains only a few of these cells (Goldsberry and Calhoun, 1959). These cells contain keratohyaline granules which stain deeply with haematoxylin. Tonofibrils are difficult to see. Nuclear shrinkage and chromotolysis starts in this layer (Trautmann and Fiebiger, 1957).

Stratum lucidum is a clear, homogeneous acidophilic layer. It consists of non-nucleated flattened cells with thin cell membranes. Keratohyalin granules are changed into a specifically stainable diffuse substance called 'eleidin,' which is of unknown significance and is not essential for keratinization. It may be absent in thin epidermis (Trautmann and Fiebiger, 1957). The nucleus and all cytoplasmic structures except tonofibrils disappear from these cells (Bloom and Fawcett, 1962). In Aberdeen Angus and Hereford cattle this layer is present only where the epidermis is moderately thick, but is absent in the muzzle, margins of hoofs and horns and perianal regions (Goldsberry and Calhoun, 1959).

Stratum corneum is the outermost layer. It is composed of many strata of flattened non-nucleated cornified, closely packed cells. Intercellular bridges are absent. Peripheral cells become dead and constantly shed off as scales. Many fine filaments of fibrous protein are seen in the cytoplasm and are known as keratin (Arey, 1954; Trautmann and Fiebiger, 1957; Ham and Leeson, 1961). This layer presents numerous furrows and ridges extending in different directions. The ridges correspond to dermal papillae. These furrows and ridges are valuable for identification of an individual in the human species (Ham, 1961). Schmid and Burghart (1961) pointed out the importance of this feature for identification of cattle by their muzzle prints.

Dermis (Corium, Leather Skin)

Layers--The dermis is divided into two layers: papillary (superficial) and reticular (deep), but there is no clear demarcation between them (Goldsberry and Calhoun, 1959; Bloom and Fawcett, 1962). These two layers are distinct where rete pegs are present and indistinct where rete pegs are absent (Finerty and Cowdry, 1960). The dermis is the thickest component of the skin (Goldsberry and Calhoun, 1959).

Stratum papillare is composed of delicate reticular fibers and an admixture of fine collagenous and elastic fibers (Trautmann and Fiebiger, 1957). Dempsey (1948) stated that it contains a dense network of elastic fibers, some of which give attachment to the arrectores pilorum. This layer, in certain areas, presents numerous projections of varying shape and size, termed dermal papillae (Trautmann and Fiebiger, 1957). The papillae are fitted into corresponding depressions of epidermis (Sisson and Grossman, 1953; Copenhaver and Johnson, 1958). Papillae are absent or poorly developed where hairs are long and dense. Some may be bifurcated as in the external genitalia and planum nasale, and others may be compound as in the foot pads of dogs. They are generally numerous at various mucocutaneous junctions such as planum nasale, labial margin, teats, udder, glans penis, clitoris and the tip of the tail (Trautmann and Fiebiger, 1957). The papillae are better developed in Holstein-Friesian than oriental breeds (Yamane and Ono, 1936). The corium varies in thickness but the papillary layer remains constant (Dempsey, 1948). Wilson (1941) considered the papillary layer a thermostat layer.

Stratum reticulare consists of a network of dense collagenous and elastic fibers (Lambert, 1948). According to Goldsberry and Calhoun (1959), it is about three times thicker than the stratum papillare in cattle.

Structural components of the dermis are connective tissue fibers--collagenous, elastic and reticular (Trautmann and Fiebiger, 1957); cells--fibroblasts, histocytes, dermal chromatophores, smooth muscle cells (Copenhaver and

Johnson, 1958), mast cells (Riley, 1959), fat cells and other occasional cells (Ham and Leeson, 1961). The corium also contains numerous blood vessels, nerves, cutaneous glands and hair follicles (Sisson and Grossman, 1953).

Collagenous fibers or bundles are composed of very fine, smooth, unbranched, usually wavy fibrils united by interfibrillar mucoid substance. The fibrillae do not branch, but fibers branch and anastomose. These fibers, which are responsible for the tensile strength of the skin, are soft, flexible, slightly extensible, vary in thickness, width and length, and follow a wavy course (Trautmann and Fiebiger, 1957; Ham and Leeson, 1961). The collagenic fibers of the papillary layer are finer than in the reticular layer (Ham and Leeson, 1961). They are arranged irregularly in the papillary layer and are mostly parallel to the surface in the reticular layer (Lever, 1961). In cattle, they are arranged in bundles extending in all directions, but fibers parallel to the surface of the skin predominate (Goldsberry and Calhoun, 1959). Chemically, these fibers consist of an albuminous substance, 'collagen,' which, on boiling, yields gelatin (Copenhaver and Johnson, 1958).

Elastic fibers are homogenous and highly refractile. They are thinner than collagenous fibers. They branch and anastomose, but do not form bundles (Copenhaver and Johnson, 1958). Some elastic fibers join the collagenous bundles, while others form a network with them specially in the superficial layer of the corium of cattle, sheep and dogs. They also surround blood vessels and hair follicles. They are highly elastic and responsible for tension of the skin (Trautmann and Fiebiger, 1957). The elasticity of the skin varies according to age, sex and body regions. Generally, females have more elastin than males. The elastic network of the papillary layer is fine and lies close to the epidermis, but it is not continuous with the basement membrane. Elastic fibers of the deep corium are coarse (Dick, 1947). They are arranged loosely, irregularly and vertically in the superficial corium and compactly in the deep corium (Lever, 1961). They develop similarly to collagenous fibers, but at a later stage (Copenhaver and Johnson, 1958). Lowery _et al_. (1941) studied collagen and elastin in tissues in different species. Elastic fibers contain protein (elastin) which is resistant to boiling, acids and alkalies (Trautmann and Fiebiger, 1957).

Reticular fibers (argyrophilic fibers) are of varying thickness, but usually very fine. They branch, anastomose and form a lattice-like network (Trautmann and Fiebiger, 1957). They lie below the epidermis (Robb-Smith, 1945, 1957). They are components of the basement membrane, papillary corium, connective tissue sheaths of sweat glands and hair follicles (Montagna, 1956). Reticular networks are generally connected to and supported by thicker collagenous fibers. Electron microscopic study reveals that reticular fibers are similar in nature to the fibrils of collagen bundles, but finer in texture (Ham and Leeson, 1961). Their origin is also like collagen fibers (Copenhaver and Johnson, 1958). Maximow (1930) demonstrated that in tissue culture the first extracellular fibrils that appeared were argyrophilic which later were transformed into less argyrophilic, thicker, collagen fibers (cited by Copenhaver and Johnson, 1958).

Fibroblasts are the predominating cells of the dermis (Ham and Leeson, 1961). They are large, flat, branching cells with extensive processes. The cell wall is very delicate. The cytoplasm is homogenous or finely granular and faintly stainable. The nuclei may be elliptical, oval or spherical and are poor in chromatin. They lie upon the collagen fibers and sometimes form a sheath around them (Trautmann and Fiebiger, 1957; Copenhaver and Johnson, 1958). According to Ham and Leeson (1961), older fibroblasts may be called fibrocytes. They are large and branching in the papillary layer and long and thin in the reticular layer (Montagna, 1956).

Histiocytes or fixed macrophages or resting wandering cells are multiform cells with short processes. They resemble fibroblasts, but have distinct outlines and stain better than fibroblasts. The cytoplasm is granular and the nuclei are richer in chromatin. They are found in all loose connective tissue especially near the small blood vessels (Trautmann and Fiebiger, 1957).

Mast cells or histogenous mast cells were first described by Erlich in 1887 (cited by Ham and Leeson, 1961).

They resemble basophilic leukocytes (hematogenous mast cells). They are large, rounded, oval cells with spherical nuclei and coarse cytoplasmic granules (Trautmann and Fiebiger, 1957). According to Hansen (1957), the cytoplasmic granules are metachromatic. Mast cells may be seen scattered in the entire dermis (Holmes, 1960; Marcarian, 1962), but they are mainly found along the blood vessels, hair follicles (Holmes, 1960; Marcarian, 1962) and along the lymphatics and nerves (Ham and Leeson, 1961). They are more abundant in children than adults (Brach, 1925, cited by Hellstrom and Holmgren, 1950). They are more numerous in the papillary layer than the reticular layer (Montagna, 1956; Marcarian, 1962) and increased in female swine (Marcarian, 1962). According to Hellstrom and Holmgren (1950), the mast cells are of two types: the first ones are small and multiform, with small nuclei and metachromatic fine granules which are generally formed just below the dermis; the second ones are larger and contain larger nuclei, more cytoplasm and more granules. They are formed in the deeper layer of the dermis. Metachromasia in these cells is due to the storage of mucopolysaccharides in them (Montagna, 1956).

The dermal-epidermal junction (basement or basal or dermal membrane) has been studied by many authors--Dick (1947), Medawar (1953), Odland (1950, 1958), Montagna (1956), Cooper (1958) and Haines (1958), but its structure is not fully settled. Odland (1950) investigated it in human skin and reported that the cytoplasmic processes of the basal cells of the epidermis interdigitated with a continuous network of dermal reticulum in the spaces of the basement membrane. Medawar (1953) and Montagna (1956) reported that it is composed of a complex reticular network and an amorphous intercellular substance, to which cytoplasmic processes of the basal cells extend. Haines (1958) observed it in cattle and described tonofibrils of the basal cells projecting beyond their bases and interlocking with continuous subepithelial argyrophilic dermal fibers. He also observed PAS positive fibrils projecting into the epidermis from argyrophilic dermal fibers. Odland (1958), on the basis of electron microscopic study, reported that it was not pierced by dermal collagenous filaments. According to Odland, this ill-defined junction which was named 'basement membrane' by Porter (1954), 'dermal membrane' by Selby (1955), and 'dermo-epidermal junction' by other workers, is a moderately dense homogenous membrane. Schmidt (1962), on the basis of histologic and histochemical investigation of the basement membrane in the newt's skin, observed collagen superficially and reticulin deeply embedded in the polysaccharide ground substance of the membrane. He also noticed fat droplets along the internal surface of the membrane, suggesting a possible functional association.

<u>The subcutis or hypodermis</u> is not considered a part of the skin, but it gives anchorage for the skin (Ham and

Leeson, 1961). It consists of loose areolar tissue with many elastic fibers and adipose tissue (Trautmann and Fiebiger, 1957). Bohm and Hlousek (1955) and Tulloh (1961) studied the subcutaneous tissue in cattle by the skin fold technique. By the use of this method they described two types of subcutaneous tissue: one which is rich in elastic tissue and provides higher skin folds and the other which is poor in elastic tissue and gives lower or no folds. It contains fibroblasts, histiocytes, pigment cells, plasma cells and eosinophils. When more fat is present it is termed 'panniculus adiposus' (Trautmann and Fiebiger, 1957). The cell picture of the subcutis of the cow was observed by Linder (1963).

Hair

Classification, density and arrangement of hairs--Hairs are present on the entire surface of the body except a few areas such as the nose, teats, and mucocutaneous junctions (Trautmann and Fiebiger, 1957). According to Sisson and Grossman (1953), hairs may be classified into two groups: ordinary or coat hairs which cover the general body surface and special hairs which are found at certain areas with special functions such as tactile hairs of the lips, eyelashes or cilia of the eyelids, tragi of the external ear and vibrissae of the nostrils. According to Schwartzman *et al*. (1962), hairs are of three types: guard hairs (spines, bristles) which grow from primary hair follicles and have a definite nerve supply; under hairs (wool, fur, lanugo, vellus) which are fine and soft and grow from secondary or accessory follicles and have no nerve supply, and tactile hairs (feelers, whiskers, vibrissae, sinus hairs, sensory hairs) which are large and stiff, surrounded by a vascular sinus and supplied with sensory nerve fibers. Winkelmann (1959) reported that sensory hairs are surrounded by a connective tissue sheath which contains a vascular sinus and 2 to 5 nerve bundles. Facial vibrissae are tactile hairs found on lips, chin, cheeks, snout, and around the eyes. According to Holmes (1960), Davidson and Hardy (1952) stated that all vibrissae follicles in the mouse are distinguished from coat hair follicles by possessing blood sinuses and abundant nerve endings. Guard hairs are long, stiff, straight, pointed, coarse hairs which are found scattered in the fur (Hyman, 1942). Wool hairs or lanugo hairs are fine hairs and form the fleece of the sheep. They are not confined to sheep only. They are prominent in the very young animal. In man they are lost during fetal life (Trautmann and Fiebiger, 1957). Tylotrich follicles, which are found in the skin of rats, rabbits, mice and guinea pigs, are special tactile hairs (Straile, 1960, 1961).

According to Dowling (1955a), the density of the hairs per square centimeter is 650 to 780 in Afrikanders (Bonsma, 1940), 466 to 780 in Zebu and 498 in Brown Swiss (Manucarov, 1951), 1871 in *Bos taurus* (Findlay and Yang, 1948) and 2633 in Egyptian cattle (Hafez *et al*. (1955). Dowling also reported that the density of hairs is influenced by age and nutrition. According to Goldsberry and Calhoun (1959), the density of hairs per square centimeter is 1336 in Aberdeen Angus males and 1308 in females, and 1010 in Hereford males and 1194 in females.

The pattern of distribution and the arrangement of hair varies greatly according to species. Hairs are distributed evenly in cattle and horses and in groups in dogs, cats and pigs. Each group usually consists of one main large hair and two small hairs. Each of these three cover hairs is surrounded by varying numbers of wool hairs. The follicles of wool hairs branch off from the follicle of the cover hair at the sebaceous gland opening level (Trautmann and Fiebiger, 1957). Coat hairs are arranged in groups of three in swine (Ellenberger, 1906; Smith, 1960; Marcarian, 1962) and dogs (Webb and Calhoun, 1954), in groups of 12 to 20 in cats (Strickland and Calhoun, 1960, 1963), and in groups of 75 in chinchilla (Wilcox, 1950). A guard hair is surrounded by 3 to 9 auxillary or wool hairs in the dog (Lovell and Getty, 1957), 2 to 5 clusters of hairs with each cluster containing 3 primary hairs encircled by 6 to 12 wool hairs in the cat (Strickland and Calhoun, 1963) and 2 lateral groups of wool hairs in the chinchilla (Wilcox, 1950). Each hair follicle, generally associated with a sebaceous gland, a sweat gland, and an arrector pili muscle, is known as a hair unit (Hafez *et al.*, 1955).

Microscopic structure--Each hair consists of a shaft which projects above the skin and a root which is embedded within the hair follicle. The distal end of the root is expanded into a knoblike structure called bulb of the hair. It has a conical indentation (papilla) filled with connective tissue (Copenhaver and Johnson, 1958).

Hair--According to Trautmann and Fiebiger (1957) and Copenhaver and Johnson (1958), a hair consists of the medulla, the cortex and the cuticle. The medulla is the central core of the hair and is composed of cells which are generally cuboidal and solid in the root and flattened from top to bottom and vacuolated in the shaft. It gradually disappears towards the tip of the hairs. The medullary cells of the shaft become cornified and shrunken and their nuclei become rudimentary or absent. The intercellular spaces are generally filled with air which makes hair gray. These cells also contain pigment, but do not impart color to the hairs. The medulla is absent in finer coat hairs, wool hairs and hair of the human scalp. The cortex surrounds the medulla and consists of cornified, elongated, fusiform cells arranged parallel to the hair. Toward the bulb, the cells become cuboidal. These cells contain pigment which imparts color to the hairs. Intercellular spaces of the cortex also contain air which modifies hair color. The cuticle of hairs is composed of a single layer of thin, flat, cornified and nonnucleated cells which are arranged like shingles with their edges directing upward and outward. The bulb of hairs consists of a central dermal papilla and surrounding active cells, the matrix of the hair.

The hair follicle consists of a connective tissue sheath and an epithelial sheath, which is further subdivided into outer and inner epithelial root sheaths. The connective tissue root sheath is derived from the dermis and is composed of outer, middle and inner layers. The outer layer is composed of longitudinal collagenous fibers and fine elastic fibers and contains a rich blood and nerve supply. The middle layer is composed of fine circular collagenous fibers. The inner or glassy layer is a homogenous membrane, closely applied to the epithelial root sheath (Trautmann and Fiebiger, 1957; and Copenhaver and Johnson, 1958). The outer epithelial root sheath is the direct continuation of the stratum germinativum. It contains peripheral columnar and inner polygonal cells (Copenhaver and Johnson, 1958). The inner epithelial root sheath grows upward from the papilla and extends to the opening of the sebaceous gland (Trautmann and Fiebiger, 1957). It consists of three distinct layers from without inward: Henle's layer, Huxley's layer and the cuticle of the sheath (Copenhaver and Johnson, 1958). Henle's layer consists of a single layer of nonnucleated cells while Huxley's layer contains one to three rows of nucleated cells. The cuticle of the sheath is similar to the hair cuticle and the free edges of cells are directed downward and interlocked with the cells of hair cuticle (Ham and Leeson, 1961).

Follicular folds are corrugations in the hair follicle near the sebaceous gland opening. They were observed in the cat by Strickland and Calhoun (1963), in cattle by Goldsberry and Calhoun (1959), in swine by Smith (1960), Fowler (1962) and Marcarian (1962), and in goats by Sar (1963). Montagna (1956) observed these corrugations in the rat, mouse and sheep, but not in man.

Tactile hairs--The structure of tactile hairs in rats was described by Vincent (1913) and Holmes (1960), in mice by Melaragno and Montagna (1953), in pigs by Smith (1960), Fowler (1962) and Marcarian (1962), and in goats by Sar (1963). According to these investigations, the structure of the follicles of tactile hairs is similar to that of coat hairs except that the connective tissue sheath is well developed and encloses a blood sinus. The sinus is traversed by trabeculae composed of collagenous and elastic fibers. The tactile hairs lack arrector pili muscles (Trautmann and Fiebiger, 1957).

Mm. arrectores pilorum are oblique bundles of smooth muscle and numerous elastic fibers extending from the hair follicles to the papillary dermis (Ham and Leeson, 1961). The excretory ducts of the sweat glands frequently perforate the arrectores pilorum. Sometimes these muscles divide into several branches near the body of the sebaceous gland and extend in fan shaped manner toward the epidermis. They are absent from eyelashes in carnivores and man (Trautmann and Fiebiger, 1957).

Development of hair--Lyne and Heideman (1959) observed the development of the skin and hair in cattle fetuses.

Follicular development starts from the 77th to 166th day of gestation. The development of hairs is divided into three phases, '_first-_,' '_later-_,' and '_last-formed_' follicles. The early formed follicles are the largest and are characterized by formation of an 'ental' swelling for attachment of the arrector pili muscle. They give rise to medullated hairs. The follicles formed later produce pairs of unequal size and contain a common epidermal hair canal. These follicles form non-medullated hairs. The follicles formed in the last phase of pregnancy include some of the smaller of the paired follicles as well as solitary ones. They also form non-medullated hairs during the first hair cycle. Hairs develop on the lips and the upper eyelids first in the 5th fetal month, at the tip of the tail, horn root, and coronet in the 6th fetal month, on the back and at the margin of the ears in the 8th fetal month, and throughout the body in the 9th fetal month (Sato, 1932, cited by Yasuda, _et al_., 1957).

Hair regeneration (hair cycle)--According to Berman and Volcani (1961), Heart (1956) reported that the coat of cattle shows an annual cycle of growth similar to that found in other mammals. In the hair cycle the dull colored, long haired and dark winter coat is replaced by the bright, light colored and short haired summer coat. With the exception of a few species like the Angora rabbit and sheep, hair follicles of most animals do not produce hairs continuously but show alternate periods of activity and rest.

During these stages, the hair follicles and hair roots undergo characteristic changes (Rothman, 1954). In the process of replacement of hairs, the hair bulb cornifies, separates from the papilla, becomes solid and moves upward. The hair papilla also moves upward to some extent, but remains connected with the collapsed original dermal hair sheath. Even before the old hair is shed, the matrix cells surrounding the papilla give rise to a new hair. The papilla again descends to its original position (Trautmann and Fiebiger, 1957).

Sebaceous Glands

Distribution--Sebaceous glands are always associated with hair follicles in the entire skin (Carter and Dowling, 1954; Hafez et al., 1955) except the ear canal, eyelids, glans penis, prepuce, vulva and anus (Trautmann and Fiebiger, 1957; Dukes, 1955), where they open independently on the surface of the skin. These independent sebaceous glands are called 'free sebaceous glands' (Rothman, 1954). Sebaceous glands are absent in certain areas such as the planum nasolabiale, teat, horn, hoof and dewclaws of ox (Trautmann and Fiebiger, 1957).

Morphology--Sebaceous glands of cattle are described as lobulated glands by Hafez et al. (1955) and Goldsberry and Calhoun (1959), and as a compound tubular gland by Sisson and Grossman (1953). In 1955, Hafez et al. observed sebaceous glands along the upper third of the associated hair follicles; while in 1959, Goldsberry and Calhoun marked their situation along the middle third of the follicle lying between the angle formed by the arrector pili muscle and the hair follicle. The size of the sebaceous glands varies inversely with the diameter of the associated hair follicle (Lobitz, 1957). Their size and number vary according to species, body regions and density of hairs. They are long and narrow in densely populated hairy regions and spheroidal in less densely populated hairy areas. The largest sebaceous glands are found at mucocutaneous junctions (Trautmann and Fiebiger, 1957). Sebaceous glands of cattle were larger in abdominal, axillary and groin regions (Hafez *et al*., 1955), and on the udder and about the natural openings (Sisson and Grossman, 1953). They are abundantly present between cattle dewclaws (Trumbower, 1904). Palpebrae tarsal and preputial glands are modified sebaceous glands (Dukes, 1955).

Histological structure--Sebaceous glands consist of a body and a duct (Rothman, 1954; Trautmann and Fiebiger, 1957). The body of the sebaceous glands in cattle generally contains two lobes (Hafez *et al*., 1955). It may contain two to twenty lobes (Goldsberry and Calhoun, 1959). It is composed of glandular cells resting on a basement membrane which is finally surrounded by a delicate connective tissue sheath. According to the position, the glandular cells are grouped into peripheral or stratum germinativum or basal cell layer and central cells (Trautmann and Fiebiger, 1957; Ham and Leeson, 1961). The peripheral cells are smaller and cuboidal in shape, occasionally present mitotic figures (Copenhaver and Johnson, 1958) and give rise to central cells (Ham and Leeson, 1961). Montagna and Hamilton (1949b) also observed mitosis in the basal cells in the hamster sebaceous glands. The central cells progressively become larger and polyhedral and show different stages of degeneration containing shrunken or disintegrated nuclei and fat droplets (Trautmann and Fiebiger, 1957; Goldsberry and Calhoun, 1959; Copenhaver and Johnson, 1958; and Ham and Leeson, 1961). Some cells may contain keratohyaline granules (Ham and Leeson, 1961). The duct is short and wide and connects the body with the hair follicle in cattle (Goldsberry and Calhoun, 1959). It is lined by stratified squamous epithelium which is continuous with the stratum germinativum of the outer root sheath. The larger duct resembles the epidermis and the stratum corneum as well (Trautmann and Fiebiger, 1957).

Biochemistry and histochemistry--Montagna and his associates, studying the histochemical aspects of sebaceous glands of the rat (Montagna and Noback, 1947), of the hamster (Montagna and Hamilton, 1947), and of the external ear canal of man (Montagna *et al.*, 1948), reported the presence of phospholipids in basal cells and their gradual replacement by fatty infiltration. Glyceride with unsaturated fatty acids and cholesterol esters were also seen (Montagna *et al.*, 1948). These same authors also reported abundant alkaline phosphatase in the sebaceous glands of rat and man and very little in the hamster. The sebum consists of esters of higher fatty acids and alcohols including cholesterol (Dukes, 1955).

Development of sebaceous glands--Sebaceous glands, as well as apocrine sweat glands, develop as an outgrowth of the hair follicles (Trautmann and Fiebiger, 1957). Two swellings of the external epithelial sheath appear on the lower face of the inclined hair follicle. The upper one develops into the sebaceous gland and the lower one into an epithelial bed which gives attachment to the arrector pili muscle (Arey, 1954).

Sweat (Sudoriparous) Glands

Distribution and density--The skin of cattle is richly supplied with sweat glands (Ellenberger, 1906; Muto, 1925; Yamane and Ono, 1936; Findlay and Yang, 1950; Carter and Dowling, 1954; Dowling, 1955a; Nay and Hayman, 1956; Nay, 1959; and Findlay and Jenkinson, 1960). Density of sweat glands of cattle varies according to breed, for example, 250 per square centimeter in Friesian, 600 per square centimeter in Shorthorn cattle and 1600 per square centimeter in Zebu (Dowling, 1955a). Walker (1957) stated that a decrease in the density of sweat glands with age is due to expansion of the skin. He pointed out that there is a very close correlation between the density of the apocrine glands and the heat-toleration.

Morphology--Morphologically the sweat glands are grouped into two forms: coiled and saccular. The coiled form occurs in horses, sheep, pigs and cats. The saccular form generally occurs in cattle and dogs (Ellenberger, 1906; Trautmann and Fiebiger, 1957, and Sisson and Grossman, 1953). The coiled glands are also seen at certain areas such as the muzzle, points of the hock, flexor surfaces of the fetlocks and natural openings in ox (Goldsberry and Calhoun, 1959; Sisson and Grossman, 1953). Goldsberry and Calhoun (1959) reported saccular-coiled, saccular-noncoiled and compound tubular apocrine sweat glands in Hereford and Aberdeen Angus cattle. Sweat glands of the Zebu are longer, greater, sac-like and convoluted, while in European cattle they are quite convoluted and rarely sac-like (Nay and Hyman, 1956). The shape and size of sweat glands in the same animal vary greatly. According to shape, they can be categorized into three types, tubular, baglike and club shaped (Nay, 1959). The size of sweat glands differs in cattle of the same breed and in cattle of different breeds (Nay and Dowling, 1957). Ceruminous glands of the ear are regarded as modified sweat glands (Ham and Leeson, 1961) and their secretions resemble sebum (Dukes, 1955). Quantitative and morphological variation of sweat glands of Sahiwal Zebu and Jersey cattle were reported by Pan (1963).

<u>Histological structure</u> of cattle sweat glands has been reported by several authors, Ellenberger (1906), Yamane and Ono (1936), Findlay and Yang (1950), Dowling (1955a), Nay (1959), Goldsberry and Calhoun (1959) and Ato (1961a, b). A sweat gland consists of a lower secretory and an upper excretory part. The secretory part lies deeply in the

corium, while the excretory duct passes through the corium and epidermis and opens either into hair follicle or skin surface (Ellenberger, 1906; Sisson and Grossman, 1953; Goldsberry and Calhoun, 1959). The secretory part of both types of glands is composed of three layers--a single layer of glandular epithelium, a layer of myoepithelial cells and basement membrane (Trautmann and Fiebiger, 1957). Goodall and Yang (1952) studied the myoepithelial cells in bovine sweat glands. Goldstein (1961) reported the origin of myoepithelial cells of apocrine sweat glands from their duct cells.

Merocrine tubular glands are found chiefly over the entire skin surface of primates (Trautmann and Fiebiger, 1957), in the foot pad of dogs (Nielsen, 1953) and in the metacarpal pads of cats (Strickland and Calhoun, 1963). Their secretory part is narrow, uniform and coiled. It is lined by columnar or cuboidal epithelium. The excretory duct is composed of double layered cuboidal to squamous cells with cuticular border, a basement membrane and a layer of longitudinal collagenous bundles. The duct passes up through the corium, between the dermal papillae and epidermis and finally opens on the skin surface. In thicker epidermis it pursues a spiral course (Trautmann and Fiebiger, 1957).

Apocrine tubular glands are found as main tubular glands in domestic animals. In man and other primates, they are limited to few areas such as the axilla. In ox, the secretory part is much wider, up to 150u and serpentine or coiled in form. It is lined by low cuboidal to columnar cells depending upon the phase of secretion. In some tubules, columnar cells present apical projections and contain fine basophilic granules and few pigment granules in their cytoplasm. Myoepithelial cells are denser in apocrine than in merocrine glands. The apocrine gland is associated with all hair follicles except the tactile hair follicies (Trautmann and Fiebiger, 1957). The duct always opens into the neck of the hair follicle (Ellenberger, 1906; Carter and Dowling, 1954; and Trautmann and Fiebiger, 1957).

Biochemistry and histochemistry--The nature of secretion of sweat glands of cattle is still controversial. Yang (1952), using a histochemical technique, found that the skin of Ayrshire cattle gave positive reactions for ribonucleoprotein, arginine and alkaline phosphatases and negative reactions for desoxyribonucleoprotein and acid glycerophosphotase. Sweat glands of Ayrshire and Zebu cattle give negative reactions for fat lipids or cholesterol and their esters. The bovine sweat glands do not contain glycogen, lipids and associated compounds of iron, which are present in human eccrine or apocrine sweat glands. Ato (1961a, b), using histochemical techniques, concluded that cattle sweat glands were apocrine. Their basement membrane, brush border and luminal contents gave positive reactions for mucopolysaccharides. He identified mucopolysaccharides, small amounts of protein and fatty substances in the luminal content.

Development of sweat glands--Embryological aspects of cattle sweat glands were reported by Ellenberger (1906), Yasuda *et al.* (1957) and Sugiyama (1958). According to Ato (1961), Schaffer (1940) and Maeda (1958) were also concerned with the embryology of the sweat glands of cattle. Apocrine sweat glands develop as an outgrowth of the hair follicle and on the other hand merocrine sweat glands develop as an invagination of surface epithelium (Trautmann and Fiebiger, 1957).

SPECIAL AREAS

External Ear

The skin on the convex surface of the pinna resembles general skin, but the concave surface presents a few special features. There are four longitudinal ridges in cattle. The upper and marginal parts of the ridges contain long hairs, but the skin between them and towards the ear canal is thin and covered with very fine hairs. The external ear canal of the horse is lined by thinner skin containing sparse fine hairs (Sisson and Grossman, 1953). The external ear canal of man has two kinds of cutaneous glands--true sebaceous glands and large alveolar apocrine "ceruminous" glands (Montagna *et al.*, 1948a). In 1949, Montagna and Hamilton described coiled dilated tubular sweat glands and sebaceous glands opening either into the upper part of the hair follicle or directly on the skin surface of the external ear of the cat. The ceruminous glands of man open upon the surface of the skin by long narrow ducts (Copenhaver and

Johnson, 1958). The ceruminous glands are lined by simple columnar epithelium which produces a mucus fluid and yellow pigment granules. The mixed product of ceruminous and sebaceous glands is called ear wax or cerumen (Trautmann and Fiebiger, 1957). Findlay, Goodall and Yang (1950) studied the number of sweat glands in the helix of the cow's ear. Sugiyama (1958) made a comparative anatomical investigation of the sweat glands in the auricula of several animals.

Eyelids

Eyelids are protective, movable folds of skin which carry cilia or eyelashes at their margins. They are composed of the skin, the connective tissue, muscles, glands and conjunctiva (Copenhaver and Johnson, 1958). The skin is covered by fine hairs and is provided with small sebaceous and small tubular glands (Trautmann and Fiebiger, 1957). The keratin of the epidermis gradually thins out as the skin of the margin changes into the palpebral conjunctiva (Ham and Leeson, 1961). The palebral fascia becomes thicker and denser towards the free margin and forms the tarsal plate or tarsus which contains the tarsal glands. This fascia also contains slips of the aponeuroses of the levator palpebrae superioris in the upper eyelid and the ventral rectus in the lower eyelid. The tarsus is covered outside by skeletal muscle fibers of the orbicularis oculi and inside by the palpebral conjunctiva. Some of the fibers of the orbicularis oculi which are separated from the rest by eyelashes are called muscles of Riolan which are distinct in the ox. Some smooth muscle fibers are also seen extending from the tarsal plate to the follicles of the eyelashes which are known as <u>muscles of Muller</u> (Trautmann and Fiebiger, 1957). Muller muscles are present in both upper and lower eyelids of cattle. They are supplied with sympathetic nerve fibers. <u>Meibomian glands</u> are the largest, modified sebaceous glands. They are not associated with cilia. There are about 34 in the upper and 28 in the lower eyelids of cattle. In both eyelids, they are arranged in vertical columns with their axes parallel to the lid margins. Each gland consists of a central duct surrounded by a number of alveoli which open into it. The main ducts are composed of a basement membrane and rows of epithelial cells. They open into the ventral aspect of the lid margins. The cilia of upper eyelids are about 100 in number and are densely situated laterally and medially. The cilia of the lower eyelid are finer, evenly distributed and about 60 in number. Follicles of eyelashes of cattle are associated with rudimentary sebaceous glands known as <u>Zeis glands</u>. Between the follicles are modified, large spiral sweat glands called <u>glands of Moll</u>. Ducts of these glands open anterior to the openings of Meibomian glands. Not all, but many of these ducts open into the follicles of the cilia (Prince <u>et al</u>., 1960).

<u>Hoof</u>

The skin at the end of the digits undergoes a great modification. The epidermis becomes highly keratinized and transforms into a horny box, the hoof. The corium becomes highly vascular and sensitive and the basal cells become highly columnar. The spinosal cells show many tonofibrils and the stratum germinativum on the dermal papillae produces horny tissue. The wall of the hoof is composed of three layers--external, middle and internal. The external layer consists of periople proximally and stratum tectorium distally. The middle layer is the thickest and the longest part of the hoof. It is composed of horny tubules running parallel to the surface of the wall and the tubules are united by intertubular horn. The internal layer consists of non-pigmented horny laminae. Secondary laminae are generally absent in the ox. The horny sole is composed of firmly united horny tubules and intertubular horns. The horny bulb consists of incompletely cornified horny tubules and intertubular horn (Trautmann and Fiebiger, 1957; Sisson and Grossman, 1953). Tohara _et al._ (1955), Oda (1956) and Wagai and Tohara (1962) reported the histological structures of cattle hoof. Kawata (1943), Wagai and Tohara (1962) investigated the innervation of cattle hoof and reported the presence of free and capsulated nerve endings in the corium and a few free nerve endings in the epithelium of the laminae.

Horn

The horn is a modification of the epidermis. The stratum corneum which remains soft at the base of the horn and resembles periople of the hoof forms epikeras. The main substance of the horn is composed of fine horny tubules united by loose intertubular horn. These tubules contain medullary substance or sometimes air. The intertubular horn is abundant in sheep and less abundant in goats and scarce in cattle (Trautmann and Fiebiger, 1957). George (1956) studied the development of the os cornu of horns of sheep.

Interdigital Skin

The skin on the interdigital pouch of the sheep contains fine hairs, well-developed sebaceous glands and large compound tubular apocrine glands. Tiniakov (1958) investigated the histology and development of interdigital glands of the sheep. He described them as looped sudoriferous types of tubular glands.

Labia Vulvae

The labia is covered by thin, pigmented, wrinkled skin. The ventral commissure presents a number of long hairs (Sisson and Grossman, 1953). They are rich in sebaceous and tubular glands and elastic tissue. Free sebaceous glands are also present (Trautmann and Fiebiger, 1957).

Muzzle (Planum Nasolabiale)

The hairless part of the upper lip and between the nostrils is termed muzzle or planum nasolabiale. The surface is smooth and divided by irregular furrows into small polygonal areas which present orifices of the nasolabial glands at their centers. In health, it is kept cool and moist by a clear fluid secreted by nasolabial glands. The skin adjacent to the muzzle contains ordinary and tactile hairs (Sisson and Grossman, 1953). According to Goldsberry and Calhoun (1959), the muzzle skin is the thickest skin in the body. Nasolabial glands were described as highly specialized, modified, serous, tubular glands with intercellular canaliculi by Trautmann and Fiebiger (1957), multilobular, compound, tubuloacinar glands by Ellenberger (1906) and Mackie and Nisbet (1959) and compound tubular glands by Goldsberry and Calhoun (1959). Ellenberger (1906) and Trautmann and Fiebiger (1957) regarded them as serous glands, but Mackie and Nisbet (1959), by histochemical tests, concluded that they were mucous glands. Nasolabial glands of the goat were reported as serous glands (Sar, 1963). Moriconi and Renzoni (1955) pointed out that muzzle prints might be utilized for identification of cattle. Zimmermann (1934) studied the histological structure of the muzzle of cattle.

Scrotum

The scrotal skin of bulls is generally nonpigmented. It contains few short fine hairs. It is abundantly supplied with very large sebaceous and sweat glands (Sisson and Grossman, 1953; Trautmann and Fiebiger, 1957). The skin is attached to the tunica dartos by a thin layer of areolar tissue. The dartos contains collagenous, elastic and smooth muscle fibers (Trautmann and Fiebiger, 1957). Sebaceous glands of the scrotum of the cat are large and dense and are usually associated with hair follicles (Strickland and Calhoun, 1963).

Udder and Teat

The skin of the udder contains a dense layer of stratified squamous epithelium. Its thickness varies at different parts of the udder, being the thickest on the teats which are exposed to greatest mechanical friction (Turner, 1952). Mankowski (1903) recognized six layers--stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum and stratum mortificatum (cited by Turner, 1952). The papillary layer of the dermis presents numerous papillae projecting into the corresponding cavities in the epidermis. The papillae are richly supplied with blood capillaries and some papillae contain tactile organs.

The wall of the teat is composed of five layers, the skin, the outer fibrous layer, the intermediate layer, the inner fibrous layer and the mucosa of the teat canal (Sisson and Grossman, 1953). Mankowski (1903) (cited by Turner, 1952) believed that dermal papillae and columns of epithelial cells gave elasticity to the teat. The teat skin contains elastic tissue. It is hairless and nonglandular (Trautmann and Fiebiger, 1957). Ziegler and Mosimann (1960) studied the anatomy and physiology of the udder of the cow. St. Clair (1942) and Niggli-Stokar (1961) investigated the nerve supply of the mammary gland of cows.

Tail

The thickness of the skin of the root of the tail is about 5 mm (Sisson and Grossman, 1953). Holmes (1960) reported that the thickness of the dermis of rat tail decreased from the proximal to the distal end, while the germinativum increased in thickness from the distal to the proximal end. According to Goldsberry and Calhoun (1959), the skin from the tail root was usually one of the thickest skins of the body in both sexes of cattle.

MATERIALS AND METHODS

Source of Animals

Skin specimens from healthy normal Holstein cattle were obtained from 36 different body areas of two bull calves 9 to 10 months old and four cows 3 to 6 years of age. A few additional samples from special areas were taken from another three cows, 4 to 6 years old; from one calf about 3 weeks old, and from two fetuses, 11 weeks and 5½ months old. Two cows were in heat on the slaughter day. These animals were obtained from the Dairy, and Surgery and Medicine departments at Michigan State University.

Technique

When the animals were slaughtered, skin specimens of about one inch square were collected. They were preserved in FAA mixture (Lavdowsky's mixture, Guyer, 1949) in less than half an hour after slaughter. The mixture contained 10 parts of commercial formalin, 50 parts of 95% alcohol; 2 parts of glacial acetic acid; and 40 parts of distilled water. After two to three days the specimens were trimmed to about 2 by 10 mm for vertical sections and to 5 by 5 mm for horizontal sections. They were left in the FAA solution for 10 to 30 days. After this they were transferred to 70% alcohol.

The specimens were dehydrated by dioxane (Bucher and Blackely, 1936) in three changes: the first and second change for 4 hours and the last change over night, about 15 hours. The tissues were infiltrated with paraffin in 5 to 6 changes of 15 minutes each. They were alternately kept in vacuum and under pressure (15 lbs. atm.). Sections were cut at 6 microns. They were stained with (1) Harris hematoxylin and eosin (Malewitz and Smith modification, 1955) for general study, (2) Weigert and Van Gieson's stain for elastic fibers, (3) Mallory's triple stain (Crossmon modification, 1937) for connective tissue, (4) Toluidine Blue (1:1000) for mast cells, mucin and metachromasia, (5) Alcian Blue and Nuclear Fast Red for nasolabial glands, (6) Periodic Acid Shiff (PAS) reagent for mucin and glycogen in muzzle glands, (7) Bodian's stain for nerve fibers and nerve endings, (8) Gomori's reticular stain (Gridley, 1957) and (9) Modified Bielschowsky-Gros silver stain for nerve endings (Cauna, 1959).

Measurements

The thickness of the epidermis, the dermis, the hairs, the connective tissue fibers, cutaneous grooves, the depth of the grooves of the skin, the dimensions of the hair follicles, the sebaceous and sweat glands and the mast cells were measured in microns with an ocular micrometer. The density of hairs and sweat glands per square centimeter were observed by counting number of hairs and sweat glands in one square millimeter with the help of the ocular micrometer and then calculating for one square centimeter.

PLATE I

Body Areas From Which Skin Specimens Were Taken

Regions with section number

Head
- Forehead.................. 1
- Horn......................41
- Ear canal.................25
- Ear tip...................26
- Upper eyelid..............27
- Lower eyelid..............28
- Muzzle....................24
- Submaxillary space........29

Neck
- Dorsal neck............... 2
- Lateral neck..............12
- Ventral neck (dewlap).... 6
- Jugular area..............32

Thorax
- Dorsal thorax............ 3
- Lateral thorax...........13
- Ventral thorax (brisket). 7

Abdomen
- Dorsal abdomen (lumbar).. 4
- Lateral abdomen..........14
- Ventral abdomen.......... 8

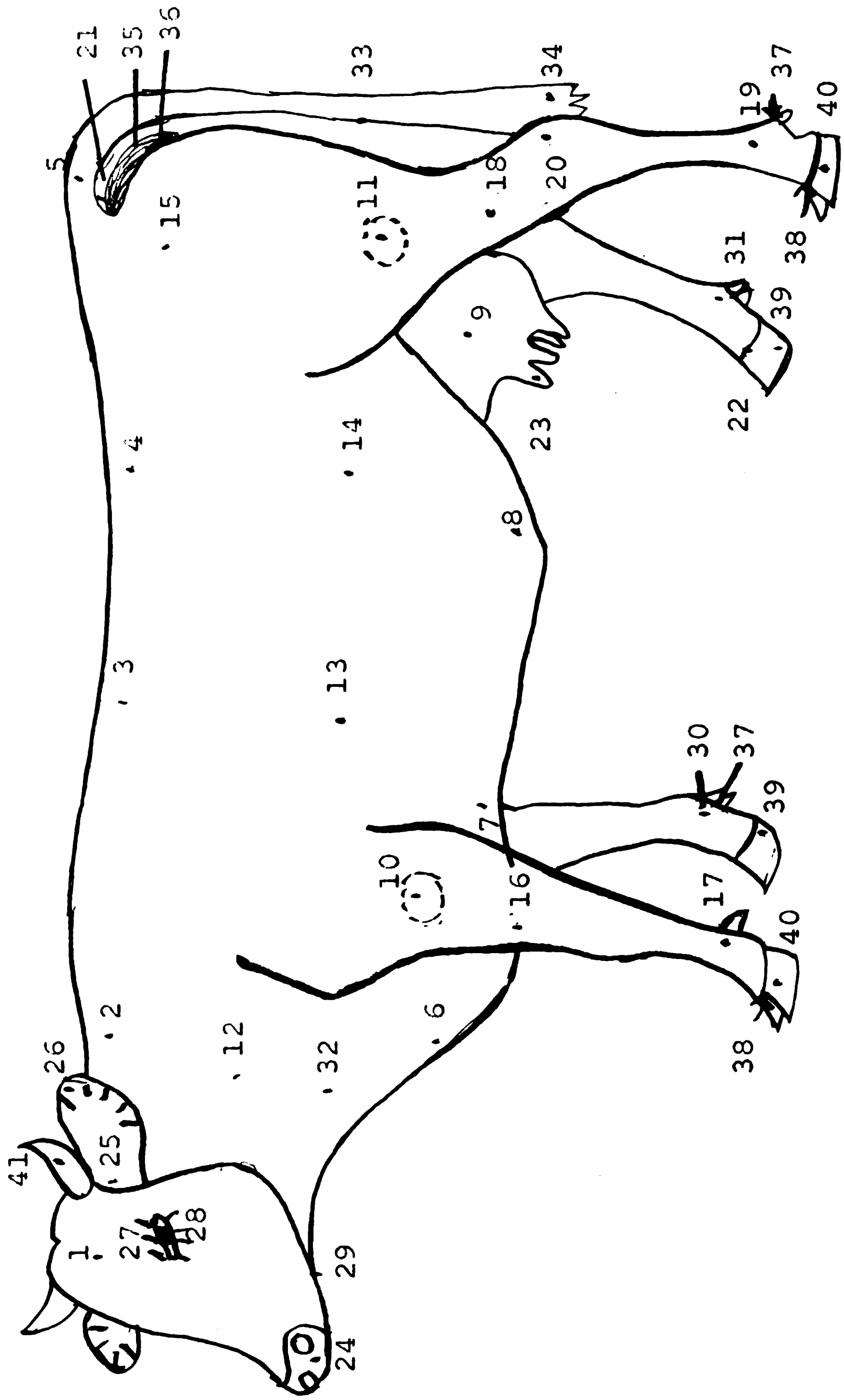
External genitalia & perineum
- Udder/scrotum............ 9
- Teat.....................23
- Vulva....................35
- Clitoris.................36
- Perineum.................21

Tail
- Tail root.................. 5
- Tail middle...............33
- Tail tip..................34

Pectoral limb
- Axilla....................10
- Outer brachium............16
- Fetlock fore outer........17
- Fetlock fore inner........30
- Interdigital space........38
- Interdewclaws.............37

Pelvic limb
- Gluteal region............15
- Outer crus (tibial region).18
- Groin.....................11
- Achilles tendon insertion..20
- Fetlock hind outer........19
- Fetlock hind inner........31
- Interdigital space........38

Hoof
- Bulb of hoof (heel)........39
- Hoof wall.................40
- Hoof skin junction........22



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RESULTS AND DISCUSSION

GENERAL FEATURES

Skin Thickness

The average total skin thickness in the Holstein cattle under investigation was about 3.8 mm in general; about 4 mm in cows, 3 to 6 years of age, and about 3.5 mm in bull calves, 9 to 10 months old. The thickest skin from the general body area was found on the forehead (4.5 mm), on the dorsal neck (4.5 mm), on the lateral abdomen (4.5 mm) and on the ventral aspect of the tail root (4.5 mm) (table 2). The thickest skin from the special areas was found on the muzzle (7.4 mm), on the lower eyelid (6.2 mm), on the teat (6.1 mm) (table 3) and on the udder (5.1 mm) (table 2). The thinnest skin from the general body area was observed on the axilla (2.9 mm), lateral thorax (3.1 mm) and brisket (3.1 mm) (table 2). From special areas, the thinnest skin was found on the tip of the external ear (0.6 mm) (table 3). Comparing the present investigation with the work of Goldsberry and Calhoun (1959), the skin of Holstein cattle appeared thinner than that of Hereford and Aberdeen Angus beef cattle.

The thickest epidermis was found on the muzzle (0.8 mm), forehead (0.1 mm), fetlock of the fore limb (0.1 mm), scrotum (0.1 mm) and udder (0.09 mm). The average epidermal thickness in cows was 0.08 mm and in bull calves 0.06 mm.

The thickest dermis was found on the udder (5.0 mm), forehead (4.5 mm), tailroot (4.4 mm), and dorsal neck (4.4 mm) (table 1). The average dermal thickness in cows was 3.8 mm, and in bull calves 3.5 mm. These findings suggest that the skin is thinner in younger animals than in adults and the thickness of the skin varies according to age and region of the body. With a few variations, these findings agree with the observations of Sisson and Grossman (1953) and Goldsberry and Calhoun (1959) in cattle. The thick skin of the dorsal and lateral aspects of the body probably protects the animal from cold.

Pigmentation

In both sexes, yellow to brown pigment granules were observed within and between cells of all layers of the epidermis, but the amount of the pigment decreased from the stratum cylindricum to the stratum corneum. They occurred in abundance in the stratum germinativum, and occasionally were seen in all four layers. A few shrunken clear cells, which are regarded as pigment producing cells and are called melanocytes, were found in the stratum basale throughout the skin. An increase in pigment granules was always found in cells at the mouth of hair follicles and skin furrows and in the skin of the forehead, dorsal neck, dorsal thorax, dorsal lumbar, lateral neck, lateral thorax, lateral abdomen, hip, outer brachium, perineum, muzzle and tip of the pinna. Generally they were decreased in number or absent in the dewlap, brisket, udder, teat, scrotum, axilla, fetlock, ear canal, submaxillary space, ventral tail root and interdigital space. These findings agree with the report of Goldsberry and Calhoun (1959) that more pigment occurs in the dorsal and lateral aspects of the body than ventrally. Arrangement of pigment granules in the cells of the stratum cylindricum gave an appearance of a supranuclear cap. The pigment granules were also found in the duct of some sweat glands up to the sebaceous gland level.

Keratinization (Cornification)

Cytoplasmic fibrils and keratohyaline granules which are considered to play an important role in keratinization of the epidermis, were distinct and greater in number at the zone where the skin was changing into horny structures such as the margins of hooves, horns and dewclaws. They were also abundant in thick skin, muzzle, interdigital skin and udder and teat. At the margins of horny structures, cells of the stratum granulosum gradually increased from one cell layer to fifteen cell layers. This observation suggests that they are positively related to the formation of keratin. Cytoplasmic fibrils (tonofibrils) were noticed chiefly in cells of the stratum germinativum and stratum granulosum. Keratohyaline granules were found only in cells of the stratum granulosum, whereas keratin granules in varying degree were found in all the layers of epidermis specially in areas of horny structures (Plate XI). Cornified structures appeared deep pink with alcian blue and nuclear fast red stain. This stain revealed cornification of the roots and shafts of hairs, innerepithelial sheaths of hair follicles, ducts of sweat glands up to the sebaceous gland level and ducts of sebaceous glands.

Aging of Skin

The thickness of the epidermis, dermis and total skin was less in bull calves, 9 to 10 months old, than in cows of 4 to 6 years of age (tables 1, 2). Elastic fibers were more abundant in young animals than in adults. Collagen bundles were thicker and denser in the adults than in young ones. Hairs were more coarse and less dense in cows than in calves (table 4). Mast cells and metachromatic fibers were prominent and greater in number in bull calves (Plate XXXIV) than in cows.

Functional Anatomy of the Skin

Microscopic structures of tissues vary according to their functions and stages of their activities, as for example, endometrium, mammary glands, sweat glands, sebaceous glands, hair follicles and many other structures. The heading "functional anatomy" has been used here in the same sense. The epidermis shows great cornifications at extremities and prominences of the body which undergo constant friction and pressure such as muzzle, tip of tail, hooves, brisket, point of hock, ventral abdomen and teats.

Well developed sebaceous glands, and large, highly convoluted apocrine sweat glands were observed in the labia vulvae of two cows which were in heat at the time of slaughter (Plate VI). These glands were better developed in these two cows than in the other cows and are probably functionally related to sex hormones. Possibly these glands secrete some odorous substance during the heat period which enables bulls to recognize the cow in estrus.

As the temperature of the body is mainly regulated with the help of sweat glands and hairs, the relation between these two structures seems constant in the skin of different species. Where hairs were less dense and fine, such as in the external ear canal, labia vulvae (Plate V), udder, and perineum (Plate XXXII), better developed sweat glands were observed.

Blood Supply

Distribution of blood vessels in the skin of Holstein cattle coincide with the findings of Findlay and Yang (1948) and Sisson and Grossman (1953). Large blood vessels were seen in the subcutis, the branches of which ascended in the dermis. Vascular plexuses were seen in the deep corium and in the papillary corium. From the papillary plexus, blood capillaries were seen running vertically up in the dermal papillae (Plate XI). Blood vessels were more numerous in the skin of the vulva, udder, teat and sensitive corium of the hooves.

Nerve Supply

Nerve supply to the skin was demonstrated in the muzzle skin. With modified Bielschowsky-Gros silver stain (Cauna, 1959), free nerve endings and pacinian like corpuscles were observed in the upper part of the corium (Plate XXIII). Many nerve trunks and nerve fibers of varying thickness were noted throughout the corium of skin. Numerous nerve trunks were observed in the hornbud.

COMPONENTS OF THE SKIN

Epidermis

The epidermis, as usual, was composed of stratified squamous epithelium. Its free surface was marked with fine ridges, grooves and openings of hair follicles and certain cutaneous glands. Out of its four layers, three layers--the stratum corneum, the stratum granulosum, the stratum germinativum, were present everywhere. The stratum lucidum was found occasionally at certain specialized non-hairy areas.

Stratum corneum was composed of flat, stretched, nonnucleated, keratinized, closely packed squamous cells. Cell boundaries were indistinct in the superficial layer, but distinct in cells near the stratum granulosum. This layer was thickest at places which were devoid of hairs and exposed to much pressure and friction such as hoof (Plate XII), horn, muzzle (Plate XXXVI), teat (Plate III), ventral abdomen, interdigital skin and tip of tail. Intercellular bridges were absent in this layer. Keratins in the form of fine filaments were observed in cornified cells. Its free surface was not smooth, but was marked with some elevations and depressions in the manner of water waves (Plate XXII). This feature was pronounced where rete pegs and dermal papillae were highly developed.

Stratum lucidum--According to Goldsberry and Calhoun (1959), this layer was absent in general skin as well as in the muzzle and margins of the hoof and horn in Aberdeen Angus and Hereford cattle. In Holstein cattle, the stratum lucidum was absent in the muzzle, but present in horn buds and hoof margins. These variations may be due to breed differences. It was distinct in the teat of a lactating cow (Plate III). It was composed of homogeneous acidophilic, nonnucleated cells, generally with indistinct walls. Keratohyaline granules were absent. Tonofibrils were marked at certain places.

Stratum granulosum was present throughout the skin in varying degrees. It generally consisted of fusiform, widely separated, highly granular cells arranged in rows parallel to the skin surface (Plates XIV, XXII). The density and rows of cells increased as the thickness of the skin increased. At the hoof margin up to 15 layers of cells were observed. In the teat (Plate XIV), scrotum (Plate XXII), muzzle and interdigital skin, these cells were arranged closely and generally in one to two layers. These cells were packed with basophilic keratohyaline granules. Nuclear shrinkage and chromatolysis were observed.

Stratum germinativum--On the basis of type of cells and their arrangements, it was subdivided into two layers--stratum spinosum and stratum cylindricum. Stratum spinosum was composed of several layers of polygonal cells with spherical centrally placed nuclei. Intercellular bridges were distinctly marked in thicker epidermis where rete pegs were developed such as the muzzle, tip of tail, interdigital skin and hoof margins. Some cells contained pigment granules. Stratum cylindricum was composed of a single layer of cuboidal to columnar cells with oval nuclei in their lower third segments (Plates XIV, XXII). The height of these cells varied with the thickness of the epidermis. They were highly columnar in the muzzle, brisket, ventral abdomen, udder, teat, scrotum and hock. They were cuboidal to low columnar in the skin of the ear tip, the brachium and the outer crus. Their cytoplasmic processes were marked penetrating the faintly stained basement membrane. A few clear cells (melanocytes) were present in varying degrees throughout the skin (Plate III). Mitotic figures were observed.

Dermis (Corium, Leather Skin)

Layers--The dermis, the connective tissue part of the skin, lies below the epidermis. It is the thickest component of the skin. According to the thickness of connective tissue fibers and varying degree of waviness in the superficial surface of the corium, it is divided into two layers--stratum papillare and stratum reticulare. According to Findlay *et al*. (1948), the corium may be divided into five levels--subepidermal level, sebaceous gland level, subsebaceous gland level, sweat gland level and subsweat gland level.

Stratum papillare was composed of fine reticular, collagenous and elastic fibers. It is a narrow zone situated below the epidermis. In agreement with the work of Dempsey (1948), thick elastic fibers gave attachments to the arrector pili muscles (Plate XXVI). Where the skin was thick and hairs were scanty or absent, dermal papillae of various shapes and sizes were present. The papillae were generally long and bifurcated or trifurcated in the labia vulvae, teat (Plate III), muzzle (Plate XXXVI), hoof margin (Plate XXXV), and tip of the tail. Small papillae were also found in the udder, fetlock, forehead and other thick skin.

Stratum reticulare was continuous with the stratum papillare. There was no line of demarcation except the fibers of this layer were coarse. It was composed of a network of coarse collagenous and thick elastic fibers.

Structural components--The corium was composed of connective tissue fibers--collagen, elastic and reticular and cells--fibroblasts, mast cells, smooth muscle cells, and occasional fat cells and leukocytes. The corium also contained hair follicles, hair roots, sebaceous glands, sweat glands, modified cutaneous glands, blood vessels, lymphatics and nerves.

Collagenous fibers were composed of fine, unbranched and usually wavy fibrils. Collagen bundles were of varying thickness (8-40 u), length and density. They were found branching and following a wavy course. They extended in all directions, but chiefly parallel to the skin surface (Plate XXVII).

<u>Elastic fibers</u> were thinner and straighter than collagenous bundles. Their thickness varied from 1 to 8 u. They formed a fine network in the papillary corium. Some fibers joined and followed the course of the collagen fibers in the reticular corium. The thickness of these fibers increased from the upper to lower strata of the corium. In the deep corium these fibers were very thick (8 u), abundant, and generally paralleled the surface of the skin (Plate XXVII). They were numerous around hair follicles and blood vessels. These findings are in agreement with the findings of Dick (1947). Rothman (1954) stated that elastic fibers were more numerous in children than in adults. This investigation revealed more elastic fibers in ten-month-old bull calves than in cows of 3 to 6 years of age. They were present abundantly in thinner skin such as the lateral thorax, brachium and tibial regions.

<u>Reticular or argyrophilic fibers</u> were thinner than elastic fibers. Their thickness varied from 0.5 to 1 u. They also varied in length and density. They branched and formed networks around blood vessels, arrector pili, cutaneous glands, and hair follicles. These fibers were the main structure of the dermal-epidermal junction, the basement membrane (Plate XXV). Although they formed a network in the papillary corium, vertical fibers predominated at the dermal-epidermal junction (Plate XXV).

<u>Fibroblasts</u> were the major cells in the dermis. They were of various shapes and sizes, but were generally

fusiform cells with light staining cytoplasm and deeper staining elliptical, oval or spherical nuclei. As observed by Montagna (1956) in the human species, fibroblasts were large and branching in the papillary layer and long and thin in the reticular layer of these animals.

Mast cells or histogenous mast cells were found throughout the dermis, but more around capillaries (Plates XXXIV, XXIX). They were of various shapes and sizes, but generally large, rounded or oval cells with spherical nuclei and coarse granular cytoplasm. As observed by Hellstrom and Holmgren (1950) in human skin, two types of mast cells were present in Holstein cattle. Smaller multiform cells with oval nuclei and metachromatic granules were found in the papillary layer and larger ones with large oval nuclei and coarse metachromatic granules were found in the reticular layer mostly around blood vessels. Mast cells were found abundantly in the forehead, the dorsal neck, the dewlap and dorsal thorax in bull calves and in the perineal skin of cows. They were more abundant in young animals than in adults.

Attachments of the dermis--The dermis is attached externally to the epidermis at the dermal-epidermal junction and internally to the subcutis.

Dermal-epidermal junction--In sections stained with Gomori's reticular stain, cytoplasmic processes of the basal cells interdigitated with the reticular fibers which make up the basement membrane uniting the epidermis and dermis

(Plate XXV). It was lightly stained with H and E. The matrix was homogenous.

Subcutis or hypodermis was composed of areolar tissue with varying amounts of elastic fibers and fat cells. It also contained large blood vessels and nerve trunks. It was thick where the skin could be easily lifted into folds.

Hair

Classification, density and arrangement of hairs-- On the basis of the description given by Sisson and Grossman (1953) and Schwartzman (1962), hairs of the Holstein cattle were divided into two general groups: ordinary or coat hairs which covered the general body surface, and special hairs which occur on particular areas with specific functions. Coat hairs varied greatly in their length, breadth and texture according to the region and the age of animals. A few giant coat hairs were scattered throughout the skin. They were deeply rooted in the corium and extended beyond the level of the sweat glands (Plate XXXIII). While most of the hairs were medullated, a few nonmedullated hairs were also observed. Fine nonmedullated coat hairs were fairly numerous. Guard hairs, wool hairs and secondary follicles which occur in dogs, cats, swine, sheep, goats, chinchillas and other animals were not found in the cattle under investigation. Special hairs, according to their structures, morphology and functions, were divided into five groups: tactile (sensory or sinus) hairs which were found mostly on the lips (Plate XXXVII) and a few on the eyelids; eyelashes or cilia; tragi hairs which occur on the ridges and margins of the pinna; urine directing hairs at the preputial orifice and ventral commissure of vulva which were longer than ordinary hairs; tail tip hairs which were the longest and the thickest hairs on the body (Plate X).

The density of hairs in these animals varied from 1943 to 2271 per square centimeter (table 4). The average number of hairs per square centimeter was 2221 in bull calves and 2082 in cows. Although the density of hairs in young animals appeared greater than in adults, the number of young animals used was too small to form definite conclusions. According to Dowling (1955), as the age of animals increases, density of hairs decreases. The number of hairs per unit was greater on the ventral neck, ventral thorax and ventral abdomen than on the forehead, dorsal neck and dorsal lumbar region.

Although Trautmann and Fiebiger (1957) reported an even distribution and arrangement of hairs in the ox, the present findings differ. In these cattle, hairs of different sizes seemed to be arranged in groups of varying number from one to seven. These groups were surrounded by coarse collagenous bundles generally into polygonal areas which were further subdivided into smaller areas by thinner collagen fibers (Plate XXVIII). Each hair follicle, which was associated with a sweat gland, a sebaceous gland, an arrector pili muscle and finally surrounded by collagen fibers, constituted a hair unit.

Microscopic structure--On the structural basis and for the convenience of description, a complete hair was divided into the hair shaft, the hair bulb, and the hair follicle. The part of the shaft which was embedded in the skin, and the bulb of the hair formed the root of the hair. Except for the follicular folds, the general microscopic structures of hair shafts, bulbs and follicles were in agreement with the description given by Trautmann and Fiebiger (1957), Copenhaver and Johnson (1958) and Ham and Leeson (1961).

Follicular folds, which appeared to be a special feature, were found in many hair follicles, but not in all. They were formed by foldings in the inner epithelial sheath of the hair follicle just below the opening of the sebaceous gland (Plates II, XXIX). In vertical section they generally appeared on both edges of the follicle but occasionally only on one side, but always below the opening of the sebaceous gland. In frontal section they surrounded 2/3 to 3/4 of the follicle (Plate XXVIII). Due to the fact that they do not completely surround the follicle, these folds did not appear in the sections of all follicles. Follicular folds were observed in almost all domestic animals by various authors. Functions of these folds are not exactly known. Strickland and Calhoun (1960) stated that these folds may be related to propulsion of the sebum. Holmes

(1960) dèscribed them simply as a relaxed form of the follicle. Smith (1960) reported them as a structure responsible for emergency supply of sebum. Marcarian (1962) speculated they were an artifact. In the author's opinion, they appeared to be related to the accommodation and expulsion of the sebum. They seemed to be prominent near the lobes of the sebaceous gland. They might be giving accommodation for enlarged sebaceous glands at their highest phase of activity. After production and expulsion of the sebum, they might be assuming the original corrugated appearance.

Tactile hairs were found on the upper lip but were not components of the muzzle. They were longer and thicker than ordinary hairs and more deeply rooted. They were surrounded by a thick connective tissue sheath which contained a blood sinus. The sinus was in cavernous form because it was traversed by trabeculae composed of collagenous and elastic bundles. The ringwulst and annular sinus, which are characteristic features of tactile hairs of rats, were absent in tactile hairs of these cattle. The tactile hairs at the muzzle margin were surrounded by ordinary hairs in a circular manner (Plate XXXVII). No arrector pili muscles were observed with them, but skeletal muscles were noticed in their vicinity. These findings coincided with those of Vincent (1913) and Holmes (1960) in rats, and Strickland and Calhoun (1960) in the cat.

The arrectores pilorum were well developed. A few of them divided into 2 to 4 branches. The bellies of these muscles were rich in reticular fibers and both ends terminated in elastic fibers. The hairs at the margin of the muzzle, tip of tail, and the ordinary hairs of the eyelids lacked these muscles.

Development of hairs was noticed in a fetus about 165 days old. Solid epithelial hair pegs from the stratum germinativum descended into the corium (Plate XIII). One hair peg gave rise to only one primordium of the sweat gland.

Regeneration of hair (hair cycle) was noticed throughout the skin. Degenerative changes were observed in the hair bulb and hair dermal papilla. Due to atrophy of hair bulb, hair follicle of that part collapsed and became irregular in contour. Cellular distinction of the hair root was progressively lost. Regeneration started from the matrix cells surrounding the papilla. Due to the presence of the degenerated hair root, regenerated hair moved upward obliquely (Plate XXXII).

Sebaceous Glands

Distribution and situation--Sebaceous glands were generally associated with hair follicles even in the ear canals, eyelids and vulva where, according to Trautmann and Fiebiger (1957) and Dukes (1955), they are supposed to open separately on the surface of the skin. A few sebaceous glands of the ear canal and the tarsal glands of the eyelids opened independently. In agreement with Trautmann and Fiebiger (1957), sebaceous glands were not found in the muzzle, teat, horn, hoof and claw. Usually, only one sebaceous gland opening was seen in a hair follicle; however, openings of two sebaceous glands were not uncommon (Plate XXIX). They were situated mostly along the upper third of the hair follicles in the angle formed by the arrector pili muscle and follicles. In cross section many sebaceous glands were seen completely surrounding hair follicles.

Morphology--Sebaceous glands in these cattle appeared to be simple branched alveolar glands in which the common duct gave rise to a number of alveoli of varying sizes. The number of alveoli varied from 2 to 20, but in a majority of glands, there were only two. Due to many large alveoli, the glands appeared to be multiloculated. The shape, size, and number of these glands varied according to the regions of the body. Well developed, multilobulated sebaceous glands were found at mucocutaneous junctions such as lips (Plates II, XV) and eyelids; horny-cutaneous junctions such as the hoof margins, horns and claws (Plates XXXV, XXXI); and at thin hairy areas such as the perineum. They were less developed in bull calves than in cows. They were largest in the labia vulva of cows which were in heat during the time of slaughter.

Histological structure--A sebaceous gland was composed of a lobulated body (the secretory part) and a short, wide duct (the excretory part). The body generally contained two lobes; but lobes up to 24 were also noticed. They were composed of mucous cells resting on a basement membrane, surrounded by a connective tissue sheath. Peripheral cells were small, and cuboidal with large oval nuclei; while central cells were large and polyhedral. In some of these cells, shrunken or degenerating nuclei were marked. Many glands contained vacuolated cells and large fat droplets (Plate V). Embryologically, sebaceous glands were observed as an outgrowth from the neck of the developing hair follicle (Plate XIII).

Sweat Glands

Distribution and density--Since generally only one sweat gland opens into a hair follicle, the density of sweat glands remains the same as the density of hairs. A fairly large number of sweat glands, about 2123 per square centimeter in the densest area, were present in the skin of these cattle. Their density varied according to the population of the hairs and the region of the body. Generally, it was observed that where the hairs were thinner these glands were better developed, such as the ear canals and eyelids (Plate XIX), perineum (Plate XXXII) and labia vulva (Plate V). They were greater in number on the ventral aspect of the neck, the chest and the abdomen.

Morphology and types--Sweat glands in these animals were simple tubular glands. On the basis of toluidine blue staining and their positive PAS reaction, these glands appeared to be apocrine. This agrees with the work of Ato (1961). On a morphological basis, the sweat glands of cattle were divided into tubular, saccular, and saccular coiled glands. Tubular glands were generally straight and uniform in caliber except at their mouth which was funnel shaped. Saccular glands appeared to have a bag-like body and a narrow excretory duct. Both tubular and saccular glands occurred in the skin of the general body area. Although, with histochemical tests, they reacted as apocrine glands, no apical projections were marked in them. It suggests that they might be chemically like apocrine glands, but their mode of secretion may be like that of eccrine glands. The saccular-coiled sweat glands were generally lined by columnar cells and were deeply placed. They were found at special body areas such as eyelids (the glands of Moll--Plate XIX), ear canals (ceruminous glands--Plate IX), the muzzle (nasolabial glands--Plate XVI), and labia (labial sweat gland). In addition to histochemical similarities, morphologically they were like true apocrine sweat glands since they contained distinct apical projections. The thickness of the wall and the volume of the gland may be related to the production of sweat and regulation of heat of that part of the body, as for example, visible clear fluid droplets were observed on the muzzle which contained thick walled, highly convoluted modified sweat glands, the nasolabial glands.

Histological structures--Sweat glands were composed of a lower secretory part (the body), and an upper excretory duct. The body of both types of glands was composed of three layers--a single layer of glandular epithelium of varying heights, a layer of myoepithelial cells lying parallel to the axis of the tube and a basement membrane. The duct was lined by flattened cuboidal epithelium, but at the orifice mouth the lining changed to stratified squamous epithelium. Myoepithelial cells were generally absent in the duct, but in some thick walled glands they extended a short distance on the duct. Embryologically sweat glands developed as an outgrowth from the uppermost part of the developing hair follicle (Plate XIII).

SPECIAL AREAS

External Ear

The skin on the apex of the pinna contains fewer hairs outside than inside. The skin of this area was the thinnest, about 0.6 mm. The skin of the external ear canal became thicker than at the apex and measured about 1.4 mm in thickness (table 3). It was covered by fine hairs. Sebaceous glands were well developed in the ear canal and contained up to 16 lobules, but towards the apex they were more like those of the general body skin. Almost all sebaceous glands opened into the hair follicles. The sweat glands on the apex of the pinna were saccular and convoluted. Their narrow ducts passed through the lobules of sebaceous glands and opened in the hair follicles by funnel shaped openings (Plate XX). The ceruminous glands which were classified as modified sweat glands by Ham (1961) and alveolar apocrine glands by Montagna (1948) were more convoluted in the deeper part of the corium than in the upper part and opened into hair follicles (Plate IX). They were lined by cuboidal cells in most cases, but some were lined by low cuboidal or flattened cells also. Myoepithelial cells were distinct. The luminal contents were also present, suggesting apocrine sweat glands. The elastic fibers were finer towards the apex of the pinna than in the ear canal. Pigment granules were generally absent in the canal, and only rarely present at the opening of a few hair follicles.

Eyelids

Skin of both eyelids was thicker than general skin. The average total thickness was 3.7 mm in the upper eyelid and 6.2 mm in the lower eyelid (table 3). Abundant pigment granules were found in and between the cells of the stratum germinativum. Elastic and collagenous fibers were fine. According to Trautmann and Fiebiger (1957), the skin is covered by fine hairs and is provided with small tubular and small sebaceous glands, but in this investigation, hairs were provided with well developed sebaceous glands completely surrounding the ordinary hair follicles. Scattered skeletal muscle fibers of the orbicularis oculi (muscles of Riolan) were seen around the eyelashes (Plate VII). A few smooth muscle fibers (Mullers muscles) extending from tarsal plate to the follicles of the eyelashes were also seen. Arrector pili muscles were lacking from ordinary hairs. Tarsal glands were the largest modified sebaceous glands in both eyelids (Plates IV, VII). They were covered dorsally and ventrally by skeletal muscles. Sebaceous glands of the eyelashes

(Zeis glands) were also better developed. Glands of Moll were convoluted, lined by columnar cells having apical projections and rested on the basement membrane. Myoepithelial cells were also distinct. These features suggest that the glands of Moll are modified apocrine sweat glands.

Hoof

The hoof of cattle consists of three parts, wall, sole and bulb. The frog which is a characteristic feature of the horse hoof, is absent in cattle. The structure of the hoof was studied in mature cattle and in a fetus about 165 days old. The components of the wall (horny tubules, intertubular horns and horny laminae) and highly vascular, long, sensitive laminae (dermal papillae) (Plates XI, XII) were observed. Cells of the stratum germinativum, which surround the dermal papillae and give rise to horny tubules, were observed at the margin of the hoof. The horn tubules in cross section appeared elliptical in shape. The interpapillary portion of the germinativum gave rise to the intertubular horn. The basal cells of the stratum germinativum were highly columnar (Plate XII). The dermal papillae, which were composed of fine connective tissue and supplied with blood capillaries, interdigitated with the horny laminae which keeps these two structures united tightly. No secondary laminae were present in the horny laminae of the fetal hoof (Plate XII). The horn of the dew claws was similar in structure to that of the hoof.

Horn

To investigate the microscopic structure of horns during their development, the horn buds of a three-week-old calf and an eleven-week-old fetus were examined. All four layers of the epidermis were marked in both subjects. The horn bud of the fetus presented a local thickening and greater cornification of the epithelium. In the three-week-old calf the stratum corneum had increased in width, the stratum lucidum was more distinct and the stratum granulosum had increased from one or two layers to eight layers of cells. Rete pegs were shorter in comparison to that of fetus but were more numerous. Sebaceous and sweat glands and hair follicles were present in the fetus but hair was absent in the three-week-old calf. Cells of the stratum germinativum, surrounding the dermal pegs and lying between them, produce horny tubules and loose intertubular horn. The dermis contained many nerve bundles and blood vessels.

Interdigital Skin

The interdigital skin was one of the thickest of the body (5.3 mm). The stratum corneum was thick and highly cornified. The rows of cells of the stratum granulosum increased up to 15 cell layers at the junction of the skin and hoof. The stratum lucidum was absent. Long rete pegs of varying shapes and sizes were typical. Large modified sebaceous glands surrounded the hair follicles (Plate XXI) and large, convoluted, modified sweat glands resembling the ceruminous glands, glands of Moll, and the apocrine sweat glands of man were present (Plate XXI). A few fine hairs were also observed.

Labia Vulvae

The average skin measurement of the labia (3.7 mm, table 3) was thinner than that of the general body skin. The epithelium was marked by fine grooves and contained pigment and rete pegs of varying sizes. The stratum granulosum contained one to two cell layers. Well developed sebaceous and apocrine sweat glands were present (Plate VI). Morphologically, apocrine sweat glands resembled ceruminous glands, glands of Moll, and interdigital sweat glands. These glands, specially the sweat glands, were better developed in the two cows which were in heat than in those cows which were not in heat.

Muzzle (Planum Nasolabiale)

The average total thickness of the muzzle skin was 7.4 mm, the thickest in the body. This result coincided with the report of Goldsberry and Calhoun (1959) on Hereford and Aberdeen Angus cattle. The muzzles of both ten-month-old males were thinner than those of the females. The average epidermal thickness was 1 mm. Out of the four layers of the epidermis, only three--stratum corneum, stratum granulosum, and stratum germinativum--were well marked, the stratum lucidum being indistinct. Rete pegs were long (1325 u). The pigment granules were dense in the deep cells of the stratum germinativum and dendritic cells (melanocytes) were present in the basal layer. Intercellular bridges were distinct in the stratum spinosum. At the margin of the muzzle, the openings of hair follicles were wide (336 u). Two well developed sebaceous glands were found opening in a large hair follicle. Many skeletal muscle fibers were in the vicinity of the hair follicles extending vertically in the muzzle. Arrector pili muscles were lacking in both coat and tactile hairs in this area. High dermal papillae extended between the long rete pegs.

Nasolabial glands were situated in the deep corium. They were surrounded by collagenous fibers. Some skeletal muscle bundles were seen in the interglandular septa and in the vicinity of the glands. As Ellenberger (1906) and Mackie and Nisbet (1959) stated, these glands were multilobular compound tubulo-acinar glands resembling salivary glands in structure. They were composed of a single layer of glandular cells, a layer of myoepithelial cells and a basement membrane. The nuclei of the secretory cells were elongated and peripherally placed like mucous cells. The ducts were lined with cuboidal cells with large, spherical, centrally placed nuclei (Plate XVI). They were also surrounded by myoepithelial cells. The reaction of these glands to toluidine blue and Alcian blue nuclear fast red stains and to periodic acid shift (PAS) indicated a mucous secretion. These conclusions coincide with those of Mackie and Nisbet (1959), but differ from those of Ellenberger (1906) and Trautmann and Fiebiger (1957) who considered them serous in nature. Many lamellated and free nerve endings in the dermis (Plate XXIII) and a few free intraepithelial nerve fibers were seen.

Scrotum

The average total skin thickness of the scrotum was 4 mm. The surface epithelium was marked by fine grooves with a few prominent short rete pegs at the bottom. The stratum granulosum presented two rows of cells (Plate XXII). Pigment granules were not present. Sisson and Grossman (1953) and Trautmann and Fiebiger (1957) reported well developed sebaceous and sweat glands, but in the two ten-month-old bull calves these glands were not well developed. Many mast cells and several smooth muscle fibers were present in the corium.

Udder and Teat

The skin of the udder was thicker than the general body skin (5.1 mm, table 2) and that on the teat was one of the thickest skins (6.1 mm, table 3) of the entire body. The stratum corneum of the teat was thicker than that of the udder. The stratum lucidum was distinct in the skin of a lactating cow (Plate III), while it was not clear in the others. The stratum granulosum generally contained two closely arranged layers of granular cells. The rete pegs and dermal papillae were more highly developed in the teat than in the udder. Pigment granules were absent in the teat skin. A network of elastic fibers was present in both udder and teat and fine elastic fibers characterized the teat. Well developed sweat and sebaceous glands were present in the skin of the udder, but they were entirely absent in the teat. Fine hairs were present on the udder but the teat was hairless (Plates III, XIV). Wrinkles in the skin of the udder and teat were marked grossly as well as microscopically. Many smooth muscle fibers and blood vessels were seen in the connective tissue between the skin and epithelium of the teat canal.

Tail

The skin on the ventral aspect of the root of the tail, where blood samples are sometimes taken (Brown and Carrow, 1963), was thicker than the skin of the general body area. The average total thickness of this particular area was 4.5 mm (table 2). The average thickness of the epidermis was 0.07 mm, while that of dermis 4.4 mm (table 1). The thickness of the stratum corneum was nearly the same through the middle of the tail but greatly increased at the tip. The stratum germinativum gradually increased from the root to the tip of the tail. The tip of the tail had one of the thickest skins (4.3 mm) of the body. The ventral aspect of the root of the tail contained fine, sparsely scattered hairs, while the end of the tail contained the coarsest hairs on the body. These hairs were deeply rooted and closely packed. The sebaceous glands were small, but sweat glands were well developed, convoluted, and deeply placed. Generally, the stratum granulosum was composed of two rows of cells. The stratum lucidum was not marked. The arrectores pilorum which were generally divided into two to four branches in the upper part of the tail were absent at the tip of the tail (Plates X, XVII and XXXIII).

Table 1
Average measurements (microns) of total epidermal and dermal thickness in Holstein cattle

	Epidermis							Dermis						
	MF 10 mo	MG 10 mo	FA 6 yrs	FB 5 yrs	FC 3 yrs	FE 3 yrs	Average	MF 10 mo	MG 10 mo	FA 6 yrs	FB 5 yrs	FC 3 yrs	FE 3 yrs	Average
Forehead	66	98	100	120	111	114	102	4998	5108	4079	4410	4775	3381	4458
Dorsal neck	44	70	66	86	78	82	71	3491	4329	4587	4477	4705	4814	4401
Dorsal thorax	36	72	64	62	62	64	60	2793	3234	4404	4410	4550	5182	4096
Dorsal lumbar	40	60	62	98	56	68	64	4249	3528	4403	2485	4433	4116	3869
Tail root	89	70	60	74	58	76	71	3638	4484	4778	4851	4477	4327	4426
Dewlap	46	56	60	60	46	54	54	4022	4557	3308	3087	2382	3749	3517
Brisket	40	66	122	60	64	60	69	3014	2566	3778	2632	2632	3344	2994
Ventral abdomen	76	70	112	62	64	86	78	2779	4602	4477	2566	2885	4116	3571
Scrotum/udder	92	112	112	96	56	100	102/91	5028	2713	5269	2646	5120	6799	3871/4959
Axilla	58	52	86	70	62	64	65	3528	2713	2632	2499	2721	2719	2802
Groin	36	70	80	70	82	62	67	2933	3014	3161	3161	4036	3014	3220
Lateral neck	40	52	76	80	64	62	62	4249	3482	4329	3749	3852	3234	3816
Lateral thorax	46	58	52	84	52	56	58	2632	2315	3234	3087	3740	3007	3003
Lateral abdomen	68	68	70	70	70	54	67	3602	4329	3785	5159	4169	5255	4383
Gluteus	48	70	80	72	60	70	67	3675	2933	4102	4778	4506	4403	4066
Outer brachium	42	50	62	57	54	56	54	2853	2713	2632	3014	3014	3161	2898
Fetlock fore	88	116	138	128	116	80	111	3418	3087	3852	2779	3234	2779	3192
Outer crus	54	60	80	58	78	80	68	3918	2779	4403	3234	3945	3565	3641
Fetlock hind	72	74	114	128	60	58	84	3418	2419	4168	2774	3161	5469	3568
Hock	70	86	72	86	44	106	77	2853	2859	4184	4410	3087	3675	3511
Average	58	72	83	81	67	73	73	3530	3388	3978	3510	3771	4005	3705

Key: M--male, F--female, A-G--animal designation.

Table 2

Average measurements in millimeters of total skin thickness in Holstein cattle

Regions / Age	MF 10 mo.	MG 10 mo.	FA 6 yrs.	FB 5 yrs.	FC 3 yrs.	FE 3 yrs.	Average
Forehead	5.1	5.2	4.2	4.2	4.9	3.5	4.5
Dorsal neck	3.5	4.4	4.7	4.6	4.8	4.9	4.5
Dorsal thorax	2.8	3.3	4.5	4.5	4.6	5.2	4.2
Dorsal lumbar	4.3	3.6	4.5	2.5	4.5	4.2	3.9
Tail root	3.7	4.6	4.8	4.9	4.5	4.4	4.5
Dewlap	4.1	4.6	3.4	3.1	2.4	3.8	3.6
Brisket	3.1	2.6	3.9	2.7	2.7	3.4	3.1
Ventral abdomen	2.9	4.7	4.6	2.6	2.9	4.2	3.7
Scrotum/udder	5.1	2.8	5.4	2.7	5.2	6.9	3.9/ 5.1
Axilla	3.6	2.8	2.7	2.6	2.8	2.8	2.9
Groin	3.0	3.1	3.2	3.2	4.1	3.1	3.3
Lateral neck	4.3	3.5	4.4	3.8	3.9	3.3	3.9
Lateral thorax	2.7	2.4	3.3	3.2	3.8	3.1	3.1
Lateral abdomen	3.7	4.4	3.9	5.2	4.2	5.3	4.5
Gluteus	3.7	3.0	4.2	4.9	4.6	4.5	4.2
Outer brachium	2.9	2.8	2.7	3.1	3.1	3.2	3.0
Fetlock, fore	3.5	3.2	3.9	2.9	3.3	2.9	3.3
Outer crus	4.0	2.8	4.5	3.3	4.0	3.6	3.7
Fetlock, hind	3.5	2.5	4.3	2.9	3.2	5.5	3.7
Achillis insertion	3.0	2.9	4.3	4.5	3.1	3.8	3.6
Average	3.6	3.5	4.1	3.9	3.8	4.1	3.8

Key: M--male, F--female, A-G--animal designation.

Table 3

Average measurements of total skin thickness of special areas in millimeters

Areas / Age	MF 10 mo.	MG 10 mo.	FA 6 yrs.	FB 5 yrs.	FC 3 yrs.	FE 3 yrs.	Aver-age
Muzzle	6.4	6.2	8.0	9.0	7.1	7.8	7.4
Upper eyelid	4.3	2.4	3.4	4.3	3.3	4.3	3.7
Lower eyelid	3.9	3.2	2.0	6.0	3.5	6.4	6.2
Ear canal	1.4	1.7	1.0	1.5	1.4	1.4	1.4
Ear tip ext.	0.6	0.8	0.5	0.6	0.6	0.5	0.6
Tail tip	3.8	4.4	4.6	4.4	4.2	4.4	4.3
Teat	---	---	4.7	6.8	6.2	6.5	6.1
Vulva	---	---	3.1	3.8	4.8	3.0	3.7

Table 4

Density of hair and sweat glands per square centimeters

Areas / Age	MF 10 mo.	MG 10 mo.	FA 6 yrs.	FB 5 yrs.	FC 3 yrs.	FE 3 yrs.	Aver-age
Forehead	2400	2500	2000	1600	1500	1300	1883
Dorsal neck	1600	1800	1800	2000	1700	2000	1816
Dorsal thorax	2500	3000	2100	1600	1800	2000	2167
Dorsal lumbar	1600	1600	2200	1600	1800	2000	1800
Ventral neck	3100	2900	2600	2600	3000	2500	2750
Ventral thorax	1800	2000	2000	2400	2900	2800	2317
Ventral abdomen	2200	2100	1900	1800	2000	2800	2133
Average	2171	2271	2086	1943	2100	2200	2124

Key for both tables: M--male, F--female, A-G--animal designation.

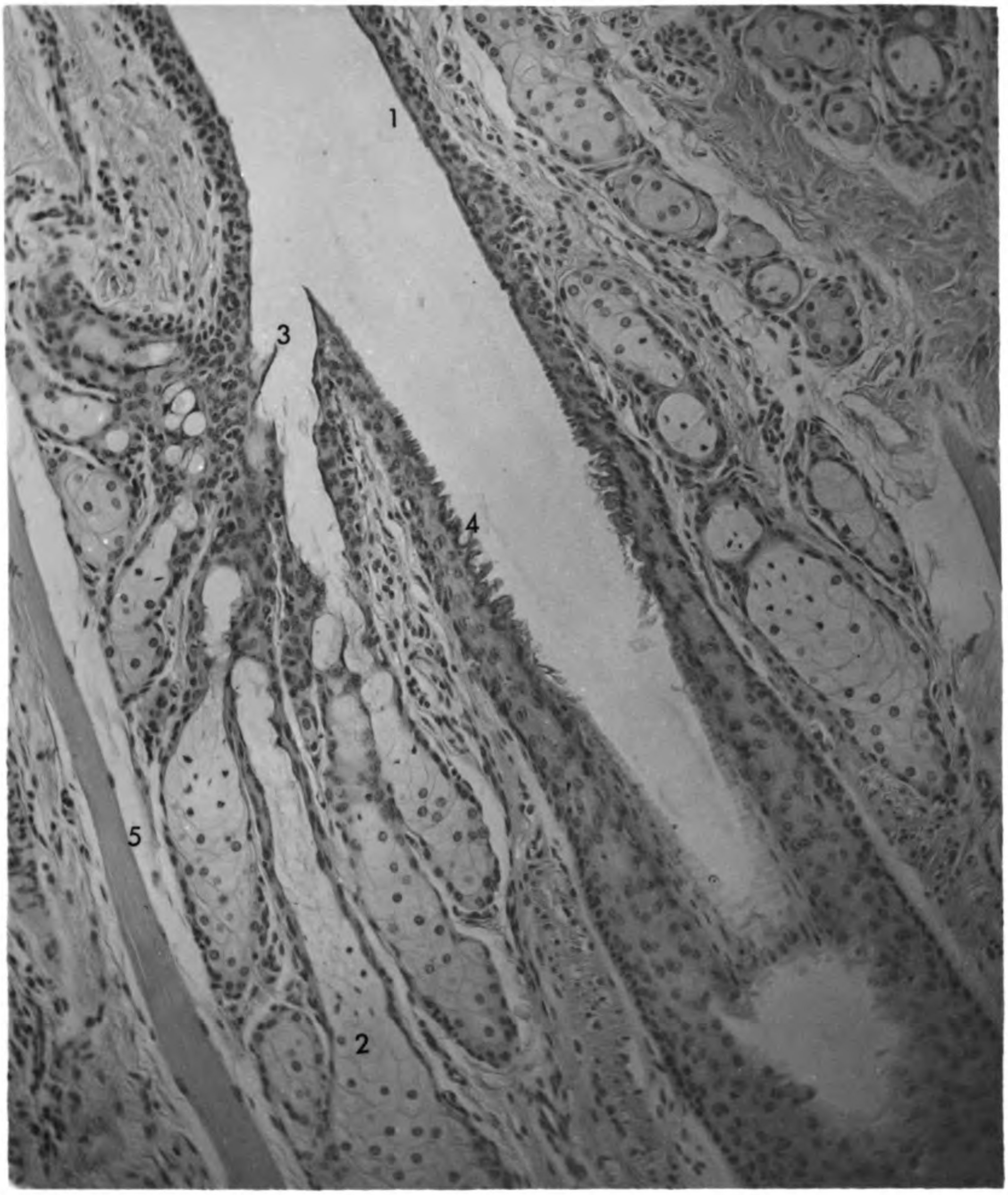

PLATE II

Follicular folds and sebaceous gland (muzzle margin) vertical section.

H. & E. Stain, 135 X

1. Hair follicle, 2. Sebaceous gland, 3. Opening of sebaceous gland, 4. Follicular folds, 5. Skeletal muscle (orbicularis oris).

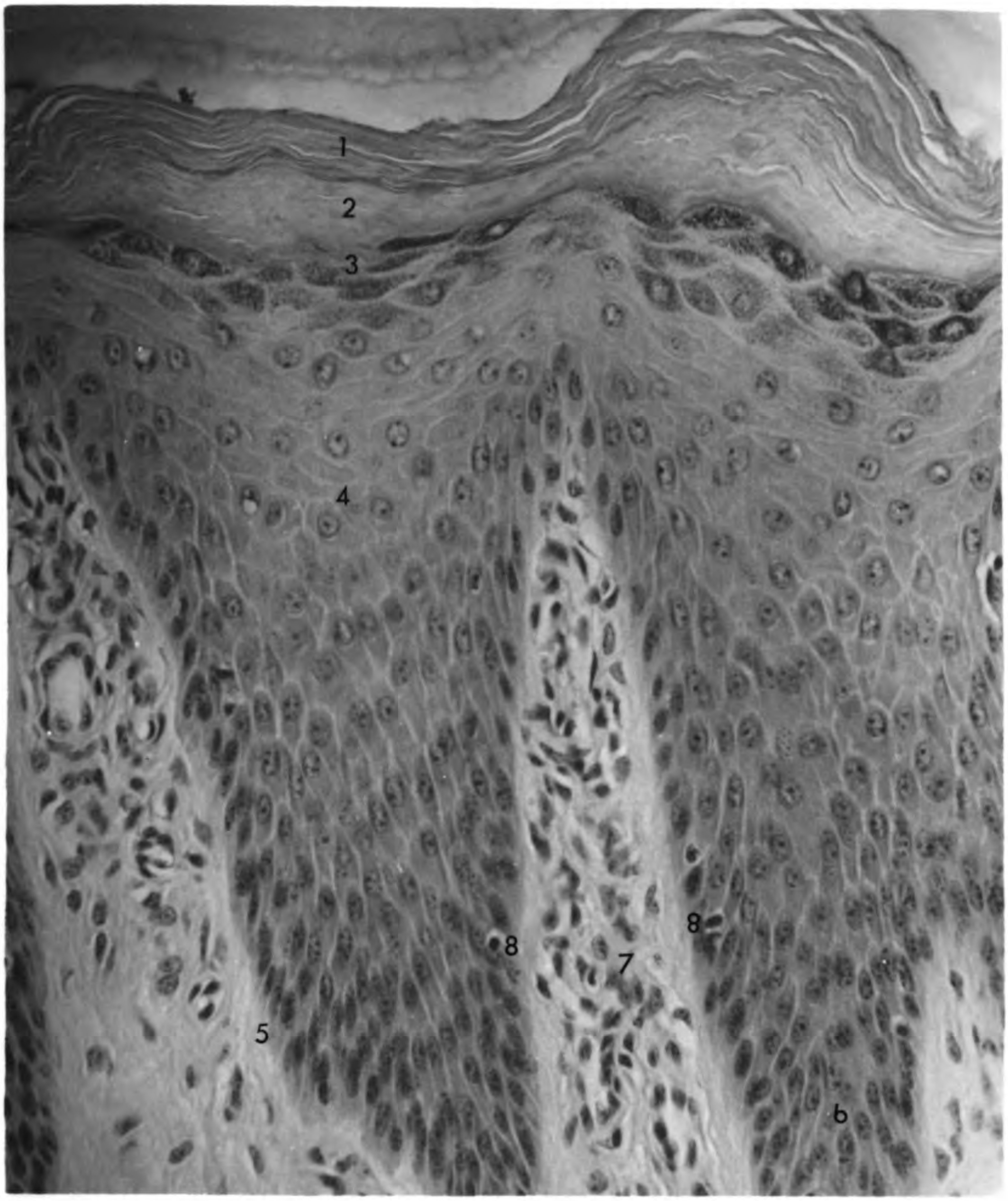

PLATE III
Teat epidermis with stratum lucidum, vertical section.
H. & E. Stain, 270 X
1. Stratum corneum, 2. Stratum lucidum, 3. Stratum granulosum, 4. Stratum spinosum, 5. Stratum cylindricum (stratum germinativum), 6. Rete peg, 7. Dermal papilla, 8. Clear cell (melanocyte).

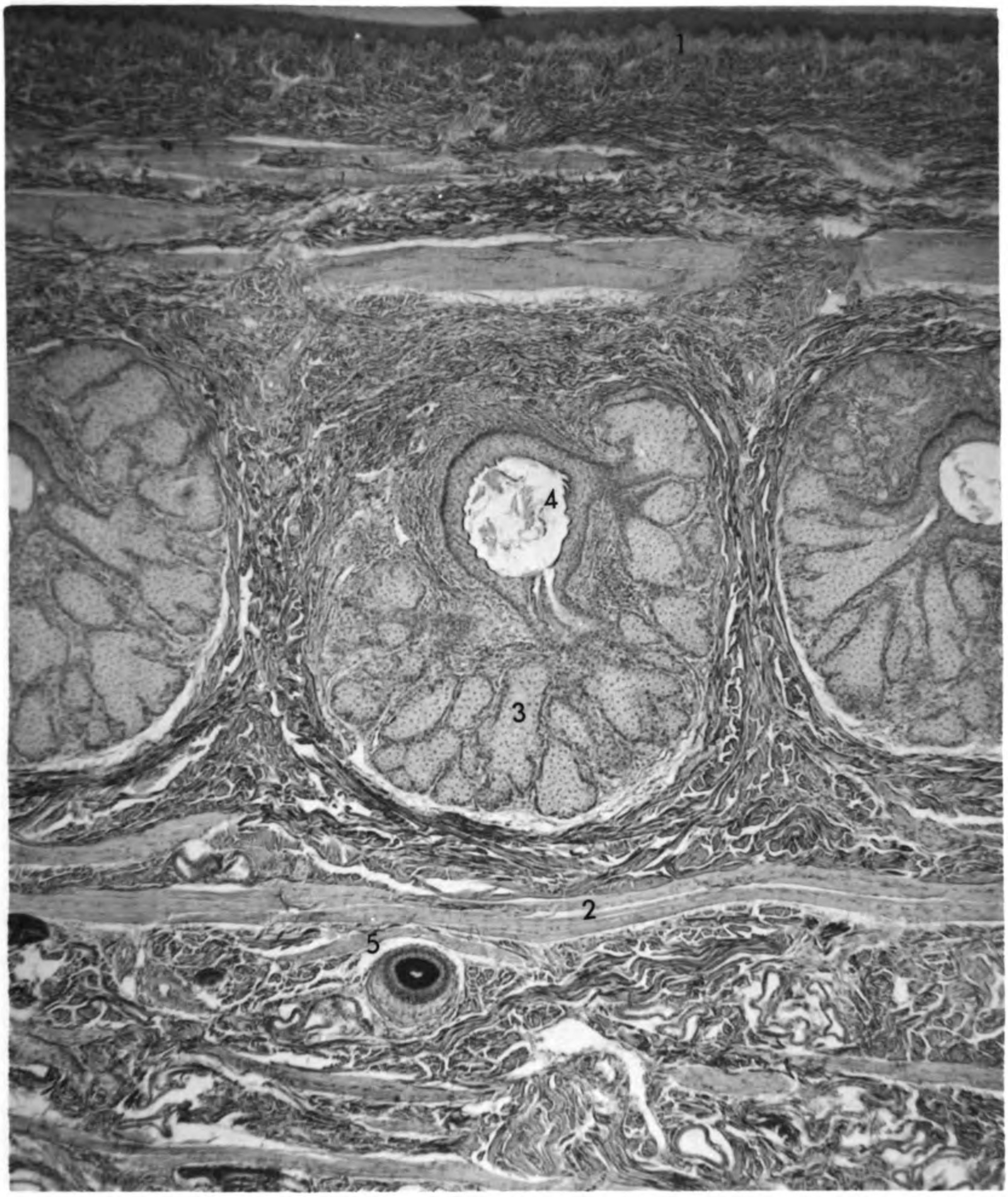

PLATE IV
Lower eyelid, vertical section
H. & E. Stain, 76 X
1. Palpebral conjunctival epithelium, 2. Mm orbicularis oculi, 3. Tarsal gland, 4. Excretory duct of tarsal gland lined by stratified squamous epithelium, 5. Hair follicle.

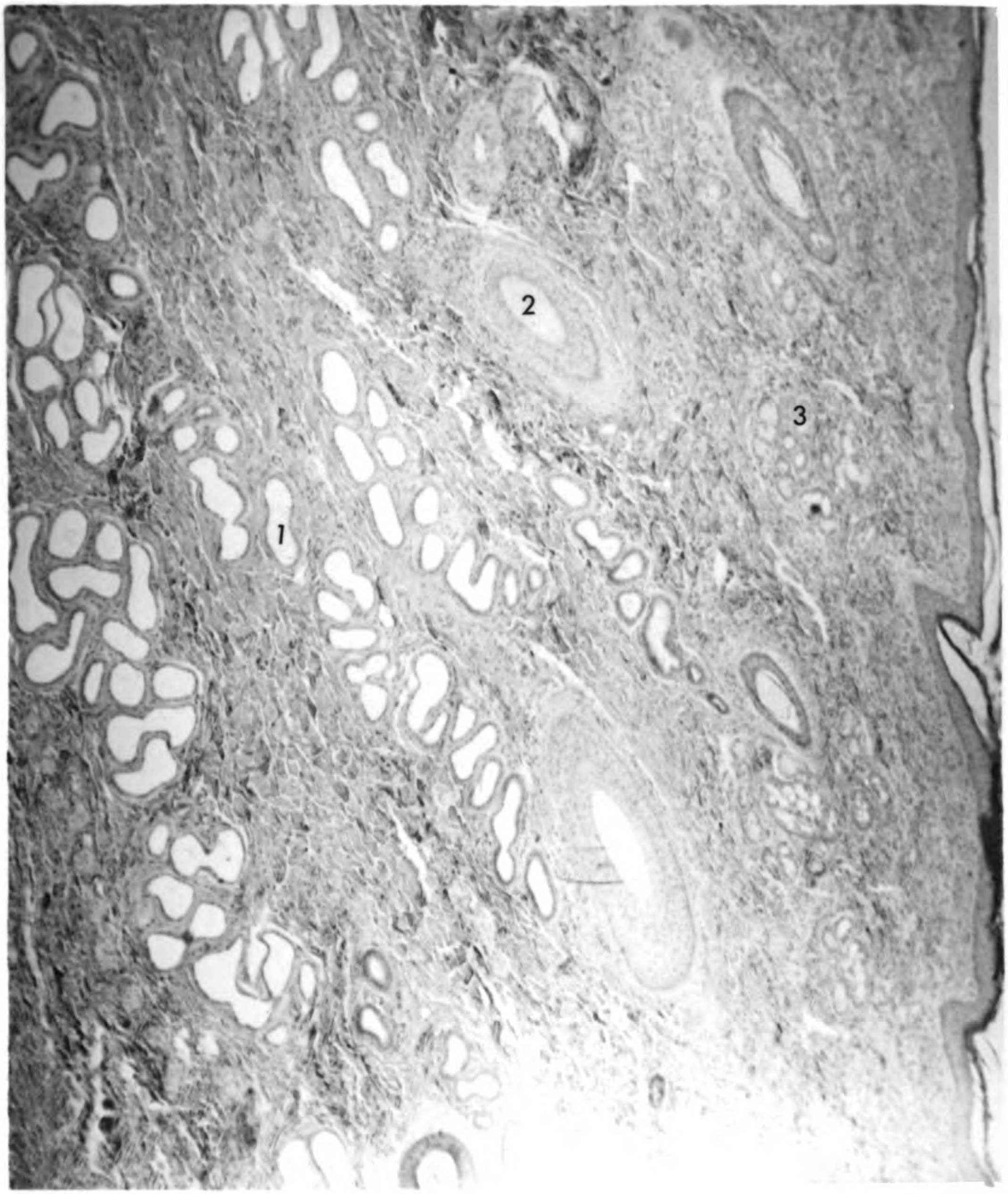

PLATE V
Labia vulva, vertical section
H. & E. Stain, 61 X
1. Sweat glands in rows, 2. Hair follicle, 3. Sebaceous gland.

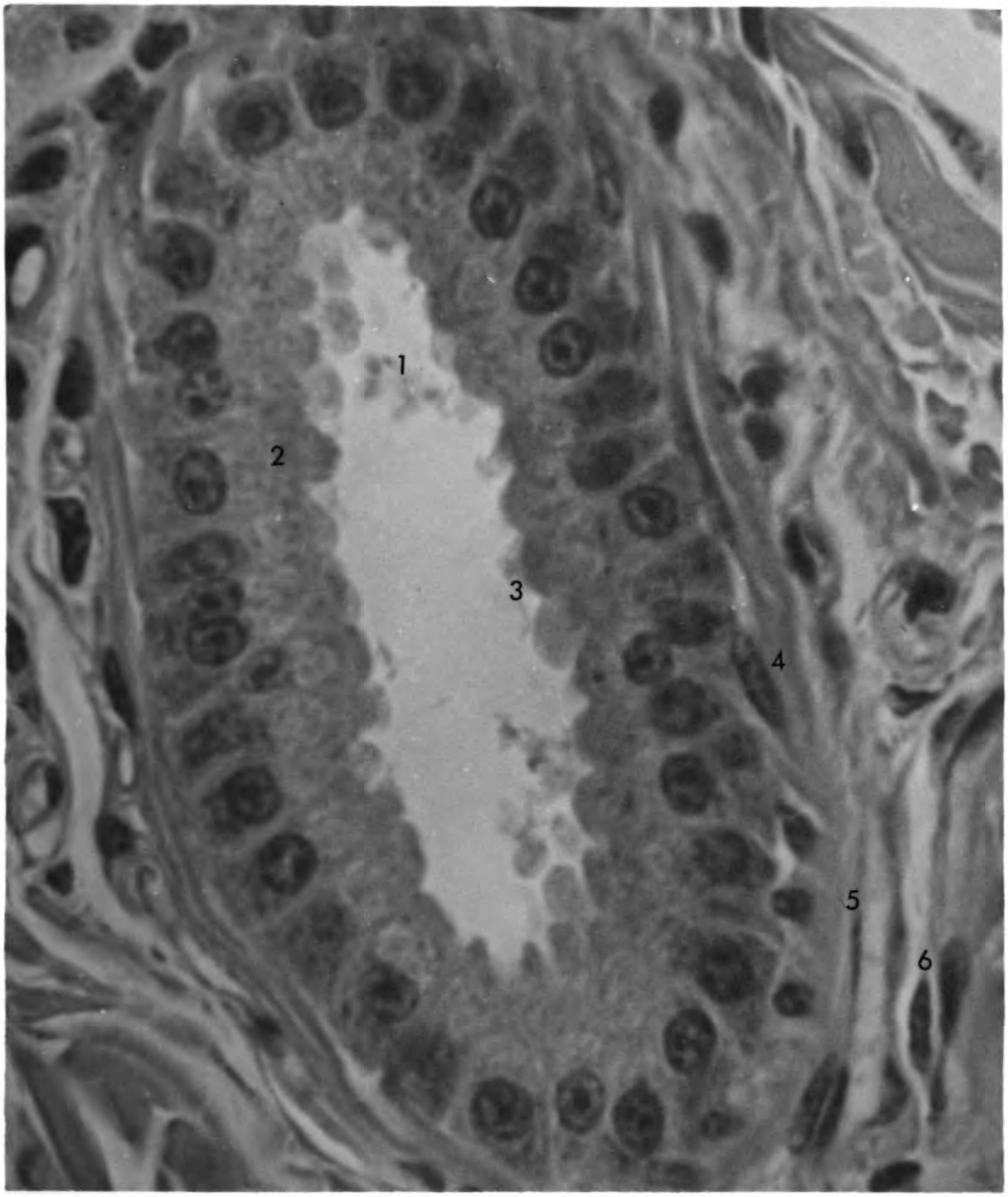

PLATE VI
Active apocrine sweat gland (labia vulva), cross section

H. & E. Stain, 1160 X

1. Lumen with luminal content, 2. Active glandular cells, 3. Apical projections, 4. Myoepithelial cell, 5. Basement membrane, 6. Fibroblast.

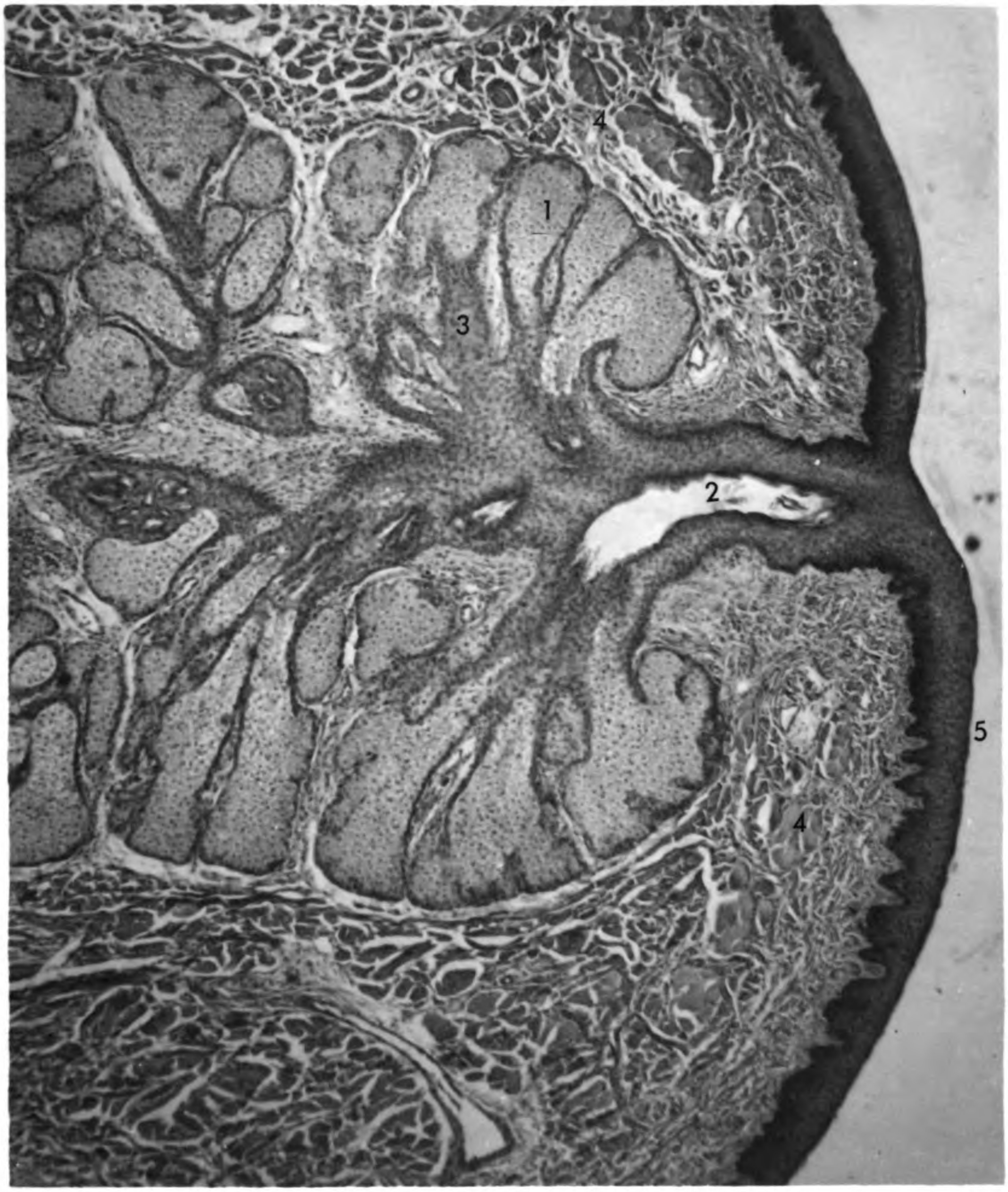

PLATE VII
Upper eyelid, vertical section
H. & E. Stain, 350 X
1. Tarsal gland, 2. Excretory and 3. Alveolar ducts of tarsal gland lined with stratified squamous epithelium, 4. Mm orbicularis oculi (Riolan's muscle), 5. Palpebral conjunctival epithelium.

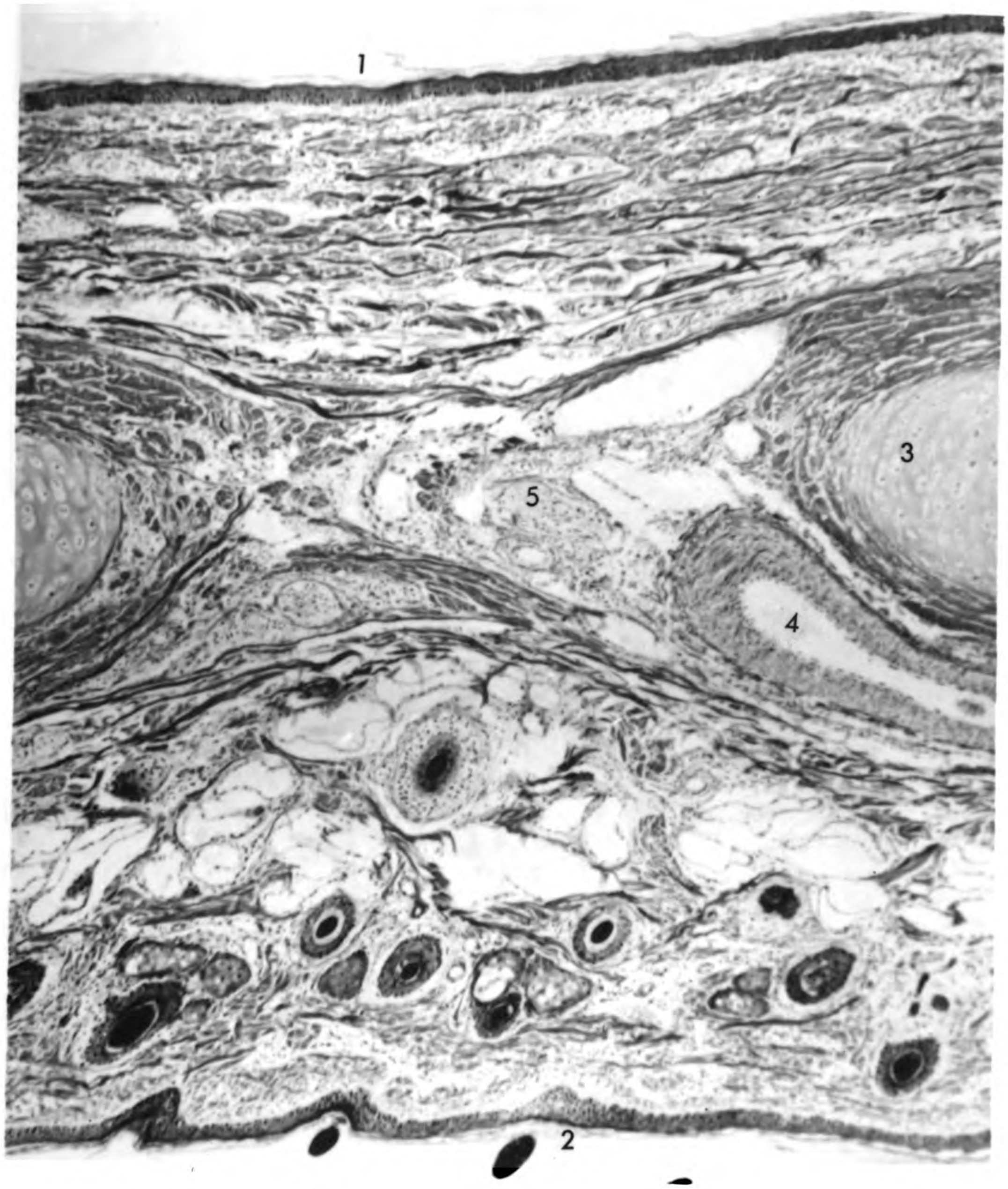

PLATE VIII
Pinna near tip, vertical section
H. & E. Stain, 90 X
1. External epithelium, 2. Internal epithelium, 3. Conchal cartilage, 4. Artery and 5. Nerve in chondral perforation.

PLATE IX

External ear canal, vertical section

H. & E. Stain, 195 X

1. Epithelium, 2. Hair follicle, 3. Opening of sebaceous gland, 4. Opening of ceruminous gland at the mouth of the hair follicle, 5. Deep part of ceruminous gland, 6. Duct of ceruminous gland.

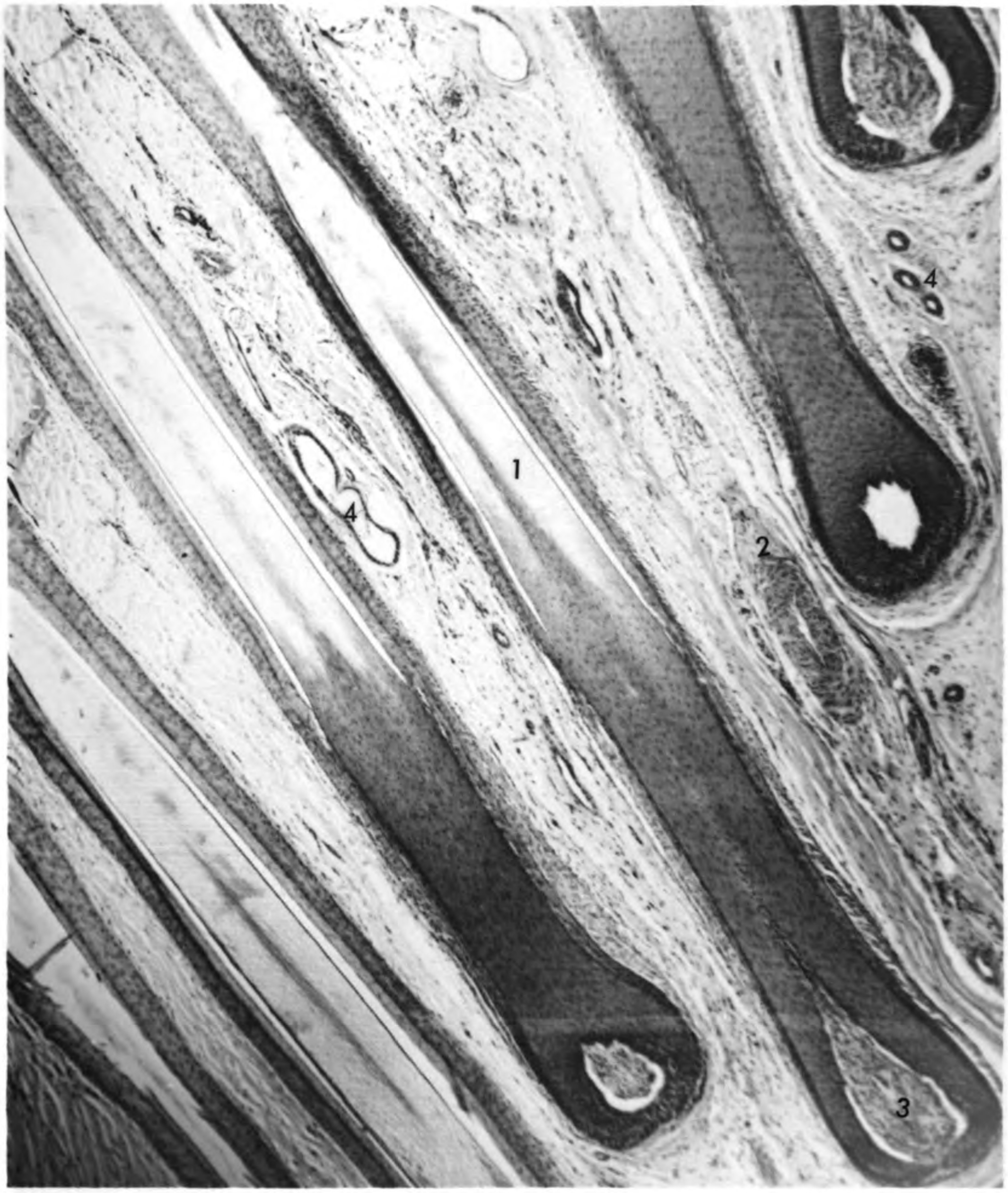

PLATE X
Tail tip hairs, vertical section
H. & E. Stain, 64 X
1. Deeply rooted thick hair, 2. Artery, 3. Hair papilla, 4. Ducts of sweat glands.

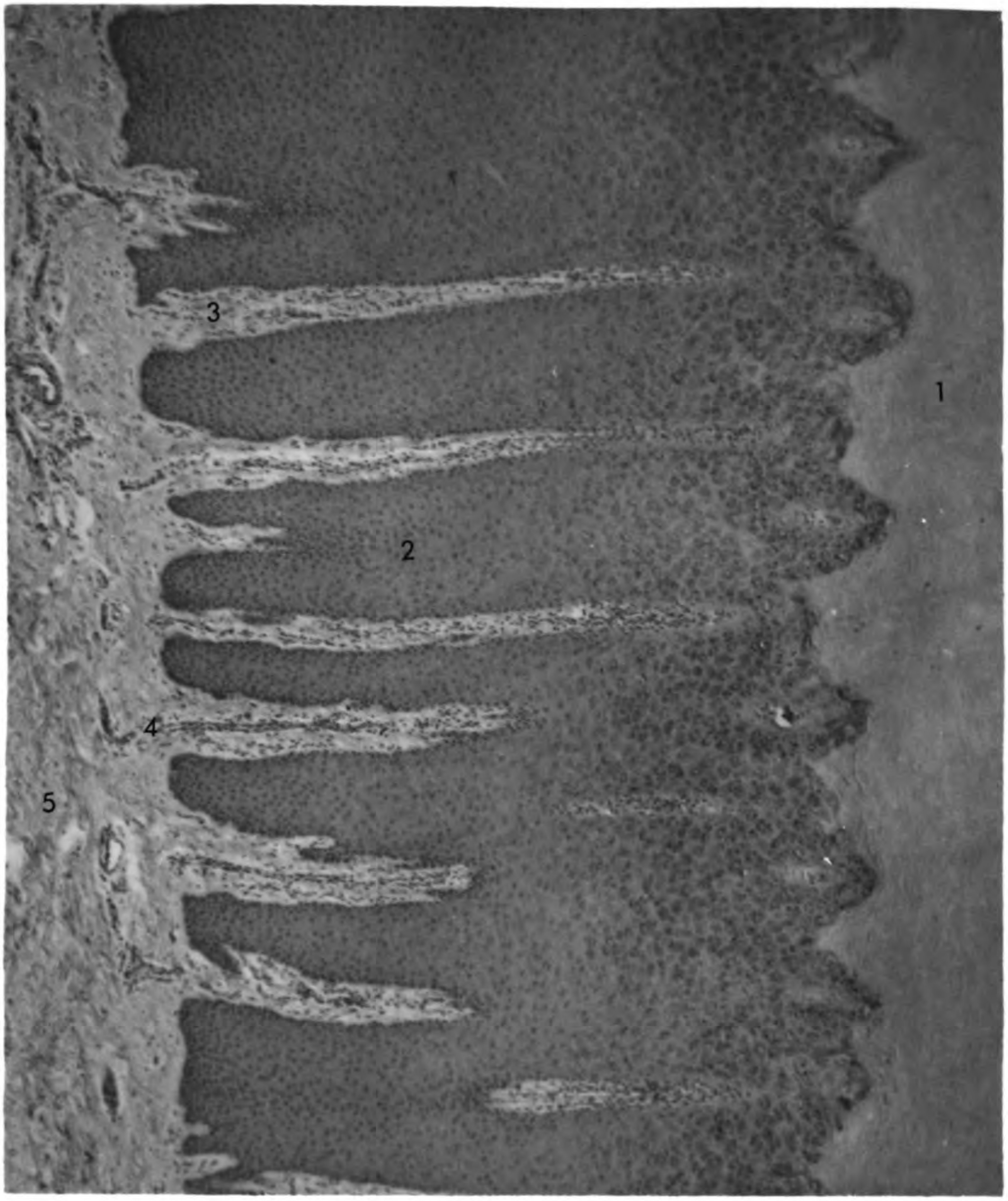

PLATE XI
Hoof and interdigital skin junction, vertical section
H. & E. Stain, 71 X
1. Stratum corneum (faint striation of intertubular horn), 2. Horny laminae, 3. Dermal papilla, 4. Blood capillary in dermal papilla, 5. Corium.

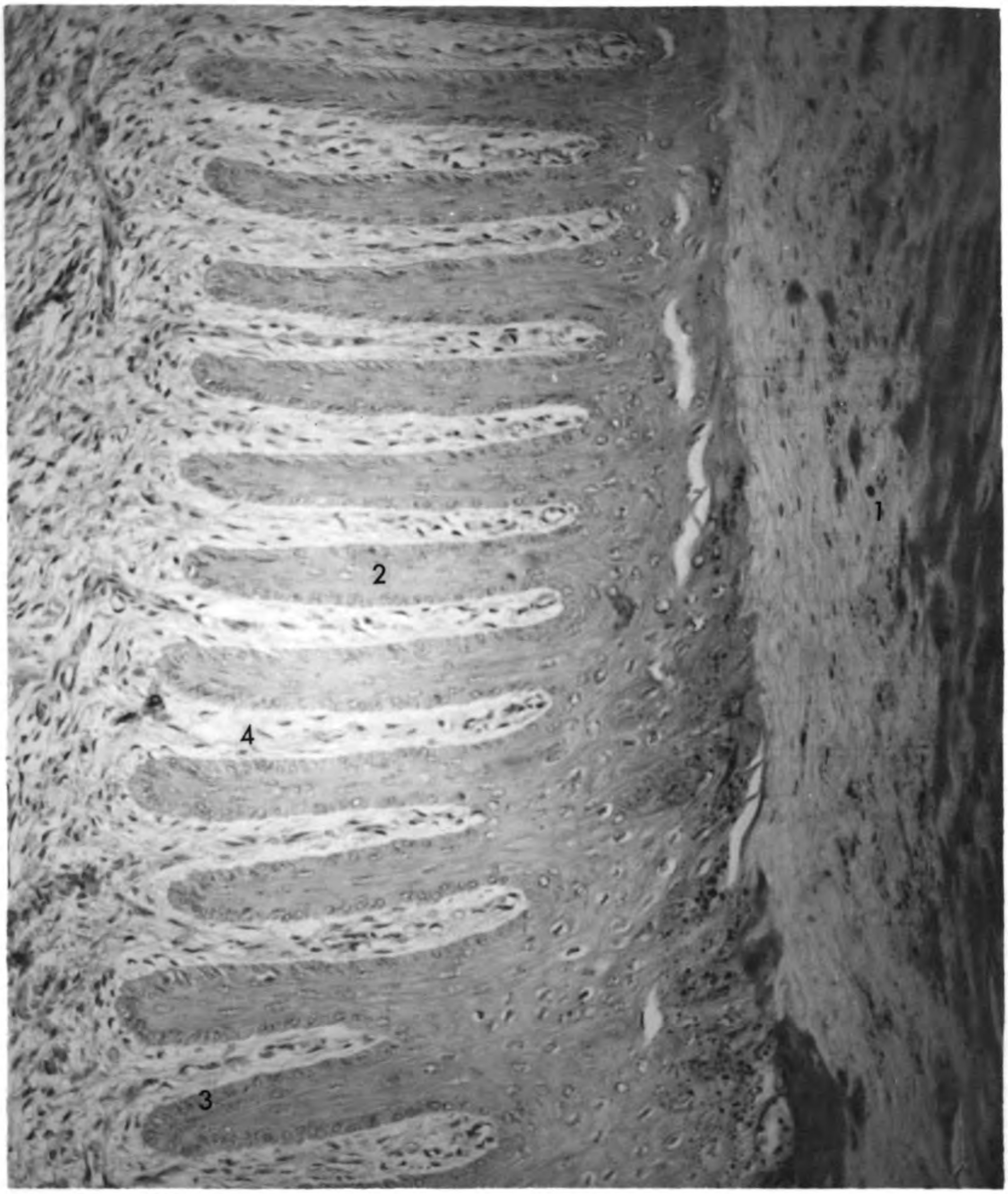

PLATE XII
Fetal hoof, transverse section
H. & E. Stain, 190 X
1. Stratum corneum transforming into horny wall, 2. Horny laminae (primary only), 3. Stratum cylindricum, 4. Dermal papilla.

PLATE XIII
Fetal digital skin ($5\frac{1}{2}$ months), vertical section
H. & E. Stain, 156 X
1. Developing hair follicle, 2. Opening of hair follicle, 3. Developing sweat gland, 4. Developing sebaceous gland.

PLATE XIV
Teat epidermis without stratum lucidum
H. & E. Stain, 920 X
1. Stratum corneum, 2. Stratum granulosum (two cell layer), 3. Stratum germinativum, 4. Rete pegs, 5. Dermal papilla.

PLATE XV
Large multilobulated sebaceous gland (muzzle margin), cross section
H. & E. Stain, 140 X
1. Hair follicle, 2. Large sebaceous gland, 3. Rete peg (transversely cut), 4. Duct of sweat gland.

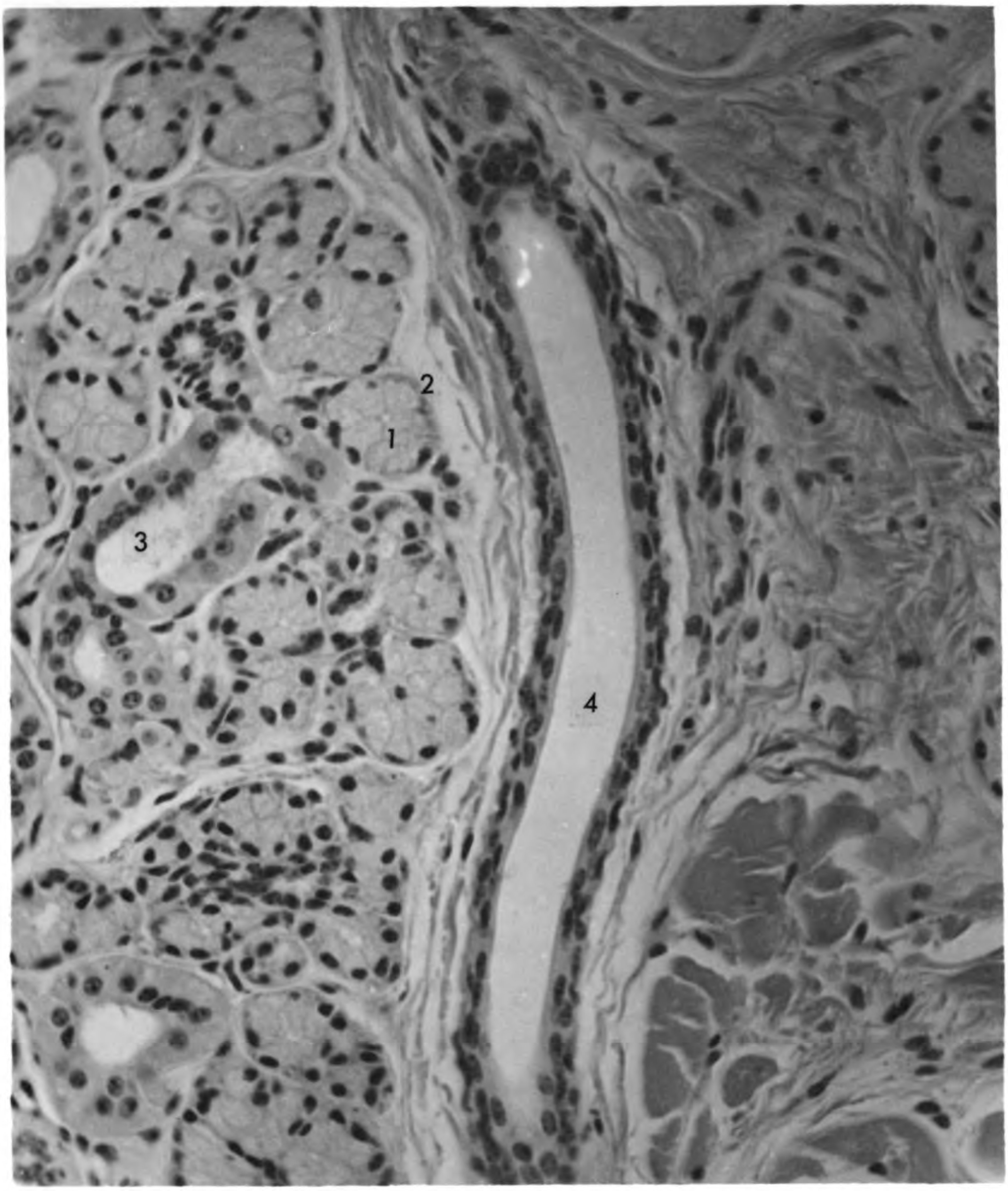

PLATE XVI
Nasolabial glands (planum nasolabiale), vertical section
H. & E. Stain, 350 X
1. Acinus lined with mucous cells, 2. Basement membrane, 3. Intercalated duct, 4. Excretory duct lined by stratified low cuboidal epithelium.

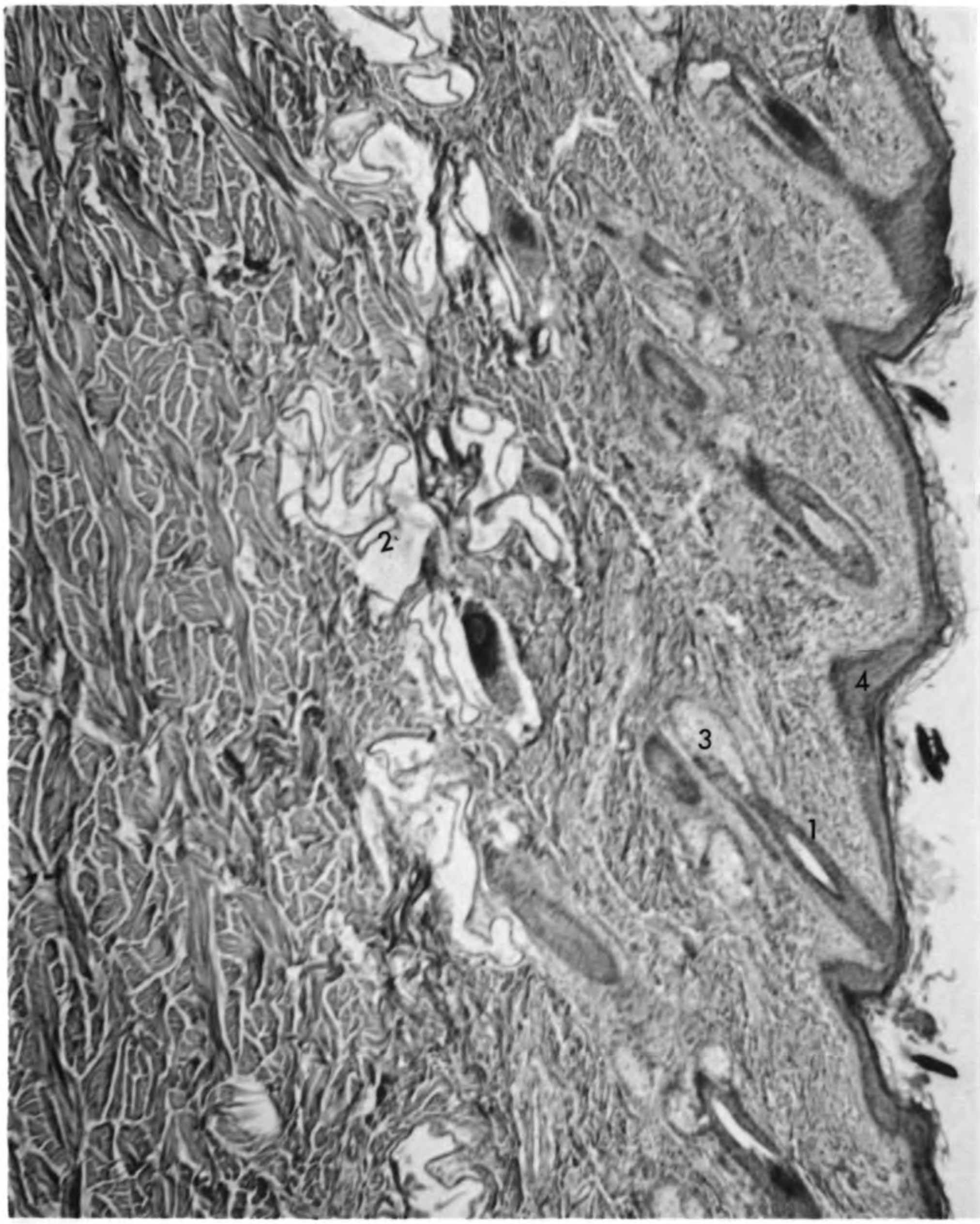

PLATE XVII
Ventral tail root scarce fine hairs (blood sample site), vertical section
H. & E. Stain, 67 X
1. Hair (note scarce fine hairs compared with Plate XXXIII), 2. Sweat gland, 3. Sebaceous gland, 4. Epidermis.

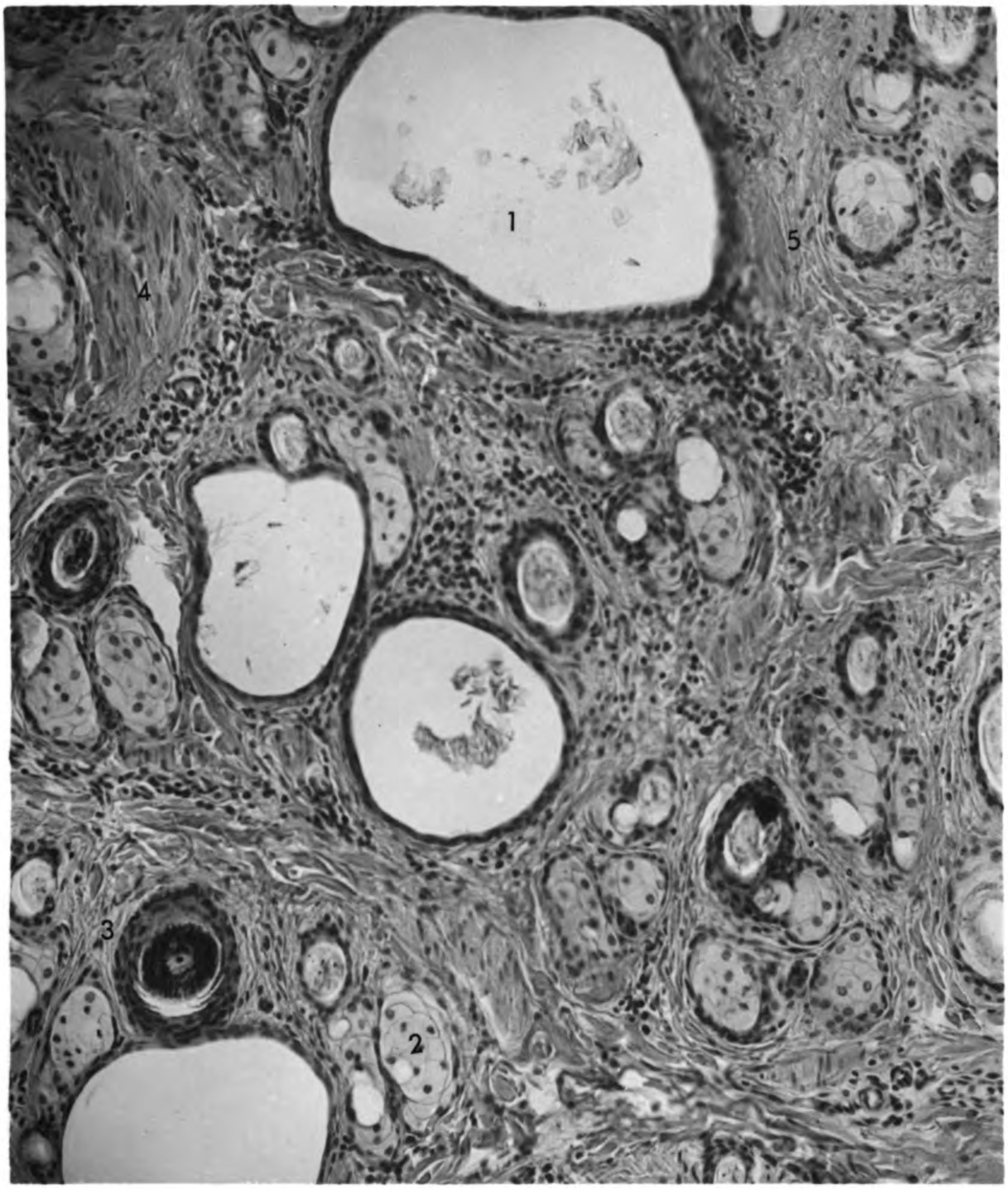

PLATE XVIII
Large saccular sweat glands (gluteal region), cross section

H. & E. Stain, 142 X

1. Large saccular sweat gland, 2. Sebaceous gland, 3. Hair follicle, 4. Arrector pili muscle, 5. Collagenous bundle.

PLATE XIX
Modified apocrine sweat gland (gland of Moll of lower eyelid), cross section
H. & E. Stain, 970 X
1. Luminal content of the gland of Moll, 2. Glandular epithelium, 3. Myoepithelial cell (transversely cut), 4. Myoepithelial cell (longitudinally cut), 5. Skeletal muscle fibers from orbicularis oculi, 6. Vein.

PLATE XX
Sweat gland duct (ear tip), oblique section
H. & E. Stain, 149 X
1. Hair follicle (cut obliquely), 2. Duct of sweat gland passing between sebaceous gland lobules, 3. Alveolus of saccular sweat gland, 4. Sebaceous gland, 5. Arrector pili muscle.

PLATE XXI
Interdigital multilobulated sebaceous gland, cross section

H. & E. Stain, 145 X

1. Multilobulated sebaceous gland, 2. Fat droplets, 3. Hair, 4. Collagen fibers.

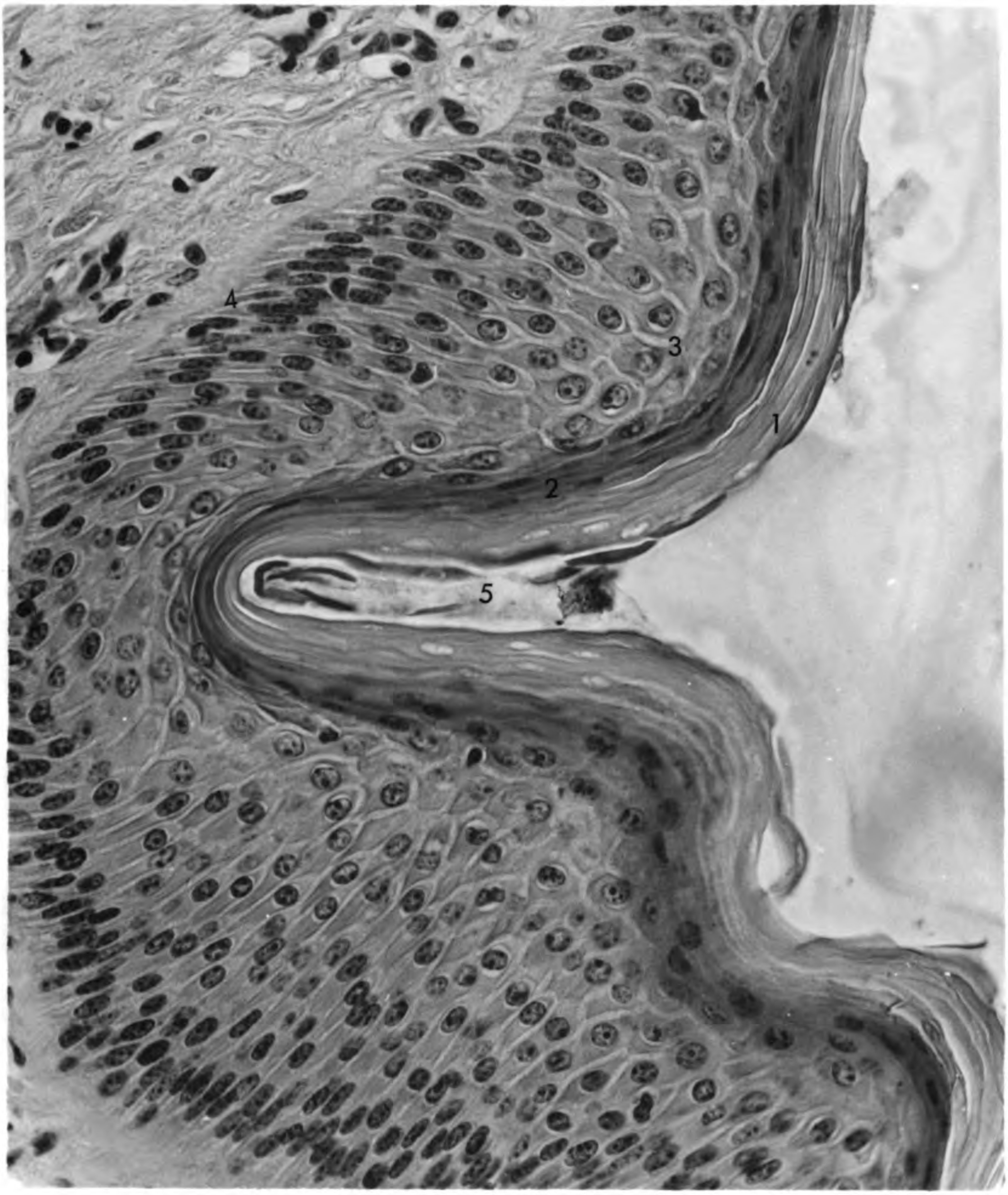

PLATE XXII
Scrotal epithelium, vertical section
H. & E. Stain, 340 X
1. Stratum corneum, 2. Stratum granulosum (two rows of cells), 3. Intercellular bridges in stratum spinosum, 4. Stratum cylindricum, 5. Scrotal groove.

PLATE XXIII
Lamellated and free nerve endings (muzzle), vertical section
Modified Bielschowsky Gros Silver Stain, 430 X
1. Axis cylinder, 2. Connective tissue lamillae, 3. Free nerve endings, 4. Nerve, 5. Internal edge of epithelium.

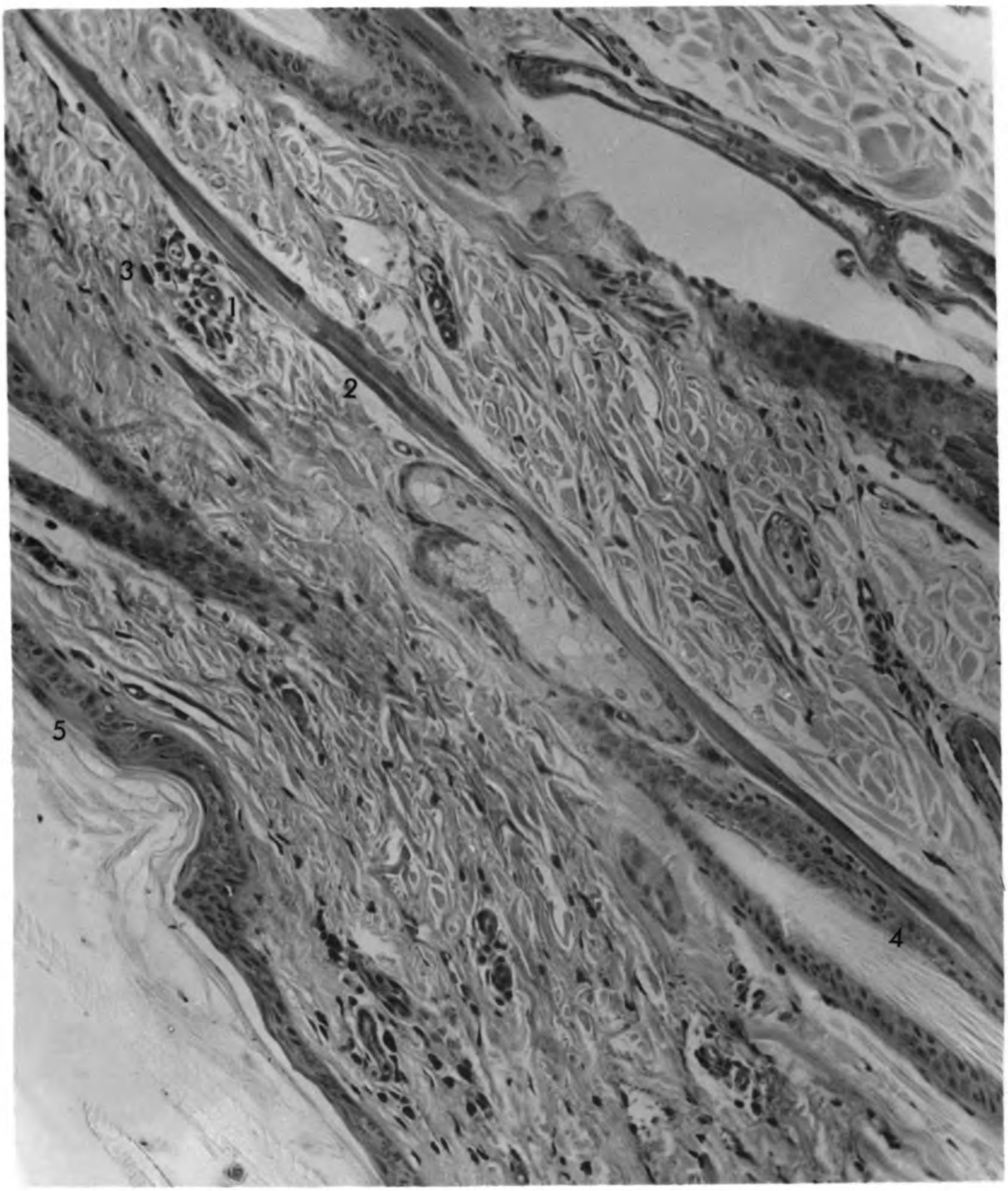

PLATE XXIV

Dewlap: Mast cells, blood capillaries and arrector pili muscle relations, vertical section

Toluidin blue stain, 180 X

1. Blood capillary surrounded by mast cell, 2. Arrector pili muscle, 3. Mast cell, 4. Hair follicle, 5. Epidermis.

PLATE XXV
Dermal-epidermal junction showing reticular fibers (dorsal neck), vertical section
Gomori's reticular stain, 840 X
1. Reticular fibers, 2. Epidermis, 3. Blood capillary.

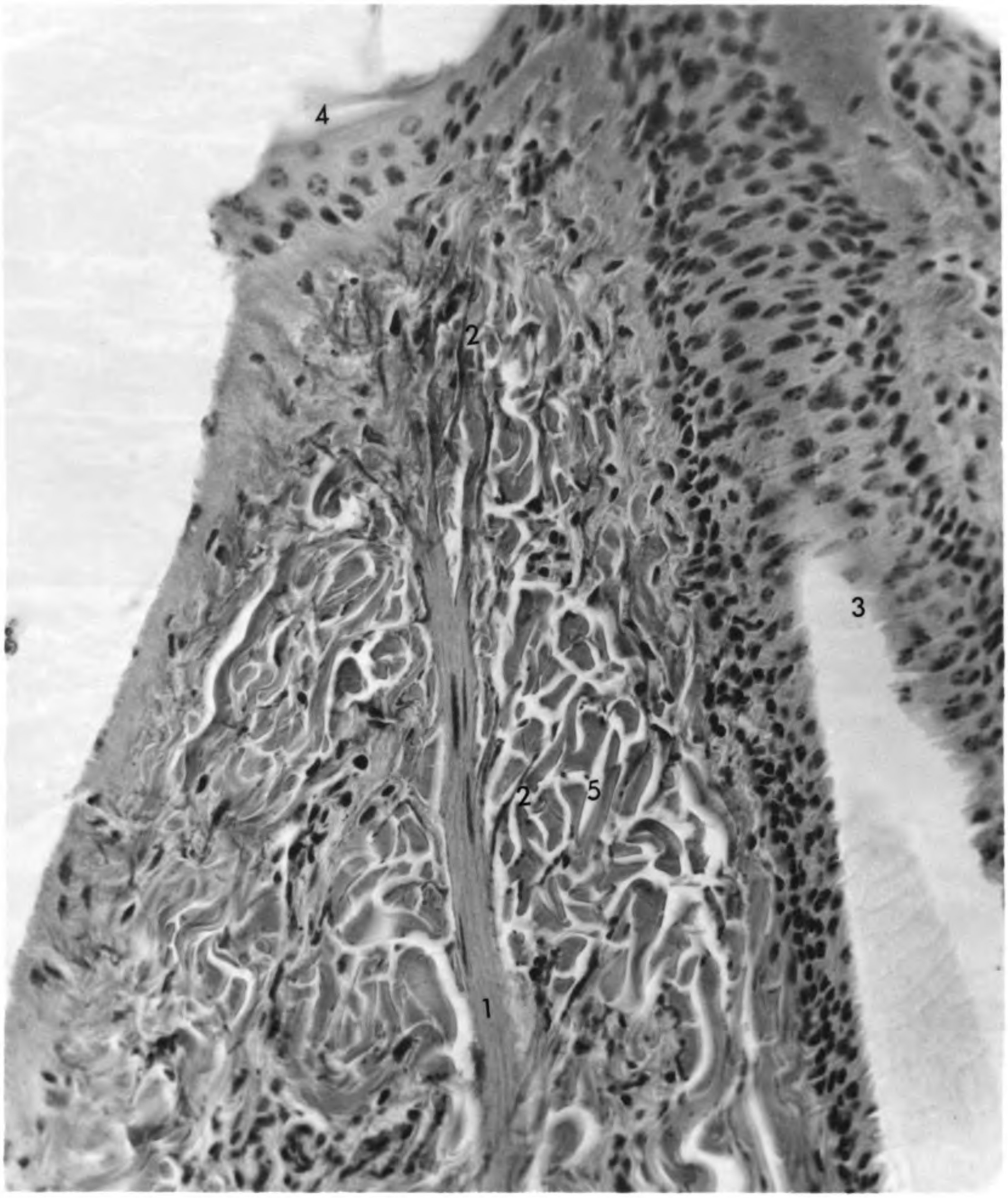

PLATE XXVI
Arrector pili muscle ending in elastic fibers (ventral tail root), vertical section
Weigert & Van Gieson's connective tissue stain, 320 X
1. Arrector pili muscle, 2. Elastic fibers, 3. Hair follicle, 4. Epithelium, 5. Collagen fibers.

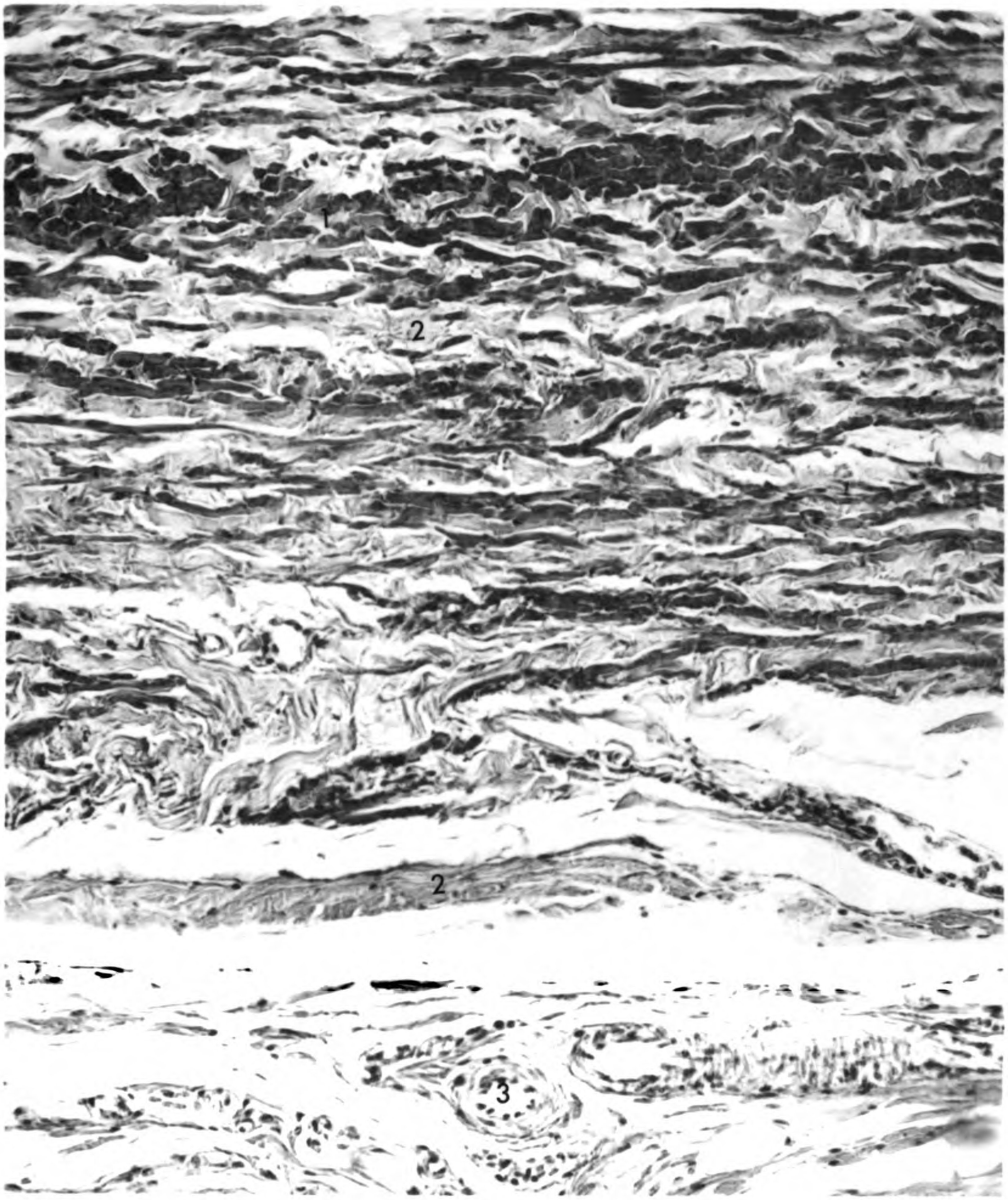

PLATE XXVII
Elastic fibers (dorsal thorax), vertical section
Weigert & Van Gieson's connective tissue stain, 160 X
1. Elastic fibers (all dark stained fibers), 2. Collagenous fibers, 3. Artery in subcutis.

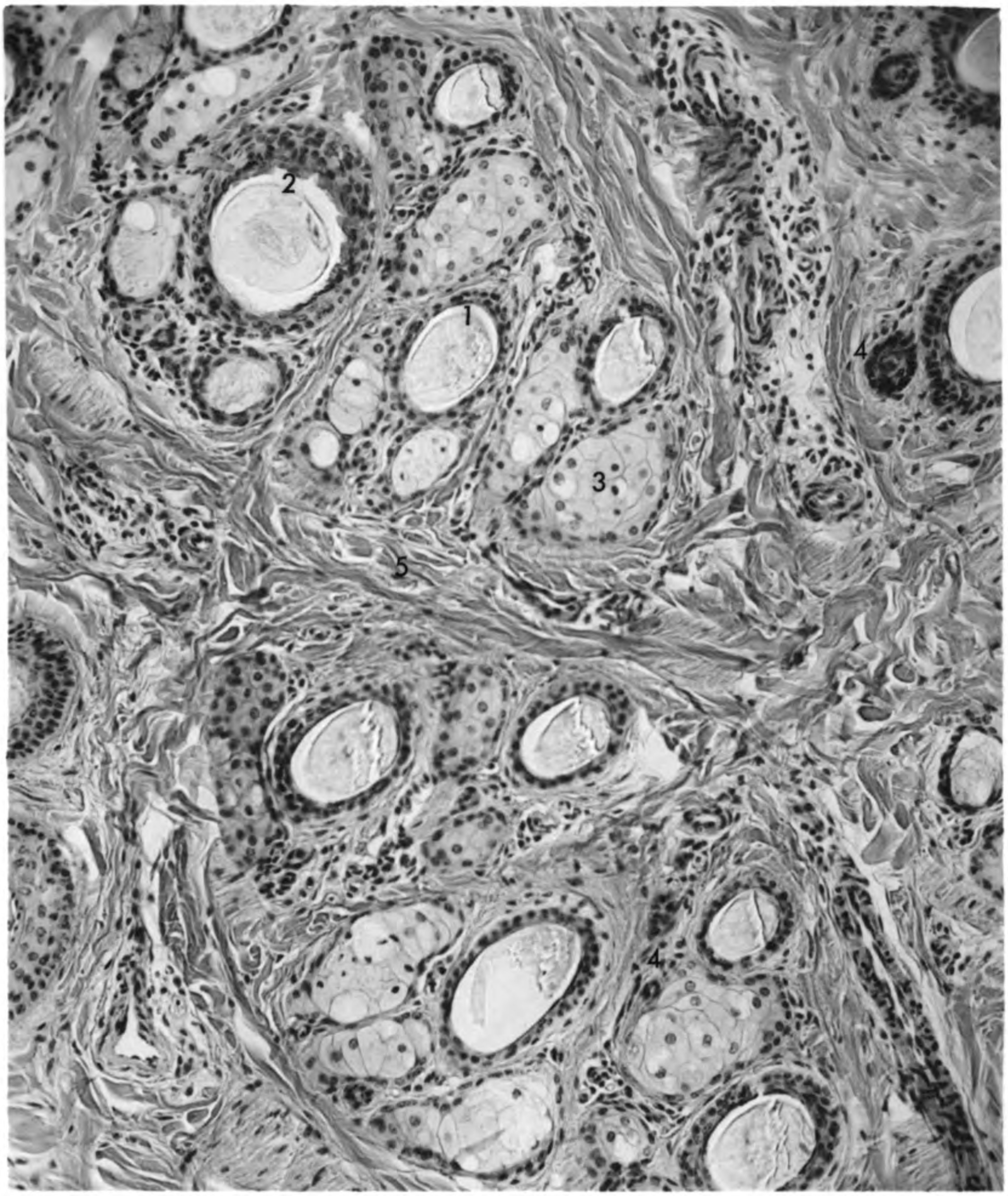

PLATE XXVIII
Hair groups and follicular folds (hock), transverse section
H. & E. Stain, 145 X
1. Hair follicle, 2. Follicular folds transversely cut and surrounding 2/3 of the follicle, 3. Sebaceous gland, 4. Duct of sweat gland, 5. Heavy collagenous bundles surrounding hair group.

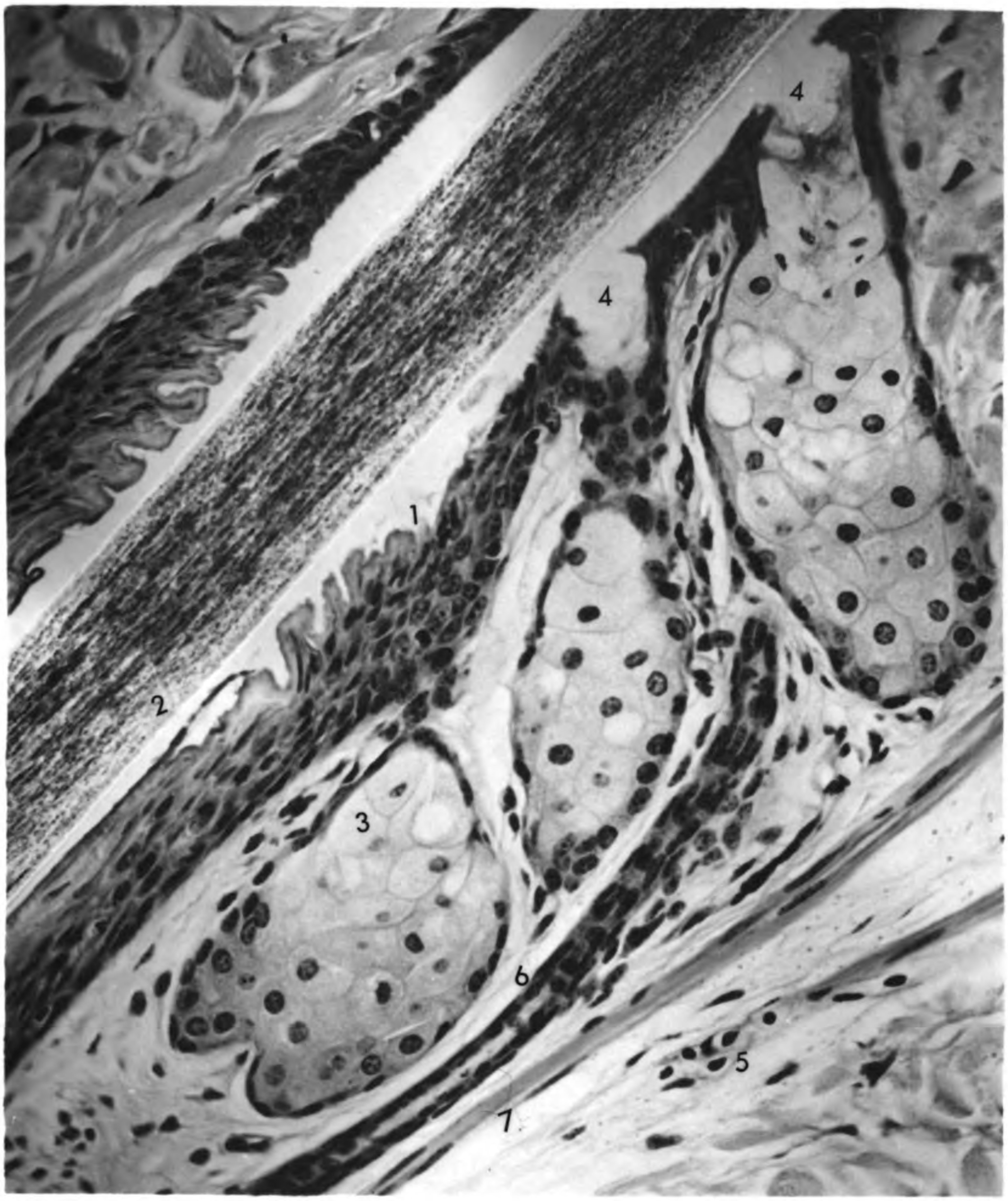

PLATE XXIX
Follicular folds and openings of two sebaceous glands in a follicle (forehead), vertical section
Toluidin blue stain, 277 X
1. Follicular folds, 2. Hair shaft, 3. Sebaceous gland, 4. Openings of sebaceous glands, 5. Mast cells, 6. Duct of sweat gland, 7. Arrector pili muscle.

PLATE XXX
Hair shaft and hair follicle structures (lateral abdomen), vertical section
H. & E. Stain, 135 X
1. Hair cuticle, 2. Cortex of hair, 3. Medulla of hair, 4. Hair papilla, 5. Cuticle of inner root sheath, 6. Huxley's layer, 7. Henle's layer, 8. Connective tissue sheath, 9. Duct of sweat gland.

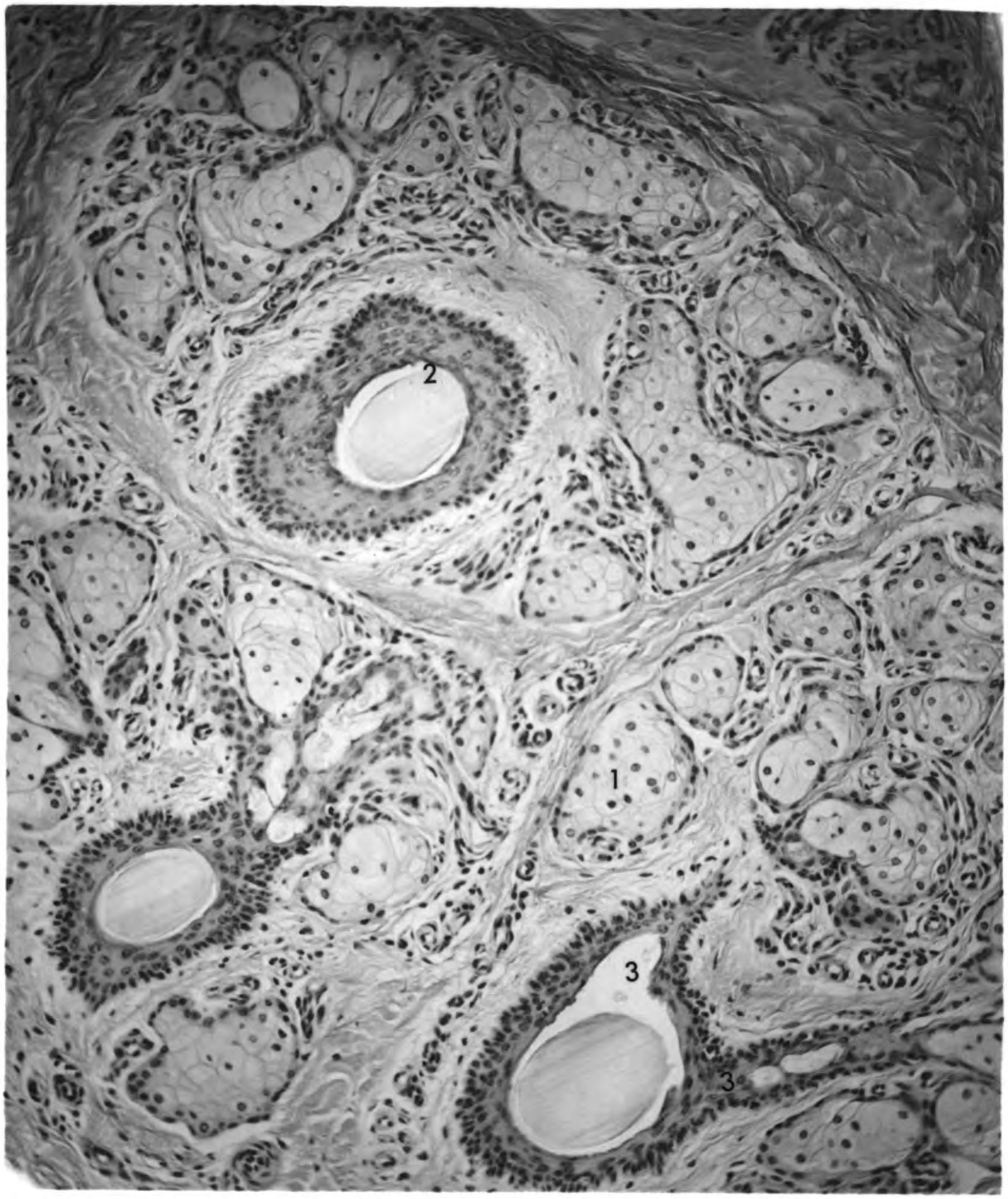

PLATE XXXI
Interdewclaws: multilobulated sebaceous gland, oblique section
H. & E. Stain, 142 X
1. Sebaceous glands, 2. Hair follicle, 3. Openings of sebaceous glands.

PLATE XXXII
Perineum, vertical section
Weigert and Van Gieson's connective tissue stain, 76 X
1. Regenerating hair, 2. Saccular sweat glands, 3. Arrector pili muscle, 4. Elastic fibers attaching muscle to hair follicle.

PLATE XXXIII
Giant and small coat hair (mid tail), vertical section
H. & E. Stain, 58 X
1. Giant coat hair, 2. Small coat hair, 3. Sweat gland.

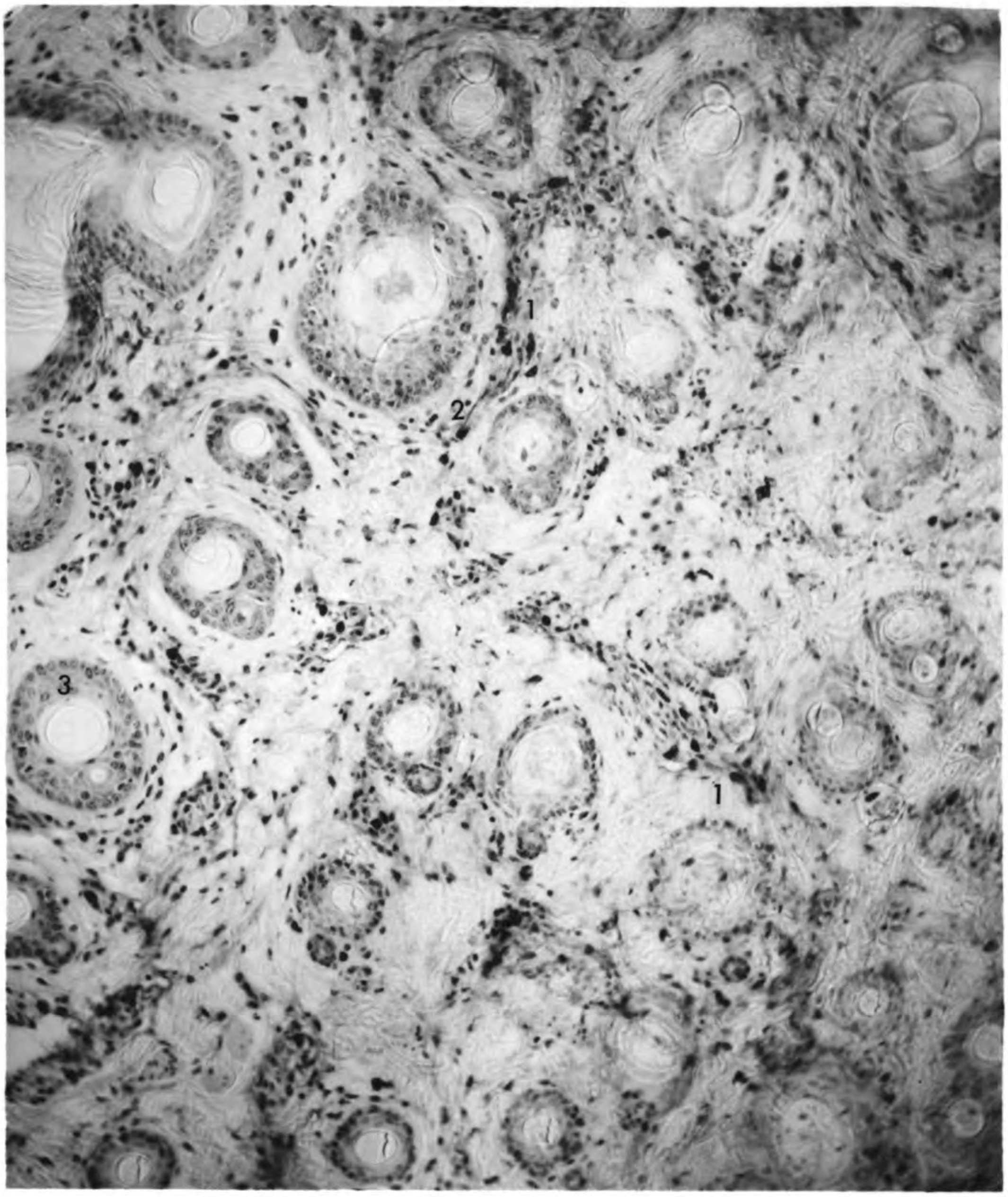

PLATE XXXIV
Metachromatic fibers and mast cells (dorsal thorax), oblique section
Toluidin blue stain, 140 X
1. Metachromatic mast cells, 2. Metachromatic fibers, 3. Hair follicle.

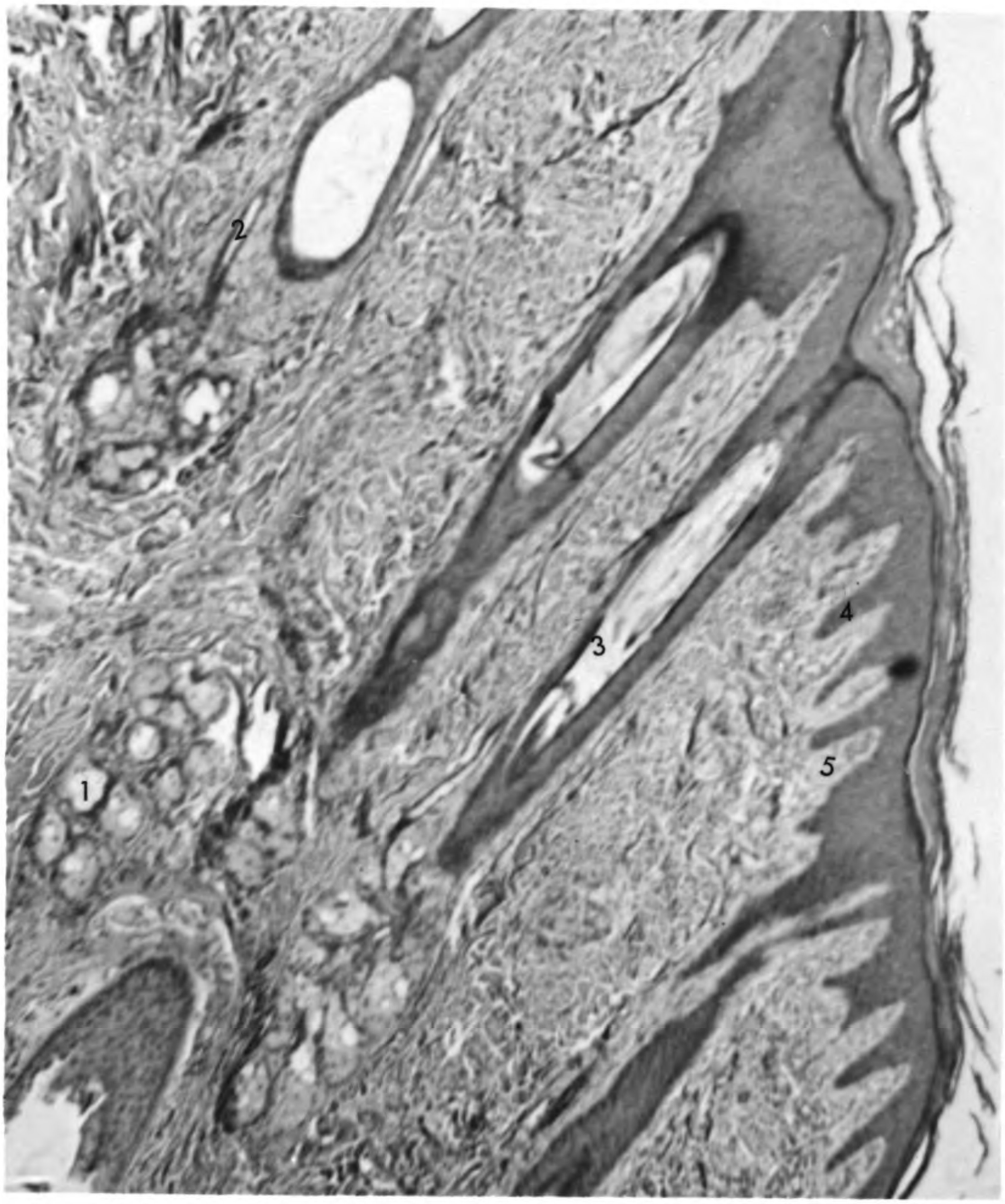

PLATE XXXV
Hoof margin, vertical section
H. & E. Stain, 92 X
1. Multilobulated sebaceous gland, 2. Duct of the sweat gland, 3. Hair follicle, 4. Rete pegs, 5. Dermal papillae.

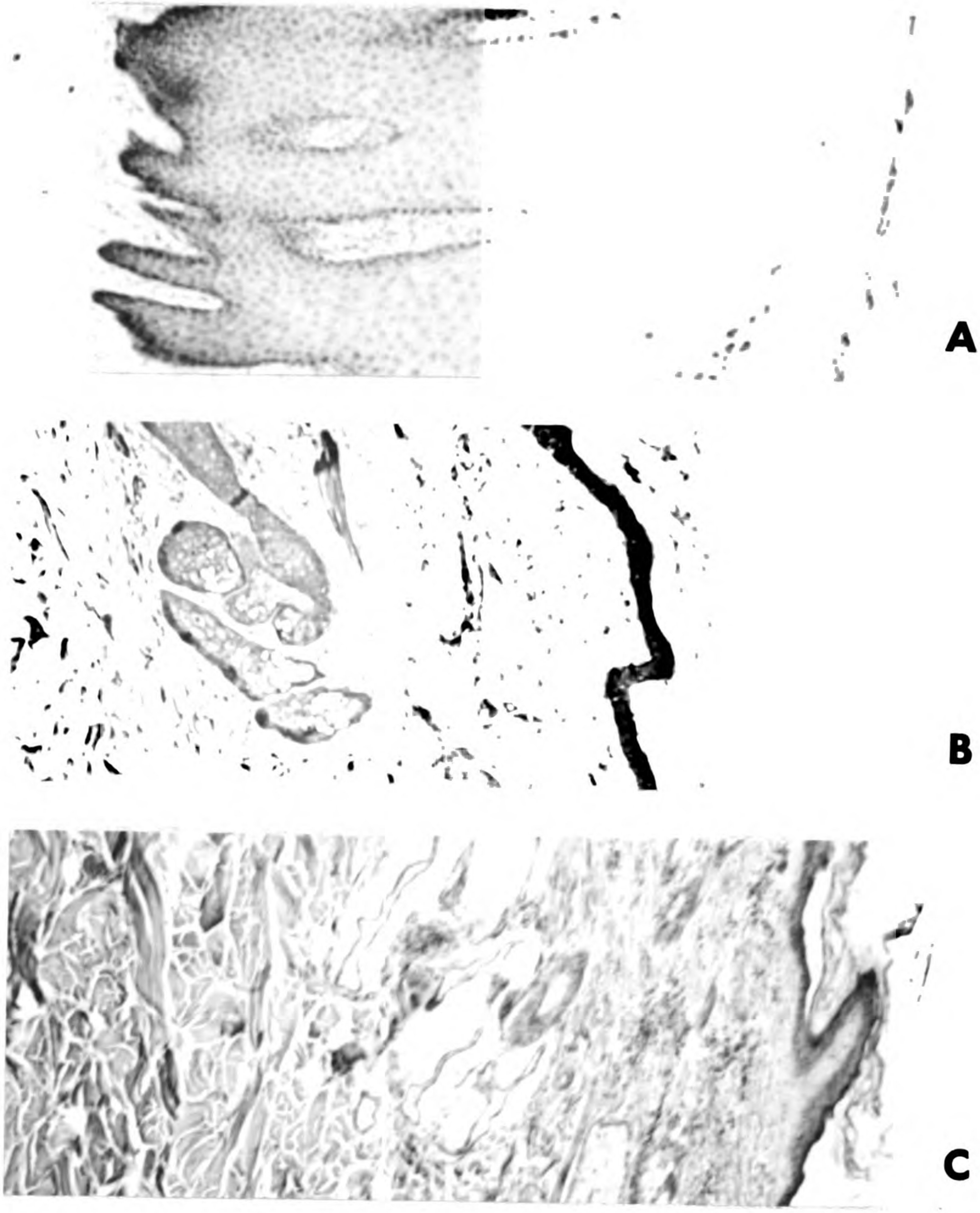

PLATE XXXVI

General and modified epidermal thickness, vertical section

A. Muzzle--H. & E. Stain, 99 X, the thickest modified epidermis

B. Forehead--Mallory's triple stain, 96 X, the thickest general body epidermis

C. Axilla--H. & E. Stain, the thinnest general body epidermis, 90 X.

PLATE XXXVII
Tactile hair (muzzle), transverse section
Modified Bielschowsky-Gros Silver Stain, 61 X
1. Sensory hair, 2. Epithelial sheath, 3. Fibroelastic trabeculae in blood sinus, 4. Outer connective tissue sheath, 5. Coat hairs, 6. Sebaceous gland.

SUMMARY AND CONCLUSIONS

A microscopic investigation of the integument of Holstein cattle revealed an average total skin thickness of 3.8 mm, ranging from 0.6 mm at the tip of the external ear to 7.5 mm on the muzzle and 3.5 mm in the two bull calves to 4 mm in the four cows. The skin of Holstein cattle was thinner than that of Hereford and Aberdeen beef cattle investigated by Goldsberry and Calhoun in 1959. Pigment granules were abundant at the opening of hair follicles, in furrows and grooves on the skin and on the dorsal aspect of the body. Cytoplasmic fibrils and keratohyaline granules were most numerous at the horny-cutaneous junctions and in thick skin. Mast cells and metachromatic fibers were abundant in young animals.

The stratum lucidum was occasionally present at certain specialized non-hairy areas such as the teat, hoof margin and horn bud. Density and rows of cells of the stratum granulosum increased as the thickness of the skin increased. The stratum papillare was composed of fine reticular, collagenous and elastic fibers, while the stratum reticulare contained coarse collagenous and thick elastic fibers. Mast cells were small in the papillary layer and large in the reticular layer. Guard and wool hairs and secondary hair follicles were not present. The average number of hairs per square centimeter was 2221 in bull calves and 2082 in cows. Hairs were arranged in groups varying in number from 1 to 7. Although follicular folds were not seen in all follicles, when present they surrounded 2/3 to 3/4 of the follicle. Almost all sebaceous glands, including those of the ear canal and vulva, opened into the hair follicle. They were well developed at horny and mucocutaneous junctions and were absent from the muzzle, teats, horns, hooves and dew claws. Morphologically sweat glands were tubular, saccular and coiled saccular.

The histology of special areas such as the external ear, hoof, horn, interdigital area, labia vulva, muzzle, scrotum, udder, teat and tail was also investigated.

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