MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE SKIN OF THE PIG (*Sus domesticus*)

SUMENA. K. B.

Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2006

Department of Veterinary Anatomy and Histology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled **"MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE SKIN OF THE PIG (*Sus domesticus*)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Sumena 22-09-06

Mannuthy
22.09.2006

SUMENA. K. B.

CERTIFICATE

Certified that this thesis, entitled **"MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE SKIN OF THE PIG (*Sus domesticus*)"** is a record of research work done independently by **Sumena. K.B.,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Mannuthy
22.09.2006

22/9/06
Dr. K.M. Lucy
(Chairperson, Advisory Committee)
Assistant Professor (SS),
Department of Veterinary Anatomy & Histology,
College of Veterinary and Animal Sciences,
Mannuthy.

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sumena. K.B,** a candidate for the degree of **Master of Veterinary Science** in **Veterinary Anatomy**, agree that this thesis entitled **"MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE SKIN OF THE PIG (*Sus domesticus*)"** may be submitted by Sumena. K.B. in partial fulfillment of the requirement for the degree.

22/9/06

Dr. K.M. Lucy
(Chairperson, Advisory Committee)
Assistant Professor (SS),
Department of Veterinary Anatomy and Histology,
College of Veterinary and Animal Sciences,
Mannuthy.

22/9/06

Dr. Jose John Chungath
Associate Professor & Head
Department of Veterinary Anatomy
and Histology, College of Veterinary &
Animal Sciences, Mannuthy (Member)

22.09.06

Dr. N. Ashok
Associate Professor
Department of Veterinary
Anatomy and Histology,
College of Veterinary &
Animal Sciences, Pookot
(Member)

22/9/2006

Dr. P. Kuttinarayanan
Associate Professor & Head,
Department of Livestock Products
Technology, College of Veterinary &
Animal Sciences, Mannuthy (Member)

22/9/06

Smt. K.S. Sujatha
Assistant Professor (SG) & Head
Department of Statistics, College
of Veterinary & Animal Sciences,
Mannuthy
(Member)

10/10/06

External Examiner

Dedicated to....
*the eternal **LOVE** of my Parents, Sisters and Brothers*
*the meticulous **Guidance** of my Teachers*
*the divine **Grace** of Paramekkavu Bhagavathi*

ACKNOWLEDGEMENT

I find myself on lookout for words as I place on record my sincere and heartfelt gratitude to the chairperson of the Advisory Committee Dr. K.M. Lucy, Assistant Professor (SS), Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy, for her valuable guidance, personal attention, keen interest, affectionate encouragement, persuasion and unstinted help offered to me for the ship shaping of this manuscript.

With the same spirit of gratitude I would like to express my heartfelt thanks to Dr. K.R. Harshan, Professor and Head, Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Pookot, the former chairman of the Advisory Committee. I really reckon it as a rare privilege to work under his counsel and indomitable spirit.

I deem it as my privilege in expressing my deep sense of gratitude to Dr. Jose John Chungath, Associate Professor and Head, Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy, and member of the Advisory Committee for the generous encouragement, inspiration and personal guidance in the pursuit of this research work.

My cordial and immense thanks to Dr. N. Ashok, Associate Professor, Department of Veterinary Anatomy and Histology, Pookot, member of Advisory Committee, for his professional guidance at times of difficulty, constructive suggestions, the kindness and care throughout the study.

I wish to record my sincere gratitude to Dr. P. Kuttinarayanan, Associate Professor and Head, Department of Livestock Products Technology, member of the Advisory Committee for his informative suggestions and generous help rendered to me throughout the research work.

I am extremely thankful to Smt. K.S. Sujatha, Assistant Professor (SG) and Head, Department of Statistics, member of the Advisory Committee, for her valuable help and keen interest shown at every stage of this research work.

I am grateful to Dr. C. K. Sreedharan Unni, Associate Professor, Department of Veterinary Anatomy and Histology for his generous encouragement and help rendered in all possible ways throughout the course of my study.

I express my sincere thanks to Dr. S. Maya, Assistant Professor (SS), Department of Veterinary Anatomy and Histology for her encouraging advice, timely help and moral support without which this work might have not been completed.

I sincerely owe my thanks to my respected teachers Dr. G. Krishnan Nair, Dr. K.A. Mercy, Dr. George. T. Oommen, Dr. V. Jayaprakasan, Dr. A.D. Mercy, Dr. C.R. Lalithakunjamma, Dr. M.R. Rajan, Dr. M. Mini, Dr. K. Karthiayini and Dr. A. Kannan, for the timely help rendered by them during my research work.

I express my extreme sense of gratitude to Dr. E. Nanu, Dean, College of Veterinary and Animal sciences, Mannuthy, for providing the necessary facilities to carry out this work.

I avail this opportunity to extend my heartfelt thanks to Dr. S. Rajathi, my friend and senior, for the timely help and advise rendered to me throughout the study.

Words and deeds would really be insufficient to owe my deep sense of gratitude to my dear friends who helped me in all possible ways amidst their busy time. I am really grateful to Dr. Shambhunath Choudhary, Dr. Ranjini Chandran, Dr. Shamughasundharam, Dr. Vivek A.K., Dr. Ranjith Ramnathan, Dr. Kishor, Dr. Aneesh. A., Dr. Rajagopal, Dr. Ajmal, Dr. Naseera, Dr. Paulson and Dr. Rana Raj. I am thankful to Dr. Murughan and Dr. Sekhar for their timely help.

With deep sense of love and gratitude I recall the constant encouragement, moral support, love and affection enjoyed from my dearest friends Jasmine Rani, Manjusha. A., Raseena Karim, Preethymol Joseph, Seena, Kavitha and Jenifer. The love and care by Deepa, Lekha, Jaibi, Rani, Bini, Raji, Aswathy, Ananthalekshmi, Rakhi Chechi, Sulekha and Rani Alex during the difficult times of my work helped me to sail smoothly to the safe shore.

My sincere thanks to Varsha, Sandhya and P.D. Antony, the non-teaching staff of the Department of Veterinary Anatomy and Histology for their help and care. I take this opportunity to thank all the Labourers of Meat Plant Unit, Mannuthy, who helped me during the sample collection.

A special thanks to Mr. A.T. Francis, the Librarian, C & B College, Vellanikkara for his sincere help. I thank Mr. Eldo, Teaching Assistant, Department of Statistics, Ms. Reji, Research Assistant, Central Instrumentation Laboratory and Ms. Annie Thomas, Technical Assistant, ARIS Cell for the sincere help rendered to me. My heartfelt appreciation goes to the Staffs of Yescom for their effort in formatting the manuscript.

With immense pleasure, I remember the love and prayers showered on me by my parents, my brothers, sisters and their children. I thank Kittumon for his help in all possible ways.

Above all, I bow before the Divine love and Blessings that helped me in this endeavour and throughout my life.

Sumena 22/09/06

SUMENA. K.B.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Skin is the largest and the heaviest organ in the body. It separates the controlled internal environment of the body from the potentially hostile external environment. The skin completely encloses the body and blends with the mucous membrane at various natural openings. It is the barrier as well as the principal organ of communication between the animal and its environment. It reflects the well-being or disorder of the organism. Skin is a turbulent tissue and it grows, differentiates and renews itself continuously. Skin is versatile and performs various functions. It produces several end products such as sweat, sebum, vitamin D, pheromones and keratin. It plays an important role in the temperature regulation of the body. Skin protects the body from loss of water and electrolytes; prevents entry of pathogens and absorption of toxic and harmful substances from external environment. A large number of sensory receptors located in the lower part of the skin make it the largest sensory organ in the body and receive four basic stimuli viz., touch, pain, temperature and pressure. The subcutaneous fat in the lower layers of the skin acts as energy store and thermal insulating layer.

Important researches on the skin are going on in the fields of pathology, medicine, surgery and cosmetic industry. Thus it makes necessary to find a suitable experimental model to be used in research studies on human skin. It is important to realise the remarkable similarities and significant differences existing between the skin of pigs and human beings. Skin of the pig grossly resembles that of man, particularly after the removal of bristles. Moreover, pig has a sparse cover of hair similar to man. According to Montagna and Lobitz (1964), the skin of pig has a remarkable number of focal specialisations, the most significant being the snout. The reported differences in the skin of pig include the presence of a unique interfollicular muscle layer, presence of apocrine sweat glands, considerably less vascularity and a thicker stratum corneum (Monteiro-Riviere and Stromberg, 1985).

The high prolificacy, short generation interval, fast growth rate and other biological advantages contribute to the selection of pig as a biological experimental model in the field of research. Pigs are considered to be supreme amongst the meat-

The high prolificacy, short generation interval, fast growth rate and other biological advantages contribute to the selection of pig as a biological experimental model in the field of research. Pigs are considered to be supreme amongst the meat-producing animals and are efficient converters of various unconventional feed stuffs to valuable animal proteins. The pig industry as a meat producing enterprise is gaining momentum at a rapid rate in India in recent years. This contributes to the easy availability of the skin of pig for its various utilities. Usually skin is used for food, cosmetic ingredients and medicinal prosthetics such as skin grafts and sutures. Porcine skin is utilised as the major source of gelatin for various snacks and sausages. It is thinner than cattle hides and is the second most common leather making raw material world wide. Largest producer of porcine skin is the People's Republic of China (Gracey *et al.*, 1999).

The skin of bacon pig is used for making excellent leather, which is suitable for saddles, handbags, gloves and appliqué works. Tanning of porcine skin is confined to Germany and Scotland where skinning of bacon pig is carried out prior to the preparation of Ayrshire roll bacon (Thornton and Gracey, 1974).

Appearance and structure of the skin alters in response to a multiple number of factors. In conditions of jaundice, glandular deficiencies and impaired circulatory and respiratory functions, the colour of skin varies. In vitamin A deficiency, the skin becomes rough with alopacia. Cutaneous diseases form a major threat in veterinary practice. Pigs are susceptible to a variety of skin lesions.

The proliferative and regenerative abilities of the epidermal cells of the skin suggest a key role of the keratinocyte stem cells and progenitor cells in the tissue maintenance, repair and renewal. Buldge cells are the stem cells recognised as molecular markers with CD34 expression and K15 promoter activity from the hair follicle. Understanding the signals directing the movement and differentiation of buldge cells into different lineages is important for developing treatments based on stem cells as well as clarifying their role in skin diseases (Costarelis, 2006). Because dermatologic, cutaneous, pharmacologic and toxicological studies utilise

the skin from swine, a thorough knowledge of its structure is very important. This study is contributory to the existing anatomical knowledge and will form a basis for further physiological, pathological and biochemical studies. Hence, the present work was undertaken with the following objectives:

1. To study the morphology, morphometry and the distribution of hair on the surface of the skin of pig.
2. To study the histology of the skin at various regions such as snout, dorsal nasal region, dorsal neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen and carpal regions.
3. To compare the sex differences if any, in the skin of pig.

2. REVIEW OF LITERATURE

2.1 THICKNESS OF THE SKIN

Structure of the skin in pigs is basically similar to that of other domestic animals. The cutaneous surface was creased by delicate intersecting sulci, which when shaved resembled the skin of man. The skin was thickest on the glabrous surface of the lips, on the snout and between the toes (Montagna and Lobitz, 1964).

According to Smith and Calhoun (1964), the porcine skin was thickest over the dorsal surface of the body and on the lateral surface of the limbs and gradually became thin towards the ventral side of the body and medial surface of the limbs. In regions with heavy protective coat of hair, epidermis was thin and in non-hairy muco-cutaneous junctions, it was thicker. Skin of the snout appeared to be thicker in sow than that of the boar. Total thickness was reported to vary from 0.50 mm at vulva to 3.50 mm on the rostral surface of the snout.

Thickness of the skin in improved breeds of pigs was 1 to 2 mm. In adult boar, the corium measured about 3.50 to 4.00 mm. Fat accumulated in the subcutis and formed a distinct and extremely thick panniculus adiposus over greater part of the body (Ellenport, 1975). The skin was remarkably loose on the dorsal aspect of the neck and trunk, where it could be raised in extensive folds.

A conspicuous thickened area on the neck, shoulders and lateral aspects of the chest, the 'shield', was reported to be an integumental peculiarity to sexually mature boars and seemed to have an influence of male sex hormone. In older animals this was extended caudally to the lower chest. It was composed of high amount of tough connective tissue with thin subcutis. The cutis was thicker in the improved German Landrace than that of English pigs (Schummer *et al.*, 1981).

In adult lion, the thickness of the skin was maximum in the leg region and minimum at the axillary region with an average thickness of 3690.50 ± 55.98 μm (Bhayani *et al.*, 1995). In lion cub, total thickness was maximum in the lower abdomen and minimum in the thoracic region.

Bhayani *et al.* (2001) found significant breed differences in the thickness of the skin of sheep and recorded thicker skin in Marwari than Patanwadi breed.

The skin thickness in Deoni cattle was found to be in the range of 3 to 4 mm. According to Mugale and Bhosle (2001), skin thickness was more in male Deoni cattle (3857.04 ± 0.95 μm) than in the female (3655.95 ± 0.79 μm).

Comparison between ultrasonographic and histologic appearance of skin revealed that layering of canine skin and the subcutaneous tissue could be recognised and measured by using high frequency ultrasonography (Diana *et al.*, 2004).

2.2 EPIDERMIS

The epidermis is a nonvascular stratified squamous epithelium that covers the entire outer surface of the body. The properties of the epidermis showed remarkable topographic differences in the mammals (Montagna, 1962).

2.2.1 Morphology

Even though the epidermis was structurally similar in most mammals, its morphological details varied in different species. Lee and Nielsen (1962) noticed that the average thickness of epidermis in camel was 0.76 mm and epidermal pegging was absent in hairy areas. Montagna (1962) reported that in the mouse and hamster, the epidermis was very thin when compared to that of the rat and guinea pig.

In the human embryo, the epidermis was two-layered until three months, each layer being one cell thick. The outer layer, the periderm, constituted a protective covering, and the lower germinative layer was proliferative. Fowler and Calhoun (1964) observed that the epidermis was two to five cells thick in 30 to 35 days-old foetal pig.

Montagna and Yun (1964) compared the skin of human and swine and found many dissimilarities appearing mainly in the dermis and appendageal structures. In the epidermis, the thickness and understructure of the ridges were similar; however the swine had a thickened and compact stratum corneum with a positive alkaline phosphatase reaction in the epidermis.

Smith and Calhoun (1964) reported that the epidermis of the pig was thickest at the snout and the interdigital areas. Marcarian and Calhoun (1966) noticed that in sow, thickness of the epidermis of the snout measured up to 900.00 μm. The interdigital skin possessed a thick stratum corneum characterised by sharp epidermal projections. Eyelid, axilla and dorsal areas of the abdomen and thorax possessed a thin cornified layer.

The epidermis varied from 0.07 to 0.12 mm in thickness over most of the human body, but it reached a thickness of 0.80 mm on the palm and 1.40 mm on the sole (Bloom and Fawcett, 1975). Morris and Hopewell (1990) reported that in pig, the thickness was between 30.00 μm and 100.00 μm and that was within a range similar to that of man. The mean thickness of the epidermis in the adult lion was 37.39 ± 1.80 μm with a maximum at the neck region (Bhayani *et al.*, 1995).

In the buffalo, Hafez *et al.* (1955) observed that the epithelial pegs were 115.00μm deep and considered them as papillomatous epidermis. Sharma *et al.* (1996) reported that the epithelial pegs were simple and shallower in the yak, when compared to the buffalo.

The epidermis was divisible into distinct layers, the number of which varied with regions of the body. Primarily, it was divisible into two layers, viz., a superficial, harder, drier and dead stratum corneum and a deeper softer and living part, the stratum malpighii that rested upon the dermis. The stratum malpighii was subdivided into the deep stratum basale, stratum spinosum, stratum granulosum and stratum lucidum (Trautmann and Fiebiger, 1957; Montagna, 1962; Ham, 1969; Banks, 1993).

2.2.2 Histology

2.2.2.1 Stratum Basale or Stratum Cylindricum

Montagna (1962) reported that stratum basale consisted of a single layer of columnar or cuboidal cells resting on the basal lamina. In early embryonic stage, they were cuboidal and possessed a clearly stainable large nucleus and a smooth basal surface, which allowed an easy separation from the dermis. Later, the basal cells became columnar and the lower surface of the cells became serrated as cytoplasmic processes grew into the layers of the dermis.

According to Smith and Calhoun (1964), pigment granules were scattered throughout the epidermis and concentrated around the cells of stratum cylindricum in the coloured breeds of pigs and were absent in the epidermis of Duroc-Yorkshire crossbreds. Clear cells were observed in the stratum basale (Smith and Calhoun, 1964 and Marcarian and Calhoun, 1966) in the pig and the yak (Sharma *et al.*, 1996). They contained clear cytoplasm and a basophilic nucleus. The stratum basale of the pig was considerably undulated and the rete ridges or papillae were seen in many areas (Morris and Hopewell, 1990).

Urmacher (1990) demonstrated four distinct regions for the basal lamina of vertebrate skin as follows: (a) the plasma membrane of the basal cells containing the hemidesmosomes and anchoring filaments; (b) the lamina lucida, an electron-lucent area composed of laminin and bullous pemphigoid antigen; (c) the lamina

densa, electron-dense area composed of type IV collagen and (d) the sublamina densa containing the anchoring fibrils that originated in the lamina densa and extended into the dermis. Melanocytes, the dentritic cells derived from the neural crest, had an elongated nucleus surrounded by a clear space. Langerhan's cells, the dentritic cells derived from the bone marrow were also seen in the upper part of the squamous layer (stratum spinosum). Merkel cells, the non-dentritic cells also occurred in the epidermis, which possessed scanty cytoplasm, invaginated nuclei, parallel array of cytokeratin filaments in the paranuclear zone and membrane-bound dense core-granules. These cells were highly concentrated in glabrous skin of digits, lips and the oral cavity.

Sharma *et al.* (1996) demonstrated a thick collagenous basement membrane in the skin of the yak. Moreover, they opined that many clear cells were seen along or below the basement membrane, where either the migratory lymphocytes or the chromatophores,.

2.2.2.2 Stratum Spinosum or Prickle Cell Layer

Stratum spinosum consisted of several layers of irregular polyhedral cells. The thickness of this layer varied according to the different anatomical sites; it was thicker in the glabrous skin. This layer filled in all the depressions between the dermal papillae. The suprabasal keratinocytes were polyhedral, somewhat basophilic and contained a round nucleus. The more superficial cells were larger, flattened, eosinophilic and oriented parallel to the surface (Trautmann and Fiebiger, 1957; Urmacher, 1990; Banks, 1993; Monteiro-Riviere, 1998).

Marcarian and Calhoun (1966) opined that the cells of the stratum spinosum were larger than the basal cells and showed definite intercellular bridges, which were prominent in the snout and interdigital skin of the pig. Morris and Hopewell (1990) found that the stratum spinosum at the interpapillary region was about four cells thick in pig. Monteiro-Riviere (1998) and Sadler (2004) reported that desmosomes connected the cells of the stratum spinosum with each other and to the

cells of the stratum basale. Tonofilaments were also prominent in this layer. According to them, the large intercellular space usually seen in this layer was the result of shrinkage artifact, which occurred while preparing the samples for light microscopic study.

The stratum basale and stratum spinosum comprised the stratum germinativum. Banks (1993) noticed that the thickness of stratum germinativum varied in different regions of the mammalian integument. It was usually thick in the hairless areas of the body and thin in heavily haired region.

2.2.2.3 Stratum Granulosum

Marcarian and Calhoun (1996) noted that stratum granulosum consisted of several layers of flat polygonal cells containing basophilic granules. Thickness of this layer was more in the regions of thicker skin and less or even absent in the areas of thin skin. Many of the cells had fragmented nuclei, and the cells varied from basal columnar or cuboidal to superficial squamous or polygonal type.

Stratum granulosum and stratum lucidum were not observed in the epidermis of the yak (Sharma *et al.*, 1996)

Ultrastructural studies of the integument in domestic animals by Monteiro-Riviere (1998) revealed the presence of lamellated bodies namely, the Odland bodies, which were smaller than mitochondria and occurred near the Golgi complex and smooth endoplasmic reticulum. These granules increased in number and size towards the surface of the epidermis. Stratum granulosum also contained irregularly shaped, non-membrane bounded, electron-dense keratohyalin granules. These granules contained a structural protein, the profilaggrin and a precursor of filaggrin those were thought to play a role in keratinization and barrier function. Banks (1993) reported that the granules of stratum granulosum were of variable occurrence and absent in regions of excessive hair coat.

2.2.2.4 Stratum Lucidum

Stratum lucidum was not apparent in the study conducted by Smith and Calhoun (1964) in the pig. Kozlowski and Calhoun (1969) opined that the presence of stratum lucidum in the skin of sheep was limited to the planum nasale, lip and margin of the hoof.

Banks (1993) noticed that stratum lucidum of domestic animals consisted of several layers of homogenous, transluscent, squamous cells with lighter staining affinity. The keratohyalin granules were not visible. The cytoplasm contained protein bound phospholipids and eleidin, a protein that was similar to keratin but had a different staining pattern (Monteiro-Riviere, 1998). Stratum lucidum was found only in specific areas of exceptionally thick skin and in hairless regions including planum nasale and footpads of carnivores. The study conducted by Mandage *et al.* (2003) in the Deccani sheep suggested the absence of stratum lucidum in epidermis.

2.2.2.5 Stratum Corneum

The superficial stratum corneum consisted of many layers of anucleated, squamous and cornified (keratinized) cells. Amakiri (1973) stated that the thickness of stratum corneum in the Nigerian breed of cattle was 13.00 μm. The average thickness of this layer in Surti buffaloes was 45.50 μm (Bhagi, 1974; Bhagi and Vyas, 1983).

Lloyd and Grathwaite (1982) reported that the stratum corneum measured 13.30 μm in adult crossbred dog. According to Bhayani *et al.* (1995), this layer was thicker in adult lion compared to lion cub.

Scanning Electron Microscopy of stratum corneum in the skin of one-horned Rhinoceros revealed hexagonal or polygonal cells at the neck fold, shoulder shield and abdomen. Cells of other regions were irregular in shape (Bhattacharya *et al.*,

1998). The highly ordered arrangement of cells in stratum corneum minimised the transepidermal water loss. Hair, nail and stratum corneum had a highly ordered, lamellar crystalline lipid structure. The structure of water clusters revealed that mainly bound water was present in the stratum corneum of the human skin and nail (Gniadecka *et al.*, 1998).

A thin stratum corneum was observed from 73 days onwards in buffalo foetuses (Panchal *et al.*, 1999). The thickness of this layer increased along with the appearance of the dermal papilla. It was thinnest at birth and reached a maximum thickness at one year of age, in sheep. The thickness was more in Marwari sheep than in Patanwadi breed at all age groups. But it was found to be same in both breeds at one year of age (Bhayani *et al.* 2001).

Mehta (2002) reported that Murrah buffalo had a thicker stratum corneum, higher concentration of melanin pigment and sparse hair than that of ox. The average thickness of stratum corneum in Murrah buffalo and ox were 48.30 μm and 8.50 μm, respectively.

The seasonal changes in the physical properties of the stratum corneum from summer to winter were accompanied by significant decrease in the levels of lactate, potassium, sodium and chloride in this layer. Nakagawa *et al.* (2004) demonstrated the importance of lactate and potassium in maintaining the physical properties of the stratum corneum in healthy animals.

2.3 DERMIS

The epidermis and cutaneous appendages grow upon the dermis and take nourishment from it. The dermis was made up of a superficial papillary layer or papillary body and a deep reticular layer. A well developed papillary body was most characteristic to man. In many mammals the distinction between the papillary and reticular layer was not clear. In the papillary body, fine collagen bundles were densely interwoven. The surface of this layer bore cone shaped papillae, which

contained loops of capillaries. Papillae were small and poorly differentiated in the hairy portions of the skin (Trautmann and Fiebiger, 1957; Montagna, 1962; Ham, 1969). Trautmann and Fiebiger (1957) reported the presence of bifurcated papillae in the external genitalia and planum nasale of the domestic animals. Compound papillae were found in the footpads of dog. Large slender papillae were seen at various hairless areas of the body. According to Montagna (1962), many papillae of man contained Meissner's corpuscles. Stratum reticulare was the deepest and thickest layer of the corium. In the reticular layer, the fibre bundles interwove mainly in the horizontal plane (Ham, 1969).

Lee and Nielsen (1962) opined that the camel possessed a relatively thick dermis with an average thickness of 2.95mm. The dermal papillae were absent in hairy areas, but were prominent in non-hairy regions, such as lip and anal areas.

Fowler and Calhoun (1964) noticed that by 95th day, the foetal pig developed all the components of the dermis and elastic fibres could be seen throughout the stratum reticulare in the ventral part of the body and the limbs.

Montagna and Yun (1964) reported that the skin of pig had a thick papillary body and a rich population of elastic fibres. Contrary to this, Smith and Calhoun (1964) opined that elastic tissue occasionally associated with a dense collagenous sheet in panniculus adiposus and was not apparent in the stratum papillare. The dermal ridges were extremely long. Sausage shaped end organs and free nerve endings were seen associated with epidermal ridges. Unlike human skin, the elastic fibre content of the porcine skin was relatively low, but was still higher than other mammalian species studied.

Smith and Calhoun (1964) stated that in pigs, the collagen fibres were thin and closely arranged in the stratum papillare but were thicker and more loosely arranged in the stratum reticulare. The thickness of the dermis varied in different regions and it was more at the snout in both male and female pigs. Dermal papillae were higher in the areas of thicker skin. Rete pegs were seen in the dermal papillae.

Marcarian and Calhoun (1966) noticed that the papillary and reticular layers blended without a distinct demarcation in the case of pigs. Sharp delimitation was noticed between the reticular layer and subcutaneous layer of panniculus adiposus. Numerous eosinophils were observed in the papillary layer of female pigs.

In sheep, Kozlowski and Calhoun (1969) observed that the papillary layer was composed of a network of elastic fibres that continued with the skeletal muscle fibres of the dermis and ended as flaring points of attachment to the collagenous fibres. Elastic fibres formed fine branches in the connective tissue sheaths of wool follicles and sebaceous glands. The fibres were less numerous in deeper parts of the dermis and extended as fine filaments into the subcutaneous tissue. Compound dermal papillae were present in sheep.

Meyer *et al.* (1982) suggested that the ability of the skin to resist the mechanical stress of tension and pressure resulted from the arrangement and tensile strength of collagen fibres. A massive three-dimensional network of collagen fibre bundles dominated in the dermis of pig. Urmacher (1990) reported that the human dermis was composed of high amount of type I collagen and small amount of type II collagen.

Saxena *et al.* (1994) had the opinion that elastin was denser in young calves that decreased with age, whereas reticulin and melanin showed a reverse trend.

Sharma *et al.* (1996) found that the dermis of paralumbar skin of yak was so thick and accounted for more than 90 percent. Different stages of fibroblasts, lymphocytes, mast cells and a few chromatophores and macrophages were also demonstrated. Vardaxis *et al.* (1997) using Confocal Laser Scanning Microscopy demonstrated that elastic fibres of porcine dermis followed the direction of collagen fibres and also occurred as focal aggregates at various depths in the dermis.

Monteiro-Riviere (1998) opined that the predominant cell types of the dermis in domestic animals were fibroblasts, mast cells and macrophages. Plasma cells, chromatophores, fat cells and extravasated leucocytes were often found. The dermis was traversed by blood vessels, lymph vessels and nerves. In the skin of horse, a third layer was present beneath the reticular layer of the dermis. In the lateral neck region, this additional layer consisted of dense parallel collagen fibre bundles, whereas in the gluteal and sacral regions, the collagen fibres were interwoven with elastic and reticular fibres.

2.4 HAIR

2.4.1 Morphology

Hairs form a coat for the skin, which act as a bad conductor of heat between the body and the atmosphere. Hence it plays an important role in the regulation of the body temperature. Lee and Nielsen (1962) found that the hairs on the skin of camel were spaced evenly and singly at lip, external nares and lower eyelid; in all other areas, they were grouped into distinct clusters, projecting from irregular depressed area or groove and were separated from adjacent clusters by elevated ridges. From each hair cluster two to three larger cover hairs emerged singly and were surrounded by two to five groups of wool hairs. Each of these groups consisted of two to nine hairs emerging from a common orifice. A delicate connective tissue sheath surrounded the entire cluster. The wool-hairs were in the compound follicles in most areas of the body.

In pig foetuses, Fowler and Calhoun (1964) noticed prominent hair follicles at the jowl and snout. By 50 to 53 days, sinus hairs could be located on the snout, eyelid and jowl. The regular hairs developed later and occurred in groups of three to five follicles, with a well-developed central hair.

In the case of pig, hairs occurred in groups of three and might vary from single hair follicle to groups of four to six hair follicles (Smith and Calhoun, 1964). In typical grouping of three follicles, the central hair was always the largest and there was only one hair per follicle regardless of the size of the hair. The hair density was greater in females than in males. The thinnest skin usually had greater hair density. Each hair was arranged approximately at an angle of 30 degree with the surface of the skin. Prominent tactile hairs were seen only in the lateral snout region. Montagna and Yun (1964) opined that pigs possessed longer and moderate to sparse hair coat on the back than on the belly. The hairs were shorter and more crowded together in those areas, where expansion of skin surface was minimal.

According to Montagna and Yun (1964), the hair follicle originated in groups, became separated while the body surface of the animal expanded and there was no real grouping. Occasionally they were encountered in groups of two or three. Grouping was predictable only where the hair grew close together (Montagna and Yun, 1964; Marcarian and Calhoun, 1966).

Ham (1969) and Sadler (2004) observed that hair follicle development occurred early in the third month of foetal life in human beings from the epidermis into the underlying dermis. It took place first in the region of eyebrows, chin and upper lip, then throughout the skin. By fifth or sixth month, the foetus became covered with delicate hairs – the lanugo of foetus. This was later replaced by the vellus.

In the case of sheep, the hair follicle group contained three primary follicles and 15 to16 secondary follicles. Tactile or sinus hairs were present in the muzzle and eyelid region (Kozlowski and Calhoun, 1969).

In pigs, Schummer *et al.* (1981) reported that the primary hair inserted into the skin at a more acute angle than the secondary hairs. The hair groups were arranged in rows transverse to the long axis of the body. Typical triple grouping was present in young piglets while in older animals two groups were equally

common. The density and type of hair were related to the extend of domestication. The wild pig had a fairly dense hair covering. Except at the eyelid and rims of ears, all natural orifices bore short hairs. The upper eyelid had two to three rows of eyelashes and none on the lower eyelid. The snout disc bore non-medullated dwarf sinus hairs, two to four millimeters in length, implanted at regular intervals. The outer hairs were in the form of stiff, fairly long bristles of varying thickness and their tip was frayed into several strands. These became progressively stiffer and more brittle with increasing age. They were thickest and more densely placed on the nape, back and lateral surfaces of the body and limbs. On the ventral thorax, belly and inner surface of the extremities, bristles occurred sparsely and were softer ones. Between bristles, more delicate and softer wool hairs occurred and were seen in appreciable numbers on the head and limbs. These were more densely packed in young animals in cold weather.

According to Saxena *et al.* (1994), the hair follicular density was greater in calves below six months of age. The greater hair follicular density during winter was due to the shrinkage of skin. The number of hair follicles was not found to increase in proportion to the skin expansion with age.

Monteiro-Riviere (1998) reported that in domestic animals, hair covered the entire body with the exception of footpads, hooves, glans penis, mucocutaneous junctions and teats of some species.

2.4.2 Histology

The hair comprised of two portions, viz., the visible dead hair shaft above the skin and an actively growing root zone inside the skin. The hidden base of the hair is surrounded by its root sheath with a complex structure.

David (1932) compared histology of the skin of the normal, heterozygous and homozygous Mexican hairless swine (*Sus scrofa).* Hair follicles of swine showed a typical outer root sheath only in the upper permanent portion. However, the lower portion showed a mesenchyme like outer sheath.

According to Straile (1960), the tylotrich follicle, a tactile organelle occurred widely in mammals including man with varying degrees of specialisation. It differed from other pelage follicles in that it possessed the annulus composed of connective tissue, capillaries and nerve endings; a band of smooth muscle seen inside border of the annulus; the Haarscheibe, and a thick epidermis surrounding the orifice of the follicle.

Hairs are dead structures, composed of keratinized cells that are compactly cemented together. They grew out of tubes of epidermis, the hair follicles that were located in the dermis. Hair follicles together with the sebaceous glands that grew from their sides formed a pilosebaceous system. The small mass of cells at the base of hair follicles, the matrix, produced the hair. Follicles periodically did not produce the hair; the major portion of the bulb would disappear and remaining cells in the follicle would enter a period of quiescence. After some period, the dormant follicle would burst into activity again and a new follicle would develop. Growing hair follicle were said to be anagen, quiescent ones the telogen and the period of transition between the two was the catagen (Montagna, 1962; Ham, 1969; Urmacher, 1990; Sadler, 2004).

Fowler and Calhoun (1964) reported the presence of multiple medullated hairs in the skin of the pig. Groups of four to six follicles occurred in swine, each group being separated by dense collagen fibres. The sinus hairs were readily distinguished from the regular hairs by the thicker connective tissue sheath.

According to Montagna and Yun (1964), the hairs of pigs composed entirely of the cortex with a rare indication of a medulla. The cuticle cells were very small and adhered close to the shaft. In pigmented hairs, melanin was found only in the

cortex and the cuticle cells were non-pigmented. The bulb of growing follicles rested either in the upper portion of the hypodermal fat, or entirely within the lower part of reticular layer of the dermis, seldom extending to its lower portion. Hair follicles were peculiar in having a thin outer root sheath and thick inner root sheath. No bulge for attachment of muscles could be seen.

The study made by Smith and Calhoun (1964) in pigs revealed that follicular folds in the inner epithelial root sheath were situated both inferior and superior to the sebaceous gland opening. The skeletal muscle fibres were found in the snout near the tactile hairs, some of these attached to the follicle wall and others formed horizontal bands enclosing the follicle. Two types of tactile hairs were reported, one type with annular sinus and other without it.

Follicular folds consisted of seven to twenty folds in the inner epithelial root sheath and these projected into the lumen of the hair follicle below the opening of sebaceous glands in the case of cattle. In pigs, one to twenty-three corrugations were located immediately superior and inferior to sebaceous gland opening (Marcarian and Calhoun, 1966). Localised thickening of epithelial root sheath was observed at the point of attachment of arrector pilorum. Follicular folds were reported to be specialised follicular structures of domestic animals and the cells were oval to flat elongated ones with pyknotic nuclei. The cells within the corrugations were smaller than those of stratum cylindricum and were irregularly placed.

Urmacher (1990) reported the presence of hair disc or Haarcheibe, a touch receptor in close vicinity of hairs and the epidermis, in the skin of vertebrates. Above this area possessed a large number of Merkel cells in the basal layer.

Bhayani *et al.* (1995) noticed that the hair follicles of the lion were situated deep into the reticular, but not extended into the subcutaneous tissue and possessed compound hair follicles.

The hair follicle was reported to be a specialised immune compartment of the skin that served as an intermediate reservoir of Langerhans cells between bone marrow and epidermis and played a critical role in immune surveillance (Gilliam *et al.*, 1998).

As its base, the hair was shaped like a bell, the hair bulb, surrounding a small dermal papilla. The cells next to the papilla represented the germinative zone of the hair and its surrounding tissue. This tissue consisted of five different concentric layers. The first three innermost layers were united to form the inner root sheath. Directly in contact with the cuticle of hair was the cuticle of inner root sheath, followed by next two layers – the Huxley's layer and the Henle's layer. Outer most layer was the outer root sheath that surrounded the other layers, hair in the lower part of hair follicle and the hair channel. Wagner and Bailey (2006) demonstrated the ultrastructure of hair root-epidermis of bovine by scanning electron microscopy and found that each layer consisted of cells that differed from the neighbouring cell layer.

2.5 ARRECTORES PILORUM

Trautmann and Fiebiger (1957) opined that in pigs, the arrectores pilorum of ordinary hairs were thick and that of bristles were thin. Fowler and Calhoun (1964) demonstrated that the arrectores pilorum completely encircled the hair follicle like a sling. These muscles attached halfway down the follicle to its connective tissue sheath. A single arrectores pilorum extended to two of the follicles in a group. Sometimes two arrectores pilorum muscles attached to a single follicle (Marcarian and Calhoun, 1966).

Kozlowski and Calhoun (1969) reported that in sheep the skeletal muscle fibers were conspicuous in the areas of muzzle, forehead, eyelid and perianal regions. These were originated from deeper structures and attached to connective tissue capsule of tactile hair. Large arrectores pilorum occurred in perianal, dorsal cervical and thoracic regions.

Stromberg *et al.* (1981) and Monteiro-Riviere and Stromberg (1985) noticed that the arrectores pilorum muscles were well developed and often forked in vertebrates. Each follicle was supplied with at least one arrectores pilorum in addition to interfollicular muscle. Arrectores pilorum of mammalian skin was described as a small bundle of smooth muscle fibres attaching to the dermal root sheath of the hair follicles just below the sebaceous gland and extending obliquely towards the skin surface, where it attached to the collagenous fibre network in the reticular layer of the dermis. Their function is reported to be the erection of the hairs that emerged from the follicle and emptying of the sebaceous glands.

2.6 INTERFOLLICULAR MUSCLE

Langley (1904) described the highly complex arrangement of the smooth interfollicular muscles responsible for ruffling the feathers of the birds. Striated interfollicular muscles extending from the base of one follicle to the top of an adjacent one have been described by Vincent (1913) for the sinus hairs of the rat and by Yohro (1977) for the shrew. Interfollicular muscles were seen on the side of the mature follicle opposite to the attachment of the arrectores pilorum muscle, on the side opposite to the occurrence of sweat duct and sebaceous gland. These muscles connected each hair follicle of its characteristic hair group. It was found about midway between the level of sebaceous gland and apocrine sweat gland. The broadest portion of each interfollicular muscle was attached primarily to the two outer follicles of the aligned triad (Stromberg *et al.*, 1981).

Among mammals, interfollicular muscles were identified only in swine. The fine structure of interfollicular muscle resembled that of the arrector pili and to the smooth muscles found in other parts of the body. But the nuclei appeared elongated and surrounded by a clear zone lacking myofilaments. The nuclei of interfollicular muscle were fusiform and the sarcoplasm was filled with myofilaments. Nerve endings with numerous mitochondria were frequently seen within the interfollicular muscle (Monteiro-Riviere and Stromberg, 1985). Presence

of smooth muscle fibres in the dermis of specialised areas such as the scrotum, teat and penis is also reported by Monteiro-Riviere (1998) in the pig.

2.7 SWEAT GLANDS

Sweat glands were found in association with each hair follicle. David (1932) opined that the reduction in the number of sweat glands was directly proportional to the reduction in the number of follicles. Sweat glands were generally located around the base of the follicle, while the duct ran parallel with the follicle, on its side forming an obtuse angle with the surface of the skin and opened near the opening of the hair canal. In hairless swine, the end of the follicle and sweat gland was considerably distant from one another.

Horse, sheep, pig and cat had glomiform secretory tubule; while in ox, goat and dog, it was serpentine type. Tubular sweat glands were largest at the margins of the haired skin, in the cutaneous sinuses in the case of sheep and pig and around the teats of sow. Dogs normally did not produce liquid sweat, horse produced albuminous sweat that lathered easily, while the ox, sheep and goat produced fatty secretion with characteristic odour (Trautmann and Fiebiger, 1957).

Sweat glands of camel were simple, coiled tubular and were found to be associated with the larger cover hairs and not with the small wool hairs. The duct was narrow and lined by a two-layered cuboidal epithelium. The secretory portion was lined by a low columnar epithelium with numerous myoepithelial cells, which lay inner to the basement membrane. The secretory granules seen in the apices as the bleb like protrusion indicated apocrine mode of secretion (Lee and Nielsen, 1962).

Montagna and Yun (1964) reported that the sweat glands in the snout, lip and carpal organ were aggregations of serous glands and were located deep in the subdermal fat. They were attached to the underside of epidermis by stout, funnel

like dilatations and passed through the epidermis in a straight path. These glands had highly branched ducts and secretory portion. Secretory part was lined with a cuboidal or columnar epithelium and had an outer layer of giant myoepithelial cells. A distinct population of clear and dark cells was also reported. Dark cells had oval dense nucleus and were full of periodic acid Schiff (PAS) positive, non-glycogen granules. Glycogen was absent in secretory cells. The clear cells had a spheroidal and lightly stained nucleus. No apocrine glands were reported on the glabrous surfaces of the lips and on the snout. The size varied everywhere on the body surface. Secretory coil was lined by cuboidal or columnar secretory epithelium with blebs projecting into the lumen.

Smith and Calhoun (1964) observed that the sweat glands of pig were coiled tubular in all areas except the snout, where it was compound tubular type. The lumen of sweat glands was larger and was lined by simple cuboidal cells in Duroc-Yorkshire crossbreds, while in Duroc-Hampshire crossbreds, it was smaller and the wall of gland appeared stratified.

Marcarian and Calhoun (1966) opined that the sweat glands of Yorkshire pigs were functional. Largest sweat glands were found in the axilla and anal region, while the smallest ones in the eyelid and external ear.

In sheep, apocrine sweat glands occurred in all hairy skin areas and dominated in the interdigital and inguinal areas. Large coiled apocrine gland was located in the skin of scrotum, perianal region, prepuce and lateral metatarsal region (Kozlowski and Calhoun, 1969).

Ellenport (1975) observed that the sweat glands were yellowish or brownish in colour in pigs and were in many places visible to naked eye. At the medial palmar side of carpus, were found the carpal glands in a small diverticulum. Large glands were present in the skin of digits and interdigital spaces. Compound tubular glands were present on the skin of snout. Large sebaceous and sweat glands were found at the entrance of prepucial diverticulum.

According to Schummer *et al.* (1981), the density of sweat glands was dependent on the age. Newborn piglets had maximum density, which got reduced as age advanced. Their mode of secretion was said to be apocrine in summer and merocrine in winter.

The sweat gland duct generally approached and penetrated the follicle in a direction almost parallel to it and measured about 493±46μm in cow, 615±30μm in sheep and 517±31μm in goat (Montogmery *et al.*, 1982a). Equine sweat gland duct measured an average of 563±64μm in length (Montgomery *et al.*, 1982b). In the outer portion of hair follicle, the luminal surface was keratinized but the presence of microvilli with closely associated vesicles deeper with in this layer suggested the possibility of reabsorptive role.

Urmacher (1990) noticed that the eccrine sweat gland of man possessed spiral intraepidermal portion, the acrosyringium that opened on to the surface.

According to Bhayani *et al.* (1995), sweat glands were longer and saccular in lion. The average length and diameter of sweat gland were 297.20±15.19μm and 100.13±4.44μm, respectively in adult lion and 189.58±11.39μm and 64.56±3.15μm, respectively in the cub.

In yak, the glands were coiled tubular, branched and rarely sac-like with a diameter of 90 to100μm. They were poorly developed and non-functional and lined with either low cuboidal or squamous type of epithelium. Some of the glands possessed an eosinophilic secretion in lumen with apocrine mode of secretion (Sharma *et al.*, 1996).

Mugale and Bhosle (2001) reported that the sweat gland density in Deoni cattle was higher in males (1004.91±21.00) than in females (840.37±20.10). These glands were deeper in position in males when compared to the females.

The ducts of sweat glands were flexuous in buffalo and straight in ox. The average number of sweat glands per centimeter of skin as 161.23 in Murrah buffalo and 2527.85 in non-descript Indian cattle (Mehta, 2002).

2.8 SEBACEOUS GLANDS

Sebaceous glands are the appendages of hair follicles and open inside the pilosebaceous canal. According to David (1932), sebaceous glands of homozygous and heterozygous Mexican hairless pigs were rudimentary and less developed compared to the normal subjects.

The sebaceous glands developed in foetus by proliferation of outer hair root sheath. They formed the superficial layer of glands and occurred in the middle part of the corium and opened near the neck of the hair follicle. Independent sebaceous glands occurred only in the glans penis, prepuce, labia vulvae, anus, external ear canal and tarsal glands of the eyelids. The sebaceous glands were simple, alveloar holocrine glands. The glandular body was filled with epithelial cells. In regions, where hair growth was dense, sebaceous glands were long and narrow, whereas in places where hairs were placed distantly, the sebaceous glands were spheroid. Smaller the hairs, larger the sebaceous glands. Horse and dog had largest sebaceous glands, while those of the pig were rudimentary. In ungulates, two to six glands emptied into one hair follicle. In those animals having complex hair follicle, same number of sebaceous glands surrounded the hair complex. Largest sebaceous glands usually occurred in the areas around the mucocutaneous junctions. Secretion of the sebaceous glands consisted of cellular debris and a lipoid mixture high in cholesterol (Trautmann and Fiebiger, 1957).

The sebaceous glands of camel were found to be associated with each cover hair follicle and each branch of the compound hair follicle was found to be surrounded by a ring of typical simple or branched saccular glands of holocrine type (Lee and Nielson, 1962).

Sebaceous glands were smaller in sows (Marcarian and Calhoun, 1966; Montagna and Yun, 1964; Ellenport, 1975). According to Smith and Calhoun (1964), these glands were located in the angle between the hair follicle and arrector pili muscles. They were particularly abundant surrounding the upper third of tactile hairs of snout where they were arranged in a rosette pattern, about six per tactile hair follicle.

According to Marcarian and Calhoun (1966), sebaceous glands of the pigs were branched alveolar type. They generally opened by one duct and sometimes by two ducts – one on either side of the follicle.

Kozlowski and Calhoun (1969) demonstrated the occurrence of small sebaceous glands at the superior portion of the tactile hair in sheep, and branched ones in the infraorbital pouch and perianal area. Tarsal gland was a large multilobulated sebaceous gland.

In pigs, sebaceous glands of the bristles were in a rosette arrangement around the hair follicle, the shape varied from semicircular to extremely elongated. Wool hairs had only two apposed sebaceous glands. In sinus hairs, the glands were very small and referred to as the dwarf sebaceous glands (Schummer *et al.*, 1981).

Sharma *et al.* (1982) reported that the sebaceous glands in the neck and body of the scrotum of buffaloes were large, lobulated sac-like structures always associated with the hair follicle, but could independently occur in the ventral part. There was no correlation between the size of the gland, depth and diameter of hair follicle and also the total area of the follicular unit.

The sebaceous glands were round to oval, rectangular, triangular or irregular in shape and were single, bilobed or branched depending on the area of skin in yak (Barari *et al.*, 1994). Saxena *et al.* (1994) reported that the number of sebaceous glands per unit area in yak was higher during winter than in summer and monsoon. It was due to the shrinkage of skin during winter. The mean sebaceous gland

number was 268.55 per square centimeters of skin in yak with average length and diameter of 103.45 ± 3.5 µm and 57.89 ± 1.76 µm, respectively. Sharma *et al.* (1996) opined that, in yak, the sebaceous glands were poorly developed and were generally associated with small hair follicles and were double lobed.

According to Mugale and Bhosle (2001), there was not much difference in the position of the sebaceous glands between male and female Deoni cattle unlike in the case of sweat glands.

2.9 SUBCUTANEOUS TISSUE

David (1932) reported that subcutaneous layer of the homozygous hairless Mexican pig was thinner as against to the normal ones.

Trautmann and Fiebiger (1957) reported that the subcutis (hypodermis) in the pig was composed of loose collagenous trabeculae containing many elastic fibres that crossed each other to form a meshwork. Homogenous adhesive ground substance converted the fibre nets to thin membranes. Between the membranes were narrow tissue spaces, which were the area of subcutaneous oedema or emphysema in pathological conditions. The degree to which the skin could be folded depended on the development of subcutis. The spaces of subcutis were often filled with adipose tissue. In well-finished animals panniculus adiposus extended to the deep surface of cutaneous muscles. Subcutis and corium contained fibroblasts, histiocytes, melanophores, plasma cells and eosinophilic granulocytes. Panniculus adiposus was heaviest in the pig and was absent or sparse in those areas, where it anchored tightly to subjacent tissue.

Strands of connective tissue from the deeper layers of dermis extended throughout the subcutaneous fat layer, generally perpendicular to the surface of the skin (Marcarian and Calhoun, 1966).

In some breeds of swine, the subcutis was clearly demarcated from the corium, but in fatty animal, this layer extended far into the dermis. The panniculus adiposus was connected to the corium by stout fibre bundles (Schummer *et al.*, 1981).

According to Vardaxis *et al.* (1997), both the human and the swine relied on fat and not on fur or hair for insulation. The fat layer was pronounced in swine. The amount of subcutaneous fat layer varied with anatomical site examined and nutritional status of the animal, so also with age and sex.

Monteiro-Riviere (1998) opined that the hypodermis anchored the dermis to the underlying muscles or to the bone. The loose arrangement of collagen and elastic fibres allowed the skin flexibility and free movement over the underlying structures. Panniculus adiposus in pigs could be seen either as small clusters of cells or as large masses of fat. Pork bacon and fat back were derived from panniculus adiposus. Large fat deposits of carpal, metacarpal and digital pad acted as shock absorbers.

2.10 HISTOCHEMISTRY

2.10.1 Carbohydrates

According to Yang (1952), the sweat glands of cattle did not contain glycogen. Montagna (1962) opined that more glycogen was encountered in the sweat glands of older men than younger individuals. A band of glycogen was demonstrated in the upper cells of malpighian layer of epidermis of pig (Montagna and Yun, 1964). They suggested that glycogen was absent in the stratum basale and stratum spinosum. Both apocrine and eccrine sweat glands contained periodic acid Schiff (PAS) reactive substance in their lumina (Montagna and Yun, 1964). The basement membrane of glandular epithelium was PAS positive in reaction (Urmacher, 1990). Glycogen was absent in secretory cells of sweat glands in the snout, lip and carpal organ (Montagna and Yun, 1964).

In the pig, glycogen was absent in the secretory portion of the sebaceous glands, however moderate reaction was noticed in their ducts (Montagna and Yun, 1964). The alveolar cells of the sebaceous glands of the buffalo did not contain glycogen, but the capsule was strongly reactive for PAS and the peripheral cells were slightly alcianophilic (Sharma *et al.*, 1982).

Monteiro-Riviere and Stromberg (1985) opined that in pigs, glycogen was seen only in keratinocytes at one week of age. Hafez *et al.* (1955) reported that mucopolysaccharides were present in the prepucial skin of cattle. Its stratum corneum and collagen showed a positive PAS reaction and indicated the presence of neutral mucopolysaccharides.

According to Urmacher (1990), the basal layer of human epidermis showed PAS positive reaction. The outer root sheath of hair follicle was rich in glycogen and the PAS positive basement membrane separated the outer root sheath from the surrounding connective tissue. In the clear cells, glycogen was present and appeared as dense globular PAS positive material. In dark cells, the cytoplasm was finely granular, PAS positive and diastase resistant. The ductal lining cells of both apocrine and eccrine glands showed variable staining with PAS. Secretory portion of apocrine glands showed a negative to focally positive reaction to PAS. Dermal connective tissue was embedded in the ground substance that consisted mainly of nonsulfated acid mucopolysaccharides, predominantly hyaluronic acid and to a lesser degree the nonsulfated acid mucopolysaccharides and chondroitin sulphate.

2.10.2 Lipids

The skin of pigs and man possessed surface lipids composed of mainly triglycerides and free fatty acids, in contrast to the densely haired mammals. The skin surface lipid sample of pig represented primarily the hexane extractable epidermal lipids (Nicolaides and Rice, 1968).

Sharma *et al.* (1982) demonstrated sudanophilic substances in the sebaceous glands of the buffalo and opined that the central cells contained neutral fat and the peripheral cells possessed acidic fat. Dunnigan's Nile blue technique revealed pink, large fat globules of hydrophobic type neutral fat in the centre of the gland.

According to Urmacher (1990), in human, the subcutaneous tissue was arranged into lobules of mature adipocytes. Thin bands of dermal connective tissue that constituted the interlobular septae separated these lobules. Luminal cells of eccrine sweat glands showed eosinophilic cytoplasm that contained lipid, iron, lipofuscin and PAS positive diastase resistant granules and a large nucleus located near the base of the cell.

2.10.3 Phosphatases

Montagna and Yun (1964) observed a strong positive alkaline phosphatase reaction in the lower portion of the malpighian layer, mild reaction in the upper cells of spinous layer. The reaction was negative in stratum corneum of pig. Human epidermis lacked this enzyme. In the dermis, alkaline phosphatase activity was absent in the endothelium of capillary plexus inside the dermal ridges while those capillaries seen around the apocrine sweat gland showed a positive reaction. Dermal papillae of both quiescent and active follicles and basal plates of active follicles were rich in alkaline phosphatase. Cornified portions of inner root sheath, i.e., the Henle's layer down to the middle of the bulb showed a positive reaction, while in the Huxley's layer no reaction was noticed up to the level of keratogenous zone. Peripheral cells of sebaceous glands of pigs showed an intense alkaline phosphatase activity, while the activity was absent in the sebum. Secretory epithelium as well as luminal cells of the duct of sweat glands showed alkaline phosphatase reaction.

The subepidermal capillary plexus in the pig showed a moderate reaction for alkaline phosphatase and the human skin showed a strong reaction (Vardaxis *et al.*, 1997).

Urmacher (1990) reported that no acid phosphatase activity was present in the eccrine glands and ducts of the skin of human beings. The presence of acid phosphatase in the Odland bodies of stratum granulosum was demonstrated by Monteiro-Riviere (1998).

3. MATERIALS AND METHODS

Morphological and histological studies were conducted on the skin of Large White Yorkshire pigs of six to ten months of age. Skin samples from eight body areas were collected from 12 animals (six each from either sex) from the Meat Technology unit of Kerala Agricultural University, Mannuthy. The age, sex and body weight of the animals were recorded (Table. 1). Skin samples of 2 cm^2 area were collected immediately following exsanguination from eight areas of the body viz., the snout, dorsal nasal, dorsal neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen and carpal regions (Fig. 1). Samples were washed in normal saline and mopped with a blotting paper. Thickness of the skin and subcutaneous fat was measured using Vernier Callipers. Number of hairs per square centimeter area was also recorded. The skin samples were cut into smaller pieces of 2mm thickness and fixed in 10 percent neutral buffered formalin. The tissue pieces were processed in high melting paraffin (melting point, 58-60 °C) using the following procedure:

3.1 STEPS FOR THE PROCESSING PROCEDURE:

1. Washing in running tap water – 4 hours
2. 50 per cent Alcohol – 1 hour
3. 70 per cent Alcohol – 12 hours
4. 80 per cent Alcohol – 30 minutes
5. 90 per cent Alcohol – 30 minutes
6. Absolute alcohol I – 2 hours
7. Absolute alcohol II – 2 hours
8. Absolute alcohol III – 2 hours
9. Xylene I – 20 minutes
10. Xylene II – 20 minutes
11. Xylene III – 20 minutes
12. Paraffin I – 2 hours
13. Paraffin II – 2 hours

14. Paraffin III – 2 hours
15. Paraffin IV – 2 hours
16. Embedding

Paraffin sections of 4 to 5 μm thickness were taken for histological studies. For histochemical demonstration of lipids and phosphatases, frozen sections of 10 μm thickness were used.

3.2 STAINING

The following histological staining techniques were employed in paraffin sections.

1. Haematoxylin and eosin staining technique for routine histological studies (Luna, 1968).
2. Van Gieson's method for collagen fibres (Luna, 1968)
3. Verhoeff's elastic stain (Singh and Sulochana, 1996)
4. Masson's trichrome method for muscles and collagen fibres (Luna, 1968)
5. Ayoub-Shklar method for keratin and prekeratin (Luna, 1968)
6. Mallory's phosphotungstic acid hematoxylin method (PTAH) for muscles (Luna, 1968)
7. Gridley's modification of silver impregnation method for staining reticular fibres (Sheehan and Hrapchak, 1980).

The different cytological staining techniques employed were the following:

1. Fontana-Masson silver method for melanin pigments (Luna, 1968)
2. Toludine blue method for mast cells (Singh and Sulochana, 1996)

For histochemical studies, the following methods were employed.

1. PAS Alcian blue method for mucosubstance (Singh and Sulochana, 1996)
2. Best's carmine method for glycogen (Luna, 1968)
3. Oil red O in propylene glycol method for fats (Luna, 1968)
4. Modified Gomori's method for alkaline phosphatase (Singh and Sulochana, 1996)

5. Modified Gomori's method for acid phosphatase (Singh and Sulochana, 1996)

3.3 STATISTICAL ANALYSIS

3.3.1 Morphometry

The following morphometric parameters were measured from eight areas of the body viz., the snout, dorsal nasal, dorsal neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen and carpal regions in both male and female pigs and Student's-t-test was done to test the difference between the following parameters in males and females.

1. Skin thickness
2. Subcutaneous fat thickness
3. Number of hairs per square centimeter

3.3.2 Micrometry

The following micrometrical parameters were measured using an ocular micrometer. Analysis of these parameters was done by one-way analysis of variance technique (Cochran and Cox, 1992).

1. Thickness of stratum corneum
2. Thickness of stratum lucidum
3. Thickness of stratum granulosum
4. Thickness of stratum spinosum
5. Thickness of stratum basalis
6. Total thickness of epidermis
7. Thickness of papillary layer
8. Thickness of reticular layer
9. Thickness of the dermis
10. Thickness of subcutaneous fat

11. Diameter of rete peg
12. Length of rete peg
13. Diameter of the secretory end piece of the sebaceous gland
14. Diameter of the secretory end piece of the sweat gland
15. Length of the duct of sweat gland

In order to find out the relationship if any, between the following parameters, correlation and regression analyses were done (Snedecor and Cochran, 1985).

1. Thickness of epidermis and thickness of skin
2. Thickness of dermis and thickness of skin
3. Thickness of subcutaneous fat and thickness of skin

4. RESULT

4.1 MORPHOLOGY OF THE SKIN

The skin of Large White Yorkshire pigs was white with creased surface. Even though the entire skin was white, areas of black spots could be seen occasionally. Thickness of the skin and subcutis and hair distribution of different body regions in the animals used for the study are given in the table 2. In general, skin of male animals was slightly thicker than that of the females (Fig. 2). When compared using Student's-t-test, there was no significant difference in the skin thickness of the males and females except in the dorsal nasal region (Table. 3).

Thickness of the skin varied considerably in different regions of the body. Maximum thickness was noticed in the snout region with an average value of 6.43 ± 0.06 mm in female pigs and 6.58 ± 0.08 mm in males. Minimum thickness was noticed at the ventral abdominal region, which measured 2.15 ± 0.07mm in females and 2.52 ± 0.14mm in males. In general, skin was thicker on the dorsal surface of the body than on the ventral surface. Snout showed the maximum thickness followed by the dorsal nasal area, carpal, dorsal neck, dorsal abdomen, lateral abdomen, ventral neck and the ventral abdomen regions (Fig. 2).

Skin was composed of the superficial epidermis and the deeper dermis. Contribution of the epidermis to the total skin thickness was maximum in the snout region (14.33% in females and 14.99% in males). Comparison of the thickness of the epidermis and dermis in different body regions in female and male animals are shown in the figures 3 and 4. Both the layers were thickest in the snout region, that too in the male pigs.

Beneath the dermis was the subcutaneous tissue that was massively infiltrated with adipose tissue to form the panniculus adiposus. Thickness of the subcutaneous fat layer in different body regions is given in table 2. Maximum

thickness was noticed in the dorsal neck region (Fig. 5). Subcutaneous fat layer was slightly thicker in females.

In the snout region, subcutaneous fat was less and contained connective tissue fibres and cutaneous muscle bundles (panniculus carnosus). Cutaneous fascia was pale, thin and closely adherent to the skin. The subcutaneous fat was diffusely arranged between the dermis and underlying muscle tissue. It measured 0.98 ± 0.06 mm and 0.90 ± 0.02 mm in females and males, respectively.

In the case of dorsal neck and dorsal and lateral abdominal regions, subcutaneous fat layer was found as a separate sheet under the dermis. In the dorsal and lateral abdominal regions, the fat was occasionally arranged in two layers. Large blood vessels supplying the subcutaneous tissue were grossly visible in certain regions. A thin muscular layer was interposed between the layers of fat in the lateral abdominal area. In the ventral neck and abdominal regions, the subcutaneous fat was arranged loosely in small lobules.

Hair was sparsely arranged in the swine. The number as well as size of hair varied from region to region (Fig. 6). Straight stiff guard hairs, the bristles, provided the hair coat. Longer ones were seen in the neck and abdominal regions. Compared to the ventral and lateral body regions, lengthier ones were found on the dorsal aspect.

4.2 EPIDERMIS

4.2.1 Morphology

In all the regions studied, the epidermis was very thin compared to the dermis. The epidermal thickness at the different body regions in male and female pigs are given in table 3. Epidermis was thickest in the snout and thinnest in the lateral abdominal region (921.50 ± 1.53 μm and 111.62 ± 6.31μm in females and 986.67 ± 3.41 μm and 112.47 ± 3.52 μm in males, respectively). A highly

significant positive correlation was noticed between the total skin thickness and the thickness of the epidermis in the snout, dorsal nasal and carpal regions (Table. 4) in both male and female animals. In the other regions (neck dorsal, neck ventral, dorsal, lateral and ventral abdominal regions), there was no significant correlation between these parameters. Similarly, no significant correlation was observed between the thickness of epidermis and dermis throughout the body in both sexes. The Analysis of Variance technique (ANOVA) showed that epidermal thickness in the ventral neck and dorsal, lateral and ventral abdominal regions came under same group in male and female pigs (Table. 3).

4.2.2 Histology

The epidermis was formed of four layers, viz., stratum basalis, stratum spinosum, stratum granulosum and stratum corneum (Figs. 7, 8 and 9) except in the snout, dorsal nasal and ventral abdominal regions, where an additional layer, the stratum lucidum was noticed (Fig. 10).

Downward projections of the epidermis into the dermis, the rete pegs, were abundant in the snout and dorsal nasal regions (Fig. 8). The papillary like elevations of the dermis, the dermal papillae also showed a similar pattern. The average number of rete pegs and dermal papillae per field under low power magnification of the microscope, and their micrometrical parameters are given in the table 5. In the snout region, they were abundant, very deep and took part in the formation of compound papillae. The dorsal nasal region possessed deeper and pointed rete pegs. The carpal region also showed abundance of rete pegs and dermal papillae. These were found to be minimum in the dorsal abdominal region in both male and female pigs. In general, the extremities like snout, dorsal nasal and carpal regions showed more number of rete pegs and dermal papillae than the neck and trunk regions.

4.2.2.1 Stratum Basalis

The thickness of the stratum basalis in different regions of the body in both males and females are given in table 3. Stratum basalis was made up of a single layer of columnar cells with their axes perpendicular to the basal lamina (Fig. 7). The columnar cells measured about 18.50 μm in height and 9.25 μm in width. The nucleus was oval in shape with a prominent nucleolus of 11.10 μm in length and 5.55 μm in width (Fig. 8). Height of these cells gradually reduced towards the apex of the dermal papilla and they became cuboidal in shape (Fig. 7). These cuboidal cells measured 14.80 μm in height and 11.10 μm in width. Nucleus became almost spherical with a diameter of 4.44 μm. From the lower surface of the stratum basalis cells, cytoplasmic processes grew into the papillary layer of the dermis (Fig. 9). In the carpal region, all the basal cells were almost cuboidal in shape.

Cells of stratum basalis rested on a basement membrane made up of collagen (Fig. 9) and reticular fibres. The basement membrane showed a positive reaction for PAS-alcian blue staining. The basement membrane separated the basal layer from the dermis. No blood vessels could be noticed in the epidermis.

Clear cells could be located in the stratum basalis and stratum spinosum (Figs. 10 and 11). These cells were relatively larger and appeared lighter than the keratinocytes (cells of the stratum basalis and stratum spinosum). Cytoplasm of these cells was clear and the nucleus was indented, sometimes had a reniform appearance (Fig. 10). These cells possessed the characteristics of the Langerhan's cells. Among the regions under study, the dorsal and ventral neck regions possessed a higher concentration of the clear cells compared to other regions.

4.2.2.2 Stratum Spinosum

Stratum spinosum was the thickest layer of the epidermis. Cells of this layer were large, irregular and polyhedral with distinct cell boundaries and had an average diameter of 19.20 μm (Figs. 8 and 12). A clear zone separated adjacent

cells of this layer from one another. The fine lines across this clear zone formed the intercellular connections and gave a prickled appearance to this layer (Fig. 13). The cells of stratum spinosum possessed a large, round to oval vesicular nucleus with a diameter of 11.10 μm. Nucleus possessed a distinct nucleolus (Fig. 13). Nuclei of the some of the cells of the deeper layers were in various stages of mitosis. These cells became flattened as they moved towards the surface with their long axes parallel to the surface (Figs. 8 and 9) and the cells measured 25.90 μm in length and 13.00 μm in width. The new cells formed by mitosis migrated outward and eventually sloughed from the superficial layer of the stratum corneum.

Thickness of the stratum spinosum varied in different body regions. At the snout region, in the area corresponding to the tip of the dermal papilla, this layer was 12 to 16 cell-layers thick and at the region of rete peg the thickness of this layer varied from 45 to 50 cell-layers. In dorsal nasal region, the stratum spinosum was four to six cells thick and ten to twelve cells-thick at the region of the dermal papilla and ret peg, respectively (Fig. 8). In other body regions, thickness of this layer was comparatively less and ranged from two to four cells-thick and six to seven cells-thick, respectively (Fig. 10). The cell boundaries and the prickling appearance at the intercellular region were more distinct in the snout and carpal regions. Stratum spinosum was thinner at the dorsal and ventral neck regions and in the abdominal region. Here the cell boundaries were indistinct and the nucleus showed peripheral condensation of chromatin (Fig. 17). At the carpal region, the cells were comparatively smaller (14.80 μm), and closely packed with clear cell boundaries and possessed an oval nucleus (7.40 μm).

Cytoplasmic staining property of the cells of the stratum spinosum varied in different layers. In the deeper layers, the cells were faintly basophilic and towards the middle, they became eosinophilic and closely packed. The peripheral cells were larger and the cytoplasm showed basophilic granules (Fig. 14). Prekeratin granules could be detected in the upper layers of stratum spinosum (Fig. 9).

4.2.2.3 Stratum Granulosum

Thickness of the stratum granulosum in different body regions in both male and female Large White Yorkshire pigs are given in the table 3. Thickness of the stratum granulosum in female pigs varied from 3.39 ± 0.27 μm at ventral abdominal region to 84.67 ± 0.86 μm at the snout region. The corresponding values in male pigs were 3.41 ± 3.03 μm and 80.33 ± 5.52 μm, respectively.

Stratum granulosum consisted of two to four rows of flattened, diamond-shaped cells with their long axes parallel to the surface of the skin (Figs. 13 and 14). The cells measured about 24.00 μm with a nucleus of 5.55 μm size. The nucleus was spherical in shape. Towards the periphery, nuclei of some cells were fragmented (Figs. 14 and 15). The cytoplasm showed irregular, conspicuous, basophilic keratohyalin granules that stained intensely with haematoxylin.

In the snout and dorsal nasal regions where the stratum granulosum was thicker, three to four cell layers could be identified (Fig. 15). In the abdominal region, it was thinner with one to two cell layers. In the ventral neck and lateral and ventral abdominal regions, it was very thin and occasionally sporadic.

4.2.2.4 Stratum Lucidum

Stratum lucidum appeared as a clear, bright, homogenous, strongly eosinophilic layer (Figs. 16 and 17). This layer consisted of flattened, compact, eosinophilic cells without clear cell boundaries or the nucleus, thus forming a homogenous clear layer. It was adherent to the stratum granulosum ventrally and was continuous with the stratum corneum at the outer surface. This layer could be detected in the snout, dorsal nasal and ventral abdominal regions. Thickness of this layer in these areas was 301.67 ± 0.66 μm, 17.28 ± 0.55 μm and 24.05 ± 0.27 μm in the female pigs and 325.17 ± 4.83 μm, 9.87 ± 1.58 μm and 18.91 ± 0.27 μm, respectively in males. This layer could not be detected in the dorsal neck, ventral neck, dorsal abdomen, lateral abdomen and the carpal regions.

4.2.2.5 Stratum Corneum

Stratum corneum formed the outermost layer and was present throughout the epidermis. Number of cell layers of this stratum varied greatly in different areas of the body. The mean thickness of stratum corneum at different regions of the body is given in table 3. Maximum thickness was noticed in the snout region (103.00 ± 0.31 μm in the females and 106.50 ± 0.62 μm in the male pigs). Thickness gradually reduced in neck dorsal, neck ventral, dorsal nasal, abdomen lateral and carpal regions with a minimum thickness observed in the ventral abdomen (33.92 ± 1.53 μm in females and 44.02 ± 3.52 μm in males).

Stratum corneum consisted of scale-like polygonal, clear cells. These were keratinized dead cells and contained no nucleus and cytoplasmic organelles (Fig. 14). The cytoplasm was filled with keratin (Fig. 9). The cells became more and more flattened towards the surface and were closely packed without obvious intercellular spaces. The limiting membranes of the cells became thickened and were closely interdigitated. The most superficial layers were seen detached from the surface (stratum disjunctum) as shown in the figure 12. New cells that moved towards the surface during the process of keratinization replaced these desquamated cells.

Among the five layers of the epidermis, stratum basalis, stratum spinosum and stratum corneum were always present and formed continuous layers throughout the body surface. The stratum granulosum consisting of one to three layers of cells was a continuous layer in most of the regions. But in ventral neck and lateral and ventral abdominal regions this layer was not continuous. A definite stratum lucidum was seen only in the snout, dorsal nasal and ventral abdominal areas. The epidermis was entirely devoid of blood vessels and received nourishment from the capillaries of the papillary dermis (Fig. 13).

4.3 DERMIS

4.3.1 Mophology

Thickness of the dermis varied from 2220.78 ± 2.11 μm to 5221.00 ± 12.49 μm in females and 2567.00 ± 26.34 μm to 5336 ± 7.39 μm in males (Table. 6). The maximum thickness of dermis was noticed in the snout and minimum at the ventral abdominal region. Two layers could be distinguished in the dermis. The superficial papillary layer was thinner, while the deep reticular layer was thicker (Figs. 18 and 19). There was a significant positive correlation between the total thickness of the skin and that of dermis in all the regions under study in both the female and male pigs (Table. 4). Statistical analysis (ANOVA) showed that there was no significant difference between the thickness of the dermis in the dorsal neck and dorsal abdominal areas (Table 6).

4.3.2 Histology

4.3.2.1 Papillary Layer

Micrometrical parameters of the papillary layer are given in table 6. The papillary layer was thickest in the snout (563.33 ± 6.28 μm in the female and 570.00 ± 7.29 μm in the male pigs). In the papillary layer, the fibres were finer and more closely arranged. This layer conformed to the contour of the stratum basalis of the epidermis. The papillary layer protruded into the epidermis at certain intervals, thereby giving rise to the dermal papillae. A clear line of demarcation was absent between the papillary and reticular dermis. The papillary layer was made up of collagen fibres predominantly (Fig. 20). This layer also contained elastic and reticular fibres those were embedded in an amorphous ground substance. This amorphous ground substance gave a positive reaction to PAS alcian blue staining. The fine fibres of the papillary layer interdigitated into the stratum basalis (Fig. 9). Unlike the epidermis, this layer was highly vascular and large number of capillary loops could be seen in the dermal papillae (Figs. 13 and 21). Fibroblasts

and macrophages were the predominant cell types in the papillary layer (Fig. 17). In addition to these, mast cells were also detected (Fig. 22).

Papillary dermis formed primary ridges corresponding to the epidermal ridges. The primary dermal ridge was again divided into secondary dermal ridges or dermal papillae by the rete pegs (Fig. 23). These were numerous, tall and often branched. The measurements of the dermal papillae at different regions are cited in table 5. Highest dermal papillae could be seen in the snout (Fig. 23). Compound dermal papillae could be located in the ventral abdominal region (Fig. 24).

Dermal papillae were pronounced in the snout and carpal regions. They were single with very short rete pegs in the ventral neck and ventral abdominal regions (Fig. 25). In the dorsal and lateral abdominal regions, the dermal papillae were less developed (Figs. 11 and 26).

4.3.2.2 Reticular Layer

There was no clear demarcation between the papillary and reticular layers of the dermis. But both could be distinguished from each other because of the difference in the nature and arrangement of connective tissue fibres (Figs. 18 and 20). Reticular layer consisted of large, coarse and loosely interwoven bundles of collagen fibres.

The measurements of the reticular layer of dermis in male and female pigs in different body regions are given in the table 6. In female pigs, thickness of the reticular dermis varied from 2127.67 ± 7.21 μm to 4654.17 ± 9.80 μm whereas in the male animals the values were 2493.00 ± 21.87 μm and 4762.67 ± 3.88 μm, respectively. The reticular layer was thickest in the snout region. Average thickness of the reticular layer was about eight times than that of the papillary layer in this region.

In the reticular layer, bundles of collagen fibres were arranged mostly parallel to the surface. A few perpendicularly directed fibres could be traced down to the subcutaneous layer (Fig. 27). These bundles ran parallel to the hair follicles and contributed to the formation of the interlobular septa that separated the subcutaneous adipose layer into numerous lobules. In addition to the parallel fibres, alternate layers of collagen fibres were also observed at an angle to the former. In the carpal region, the fibres formed irregular network (Fig. 28). Besides the collagen fibres, elastic fibres were also seen in the dermis (Fig. 29). They formed thick networks between the collagen bundles and were condensed about the hair follicles, sebaceous glands and sweat glands. In the papillary layer, they were thinner. Reticular fibres were also noticed surrounding the secretory end pieces of the sweat glands and sebaceous glands (Figs. 30 and 31).

Towards the deeper aspect, size of the collagen bundles greatly reduced and they were seen as small, thin bundles. There was no clear demarcation between the dermis and subcutaneous tissue. This was noticed in the ventral neck region and lateral and ventral abdominal regions. In the dorsal aspect of the body, the dermis was clearly demarcated from the subcutaneous fat (Figs. 32 and 33). The separation between the papillary and reticular layers was more distinct in the abdomen dorsal and abdomen lateral regions (Fig.18).

Blood vessels, lymph vessels and nerves traversed the dermis (Fig. 34). Small and medium-sized arteries predominated in the reticular layer (Fig. 35). The tunica media of these arteries was composed of smooth muscle fibres. Numerous blood vessels were noticed in the reticular dermis and near the sweat glands. Most of them formed the arteriovenous anastomoses or glomi. Glomi were most numerous in the snout region (Fig. 36).

The cellular elements were less abundant in the reticular layer when compared to the papillary dermis. Fibroblasts, mast cells, macrophages and extravasted leucocytes were often found. Aggregations of lymphocytes were noticed in the snout region surrounding the ducts of eccrine sweat glands (Fig. 37).

These were found to be associated with the compound tubular sweat glands. Large number of receptors and nerve bundles could be noticed in the dermis (Figs. 38 and 39).

4.4 HAIR

4.4.1 Morphology

The hair arrangement in swine was simple, but grouping of hairs was evident. Mostly two to three hairs formed a group and they emerged out very closely but not from a single orifice unlike in the case of compound hairs. Snout region lacked hair on the rostral aspect and sparse wool hairs were found on the dorsal surface. Dorsal nasal and carpal regions bore dense population of short, stout hairs.

Maximum hair density per square centimeter area was noticed in the dorsal nasal area with an average of 58.00 ± 2.33 in females and 61.50 ± 2.60 in males (Table. 2). Hair distribution was minimum in the snout. This was followed by ventral abdomen region with an average number of 11.83 ± 0.27 in females and 12.83 ± 0.41 in males. The mean distance between adjacent hairs in the dorsal nasal region was 1.28 mm and 1.23 mm and in the abdomen ventral area was 5.48 mm and 5.18 mm in female animals and male animals, respectively.

In general, density of hair distribution was more in the male animals than the females (Table. 2). A significant sex difference ($p < 0.05$) was observed in the ventral neck region (Table. 2).

4.4.2 Histology

Hair was composed of a shaft that projected beyond the surface of the skin and a root, which was inserted obliquely into the corium. The hair root was enlarged at its ventral end to form the hair bulb that was indended by the hair papilla. The hair roots were situated in tubular pockets, the hair follicles in the epidermis, which

extended into the dermis. Hair shaft was composed of a cuticle, cortex and medulla (Figs. 40 and 41). Cuticle was formed by a single layer of flat, keratinized, anucleated squamous cells (Fig. 40). Cortex of the hair was made up of several layers of dense, compact, keratinized, spindle-shaped cells with their long axes parallel to the hair shaft (Fig. 42). These cells measured 18.50 μm in length and 5.55 μm in width. Nuclei of these cells were elongated and darkly stained (Fig. 42). Near the hair bulb, these cells became shorter to form oval cells with spherical nuclei. Medulla of the hair shaft was composed of loosely filled cuboidal or polygonal cells, with air filled spaces (Figs. 40 and 43). These cells showed some keratin granules. But in the root region, medulla was solid and did not contain air filled spaces.

Hair follicle was composed of four parts, viz. hair papilla, hair matrix, inner root sheath and outer root sheath. Hair papilla was the part of dermis encapsulated by the hair matrix cells that formed a structure called the hair bulb (Fig. 44). In some regions, the hair bulb extended deep into the subcutaneous tissue. The hair matrix cells resembled the cells of stratum basalis of the epidermis (Fig. 44). The inner root sheath was composed of inner cuticle, middle granular epithelial layer, the Huxley's layer and outer pale epithelial layer, the Henle's layer (Fig. 45). Cuticle of inner root sheath was similar to the cuticle of the hair. It was composed of thin, scale-like, overlapping cells, the free borders of which were directed towards the hair root. Cuticular layers of both the hair and the hair follicle were interlocked (Figs. 46 and 47). Huxley's layer lay between the cuticle and the Henle's layer. This layer was composed of two to three rows of elongated, granular cells (Fig. 47). Henle's layer was the outer most and was composed of a single layer of columnar cells with darkly stained nuclei (Fig.45). All the layers of inner root sheath showed a positive reaction for keratin (Fig. 48). Immediately below the opening of the sebaceous glands, internal root sheath of the large follicle became corrugated and formed several circular or follicular folds (Fig. 49). Above the level of the pilosebaceous opening, the inner root sheath became thinner and cells were in a disintegrating stage.

The external root sheath was composed of several layers of cells and was continuous with the upper portion of the hair follicle (Fig. 50). It was formed of single layer of stratum basalis and several layers of stratum spinosum cells (Figs. 48 and 50). Externally a homogenous glassy membrane covered the external root sheath (Fig. 48). This corresponded to the basal lamina of the epidermis. The entire hair follicle was enclosed by connective tissue sheath, which was composed of internal and external layers of collagen and elastic fibres (Fig. 51). The inner layer was made up of circularly arranged fibres (Figs. 52 and 53) and the outer layer of longitudinal fibres (Figs. 46 and 48). The connective tissue sheath was richly supplied with blood vessels and nerves, especially in the hair papillae.

Different stages of hair follicles were noticed in the dermis. Anagen bore mitotically active cells in the hair bulb; while catagen showed regressive type of cells (Fig. 54). The hair papilla was reduced to a ball of cells located below the capsule of the hair matrix cells of the bulb. Hair follicle at this stage was the telogen (Fig. 55).

4.5 ARRECTORES PILORUM

Arrectores pilorum muscle appeared as a small bundle of smooth muscle fibres that inserted obliquely in the connective tissue sheath of the hair follicle (Figs. 56 and 57). The outer end extended towards the epidermis. At this point, it was attached to the superficial papillary layer of the dermis. This muscle was anchored by elastic fibres at its insertion and attachment. Arrectores pilorum muscle completely encircled the hair follicle like sling (Fig. 43). Largest arrectores pilorum (143µm in diameter) were noticed in the abdomen dorsal region (Fig.58).

4.6 INTERFOLLICULAR MUSCLE

Interfollicular muscle resembled the arrectores pilorum muscles. These smooth muscle fibres connected adjacent hair follicles of its characteristic hair group (Figs. 52 and 59). It was found midway between the level of sebaceous

glands and apocrine sweat glands and attached to the hair follicle opposite to the side of attachment of the arrectores pilorum and the duct of the sweat gland.

4.7 SWEAT GLANDS

Sweat glands were located lateral and ventral to the hair follicles in the hairy areas of the skin (Fig. 33). In the case of glabrous, non-hairy regions like the snout, they found independently. Sweat glands were located mostly in the deeper dermis and in the subcutaneous tissue (Fig. 60). In the snout region, sweat glands were smaller in diameter (32.50 to 39.00 μm) and occurred in clusters. At the neck dorsal and abdomen dorsal regions, the sweat glands were very large and distributed abundantly (Fig. 60). The mean diameter of the secretory end piece was 104.00 to 234.00 μm and 182.00 to 260.00 μm, respectively in the dorsal neck and abdominal regions. In the trunk region, they were minimum at the lateral and ventral areas compared to the dorsal region. In the lateral abdominal region, they had a diameter of 104.00 μm to 117.00 μm. In the ventral abdominal region, the diameter ranged from 65.00 μm to 91.00 μm. In the dorsal nasal, ventral neck, ventral abdomen and carpal regions, the sweat glands were relatively less abundant. In the regions under study, maximum number of sweat glands was observed in the snout region.

Sweat glands were apocrine type in all the regions under study except in the snout and dorsal nasal regions, where it was of eccrine type. Apocrine sweat glands showed wider secretory end pieces than the eccrine glands. It was lined by simple columnar epithelium (Fig. 61). Height of the cells varied depending on the stage of secretion. During secretion, the cells showed bud like apical projections, the apical blebs (Fig. 61). The basophilic cytoplasm showed lipid droplets. The nucleus was oval in shape. Once the secretory process was over, the epithelium became flattened and the lumen was filled with secretion (Fig. 33). Just below the lining epithelium, myoepithelial cells formed a distinct layer above the basal lamina. These cells possessed elongated nucleus (Fig. 61). The duct of apocrine sweat gland coarsed parallel to the hair follicle wall, towards the epidermis. Most of them opened directly on to the surface of the epidermis (Fig. 62). Others opened into the upper

portion of the hair follicle above the opening of the sebaceous gland (Fig. 57). The duct was made up of a single layer of cuboidal cells, and possessed a narrow lumen (Fig. 63). The nucleus of these cells was spherical in shape.

Secretion of the sweat gland gave a positive reaction to hyaluronic acid, sialomucins, strongly acidic sulfated mucosubstances and lipids (Fig. 64).

The sweat glands in the snout and dorsal nasal regions were of compound tubular eccrine type with branched secretory portion (Fig. 65) and branched ducts (Fig. 66). Unlike apocrine sweat glands, these were embedded in the reticular layer of the dermis and were not associated with the hair follicles.

Histologically, the secretory portion consisted of a single layer of cuboidal to pyramidal cells resting on myoepithelial cells and a basement membrane (Figs. 67 and 68). In some regions, two types of secretory cells were visible, viz., the 'clear cells' and 'dark cells' (Fig. 69). Dark cells had an oval dense nucleus, while the clear cell nucleus was spheroidal and lightly stained. The clear cells showed a broad base that rested on the myoepithelial cells and the basement membrane. The dark cells had an inverted pyramidal shape with a broad apex.

Duct of the eccrine sweat gland was composed of stratified cuboidal epithelium (Fig. 37). The nuclei were spherical in shape. The adluminal cells had a more granular eosinophilic cytoplasm than the peripheral rows of cells. The duct of these glands was composed of convoluted portion in close association with the secretory unit, a straight dermal component, and a spiral intraepidermal portion, the acrosyringium, which opened onto the surface (Fig. 70). The compound ducts opened directly through the epidermis (Fig. 66).

The sweat glands of ventral abdomen, dorsal nasal area and lateral abdomen areas bore long ducts with a length of 3000 to 3500 μm, 2500 to 2900 μm, and 2000 to 2300 μm, respectively. In the snout and carpal regions, ducts were shorter (1300 to 1500μm).

4.8 SEBACEOUS GLANDS

Sebaceous glands appeared as large, lobulated, sac-like structures associated with the hair follicles. They were of simple branched alveolar type (Fig. 71). Around the bristles, they were arranged in a rosette fashion (Fig. 72). The lobes appeared as round, oval, triangular, quadrilateral or elongated ones. All these were embedded in the dermis and not extended into the subcutaneous tissue unlike the apocrine sweat glands. The secretory units consisted of a solid mass of epidermal cells, enclosed by a connective tissue sheath that blended with surrounding connective tissue of the dermis (Fig. 72). At the periphery of the glandular mass, a single layer of low cuboidal cells with round nucleus rested on the basal lamina (Fig. 73). Mitotic figures could be observed in these cells. As they moved inward, they became polygonal or spheroidal and accumulated numerous lipid droplets (Figs. 73 and 74). The nuclei gradually shrunk and disappeared. The smaller peripheral cells contained only a few small fat droplets or none at all. The inner cells of secretory unit were larger. The cytoplasm of the most central ones and those in the lumen of the duct almost converted into fat and their nuclei were disintegrated (Fig. 73). Necrosis of these cells happened as they were pushed further towards the centre of the gland (Fig.72). In the middle zone, the cells were in intermediate stages.

The alveoli of the sebaceous glands opened into a short duct. These ducts were opened through pilosebaceous canal into the upper portion of the hair follicle (Figs. 75 and 76). They were most abundant in neck dorsal region among the different areas under study. Most of them opened by one duct. It was lined by stratified squamous epithelium and was continuous with the outer root sheath of the hair follicle.

4.9 SUBCUTANEOUS TISSUE

The subcutaneous tissue was composed of a loose meshwork of connective tissue fibres, predominantly the collagen fibres and small quantities of elastic and

reticular fibres (Fig. 33). Homogeneous adhesive ground substance held these fibres. The spaces in the network were filled with adipose tissue (Fig. 81).

Subcutaneous tissue also showed fibroblasts, lymphoid aggregations, lymphatics, large blood vessels and nerve bundles. The collagen fibre bundles from reticular dermis extended down to the subcutaneous tissue. These bundles ran perpendicular to the surface of the skin.

Thickness of the subcutaneous fat ranged from 0.98 ± 0.06 mm to 29.60 ± 0.99 mm in the females and 0.90 ± 0.02 to 29.10 ± 0.72 mm in the males (Table. 2). Maximum thickness was noticed at the neck dorsal region in both male and female pigs. It was thinnest in the snout region. In general, the subcutaneous fat thickness was slightly more in the female animals than in the male animals (Table. 2). But no significant difference was observed by Student's-t-test (Table. 2).

In the snout, dorsal nasal and carpal regions, the subcutaneous tissue was comparatively thin. Adipose tissue was minimum and sheets of cutaneous skeletal muscles were embedded in the subcutis in the dorsal nasal region (Fig. 77).

Large Pacinian corpuscles were noticed in the subcutaneous tissue (Fig. 78). Numerous nerve fibre bundles were also present. Root of the hair follicles reached deep in the subcutaneous tissue and was surrounded by lobules of fat. Apocrine sweat glands were also embedded in the subcutaneous tissue.

Subcutaneous fat lobules were very large in the dorsal neck and dorsal and lateral abdominal regions and showed a sharp distinction from the dermis. In the ventral aspect of neck, these lobules were smaller and were separated by very fine collagen meshwork. Adipocyte size was smaller immediately beneath the skin than in the middle layers of the subcutaneous depots. Subcutaneous adipocytes of the inner layers of the depot were nearer in size to those on the superficial layer.

4.10 HISTOCHEMISTRY

4.10.1 Carbohydrates

PAS-alcian blue positive areas were detected in the upper stratum spinosum and stratum granulosum of the epidermis (Fig. 18). No activity could be detected in the stratum basalis, lower layers of stratum spinosum, stratum lucidum and stratum corneum. The same pattern was also observed for Best's carmine staining procedure for glycogen. The epidermal cells around the pilosebaceous opening and around the orifices of sweat glands showed the presence of glycogen. In the dermis, the ground substance was positive for Best's carmine and PAS-alcian blue. Cytoplasm of the fibroblasts gave a negative reaction, while the nucleus showed PAS- alcian blue positive granules. The cells of the outer root sheath of hair follicle around the keratogenous zone showed an intense PAS-alcian blue positive activity. Active hair follicles also gave a positive PAS- alcian blue reaction (Fig. 79). The upper and lower portions of the follicle showed a weak reaction compared to the middle third. The cells of internal root sheath showed a negative reaction to Best's carmine and was PAS-alcian blue positive (Fig. 56). The cuticle cells just above the hair bulb gave a positive reaction to Best's carmine and the cells at the middle third of the follicle gave a negative result. Dermal papilla of the hair follicle showed PAS-alcian blue positive reaction. But no glycogen could be detected in this area.

Both eccrine and apocrine sweat gland epithelia showed a positive reaction to Best's carmine. Glycogen granules were detected in the apical portion of the cells (Fig. 80). Secretion of the sweat glands was positive for both Best's carmine and PAS-alcian blue (Figs. 64 and 80). Epithelium of the duct also showed glycogen granules.

Secretory portions of sebaceous glands showed negative results for both Best's carmine and PAS-alcian blue. Ductular epithelium was weakly positive.

4.10.2 Lipids

Epidermal cells of stratum corneum and stratum spinosum showed a positive reaction to Oil red O. Dermal fibroblasts showed delicate clusters of lipid granules in the cytoplasm at both poles of the cells. The sebaceous glands were strongly positive (Fig. 74). The sebaceous gland duct also showed a positive reaction. Clear cells of the eccrine sweat glands showed the presence of lipid granules. The hair follicle also gave a positive reaction. Figure 81 shows a highly positive reaction for Oil red O in the subcutaneous fat but the interlobular septa gave a negative reaction.

4.10.3 Phosphatases

In the epidermis, the stratum basalis, stratum spinosum and stratum granulosum showed a positive alkaline phosphatase reaction, while stratum corneum was negative. The cortex of the hair follicle gave a slight positive reaction. The cuticle and inner root sheath possessed alkaline phosphatase (Fig. 82). The dermal papillae, blood vessels surrounding the hair follicles and the sweat glands showed a positive reaction to alkaline phosphatase (Fig. 83).

Epidermis gave a highly positive reaction to the acid phosphatase(Fig. 84). Stratum basalis showed a negative result, while the cells of the stratum spinosum showed a gradual increase in reactivity as proceeded upward. Stratum granulosum and stratum corneum showed an intense reaction. Sebaceous glands showed a positive reaction in the acini, while the periphery as well as degenerating region of the acinar cells showed a negative reaction. The apical portion of the secretory cells of the apocrine sweat glands showed moderate positive reaction.

Tables

Table 1. Body weight and sex of the animals used for the study

Sl No.	Sex	Body weight (kg)
1	Male	75
2	Male	70
3	Male	79
4	Male	80
5	Male	84
6	Male	75
7	Female	66
8	Female	75
9	Female	75
10	Female	71
11	Female	75
12	Female	70

Table 2. The skin thickness, subcutaneous fat thickness and hair distribution of the different body regions of the pigs

Sl. No.	Body regions	Skin thickness (mm) Mean ±S.E.		Subcutis fat thickness (mm) Mean ±S.E.		No. of hairs per cm^2 Mean ±S.E.	
		Female	Male	Female	Male	Female	Male
1	Snout	$6.43\pm 0.06^{a}_{ns}$	$6.58\pm 0.08^{a}_{ns}$	$0.98\pm 0.06^{e}_{ns}$	$0.90\pm 0.02^{d}_{ns}$	$8.17\pm 0.47^{d}_{ns}$	$9.67\pm 0.70^{c}_{ns}$
2	Dorsal nasal	$4.00\pm 0.07^{bc}_{**}$	$4.31\pm 0.05^{b}_{**}$	$1.50\pm 0.11^{e}_{ns}$	$1.48\pm 0.12^{d}_{ns}$	$58.00\pm 2.33^{a}_{ns}$	$61.50\pm 2.60^{a}_{ns}$
3	Neck dorsal	$3.73\pm 0.10^{bc}_{ns}$	$3.71\pm 0.09^{c}_{ns}$	$25.93\pm 0.99^{a}_{ns}$	$25.98\pm 0.72^{a}_{ns}$	$18.67\pm 1.16^{b}_{ns}$	$22.17\pm 1.23^{b}_{ns}$
4	Neck ventral	$2.72\pm 0.26^{d}_{ns}$	$2.63\pm 0.10^{d}_{ns}$	$12.27\pm 0.29^{d}_{ns}$	$11.32\pm 1.28^{c}_{ns}$	$12.67\pm 0.29^{cd}_{*}$	$14.00\pm 0.31^{c}_{*}$
5	Abdomen dorsal	$3.68\pm 0.09^{bc}_{ns}$	$3.72\pm 0.11^{c}_{ns}$	$20.70\pm 0.91^{b}_{ns}$	$19.92\pm 0.65^{b}_{ns}$	$18.00\pm 1.07^{b}_{ns}$	$21.17\pm 1.23^{b}_{ns}$
6	Abdomen lateral	$3.53\pm 0.05^{e}_{ns}$	$3.65\pm 0.07^{c}_{ns}$	$14.88\pm 0.80^{c}_{ns}$	$13.52\pm 1.07^{c}_{ns}$	$17.00\pm 1.14^{bc}_{ns}$	$19.67\pm 0.91^{b}_{ns}$
7	Abdomen ventral	$2.15\pm 0.07^{e}_{ns}$	$2.52\pm 0.14^{d}_{ns}$	$12.58\pm 0.86^{d}_{ns}$	$13.38\pm 1.28^{c}_{ns}$	$11.83\pm 0.27^{d}_{ns}$	$12.83\pm 0.41^{c}_{ns}$
8	Carpal	$4.15\pm 0.24^{b}_{ns}$	$4.28\pm 0.10^{b}_{ns}$	$1.60\pm 0.11^{e}_{ns}$	$1.60\pm 0.12^{d}_{ns}$	$56.50\pm 2.25^{a}_{ns}$	$59.00\pm 2.64^{a}_{ns}$

$P < 0.01$, significant at 1% level. Means having same superscript are not significantly different in different body regions (ANOVA).

ns - Non significant difference between male and female pigs
* Significant at 5 per cent level
** Significant at 1 per cent level (Student's-t-test)

Table 3. Micrometrical parameters of the layers of the epidermis at different body regions, µm

Sl. No	Body Regions	Stratum Corneum Mean ±S.E		Stratum Lucidum Mean ±S.E		Stratum Granulosum Mean ±S.E		Stratum Spinosum Mean ±S.E		Stratum Basalis Mean ±S.E		Epidermis Mean ±S.E	
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
1	Snout	103.00^{a} ±0.31	106.50^{a} ±0.62	301.67^{a} ±0.66	325.17^{a} ±4.83	84.67^{a} ±0.86	80.33^{a} ±5.52	410.00^{a} ±0.63	445.50^{a} ±1.44	22.17^{bc} ±4.07	29.17^{ab} ±1.96	921.50^{a} ±1.53	986.67^{a} ±3.41
2	Dorsal nasal	56.45^{bcd} ±2.43	56.85^{bc} ±6.93	17.28^{b} ±0.55	9.87^{b} ±1.58	25.42^{c} ±1.07	12.95^{de} ±1.37	51.08^{bc} ±2.76	82.85^{b} ±3.50	32.93^{a} ±9.89	27.92^{ab} ±2.26	183.17^{b} ±3.73	190.43^{b} ±16.97
3	Neck dorsal	70.92^{b} ±7.65	77.70^{b} ±14.87	0.00^{d}	0.00^{d}	26.83^{c} ±7.51	24.67^{c} ±2.70	37.00^{de} ±6.77	43.17^{cd} ±2.06	24.67^{abc} ±5.83	19.12^{c} ±1.53	159.41^{c} ±10.65	164.65^{b} ±15.34
4	Neck ventral	66.17^{bc} ±2.15	44.98^{cd} ±2.83	0.00^{d}	0.00^{d}	15.53^{cd} ±0.87	18.50^{cde} ±1.85	24.60^{e} ±0.08	37.38^{cd} ±1.23	16.48^{c} ±3.48	22.82^{bc} ±2.80	122.78^{d} ±2.68	123.68^{c} ±5.31
5	Abdomen dorsal	50.58^{cde} ±9.10	64.45^{bc} ±1.92	0.00^{d}	0.00^{d}	9.87^{d} ±0.68	21.58^{cd} ±1.74	33.33^{de} ±4.30	26.52^{d} ±0.80	29.82^{ab} ±2.25	18.50^{c} ±1.17	123.60^{d} ±8.91	131.05^{c} ±2.21
6	Abdomen lateral	41.70^{de} ±2.60	33.68^{d} ±3.72	0.00^{d}	0.00^{d}	5.24^{d} ±1.68	9.25^{e} ±1.98	37.93^{cde} ±3.79	38.87^{cd} ±1.40	21.50^{bc} ±3.95	27.75^{ab} ±3.28	106.38^{d} ±8.37	109.55^{c} ±6.90
7	Abdomen ventral	33.92^{e} ±1.53	44.02^{cd} ±3.52	24.05^{c} ± 0.27	18.91^{c} ±0.27	3.39^{d} ± 0.27	3.41^{cd} ±3.03	46.25^{cd} ±3.58	26.52^{d} ±1.84	24.05^{abc} ±5.19	19.12^{c} ±1.53	111.62^{d} ±6.31	112.47^{c} ±3.52
8	Carpal	33.92^{e} ±4.41	48.83^{cd} ±1.68	0.00^{d}	0.00^{d}	43.78^{b} ± 8.37	35.02^{b} ±2.42	63.52^{b} ±4.71	51.53^{c} ±0.41	23.43^{abc} ±1.17	32.27^{a} ±1.46	169.58^{bc} ±4.00	167.65^{b} ±3.89

$P < 0.01$, significant at 1% level. Means having same superscript are not significantly different.

Table 4. Correlation and regression coefficients of different parameters on the skin thickness

Sl. No	Parameters	Correlation coefficient				Regression coefficient			
		Region I		Region II		Region I		Region II	
		Female	Male	Female	Male	Female	Male	Female	Male
1.	Thickness of epidermis	0.28^{ns}	0.30^{ns}	0.95**	0.98**	-	-	283.43	340.84
2.	Thickness of dermis	0.87**	0.88**	0.78**	0.78**	767.42	71.08	781.08	43.71
3.	Thickness of subcutaneous fat	0.60**	0.56**	-0.75**	-0.76**	4.77	5.54	-0.24	-0.28

** Significant at 1 per cent level ns Non-significant

Region I - Dorsal neck, ventral neck, dorsal abdomen, lateral abdomen and ventral abdomen regions

Region II - Snout, dorsal nasal and carpal regions

Table 5. Micrometrical parameters and the average number of rete pegs and dermal papillae per field in different body regions

Sl. No.	Regions	Rete pegs						Dermal papillae					
		Female			Male			Female			Male		
		No. per field	Depth µm	Width µm	No. per field	Depth µm	Width µm	No. per field	Height µm	Width µm	No. per field	Height µm	Width µm
1	Snout	10.00	452.00	39.00	12.00	416.00	45.00	8.00	325.00	39.00	10.00	390.00	52.00
2	Dorsal nasal	8.33	167.60	52.90	9.10	176.00	46.81	6.11	195.30	112.85	6.50	164.02	111.63
3	Neck dorsal	3.80	56.72	43.80	4.00	48.00	36.37	5.60	62.27	78.81	5.70	51.63	93.72
4	Neck ventral	3.80	28.01	23.80	4.20	32.00	36.37	5.60	26.97	54.98	5.80	31.64	93.72
5	Abdomen dorsal	2.50	40.71	38.10	3.00	29.00	41.32	3.50	52.32	83.51	3.62	31.45	101.75
6	Abdomen lateral	2.50	66.08	43.90	3.20	56.50	34.56	2.33	54.30	98.68	2.50	32.08	41.33
7	Abdomen ventral	4.80	43.77	40.70	4.80	58.00	27.12	4.50	57.28	31.45	4.60	78.92	70.30
8	Carpal	6.20	43.18	26.50	6.50	54.30	35.03	6.70	30.71	40.70	6.90	52.90	83.88

Table 6. Micrometrical parameters of the layers of dermis at different body regions in pigs, µm

Sl. No.	Regions	Papillary layer Mean ±S.E		Reticular layer Mean ±S.E		Dermis Mean ±S.E	
		Female	Male	Female	Male	Female	Male
1	Snout	563.33^{a} ±6.28	570.00^{a} ±7.29	4654.17^{c} ±9.80	4762.67^{b} ±3.88	5221.00^{a} ±12.49	5336.17^{a} ± 7.39
2	Dorsal nasal	135.67^{c} ±9.27	369.47^{b} ±29.76	3837.50^{b} ±7.71	3847.50^{b} ±7.20	3973.17^{c} ±11.49	4216.97^{a} ± 36.42
3	Neck dorsal	399.60^{b} ±26.37	155.17^{cd} ±3.66	3384.33^{e} ±21.95	3592.67^{c} ±16.05	3783.93^{d} ±15.93	3747.83^{b} ± 14.69
4	Neck ventral	47.48^{d} ±3.83	89.42^{e} ±15.02	2622.33^{f} ±3.85	2601.83^{e} ±15.95	2669.82^{f} ±0.83	2691.25^{c} ± 3.45
5	Abdomen dorsal	131.97^{c} ±22.39	117.17^{de} ±8.94	3588.00^{c} ±43.90	3606.00^{c} ±11.00	3719.97^{d} ±26.69	3723.17^{b} ±6.39
6	Abdomen lateral	72.15^{d} ±7.53	185.00^{c} ±11.70	3487.33^{d} ±7.67	3425.83^{d} ±108.17	3559.48^{e} ±1.13	3610.83^{b} ± 104.29
7	Abdomen ventral	93.12^{cd} ±8.08	74.00^{e} ±8.42	2127.67^{g} ±7.21	2493.00^{e} ±21.87	2220.78^{g} ±2.11	2567.00^{c} ±26.34
8	Carpal	88.18^{cd} ±15.33	102.37^{e} ±13.66	4000.67^{a} ±28.31	4154.00^{a} ± 13.43	4088.85^{b} ±17.28	4256.37^{a} ± 13.40

$P<0.01$, significant at 1% level. Means having same superscript are not significantly different.

Fig. 1 Regions of skin sample collection in the pig

Fig.2 Comparison of skin thickness in different areas in male and female pigs

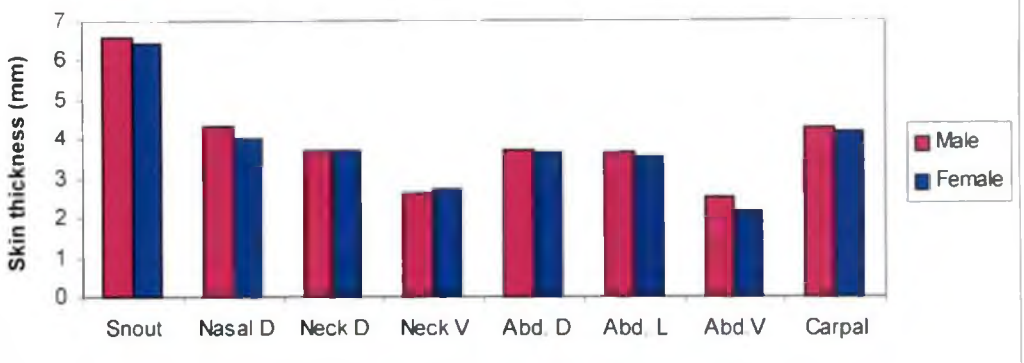


Fig. 3 Comparison of epidermal thickness in different areas in male and female pigs

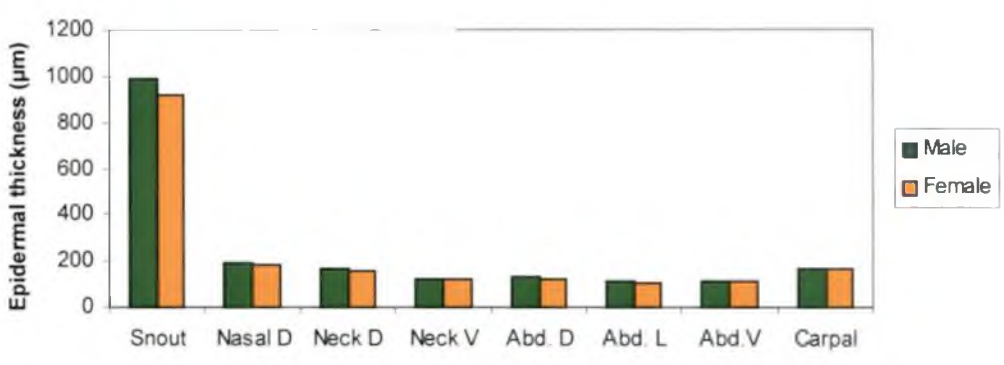


Fig. 4 Comparison of dermal thickness in different areas in male and female pigs

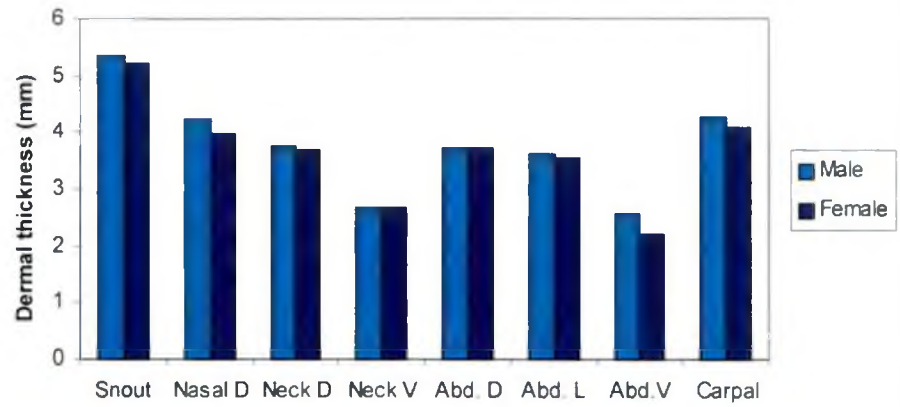
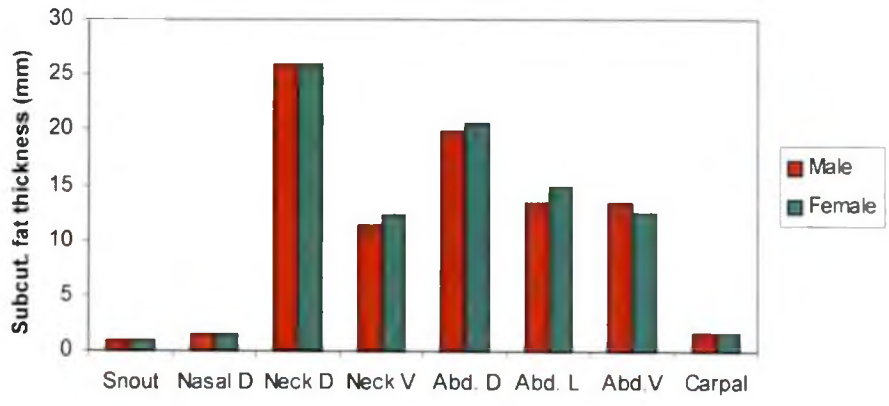


Fig. 5 Comparison of subcut. fat thickness in different areas in male & female pigs



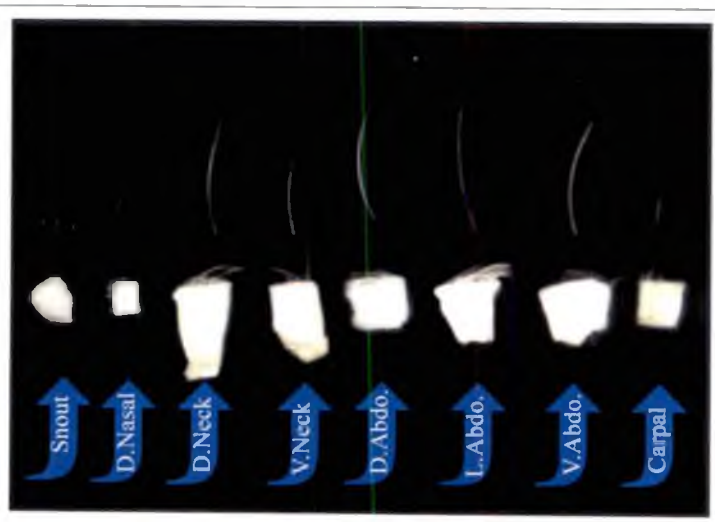
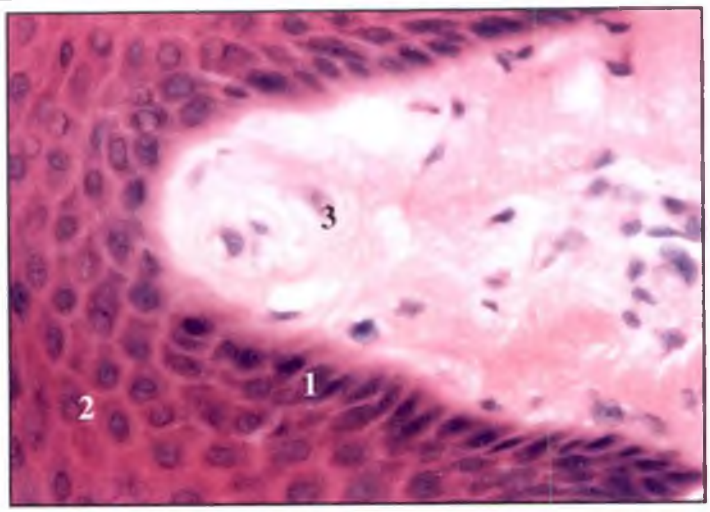
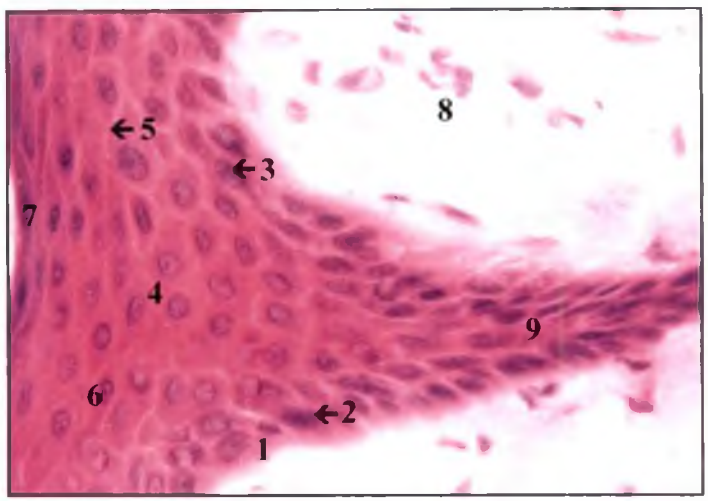


Fig. 6 Skin samples from different regions and hair from the corresponding regions



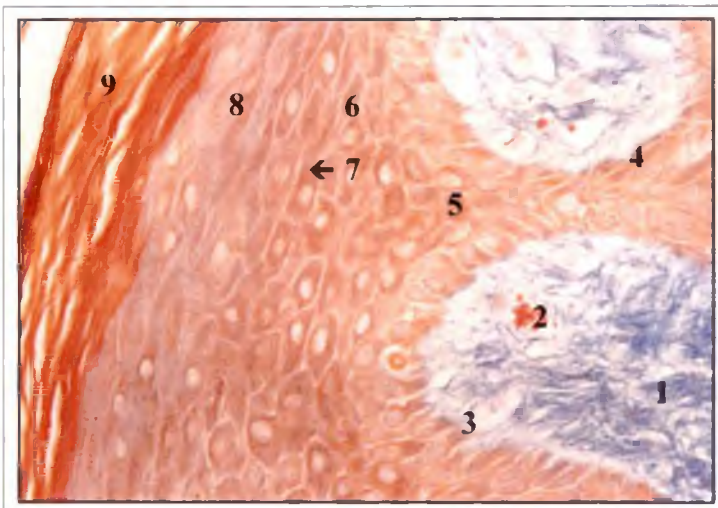
1. Stratum basalis
2. Stratum spinosum
3. Dermal papilla

Fig. 7 Section of skin in the dorsal nasal region showing the layers of the epidermis. H&E. x 400



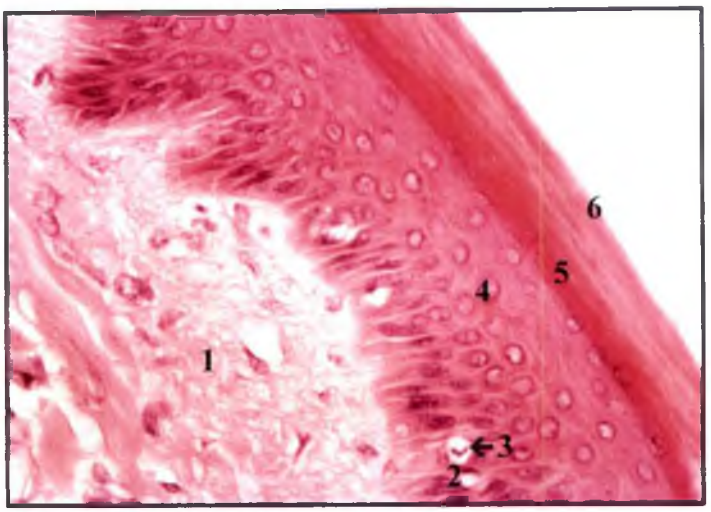
1. Stratum basalis
2. Nucleus
3. Nucleolus
4. Stratum spinosum
5. Clear zone
6. Flattened cells of stratum spinosum
7. Stratum granulosum
8. Dermal papilla
9. Rete Peg

Fig. 8 Section of skin in the dorsal nasal region showing rete peg. H&E. x 400



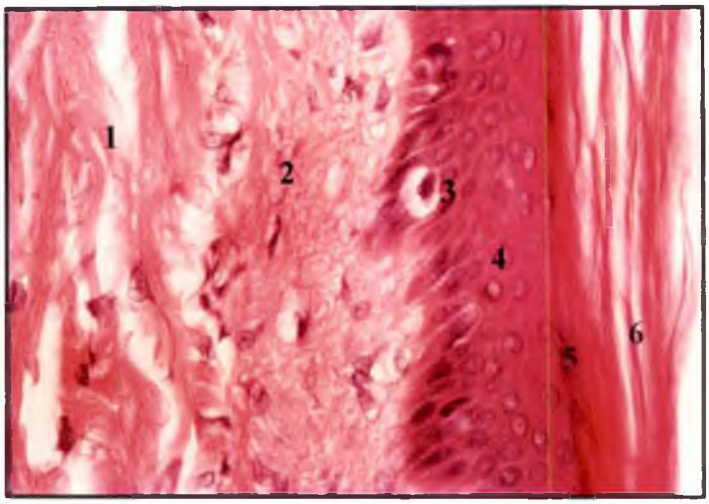
1. Dermal papilla
2. Capillaries
3. Cytoplasmic processes of the basal cells
4. Basement membrane
5. Deeper layers of stratum spinosum
6. Flattened cells of stratum spinosum
7. Prekeratin granules
8. Stratum granulosum
9. Stratum corneum

Fig. 9 Section of skin in the neck dorsal region. Ayoub-Shklar method for keratin and prekeratin x 400



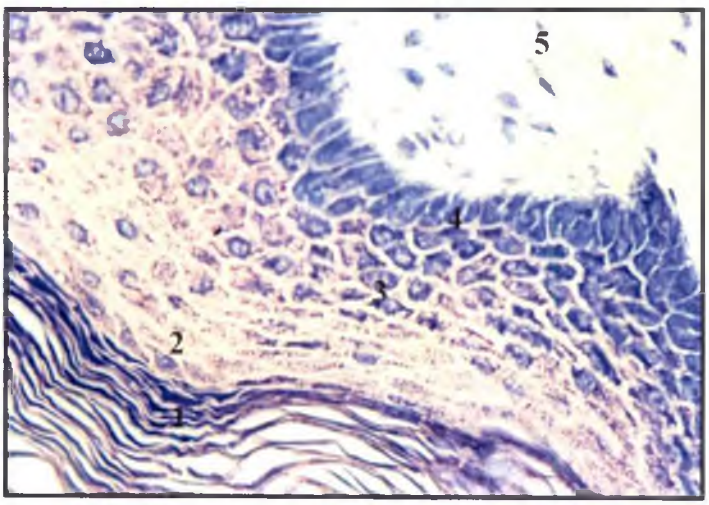
1. Papillary dermis
2. Clear cells
3. Reniform nucleus
4. Stratum spinosum
5. Stratum lucidum
6. Stratum corneum

Fig. 10 Section of skin in the ventral abdominal region. H&E. x 400



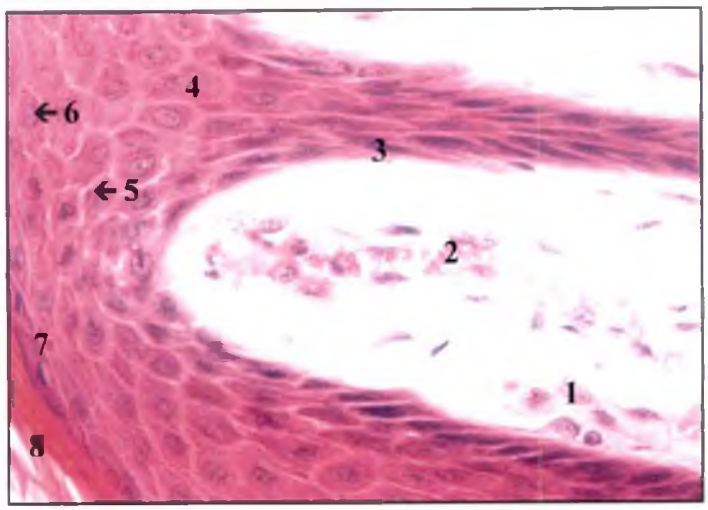
1. Reticular layer
2. Papillary layer
3. Clear cell
4. Stratum spinosum
5. Stratum granulosum
6. Stratum corneum

Fig. 11 Section of skin in the abdomen dorsal region. H&E. x 400



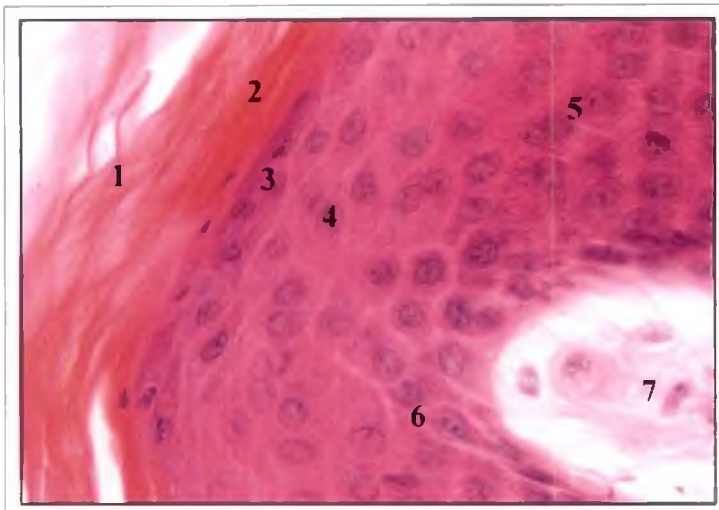
1. Stratum corneum
2. Stratum granulosum
3. Stratum spinosum
4. Stratum basalis
5. Papillary dermis

Fig. 12 Section of skin in the dorsal nasal region. PTAH x 400



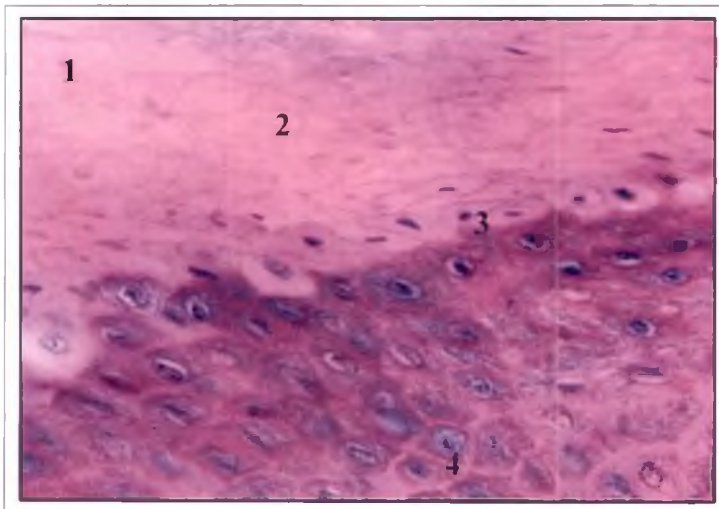
1. Dermal papilla
2. Capillaries
3. Stratum basalis
4. Stratum spinosum
5. Inter cellular processes between adjacent cells
6. Nucleolus
7. Stratum granulosum
8. Stratum corneum

Fig. 13 Section of skin in the dorsal nasal region. H&E. x 400



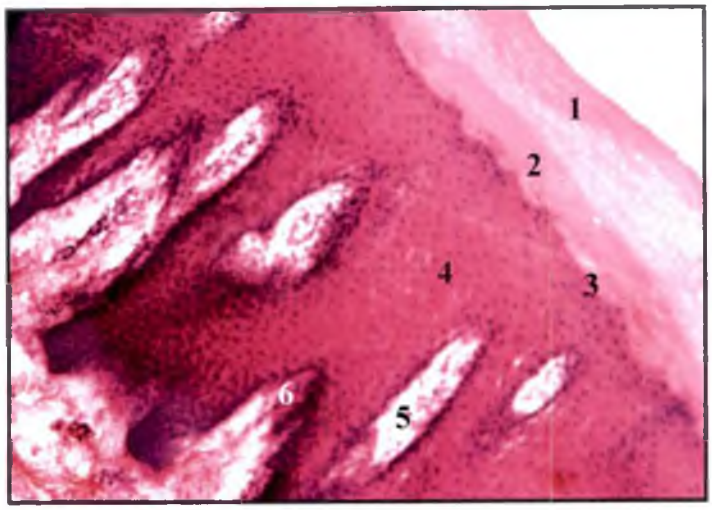
1. Stratum corneum
2. Stratum lucidum
3. Stratum granulosum
4. Peripheral cells of the stratum spinosum
5. Middle layer of the stratum spinosum
6. Deeper layer of the stratum spinosum
7. Dermal papilla

Fig. 14 Section of skin in the dorsal nasal region showing superficial layers of epidermis . H&E. x 400



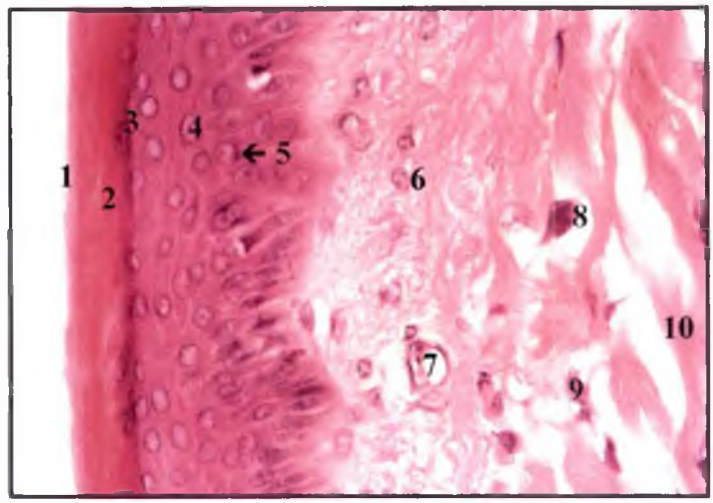
1. Stratum corneum
2. Stratum lucidum
3. Stratum granulosum
4. Stratum spinosum

Fig. 15 Section of epidermis in the snout region. H&E. x 400



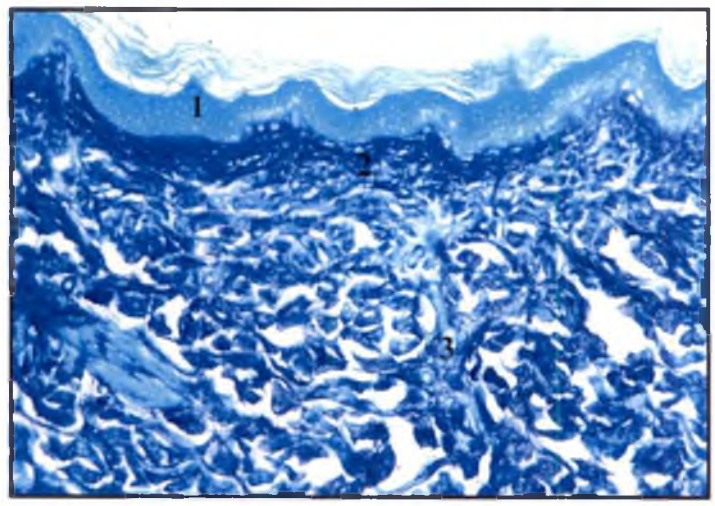
1. Stratum corneum
2. Stratum lucidum
3. Stratum granulosum
4. Stratum spinosum
5. Dermal papilla
6. Stratum basalis

Fig. 16 Section of skin in the snout region. H&E. x 100



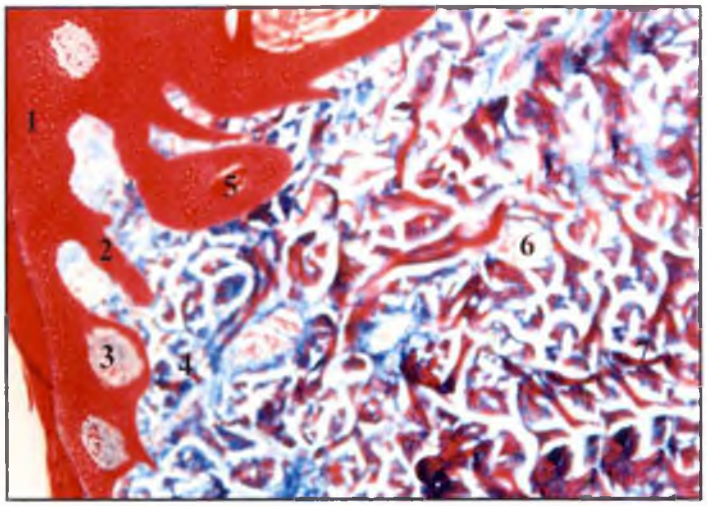
1. Stratum corneum
2. Stratum lucidum
3. Stratum granulosum
4. Stratum spinosum
5. Nucleus showing peripheral condensation of the chromatin
6. Papillary dermis
7. Capillary
8. Macrophage
9. Fibroblast
10. Reticular layer

Fig. 17 Section of skin in the dorsal abdominal region. H&E. x 400



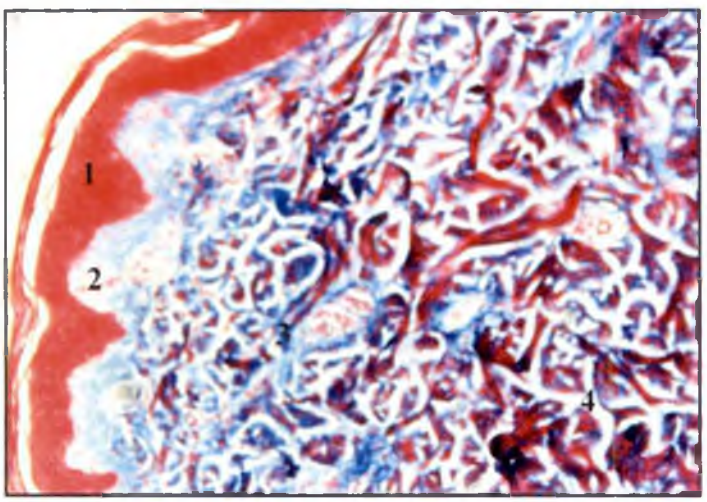
1. Epidermis
2. Papillary layer
3. Reticular layer

Fig. 18 Section of skin in the lateral abdominal region. PAS Alcian blue method for mucosubstance x 100



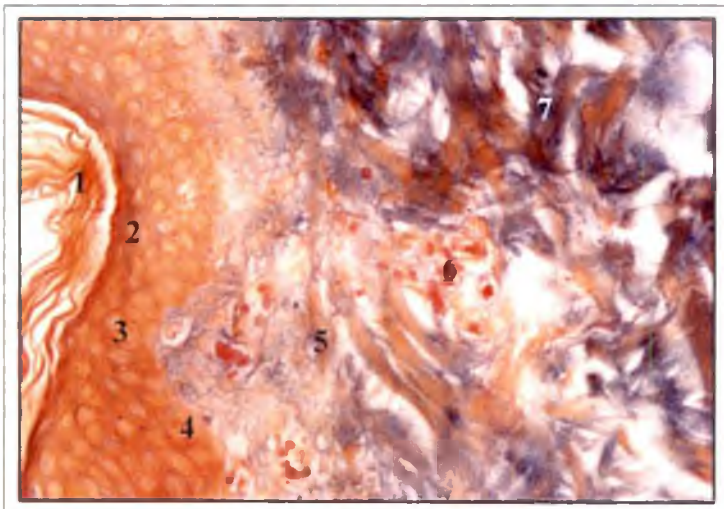
1. Epidermis
2. Rete peg
3. Dermal papilla
4. Papillary layer
5. Hair follicle
6. Blood vessel
7. Reticular layer

Fig. 19 Section of skin in the dorsal nasal region.
Masson's trichrome method x 100



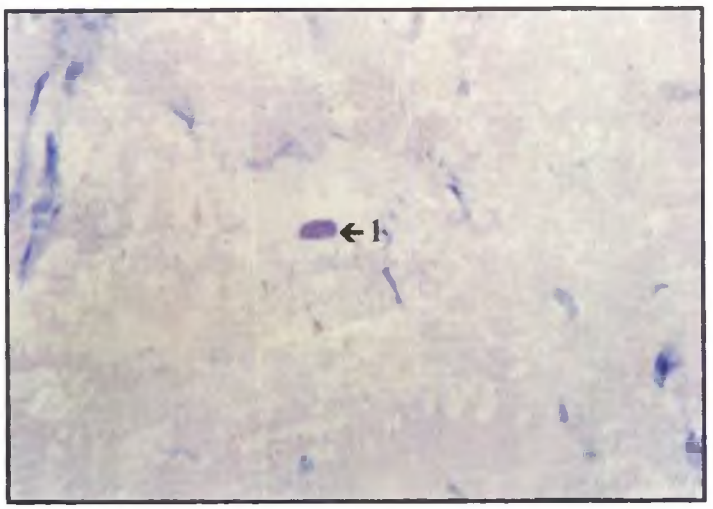
1. Epidermis
2. Papillary layer
3. Collagen fibres
4. Reticular layer

Fig. 20 Section of skin in the ventral neck region.
Masson's trichrome method x 100



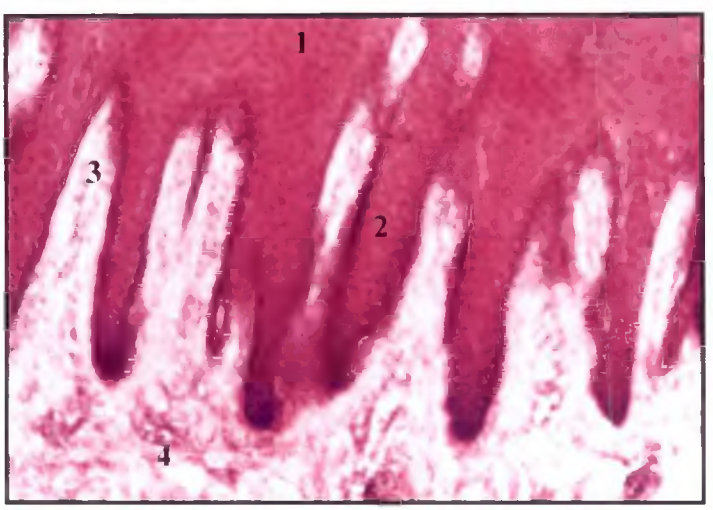
1. Stratum corneum
2 .Stratum granulosum
3. Stratum spinosum
4. Stratum basalis
5. Papillary layer
6. Blood vessel
7. Reticular layer

Fig. 21 Section of skin in the carpal region.
Ayoub-Shklar method for keratin and prekeratin x 400



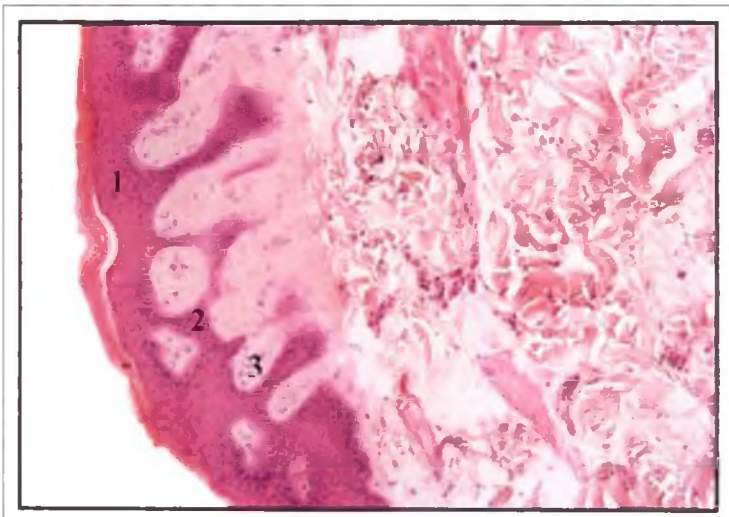
1. Mast cell

Fig. 22 Section of dermis in the carpal region. Toluidine blue method x 400



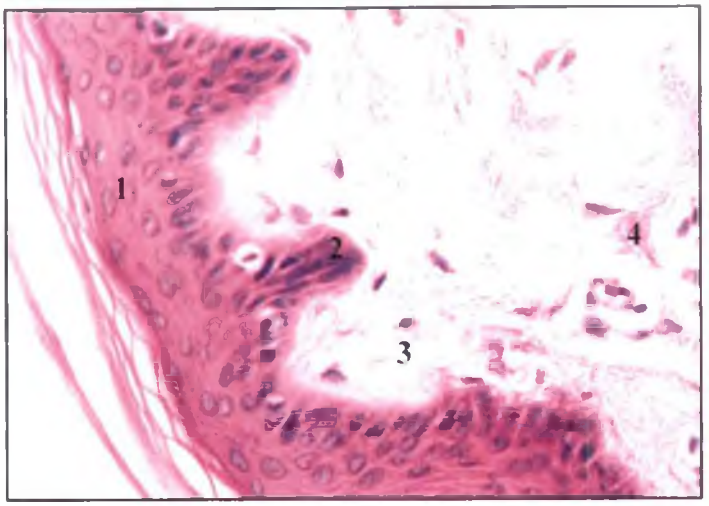
1. Epidermis
2. Rete peg
3. Dermal papilla
4. Dermis

Fig. 23 Section of skin showing numerous dermal papillae in the snout region. H&E. x 100



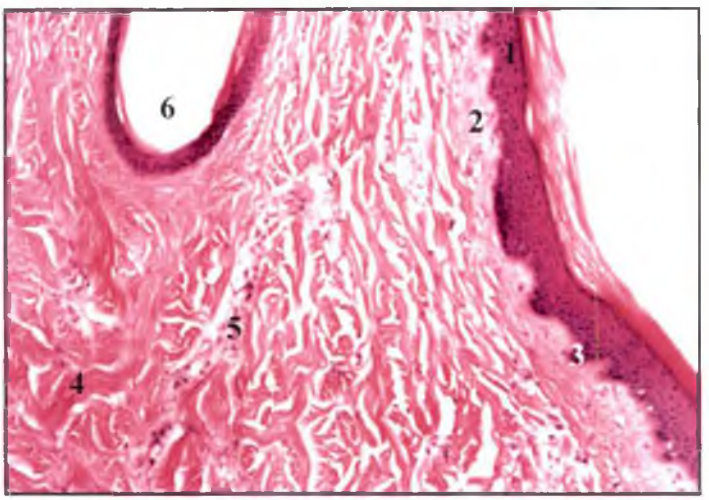
1. Epidermis
2. Compound rete peg
3. Dermis

Fig. 24 Section of skin showing compound rete peg in the ventral abdominal region. H&E. x 100



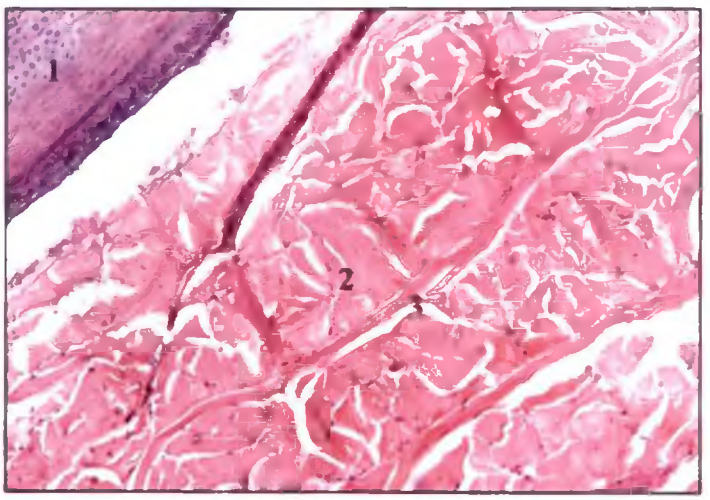
1. Epidermis
2. Rete peg
3. Dermal papilla
4. Dermis

Fig. 25 Section of skin in the ventral abdominal region. H&E. x 400



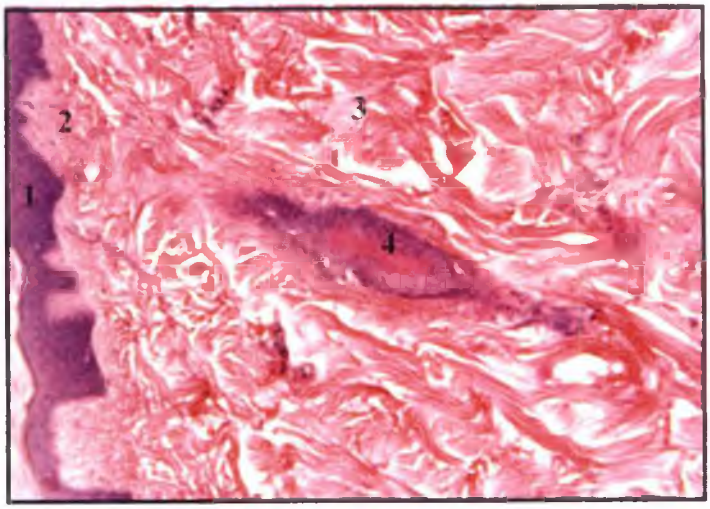
1. Epidermis
2. Papillary layer
3. Rete peg
4. Reticular layer
5. Capillary
6. Hair follicle

Fig. 26 Section of skin in the dorsal abdominal region. H&E. x 100



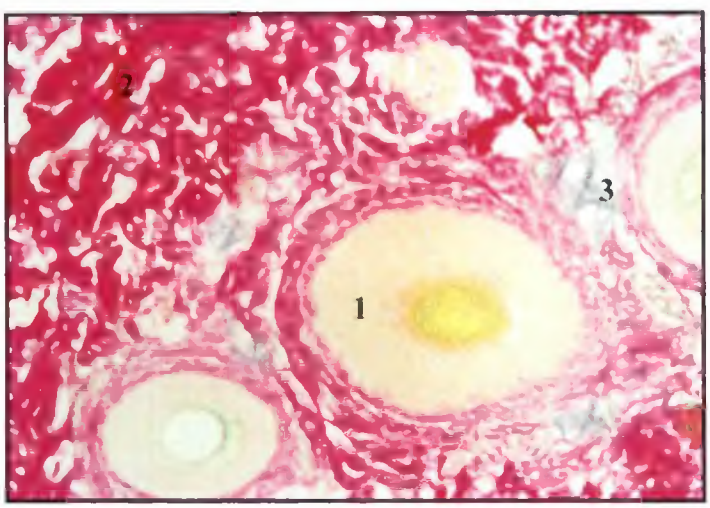
1. Hair follicle
2. Reticular layer of the dermis
3. Perpendicular bundles of collagen fibres

Fig. 27 Section of skin in the dorsal abdominal region. H&E. x 100



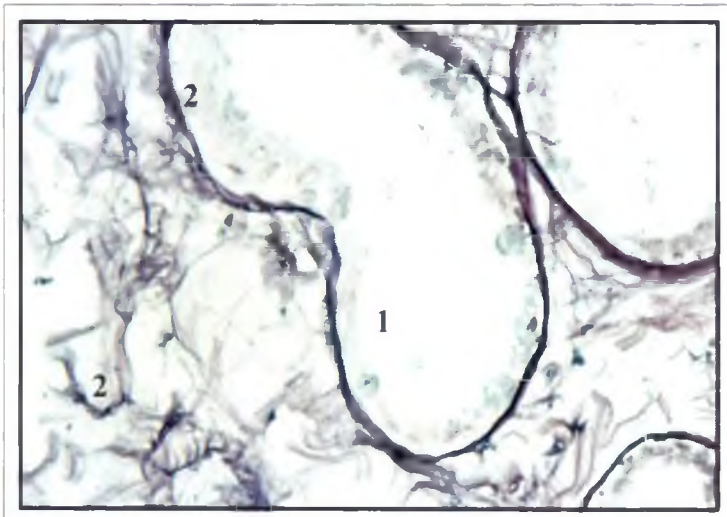
1. Epidermis
2. papillary layer
3. Reticular layer
4. Hair follicle (telogen)

Fig. 28 Section of skin in the carpal region showing irregular network of collagen fibres. H&E. x 100



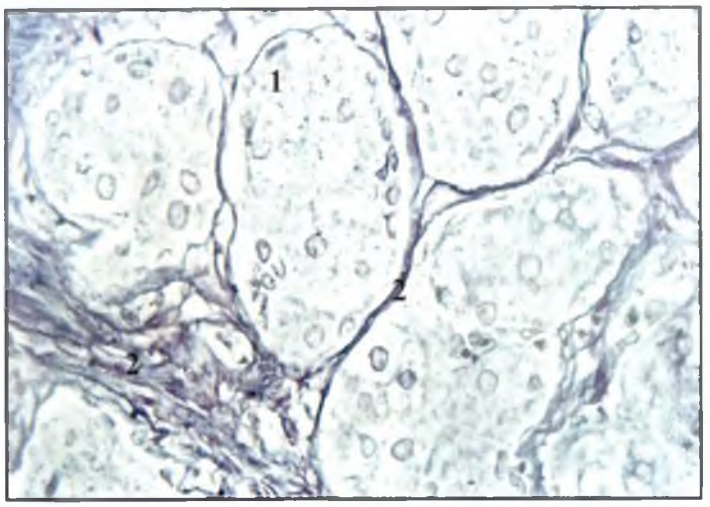
1. Hair follicle
2. Collagen bundle
3. Elastic fibres

Fig. 29 Section of skin in the dorsal nasal region. Verhoeff's elastic stain x 100



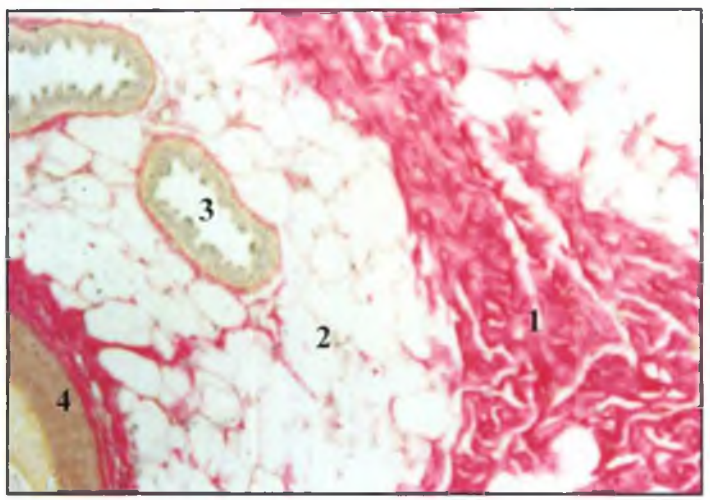
1. Secretory end piece
2. Reticular fibres

Fig. 30 C.S. of sweat gland in the carpal region. Gridley's modification of silver impregnation method x 400



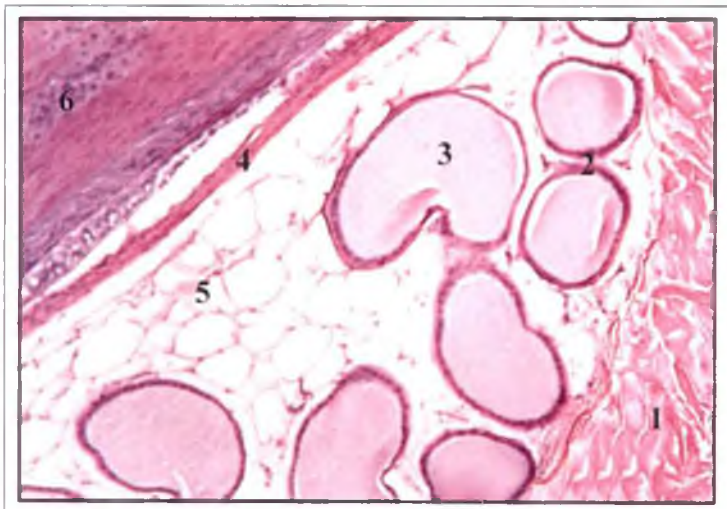
1. Secretory end piece
2. Reticular fibres

Fig. 31 C.S. of sebaceoous gland in the neck dorsal region. Gridley's modification of silver impregnation method for reticular fibers x 400



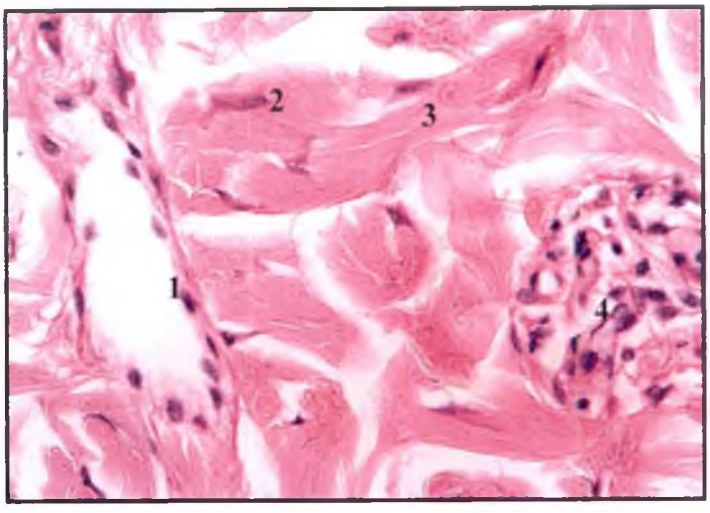
1. Collagen bundle
2. Subcutaneous fat
3. Sweat gland
4. Hair follicle

Fig. 32 Section through the deeper layers of the dermis in the neck dorsal region. Van Gieson's method for collagen fibres x 100



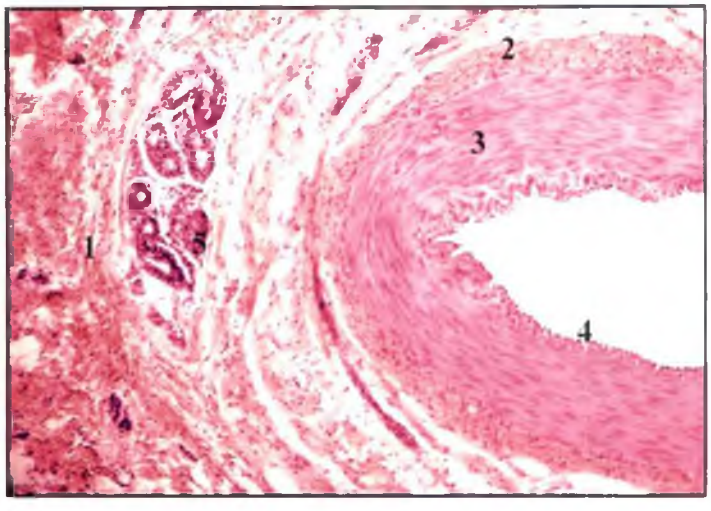
1. Reticular layer
2. Sweat gland
3. Secretion of the sweat gland
4. Arrector pilorum
5. Subcutaneous tissue
6. Hair follicle

Fig. 33 Section through the deeper layers of the dermis in the dorsal abdominal region. H&E. x 100



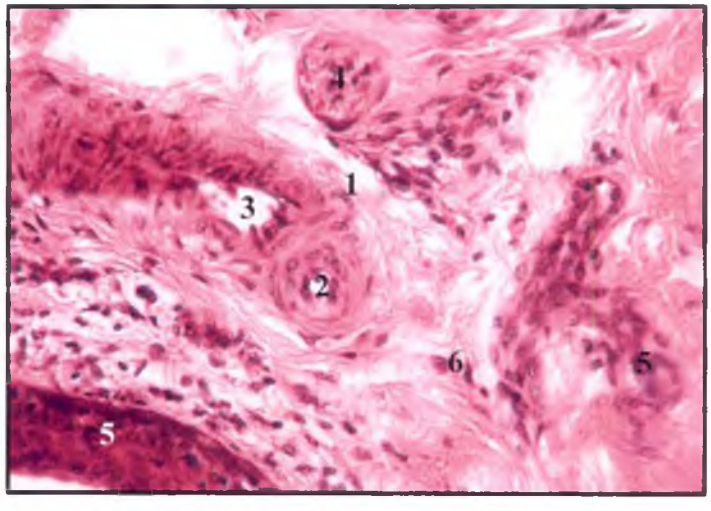
1. Lymph vessel
2. Fibroblast
3. Collagen bundle
4. Nerve bundle

Fig. 34 Section of skin in the neck ventral region. H&E. x 400



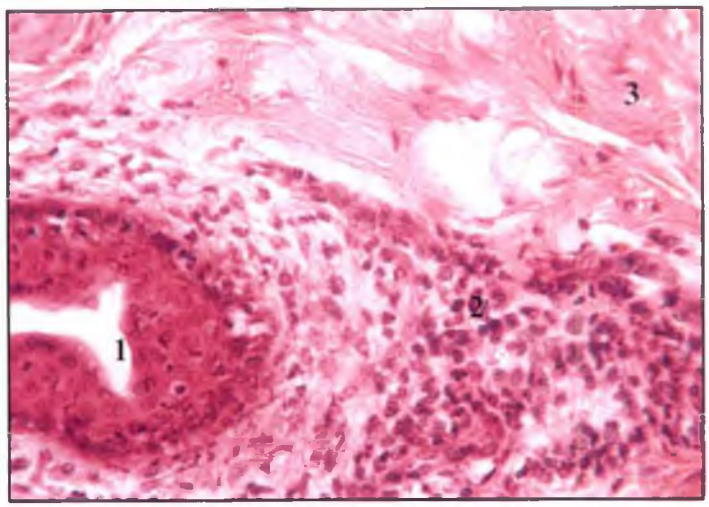
1. Reticular layer
2. Artery
3. Tunica muscularis
4. Endothelium
5. Sweat gland

Fig. 35 Section of skin of the snout region showing blood vessel in the dermis. H&E. x 400



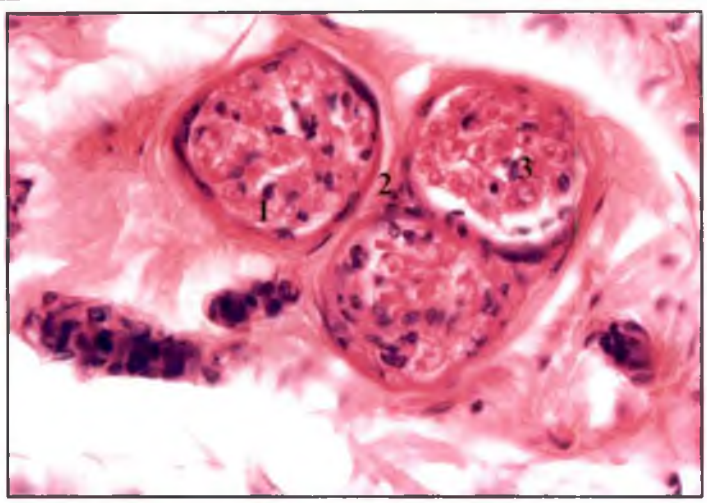
1. Glomus
2. Artery
3. Vein
4. Nerve bundle
5. Duct of sweat gland
6. Dermis

Fig. 36 Section of dermis of the snout region showing the glomus H&E. x 400



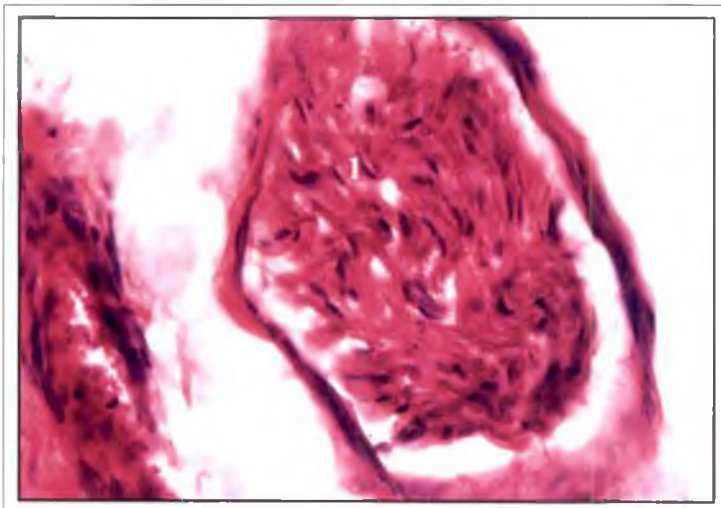
1. Duct of sweat gland
2. Lymphocyte aggregation
3. Dermis

Fig.37 Section of skin of the snout region showing lymphocyte aggregation H&E. x 400



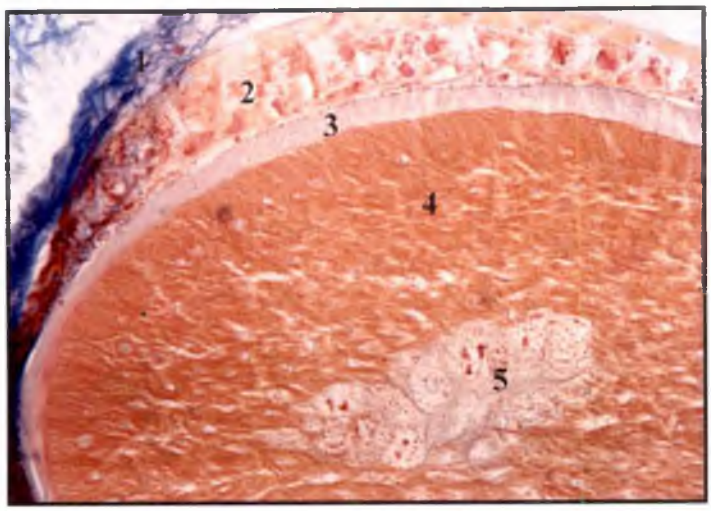
1. C.S. of axon
2. Epineurium
3. Glial cells

Fig. 38 Section of skin of the snout region showing nerve bundle H&E. x 400



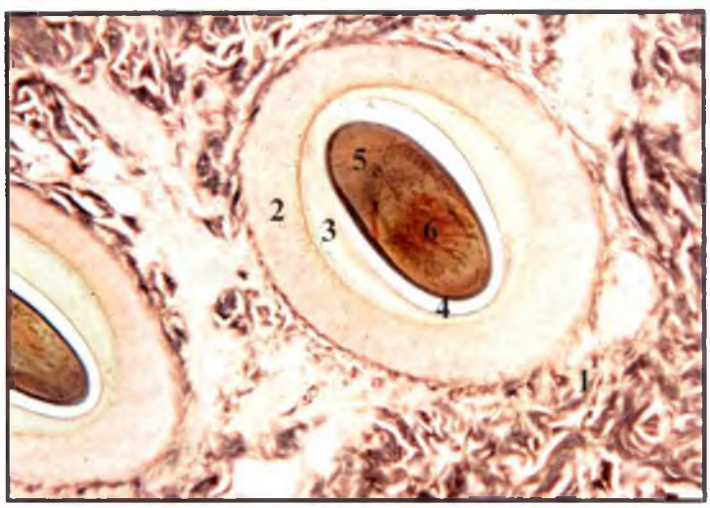
1.Meissner's corpuscles

Fig. 39 Section of skin of the snout region. H&E. x 400



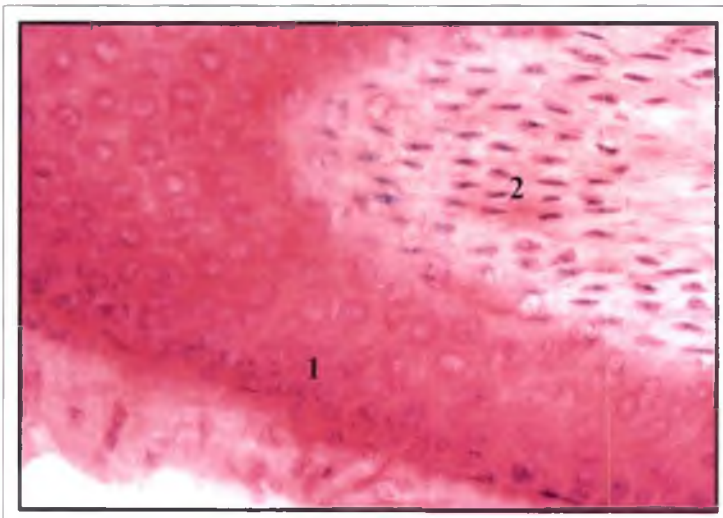
1. Connective tissue of the dermis
2. Inner root sheath
3. Cuticle
4. Cortex
5. Medulla

Fig. 40 C. S. of hair in the dorsal nasal region.
Ayoub-Shklar method for keratin and prekeratin x 400



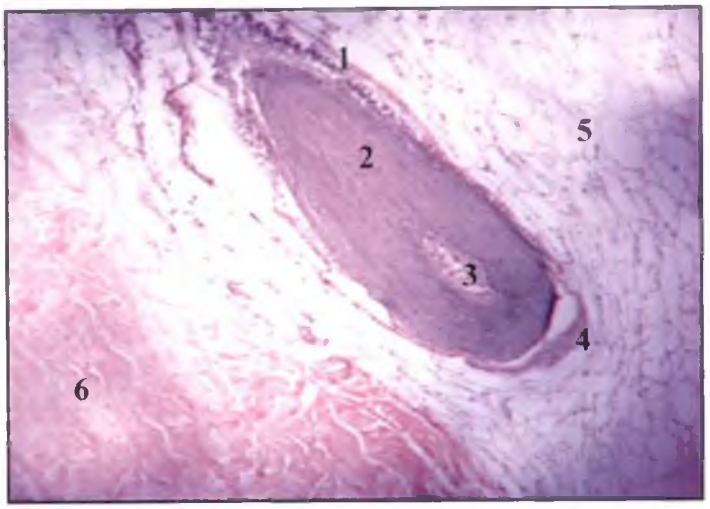
1. Connective tissue sheath
2. Outer root sheath
3. Inner root sheath
4. Cuticle
5. Cortex
6. Medulla

Fig. 41 C. S. of hair in the dorsal abdominal region
Fontana-Masson silver method x 100



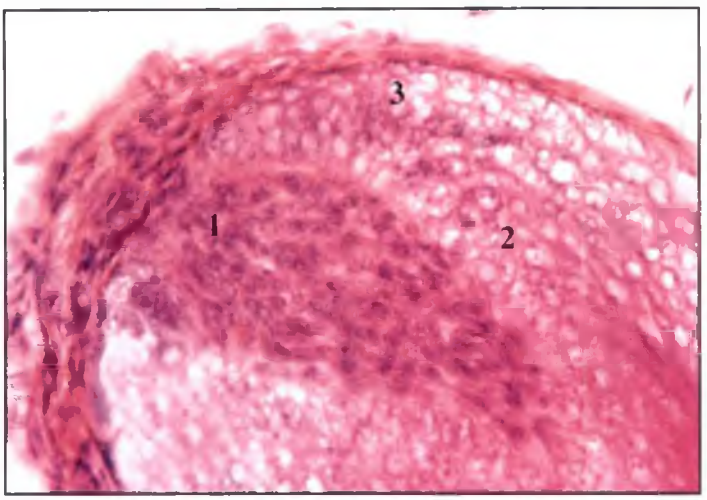
1. Outer root sheath
2. Cortex

Fig. 42 L. S. of hair in the dorsal abdominal region.
H&E. x 400



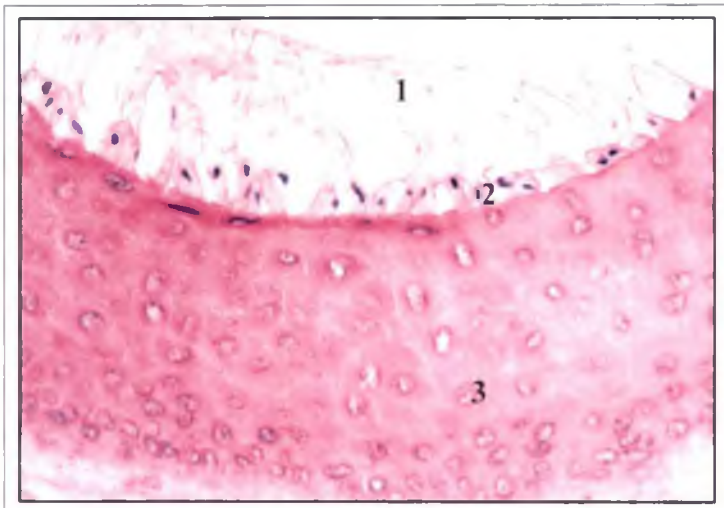
1. Hair follicle
2. Cortex
3. Medulla
4. Arrectores pilorum
5. Subcutis
6. Dermis

Fig. 43 Oblique section of the hair in the dorsal nasal region. H&E. x 100



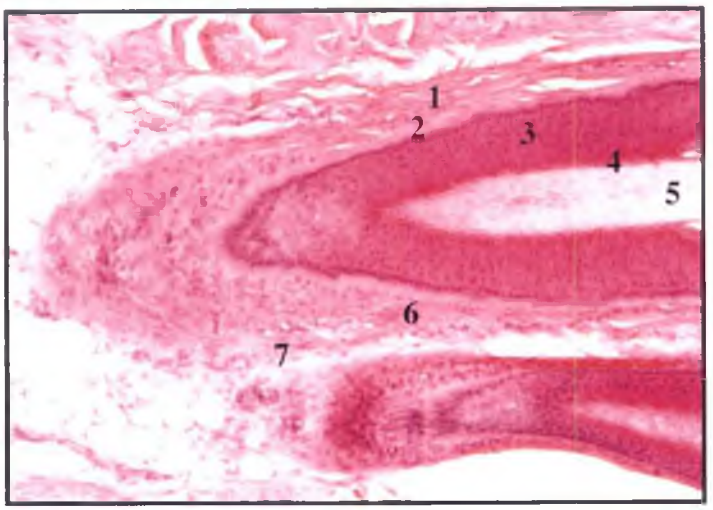
1. Hair papilla
2. Hair matrix cells
3. Hair bulb

Fig. 44 Oblique section of the root of the hair in the dorsal nasal region. H&E. x 400



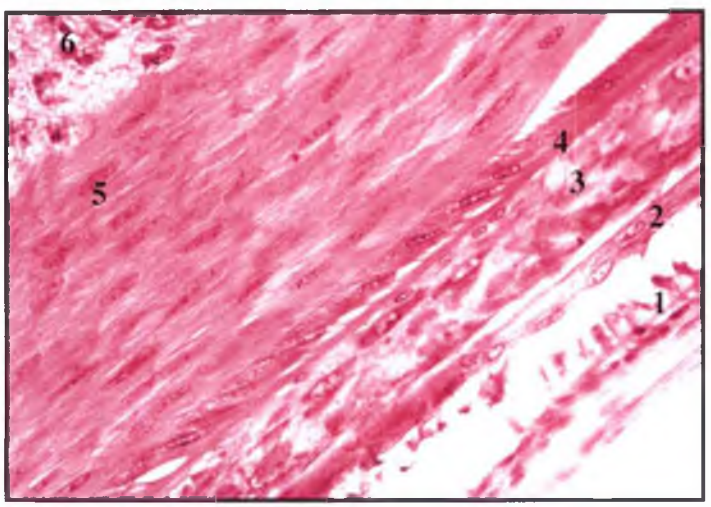
1.Huxley's layer
2.Henle's layer
3.Outer root sheath

Fig. 45 C. S. of the hair follicle in the dorsal nasal region. H&E. x 400



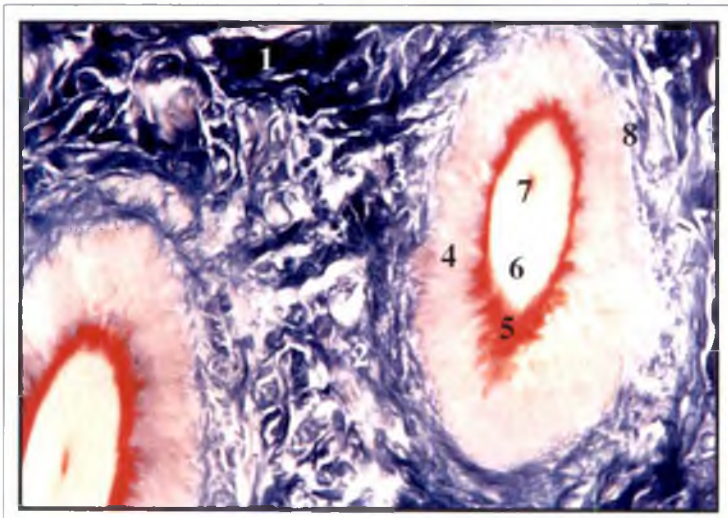
1. Connective tissue sheath
2. Stratum basalis of the outer root sheath
3. Stratum spinosum of the outer root sheath
4. Cuticular interlocking
5. Hair
6. Inner circular connective tissue layer
7. Outer longitudinal connective tissue layer

Fig. 46 L. S. of the hair in the dorsal abdominal region. H&E. x 100



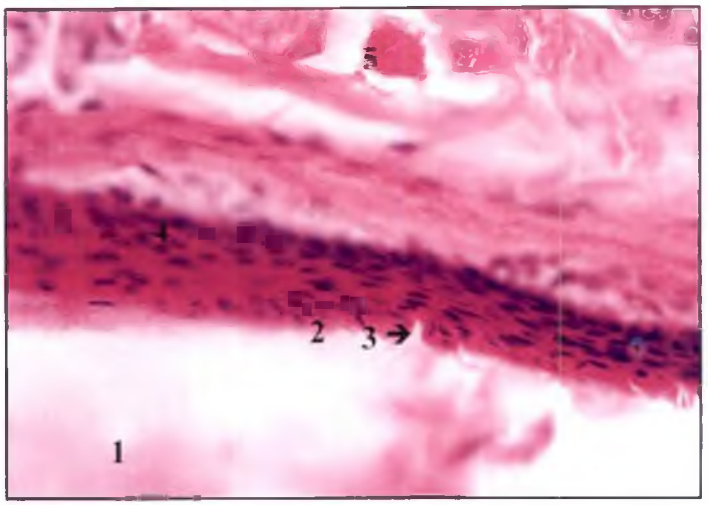
1. Outer root sheath
2. Henle's layer
3. Huxley's layer
4. Cuticle
5. Cortex
6. Medulla

Fig. 47 L. S. of the hair in the dorsal abdominal region. H&E. x 400



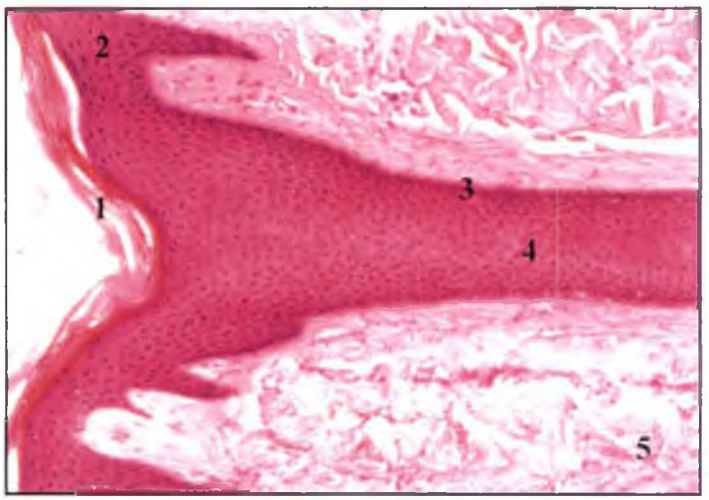
1. Dermis
2. Outer longitudinal connective tissue layer
3. Inner circular connective tissue layer
4. Outer root sheath
5. Inner root sheath
6. Cortex
7. Medulla
8. Glassy membrane

Fig. 48 Section of skin in the carpal region.
Ayoub-Shklar method for keratin and prekeratin x 100



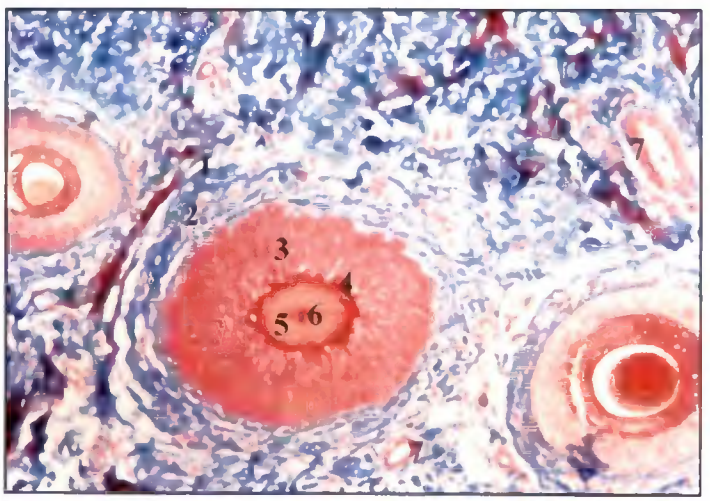
1. Hair
2. Inner root sheath
3. Follicular fold
4. Outer root sheath
5. Dermis

Fig. 49. L.S. of the hair showing follicular fold of inner root sheath in skin of the dorsal nasal region. H&E. x 400



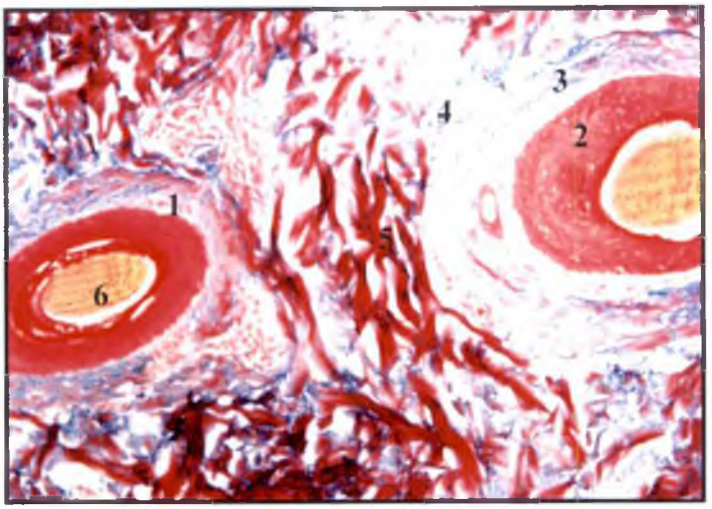
1. Stratum corneum
2. Epidermis
3. Outer root sheath
4. Stratum spinosum
5. Dermis

Fig. 50 L.S. of hair follicle through the external root sheath in the dorsal nasal region. H&E. x 100



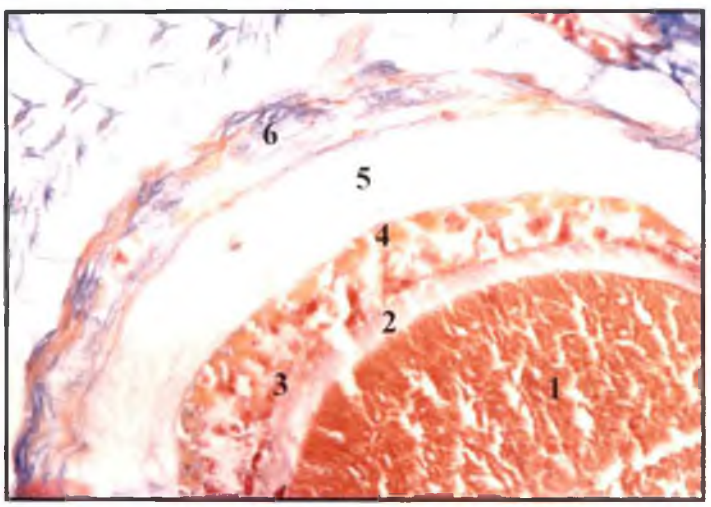
1.Outer longitudinal connective tissue layer
2. Inner circular connective tissue layer
3. Outer root sheath
4. Inner root sheath
5. Cortex
6. Medulla
7. Blood vessel

Fig. 51 C.S. of hair follicles in the dorsal nasal region of skin. Masson's trichrome method x 100



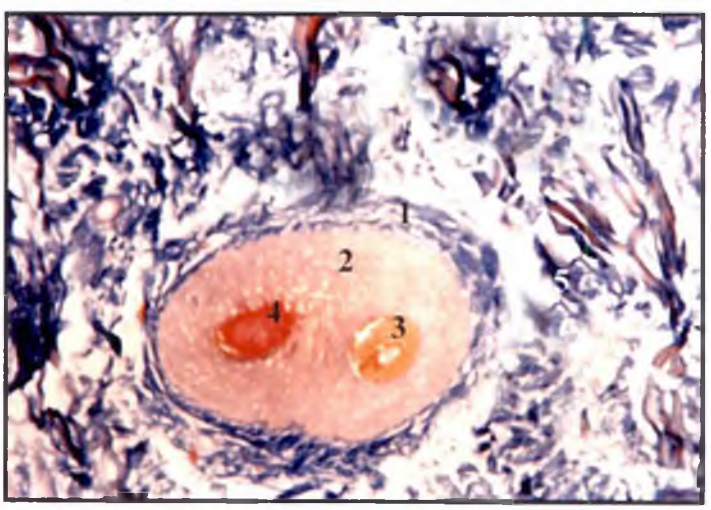
1. Hair follicle
2. Outer root sheath
3. Inner circular connective tissue layer
4. Outer longitudinal connective tissue layer
5. Interfollicular muscle
6. Hair

Fig. 52 Section of the dermis in the dorsal nasal region of skin. Masson's trichrome method x 100



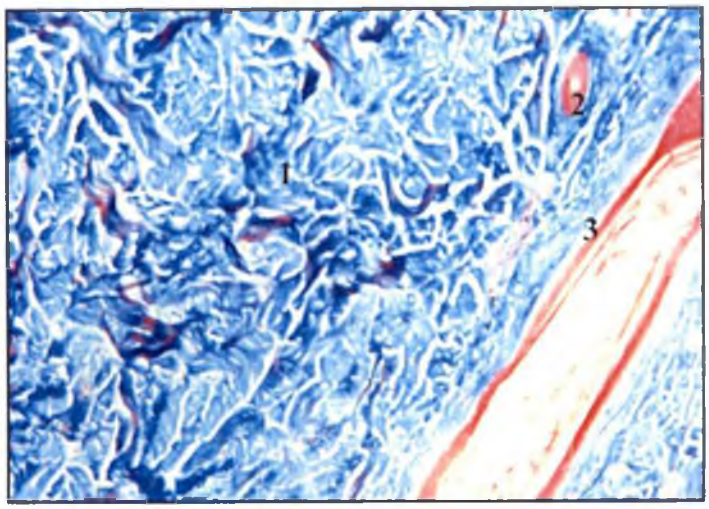
1. Cortex
2. Cuticle
3. Huxley's layer
4. Henle's layer
5. Outer root sheath
6. Inner circular connective tissue layer

Fig. 53 C. S. of hair in the dorsal nasal region. Ayoub-Shklar method for keratin and prekeratin x 400



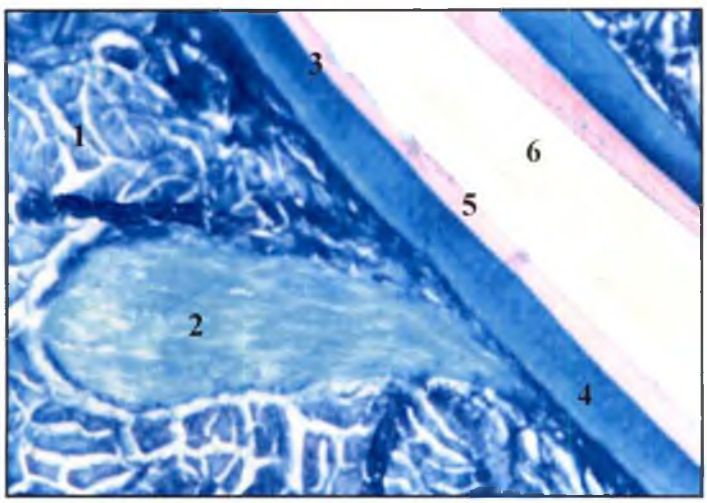
1. Connective tissue sheath
2. Outer root sheath
3. Anagen
4. Catagen
5. Dermis

Fig. 54 Section of skin in the dorsal neck region. Ayoub-Shklar method for keratin and prekeratin x 400



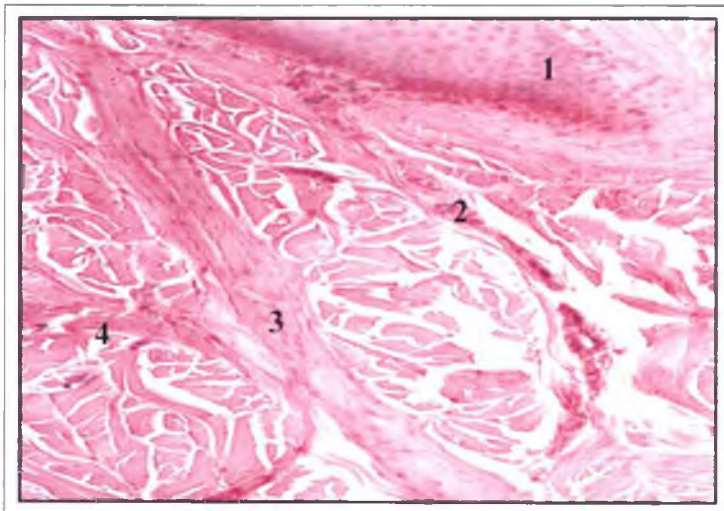
1. Dermis
2. C.S. of telogen
3. L.S. of hair

Fig. 55 Section of skin in the ventral neck region.
Masson's trichrome method x 100



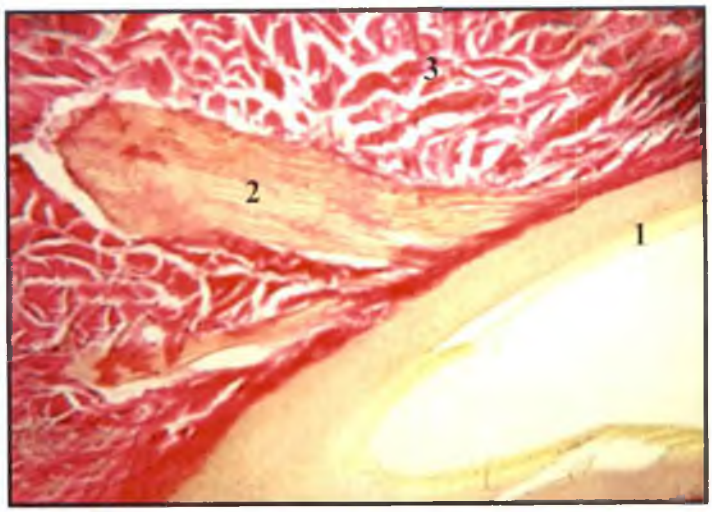
1. Dermis
2. Arrectores pilorum
3. Hair follicle
4. Outer root sheath
5. Inner root sheath
6. Hair

Fig. 56 Section of skin in the lateral abdominal region.
PAS Alcian blue method for mucosubstance x 100



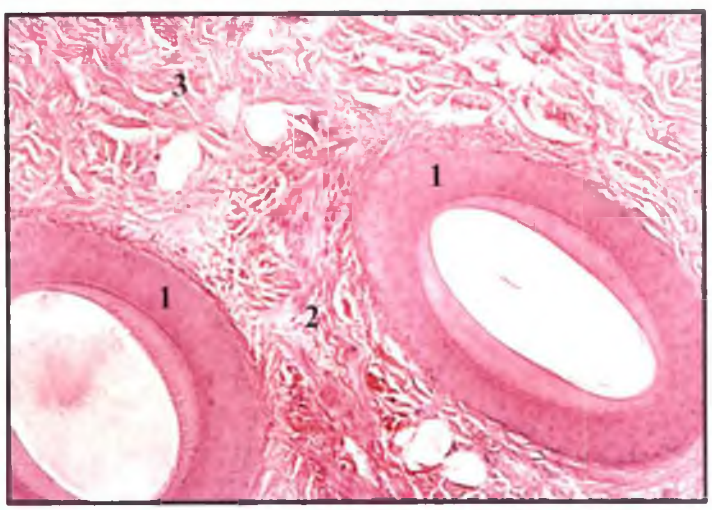
1. Hair follicle
2. Duct of sweat gland
3. Arrectores pilorum
4. Dermis

Fig. 57 Section of skin in the dorsal abdominal region. H&E. x 100



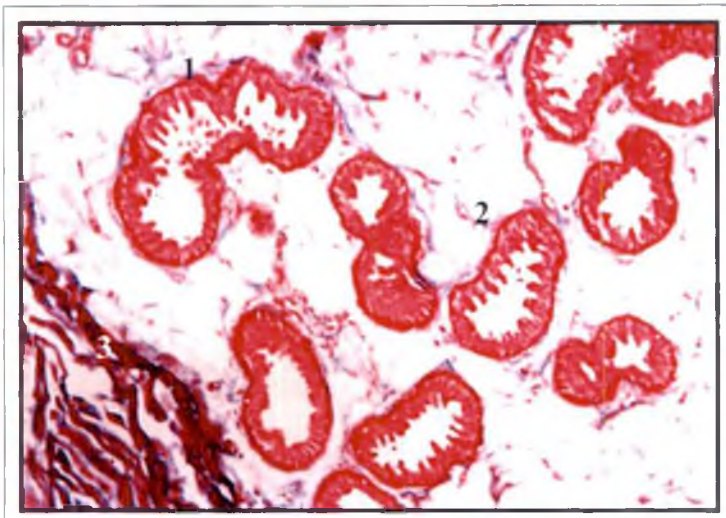
1. Hair
2. Arrectores pilorum
3. Dermis

Fig. 58 L.S. of hair follicle in the dorsal abdominal region. Van Gieson's method x 100



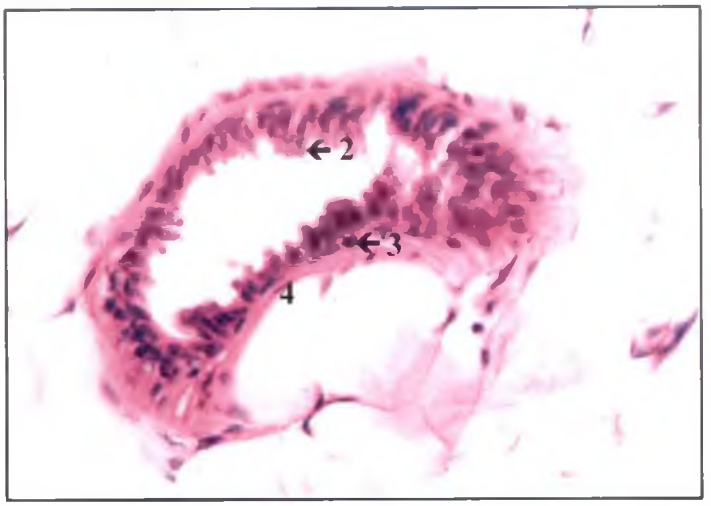
1. Hair follicle
2. Interfollicular muscle
3. Dermis

Fig. 59 Section of dermis showing the interfollicular muscle in the lateral abdominal region. H&E. x 100



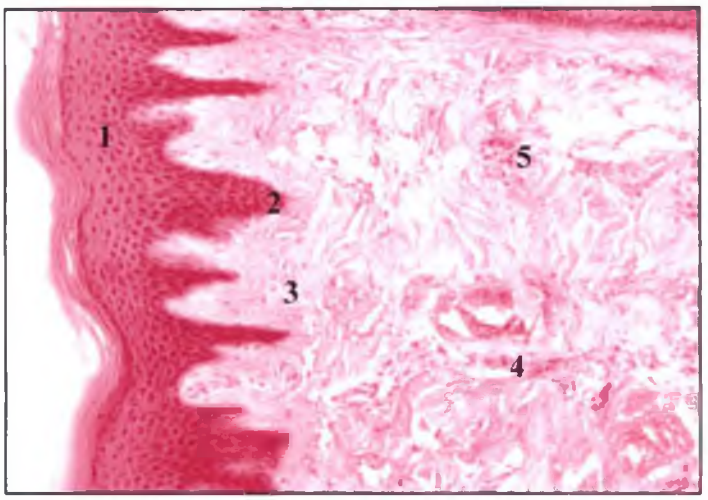
1. Secretory end piece of sweat glands
2. Interlobular connective tissue
3. Reticular layer of the dermis

Fig. 60 C. S. of the sweat glands in the neck dorsal region. Masson's trichrome method x 100



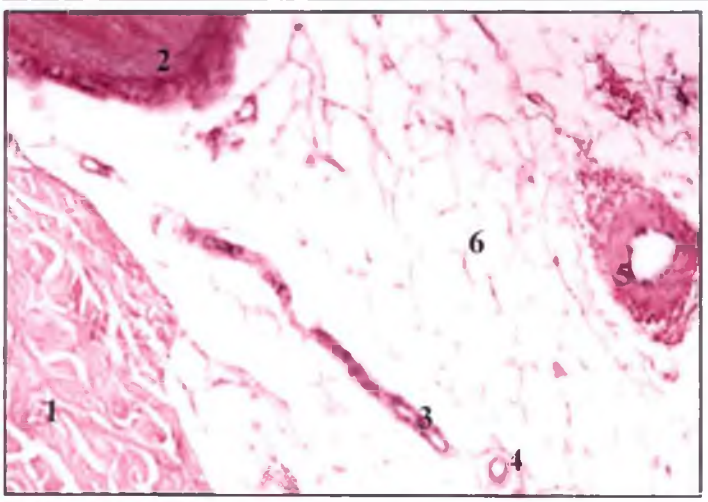
1. Lining epithelium
2. Apical bleb
3. Myoepithelial cells
4. Basement membrane

Fig. 61 C.S. of secretory end piece of the apocrine sweat gland in the neck dorsal region. H&E. x 400



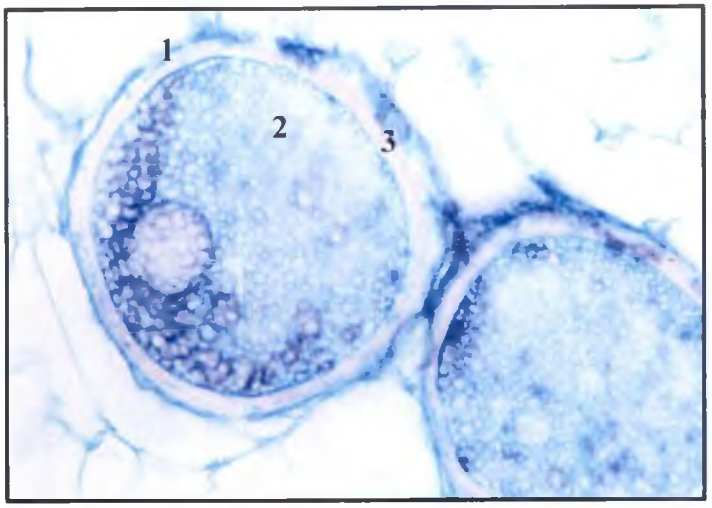
1. Epidermis
2. Rete peg
3. Papillary dermis
4. Duct of sweat gland
5. Dermis

Fig. 62 Section of skin in the dorsal nasal region. H&E. x 100



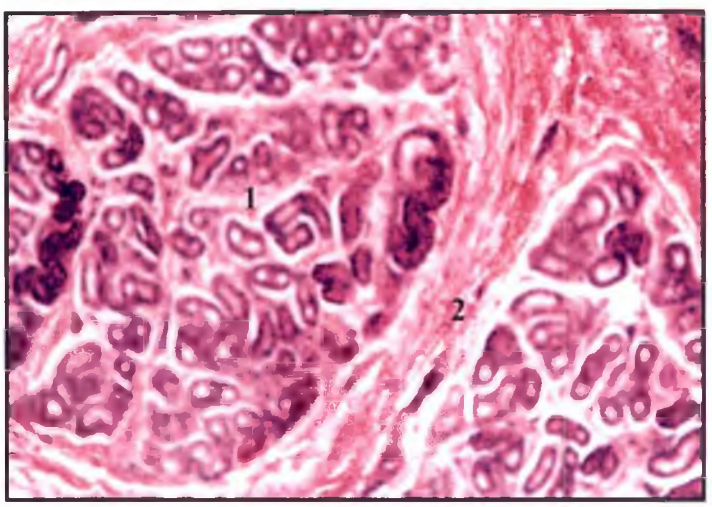
1. Reticular dermis
2. Hair follicle
3. L.S. of the duct of sweat gland
4. C.S. of the duct of sweat gland
5. Blood vessel
6. Subcutaneous tissue

Fig. 63 Section through the deeper layer of the skin and the subcutis. H&E. x 100



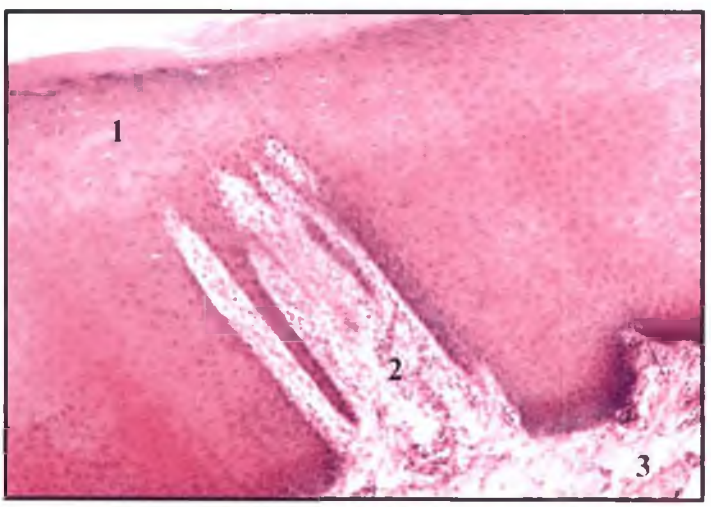
1. Secretory end piece
2. Secretion
3 Lining epithelium
4. Interlobular septum

Fig. 64 Section of sweat gland in the lateral abdominal region. PAS Alcian blue method for mucosubstance x 400



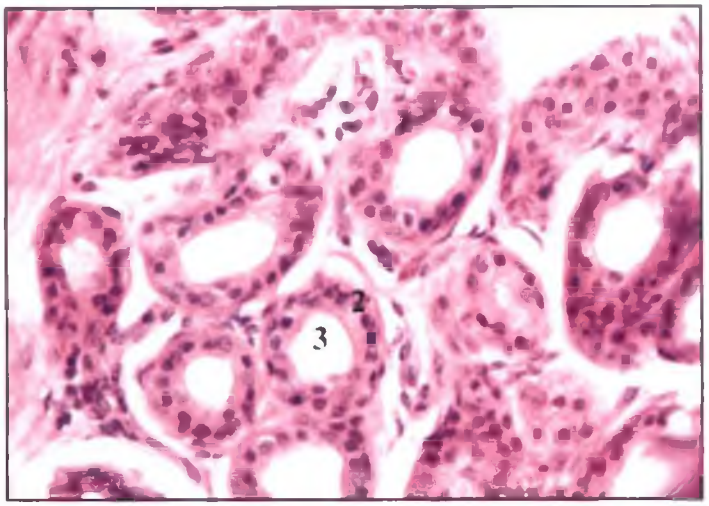
1. Eccrine sweat glands
2. Interlobular septum

Fig. 65 C.S. of sweat glands in the skin of the snout region. H&E. x 100



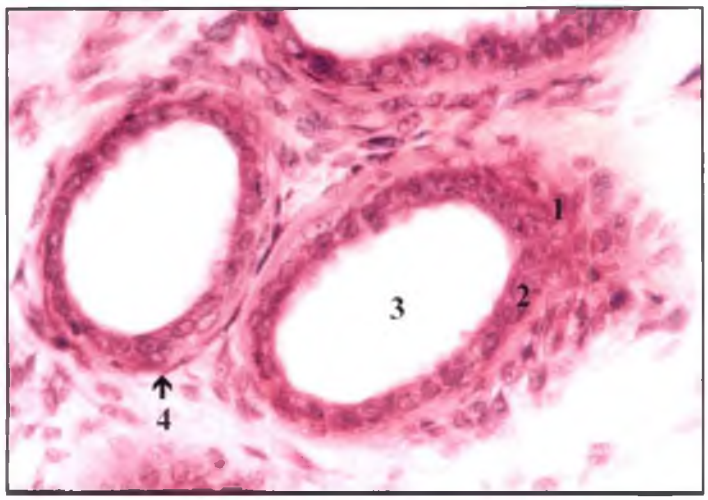
1. Epidermis
2. Branched ducts of sweat glands
3. Dermis

Fig. 66 Section of the skin of the snout region showing compound ducts of sweat glands H&E. x 100



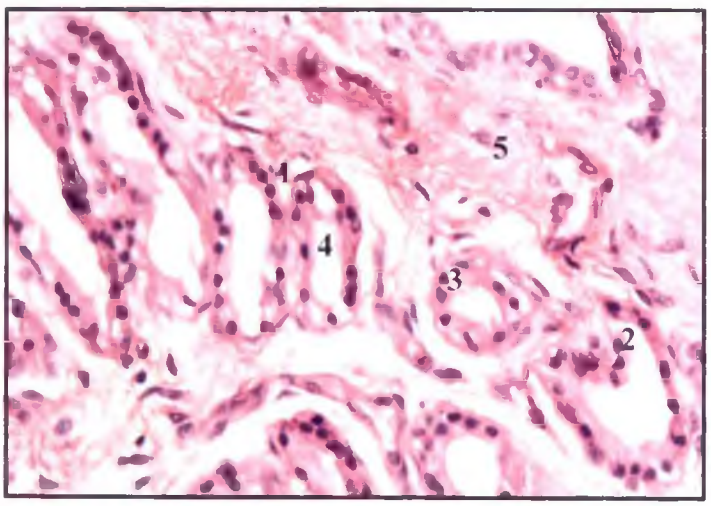
1. Secretory end piece
2. Lining epithelium
3. Lumen

Fig. 67 C.S. of secretory end piece of eccrine sweat glands in the skin of the snout region. H&E. x 400



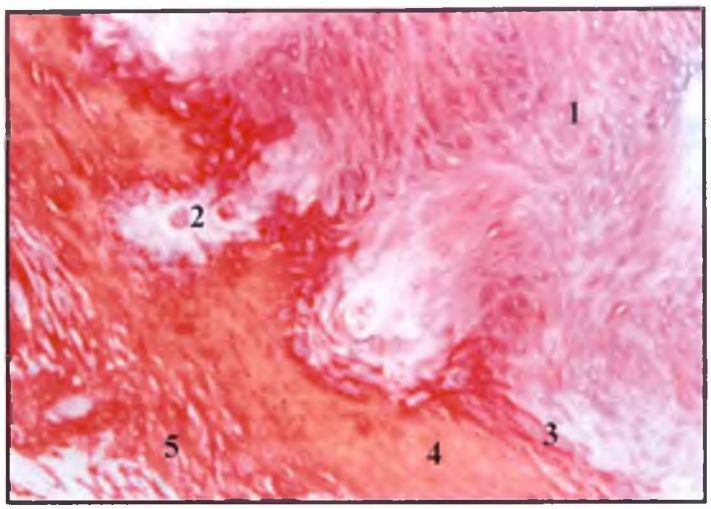
1. Secretory end piece
2. Lining epithelium
3. Lumen
4. Basement membrane

Fig. 68 C.S. of secretory end piece of eccrine sweat glands in the skin of the dorsal nasal region. H&E. x 400



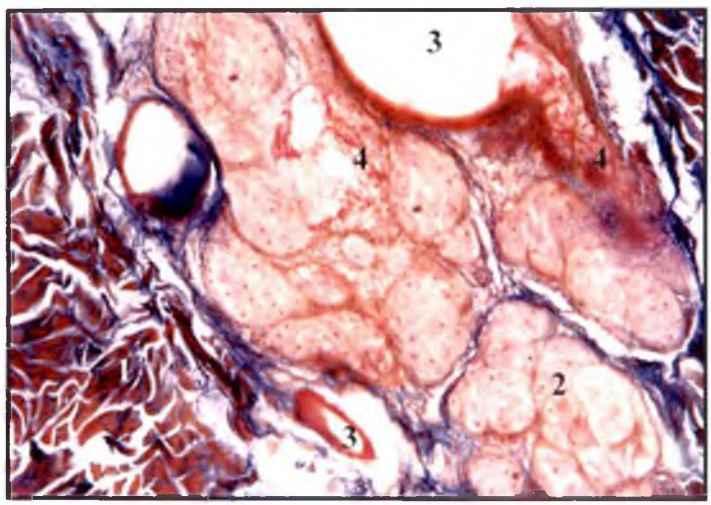
1. Secretory end piece
2. Clear cell
3. Dark cell
4. Lumen
5. Connective tissue

Fig. 69 C.S. of secretory end piece of eccrine sweat glands in the skin of the snout region. H&E. x 400



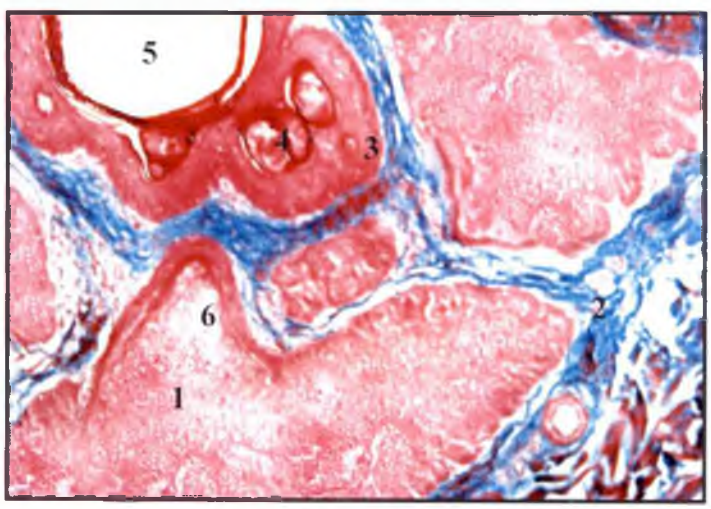
1. Stratum spinosum
2. Acrosyringium
3. Stratum granulosum
4. Stratum lucidum
5. Stratum corneum

Fig. 70 Section of epidermis in the snout region. Masson's trichrome method x 400



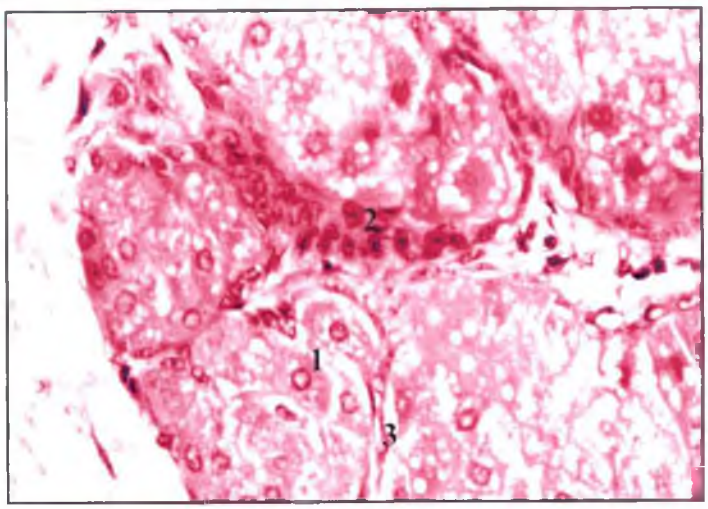
1. Dermis
2. Sebaceous gland
3. Duct of the sebaceous gland
4. Necrosed area

Fig. 71 L.S. of sebaceous gland in the skin of the dorsal neck region. Ayoub-Shklar method for keratin and prekeratin x 100



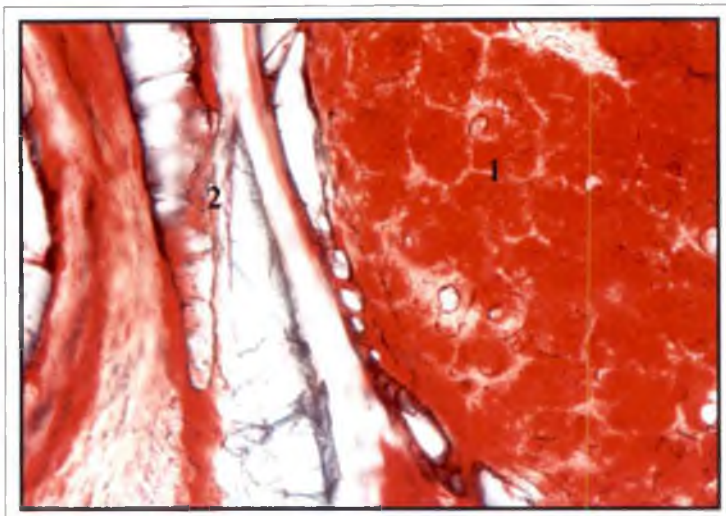
1. C.S. of the sebaceous gland
2. Connective tissue sheath
3. Duct of the sebaceous gland
4. Secretion in the duct
5. Hair follicle
6. Necrosed area

Fig. 72 Section of skin in the dorsal neck region showing the C.S. of the sebaceous gland. Masson's trichrome method x 100



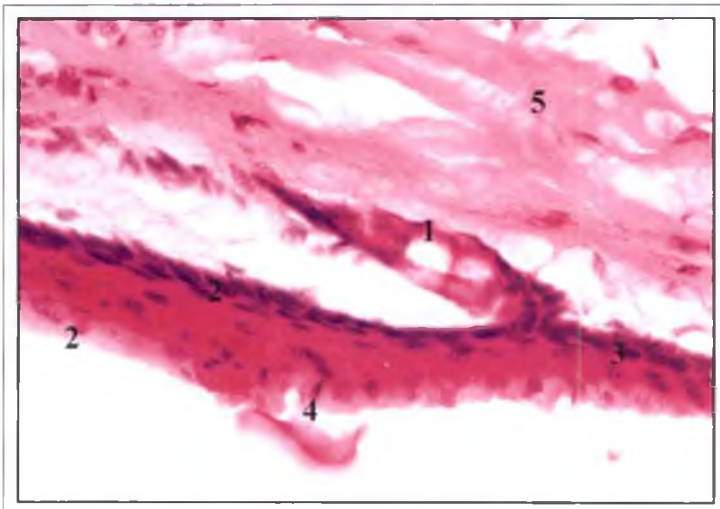
1. Large polygonal cell
2. Mitotic basal cells
3. Interlobular septum

Fig. 73 Section of skin in the dorsal neck region showing the C.S. of the sebaceous gland. H&E. x 400



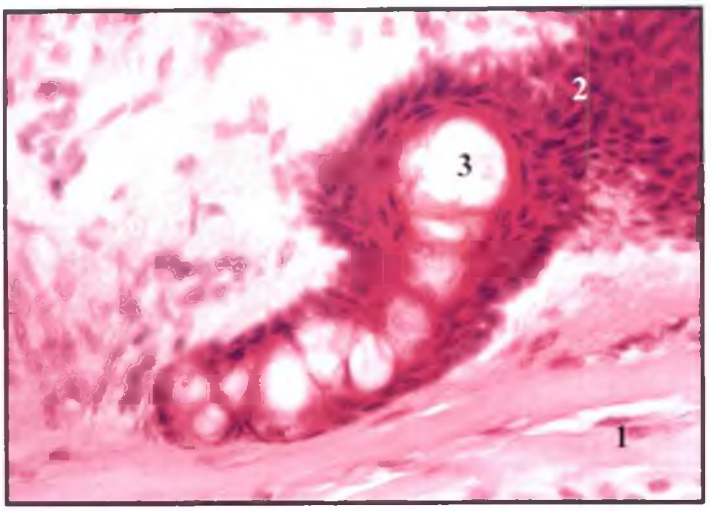
1. Sebaceous gland epithelium
2. Interlobular septum

Fig. 74 C. S. of the sebaceous gland in the dorsal neck region. Oil red O in propylene glycol method for fats x 400



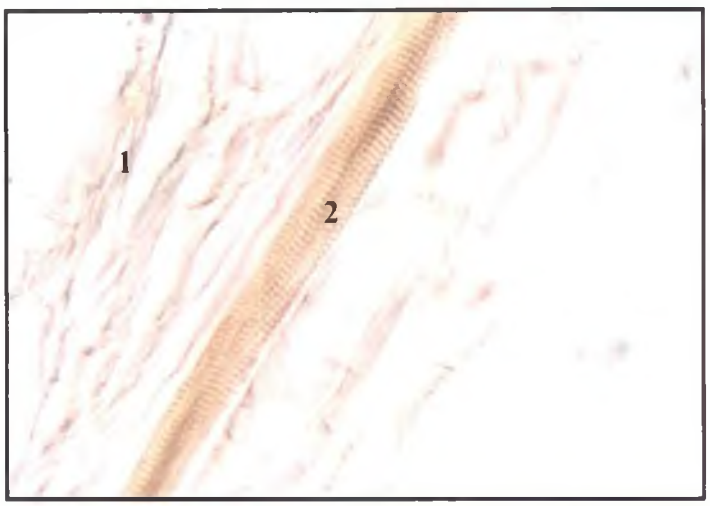
1. Pilosebaceous opening
2. Inner root sheath
3. Outer root sheath
4. Follicular fold
5. Dermis

Fig. 75 L.S. of the hair showing follicular fold of inner root sheath in skin of the dorsal nasal region. H&E. x 400



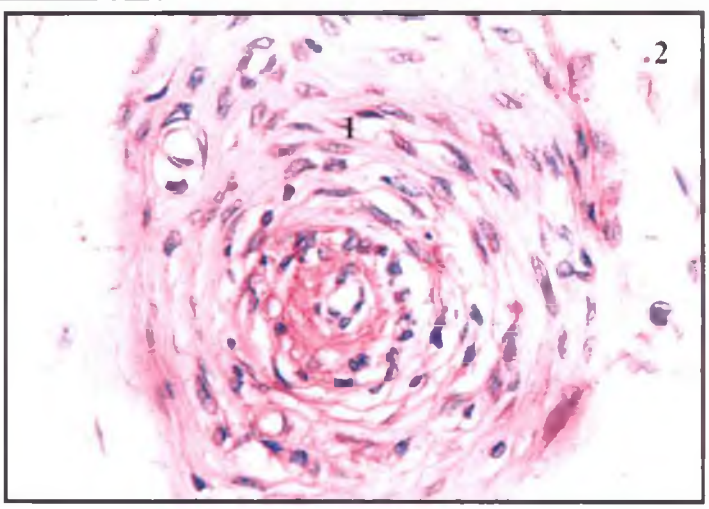
1. Dermis
2. Outer root sheath
3. Pilosebaceous canal

Fig. 76 L.S. of the hair showing the pilosebaceous canal in skin of the dorsal nasal region. H&E. x 400



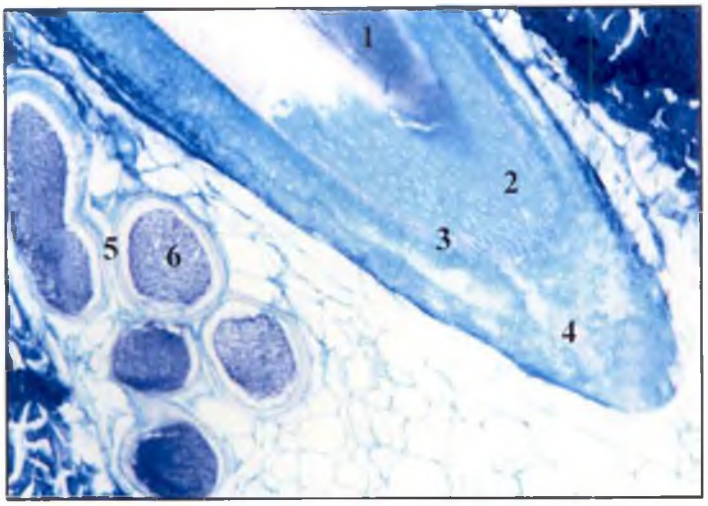
1. Subcutaneous tissue
2. Skeletal muscle fibres of cutaneous fasciae

Fig. 77 Section of skin in the dorsal nasal region. Modified Gomori's method for alkaline phosphatase x 100



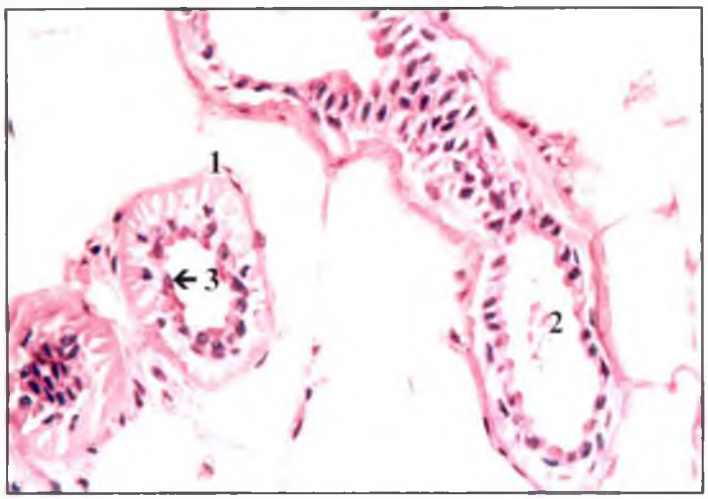
1. Pacinian corpuscle
2. Subcutaneous tissue

Fig. 78 Section of the subcutis in the dorsal nasal region. H&E. x 400



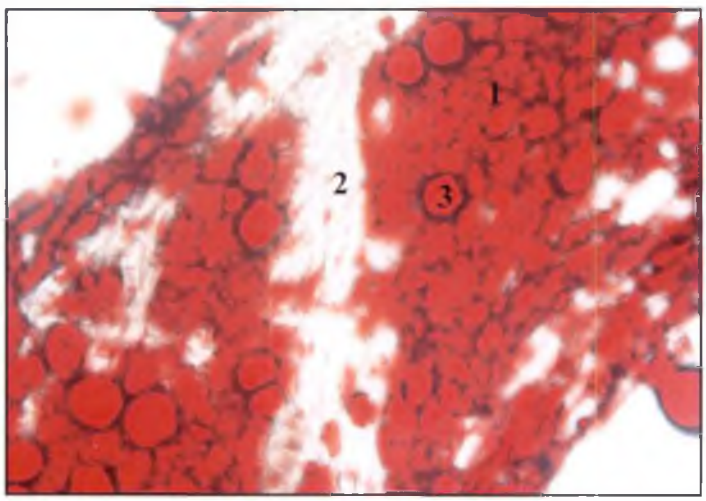
1. Medulla of the hair
2. Cortex of the hair
3. Inner root sheath of the hair follicle
4. Outer root sheath of hair follicle
5. Secretory end piece of sweat gland
6. Secretion of sweat gland

Fig. 79 Section of the deeper layers of skin in the lateral abdominal region. PAS Alcian blue method for mucosubstance x 100



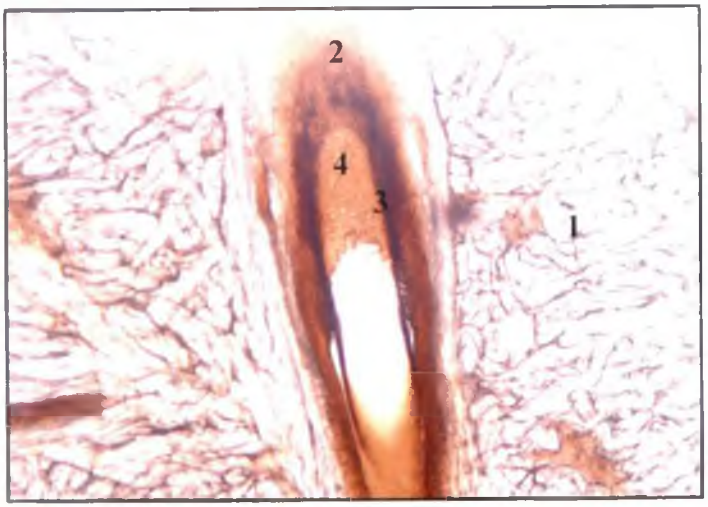
1. Secretory end piece of sweat gland
2. Secretion of sweat gland
3. Apical bleb

Fig. 80 Section of the deeper layers of skin in the lateral abdominal region. Best's carmine method for glycogen x 100



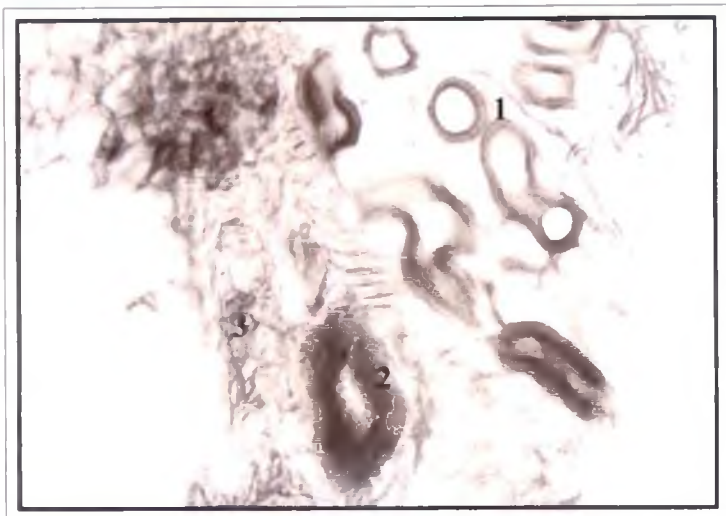
1. Fat lobules
2. Connective tissue septum
3. Adipocyte

Fig. 81 Section of skin in the dorsal neck region showing the subcutaneous fat lobules. Oil red O in propylene glycol method for fats x 100



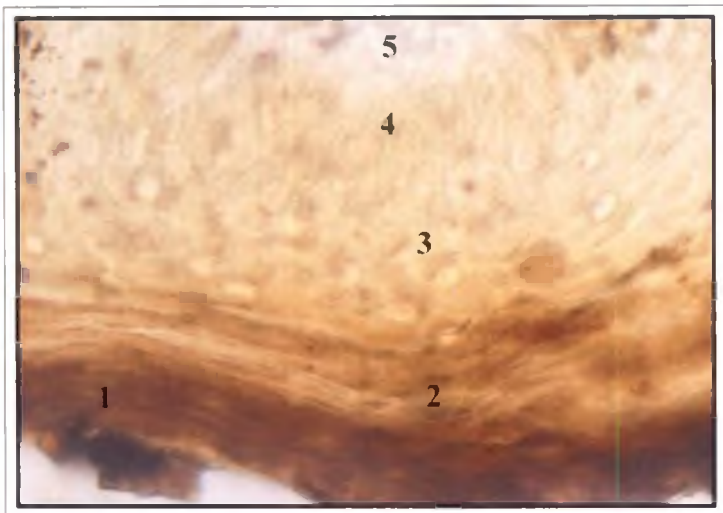
1. Dermis
2. Outer root sheath of hair follicle
3. Inner root sheath of hair follicle
4. Cortex

Fig.82 Section of skin in the dorsal neck region.
Modified Gomori's method for alkaline phosphatase activity x 100



1. Secretory end piece of sweat gland
2. Artery
3. Subcutaneous tissue

Fig.83 Section of skin in the dorsal neck region.
Modified Gomori's method for alkaline phosphatase activity x 100



1. Stratum corneum
2. Stratum granulosum
3. Stratum spinosum
4. Stratum basalis
5. Dermis

Fig. 84 Section of skin in the dorsal neck region.
Modified Gomori's method for acid phosphatase activity x 400

5. DISCUSSION

5.1 MORPHOLOGY OF SKIN

Entire skin of Large White Yorkshire pigs was white with areas of black spots occasionally. The surface of the skin was creased with sulci. Montagna and Lobitz (1964) opined that when shaved, the creased skin surface of pig resembled that of man.

The skin was slightly thicker in male pigs than in females. But the statistical analysis revealed that a significant difference in skin thickness observed only in the dorsal nasal region. Yagci *et al.* (2006) observed that skin thickness was higher in male White New Zealand rabbits than in the females.

Maximum skin thickness was noticed in the snout region with an average value of 6.43 ± 0.06mm in female pigs and 6.58 ± 0.08mm in males. This was followed by dorsal nasal, carpal, dorsal neck, dorsal abdomen, lateral abdomen, ventral neck and ventral abdomen regions. This is in accordance with the findings of Montagna and Lobitz (1964). Smith and Calhoun (1964) observed that the porcine skin was thickest over the dorsal surface of the body and on the lateral surface of the limbs, gradually became thin towards the ventral side of the body and medial surface of the limbs.

Skin was composed of the superficial epidermis and the deeper dermis. Contribution of the epidermis to the total skin thickness was maximum in the snout region (14.33 % in females and 14.99 % in males). Epidermis was thicker in male pigs. Contrary to this, Smith and Calhoun (1964) reported that the skin of snout in females was thicker than that of the male animals.

The subcutaneous fat layer was slightly thicker in the females. This layer was massively infiltrated with adipose tissue and formed the panniculus adiposus. Maximum thickness was noticed in the neck dorsal region. The subcutaneous fat was less in the snout, dorsal nasal and carpal regions. The subcutaneous tissue of the snout region contained connective tissue fibres and skeletal muscle fibres. Presence of strands of striated muscle in the skin of the neck and face as muscles of expression is reported in human beings by Urmacher (1990). Cutaneous fascia was pale and closely adherent to the skin. In the snout region, subcutaneous fat was diffusely arranged between the dermis and underlying muscle tissue whereas the subcutaneous fat formed a separate sheet under the dermis in the dorsal neck and dorsal and lateral abdominal regions. In the ventral neck and ventral abdominal regions, fat was arranged loosely in small lobules. In the dorsal and lateral abdominal regions, the fat was occasionally arranged in two layers. The panniculus adiposus of swine was pronounced in the trunk region compared to the extremities which provided an insulation. According to Vardaxis *et al.* (1997), both human and swine relied on fat and not on fur or hair for insulation and fat layer was pronounced in swine.

5.2 EPIDERMIS

5.2.1 Histology

The epidermis was very thin compared to the dermis. Epidermis was thickest in the snout and thinnest in the lateral abdominal region. A highly significant positive correlation was noticed between the total skin thickness and the thickness of the epidermis in the snout, dorsal nasal and carpal regions in both male and female animals. In the other regions (neck dorsal, neck ventral, dorsal, lateral and ventral abdominal regions), there was no significant correlation between these parameters. Schummer *et al.* (1981) reported that as a rule, the hair-bearing parts of the skin had a thin epidermis with only a moderate degree of keratinization, whereas

the hairless areas of the skin such as muzzle and snout disc had a thick epidermis with pronounced keratinization.

5.2.1.1 Stratum Basalis

Stratum basalis consisted of a single layer of columnar or cuboidal cells. From the basal surface of these cells, cytoplasmic processes grew into the papillary layer of the dermis, which helped for anchorage. Montagna (1962) opined that in early embryonic stage, the cells of stratum basalis were cuboidal with a clearly stainable large nucleus and smooth basal surface. This contributed to the easy peeling of the epidermis from the dermis. Later, the basal cells became columnar and the lower surface of the cells became serrated as the cytoplasmic processes invaded into the dermis. Copenhaver *et al.* (1971) suggested that the irregular boundaries of the epidermis and its underlying connective tissue as well as the half desmosomes accounted for the adherence between epidermis and dermis. Morris and Hopewell (1990) observed that the basal portion of the epidermis was considerably undulated in pigs.

Epidermis was limited from the dermis by the basement membrane that was made up of collagen fibres and reticular fibres. Sharma *et al.* (1996) demonstrated a thick collagenous basement membrane in the skin of the yak. Urmacher (1990) explained basement membrane as a thin PAS positively stained layer.

Nucleus of some of the cells of stratum basalis and stratum spinosum showed stages of mitosis. Monteiro-Riviere (1998) reported that some basal cells could act as stem cells with the ability to divide and produce new cells, whereas others primarily served to anchor the epidermis. Identification and functional characterisation of murine and human epidermal stem cells have been made by Kaur (2006), which

provided insights into the fundamental process of the tissue renewal and repair in the epidermis.

Clear cells, the relatively larger and lighter cells with clear cytoplasm and reniform nucleus, were detected among the keratinocytes of stratum germinativum. Similar types of cells were demonstrated in the stratum germinativum by Smith and Calhoun (1964) and Marcarian and Calhoun (1966) in the pig, Bagi (1974) in Surti buffalo and Sharma *et al.* (1996) in the yak. Ham (1969) opined that the clear cells that appeared in the epidermis were melanocytes before starting their functioning. Monteiro-Riviere (1998) opined that clear cells possessed all the characteristics of the Langerhan's cells. Copenhaver *et al.* (1971) gave detailed reports of Langerhan's cells in the upper layers of stratum Malpighii. These cells possessed a dark stained nucleus surrounded by apparently clear cytoplasm. In sections stained by the gold chloride method, these were blackened and were revealed as stellate or dentritic cells. Their slender processes penetrated the intercellular spaces among the prickle cells and were devoid of desmosomes attaching them to the neighbouring cells. Langerhan's cells were interpreted variously such as worn-out melanocytes or as distinct series of cells derived from bone marrow. Electron micrographs showed that the characteristic Birberck granules of Langerhan's cells were different from the melanin pigment.

In the present study, maximum number of clear cells was noticed in the ventral abdominal region. Urmacher (1990) reported that the Langerhan's cells were the immunologic cells of the skin, which were needed to induce proliferative and cytotoxic T-cell responses by recognizing and presenting antigen to immunocompetent T-cell lymphocytes. Langerhan's cells also possessed nonspecific esterase activity and expressed ATPase staining of the plasma membrane.

5.2.1.2 Stratum Spinosum

Stratum spinosum was the thickest layer of the epidermis. The cells were large, polyhedral with clear cell boundaries. The intercellular regions were marked by a clear zone with prickling appearance. Copenhaver *et al.* (1971) and Urmacher (1990) reported that the plasma membranes of adjacent cells were normally in close apposition throughout most of their extent, but during tissue processing, the cells shrunk and got pulled apart, leaving a clear intercellular space.

According to Bloom and Fawcett (1975), the prickling appearance of this layer was formed by the short processes or spines that were attached to similar projections from adjacent cells. Because the cell membranes at the sites of end to end junction of these processes could not be resolved with light microscopy, these structures were formerly called "intercellular bridges" in the belief that they represented open communications between the epidermal cells. But the electron microscopic studies revealed that there was no protoplasmic continuity between the cells. Instead, these short processes met end to end or side to side and were firmly attached by a well developed desmosome which appeared as a dense dot or granule in each 'bridge'. Monteiro- Riviere (1998) reported that desmosomes connected adjacent cells of stratum spinosum and these cells to the cells of stratum basalis below.

The cell boundaries and the prickling appearance were more distinct at the snout region and the carpal region. The cells in the deeper layers of stratum spinosum were faintly basophilic, while the central layers were more eosinophilic. Copenhaver *et al.* (1971) correlated the basophilia with the ribosomal content of cytoplasm, hence the cells were considered as active protein synthesising cells. Prekeratin granules were present in the upper layers. The uppermost layers of stratum spinosum contained small granules as reported by Urmacher (1990) in human

beings. These granules were composed of lipids and neutral sugars conjugated to proteins and lipids. Their function is to provide epidermal lipids, which increase the barrier properties of the cornified layer and aid in desquamation process. It is also the site of synthesis and storage of cholesterol.

The thickness of stratum spinosum varied in different areas of the body. This layer was thickest at the hairless snout region, with four to six cell-layers thick at the region corresponding to tip of papillae and 10 to 12 cells thick at the rete peg. In the case of human beings, maximum thickness of stratum spinosum was observed at palms and soles (Urmacher, 1990). In other regions under study, thickness varied from two to four and six to seven cell-layers thick at the above mentioned regions. Morris and Hopewell (1990) found that the stratum spinosum at the interpapillary region was about four cells thick in pig.

Stratum spinosum was extremely thin in some parts of the body like the ventral abdominal, dorsal neck and ventral neck regions. At the carpal region, the cells were comparatively smaller and closely packed. Banks (1993) noticed that the thickness of stratum germinativum varied in different regions of the mammalian integument. It was usually thick in hairless areas of the body and rather thin in heavily haired region.

5.2.1.3 Stratum Granulosum

Stratum granulosum consisted of flattened diamond-shaped cells with their long axes parallel to the surface of the skin. The cytoplasm showed irregular conspicuous granules that stained intensely with haematoxylin. According to Monteiro-Riviere and Stromberg (1985), these granules were non-membrane bound electron-dense keratohyalin granules. The keratohyalin granules contained

profilaggrin, a structural protein and a precursor of filaggrin and were thought to play a role in keratinization and barrier function.

Monteiro-Riviere (1998) reported presence of lamellar granules or Odland bodies in the stratum granulosum. They released their contents by exocytosis into the intercellular space between the stratum granulosum and stratum corneum, thereby coating the cell membrane of the stratum corneum cells. These granules formed the barrier, which prevented both the penetration of substances from the environment and loss of body fluids.

In the areas of thick skin like snout region, stratum granulosum was three to four cell-layers thick. This layer was thinner in the abdominal region with one to two cell layers. It was thinnest and formed a discontinuous layer at the dorsal and ventral neck regions. This is in accordance with the observations made by Marcarian and Calhoun (1966) in adult Yorkshire pigs. The stratum granulosum was thickest at snout and interdigital skin and was one layer thick or occasionally sporadic in the skin of eye and axilla. Banks (1993) reported that the granules of stratum granulosum were of variable occurrence and might be absent in regions where hair was abundant.

5.2.1.4 Stratum Lucidum

Stratum lucidum was made up of eosinophilic cells that lost their nuclei, cytoplasmic organelles and clear cell boundaries. According to Banks (1993), stratum lucidum consisted of several layers of homogenous, translucent, squamous cells that were slightly stainable. Monteiro-Riviere (1998) found that the cytoplasm lacked keratohyalin granules, but possessed protein-bound phospholipids and eleidin. Eleidin had a different staining property compared to keratin and was the precursor of keratin.

This clear, bright homogenous layer was noticed only in the snout, dorsal nasal region and abdomen ventral region. In other areas, it was absent. The regional limitation of stratum lucidum to the planum nasale, lip and margin of the hoof was reported in the case of sheep by Kozlowski and Calhoun (1969). Mandage *et al.* (2003) recorded absence of stratum lucidum in the epidermis of the Deccani sheep. Smith and Calhoun (1964) reported that this layer was not apparent in the skin of pig. In the present study, presence of stratum lucidum in the snout, dorsal nasal and ventral abdominal regions can be related to the thickness of the epidermis, pattern of hair growth and the physical stress to that region. This is in accordance with the findings of Monteiro-Riviere (1998), who found that in dog stratum lucidum was found only in specific areas of exceptionally thick skin and in hairless regions like planum nasale and footpads.

5.2.1.5 Stratum Corneum

Stratum corneum was the outermost layer of the epidermis and consisted of several layers of keratinized dead cells, which were constantly being shed. The thickness of this layer varied in different regions of the body. Snout region showed maximum thickness (103.00 ± 0.31 µm in females and 106.50 ± 0.62 µm). Minimum thickness recorded was 33.92 ± 1.53 µm and 44.02 ± 3.52 µm in female and male animals respectively, in the ventral abdominal area. The overall average thickness of stratum corneum was 57.08 ± 1.9 µm in females and 59.63 ± 1.24 µm in male pigs. Thickness of this layer recorded was 13.00 µm in Nigerian cattle breed (Amakiri, 1973); 45.50 µm in Surti buffaloes (Bagi, 1974; Bagi and Vyas, 1983); 13.30 µm in crossbred dogs; 9.74 ± 0.64 µm in lion cub and 15.64 ± 0.91 µm in adult lion (Lloyd and Grathwaite, 1982); 48.30 µm in Murrah buffalo and 8.50 µm in ox (Mehta, 2002).

The keratinized cells of stratum corneum were polygonal in shape and flattened towards the periphery. These were closely packed without obvious intercellular spaces. The thickened cell membranes of adjacent cells were found to be closely interdigitated. Bhattacharya *et al.* (1998) reported that hexagonal or polygonal cells formed the stratum corneum at neck fold, shoulder shield and abdomen in the skin of one-horned rhinoceros, whereas in other regions, the stratum corneum cells were irregular in shape. The orderly arrangement of cells in stratum corneum minimised the transepidermal water loss (Gniadecka *et al.*, 1998; Monteiro-Riviere, 1998).

In pigs, Marcarian and Calhoun (1966) recorded maximum thickness of stratum corneum at snout region, followed by interdigital skin. According to them, this cornified layer was thin in the eyelid, axilla and dorsal areas of the abdomen and thorax.

Monteiro-Riviere (1998) postulated that the stratum corneum cells were highly organised into vertical columns consisting of a tetrakaidecahedral shape. This 14 sided polygonal structure provided a minimum surface: volume ratio that allowed packing without interstices. The major component of barrier property of the skin was contributed by the intercellular substance present between stratum corneum cells. This intercellular substance derived was from the lamellated granules. But Copenhaver *et al.* (1971) opined that the intercellular matrix was derived from the keratohyalin granules. The thickened membranes or husks enveloping the horny cells were resistant to keratinolytic agents and provided integrity for the filament matrix complex within the cell.

Stratum basalis, stratum spinosum and stratum corneum were continuous throughout the epidermis in all the regions under study. Stratum granulosum was continuous in most of the regions except ventral neck and lateral and ventral

abdominal regions. In snout, dorsal nasal and ventral abdominal area, a definite stratum lucidum could be detected. According to Copenhaver *et al.* (1971), the variation in thickness of epidermal layers in different regions might be due to the fact that keratinization is not a continuous process, but occurred at certain times.

Blood vessels were absent in the epidermis. According to Bloom and Fawcett (1975), epidermis was nourished by the capillaries in the underlying connective tissue by diffusion through the tissue fluid that occupied an extensive system of intercellular spaces of the Malpighian layer.

5.3 DERMIS

5.3.1 Morphology

Maximum thickness of dermis was noticed in the snout region. The thickness of dermis varied from 2220.78 ± 2.11 μm to 5221.00 ± 12.49 μm in females and 2567.00 ± 26.34 μm to 5336.71 ± 7.39 μm in males. Schummer *et al.* (1981) opined that the dermis was generally thicker in males than in females. Bloom and Fawcett (1975) reported that in man, the average thickness of dermis was approximately 1 to 2 mm, with a minimum of 0.60 mm on the eyelids and prepuce and a maximum of 3 mm on the soles and palms. Thickness of the dermis was generally more on the extremities and dorsal surface of the body than on the lateral and ventral surfaces as reported by Bloom and Fawcett (1975) in human beings. According to Lee and Nielson (1962), camel possessed a relatively thick dermis (2.95μm). According to Schummer *et al.* (1981), the thickness of skin was determined by the thickness of the corium. Among the domestic animals, excluding the dwarf breeds of dogs and cats, the sheep had the thinnest and cattle had the thickest corium.

5.3.2 Histology

5.3.2.1 Papillary Layer

Papillary layer conformed to the contour of stratum basalis of the epidermis. This layer was composed of finer connective tissue fibres and appeared to be compactly arranged. Although collagen fibres were predominant in this layer, elastic and reticular fibres were also detected. Presence of elastic fibres in porcine dermis is reported by Vardaxis *et al.* (1997). Schummer *et al.* (1981) opined that these elastic fibres ensured that the origin and shape of the skin was regained after temporary distortion. These fibres were embedded in a ground substance. Proportionately more ground substance occurred in papillary layer than the reticular layer. It was semifluid, nonfibrillar, amorphous substance that filled the spaces between the fibres and cells. According to Montagna (1962), the papillary body was composed of wildey separated delicate collagenous, elastic and reticular fibres, enmeshed with superficial capillaries in a viscous ground substance.

The ground substance stained weakly with the PAS technique, suggesting the presence of glycoproteins. The total amount of ground substance and proportions of its component substances varied from region to region. This stained metachromatically with toludine blue indicating the presence of acid mucopolysaccharides. Montagna (1962) reported that this substance in wounds diminished as the scars matured, indicating that the mucopolysaccharides might tie up with the formation of collagen fibres. Hyaluronic acids in connective tissue occurred in greatly hydrated gels and involved in water binding. Fibroblasts, macrophages and mast cells could be detected in papillary layer as repoted by Monteiro-Riviere (1998) in domestic animals.

Papillary layer was thickest in the snout region. Primary dermal ridges were observed just beneath and following the contour of the epidermal ridges. It was divided into secondary dermal ridges, dermal papillae or papillary body by the rete peg. In the dorsal and lateral abdominal regions where mechanical demands were slight, the dermal papillae were low and less in number. They were single with very short rete pegs in the ventral neck region. In the snout, dorsal nasal and carpal regions, where more pressure is encountered dermal papillae were well developed. Copenhaver *et al.* (1971) observed numerous tall dermal papillae in lips, clitoris, penis, labia minora and nipples in human beings. Schummer *et al.* (1981) reported that in thickly haired parts of the skin in domestic animals where the epidermis had relatively few layers, the papillary body was also thin. Those areas of the skin, which carried few or no hairs, had numerous tall and closely packed pegs on the papillary body. This arrangement ensured not only the adequate nutrition and characteristic innervation of the epidermis but also provided a firm union in an area, which was usually exposed to great mechanical stress.

5.3.2.2 Reticular Layer

Eventhough a distinct line of demarcation between papillary and reticular layers was absent in most of the areas in the skin of pigs, the delimitation of two layers could be distinctly recognised in the dorsal and lateral abdominal regions. Montagna (1962) reported clear distinction of papillary and reticular layers in the skin of human beings. According to Trautmann and Fiebiger (1957) and Ham (1969), clear line of demarcation between these layers was absent in the domestic animals. Marcarian and Calhoun (1966) opined that the papillary and reticular layers blended without distinct demarcation in the pig.

The thickness of the reticular dermis in female pigs varied from 2127.67 ± 7.21 μm to 4654.17 ± 9.80 μm whereas in male pigs the values were

2493.00 ± 21.87 μm and 4762.67 ± 3.88 μm. The maximum thickness was noticed in the snout region. According to Bagi (1974), the average thickness of reticular dermis in Surti buffaloes was 5.01 mm. In human beings, Urmacher (1990) reported that the skin of the back normally showed a thick reticular dermis.

Reticular layer was composed of dense network of collagen, elastic and reticular fibres. According to Schummer *et al.* (1981), this dense fibre network accounted for the firmness of leather. The collagen fibres were arranged parallel to the epithelial surface. Towards the deeper aspect, the size of the collagen bundles greatly reduced and seen as small, thin bundles, which was more distinct at the neck ventral, abdomen lateral and abdomen ventral regions. In addition to the parallel layers, alternate layers of collagen were observed at an angle to the former. Occasionally, collagen bundles ran parallel to the hair follicle, which were oriented almost perpendicular to the majority. Meyer *et al.* (1982) opined that the massive three-dimensional networks of collagen fibres gave the resistance power against the mechanical stress of tension and pressure. Uramacher (1990) suggested that human dermis was composed of high amount of type I collagen and small amount of type II collagen.

Elastic fibres were observed as fine network among the massive network of collagen fibres. According to Kozlowski and Calhoun (1969), in sheep, elastic fibres formed fine network at the papillary layer and in the connective tissue sheaths of wool follicles and sebaceous glands. Vardaxis *et al.* (1997) reported focal aggregates of elastic fibres at various depths in the dermis of pig.

Reticular fibres were noticed surrounding the secretory end pieces of the sweat glands and sebaceous glands as reported by Kozlowski and Calhoun (1969) in sheep.

Different cell types present in the reticular dermis were fibroblasts, mast cells, macrophages and extravasated leucocytes as reported by Monteiro-Riviere (1998) in domestic animals. Urmacher (1990) reported the presence of mast cells in the dermis of human beings. These were usually located around blood vessels. Aggregations of lymphocytes were noticed in the snout region surrounding the ducts of eccrine sweat glands. Numerous nerve receptors also could be noticed in the dermis. These were most abundant in the snout region. Schummer *et al.* (1981) reported that as a general rule, skin regions which were densely covered with hair were less rich in nerve's that poorly haired or hairless areas. Sharma *et al.* (1996) reported different stages of fibroblasts, lymphocytes, mast cells and a few chromatophores and macrophages in the skin of yak.

Dermis showed a large number of glomi. In the snout region, they were most abundant. According to Copenhaver *et al.* (1971), glomus occurred normally in the sole of foot, palm of hand and skin of phalanges and in the nail bed of human beings. In other regions, it developed in pathological conditions resulting from injury, in vascular neoplasm and in developmental anomalies. The arteriovenous anastomoses were usually surrounded by a connective tissue sheath, and the arterioles followed a convoluted course, forming the glomus. The smooth muscle fibres were modified in shape and structure and were epitheloid in appearance. The internal diameter of the narrowest part of the anastomosis was usually 20 to 40 μm. Thus, the anastomoses conveyed much more blood than capillaries did. By contraction or relaxation, they influenced the amount of blood flowing through localised regions. So this had a role in controlling peripheral temperature. This was the reason contributed to their high degree of development in the feet of penguins.

5.4 HAIR

5.4.1 Morphology

Arrangement of hair was simple in swine. Mostly two to three hairs formed a group and they emerged out very closely but not from a single orifice unlike in the case of compound hairs. Eventhough single hair to groups of four to six were recorded in the skin of pig, only one hair per follicle was noted, regardless of the size (Smith and Calhoun, 1964). Montagna and Yun (1964) opined that the hair follicles might be originated in groups and became separated as skin surface expanded. Rostral surface of the snout disc lacked hairs while the dorsal surface bore sparse wool hairs. The rostral region was directly continuous with the mucous membrane of nostrils, hence this region lacked hairs. Schummer *et al.* (1981) observed non-medullated dwarf sinus hairs on the snout disc. According to Monteiro–Riviere (1998), in domestic animals, mucocutaneous junctions, footpads and hoofs lacked hairs.

In the snout region, the epidermis carried raised areas and depressions very clearly. Schummer *et al.* (1981) reported that the surface configurations in the muzzle and snout disc were genetically determined similar to those of human finger prints. Imprints of these special skin areas or nasolabiograms can be used for the identification of animals.

Maximum hair density was noticed in the dorsal nasal area (58 to 61 per cm^2) followed by the carpal region in both male and female animals. The hairs of these regions were shorter and stiff. Montagna and Yun (1964) noticed that in the areas of minimum expansion of skin surface, the hairs were shorter and crowded together. In the areas under present study, dorsal nasal and carpal regions showed minimum expansion of skin surface. Subcutaneous tissue as well as the muscles were less in

this region. Minimum numbers of hairs were observed in the snout, followed by ventral abdomen region. The mean distance between adjacent hairs were minimum in the dorsal nasal region and was maximum at the ventral abdominal region.

In general, density of hair distribution was more in the male animals than the females. But a significant statistical sex difference was observed only in the ventral neck region. Contrary to this, Smith and Calhoun (1964) observed greater hair density in females than male pigs.

5.4.2 Histology

Hair consisted of a shaft and a root. Shaft projected beyond the surface of the skin while the root was placed at an angle in the reticular layer of the dermis. The distal end of the hair root possessed an expanded portion, the hair bulb. Hair papilla was situated inside the hair bulb. Hair was composed of cuticle, cortex and medulla. Single layer of flat keratinized, anucleated squamous cells formed the cuticle. Cuticle of inner root sheath of hair follicle was similar to that of the hair. But the cells of these two layers were overlapping and scale like, lay in opposite direction, so that free borders of these layers were interlocked. The pain felt during plucking of hair resulted from the difficulty in detachment of these two layers (Ham, 1969).

Cortex of hair was composed of spindle-shaped keratinized cells with their long axes parallel to the hair shaft. The cells were arranged in several layers and were dense and compact. Their nuclei were elongated and darkly stained. Those cells towards the hair bulb were oval in shape with spherical nuclei. Medulla of the hair was slender than the cortex and made up of loosely filled cuboidal or polygonal cells with air filled spaces, but towards the root region, the medulla became solid and lacked air filled spaces. Schummer *et al.* (1981) reported that hairs containing much medulla stood erect and were brittle and those containing more cortex were stronger.

The forensic identification of hair makes use of the relative thickness of medulla and cortex, the stratum of the medullary cells and the appearance of the free borders of the cuticular cells. He also reported that medulla was the most valuable part of the hair. Cells of the medulla showed keratin granules. According to Bloom and Fawcett (1975), keratinization of medulla, cortex and cuticle occurred in the keratogenous zone just above the dome of the hair papilla. Keratinization of epidermis was continuous and here the keratin formed was soft while that of the hair follicle was an intermittent process and localised to a particular portion of the dermis, the hair papilla. The product was hard, cohesive, and non-shedding and accumulated in concentric layers. Keratinization of the hair papilla was the inductive force for the hair formation. Thus any reason leading to the damage of hair papilla in postnatal life caused cessation of hair formation.

Hair follicle was composed of four parts – hair papilla, hair matrix, inner root sheath and outer root sheath. Hair papilla was the part of dermis encapsulated by the hair matrix cells that formed a structure called the hair bulb. This was located in the deeper reticular layer and in some cases extended deep into the subcutaneous tissue. Montagna and Yun (1964) opined that the hair follicles seldom extended to deeper layers of hypodermal fat in pigs. Hair matrix cells resembled the cells of the stratum basalis of the epidermis, as its life cycle ended with the formation of cornified cells. The inner root sheath was composed of inner cuticle, middle granular epithelial layer, the Huxley's layer and outer pale epithelial layer, the Henle's layer. Cuticle of inner root sheath was similar to the cuticle of the hair. Huxley's layer of inner root sheath was composed of two to three rows of elongated, granular cells. Henle's layer was the outer one and was made up of a single layer of columnar cells with darkly stained nuclei. Similar observations were made in the hair of domestic animals by Monteiro-Riviere (1998). Immediately beneath the opening of the sebaceous glands in the inner root sheath of large hair follicles were several circular or follicular folds and the cells above the pilo-sebaceous opening became thinner and were in a stage of

disintegration. Eventhough the follicular folds were noticed inferior to pilo-sebaceous opening, their occurrence at both inferior and superior aspects was reported by Smith and Calhoun (1964) and Marcarian and Calhoun (1966) in pigs.

The external root sheath was composed of single layer of basal cells and several layers of stratum spinosum cells. This was continuous with the epidermis at the upper portion. Externally a homogenous glassy membrane covered the external root sheath, which was continuous with the basal lamina of the epidermis. Entire hair follicle covered by a connective tissue layer was composed of inner circularly arranged connective tissue fibres and outer longitudinal layer. This was formed of collagen and elastic fibres with large number of blood vessels and nerves. These blood vessels carried nutrients to the hair follicle as reported by Monteiro-Riviere (1998).

Dermis possessed different stages of hairs such as anagen, catagen and telogen. Anagen bore mitotically active cells in the hair bulb, while catagen showed regressive type of cells. The hair papilla was reduced to a ball of cells located below the capsule of the hair matrix cells of the bulb. Hair follicle at this stage was the telogen. According to Monteiro-Riviere (1998), hair matrix cells underwent periods of quiescence during which no mitotic activity occurred. When the matrix cell proliferation was resumed, in the anagen stage, a new hair was formed. This cyclical activity of the hair bulb accounted the seasonal changes in the hair coat of domestic animals. Hair required approximately three to four months to regrow after shaving in normal and short coats and up to 18 months in long coats. The length of time varied with the growth stage of the hair follicle.

5.4 ARRECTORES PILORUM

Arrectores pilorum was a small bundle of smooth muscle fibres seen in close association with the hair follicle. It was inserted obliquely in the connective tissue sheath of the hair follicle. The upper end extended towards the epidermis and attached to the superficial papillary dermis. This muscle was anchored by elastic fibres at its insertion and attachment. Arrectores pilorum completely encircled the hair follicle like a sling. The sling formation around hair follicle was also reported by Fowler and Calhoun (1964) in pig. Largest arrector pili muscles were noticed in the abdomen dorsal region. The functions attributed to these muscles were the errection of hairs that emerged out from the follicle and emptying of sebaceous glands. According to Kelly *et al.* (1984), the arrector pili muscle was situated in the obtuse angle between the hair follicle and surface and when the muscle contracted, the hair became more vertical to the surface and a small groove appeared in the skin at the place, where the muscle was attached. This gave rise to the appearance of goose flesh.

5.5 INTERFOLLICULAR MUSCLE

Interfollicular muscle connected adjacent hair follicles and its characteristic hair group. These were smooth muscles, resembled arrector pili muscle, but were located at a level midway between the level of sebaceous glands and apocrine sweat glands. These were attached to the hair follicle opposite to the attachment of Arrectores pilorum and the duct of sweat gland. These muscles connected each hair of its characteristic hair group. The broadest portion of each interfollicular muscle was attached primarly to two outer follicles of the aligned triad. Monteiro-Riviere and Stromberg (1985) reported that among the mammals, interfollicular muscles were identified only in swine and observed that the nuclei of interfollicular muscles were

fusiform and the sarcoplasm was filled with myofilaments, while other smooth muscles had elongated nuclei with a clear sarcoplasm.

5.7 SWEAT GLANDS

Sweat glands were abundant in the skin of pigs and were found in all regions under study. Similar observations were made in pigs by Schummer *et al.* (1981). They reported that there were as many as half a million of these glands and their density was dependent on age. The number reduced as age advanced.

In the hairy regions of the skin, sweat glands were located lateral and ventral to the hair follicles. David (1932) reported that sweat glands of swine were generally located around the base of the hair follicle and the duct ran parallel to the follicle, and opened near the hair canal at the skin surface, forming an obtuse angle.

Secretory portion of the sweat glands appeared tubular in most of the regions. Trautmann and Fiebiger (1957) reported glomiform type of secretory tubule in the pig. This can be attributed to the angle of sectioning.

Sweat glands were apocrine in nature in all the regions under study except in the snout and dorsal nasal regions where it was of eccrine type. Schummer *et al.* (1981) reported that in domestic animals eccrine sweat glands were mainly found in regions which had few or no hairs and they produced a watery secretion. Apocrine sweat glands showed wider secretory end pieces than the eccrine glands. It was lined by simple columnar epithelium. Height of the cells varied depending on the stage of secretion. During secretion, the cells showed bud-like apical projections, the apical blebs. Similar observations were recorded by Lee and Nielsen (1962) in camel. Schummer *et al.* (1981) reported that the apocrine glands comprised the principal type of sweat gland in the hair-covered skin of domestic animals and produced a

viscous, concentrated secretion which contained scent that was characteristic of the individual animal. In the snout and carpal regions, sweat glands and hair follicles were located in the upper dermis. According to Lee and Nielsen (1962), in camel, sweat glands were associated with larger cover hairs and not with the wool hairs.

In some regions, two types of secretory cells were visible, viz., the 'clear cells' and 'dark cells'. Dark cells had an oval dense nucleus while the clear cell nucleus was spheroidal and lightly stained. The clear cells showed a broad base that rested on the myoepithelial cells and the basement membrane. The dark cells had an inverted pyramidal shape with a broad apex. It has been suggested that the dark cells of eccrine sweat glands permitted reabsorption of sodium, potassium and chloride and might contribute sialomucin to the sweat (Urmacher, 1990).

The basophilic cytoplasm of the secretory cells showed lipid droplets. The nucleus was oval in shape. Once the secretory process was over, the epithelium became flattened and the lumen was filled with secretion. Just below the lining epithelium, myoepithelial cells formed a distinct layer above the basal lamina. These cells possessed elongated nucleus. The secretory portion of sweat gland in the pig was similar in structure to other animals as reported by Trautmann and Fiebiger (1957).

In the snout region, the secretory portion was tubular and smaller with a diameter of 32.50 to 39.00 μm. Larger sweat glands were located at neck dorsal and abdomen dorsal areas (104.00 to 234.00 μm and 182.00 to 260.00 μm, respectively). The size of sweat glands in these areas was similar to that of adult lion as reported by Bhayani *et al.* (1995). In adult lion, the saccular type of sweat glands measured about 297±15.91 μm x 100.13±4.44 μm.

The duct of sweat gland was made up of a single layer of cuboidal cells and possessed a narrow lumen. The sweat gland duct persuaded the coarse of hair follicle

and opened at the epidermis. In the hairy areas, they opened along the hair follicle to the skin surface. In the non-haired and areas of less haired regions, the duct independently opened to the skin surface. In addition to participating in the reabsorption of electrolytes, the excretory duct also had the important function of delivering parentral or orally administered drugs to the surface of the skin (Urmacher, 1990). In addition, the ductular epithelium also participated in the process of wound healing.

Abdomen ventral, dorsal nasal and abdomen lateral areas bore long ducts. In the snout and carpal regions, the ducts were shorter. In swine, the sweat gland ducts were longer compared to cow, sheep and goat (Montgomery *et al.*, 1982a) and equine (Montgomery *et al.*, 1982b).

5.8 SEBACEOUS GLANDS

Sebaceous glands of pigs were of simple, branched alveolar holocrine type, and their lobes were of different shapes. They were located in the dermis. Among the different areas under study, sebaceous glands were most abundant in the dorsal neck region. According to Trautmann and Fiebiger (1957), the sebaceous glands of pigs were rudimentary. But well developed branched alveolar type sebaceous glands were observed in the present study. Marcarian and Calhoun (1966) also reported that these glands were of branched alveolar type.

The secretory units consisted of solid mass of epidermal cells separated from the dermis by a connective tissue sheath. The peripheral cells of the gland were low cuboidal with round nucleus and these rested on the basal lamina. Rest of the cells were in various stages of differentiation. Towards the center, the cells showed changes in shape and in their cellular contents. They became polygonal or spheroidal and accumulated numerous lipid droplets. The nuclei of these cells gradually shrunk

and disappeared. Similar observations were made in domestic animals by Trautmann and Fiebiger (1957), Borysenko *et al.* (1979) and Monteiro-Riviere (1998). In the centre of the acini, the larger secretory cells might rupture due to the lipid accumulation. The detritus and lipid secretion together formed the sebum. This got excreted with the help of arrector pili muscles. The cells lost in secretion were replaced by mitotic divisions of the indifferent cells at the periphery of the gland.

The alveoli of sebaceous glands opened into a short duct, which in turn opened into the pilosebaceous canal at the upper portion of the hair follicles. The duct was lined by stratified squamous epithelium and was continuous with the outer root sheath of the hair follicle. Most of the sebaceous glands opened by one duct to the hair follicle. Similar observations were made by Marcarian and Calhoun (1966) in pigs. They could also find two ducts per gland that opened on either side of the hair follicle. Urmacher (1990) opined that the short excretory duct of sebaceous gland was shared by several lobules and was lined by cornified squamous epithelium.

5.9 SUBCUTANEOUS TISSUE

The subcutaneous tissue or hypodermis anchored the dermis to the underlying muscles or bone. Loose network of collagen fibres along with homogenous adhesive ground substance formed thin membranes. Monterio-Riviere (1998) reported loose arrangement of collagen and elastic fibres in the subcutis of domestic animals. This allowed the skin flexibility and free movement over the underlying structures. The spaces between collagen networks were filled with adipocytes, which were arranged in small lobules in the ventral neck and ventral abdominal regions, whereas in the neck dorsal, abdomen dorsal and abdomen lateral areas these occurred in large lobules and were sheath-like. In the ventral neck and ventral abdominal regions, fat was arranged loosely in small lobules. In the dorsal and lateral abdominal regions, the fat was occasionally arranged in two layers. According to Vardaxis *et al.* (1997),

both the human and the swine relied on fat and not on fur or hair for insulation. The fat layer was pronounced in swine. The amount of subcutaneous fat layer varied with anatomical site examined and nutritional status of the animal, so also the age and sex. Schummer *et al.* (1981) opined that increased number of fat cells in the subcutis appeared to have a special predisposition for this phenomenon and this fat accmulation need not be dependent on a calory- rich diet. Even with normal diet, the fat formed a solid layer in pigs, which was grayish-white and firm, and its consistency was between that of horses and ruminants. In pigs, layering of fat by the interposition of fasciae was also prominent. In other domestic animals on a normal diet, fat accumulated only in certain regions such as the lower chest in the ox, the nape in horse and the lumbar and inguinal regions in the dog.

The subcutaneous tissue was less in the snout, dorsal nasal area and carpal regions as compared to the other regions under study. According to Ham (1969), this layer was absent in the soles, palms and fingertip of man, where the skin is denser. Schummer *et al.* (1981) opined that in domestic animals the subcutis might be lacking altogether in certain regions for functional reasons and in this locations the musculature was in direct contact with the skin. The strands of connective tissue from the deeper reticular dermis extended throughout the subcutaneous fat layer and were perpendicular to the surface of the skin. Similar observations were reported by Marcarian and Calhoun (1966) in pigs. Monteiro-Riviere (1998) reported that the panniculus adiposus in pigs could be seen either as small clusters of cells or as large masses of fat. Pork bacon and fat back were derived from panniculus adiposus. According to Monteiro-Riviere (1998), large fat deposits were noticed in the carpal, metacarpal and digital pads of mammals which acted as shock absorber.

Numerous nerve fibre bundles and Pacinian corpuscles were observed in the subcutis of the pig. In human beings, Kelly *et al.* (1984) reported that larger trunks of nerve fibres lay in the subcutis that gave off branches to the dermis, where they

formed a rich subpapillary plexus of both myelinated and non-myelinated fibres. The branches from the subcutaneous nerve trunks and subpapillary plexus formed more or less elaborate special nerve endings. The pacinian or lamellar corpuscles located in the subcutaneous tissue was reported to be most numerous in palms and soles. Tactile corpuscles of Meissner were found in the papillae, especially in the fingertip, palm and sole. Krause's end bulbs were usually found in the dermis just beneath the papillae and rarely with in the papillae. Free nerve endings were found in the intercellular spaces of the epidermis (Kelly *et al.*, 1984).

Large number of blood vessels and lymphatics were found in the subcutis. Schummer (1981) reported that in the domestic animals, the rich capillary supply of the subcutaneous connective tissue and the presence of considerable amount of connective fluid associated with the cutaneous lymphatic system is the reason for the efficacy of subcutaneous therapeutic injections and vaccinations

5.10 HISTOCHEMISTRY

5.10.1 Carbohydrates

In the epidermis, upper stratum spinosum and stratum granulosum gave a positive reaction for both PAS-alcian blue and Best's carmine indicating the presnce of glycogen, hyaluronic acid, sialomucin and strongly acidic sulfated mucosubstances. Epidermal cells around the pilosebaceous opening and those around the orifies of sweat glands also showed glycogen. Montagna and Yun (1964) demonstrated a band of glycogen in the upper cells of Malpighian layer of the epidermis of pigs. Ground substance of the dermis also gave a positive reaction to glycogen and PAS-alcian blue substances. Urmacher (1990) reported the presence of nonsulfated acid mucopolysaccharides, predominantly hyaluronic acid and to a lesser

degree, chondroitin sulfate in the ground substance. Cytoplasm of fibroblasts lacked PAS-alcian blue substances, while the nucleus showed a positive reaction.

Outer root sheath cells of hair follicle around the keratogenous zone and active hair follicles showed an intense PAS-alcian blue positive activity as reported by Urmacher (1990) in man. Internal root sheath cells lacked glycogen, but contained polysaccharides and mucosubstances. Cuticle cells just above the hair bulb contained glycogen, while the cells in the middle third of the follicle gave a negative result. Hair papilla gave PAS-alcian blue positive reaction. Monteiro-Riviere and Stromberg (1985) demonstrated glycogen in the keratinocytes at one week of age in pigs.

Apocrine and eccrine sweat gland epithelial cells and the basal cells of the duct of the eccrine glands showed a positive reaction for glycogen. In the apocrine cells, glycogen granules were demonstrated in the apical blebs. Secretion of sweat glands contained glycogen and mucopolysaccharides. Montagna and Yun (1964) demonstrated PAS-reactive substances in the lumina of eccrine and apocrine sweat glands of pigs.

Secretory portions of sebaceous glands lacked glycogen and mucopolysaccharides while the ductular epithelium contained traces of these substances as reported by Montagna and Yun (1964) in pigs.

5.10.2 Lipids

The cells of stratum corneum and spinosum contained lipid granules. Their function is to provide the epidermal lipids, increase the barrier properties of the cornified layer, and aid in desquamation process as reported by Urmacher (1990) in human beings. The lipid droplets were arranged at both poles of the cells in the case

of dermal fibroblasts. The sebaceous glands and their ducts were strongly positive. Sharma *et al.* (1982) demonstrated presence of neutral fat in the central cells and acidic fat in the peripheral cells of sebaceous glands of buffalo.

Clear cells of sweat glands possessed lipid granules. Adipocytes of the subcutaneous fat gave strong positive reaction to Oil Red O while the interlobular septa gave a negative reaction. Urmacher (1990) reported that bands of dermal connective tissue formed the interlobular septa, which was negative to Oil Red O.

5.10.3 Phosphatases

The stratum basalis, stratum spinosum and stratum granulosum of the epidermis showed a positive alkaline phosphatase reaction while the stratum corneum was negative. The dermal papillae, blood vessels surrounding the hair follicles and the sweat glands showed a positive reaction to alkaline phosphatase. Similar observations were made by Montagna and Yun (1964) in porcine skin. Cuticle and inner root sheath also showed a strong alkaline phosphatase reaction while the cortex gave a moderate reaction. Montagna and Yun (1964) reported the presence of alkaline phosphatase in the Henle's layer of the hair follicle down to the middle of the hair bulb.

Epidermis gave a highly positive reaction to the acid phosphatase. Stratum basalis showed a negative result while the cells of the stratum spinosum showed a gradual increase in reactivity towards the periphery. Stratum granulosum and stratum corneum showed an intense reaction. Sebaceous glands also showed a positive reaction in the acini, while the periphery as well as degenerating region of the acinar cells showed a negative reaction. The apical portion of the secretory cells of the apocrine sweat glands showed moderate positive reaction. Acid phosphatase was absent in the eccrine glands and ducts of human skin (Urmacher, 1990).

Summary

6. SUMMARY

Morphological and histological studies were conducted on the skin of Large White Yorkshire pigs of six to ten months of age. Skin samples were collected from eight areas of the body viz., the snout, dorsal nasal, dorsal neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen and carpal regions. In general, skin of male animals was slightly thicker than that of the females. When compared using Student's-t-test, there was no significant difference in the skin thickness of the males and females except in the dorsal nasal region. Thickness of the skin varied considerably in different regions of the body. Maximum thickness for the skin, epidermis and the dermis was noticed in the snout and the minimum in the ventral abdomen. Skin was thicker on the dorsal surface of the body than on the ventral surface.

Contribution of the epidermis to the total skin thickness was maximum in the snout region. A highly significant positive correlation was noticed between the total skin thickness and the thickness of the epidermis in the snout, dorsal nasal and carpal regions in both male and female animals. The epidermis was formed of four layers, viz., stratum basalis, stratum spinosum, stratum granulosum and stratum corneum except in the snout, dorsal nasal and ventral abdominal regions, where an additional layer, the stratum lucidum was noticed. The rete pegs and the dermal papillae were most abundant in the snout region and minimum in the lateral abdominal region. In general, the extremities like snout, dorsal nasal and carpal regions showed more number of rete pegs and dermal papillae than the neck and trunk regions.

Stratum basalis was made up of a single layer of columnar cells. These cells became cuboidal towards the apex of the dermal papilla. Cells of stratum basalis rested on a basement membrane made up of collagen and reticular fibres. Clear cells could be located in the stratum basalis and stratum spinosum. These cells were relatively larger and appeared lighter than the keratinocytes. Cytoplasm of these cells was clear and the nucleus was indented. These cells possessed the

characteristics of the Langerhan's cells. The dorsal and ventral neck regions possessed a higher concentration of the clear cells compared to other regions.

Stratum spinosum was the thickest layer of the epidermis. Cells of this layer were large, irregular and polyhedral with distinct cell boundaries. The fine lines across the clear intercellular zone formed the intercellular connections and gave a prickled appearance to this layer. Thickness of this layer was maximum in the snout. Stratum spinosum was thinner at the dorsal and ventral neck regions and in the abdominal regions. Here the cell boundaries were indistinct and the nucleus showed peripheral condensation of chromatin. Cytoplasmic staining property of the cells of the stratum spinosum varied in different layers. Prekeratin granules were detected in the upper layers of stratum spinosum.

Stratum granulosum consisted of two to four rows of flattened, diamond shaped cells with their long axes parallel to the surface of the skin. Cytoplasm showed irregular, conspicuous, basophilic keratohyalin granules that stained intensely with haematoxylin. In the snout and dorsal nasal regions the stratum granulosum was thicker. In the ventral neck and lateral and ventral abdominal regions, it was very thin and occasionally sporadic. Stratum lucidum appeared as a clear, bright, homogenous, strongly eosinophilic layer consisting of flattened, compact, eosinophilic cells without clear cell boundaries or the nucleus, thus forming a homogenous clear layer. This layer was detected only in the snout, dorsal nasal and ventral abdominal regions. Stratum corneum formed the outermost layer and was present throughout the epidermis. It consisted of scale-like polygonal, clear cells, which were keratinized and contained no nucleus or cytoplasmic organelles. Maximum thickness was noticed in the snout region.

Among the five layers of the avascular epidermis, stratum basalis, stratum spinosum and stratum corneum were always present and formed continuous layers throughout the body surface. The stratum granulosum was a continuous layer in most of the regions. But in ventral neck and lateral and ventral abdominal regions this layer was not continuous.

The superficial papillary layer of the dermis was thinner while the deep reticular layer was thicker. There was a significant positive correlation between the total thickness of the skin and that of dermis in all the regions under study in both the female and male pigs. The papillary and reticular layers of dermis were thickest in the snout. A clear line of demarcation was absent between these layers. The highly vascular papillary layer was made up of collagen fibres predominantly and were finer and more closely arranged. In addition, elastic and reticular fibres were also seen, which were embedded in an amorphous ground substance, which gave a positive reaction to PAS-alcian blue staining. Fibroblasts, macrophages and mast cells were detected in this layer.

Reticular layer consisted of large, coarse and loosely interwoven bundles of collagen fibres with some elastic and reticular fibres. Average thickness of the reticular layer was about eight times than that of the papillary layer in the snout region. Blood vessels, lymph vessels and nerves traversed the dermis. Glomi were most numerous in the snout region. The cellular elements were less abundant in the reticular layer when compared to the papillary layer.

The hair arrangement was simple, but grouping of hairs was evident. Mostly two to three hairs formed a group and they emerged out very closely. Snout region lacked hair on the rostral region. Maximum hair density per square centimeter area was noticed in the dorsal nasal area. Density of hair distribution was more in the male animals than the females.

Hair shaft was composed of a cuticle, thicker cortex and slender medulla. Cuticle was formed by a single layer of flat, keratinized, anucleated squamous cells. Cortex of the hair was made up of several layers of dense, compact, keratinized, spindle shaped cells. Medulla was composed of loosely filled cuboidal or polygonal cells. Hair follicle was composed of four parts, viz., hair papilla, hair matrix, inner root sheath and outer root sheath. The inner root sheath was composed of inner cuticle, middle Huxley's layer and outer Henle's layer. Cuticle of inner root sheath was composed of thin, scale-like, overlapping cells, the free

borders of which were directed towards the hair root. Immediately below the opening of the sebaceous glands, internal root sheath of the large follicle became corrugated and formed follicular folds. The external root sheath was composed of single layer of stratum basalis and several layers of stratum spinosum cells.

Arrectores pilorum muscle appeared as a small bundle of smooth muscle fibres, which inserted obliquely in the connective tissue sheath of the hair follicle. The outer end was attached to the superficial papillary layer of the dermis. Largest arrector pili muscles were noticed in the dorsal abdominal region. Interfollicular smooth muscle connected adjacent hair follicles of its characteristic hair group. It was found midway between the level of sebaceous glands and sweat glands and attached to the hair follicle opposite to the side of attachment of the arrector muscle and the duct of the sweat gland.

Maximum number of sweat glands was observed in the snout region. Sweat glands were of apocrine type in all the regions under study except in the snout and dorsal nasal regions where it was of eccrine type. Apocrine sweat glands showed wider secretory end pieces that were lined by simple columnar epithelium. Height of the cells varied depending on the stage of secretion. Just below the lining epithelium, myoepithelial cells formed a distinct layer above the basal lamina. Ducts of these glands opened directly on to the surface of the epidermis. Secretion of the sweat gland gave a positive reaction to hyaluronic acid, sialomucins, strongly acidic sulfated mucosubstances and lipids. The sweat glands in the snout and dorsal nasal regions were of compound tubular eccrine type and were embedded in the reticular layer. These were not associated with the hair follicles. Two types of secretory cells were visible, viz., the 'clear cells' and 'dark cells'.

Sebaceous glands appeared as large, lobulated, sac-like structures associated with the hair follicles. They were of simple branched alveolar holocrine type. All these were embedded in the dermis and not extended into the subcutaneous tissue unlike the apocrine sweat glands. The secretory units consisted of a solid mass of epidermal cells. At the periphery, a single layer of low cuboidal cells with mitotic

figures could be observed. As they moved inward, they became polygonal and accumulated numerous lipid droplets. The most central ones were disintegrated and necrosed. The ducts were opened through pilosebaceous canal into the upper portion of the hair follicle.

The subcutaneous tissue was composed of a loose meshwork of connective tissue fibres, fibroblasts, lymphoid aggregations, lymphatics, large blood vessels, nerve bundles and pacinian corpuscles. Subcutaneous fat layer was slightly thicker in females. Maximum thickness was noticed at the neck dorsal region in both sexes. It was thinnest in the snout region.

PAS - alcian blue positive areas were detected in the upper stratum spinosum and granulosum of the epidermis and ground substance of the dermis. The cells of the outer root sheath of hair follicle around the keratogenous zone showed an intense PAS - alcian blue positive activity. Both eccrine and apocrine sweat gland epithelia showed a positive reaction to Best's carmine. Cells of stratum corneum, spinosum, sebaceous glands, their ducts and clear cells of eccrine sweat glands showed a positive reaction to Oil Red O. Stratum basalis, spinosum and granulosum of the epidermis and the dermal papillae, blood vessels surrounding the hair follicles and the sweat glands showed a positive reaction to alkaline phosphatase. Epidermis and sebaceous glands gave a positive reaction to the acid phosphatase.

References

REFERENCES

Amakiri, S.F. 1973. A comparative study of the thickness of the stratum corneum in Nigerian breeds of cattle. *Br. Vet. J.* 129: 277-281

Bagi, A.S. 1974. Study of histological structure of skin of Surti buffalo. M.V.Sc. thesis, Gujarat Agricultural University, Anand, 87 p.

Bagi, A.S. and Vyas, K.N. 1983. Morphology of skin of Surti buffalo as compared to that of the other breeds of buffalo and the ox and its role in thermoregulation. *Gujarat agric. Univ. Res. J.* 9(1): 52-56

Banks, W.J. 1993. *Applied Veterinary Histology.* Williams & Wilkins, Baltimore, 572 p.

Barari, S.K., Biswas, D., Pal, R.N. and Basak, D.K. 1994. Histology of sebaceous glands of yak (*Poephagus grunniens*). *Int. J. Anim. Sci.* 9: 273-274

Bhattacharya, M., Chakraborty, A. and Day, S. 1998. Scanning electron microscopy of stratum corneum in the skin of one horned rhinoceros (*Rhinoceros unicornis*). *Indian J. Vet. Anat.* 10(1/2): 90-91

Bhayani, D.M., Vyas, Y.L. and Panchal, K.M. 1995. Histomorphological study on the skin of the Lion (*Felis leo*). *Indian J. Vet. Anat.* 7(1/2): 44-51

Bhayani, D.M., Vyas, K.N., Vyas, Y.L. and Pandya, S.P. 2001. Post-natal changes in the thickness of various layers of the skin after birth in Patanwadi and Marwari sheep (*Ovis aries*). *Indian J. Vet. Anat.* 13(2): 142-144

Bloom, W. and Fawcett, D.W. 1975. *A Textbook of Histology.* Tenth edition. W.B. Saunders Company, Philadelphia, 1033 p.

Borysenko, M., Borysenko, J., Beringer, T. and Gustafson, A. 1979. *Functional Histology.* Little, Brown and Company, Boston, 251 p.

Cochran, W.G. and Cox, G.M. 1992. *Experimental Designs.* Second edition. John Wiley & Sons Inc., New York, 611 p.

Copenhaver, W.M., Bunge, R.P. and Bunge, M.B. 1971. *Bailey's Text book of Histology.* Sixteenth edition. Williams & Wilkins Company, Baltimore, 745 p.

Costarelis, G. 2006. Epithelial stem cells: A folliculocentric view. *J. invest. Dermatol.* 126: 1459-1468

David, L.T. 1932. Histology of the skin of the Mexican hairless swine (*Sus scrofa*). *Am. J. Anat.* 50: 283-292

Diana, A., Preziosi, R., Guglielmini, C., Degliesposti, P., Pietra, M. and Cipone, M. 2004. High frequency ultrasonography of the skin of clinically normal dogs. *Am. J. Vet. Res.* 65(12): 1625-1630

Ellenport, C.R. 1975. Common integument. *Sisson and Grossman's The Anatomy of Domestic Animals, Vol. 2* (ed. Getty, R.). Fifth edition. W.B. Saunders Company, Philadelphia, pp. 1420-1422

Fowler, E.H. and Calhoun, M.L. 1964. The microscopic anatomy of developing foetal pig skin. *Am. J. Vet. Res.* 25(104): 156-164

Gilliam, A.C., Kremer, I.B., Yoshida, Y., Stevens, S.R., Tootell, E., Teunissen, M.B.M., Hammerberg, C. and Cooper, K.D. 1998. The human hair follicle: a reservoir of CD 40^{+} B7 – deficient langerhans cells that repopulate epidermis after UVB exposure. *J. invest. Dermatol.* 110: 422-427

Gniadecka, M., Nielsen, O.F., Christensen, D.H. and Wulf, H.C. 1998. Structure of water, proteins and lipids in intact human skin, hair and nail. *J. invest. Dermatol.* 110: 393-398

Gracey, J., Collin, D.S. and Huey, R. 1999. *Meat Hygiene.* Tenth edition. W.B. Saunders Co. Ltd., London, 758 p.

*Hafez, E.S.E., Badreldin, A.L. and Shafei, M.A. 1955. Skin structure of Egyptian buffaloes and cattle with particular reference to sweat glands. *J. agric. Sci. Camb.* 42: 126-133

Ham, A.W. 1969. *Histology.* Sixth edition. J.B. Lippincott Co., Philadelphia, 1036 p.

Kaur, P. 2006. Interfollicular epidermal stem cells: Identification, challenges, potential. *J. invest. Dermatol.* 126: 1450-1458

Kelly, D.E., Wood, R.L. and Enders, A.C. 1984. *Bailey's Textbook of Microscopic Anatomy.* Eighteenth edition. Williams & Wilkins, Baltimore, 909 p.

Kozlowski, G.P. and Calhoun, M.L. 1969. Microscopic anatomy of the integument of sheep. *Am. J. Vet. Res.* 30 (8): 1267-1279

*Langley, J.N. 1904. On the sympathetic system of birds, and on the muscles which move the feathers. *J. Physiol.* 30: 221-252

Lee, D.G. and Nielsen, S. K. 1962. The skin, sweat glands and hair follicles of the camel (*Camelus dromedarius*). *Anat. Rec.* 143(1): 71-77

Lloyd, D.H. and Garthwaite, G. 1982. Epidermal structure and surface topography of canine skin. *Res. Vet. Sci.* 33(1): 99-104

Luna, L.G. 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology.* Third edition. Mc Graw Hill Book Company, New York, 258 p.

Mandage, S.T., Bhosle, N.S., Kapadnis, P.J. and Mamde, C.S. 2003. Microscopic anatomy of the integument of the Deccani sheep. *Indian J. Vet. Anat.* 15(1 & 2): 68-69

Marcarian, H.Q. and Calhoun, M.L. 1966. Microscopic anatomy of the integument of adult swine. *Am. J. Vet. Res.* 27(118): 765-772

Mehta, S. 2002. Comparative morphological studies on the skin of Murrah buffalo and ox. *Indian J. Vet. Anat.* 14(1 & 2): 22-24

Meyer, W., Neurand, K. and Radke, B. 1982. Collagen fibre arrangement in the skin of the pig. *J. Anat.* 134(1): 139-148

Montagna, W. 1962. *The Structure and Function of Skin.* Second edition. Academic Press, New York, 454 p.

Montagna, W. and Lobitz, W.E. 1964. *The Epidermis.* Academic Press, New York, 415 p.

Montagna, W. and Yun, J.S. 1964. The skin of the domestic pig. *J. invest. Dermatol.* 43: 11-21

Monterio-Riviere, N.A. 1998. Integument. *Text book of Veterinary Histology* (eds. Dellmann, H.D. and Eurell, J.A.). Fifth edition. Lippincott Williams and Wilkins, Philadelphia, pp. 303-332

Monteiro-Riviere, N.A. and Stromberg, M.W. 1985. Ultrastructure of the integument of the domestic pig (*Sus scrofa*) from one through fourteen weeks of age. *Anat. Histol. Embryol.* 14: 97-111

Montgomery, I., Jenkinson, D.M. and Elder, H.Y. 1982a. The ultrastructure of the sweat gland duct of the ox. sheep and goat before and during sweating. *J. Anat.* 134(4): 741-755

Montgomery, I., Jenkinson, D.M. and Elder, H.Y. 1982b. The effects of thermal stimulation on the ultrastructure of the fundus and duct of the equine sweat gland. *J. Anat.* 135(1): 13-28

Morris, G.M. and Hopewell, J.W. 1990. Epidermal cell kinetics of the pig: a review. *Cell Tissue Kinet.* 23: 271-282

Mugale, R.R. and Bhosle, N.S. 2001. Sexual difference in the morphology of the skin in Deoni cattle. *Indian J. Vet. Anat.* 13(1): 85-86

Nakagawa, N., Sakai, S., Matsumoto, M., Yamada, K., Nagano, M., Yuki, T., Sumida, Y. and Uchiwa, H. 2004. Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects. *J. invest. Dermatol.* 122: 755-763

Nicolaides, N. and Rice, G.R. 1968. Skin surface lipids of man compared with those of 18 species of animals. *J. invest. Dermatol.* 51(2): 83-89

Panchal, K.M., Vyas, K.N. and Vyas, V.L. 1999. Histogenesis of skin and its adenexa of Surti buffalo. *Indian J. Vet. Anat.* 11(2): 158-160

Sadler, T.W. 2004. *Langman's Medical Embryology*. Ninth edition. Lippincott Williams and Wilkins, Philadelphia, 534 p.

Saxena, S.K., Malik, M.R. and Parekh, H.K.B. 1994. Histological character of skin of crossbred cattle. *Indian J. Vet. Anat.* 6(1): 8-11

Schummer, A., Wilkens, H., Vollmerhaus, B. and Habermehl, K. 1981. *The Circulatory System, the Skin and the Cutaneous Organs of the Domestic Mammals*. Vol. 3. Verlag Paul Parey, Berlin, 610 p.

Sharma, D.N., Bharadwaj, R.L. and Archana. 1996. Histoarchitechure of skin of Yak (*Bos grunniens*). *Indian J. Vet. Anat.* 8(1/2): 7-10

Sharma, D.N. Dhingra, L.D. and Singh, Y. 1982. Regional histomorphological and histochemical studies on the scrotal sebaceous glands of buffaloes. *Indian J. Anim. Sci.* 52(11): 1038-1043

Sheehan, D.C. and Hrapchak, B.B. 1980. *Theory and Practice of Histotechnology*. Second edition. The C.V. Mosby Company, St. Louis, 481 p.

Singh, U.B. and Sulochana, S. 1996. *Handbook of Histological and Histochemical Techniques*. Premier Publishing House, Hyderabad, 111 p.

Smith, J.L. and Calhoun, M.L. 1964. The microscopic anatomy of the integument of newborn swine. *Am. J. Vet. Res.* 25(104): 165-173

Snedecor, G.W. and Cochran, W.G. 1985. *Statistical Methods.* Seventh edition. Iowa State University Press, USA, 313 p.

Straile, W.E. 1960. Sensory hair follicles in mammalian skin: The tylotrich follicle. *Am. J. Anat.* 106: 133-141

Stromberg, M.W., Hwang, Y.C. and Monterio-Riviere, N.A. 1981. Interfollicular smooth muscle in the skin of the domesticated pig (*Sus scrofa). Anat. Rec.* 201: 455-462

Thornton, H. and Gracey, J.F. 1974. *Textbook of Meat Hygiene.* Sixth edition. Macmillan publishing Co. Inc., New York, 596 p.

Trautmann, A. and Fiebiger, J. 1957. *Fundamentals of Histology of Domestic Animals.* Comstock Publishing Associates, New York, 426 p.

Urmacher, C. 1990. Histology of normal skin. *Am. J. Surg. Pathol.* 14(7): 671-686

Vardaxis, N.J., Brans, T.A., Boon, M.E., Kreis, R.W. and Marres, L.M. 1997. Confocal laser scanning microscopy of porcine skin: Implication for human wound healing studies. *J. Anat.* 190: 601-611

*Vincent, S.B. 1913. The tactile hair of the white rat. *J. comp. Neurol.* 23: 1-38

Wagner, M. and Bailey, D.G. 2006. Structure of Bovine skin and hair root – A scanning electron microscope investigation. *http://www.tfl. com/pdfs/others/ structrue of bovine skin. pdf (12/05/06)*

Yagci, A., Zik, B., Uguz, C. and Altunbas, K. 2006. Histology and morphometry of white New Zealand rabbit skin. *Indian Vet. J.* 83: 876-880

Yang, S.H. 1952. Histochemical studies on the bovine sweat glands. *J. agric. Sci. Camb.* 42: 155-157

*Yohro, T. 1977. Arrangement and structure of sinus hair muscles in the big-clawed shrew (*Sorex unguiculatus*). *J. Morphol.* 153: 317-332

* Originals not consulted.

MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE SKIN OF THE PIG (*Sus domesticus*)

SUMENA. K. B.

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2006

Department of Veterinary Anatomy and Histology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

Studies on the skin of Large White Yorkshire pigs were conducted using 12 animals of six to ten months of age. The project was undertaken to study the morphology, morphometry, histology and the distribution of hair and to compare the sex differences if any, in the skin of pig. Skin samples were collected from eight areas of the body viz., the snout, dorsal nasal, dorsal neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen and carpal regions. After recording gross parameters, material was fixed in 10 per cent neutral buffered formalin and standard procedures were adopted for histoarchitectural and histochemical studies.

In general, skin of male animals was slightly thicker than that of the females. Maximum thickness for the skin, epidermis and the dermis was noticed in the snout and minimum in the ventral abdomen. Skin was thicker on the dorsal surface of the body than on the ventral surface. Contribution of the epidermis to the total skin thickness was maximum in the snout region. Subcutaneous fat layer was slightly thicker in females.

A highly significant positive correlation was noticed between the skin thickness and the thickness of the epidermis in the snout, dorsal nasal and carpal regions in both male and female animals. Among the five layers of the epidermis, stratum basalis, spinosum and corneum were always present and formed continuous layers throughout the body surface. The stratum granulosum was not continuous in ventral neck and lateral and ventral abdominal regions. A definite stratum lucidum was seen only in the snout, dorsal nasal and ventral abdominal areas. The rete pegs and the dermal papillae were most abundant in the snout region and minimum in the lateral abdominal region. Stratum basalis was made up of a single layer of columnar to cuboidal cells. Clear cells could be located in the stratum basalis and stratum spinosum. Stratum spinosum was the thickest layer of the epidermis consisting of large, irregular and polyhedral cells with distinct boundaries. Prekeratin granules were detected in the upper layers of stratum spinosum. Thickness of this layer was maximum in the snout.

Stratum granulosum consisted of two to four rows of flattened, diamond-shaped cells. Cytoplasm showed keratohyalin granules. Stratum lucidum

appeared as a clear, bright, homogenous, strongly eosinophilic layer. Stratum corneum consisted of keratinized, scale-like polygonal, clear cells.

There was a significant positive correlation between the thickness of the skin and that of dermis in all regions under study in both sexes. Papillary layer of the dermis was made up of collagen fibres predominantly, which were finer and more closely arranged. Reticular layer consisted of large, coarse and loosely interwoven bundles of collagen fibres. Glomi were most numerous in the snout.

Hair arrangement in swine was simple, but grouping of hairs was evident. Maximum hair density was noticed in the dorsal nasal area. Density of hair distribution was more in the male animals. Hair shaft was composed of a cuticle, thicker cortex and slender medulla. Hair follicle was composed of four parts, viz., hair papilla, hair matrix, inner root sheath and outer root sheath. Largest arrector muscles were noticed in the abdomen dorsal region. Interfollicular muscle connected adjacent hair follicles of its characteristic hair group.

Sweat glands were of apocrine type in all the regions under study except in the snout and dorsal nasal regions where it was of eccrine type. In the latter, both clear and dark cells were identified. Sebaceous glands appeared as large, lobulated, sac-like structures associated with the hair follicles. The secretory units consisted of a solid mass of epidermal cells. Maximum subcutaneous fat thickness was noticed at the neck dorsal region. The subcutaneous tissue was composed of a loose meshwork of connective tissue fibres, cells, blood vessels and nerve fibres.

PAS - alcian blue positive areas were detected in middle region of the epidermis and ground substance of the dermis. Cells of stratum corneum, stratum spinosum, sebaceous glands, their ducts and clear cells of eccrine sweat glands showed a positive reaction to Oil Red O. Most of the layers of the epidermis and the dermal papillae, blood vessels surrounding the hair follicles and the sweat glands showed a positive reaction to alkaline phosphatase. Epidermis and sebaceous glands showed a positive reaction for acid phosphatase.