Histomorphological studies on the skin of rabbit (*Oryctolagus cuniculus*)

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(BIHAR)

(Faculty of Post-Graduate Studies)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(Veterinary ANATOMY)

By

Sukhdeo Mukhiya

Registration No. M/Vety. Anat./24/1998-99

Department of Veterinary Anatomy & Histology

BIHAR VETERINARY COLLEGE

PATNA

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Dedicated To

My

Grand Parents

DEPARTMENT OF VETERINARY ANATOMY AND HISTOLOGY,
Bihar Veterinary College, Patna – 800 014
Rejendra Agricultural University
Pusa (Samastipur), Bihar

CERTIFICATE – I

This is to certify that the thesis entitled "**HISTOMORPHOLOGICAL STUDIES ON THE SKIN OF RABBIT (*Oryctolagus cuniculus*)**" submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Veterinary Anatomy)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa, is the record of bonafide research carried out by **Dr. Sukhdeo Mukhiya**, Registration No. – M/Vety. Anat./24/1998-99 under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

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We, the undersigned members of the Advisory Committee of **Dr. Sukhdeo Mukhiya**, Registration No. M/Vety. Anat./24/ 1998-99 a candidate for the degree of Master of Veterinary Science with major in Veterinary Anatomy, have gone through the manuscript of the thesis and agree that the thesis entitled "**HISTOMORPHOLOGICAL STUDIES ON THE SKIN OF RABBIT (*Oryctolagus cuniculus*)**" may be submitted by **Dr. Sukhdeo Mukhiya** in partial fulfilment of the requirements for the degree.

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Last but not least, I thank god for giving me patience and strength to overcome the difficulties which crossed my way in accomplishment of this endeavour.

Place : Patna

Date :

(**SUKHDEO MUKHIYA**)

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Introduction

INTRODUCTION

In the recent past the special emphasis has been given to maintain Rabbitory more scientifically in India and abroad. There has been emerging interest in rabbit farming. Considering it as a self-contained industry for persons having small means and small holdings, it can be started with a limited capital and can be expanded to a full time occupation as fast as one desires.

The high reproductive rate, small body size and ability to use fibrous plant materials. Some green forage, edible weeds and kitchen waste, vegetable/fruits can be given to rabbits at almost no cost. Handling and caring may be extended by even women and children of a family.

Rabbits, as laboratory mammals, have been used extensively for basic researches in drug and bacterial screening, toxicology, healing, tissue and organ culture, mycology, skin sensitivity, immunology, ophthalmology, oncology and reproductive biology.

Rabbit wool is processed directly for carding/combing without scouring process.

As a substitute the muchless expensive furpelt hat industries are used in the majority.

The rabbit is the only farm animal which produces meat @ 10-15 times or more its own weight in a year through progenies. Being such a prolific multiplier, it is expected to ease the demand of pressure on chicken and mutton. Rabbit grows rapidly and the growth rate is similar to that of broiler fowls (Rahumthulla *et al.*, 1987).

Being a good source of white meat which is "pearly white" and low in fat and cholesterol, high class protein can be used for heart patient. Rabbit skin can be use to make gloves, caps, dolls and other articles. Rabbit skin can fetch money by selling.

The importance of the skin is stressed as an 'organ of adjustment' between the animal and its environment. The skin is one of the largest organs in the body. The thickness of the skin and its various layers, number of sweat gland characters and blood supply to the periphery, all play a significant role in heat balance of the animal body. Functionally, it protects against mechanical injuries, noxious agents and irradiation, acts as sensory organ, elaborates vitamin D, and helps in excretion.

Considering the above facts and the figures, the anatomical studies on the rabbit have been included in the National Veterinary Curriculum under the instruction of Veterinary Council of India.

An attempt was made in the present study on histomorphology of skin to characterize the skin qualities like epidermis and distribution hair follicles, cutaneous glands etc and to compare between male and female rabbits.

The findings of the present project can be well utilized by the scientist in the allied fields of biological science.

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Review of
Literature

REVIEW OF LITERATURE

According to many authors, the skin is composed of two layers outer epidermis and inner dermis.

A subcutaneous layer of loose and adipose connective tissue binds the dermis to the underlying fascia and skeletal muscles.

EPIDERMIS

The epidermis, the outermost layer of the hairy skin was a keratinized stratified squamous epithelium. The epidermis contained from inside out cell layers as stratum basale, stratum spinosum, stratum granulosum and stratum corneum.

Trautmann and Fiebiger (1957) mentioned that the epidermis of domestic animals were stratified squamous epithelium covered by a stratum corneum and rested on the papillae of the dermis. Its free surface appeared smooth or showed elevations caused by the underlying papillae. A nucleus was absent, and the interior of the cell often appeared empty or dried to a fine meshwork.

Brody (1959) observed in normal guinea pig skin, keratin of epidermal cell was formed from tonofilaments and keratohyaline granule-material partly through a gradual incorporation of tonofilaments into the keratohyaline granules. The specific keratohyaline material would then form the interfilamentous component.

Von Bargen (1959) reported that in skin of Rabbit epidermis, the cells of the stratum granulosum were flattened. The zones of contact were parallel to the surface of the epidermis. In there cytoplasm were masses of keratohyaline, for which no signs of mitochondrial origin could be found. A participation of the nucleus in the production of this keratohyaline seemed probable.

Wolf (1959) Worked on planigraphic aspects of lipid film on the skin surface and considered the formation of lipid film exclusively by the sebum from the hair follicle.

Breathnach and Goodwin (1965) revealed submicroscopically that the epidermis of guinea pig showed presence of non-keratinizing 'clear cells' in the basal layer of the surface epidermis, and of the outer root sheaths of hair follicles in white skin.

Sar and Calhoun (1966) reported in goat the epidermis generally consisted of only four layers, since there was stratum lucidum only in the thick epidermis of the muzzle, planum nasale and junction of the hoof with the skin. The epidermis was somewhat thinner on the ventral abdomen, thorax, neck, axila, flank and it was thinnest on the pinna.

Banks (1981) described that in domestic animal the epidermis was composed of distinct layers that comprised the outer layer of the body. The cells of stratum basale varied from cuboidal to

columnar. A stratum spinosum was located peripheral to the basal layer. The cells gradually changed from a polyhedral to a squamus configuration. The stratum granulosum contained flattened rhomboid cell which possessed keratohyaline granules. The superficial stratum corneum consisted of several to many layers of anucleated squamous, cornified cells.

Colhoun and Stinson (1987) mentioned that in domestic animal, the outermost layer of the skin, was a keratinized stratified squamous epithelium. The epidermal surface was smooth in some areas, but in others it had ridges or fold, that reflected the contour of the underlying superficial dermal layer.

Kumar *et al.* (1992) observed that the epithelium at commisures and alae of the external nares in goat was stratified squamous keratinized type consisted of usual four layers. The stratum lucidum was absent.

Shravan Kumar and Thiagrajan (1992) reported that surti buffalo had significantly ($P < 0.01$) thinner epidermis and it might be possible that they had taken advantage of this thinner layer of epidermis for heat dissipation by simple diffusion of excess heat from the body tissue.

Patil *et al.* (1997) described histological structure of the skin in Mehsana buffalo that the skin consisted of superficial

epidermis comprised of four strata. The stratum lucidum was absent in all over the body regions. The inner most stratum of the epidermis rested on the papillary layer formed by the dermis.

DERMIS

Trautmann and Fiebiger (1957) mentioned that the dermis of domestic animals were condensed into a feltwork of delicate reticular fibres with an admixture of fibroelastic elements. The remainder of the dermis consisted of bundles of collagenous fibres. The papillae were small and poorly differentiated in the hairy portion of skin.

Durward and Rudall (1958) demonstrated that in rat and guinea pig the upper levels of the dermis, stouter vessels represented portions of the dermal plexus. The main regions of the dermis were seemingly without capillaries and smaller vessels. Large vessels passed from the subcutaneous plexus to the dermal plexus, being connected by smaller side branches to the hypodermal plexus as they entered the dermis from below.

Straile (1960) reported that in rabbit skin a specialized region of dermis contained the harrscheibe, which was a thick area of epidermis surrounding the orifice of the hair follicle.

Sar and Calhoun (1966) showed that in goat skin the dermis contained collagenous fibres in superficial layer. They were

fine, loosely arranged, and irregularly distributed. These fibres were thick and densely arranged in the reticular layer, where they formed bundles which usually paralleled the skin surface.

Cunningham and Fitzgerald (1972[a]) demonstrated Bulbous corpuscles occupied the inter follicular dermis in the hairy skin of rabbit, lying within 50 μm of the epidermis.

Cunningham and Fitzgerald (1972[b]) observed Bulbous corpuscles in the hairy skin of mouse, rat, cat and rabbit. They discussed their mechanoreceptor functions.

Banks (1981) described that in domestic animal the dermis was separated from the epidermis by a typical basement membrane. Two zones were described in the dermis, a papillary and a reticular area. They were however quite similar and blended insensibly with each other. The connective tissue of the papillary region was areolar and varied to DWFCT in the reticular zone. Dermal papillae extended into the epidermis. Corresponding epidermal pegs were also evident in such zone. Lymphatics tissue, glands and smooth muscle also occurred in the dermis.

Calhoun and Stinson (1987) described in domestic animal that dermis was a feltwork of collagen, elastic and reticular connective tissue fibres. Hair follicles, sweat and sebaceous glands, blood and lymph vessels, and nerves were embedded at various levels throughout the dermis.

Patil *et al.* (1989) observed that the dermis of water buffalo calves consisted of feltwork of collagen and elastic fibres. The elastic fibres were more prominent at both ends of arrector pili muscle. The blood vessels arising from a deeper dermis branched into smaller arteriole and capillaries reaching up to the level of the last layer of epidermis.

Saxena *et al.* (1994) demonstrated that the connective tissue in the dermis of cattle comprised of collagenous, reticular and elastic fibres, not only to support various structures of the skin but also to play an important role in the body heat regulation, defence and repair mechanism.

Patil *et al.* (1997) reported that the dermis was made up of collagen fibres in between which the interfacing strand of elastic fibres were seen in Mehsana buffalo.

Hairs and hair follicles

Trautmann and Fiebiger (1957) mentioned that the skin of domestic animals was covered with hairs. These were epidermal structures, the free portion of which was called the hair shaft. Root was almost always set obliquely in the skin. The hair root was attached to an underlying dermal papilla. The hair roots were inserted into pits in the skin, the hair follicles. The hair was composed of medulla, cortex and cuticle.

Montagna and Eugene (1958) described that the human hairs had a cuticle on the outside, and a cortex, some of them a medulla in the centre. The cuticle was a single layer of imbricated scales, with the free margins directed up. They were transluscent and free of pigment. The cells of the cuticle of the hair were interlocked with those of the inner root sheath firmly anchoring the hair in the follicle. The cuticle bound the cortex. The mass of hairs was formed by the cortex. They were continuous, discontinuous or fragmented.

The human hair follicle were fairly simple organs which consisted primarily of sleeves of epithelium continuous with the surface epidermis, follicle grew slanted into dermis, the follicle attained its greatest diameter at base. The arrector pilorum muscles were attached to the buldge of follicle, clusters of sebaceous glands were couched above the muscles. In the centre of the follicle was the hair.

Straile (1958) reported that the skin of rabbit contained atypical guard hair follicles. Unlike normal guard hair follicles, these atypical follicles were part of an organelle which might have a tactile function.

Pospisil and Kralove (1959) showed that the rings containing fat, PAS positive materials, acid mucopolysaccharides, aliesterase and alkaline phosphatase in the lower third of the hairs of rabbit at the end of the internal epidermal sheath.

Sar and Calhoun (1966) showed in goats, there were both primary and secondary hair follicles. Sweat gland, sebaceous glands, and the arrector pili muscle were associated with each primary follicle. In tactile hair there was a blood sinus between the outer and the inner layers of the connective tissue sheath.

Calhoun and Stinson (1987) described that in domestic animals the follicle was embedded in the dermis, usually at an angle, and the bulb may extended as deep as hypodermis. The inner most layer, next to the hair root, was the internal root sheath. It was composed of the inner cuticle, middle Huxley's and outer Henle's layer.

A primary hair follicle was one of large diameter, was rooted deep in the dermis, and was usually associated with sebaceous and sweat glands and an arrector muscle.

A secondary follicle was smaller in diameter than a primary follicle and the root was near the surface. It may had a sebaceous gland but lacked sweat gland and an arrector muscle. Those follicle with only one hair emerged to the surface were called simple follicle. Compound follicle had several hairs emerged from a single opening on to the surface of the skin.

Sinus hair follicle of the head were very large single follicles. Characterized by a blood filled annular sinus between inner and outer layer of the dermal sheath.

Rao (1992) showed that the skin of Impala lips had evenly distributed hairs except over the region of planum nasolabiale. Section of the labial skin revealed ordinary and occasional tactile hairs. The ordinary hair had a typical structure with the connective tissue hair follicle lodging the ectodermal hair shaft. In the tactile (sinus) hair the connective tissue sheath was separated from the adjacent structures by connective tissue trabeculae. The intratrabecular spaces were filled with blood. The hairs abruptly ended at the mucocutaneous junction.

Smallwood (1992) wrote that like the rodents, the rabbit had many prominent vibrissae or tactile hairs associated with upper lip. The upper lip was divided by a distinct cleft and philtrum that were confluent dorsally with external nares. Each half of scrotum was represented by relatively hairless pink skin.

Baba *et al.* (1994) observed histology of palpebral hairs of goat and sheep. The palpebral dermis of animals had three types of hairs, coat hairs, sinus hairs and eye lashes. Coat hairs of both the species were densely distributed towards the base but were scarce towards the free edge of the lips. Each hair consisted of a shaft, a root and a hair bulb. The hair had three layers of cells : cuticle, cortex and medulla. The cuticle formed the outermost layer of flat cells. The cortex was composed of dense and compact flat cells located inside the

cuticle. The central portion of the medulla consisted of loosely filled cuboidal cells. Air vacuoles were present among these cells in the hair shaft.

In goat and sheep sinus hairs were deeply rooted. Large single follicles containing blood filled annular sinuses located between inner and outer layers of dermal sheath and traversed by fibroelastic trabeculae were found in both the eye lids. Some skeletal muscle fibres were found attached to the outerdermal sheath of the follicles. Large sebaceous glands were also seen encircling the tactile hair follicle.

Patil *et al.* (1997) observed distribution of hair follicle between male and female adult Mehsana buffaloes. The average number of hair follicles per sq. cm. was 212 (male 185.47 and female 239.38). The regional variation was significant in female. In forehead, mandibular, lumbar back, lateral abdomen, umbilical, medial and lateral thigh.

Mahajan (1998) described angora wool comprised wooly fibres and guard hair. The guard hairs were of different length and thickness. The guard hairs might be classified into two types – the finer type had uniserial type medulla of ladder shape whereas the coarser type had one or two medullary rows on the base and becomes multiserial at middle or sheath portion having largest diameter.

Sebaceous gland

Trautmann and Fiebiger (1957) described in domestic animals that sebaceous glands occurred in middle depth of the dermis. The sebaceous glands were associated with hairs except few places, viz glans penis, prepuce, labia vulvae, anus, external ear canal and tarsal gland. The sebaceous glands were simple alveolar holocrine glands and appeared as evaginations of the follicle, the glassy membrane of which was continuous with the basement membrane of the gland. The glandular body was filled with epithelial cells.

Whenever the hair was dense, the sebaceous glands were long and narrow. A ring of sebaceous glands also opened into the follicles of the tactile hairs. The largest sebaceous glands usually occurred area around mucocutaneous junctions.

The secretion of sebaceous glands, sebum, consisted of cellular debris and a lipoid mixture.

Montagna and Eugene (1958) mentioned in human that clusters of sebaceous glands were couched above the arrector pilorum muscles and entered the upper part of the follicle through a duct of varying length. More than one sebaceous gland grew from some follicles.

Otto Braun-Falco (1958) reported in human that the fragmented inner root sheath became strongly positive for sudan

stain because it was impregnated with sebum at the orifice of the sebaceous gland.

Kayashima (1959) showed in laboratory mammals including rabbit that sebaceous gland was present but sweat glands were lacking in the external part of ear canal. The largest number were found in the lower wall and smallest number in the upper wall.

Sar and Calhoun (1966) showed structure of sebaceous gland in goat that the sebaceous glands were simple or branched alveolar glands distributed throughout all body areas. With few exceptions, the glands were associated with hair follicles and opened into the follicular lumen through a duct below the opening of the sweat duct.

The glandular portion of the sebaceous acinus was composed of several layers of cells bound peripherally by a basement membrane.

Banks (1981) described in domestic animal that sebaceous gland in association with hair were envagination of the epithelial lining of the root canal in the form of simple, branched alveolar glands. Sebaceous gland deposited their excretory product into the follicle.

Calhoun and Stinson (1987) described sebaceous glands in domestic animal that sebaceous glands were simple, branched or

compound alveolar glands that release their secretory product, sebum, by the holocrine mode. They originated from the external root sheath of the hair follicle and invaded the dermis. They were most frequently associated with hair follicles into which their ducts empty to form the pilosebaceous canal of the hair follicle.

Rao (1992) observed in Impala lip that the sebaceous glands were simple, branched alveolar glands. The alveoli consisted of a basal layer of squamous, undifferentiated (germinal) cells resting on a basal lamina. The centrally located polyhedral cells contained lipid.

Dyce *et al.* (1996) reported that in sheep inguinal pouches, found near the base of udder or scrotum, contained sebaceous glands.

Gupta *et al.* (1996) described sebaceous gland of rabbit that sebaceous glands were large, pear-shaped and simple or branched alveolar glands, located in outer region of dermis. Each gland had a single duct opening into a hair follicle in hairy skin. Sebaceous glands were holocrine.

Cutaneous tubular gland

Yasuda *et al.* (1960) observed sweat gland in the sole of the guinea pig. Two cell types were described : dark coloured cell and some clear cells. They were generally in single layer. No coarse 'Secretory granules' were observed. The apical portion and the boundaries of the cells were PAS positive.

Jordan (1965) described that the sweat glands were not uniformly distributed. In man they were more numerous on palms, sole and armpits. In rats and mice they were confined to soles of feet. In rabbit they were around lips.

Banks (1981) described in domestic animals that sweat glands of the skin were simple, coiled, tubular structures. They were two general types : Merocrine and apocrine. The adenomeres consisted of a low cuboidal epithelium in the merocrine glands, whereas the epithelium lining of the adenomere of apocine glands was a low columnar type.

Calhoun and Stinson (1987) described sweat glands in domestic animals based on their morphologic and functional characteristic, sweat glands were separated into two types : apocrine and merocrine. The apocrine types was the most extensively developed. They were simple saccular or tubular glands with a coiled secretory portion and a straight duct.

The merocine glands were also coiled, simple tubular glands found mainly in the foot pad of dog and cat and the nasolabial of ruminants and pig. The secretory portion was composed of cuboidal epithelium with two distinct cell types. The dark or mucoid cell had more ribosome than the clear cells, and numerous droplets occurred in the apical part of the cell. Myoepithelial cells surrounded the

excretory units. The duct was relatively straight and opened directly onto the surface of the epidermis. It was composed of two layers of cuboidal epithelial cells resting on a basement membrane.

Rao (1992) observed sweat glands in Impala lip. That was of merocrine type. The adenomeres consisted of low cuboidal epithelium resting on a well defined basement membrane. Myoepithelial cells were noted around the adenomeres. The secretory units were drained by the loosely coiled, unbranched excretory duct which opened into an adjacent hair root canal. The duct was lined by a single layer of cuboidal cells.

Smallwood (1992) showed that rabbit had sweat glands that opened on its foot pads or sole. Like the rodents, the rabbit had many prominent vibrissae or tactile hairs associated with upper lip. Deep pouches between penis and each half of scrotum were the inguinal sinuses. The inguinal glands opened into each sinuses and some of the yellowish sebaceous type secretion of these glands were visible.

Dyce *et al.* (1996) reported that in sheep inguinal pouches found near the base of udder or scrotum contained sweat glands.


Materials &
Methods

MATERIALS AND METHODS

The present investigation was conducted on the skin of twelve mature rabbits, six male and six female, between 7-8 months of age. The animals were procured locally and were apparently healthy and free from diseases.

The skin samples were collected along either side of the body from 22 anatomically distinct areas viz. upper lip, lower lip, angle of mouth, face, cheek, ear tip (pinna), dorsal neck, lateral neck, ventral neck, dorsal thoracic, lateral thoracic, ventral thoracic, dorsal lumbar, lateral lumbar, ventral abdominal wall, lateral thigh, lateral forearm, dorsal tail (root), ventral tail, anal skin, scrotal skin and sole. Preputial skin in male and vulvar skin in female were not collected during present study. The tissue blocks of 3-5 mm thickness were secured from above mentioned regions of skin and were collected immediate after death of the animals by humane slaughter. They were then stored in the desired fixatives (Luna, 1968). The following fixatives were used during this study.

1. 10% Neutral buffered formalin.
2. Zenker's solution.
3. Bouin's solution.
4. Chilled alcohol.

After proper fixation, the tissues were then processed for various histological processes such as washing, dehydration, clearing, paraffin infiltration, embedding and sectioning (Humason, 1967 and Luna, 1968). Paraffin sections were cut at 5-7 μm thickness with the help of rotary microtome. For demonstration of lipids, frozen sections of neutral buffered formalin fixed tissues were cut at 10-15 μm. The sections were stained with different staining procedures as desired.

The following stains and staining methods were employed for histological studies :-

1. Haematoxylin and Eosin stain for routine studies (Luna, 1968).
2. Van Gieson's stain for collagen and muscle fibres.
3. Verhoeff's elastin stain for elastic fibres (Humason, 1967).
4. Gomori's retuculin stain for reticular fibres. (Humason, 1967).
5. Modified Mallory's trichrome stain for connective tissue fibres (Crossman, 1937).

The following stains and staining methods were used for certain histochemical observations :-

1. Periodic Acid Schiff stain. With or without saliva digestion for glycogen and mucosubstances (Pearse, 1968).
2. Oil red-O in propylene glycol method for fat (Luna, 1968).

3. Modification of Muller's colloidal iron stain for acid mucopolysaccharide (Luna, 1968).

4. Feulgen and Rossenbeck reaction for DNA (Davenport, 1969).

The stained sections were mounted with D.P.X. For demonstration of lipids, the stained sections were mounted with glycerine jelly.

Micrometry of epidermal thickness of following regions were conducted with the help of caliberated occular micrometer.

(1) Upper lip (2) Face (3) Lower lip

(4) Dorsal thoracic region (5) Ventral abdominal region

(6) Dorsal lumbar region and (7) Sole

The average population distribution of guard hair and secondary hair follicles were also derived as per sq. mm. area after standardizing the field area of the microscope at definite magnification.

The data so collected of male and female animals were calculated for different statistical interpretations (Snedecor and Cochran, 1967).


Results &
Discussion

RESULTS AND DISCUSSION

Like those of other mammals the skin of rabbit was comprised of outer epidermis and inner dermis. The dermis rested over the hypodermis which was made up of loose connective tissue containing numerous adipocytes. Similar reports were made in the skin of domestic animal by Banks (1981) and Calhoun and Stinson (1987).

EPIDERMIS

The epidermis of rabbit's skin was comprised of keratinized stratified squamous epithelium (Figs. – 1,6,18 and 19). In most of the area of the body the epidermis was made up of three distinct cell layers from inside out as stratum basale, stratum spinosum and stratum corneum. The stratum granulosum was present between the stratum corneum and stratum spinosum in some of the body areas and was thickest at the region of angle of mouth. However, this layer was not clearly defined in the epidermis of lateral neck region (Fig. –1). The stratum lucidum was altogether absent in the epidermis of rabbit's skin. Trautmann and Fiebiger (1957) and Calhoun and Stinson (1987) described at least four layers in the keratinized stratified squamous epithelium of epidermis namely stratum basale, stratum spinosum, stratum granulosum and stratum

corneum in case of different domestic animals. They further detailed the presence of stratum lucidum, interposed between stratum granulosum and stratum corneum of planum nasale and foot pad.

The stratum basale was made up of single layer of cuboidal to columnar cell resting over faintly PAS positive basement membrane. Several mitotic figures were observed in this cell layer. The peripheral stratum spinosum was composed of variable layers of oval or polyhedral cells closely attached with each other. The nuclei were usually oval. The cells of deeper part of this region occasionally presented mitotic figure. The mitotic figures observed in the cells of stratum basale and stratum spinosum suggested the continuity of cell proliferation. The cells of superficial layer of stratum spinosum were comparatively flattened in outline from those of the deeper layer.

The stratum granulosum, when recorded, varied in thickness at different regions of skin and was thickest in the angle of mouth region. The cells of the stratum granulosum appeared rhomboid or flattened containing highly basophilic keratohyaline granules. Due to the intense basophilic character of these granules the nuclei were not clearly visible in these cells.

The stratum corneum was made up of flattened keratinized cells arranged parallel to epidermal surface. The cells were non-nucleated and acidophilic in character (Figs. – 1, 6, 12, 14,

16, 17, 18 and 19). Brody (1959) described the formation of keratin in the epidermis of normal guinea pig skin from tonofilaments and keratohyaline granules partly through a gradual incorporation of tonofilaments into keratohyaline granules. Von Bargen (1959) reported that the epidermis of rabbit's skin contained stratum granulosum formed by flattened cells. He opined the probable participation of nucleus in the production of keratohyaline granules. In contrast to the present findings Breathnach and Goodwin (1965) observed some non-keratinizing "clear cells" in the basal layer of epidermis. The absence of stratum lucidum in those skin areas considered during present investigation might be correlated with the reports made by several authors in various domestic animal as the layer was present at the non-hairy skin and at the junction of hoof with the skin (Sar and Calhoun, 1966; Banks, 1981 and Calhoun and Stinson, 1987) Kumar *et al.* (1992) also recorded absence of stratum lucidum in the epithelium of commisures and alae of external nares which was lined with stratified squamous keratinized type. Patil *et al.* (1997) also reported the absence of stratum lucidum in all over the body regions in case of Mehsana buffalo.

The epidermal pegs were distinctly prominent in the region of upper and lower lip than those of other body parts and these pegs were flanked by dermal papillae. Occasionally few nerve

filaments appeared in the deeper part of the epidermal pegs from adjoining dermis (Fig.-6). These fine nerve terminals could be correlated with tactile activities of the epidermis as described by Calhoun and Stinson (1987).

The epidermal thickness as recorded from seven representative areas of the body revealed that the male rabbits had comparatively thicker epidermis than the female ones (Table-1). At the upper lip level the epidermis measured an average of 87.0 ± 5.3913 μm in male and 79.17 ± 7.8034 μm in female which however, did not show significant difference from each other. At the lower lip region it measured 77.17 ± 6.9685 μm in male and 51.17 ± 3.4777 μm in female showing highly significant difference between them ($P < 0.01$). At the face region the epidermis measured 86.5 ± 2.2912 μm in male and 39.0 ± 2.7202 μm in female which differed highly significantly from each other at 1 percent level. The epidermal thickness of dorsal thoracic region measured 41.17 ± 2.6130 μm in male and 37.67 ± 1.3824 μm in female which did not differed significantly from each other. In the ventral abdominal region epidermal thickness of male measured 38.50 ± 3.4713 μm and in female 36.17 ± 3.3804 μm without showing significant difference. At dorsal lumbar region the epidermal thickness measured 49.17 ± 4.5491 μm in male and 34.67 ± 2.0923 μm in female which differed

significantly from each other ($P < 0.05$). At the sole region the epidermal thickness measured 63.83 ± 3.7006 μm in male and 43.33 ± 1.3824 μm in female which similarly differed significantly at 5 percent level between two sexes as detailed alongwith c.v. percentage in Table-1. In the same sex even outer convex surface of pinna revealed thinner epidermis than the inner concave surface. The variation of the thickness of epidermis were thought to be associated with regional regulation of temperature dissipation as suggested by Shravan Kumar and Thiagrajan (1991) who reported highly significant thinner epidermis in the surti she-buffaloes as compared to other she-buffaloes and opined to have an advantage of simple diffusion of excess heat from body tissue due to thinner epidermis in that particular species.

Histochemically as detailed in Table-4 the epidermis was mildly reactive for PAS and negative for Colloidal iron reaction, alkaline phosphatase and intracellular lipid droplets. However, a thin layer of Oil red-O positive substances were observed over the stratum corneum in the areas rich in sebaceous gland (Figs.-29, 30, 31, 33, 34 and 35). With Feulgen reaction the nuclei of epidermal cells of stratum basale and stratum spinosum revealed mild to moderate positivity for DNA (Fig.-32). The Oil red-O positive materials spreaded over the stratum corneum of epidermis were probably due to

smearing of sebaceous secretion from underlying dermis. Wolf (1959) demonstrated on human epidermis a lipid film which was almost exclusively formed by sebum from sebaceous glands.

DERMIS

The dermis was the subepidermal connective tissue layer of skin resting over the underlying subcutis. It was indistinctly divided into two sublayers as superficial papillary layer and deep reticular layer (Figs.-2, 6, 14, 15, 16, 17 and 18). The superficial papillary layer was made up of loose connective tissue containing fine collagen fibres and a few reticular fibres. (Figs. – 4 and 5). The elastic fibres were not recorded. This superficial layer was predominated with fibroblasts with isolated distribution of macrophages and plasma cells. The fat cells were not observed in papillary layer. Since this layer was adjacent to stratum basale of epidermis, the peripheral extension of this sublayer formed dermal papillae between the epidermal pegs. The dermal papillae were comparatively wider and branched in the regions of upper lip, lower lip and angle of mouth as compared to other region of the body. The papillary layer was however least prominent in the dermis of tail and scrotal region (Figs.- 24 and 25).

The deeper reticular layer of dermis was predominantly made up of irregularly oriented coarse collagen fibres with few

occasional distribution of elastic fibres. The fibrocytes were randomly distributed between the connective tissue fibres. The coarse fibres of this layer usually formed fibrous sheath to accommodate compound hair follicles throughout body. Occasionally few skeletal muscle fibres entered in the deeper part of reticular layer from underlying subcutis. However the dermal reticular layer of skin of perioral area usually contained skeletal muscle fibres derived from orbicularis oris muscle (Figs. – 2, 4, 10 and 17).

In general the dermis was highly vascularized and invariably showed arteriovenous anastmoses. Nerve fibres and plexuses were widely distributed in the dermis particularly in the superficial papillary layer. The dermis contained hair follicles and sebaceous glands in general body areas. However the tubular sweat glands were restricted only at certain areas of body namely the sole and the region close to inguinal pouch. Modified tubular coiled glands were also found in the dermis of circumoral region. (Figs. – 6, 16, 17, 22 and 24).

The dermis of ear pinna appeared most narrow among different body regions except in case of male where dermis was narrowest in the skin of scrotum (Figs. – 19 and 20). The dermis of convex external side of pinna presented numerous thick walled blood vessels. Trautmann and Fiebiger (1957) described the superficial

layer of dermis as a feltwork of delicate reticular fibres with an admixture of fibroelastic elements. The remainder of the dermis consisted of bundles of collagen fibres. Durward and Rudall (1958) demonstrated that in rat and guinea pig, the upper level of dermis contained stouter vessels representing the portion of dermal plexus. Straile (1960) recorded a specialized region of dermis having a thick area of epidermal cell surrounding the orifice of hair follicle and designated it as Harrscheibe. In the present observation similar epidermal cell areas were observed in the region of papillary layer of dermis for the exit of hair shafts in continuity with the hair follicle. The distribution of different varieties of connective tissue fibres in the dermis of rabbit skin could be well compared with dermis of goat skin (Sar and Calhoun, 1966), dermis of different domestic animal (Banks, 1981 and Calhoun and Stinson, 1987), in water buffalo calves (Patil *et al.*, 1989), cattle (Saxena *et al.*, 1994) and in Mehsana buffalo (Patil *et al.*, 1997). The occasional presence of chromatophores and fat cells as described by Calhoun and Stinson (1987) could not be recorded in the dermis of rabbit's skin during present study. The occasional penetration of skeletal muscles in the dermis from underlying areas of the body might be responsible for voluntary movement of skin at that particular area as opined by Calhoun and Stinson (1987).

The predominant distribution of nerve fibres, nerve plexuses and encapsulated tactile corpuscles in the dermis, particularly in papillary layer could be correlated with the distribution of bulbous corpuscles in the inter follicular dermis and in the upper dermis of hairy skin in rabbit (Cunningham and Fitzgerald; 1972[a,b]).

Histochemically (Table – 4), the dermis showed negative to mild reaction for PAS positive substances and negative for lipid (Figs. – 29, 30 and 31). Occasionally mild reaction for acid mucopolysaccharide was recorded close to the hair follicles. The fibroblasts presented mild to moderate Feulgen positive nucleus suggesting the presence of DNA, (Fig.-32).

Hairs and hair follicles

Like other mammals the rabbit presented wide spread hair coat over the skin. The hairs were distributed throughout the body including that of foot pad region. The hair shafts, the part of the hair outside the skin surface, were of variable length and thickness. The deeper part of hair entrapped in the dermis constituted the root which remained distributed within the hair follicle. Histomorphologically the hair shaft appeared to be of mainly two varieties. The long hair shafts were comparatively coarser and were

termed as guard hairs. The fine hair shafts were termed as secondary hairs or under hairs (Figs. – 3, 27 and 30). The guard hair presented an outer smooth margined epithelial layer of cuticle followed by comparatively closely packed keratinized cell layer of cortex, In the centre of shaft the medulla was present which usually showed ladder-like arrangement of flattened cells. The ladder-like arrangement were occasionally present in one or two rows in the medulla of some of the guard hair, However at the tip region a single row of such arrangement was observed. The secondary hair shaft however showed an outer cuticular layer with comparatively thin cortex. The central medulla was occasionally lacking in secondary hair. The secondary hair containing central medulla invariably showed single row ladder-like arrangement. Trautmann and Fiebiger (1957) in domestic animals and Montagna and Eugene (1958) in human being also described three distinct layers of hair as outer cuticle, middle cortex and central medulla. Montagna and Eugene (1958) further reported medulla might be continuous, discontinuous or fragmental. The large intra and intercellular air spaces in medulla determined to a large extent sheen and colour tones of hairs by influencing the reflection of light. Mahajan (1998) also described wooly hairs and guard hairs in angora rabbit. He further reported that finer hair presented ladder shaped uniserial medulla whereas multiserial medulla in coarser hairs.

The distribution of hair cover at the sole of the rabbit did not agree with the report made by Sar and Calhoun (1966) who recorded absence of hair cover at the foot pads of different domestic animals.

The root of the hair was located in the dermis to constitute hair follicles. The hair follicles in rabbit appeared to be compound hair follicles containing guard hair and secondary hair follicles, surrounded by epithelial sheaths descended into the dermis from surface epidermis. Each compound hair follicle was surrounded by densely packed connective tissue sheath of dermal origin. At the deepest part of hair root the dermal papillae entered into the centre of enlarged hair bulb from the deeper end. The outer connective tissue sheath of hair follicle was made up of outer and inner layer of collagen fibre with few elastic fibre. The epithelial root sheath was comprised of inner epithelial and outer epithelial root sheaths. The inner epithelial root sheath appeared to be made up of keratinized cells whereas outer epithelial root sheath was comprised of stratified layers of polyhedral cells very much similar to superficial cells of stratum spinosum of epidermis (Figs. – 4, 6, 13, 15, 16, 17 and 23). The hair root as usual presented outer cuticular layer middle cortex and central medulla (Fig. – 13). Some of the secondary hair follicles did not reveal the presence of medulla. The aggregation of hair follicles in

clusters presented several primary hair follicles or guard hair follicles in association with secondary hair follicles. The average population of these two varieties per sq.mm. of dermis varied differently at different regions of skin as shown in Tables-2 and 3.

In the upper lip region average population of primary hair follicle appeared as 4.93 ± 0.395 per sq.mm. in male and 3.29 ±0.354 per sq.mm. in female. The difference between the average population of guard hair follicle in two sexes was highly significant ($P<0.01$). In the lower lip region the average population of primary follicles was recorded 3.42 ± 0.22 per sq.mm. in male and 2.37 ± 0.26 per sq.mm. in female. The intersexual difference was highly significant. In the face region the total numbers of primary follicle were 2.50 ± 0.22 per sq.mm. in male and 1.45 ± 0.22 per sq.mm. in female showing significant difference at 1% level. In dorsal thoracic region the average population of guard hair follicles recorded 1.91 ± 0.20 per sq.mm. in male and 1.38 ± 0.15 per sq.mm. in female. In the dorsal lumber region average population was 2.039±0.229 per sq.mm. in male and 2.039 ± 0.249 per sq.mm. in female. No significant difference was noted between the population of guard hair follicle in two sexes in dorsal thoracic and dorsal lumbar region of skin. At the ventral abdominal skin the population of primary hair follicle appeared as 1.84 ± 0.16 per sq.mm. in male and 1.25 ± 0.15 per

sq.mm. in female showing significant difference at 5% level. At the sole region the average population of these hair follicles recorded 1.907 ± 0.249 per sq.mm. in male and 1.512 ± 0.278 per sq.mm. in female showing no significant difference between two sexes.

The secondary hair follicle population also varied at the different regions of rabbit's skin (Table-3). In the upper lip region the average population of secondary hair follicle was 13.185 ± 0.5371 per sq.mm. in male and 9.539 ± 0.6222 per sq.mm. in female the intersexual difference was significant at 1% level. In the face region average population was 12.171 ± 0.4302 per sq.mm. in male and 10.855 ± 0.6449 per sq.mm. in female they did not differ significantly. At the lower lip region the population of secondary hair follicle remained 8.486 ± 0.6539 per sq.mm. in male and 8.815 ± 0.3563 per sq.mm. in female without showing significant difference between them. The dorsal thoracic region of the skin contained 21.315 ± 0.9262 secondary hair follicle per sq.mm. area in male whereas in female 13.486 ± 0.5904 per sq.mm. area they showed significant difference between them at 1% level. At ventral abdominal region the population recorded 17.763 ± 0.6651 per sq.mm. in male and 13.749 ± 0.6464 per sq.mm. in female showing sexual variation at 1% level statistically. The population of secondary hair follicle at dorsal lumbar region recorded 23.03 ± 0.8437 per sq.mm. in male and 22.96 ± 0.77

per sq.mm. in female showing no significant difference between them. In the sole region the population of secondary hair follicle remained 11.57 ± 0.9211 per sq.mm. in male and 9.868 ± 0.628 per sq.mm. in female without showing significant difference between two sexes.

The guard hair follicles were usually associated with sebaceous glands and arrector pilorum muscle. The secondary hair follicles usually lacked the association of sebaceous glands. The arrector pilorum muscle never appeared to be associated with secondary hair follicle. The presence of guard hair follicles in association with the secondary hair follicle in the compound hair follicle of the several animals have been described by Straile (1958) in rabbit, Calhoun and Stinson (1987) in domestic animals. The atypical guard hair follicles as reported by Straile (1958) could not be recorded during the present observation. The association of sebaceous glands alongwith arrector pilorum muscle with the guard hair follicle of the rabbit's skin was inagreement with the report made by Calhoun and Stinson (1987) in several domestic animals. In contrast to the higher distribution of hair follicle in female than male adult Mehsana buffalo as reported by Patil *et al.* (1997), the present observation in rabbit skin revealed higher distribution of both guard hair follicle as well as secondary hair follicles per unit area in male than female in general.

Sinus hair follicle

The upper and lower lip of rabbit's skin presented sinus hairs of tactile nature (Figs. - 7, 8 and 9). Each sinus hair consisted of a shaft, a root and a hair bulb. Hair shaft presented typical cuticle, cortex and medulla from periphery to centre. The hair roots of sinus hair appeared as enlarged single follicles containing annular sinus filled with blood. The annular sinus was formed by inner and outer layers of dermal sheath. The interconnecting trabeculae between these two layer of connective tissue sheath were made up of fibroelastic tissue. The number of these trabeculae gradually decreased as the sinus hair ascended towards the epidermal margin of the dermis. The sinus hair follicles were occasionally associated with sebaceous glands however no arrector pilorum muscle was recorded in association with bulb of the sinus hair. Occasionally few skeletal muscle fibres were observed to be associated with the outer root sheath of sinus hair follicle. Similar structures of sinus hair and sinus hair follicle have been described by Calhoun and Stinson (1987) in different domestic animals Rao (1992) in Impala, Smallwood (1992) in rabbit and Baba *et al.* (1994) in the eyelid of goat and sheep. Smallwood (1992) however described the presence of tactile hair with upper lip of rabbit only like those of rodents. The present study in contrast, recorded distribution of sinus hair both in upper and lower lip region.

Histochemically (Table – 4), the cortex of hair in the follicle as well as in the shaft showed presence of PAS positive mucopolysaccharides. The saliva digestion followed by PAS stain revealed diminished reaction showing the presence of glycogen in the cortex of hair follicles. The cortex in hair follicle also reacted moderately for colloidal iron stain suggesting the presence of acid mucopolysaccharides. (Figs. – 29, 30, 31, 35, 36 and 37). The cells of hair follicles also reacted moderately for Feulgen reaction denoting the presence of DNA in nuclei (Fig. – 32). The hair follicles did not revealed Oil red-O positivity for fat however occasional adhesion of Oil red-O positive material was recorded which might be due to adhesion of sebaceous secretion over the cuticular layer of hair at different regions (Fig. - 33). Pospisil and Kralove (1959) however reported a ring containing fat, PAS positive material and acid mucopolysaccharides in the lower third of hairs of rabbit at the end of internal epidermal sheath.

Sebaceous gland

The dermis of rabbits skin was richly distributed with sebaceous glands usually associated with hair follicles (Figs. – 2, 4, 6 and 25). They normally appeared as simple alveolar type of gland lined with glandular epithelial cells. The alveoli occasionally showed branchings at different regions. These sebaceous glands opened

exteriorly through a duct which constituted pilosebaceous canals just before the emergence of hair shaft below the level of epidermis. The cuboidal type of cells resting over the basement membrane normally showed mitotic figures. The centre of alveolus contained secretory product of denser variety. The histomorphology of the glandular alveoli was suggestive for holocrine activities of the gland therefore prevalence of mitotic figures of basal cell layer were noticed. The branched and multilobated sebaceous glands were also observed in the dermis of perianal region where the glandular duct opened directly over the skin surface without having association with hair follicle (Fig. – 11). In the scrotal skin also the sebaceous gland of a smaller variety, opened directly over the skin surface through a long duct (Fig. – 21). In the sole region the sebaceous gland were poorly developed. The present observations were in agreement with Trautmann and Fiebiger (1957), Montagna and Eugene (1958), Kayashima (1959), Sar and Calhoun (1966), Banks (1981), Rao (1992), Dyce (1996) and Gupta (1996). Sar and Calhoun (1966) however reported well developed sebaceous glands at circumanal region of different domestic animals. They further reported that low cuboidal cell resting over the basal lamina showed mitotic activities and opined that these cells moved inward to grow into enlarged polygonal cells to accumulate numerous lipid droplet.

Histochemically, the sebaceous glands were negative for PAS and Colloidal iron reaction, However they were mild to moderately reactive for Oil red-O, suggesting the presence of lipid (Figs. – 33 and 34). With Feulgen reaction the nuclei of the basal cells of the gland reacted mild to moderately for DNA. Towards the centre the reactivity was lacking. Otto Braun-Falco in (1958) reported sudan black-B positive layer over the inner root sheath due to impregnation by sebum at the orifice of sebaceous gland. Rao (1992) similarly recorded positive reaction for fat in centrally located polyhedral cells of sebaceous gland in Impala lip. Calhoun and Stinson (1987) also reported accumulation of lipid droplets in the enlarged polygonal cells of sebaceous gland.

Cutaneous tubular gland

During the present study the tubular cutaneous gland were lacking at different regional skin samples of rabbit except for in the skin of perioral areas including upper lip, lower lip and angle of the mouth, the sole and the skin of ventral abdominal region close to prepubic area. The cutaneous tubular glands at perioral areas were simple coiled tubular gland of merocine variety (Fig. -10). The lining epithelium appeared to be of cuboidal type surrounded by isolated distribution of myoepithelial cells. The ducts of these gland opened over the epidermal surface which were lined with stratified squamous

epithelium close to the epidermal cell layer. These glands were comparatively deeply placed in the dermis much below the level of sebaceous gland. Histochemically the cells of perioral glands were negative for Oil red-O and Colloidal iron reactions. However, few of the cells of glandular tubule reacted mildly for PAS at their apical margin. Jordan (1965) also reported presence of sweat gland around the lips of rabbit. Banks (1981) reported the adenomeres consisted of a low cuboidal epithelium in the merocrine gland whereas the epithelial lining of the adenomere of apocrine gland was a low columnar type. Calhoun and Stinson (1987) reported merocrine variety of cutaneous gland in the planum nasolabiale of ox and planum nasale of pig. Rao (1992) reported the presence of merocrine variety of sweat gland in Impala lip. He also reported the presence of myoepithelial cells around the adenomeres.

In the sole region small tubular glands were observed in the superficial part of reticular layer of dermis in the rabbit. The tubular lumen of these glands were much narrower and their glandular tubules were arranged in small bunches (Fig. - 28). The lining epithelium was usually made up of flattened or low cuboidal epithelial cells. The myoepithelial cells however could not be observed clearly. Yasuda *et al.* (1960) also observed sweat gland in sole of guinea pig. However, they observed two cell types in the single

layered epithelium of such gland. Jordan (1965) however reported that the tubular sweat glands were confined to the sole feet in cats, rats and mice. Calhoun and Stinson (1987) also reported merocrine types of cutaneous glands in the foot pads of dog and cat and frog of ungulates. They further opined that low level activity of sweat gland in foot pad might be indicating hydration of epidermis during friction. Smallwood (1992) also reported presence of sweat glands opening on the foot pads of rabbit. However, he did not classify the glands according to mode of secretion.

Apocrine variety of coiled tubosaccular glands were also recorded in the dermis of ventral abdominal region close to prepubic area. The glandular epithelium though not very distinctly observed histologically, was surrounded by myoepithelial cells (Fig-26). The apical surface of glandular epithelium presented non-uniform projection which were considered to be apical blebs of apocrine gland. These glands were histochemically negative for Oil red-O reaction. However, the basophilic secretory product attached with the luminal margin reacted mildly for PAS reaction. These secretory material attached to the luminal margin of epithelium also reacted mildly for Colloidal iron stain (Fig.–38). These glands were supposed to drain their secretion in the inguinal pouches in the rabbit of both sexes. Small wood (1992) reported presence of inguinal pouches in male

rabbit between penis and each half of the scrotum whereas in female on either side of the vulva. He further reported the opening of inguinal glands in each sinus. Dyce *et al.* (1996) also reported the presence of inguinal pouches near the base of udder in ewe and near scrotum of ram, containing both sebaceous and sweat gland. The secretory product of this apocrine variety of sweat gland in rabbit might be working as marker for the flock.

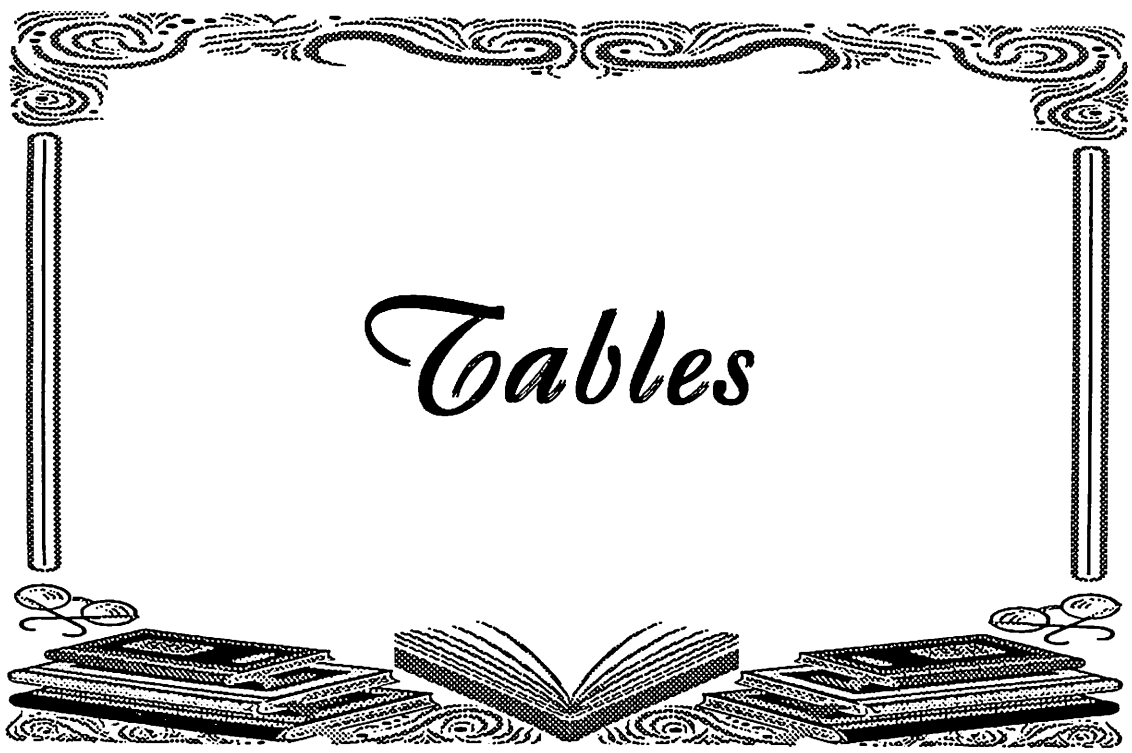

Tables

Table – 1. ***Mean ± S. E. along with C.V.% of thickness (μm) of epidermis at different regions of skin in rabbit.***

Regions	Mean ± S. E.	C.V.%
Upper lip	Male – 87.0^{a} ± 5.3913	15.1793
	Female – 79.17^{a} ± 7.8034	24.1447
Face	Male – 86.50^{a} ± 2.2912	6.4884
	Female – 39.0^{b} ± 2.7202	17.0854
Lower lip	Male – 77.17^{a} ± 6.9685	22.1202
	Female – 51.17^{b} ± 3.4777	16.6487
Dorsal thoracic region	Male – 41.17^{a} ± 2.6130	15.5478
	Female – 37.67^{a} ± 1.3824	8.990
Ventral abdominal region	Male – 38.50^{a} ± 3.4713	22.0855
	Female – 36.17^{a} ± 3.3804	22.8953
Dorsal lumbar region	Male – 49.17^{a} ± 4.5491	22.6637
	Female – 34.67^{b} ± 2.0923	14.7839
Sole	Male – 63.83^{a} ± 3.7006	14.2003
	Female – 43.33^{b} ± 1.3824	7.8144

Means with different superscripts (region wise) differed significantly ($P<0.05$).

Table – 2. ***Mean ± S. E. alongwith C.V.% of population of guard hair follicles/mm^2 in dermis at different body regions of skin in rabbit.***

Regions	Mean ± S. E.	C.V.%
Upper lip	Male – 4.93^a ± 0.395	25.33
	Female – 3.29^b ± 0.354	34.04
Face	Male – 2.50^a ± 0.22	27.20
	Female – 1.45^b ± 0.22	46.90
Lower lip	Male – 3.42^a ± 0.22	19.88
	Female – 2.37^b ± 0.26	35.02
Dorsal thoracic region	Male – 1.91^a ± 0.20	34.03
	Female – 1.38^a ± 0.15	34.78
Ventral abdominal region	Male – 1.84^a ± 0.16	28.26
	Female – 1.25^b ± 0.15	38.40
Dorsal lumbar region	Male – 2.039^a ± 0.229	35.50
	Female – 2.039^a ± 0.249	38.64
Sole	Male – 1.907^a ± 0.249	41.32
	Female – 1.512^a ± 0.278	58.20

Means with different superscripts (region wise) differed significantly ($P<0.05$).

Table – 3. ***Mean ± S. E. alongwith C.V.% of population of secondary hair follicles/mm^2 in dermis at different body regions of skin in rabbit.***

Regions	Mean ± S. E.	C.V.%
Upper lip	Male – 13.815^a ± 0.5371	12.29
	Female – 9.539^b ± 0.6222	20.63
Face	Male – 12.171^a ± 0.4302	11.18
	Female – 10.855^a ± 0.6449	18.79
Lower lip	Male – 8.486^a ± 0.6539	24.36
	Female – 8.815^a ± 0.3563	12.78
Dorsal thoracic region	Male – 21.315^a ± 0.9262	13.74
	Female – 13.486^b ± 0.5904	13.84
Ventral abdominal region	Male – 17.763^a ± 0.6651	11.84
	Female – 13.749^b ± 0.6464	14.86
Dorsal lumbar region	Male – 23.03^a ± 0.8437	11.59
	Female – 22.96^a ± 0.77	11.59
Sole	Male – 11.57^a ± 0.9211	25.15
	Female – 9.868^a ± 0.6280	20.13

Means with different superscripts (region wise) differed significantly ($P<0.05$).

Table – 4. ***Showing histochemical characters of different structures in Rabbit's skin.***

Structures	PAS	PAS with saliva digestion	Colloidal iron stain	Oil red- O
Epidermis	+	+	-	-
Dermis	±	±	±	-
Cortex of hair follicles	++	+	++	-
Sebaceous gland	-	-	-	+ to ++
Cutaneous tubular gland				
• Perioral gland	+	+	-	-
• Apocrine gland of inguinal area	+	+	+	-

+ Mild reaction ; ++ Moderate reaction ;

- Negative reaction ; ± Occasional.


Summary &
Conclusions

SUMMARY AND CONCLUSIONS

The present investigation was conducted on twelve healthy rabbits, six male and six female, for the histological and certain histochemical studies of the skin. They were between 7-8 months of age.

The skin of rabbit was comprised of outer epidermis and inner dermis. The dermis rested over the hypodermis which was made up of loose connective tissue containing numerous adipocytes.

EPIDERMIS

The epidermis was comprised of keratinized stratified squamous epithelium. The epidermis was usually made up of three distinct cell layers from inside out as stratum basale, stratum spinosum and stratum corneum. The stratum granulosum when present, was thickest at the region of angle of mouth. The stratum lucidum was altogether absent in the epidermis of rabbit's skin. The stratum basale was single layer of cuboidal to columnar cell resting over faintly PAS positive basement membrane. Several mitotic figures were observed in this cell layer. Stratum spinosum was composed of variable layers of oval or polyhedral cells. The cells of deeper part occasionally presented mitotic figures. The cells of stratum granulosum appeared rhomboid or flattened containing highly basophilic keratohyaline granules. The stratum corneum was made

up of flattened keratinized cells arranged parallel to epidermal surface. The cells were non-nucleated and acidophilic in character.

The epidermal pegs were distinctly prominent in the region of upper and lower lip than those of other body parts and these pegs were flanked by dermal papillae. Occasionally few nerve filaments appeared in the deeper part of the epidermal pegs from adjoining dermis.

In general the epidermal thickness in male rabbit appeared higher than the female ones.

Statistical analysis of epidermal thickness revealed significant difference ($P<0.05$) in the face, lower lip, dorsal lumbar and sole region between sexes. However there were no significant difference between sexes in upper lip, dorsal thoracic and ventral abdominal region of skin.

Histochemically the epidermis was mildly reactive for PAS and negative for colloidal iron reaction and intracellular lipid droplets. The cells of stratum basale and stratum spinosum revealed mild to moderate positivity for DNA in their nuclei.

DERMIS

The dermis was indistinctly divided into two sublayers as superficial papillary layer and deep reticular layer. The papillary layer was made up of loose connective tissue containing fine collagen and few reticular fibres. The elastic fibres were not recorded. This layer

was predominated with fibroblasts and isolated distribution of macrophages and plasma cells. The peripheral extension of this sublayer formed dermal papillae between epidermal pegs. The papillary layer was least prominent in the dermis of tail and scrotal region.

The deeper reticular layer of dermis was predominantly made up of irregularly oriented coarse collagen fibres with few occasional distribution of elastic fibres. The coarse fibres of this layer usually formed fibrous sheath to accommodate compound hair follicles throughout body. Dermal reticular layer of skin of perioral area usually contained skeletal muscle fibres derived from orbicularis oris muscle.

In general the dermis was highly vascularized and invariably showed arteriovenous anastmoses. Nerve fibres and plexuses were widely distributed. The dermis contained hair follicles and sebaceous glands in general body areas.

The dermis of pinna appeared to be most narrow except in case of male where dermis was narrowest in scrotum. The dermis of convex external side of pinna presented numerous thick walled blood vessels.

Histochemically, dermis showed negative to mild reaction for PAS and negative for lipid with occasionally mild reaction for acid mucopolysaccharide close to hair follicles. The fibroblasts presented mild to moderate Feulgen positive nucleus.

Hairs and hair follicle

Rabbit presented wide spread hair coat over the skin. The hairs were distributed throughout the body including foot pad region. The hair shafts were of variable length and thickness. The long hair shafts were comparatively coarser and were termed as guard hairs. The fine hair shafts were termed as secondary hairs. The guard hair presented an outer smooth margined epithelial layer of cuticle followed by comparatively closely packed keratinized cell layers of cortex. Medulla showed ladder-like arrangement of flattened cells. The ladder-like arrangement were occasionally in one or two rows in some of the guard hair. The secondary hair shaft showed an outer cuticular layer with thin cortex. The central medulla was occasionally lacking. The secondary hair, containing central medulla, invariably showed single row ladder-like arrangement.

The root of the hair was located in the dermis to constitute hair follicles. The hair follicle appeared compound hair follicles containing guard hair and secondary hair follicles, surrounded by epithelial sheaths. The epithelial root sheath was comprised of inner epithelial and outer epithelial root sheaths.

Rabbit skin revealed higher distribution of both guard hair follicles as well as secondary hair follicles per unit area in male than female in general.

Statistically, the population of guard hair follicles/mm^2 in region of upper lip, lower lip, face and ventral abdominal region were significantly different between sexes. In dorsal thoracic, dorsal lumbar and sole regions differences were nonsignificant between male and female rabbit ($p<0.05$).

The distribution of secondary hair follicles/mm^2 in upper lip, dorsal thoracic and ventral abdominal regions were significantly different between two sexes. But in face, lower lip, dorsal lumbar and sole region revealed nonsignificant difference ($P<0.05$).

The guard hair follicle were usually associated with sebaceous glands and arrector pilorum muscle. The secondary hair follicles usually lacked the association of sebaceous gland. The arrector pilorum muscle never appeared to be associated with secondary hair follicle.

The upper and lower lip presented sinus hairs. The hair roots of sinus hair appeared enlarged single follicle containing annular sinus filled with blood. The annular sinus was formed by inner and outer layers of dermal sheath.

Histochemically the cortex of hair in the follicle as well as shaft showed PAS positive mucopolysaccharide. The saliva digestion followed by PAS stain revealed diminshed reaction showing the presence of glycogen in the cortex of hair follicles. The cortex in hair follicle also reacted moderately for colloidal iron stain. The nuclei of

the cells of hair follicle also reacted moderately for Feulgen reaction. The hair follicle did not revealed Oil red-O positivity for fat.

Sebaceous gland

The dermis was richly distributed sebaceous glands usually associated with hair follicles. They normally appeared as simple alveolar type of gland lined with glandular epithelial cells. These glands opened exteriorly through a duct which constituted pilosebacous canal. The centre of alveolus contained secretory product of denser variety. The histomorphology of the glandular alveoli was suggestive for holocrine activities. The branched and multilobated sebaceous glands were also observed in the dermis of perianal region where the glandular duct opened directly over the skin surface without having association with hair follicle. In the scrotal skin sebaceous gland of smaller variety opened directly over the skin surface through a long duct.

Histochemically, the sebaceous glands were negative for PAS and colloidal iron reaction. However they were mild to moderately reactive for Oil red-O.

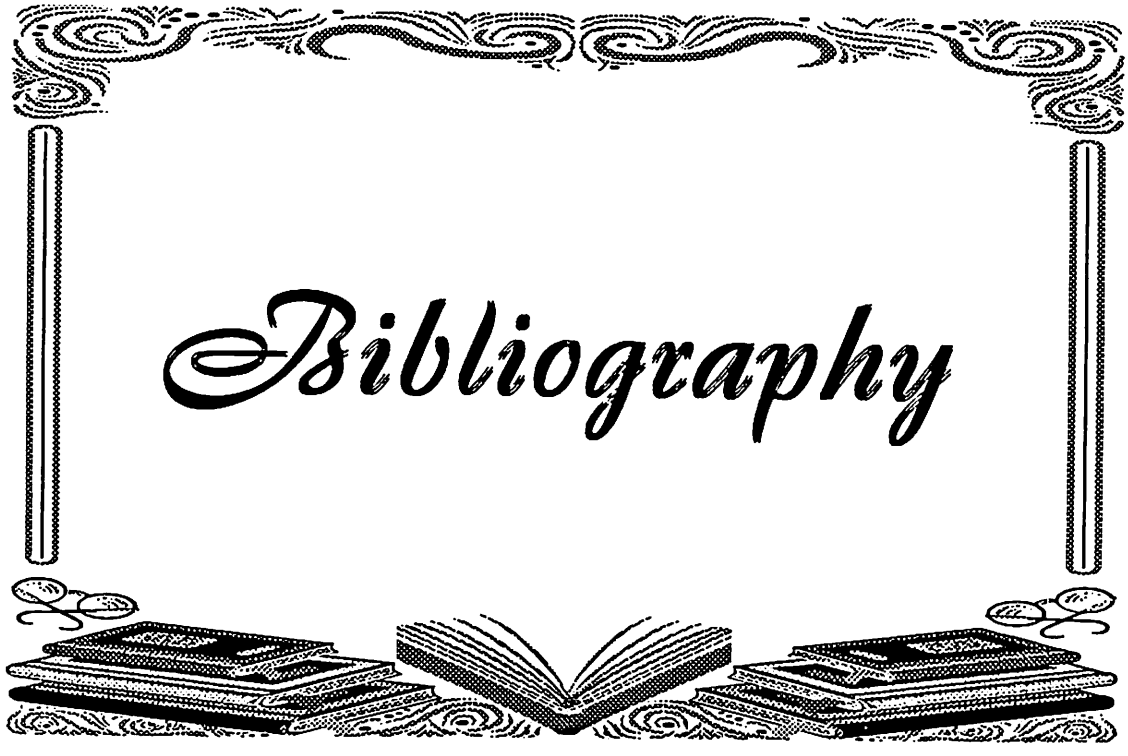
Cutaneous tubular gland

During the present study the tubular cutaneous gland were lacking at different region except for in the skin of perioral areas, the sole and ventral abdominal region close to prepubic area.

These glands at perioral areas were simple coiled tubular gland of merocrine variety. These glands were comparatively deeply placed in dermis.

In the sole region, small merocrine tubular glands were observed in the superficial part of reticular layer of dermis. The tubular lumen of these glands were much narrower and their glandular tubules were arranged in small bunches. The lining epithelium was usually made up of flattened or low cuboidal epithelial cells. Apocrine variety of coiled tubosaccular glands were also recorded in the dermis of ventral abdominal region close to prepubic area.

Histochemically, the cells of perioral glands were negative for Oil red-O and colloidal iron reactions. However, few of the cells of glandular tubule reacted mildly for PAS at their apical margin. The cells of apocrine glands found in the dermis of the skin close to prepubic area reacted mildly for PAS as well as colloidal iron reactions at their apical margins, however they did not react for fat when stained with Oil red-O.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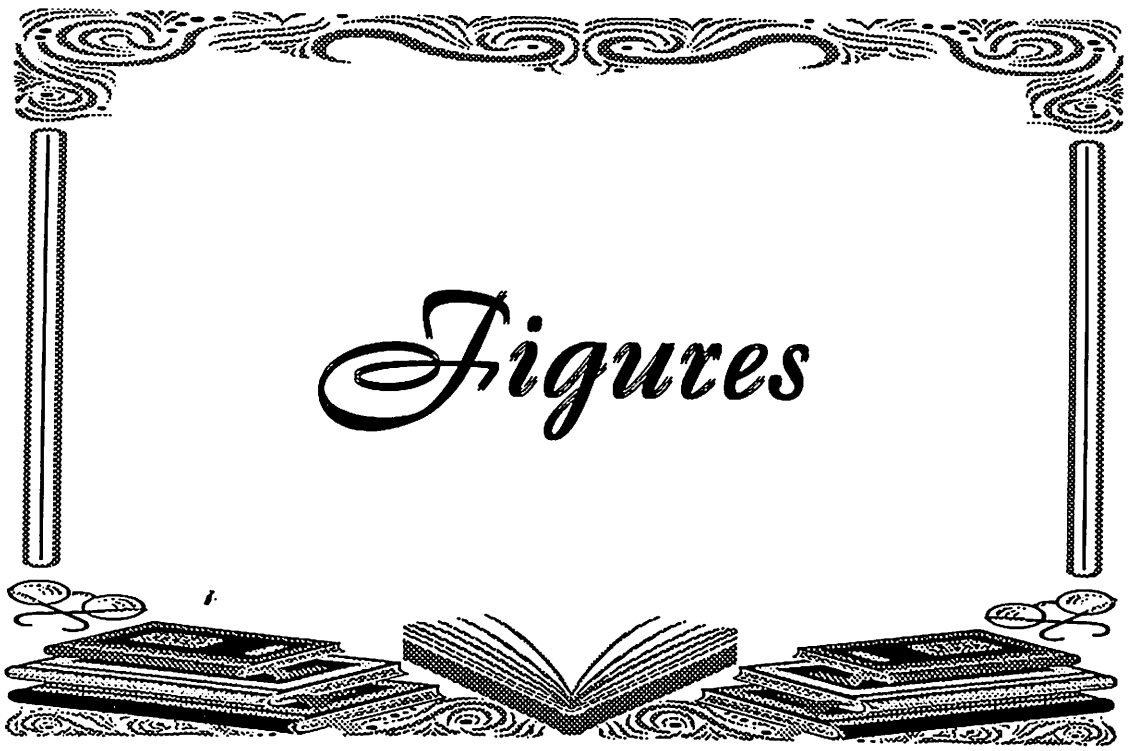
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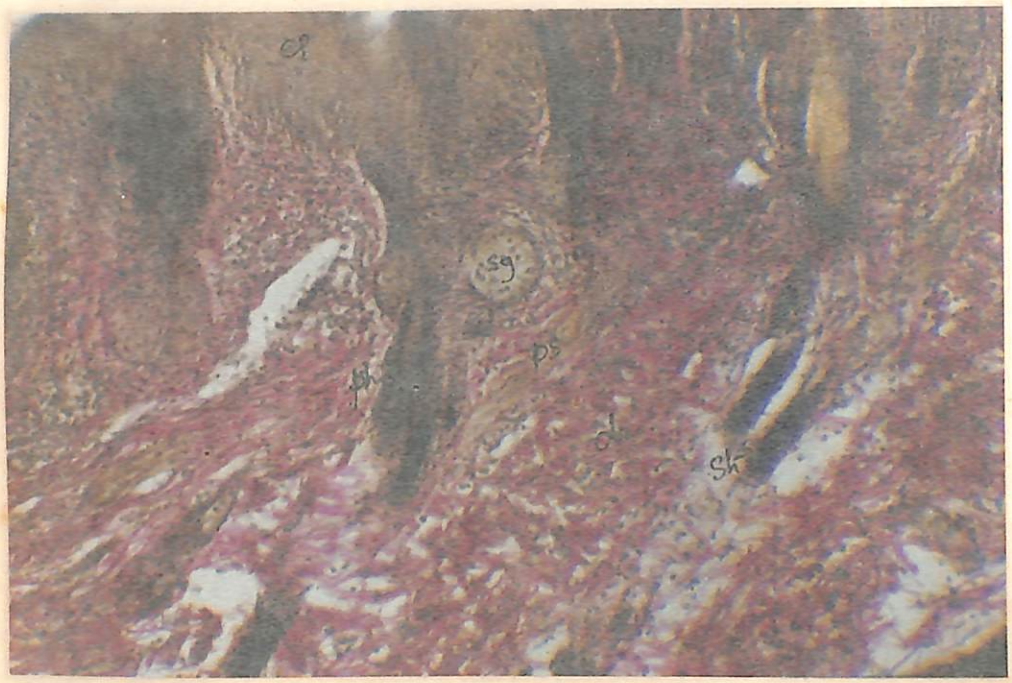
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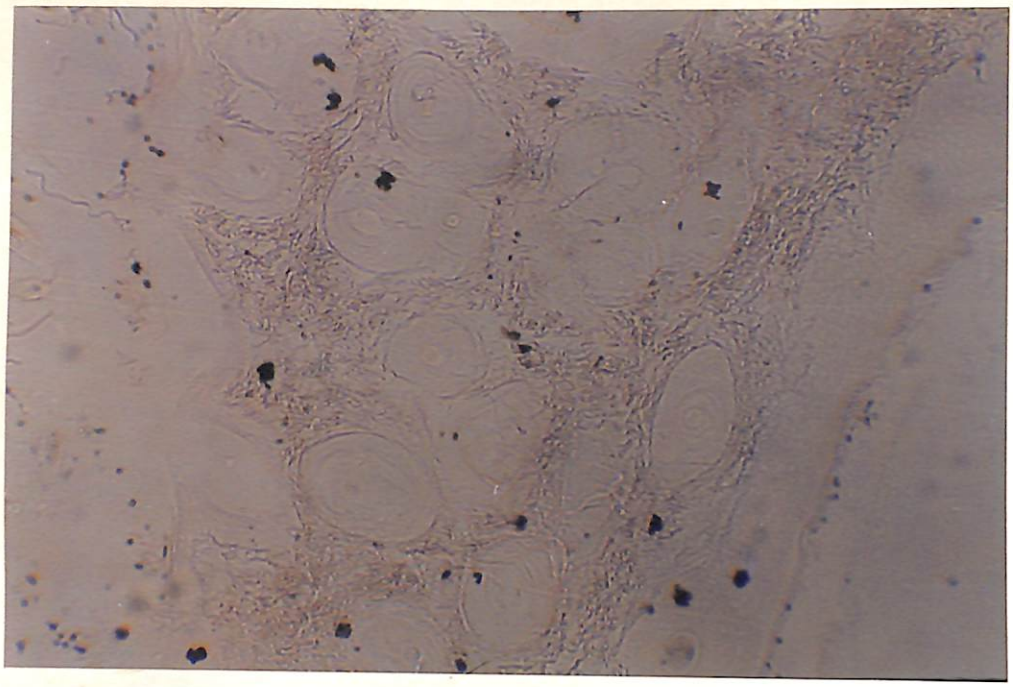

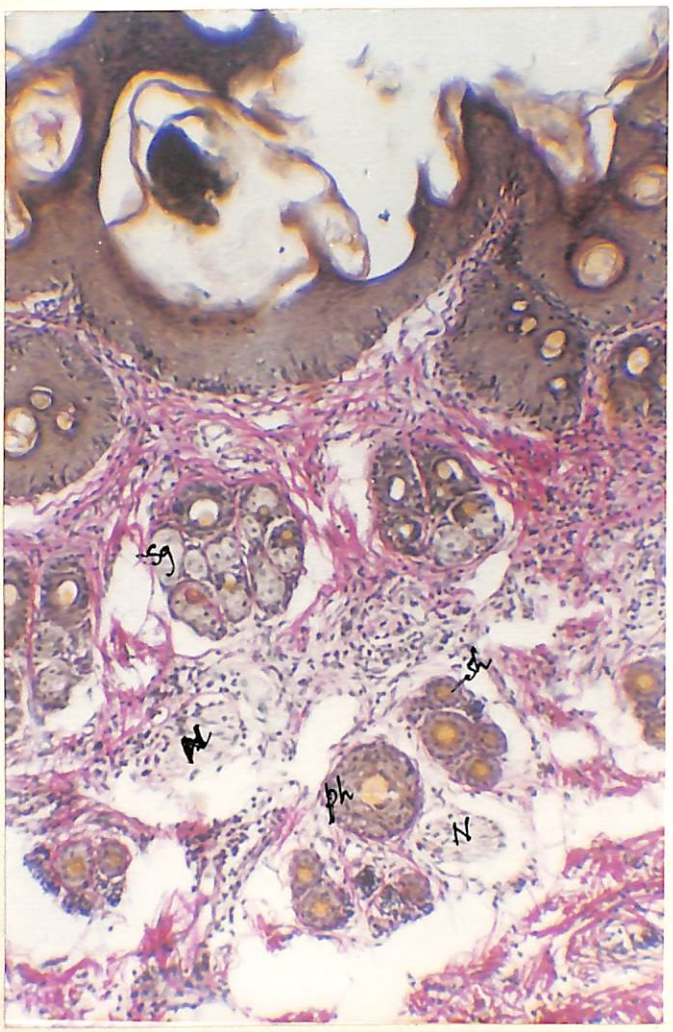
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