Studies in the Histology of Bovine Skin

By Annie Myfanwy Nisbet (nee Goodall)

In the first part of the thesis an account is given of studies on the structure of bovine sweat glands and their relation to sweat gland homologues is discussed. These glands in both Bos taurus and Bos indicus are of apocrine type. They are short sac-like structures and are lined by a layer of secreting epithelial cells enclosed within a layer of spindle shaped myoepithelial cells They were compared with the large coiled sweat glands found in the skin of the concave surface of the bovine ear and also with bovine and human ceruminous glands. Structurally all the glands have a similar structure but lipids occur only in the human ceruminous glands. It has been suggested by Russian workers that the development of bovine mammary glands, which are sweat gland homologues, may be correlated with that of the sweat glands in the ears of cows, and some of these workers have claimed that there is a highly significant correlation between the milk yield and the number of sweat glands in the pinna of the ear. This claim was investigated for Ayrshire cattle and no such correlation was found.

The second part of the thesis deals with the types of blood vessel present in bovine skin and the pattern of their arrangement. Using skin perfused with Indian ink the distribution of blood vessel was studied. It was found that in bovine skin three plexuses of blood vessels lie in planes parallel to the surface. The first lies just deep to the dermis, the second at a level between the sweat

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glands and sebaceous glands, and the third between the second plexus and the epidermis. Venae comites were observed to occur in all three plexuses. The blood supply to the bovine apocrine glands was found to be poor compared with that to the human eccrine coiled sweat glands. Using skin perfused with haematoxylin to stain the blood vessels preferentially it was found that arterio-venous anastomoses occur in the skin of the forehead, cheek, and ear and in the perichondrium of the ear. They were not observed in the skin of the trunk.

In the third part of the thesis an account of an investigation of the structure of the bovine muzzle is given. It was found that the secretion which usually keeps the surface of the muzzle moist is produced by multilobular tubulo-acinous glands which lie in the dermis. Their secretion contains mucin. The arrangement of the blood vessels differs from that found in the hairy part of the integument, and arterio-venous anastomoses occur in the dermis between the glands and the epidermis. Large numbers of sensory nerve endings occur in the muzzle. In the epidermis some nerve fibres end freely and some in menisci while in the dermis some end in organised endings and others in relation to blood vessels. The arterio-venous anastomoses have a great number of nerve fibres in their walls.

The possible importance of all the findings mentioned above is discussed, with special regard to the part which the skin plays in the dissipation of heat. The following conclusions

are reached.

1. Although it is apparent from recent studies that the sweating response of Bos indicus is greater than that of Bos taurus, the difference in function cannot be ascribed to any difference in the epithelial cells and myoepithelial coat of their sweat glands.

2. The structural and histochemical similarity between the sweat glands and sebaceous glands of the general skin surface and the glands of the external auditory meatus indicates that a mixed secretion similar to cerumen is produced all over the body surface of cattle.

3. The venae comites present in bovine skin, by allowing cooling of arterial blood before it reaches the skin surface, limit the amount of heat which may be lost to the environment when the environmental temperature is lower than body temperature.

4. Since the number of capillaries found in the third plexus of blood vessels in the skin is directly proportional to the number of hairs, a coat with a large number of fine, short hairs and consequently with a relatively poor insulation combined with a large capillary surface area, will allow maximum loss of heat.

5. The arterio-venous anastomoses in the ear may be responsible for a sudden increase in the temperature of the ear which has been shown to occur when the environmental temperature reaches 18°C.

6. The muzzle may be used by the animal in the selection of fodder, since it is well-supplied with nerve endings of various types that may serve as pressure, heat and, indirectly, humidity receptors.

STUDIES IN THE HISTOLOGY OF BOVINE SKIN

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by

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GENERAL INTRODUCTION

General Introduction

The earliest investigations into the structure of bovine skin were made by Gurlt in 1835. He studied the sebaceous and sweat glands of man and domestic animals, describing bovine sweat glands as small round sac-like structures. In 1906 Stoss gave a very full account of the structure of bovine skin and noticed that the sweat glands were always associated with hair follicles and that their long ducts opened into the hair follicle orifices.

The observations made in recent years that cattle indigenous to temperate countries fail to adapt themselves to tropical climates (Findlay, 1950) has stimulated interest in bovine skin with respect to the part which it and its appendages play in heat dissipation in both temperate and tropical breeds of cattle. Thus various workers (Yamane & Ono, 1936; Yang, 1948; Findlay & Yang, 1950; Villares & Berthet, 1951a & b; Hafez & Shafei, 1954; Hafez, Badreldin & Shafei, 1955; Carter & Dowling, 1954; Dowling, 1955a) have studied the distribution of hairs and sweat glands in the skins of different breeds while others (Schotterer, 1933; Hafez, Badreldin and Shafei, 1955; Dowling, 1955b) have measured the skin thickness. Yang (1952 a & b) has investigated the histochemistry of the sweat glands and the distribution of pigment in the epidermis. The results of much of this work may be summarised as follows.

In the skin of Bos taurus, Bos indicus and Bos bubalis each hair has associated with it a mono-, bi-

or multi-lobed sebaceous gland, a sweat gland and an arrector pili muscle. This group of structures has been termed a hair follicle unit. According to Schiefferdecker's (1922) classification the sweat glands are apocrine, developing from hair follicle anlagen. The cytoplasm of the glandular epithelium grows into the lumen of the gland forming small protuberances which break off without damaging the cells and accumulate in the lumen forming a granular mass. It has been suggested by Yang (1948) and Findlay & Yang (1950) that within the lumen the granular secretion undergoes gradual transformation into a homogeneous mass in which state it may be expelled through the long duct to the skin surface. The blood supply to the sweat glands, however, is poor. The hair population and therefore the sweat gland population varies from region to region of the body and also from breed to breed, while from birth to maturity it decreases in inverse proportion to the increase that occurs in body surface area as growth advances. This means that an individual animal is born with a definite number of hair follicles and that that number neither increases nor decreases with age. The thickness of the skin also varies from breed to breed, and that of bulls is thicker than that of cows. Within a breed there are fewer hair follicles per unit area in the skin of the bulls than in the skin of the cows but it should be noted that the bull has a greater surface area.

It was noticed (Yang, 1948; Findlay & Yang,

1950) that the wall of the bovine sweat gland showed two kinds of nuclei; round nuclei of secretory epithelium, and spindle-shaped nuclei of myoepithelium. A study of the type of myoepithelial cells found in the bovine sweat gland and the extent of the myoepithelial coat has now been made and is reported in Part I of this thesis.

It has been mentioned above that bovine sweat glands are apocrine and that they therefore resemble the apocrine sweat glands of the human axilla. Their similarity to Moll's glands of the eyelids has been noted by Findlay & Yang (1950). There is also a marked resemblance between bovine sweat glands and human ceruminous glands. Skin from human eyelids could not be obtained but a piece of human external auditory meatus was obtained and a comparison of its glands and the glands found in the bovine auditory meatus and general body surface has been made. An account of this investigation will be found in Part I of this thesis.

The generally accepted fact that mammary glands are sweat gland homologues has led several Russian workers to attempt to define the relationship existing between the development of the bovine mammary glands and the sweat glands. They have claimed that there is a positive correlation between the number of sweat glands in the pinna of the cow's ear and the milk yield. If this were so it would provide a simple and invaluable means of estimating the potential milk yield of cattle. This matter, therefore, has been

investigated in detail using animals of the Ayrshire breed. A description of this work forms a section of Part I of this thesis.

Heat lost from the skin through radiation, conduction, convection and through evaporation of sweat and of insensible perspiration is influenced by the blood flow in the skin. Nearly all the work concerned with the role of bovine skin in body temperature regulation has laid stress on only one of these factors, viz., the loss of heat through evaporation of sweat. Findlay & Yang (1948), Yang (1948) and Hafez, Badreldin & Shafei (1955), incidental to their studies on hair distribution have attempted to assess the vascularisation of bovine skin but their findings are markedly incomplete. Consequently the work recorded in Part II of the present thesis has been devoted to studying the blood supply and the types of blood vessels found in bovine skin.

The bovine muzzle in contrast to the rest of the body surface is devoid of hairs and is usually moist. It was thought that this highly differentiated area of the integument must have a special function and its structure has therefore been examined. Special attention has been given to the type of glands producing the watery secretion seen on the surface and to the arrangement of the blood vessels. Another point of interest was whether there were any specialised sensory nerve endings in the muzzle which because of its location seems likely to be a sensitive

structure. The results of these studies are recorded in Part III of the thesis.

Some of this work has already been published, (Findlay, Goodall & Yang, 1950; Goodall & Yang, 1952; Findlay & Goodall, 1953; Goodall & Yang, 1954; Goodall, 1955; Nisbet, 1955).

PART I

The sweat glands of cattle and their relation to sweat gland homologues

Chapter 1

Myoepithelial cells in the sweat glands of cattle

Introduction

Muscular elements associated with the apocrine sweat glands of various breeds of cattle have been previously observed and presumed to be myoepithelial cells (Yamane & Ono, 1936). More recently a layer of cells, with spindle-shaped nuclei, lying between the basal membrane and the glandular epithelial cells, with the longitudinal axes of the nuclei parallel to the longitudinal axis of the gland, has been described in the skin of Ayrshire cattle, and although neither the cell boundaries nor the myofibrils of these cells was demonstrated, they were presumed to be myoepithelial cells (Findlay & Yang, 1950).

Myoepithelial cells have been described in human sweat glands as flattened spindle-shaped cells, lying between the basement membrane and the glandular epithelium of the secretory portion of the gland, with the longitudinal axis parallel or slightly oblique to that of the glandular tube and possessing elongated nuclei and also longitudinal fibrils in the cytoplasm (Maximov & Bloom, 1948). However, Boeke (1934) demonstrated that the myoepithelial cells of human sweat glands may be star-shaped and form a network, the ends of the branching process being joined.

Richardson (1949) in his excellent work on the goat mammary gland has demonstrated that the myoepithelial cells of mammary glands which were

distended with milk during fixation were more elongated than those of contracted, empty alveoli which showed considerable branching and were star-shaped. The number of branching processes was also considerably greater in the contracted gland. This evidence tends to suggest the contractility of myoepithelial cells. Similar findings have been reported for the mammary glands of cats, dogs, rats, goats and humans by Linzell (1952). Recent work by Hurley and Shelley (1954) has shown that the myoepithelial cells found in the apocrine sweat glands of the human axilla are contractile and that their contraction is instrumental in expelling the secretion of the glands on to the skin surface. They described the constrictive changes which proceed along the gland tubules as being very similar to peristaltic movements in the small intestine. The contraction could be initiated by adrenaline.

Using a modification of Richardson's (1948) silver impregnation technique a comparison of the myoepithelial cells of the sweat glands of Ayrshire and Zebu cattle has been made.

Materials and Methods

Skin specimens from Ayrshire and Zebu cattle, the latter of Jamaican and Sudanese origin, were used. The specimens had been previously fixed in 10% formalin for periods ranging from 1 day to as long as 3 months. The area of each skin specimen was approximately 1 sq. cm. The specimens were transferred directly to

Weber's fixative (1944), and were left in it for 3 weeks at room temperature, though equally satisfactory results were obtained by incubating the specimens with Weber's fluid for 48 hr. at 37°C. The specimens were then washed in running water for about 4 hr. Frozen sections were cut perpendicular to the surface at 30μ thick without previous gelatin embedding. The sections were then collected in buffered sodium acetate solution of pH 5.2-5.4 and left overnight. The next day they were transferred to a silver nitrate-pyridine bath prepared by mixing 5 ml. silver nitrate (5%) with 4 ml. pyridine (50%) and 15 ml. distilled water. The pH value of the bath should be 8.5 as tested with thymol blue indicator. About six sections were incubated in this bath for 25 min. at 55°C. Each section was then individually washed for 5 sec. in absolute alcohol and reduced in a solution consisting of 0.3 g. hydroquinone, 30 ml. neutral commercial formalin and 70 ml. distilled water.

After reduction the sections were washed in distilled water, fixed in 5% sodium thiosulphate solution, and washed again in several changes of water. They were then mounted on gelatinized slides, fixed in formalin vapour, dehydrated in graded ethanol, cleared in xylol and mounted in balsam.

Experience with this method indicates that successful silver impregnation of the myofibrils depends on previous treatment with Weber's fluid and the pH of the silver nitrate-pyridine bath. The optimum value of the latter appears to be 8.5, since a

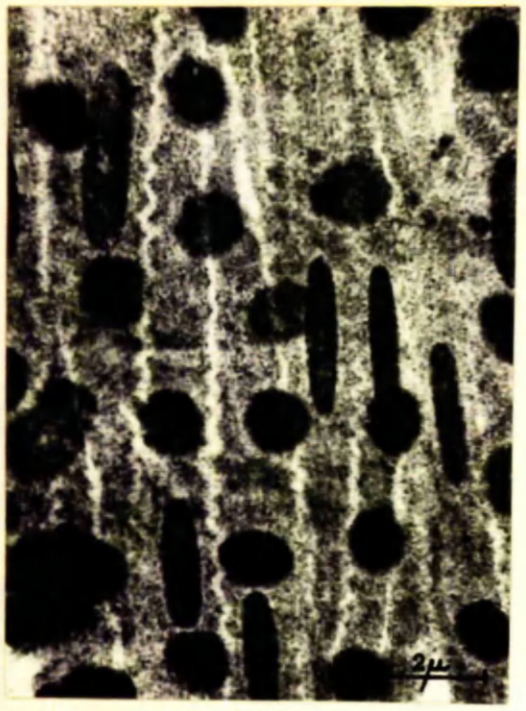


Fig.1. Spindle-shaped myo-epithelial cells in the wall of a sweat gland of a Zebu cow.

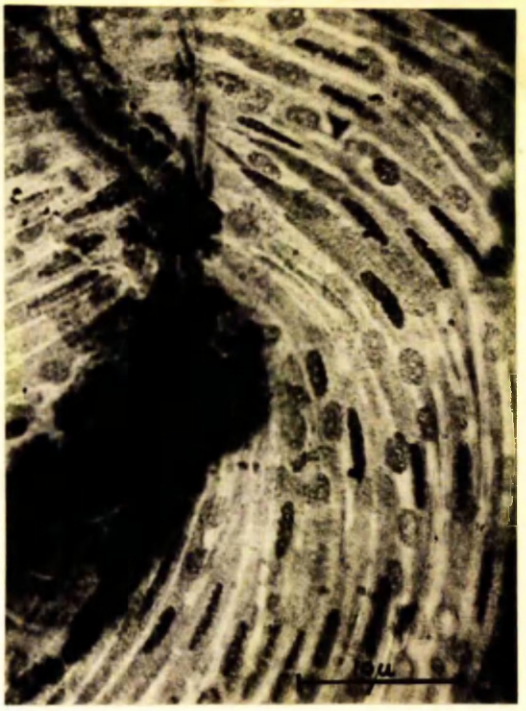


Fig.2. Spindle-shaped myo-epithelial cells in the wall of a sweat gland of an Ayrshire cow.

Fig.3. Polyhedral secretory cells and spindle-shaped myo-epithelial cells in the sweat gland of an Ayrshire cow.

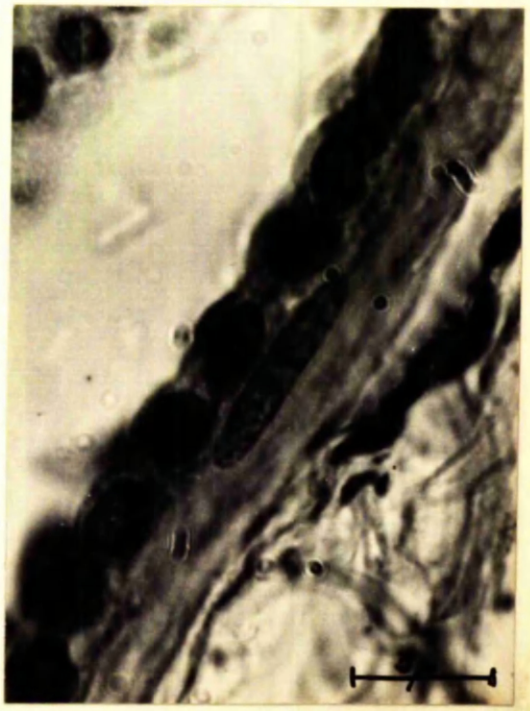


Fig.4. The spindle-shaped nucleus of a myoepithelial cell lying behind the secretory cells of a sweat gland of a Zebu cow.

difference in pH of 0.3 on either side of this value failed to give good results. It has been suggested that Weber's complex fluid could be simplified to fewer constituents (Richardson, 1949), and attempts were therefore made to use substitutes for the original fluid. Acetone was substituted for dioxane in the original formula, and chloral hydrate or cobalt nitrate solution was used alone instead of Weber's fluid. The results were unsatisfactory.

Frozen sections of formalin-fixed skin specimens were also examined under the polarizing microscope in order to study the physical nature of the myoepithelial cells.

Results

The myoepithelial cells of the so-called sweat glands of Zebu cattle as revealed by the silver impregnation technique (Fig. 1) are essentially similar in structure and arrangement to those found in the sweat glands of Ayrshire cattle (Fig. 2). This confirms the assumption made previously (Findlay and Yang, 1950).

The myoepithelial cells have spindle-shaped nuclei and are themselves spindle-shaped, whereas the secretory epithelial cells of the glands are polyhedral with round nuclei and non-fibrillar cytoplasm (Fig. 3). The myoepithelial cells, as may be seen from Fig. 4, form the outer layer of the secretory portion of the sweat gland. In both breeds of cattle, the myofibrils extend the entire length of the myoepithelial cells,

Fig.5. Longitudinal section of a sweat gland of a Zebu cow showing the birefringence of the myoepithelial layer under a polarizing microscope.

and the longitudinal axes of the cells are parallel to the longitudinal axes of the glands.

Under the polarizing microscope, when the longitudinal axis of the sweat glands is at an angle of 45° to the axis of each of the two crossed Nicol prisms, the myoepithelial layer exhibits birefringence, which is lost if the axis of the gland is rotated until it is parallel with the axis of either of the crossed prisms (Fig. 5). Treatment of the skin sections with acetone does not affect the birefringence of the myoepithelial layer (Yang, 1952a). This suggests that the myoepithelial layer consists of fibrillar structures, and that the longitudinal axes of these fibrils are parallel with the longitudinal axis of the gland.

These observations were confirmed by repeated experiments on skin specimens from four Ayrshire cows, two Ayrshire bull calves, three Zebu cattle of Sudanese origin, consisting of one female and two male animals, and one Zebu cow from Jamaica.

Discussion

In the present study the myoepithelial cells were found to be spindle-shaped, had no branching processes, and did not form a loose network covering the gland body but joined with each other in the form of a continuous sheet. Because of the effect of the histological techniques it was impossible to determine which was a contracted and which a distended sweat gland. Because of this the evidence presented here

simply establishes that the myoepithelial cells of the sweat glands of both Ayrshire and Zebu cattle contain myofibrils similar in location, arrangement and nature. It is therefore unlikely that any difference in function between the sweat glands of these two breeds of cattle, one temperate and one tropical, can be ascribed to a difference in the myoepithelial cell layer of the glands.

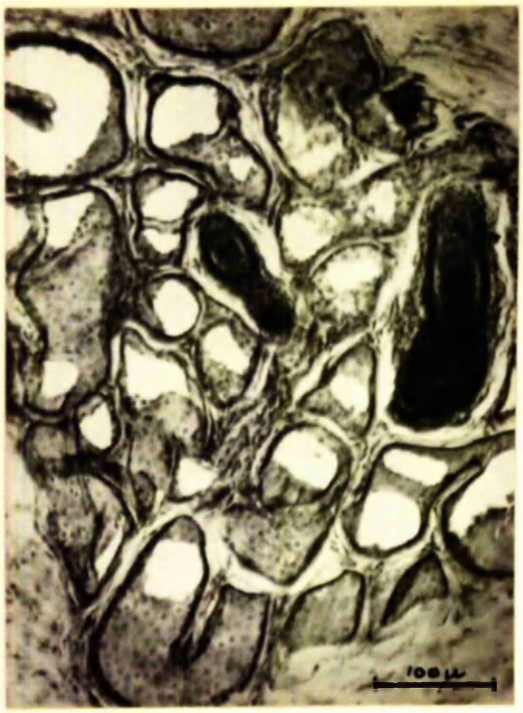


Fig.6. Large coiled sweat glands in the skin of the inner surface of the ear of a calf.

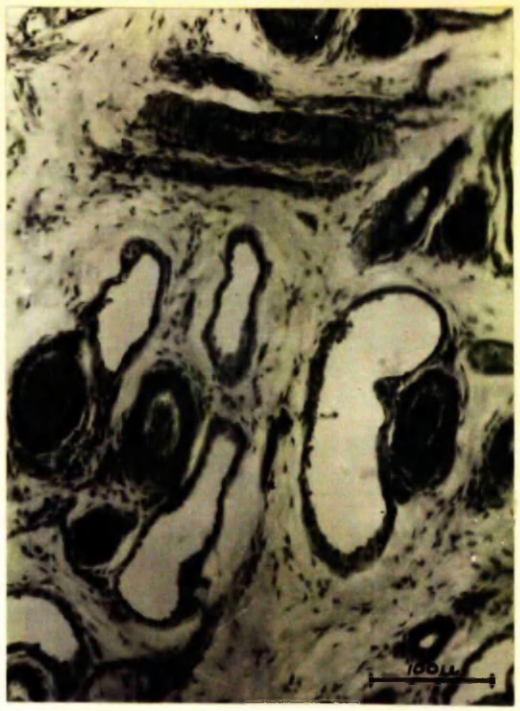


Fig.7. Small sweat glands in the skin of the outer surface of the ear of a calf.

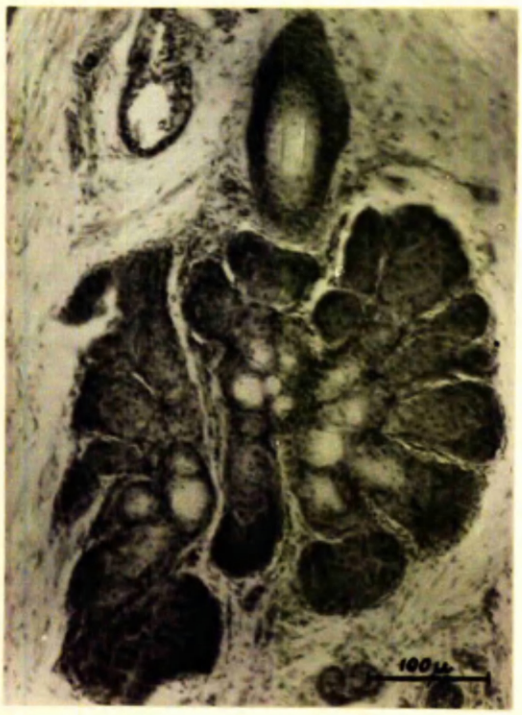


Fig.8. A large multi-lobed sebaceous gland in the skin of the inner surface of the ear of a calf.

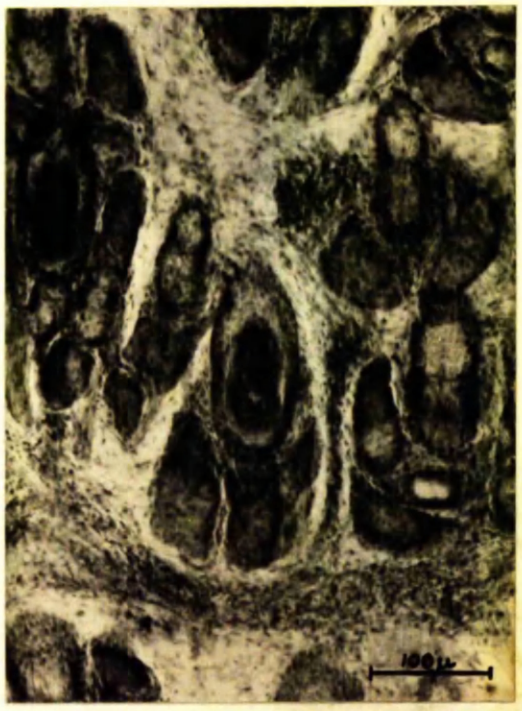


Fig.9. Small bi-lobed sebaceous glands in the skin of the outer surface of the ear of a calf.

Chapter 2

A comparison of human ceruminous glands with the ceruminous and sweat glands of cattle

Introduction

In the course of examining sections of skin from the ears of calves and cows it was noticed that the sweat glands in the skin from the inner surface (concave side) of the ear were larger and more convoluted than those in the skin from the outer surface (Figs. 6 and 7). There was a corresponding difference in the sizes of the sebaceous glands (Figs. 8 and 9). A strip of skin running from the tip to the base was cut from the inner surface of the ear of a calf. At intervals along it, blocks of tissues were removed, fixed in 10% formol saline and sectioned on the freezing microtome, at 15μ. The sections were stained with haematoxylin and eosin and were then examined. From this preliminary investigation it was found that the nearer to the external auditory meatus the location of the block of skin was, the larger were the sebaceous and sweat glands in the block. This gradual increase in the size of the glands continued until at the mouth of the external auditory meatus the largest glands were found. These sweat glands and sebaceous glands must secrete the large amounts of wax (cerumen) which is always present in the ears of cattle. It has been found by Yang (1952a) that no lipid occurs in the apocrine sweat glands of cattle. Yet lipids do occur in the

human ceruminous glands (Maximow and Bloom, 1948). The following questions therefore arise. Are there ceruminous glands in the bovine external auditory meatus and if so, are they similar to human ceruminous glands? Do the large apocrine glands found near the bovine external auditory meatus contain any lipids and if so, where in the inner surface of the ear does the transition between ordinary apocrine sweat glands and lipid-containing ceruminous glands take place? In an attempt to answer these questions the following investigation has been made.

Material and Methods

A strip of skin 1 cm. wide, running from the tip into the external auditory meatus was cut from the inner surface of the ear of a calf at autopsy. At nine points along its length blocks, of area 1 sq. cm. were cut, labelled, and fixed in 10% formol saline. After fixation each block was halved. One half of the block was embedded in paraffin and sections of it cut at 7-10μ, while the other was sectioned on the freezing microtome at 15μ.

A piece of human external auditory meatus was obtained through the good services of Dr. B. Lennox of the Pathology Department of the London Postgraduate Medical School. A block of the cartilagenous meatus was embedded in paraffin and sectioned at 7-10μ. A second block was sectioned on the freezing microtome at 15μ.

Paraffin sections of bovine and human material

were stained with Delafield's haematoxylin and eosin. Paraffin sections of bovine material were stained with Hotchkiss's (1948) periodic acid-Schiff (P.A.S.) method. The nuclear stain used was celestine blue and Mayers haemalum (Lendrum & McFarlane, 1940) with orange G as a counter stain. Control sections were stained with Schiff's reagent without previous treatment with periodic acid.

Frozen sections were stained in 1% toluidine blue for 10 min. to test for metachromasia. They were examined in water and in glycerine jelly.

To test for glycogen, sections were stained by the P.A.S. technique after treatment with saliva for 10 min. at room temperature.

Frozen sections were stained with Sudan black B in propylene glycol (Chiffelle and Putt, 1951). Carmalum was used as a nuclear stain. The sections were mounted in glycerine jelly.

Results

Examination of the sections of bovine skin stained with haematoxylin and eosin showed that the sweat and sebaceous glands of the skin nearer the external auditory meatus are much larger than those of the skin nearer the tip of the ear. Near the meatus the sweat glands, normally small straight sac-like swellings at the end of long thin ducts, are coiled and in some places branched. The sebaceous glands, normally small and bi-lobed, are large and multi-lobed. As in the skin of the general body surface each hair

Fig.10. Ceruminous glands in the external auditory meatus of a calf.

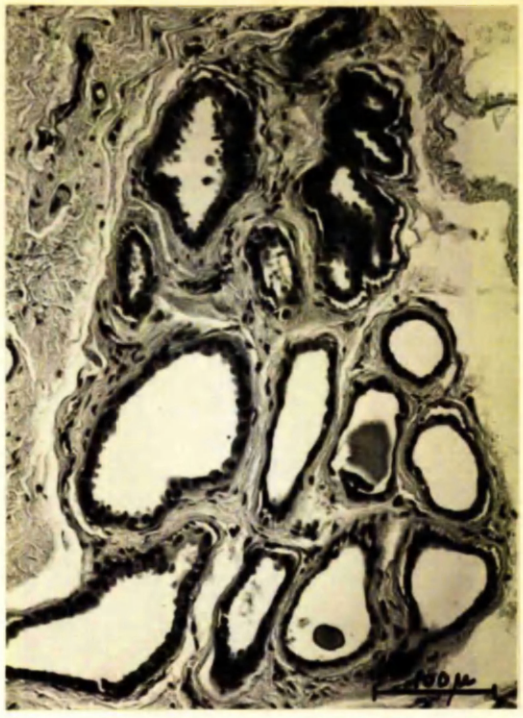


Fig.11. Ceruminous glands in the external auditory meatus of a man.

Fig.12. Sudanophilic material in human ceruminous glands.

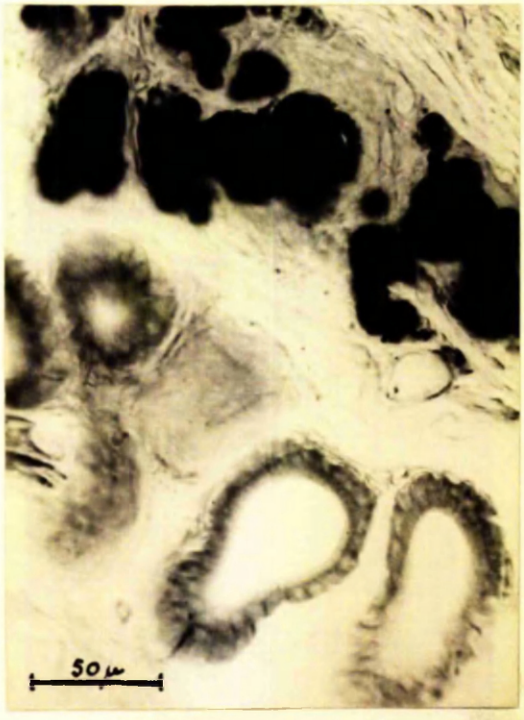


Fig.13. Bovine sebaceous and ceruminous glands. The latter display no sudanophilia.

follicle has associated with it one sweat gland and one sebaceous gland. In the meatus itself the hairs are smaller and each has associated with it a sebaceous gland and a coiled sweat gland, the ceruminous gland. The only difference between the sweat glands of the rest of the ear and those found in the meatus itself is that the latter have cubical secretory epithelial cells while the epithelium in the other regions is more flattened. These may represent two stages in apocrine secretion; the first the pre-secretory phase and the second the recovery phase. Fig. 10 shows bovine ceruminous glands in the presecretory phase of apocrine secretion.

The ceruminous glands of the human external auditory meatus are similar in structure to those of the bovine meatus. They are coiled and different phases of apocrine secretion can be seen in their epithelium. Fig. 11 shows a gland whose tubules are in different stages of apocrine secretion. Some sections of the lumen are filled with a homogeneous mass while others contain droplets of secretion and protrusions of cytoplasm from the epithelial cells.

The secretory cells of the human ceruminous glands contain large numbers of sudanophilic globules (Fig. 12). When the frozen sections were treated with lipid-solvents, acetone and chloroform, prior to staining, no large sudanophilic globules were seen but some sudanophilic material, probably pigment remained. The bovine ceruminous glands display no sudanophilia (Fig. 13). The sebaceous glands of both species show

marked sudanophilia.

The secretion of the bovine ceruminous glands and of the sweat glands of the skin of the ear stain positively with P.A.S. There is no glycogen in the glands and no metachromasia with toluidine blue. Since there is no lipid present, it is concluded that the secretion of the bovine ceruminous glands and of the sweat glands of the ear contains either a mucoprotein or a mucopolysaccharide which is not strongly acidic.

Discussion

It has already been shown by Montagna, Noback and Zak (1948) that the human ceruminous glands secrete lipids and P.A.S. positive materials, one of which is glycogen. When the glycogen is removed by the action of diastase a P.A.S. positive substance is still present in the secretion. This may be a mucoprotein or a mucopolysaccharide.

As can be seen from the above results the ceruminous glands of the calf secrete a mucoprotein or mucopolysaccharide. They secrete no lipid. In this respect they resemble the ceruminous glands of the cat (Montagna, 1949).

The apocrine sweat glands of the skin of the ear resemble those of the general body surface in containing no lipids (Yang, 1952b). Their secretion contains either a mucoprotein or a mucopolysaccharide. This is in agreement with the results of Lennox, Pearse and Symmers (1952) who found mucin in the

secretion of the bovine sweat glands. During this investigation their work has been repeated and verified for the skin of both Ayrshire and Zebu cattle.

Thus it is apparent that in the calf there is no difference between the apocrine sweat glands found in the skin of the general body surface, the large coiled apocrine sweat glands of the inner surface of the ear and the smaller coiled ceruminous glands of the external auditory meatus. All the lipid in the cerumen of the calf must be secreted by the large sebaceous glands found on the inner surface near the meatus and the smaller sebaceous glands in the meatus. The mucin secreted by the apocrine sweat glands and the ceruminous glands of the external auditory meatus must mix with the lipids on the skin surface.

Sweat and sebaceous glands, smaller than, but structurally and histochemically similar to, the glands which secrete the cerumen, occur all over the body surface of the calf. Their combined secretion must be the same as it is in the ear. It is generally believed that one of the functions of cerumen is to repel insects from entering the auditory meatus. It would be advantageous to cattle to have an insect repellent material all over the body surface to prevent infestation with parasites, and one of the functions of the apocrine sweat glands may be to secrete a portion of this insect repellent material. The mucin will be carried to the surface via the sweat gland ducts when the sweat glands are activated to expel their secretion. It has been shown by Ferguson and

Dowling (1955) that this can be brought about by a high environmental temperature and by intradermal injections of adrenaline.

Chapter 3

The relation between the number of sweat glands in the helix of the cow's ear and the milk yield

Introduction

The possibility of a relationship between the histological structure of the mammary glands and the milk production of dairy cattle has been investigated by various workers (Liskin, 1912; Nemilov, 1915; Pesciscev, 1926; Cingovatov, 1927; Lisick, 1928; Simic, 1928; Okaladnov, 1932; Podoba, 1933). It is obviously impracticable to make a histological study of the mammary tissue of living cows in order to assess their milk-producing capacity, but Nemilov (1927) claimed that there was a correlation between the development of the sweat glands in the skin of the helix of the ear and the amount of glandular tissue in the udder. This correlation was explained by him on the basis of the close phylogenetical relationship between the mammary glands and the sweat glands of the cow. Since then, other workers have endeavoured to find what correlation there is in various breeds of cattle between the milk yield and the number of sweat glands per unit area of skin in the ear (Zamjatin, 1929; Konjkov, 1930; Podoba, 1933 & 1944; Krasnokutsk, 1933; Burcev, 1937). With the exception of Krasnokutsk, they all found a significant positive correlation.

The distribution of sweat glands in the bovine ear might well be expected to vary from one area to

another, but from a study of ten right and ten left ears of cows of Red German breed, Burcev (1937) claimed that there was no difference in the number of sweat glands per unit area of skin between the two ears or between different regions of the same ear. He concluded that samples of skin from any region of the ear could be used for obtaining the sweat-gland count, and that the number so obtained would be representative of any other region of either the left or right ear. Furthermore, as a result of an investigation involving 1336 Red German cattle of both sexes and various ages, he claimed that the number of sweat glands per unit area of skin in the ear did not depend on either age or sex but was characteristic of the particular animal being studied.

If the claims made by these various workers, and particularly by Burcev, were substantiated for any of the principal breeds of dairy cattle, a relatively simple method of great value in selecting cattle for high milk production would immediately become available. The experiments described in this chapter were therefore made to determine whether Burcev's findings for Red German cattle applied also to Ayrshire cattle. Three aspects of the problem received particular attention. First, the distribution of the sweat glands in the ear was studied in detail to determine whether Burcev's conclusion was valid that any portion of either ear could be used to obtain the sweat-gland count, and that the count so obtained would be representative for any other region of either

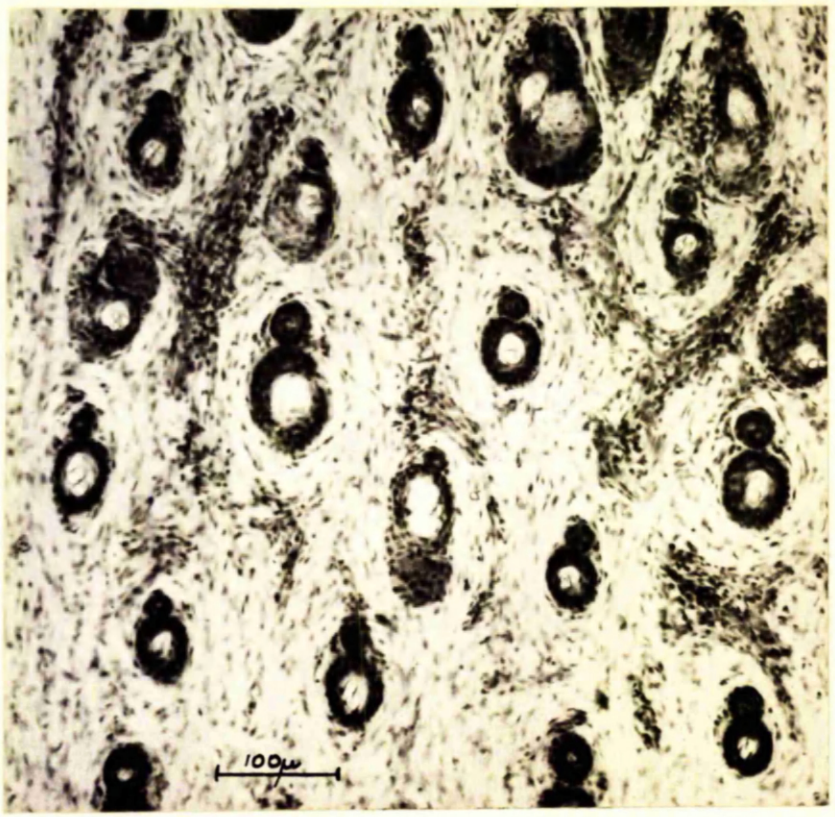


Fig.14. A section of skin, parallel to the skin surface, from the pinna of a cow's ear, showing the hair follicles and their associated sweat gland ducts.

ear. Secondly, an experiment was made to determine whether Burcev's claim could be confirmed that the sweat-gland count is independent of the age of the animal, and is the same for the very young calf as it is for the adult cow. Thirdly, attempts were made to find what correlation there is between the sweat-gland count and the milk yield.

Experimental

(1) The distribution of the sweat glands in the helix of the ear

Previous studies on the distribution of sweat glands in cattle skin have shown that each sweat gland and its duct are accompanied by one hair, so that to count the sweat glands in a given area of skin, it is necessary only to count the hair follicles (Findlay & Yang, 1950; Yamane & Ono, 1936; Yang, 1948), and it can be seen from Fig. 14 that this also holds for the skin of the ear. In fact, if attempts are made to count the sweat glands themselves, the procedure which has been adopted by Burcev (1937), large errors may arise owing to the presence of convolutions which may cause one gland to appear several times in one section. Counting the hair follicles avoids this difficulty and introduces no additional errors.

Two methods were adopted in the present work for studying the distribution of the sweat glands. In the first method the hair follicles were counted in carefully prepared sections of the skin taken from different regions of the ear, but since it was

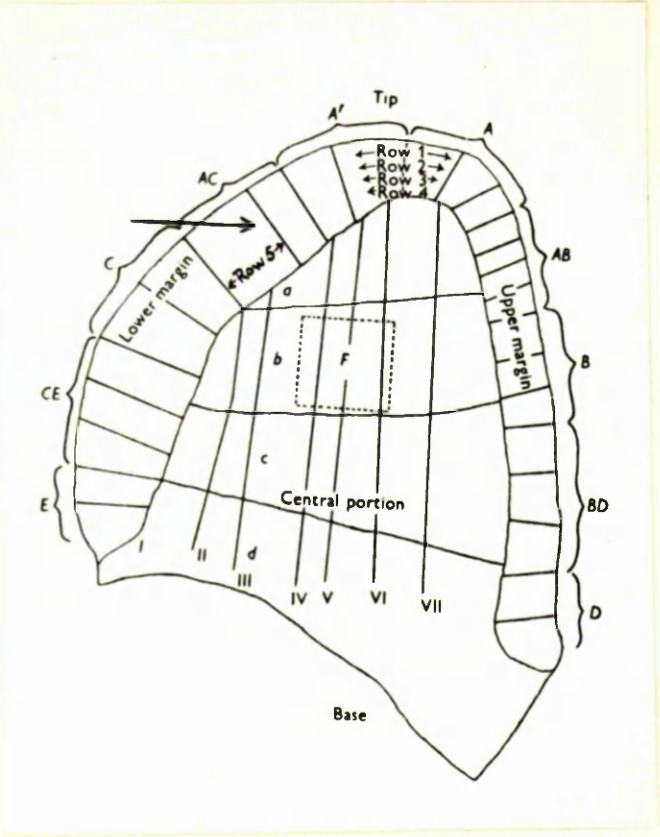


Diagram 1. The locations in the cow's ear of the columns, rows, regions and margins in which counts were made.

Table 1. *The number of hair follicles per sq.cm. of skin in different regions of the right and left ears of an Ayrshire cow, with an analysis of variance*

The counts were obtained from sections prepared as described on pp. 22 and 23. The positions of the various regions *A* to *F* are shown in Diagram 1. Two adjacent samples were taken from each region.

Region of the ear	Sample	Right ear: Surface (average of 10 counts) Inner	Right ear: Surface (average of 10 counts) Outer	Right ear: Total of the four averages	Left ear: Surface (average of 10 counts) Inner	Left ear: Surface (average of 10 counts) Outer	Left ear: Total of the four averages
A	1	990	240	2,392	1,499	204	3,087
	2	958	204		1,098	286	
B	1	411	771	2,363	668	948	2,927
	2	287	894		458	853	
C	1	787	209	1,924	661	185	1,729
	2	684	244		638	245	
D	1	1,188	1,767	5,406	451	1,380	3,337
	2	620	1,831		223	1,283	
E	1	205	194	1,093	345	292	1,428
	2	495	199		489	302	
F	1	1,261	968	4,450	706	610	3,040
	2	1,103	1,118		814	910	
Total		8,989	8,639	17,628	8,050	7,498	15,548

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Main effects:			
Ear (E)	1	90,134	90,134*
Region (R)	5	3,350,298	670,059**
Surface (S)	1	16,950	16,950
First order interactions:			
ER	5	812,395	162,479**
RS	5	4,227,248	845,449**
ES	1	850	850
Second order interactions:			
ERS	5	73,026	14,639
Error	24	455,820	18,991
Total	47	9,026,901	

Source of variation	Degrees of freedom	Right ear: Sums of squares	Right ear: Mean squares	Left ear: Sums of squares	Left ear: Mean squares
R	5	3,359,532	671,906**	803,161	160,632**
S	1	5,104	5,104	12,696	12,696
RS	5	1,897,162	379,432**	2,403,293	480,658**
Error	12	253,959	21,163	204,336	17,028
Total	23	5,515,757		3,423,486	

* Significant, $P<0{\cdot}05$. ** Highly significant, $P<0{\cdot}01$.

realized that the preparation of the sections would lead to some contraction and possibly to distortion of the skin, the distribution was investigated also by counting the hairs themselves on the shaved skin of an ear which had just been amputated from an animal immediately after slaughter. The results obtained by each of these methods are recorded briefly below.

The distribution obtained by counting the hair follicles in sections of the skin. Six different regions of both the right and left ears of a cow were chosen, A,B,C,D,E and F as shown in Diagram 1. Two adjacent pieces of skin were cut from each region a short distance from the edge of the ear and fixed in 10% formalin. Sections parallel to the skin surface, and 50μ thick, were cut by means of a freezing microtome, and were stained with toluidine blue, mounted, dehydrated and cleared in the usual way. The images of the sections were projected on white paper using a microscope whose field covered an area on the section of 20 sq.mm. Only those sections of skin near the surface level were used for counting, and ten counts in all were made from the sections from any one piece of skin. The results were averaged and expressed as the number of sweat glands per sq.cm. of skin surface area. The data thus obtained, with the analysis of variance, are given in Table 1. It can be seen from the table that the variance between the right and left ears was significant while the variances due to regions, and to the interactions of region and surface and ear and region, were highly

Table 2. *Hair counts per sq.cm. for the outer and inner surfaces of the upper and lower margins of the left ear of an Ayrshire cow*

The position of the various columns and rows can be seen in Diagram 1.

Row	Col. *A*		Col. *AB*				Col. *B*						Col. *BD*						Col. *D*				Sum
										Upper margin—outer surface													
1	924	1,050	1,410	1,570	1,490	1,550	1,420	1,270	1,684	1,674	1,468	1,290	1,042	1,068	1,095	1,354	1,300	1,115	1,057	1,190	1,304	1,330	28,645
2	420	365	405	450	509	523	558	702	835	858	904	938	790	845	903	938	948	948	1,073	938	929	1,214	16,993
3	252	232	385	360	425	480	510	640	627	701	845	691	587	700	745	854	696	839	953	988	860	800	14,170
Sum	1,596	1,647	2,200	2,380	2,424	2,553	2,488	2,612	3,146	3,233	3,217	2,909	2,419	2,613	2,743	3,146	2,944	2,902	3,083	3,116	3,093	3,344	59,808
										Upper margin—inner surface													
1	1,348	1,269	1,304	1,198	1,334	1,056	820	726	790	864	815	665	627	860	730	746	680	716	982	977	691	558	19,756
2	711	720	593	805	529	489	493	474	420	449	479	504	404	465	499	390	360	331	913	924	259	237	11,446
3	523	504	553	519	459	355	370	430	360	435	470	401	355	395	355	430	376	266	899	933	247	197	9,832
Sum	2,582	2,493	2,450	2,522	2,322	1,900	1,683	1,630	1,570	1,748	1,764	1,570	1,386	1,720	1,584	1,566	1,416	1,313	2,794	2,834	1,195	992	41,034
	Col. *A*		Col. *AC*				Col. *C*						Col. *CE*						Col. *E*				Sum
										Lower margin—outer surface													
1	859	602	592	592	663	573	624	573	701	513	612	790	735	943	914	1,017	737	913	864	682	637	523	15,659
2	351	316	286	296	247	227	183	306	291	177	874	301	330	350	276	380	356	281	291	326	276	321	7,042
3	261	301	281	227	192	291	222	326	227	143	143	177	266	202	207	227	227	192	168	187	192	222	4,881
4	237	247	242	207	207	192	197	261	247	187	163	212	177	163	202	212	271	187	163	222	276	276	4,748
Sum	1,708	1,466	1,401	1,322	1,309	1,283	1,226	1,466	1,466	1,020	1,792	1,480	1,508	1,658	1,599	1,836	1,591	1,573	1,486	1,417	1,381	1,342	32,330
	Col. *A*		Col. *AC*				Col. *C*						Col. *CE*						Col. *E*				Sum
										Lower margin—inner surface													
1	987	864	1,095	1,052	1,023	1,097	1,280	1,252	1,034	978	1,166	1,170	953	987	888	948	840	825	652	558	593	286	20,528
2	677	667	770	824	854	884	1,120	933	760	593	673	667	662	518	385	316	281	311	326	257	212	158	12,848
3	597	642	677	736	750	850	1,028	849	652	523	597	587	513	523	247	267	168	207	163	163	118	123	10,980
4	563	622	597	563	690	637	1,102	809	636	568	513	533	583	437	449	380	331	351	252	261	172	192	11,241
Sum	2,824	2,995	3,139	3,175	3,317	3,468	4,530	3,843	3,082	2,662	2,949	2,957	2,711	2,465	1,969	1,911	1,620	1,694	1,393	1,239	1,095	759	55,597

significant. The variance between the outer and inner surfaces, however, was not significant. It follows therefore that in attempting to determine the sweat-gland count very different counts will be obtained in the Ayrshire cow depending on the particular ear and region of the ear which is chosen. This is contrary to the findings of Burcev (1937) for Red German cattle.

The distribution obtained by counting the hairs on an amputated ear. The hairs on a freshly cut left ear taken from an adult Ayrshire cow were clipped and the ear divided into three portions, as shown in Diagram 1; an upper margin ABD, a lower margin ACE and a central region. The hairs of the ear were shaved sufficiently to facilitate the counting of the individual hairs. The counts were made with a magnification such that the square field covered by the eyepiece graticule represented 20.25 sq.mm. of the ear surface. A Pointolite lamp was used for illumination. The results quoted in Table 2 will help to illustrate the method adopted and the type of counts obtained. The upper and lower margins of the outer and inner surfaces of the ear were first studied. The figures in any one column of Table 2 show the number of hairs per sq.cm. from the edge of the ear inwards in adjacent fields, i.e. rows 1, 2, 3 and 4 in Diagram 1. Along the rows, the counts were also made in adjacent fields, except for short breaks between the main divisions, e.g. in passing from A to AB or from AB to B and so on (Table 2 and Diagram 1).

Table 3. *Hair counts per sq.cm. for the outer and inner surfaces of the central portion of the ear*

The position of the various columns and rows can be seen in Diagram 1.

Row	Column I	II	III	IV	V	VI	VII	Sum
				Outer surface				
a	—	—	296	306	642	459	390	
	—	—	—	252	385	420	435	5,223
	—	—	—	107	381	385	—	
	—	—	—	—	340	425	—	
b	—	272	320	242	370	415	400	
	—	257	285	350	360	474	479	
	—	—	306	370	439	385	469	11,683
	—	—	266	380	430	375	430	
	—	—	375	434	425	494	504	
	—	—	385	—	—	508	484	
c	395	276	370	533	548	587	587	
	464	266	434	548	583	652	652	
	760	286	568	662	513	716	770	
	390	326	681	691	681	1,140	933	21,532
	—	464	—	—	696	790	844	
	—	—	—	—	850	—	899	
	—	—	—	—	—	—	977	
d	682	667	720	583	790	1,220	919	
	741	617	642	677	878	982	947	17,349
	780	637	741	741	662	1,000	908	
	—	—	—	815	—	—	—	
Sum	4,212	4,068	6,389	7,691	9,973	11,427	12,027	55,787
				Inner surface				
a	—	—	—	805	755	721	415	
	—	—	—	731	666	577	518	6,955
	—	—	—	—	666	617	484	
b	—	741	603	652	677	622	474	
	—	607	568	587	617	671	494	
	—	—	568	617	647	671	568	18,895
	—	—	563	613	617	543	517	
	—	—	568	647	617	578	558	
	—	—	592	691	672	735	—	
c	737	583	617	563	736	775	558	
	597	553	647	582	701	825	642	
	548	597	587	612	721	850	593	
	—	686	642	622	775	1,010	434	
	—	647	587	637	795	1,040	499	26,486
	—	—	—	671	751	—	632	
	—	—	—	—	677	—	737	
	—	—	—	—	—	—	1,020	
d	696	751	667	810	736	849	661	
	681	800	637	755	780	825	726	15,668
	766	785	755	830	751	726	681	
Sum	4,025	6,750	8,601	11,425	13,357	12,635	11,211	68,004

It will be seen from Table 2 that in the region of the first three rows there was a marked tendency for the number of hairs to decrease from the outer edge of the ear inwards, and in rows 2 and 3 of the outer surface of the upper margin the numbers tended in general to increase from the tip of the ear to the base, but this was not so for the inner surface. On the outer surface of the lower margin there was little tendency for the counts to change from tip to base, but they were again higher at the edge (row 1) than farther towards the centre. On the inner surface of the lower margin the counts tended to increase from the tip to region C and then to decrease again towards the base.

To obtain counts for the central portion of the ear, the centre was divided into four rows a, b, c and d, and seven columns, I-VII, as shown in Diagram 1. The inner surface of the cow's ear has four cartilaginous ridges and these corresponded with columns I, III, V and the borderline dividing the upper margin from the central portion of the ear. Successive fields were counted in columns I-VII with very short breaks occurring between rows a, b, c and d. From the results which are shown in Table 3, it is clear that on the outer surface of the central portion there was a general tendency for the counts to decrease in the first few rows near the tip of the ear and then to increase towards the base. In the inner surface the same general tendency existed, but it was much less marked.

Table 4. *An analysis of variance of the results recorded in Tables 2 and 3*

	Region of the ear	Source of variation	Degrees of freedom	Sums of squares	Mean squares
1	Upper margin, outer surface	Total	65	8,555,716	—
		Rows	2	5,342,944	2,671,472**
		Columns	21	1,632,256	77,726*
		Error	42	1,580,516	37,631
2	Upper margin, inner surface	Total	65	5,273,609	—
		Rows	2	2,038,957	97,093**
		Columns	21	2,577,983	1,288,991**
		Error	42	656,669	15,635
3	Lower margin, outer surface	Total	87	4,546,441	—
		Rows	3	3,634,197	1,211,399**
		Columns	21	187,517	8,929
		Error	63	724,727	11,504
4	Lower margin, inner surface	Total	87	8,010,293	—
		Rows	3	2,756,080	918,693**
		Columns	21	4,815,891	229,328**
		Error	63	438,322	6,957
5	Central portion, outer surface	Total (for both columns and rows)	100	4,991,829	—
		Columns	6	804,039	134,007*
		Error	94	4,187,790	44,510
		Rows	3	2,600,976	866,992**
		Error	97	2,390,853	24,648
6	Central portion, inner surface	Total (for both columns and rows)	101	1,279,931	—
		Columns	6	275,874	45,979**
		Error	95	1,004,057	10,569
		Rows	3	252,287	84,095**
		Error	98	1,027,644	10,486

* Significant, $P<0{\cdot}05$. ** Highly significant, $P<0{\cdot}01$.

It has already been pointed out that according to Burcev (1937) any portion of the ear of Red German cattle can be used to obtain a reliable count of the number of sweat glands per unit area in the ear, but the present work shows very clearly that for the ear of an Ayrshire cow the distribution of sweat glands varied greatly in different regions. For the various sets of results obtained in this section of the investigation (Tables 2 and 3) an analysis of variance was made. The results are shown in Table 4. It is clear that except for one set of columns, those of the outer surface of the lower margin, the differences between counts when analysed in rows and columns were statistically significant.

(2) <u>The number and distribution of sweat glands in the ear of a calf</u>

It has already been pointed out that, according to Burcev (1937), the number of sweat glands per unit area in the ear is unaffected by the age of the animal. Burcev studied a large group of animals consisting of 515 cows, 227 bulls and 594 young cattle, the ages of the animals ranging from 1 day to 16 years. Age certainly did not appear to affect the count. Thus for twenty-five calves 1-10 days old, he obtained an average figure of 4.72 ± 0.209 sweat glands per sq.mm. ear surface, and very similar figures of 4.71 ± 0.162, 4.71 ± 0.260 and 4.70 ± 0.196 for animals of 6-9 months, 3-4 years and 10-11 years respectively. Values for the other age groups varied

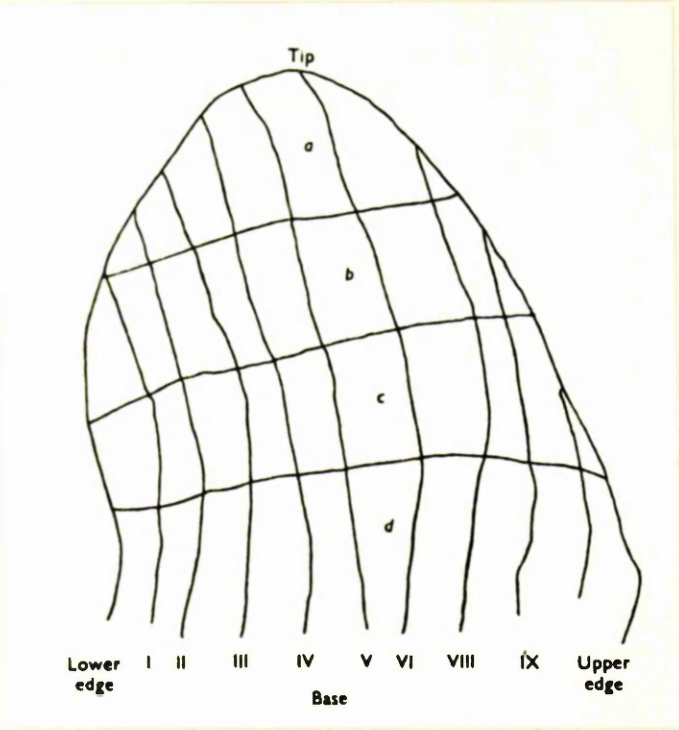


Diagram 2. The locations in the calf's ear of the columns and rows in which hair counts were made.

Table 5. *The hair counts per sq.cm. for the outer and inner surfaces of the left ear of an Ayrshire calf*

Diagram 2 shows the position of the columns, and the omission of column VII is explained on p. 26.

Row	Column I	II	III	IV	V	VI	VIII	IX	Sum
				Outer surface					
a	—	1,260	1,131	918	1,334	1,398	—	—	
	—	686	736	677	825	859	—	—	
	—	—	741	746	780	958	—	—	
	—	—	—	686	904	1,057	—	—	20,545
	—	—	—	790	943	1,161	—	—	
	—	—	—	—	859	1,096	—	—	
b	904	726	800	933	1,111	1,146	1,300	—	
	760	603	691	904	1,032	1,101	1,328	—	
	746	632	731	909	988	1,096	1,452	—	25,567
	731	820	805	938	1,062	—	1,318	—	
c	558	810	1,027	1,002	899	1,136	1,719	1,472	
	509	672	1,131	988	1,047	1,126	1,694	1,556	
	598	701	1,077	1,017	1,180	1,091	1,141	1,541	35,544
	—	—	—	—	953	1,259	1,343	1,511	
	—	—	—	—	—	1,304	1,704	1,778	
d	1,007	943	1,280	1,146	938	1,067	1,121	1,190	
	1,022	978	1,190	1,057	983	1,363	978	1,254	25,581
	—	1,180	1,210	1,165	1,220	1,244	889	1,156	
Sum	6,835	10,011	12,550	13,876	17,058	19,462	15,987	11,458	107,237
				Inner surface					
a	—	1,773	1,462	2,090	2,341	1,679	—	—	
	—	1,610	1,363	1,590	1,644	1,625	—	—	
	—	—	1,422	1,215	1,323	1,452	—	—	
	—	—	1,190	1,052	1,195	978	—	—	32,494
	—	—	—	988	938	928	—	—	
	—	—	—	—	854	1,022	—	—	
	—	—	—	—	760	—	—	—	
b	1,319	1,002	1,096	1,074	1,240	1,289	884	—	
	1,082	805	1,077	1,081	1,249	1,096	785	—	
	1,017	978	933	1,052	1,328	1,062	657	—	31,558
	894	933	1,086	1,146	1,294	1,215	696	—	
	—	—	—	—	1,215	973	—	—	
c	1,047	1,304	1,126	1,501	1,674	1,121	662	617	
	1,067	1,294	1,072	1,462	1,615	1,254	667	528	
	1,077	978	844	1,348	1,575	1,496	1,007	548	39,339
	923	894	919	1,358	1,531	1,580	1,250	736	
	—	—	—	—	1,783	1,481	—	—	
d	1,091	1,427	1,274	1,536	1,763	1,516	1,294	1,185	
	1,032	1,546	1,175	1,827	1,704	1,788	1,348	993	34,979
	1,200	1,600	1,467	1,901	—	2,040	1,432	1,151	
	—	1,689	—	—	—	—	—	—	
Sum	11,749	17,833	17,506	22,221	27,026	25,595	10,682	5,758	138,370

Table 6. *Analysis of variance for the results recorded in Table 5 for the ear of a calf (analyses nos. 1 and 2), and a comparison of the counts obtained for the calf with those for the cow (analyses nos. 3 and 4)*

Description and number of analysis	Source of variation	Degrees of freedom	Sums of squares	Mean squares
1. The outer surface of the left ear of a calf	Total for both columns and rows	102	7,417,534	—
	Columns	7	3,952,142	564,591**
	Error	95	3,465,392	36,478
	Rows	3	953,685	317,895**
	Error	99	6,463,849	65,291
2. The inner surface of the left ear of a calf	Total for both columns and rows	111	13,231,230	—
	Columns	7	3,638,240	519,748**
	Error	104	9,592,990	92,240
	Rows	3	2,739,173	913,057**
	Error	108	10,492,057	97,147
3. Comparison of the hair counts obtained for the outer surface of the calf's ear with those obtained for the outer surface of the cow's ear	Total	185	17,907,059	—
	Between cow and calf	1	10,329,130	10,329,130**
	Error	184	7,577,929	41,184
4. Comparison of the hair counts obtained for the inner surface of the calf's ear with those obtained for the inner surface of the cow's ear	Total	194	28,669,972	—
	Between cow and calf	1	16,761,887	16,761,887**
	Error	193	11,908,085	61,670

** Highly significant, $P<0{\cdot}01$.

irregularly with age from 4.06 ± 0.210 to 5.08 ± 0.198.

Theoretically it might be expected that the count per unit area would remain unaffected by age once the animal was fully grown, but it seems difficult to explain how in an ear that is growing and constantly increasing in surface area the number of sweat glands per sq.cm. can remain unchanged. A detailed study was therefore made of the ear of a 7-day-old calf. The ear was clipped and shaved, and the hairs counted by the same technique that was used for the cow. The counts were made in the columns shown in Diagram 2 and are recorded in Table 5. The columns were numbered I to IX and, as judged by the position of the cartilaginous ridges in the two ears, columns I-VI in Diagram 2 for the calf corresponded to columns I-VI in Diagram 1 for the cow, but the region corresponding to column VII in the cow's ear was too narrow in the calf for counts to be made. Column VII, therefore, does not appear in Diagram 2 and Table 5. An analysis of variance (Table 6) showed that in the ear of the calf, as in the cow, the number of hairs and therefore of sweat glands per unit area varied significantly from one region to another.

In order to obtain comparable counts for the cow's and calf's ears columns I-VI of Tables 3 and 5 were used, and since the columns in Table 3 represented only the central portion of the cow's ear, additional counts were made so that these particular columns were extended into the marginal areas to make them comparable with the columns used in the calf's ear.

From the analysis of variance in Table 6 it is clear that the differences between the counts for the calf and the cow were highly significant. When the various counts for each animal were averaged it was found that the average number of sweat glands per sq.cm. on the outer surface of the ear of the calf was 973 and for the cow 498, which gives a ratio of 1.98 to 1.0. The corresponding figures for the inner surface were 1297 and 710, giving a ratio of 1.83 to 1.0. The figure of 498 per sq.cm. for an Ayrshire cow is very close to Burcev's average of 482 per sq.cm. for a large number of cattle of the Red German breed, but the fact that the value for the calf was practically twice as great as that for the cow is completely at variance with the claims of Burcev. Nevertheless, the present finding would seem to be the more reasonable, because simple measurement has shown that the surface area of the ear of a very young calf is roughly half that of a cow, and since it is practically impossible to suppose that the total number of the sweat glands in the ear varies during growth, the number per unit area would be expected to decrease during growth.

The results which are recorded above in sections 1 and 2 have been obtained by an extensive study of one cow's ear and one calf's ear, but results obtained by less detailed examinations of the ears of other animals of the Ayrshire breed confirm the general conclusions which have been drawn.

(3) The relation between the sweat gland count and the milk yield

Experiment 1. The main object of the present work was to determine whether by adopting the procedure of Burcev (1937) the sweat-gland count could be used as an indication of the milk-producing capacity of Ayrshire cows. Burcev claimed that any part of the ear could be used. In a preliminary test, therefore, his method was followed as closely as possible, and although the pieces of ear used to obtain the counts came mainly from the tips of the pinnae of the right ears in the region marked A in Diagram 1, no attempt was made to take the pieces from precisely the same place for each of the cows involved. Two herds of Ayrshire cattle were used, the Hannah Institute herd which was denoted herd A and that of a nearby farm, herd B. Pieces of skin each 2 cm. in diameter were cut with a branding punch, a method recommended by Burcev (1937). The inner surface of each sample was clipped for identification and the pieces fixed in 10% formol saline for 72 hr. Sections 50μ thick, parallel to the skin surface, were made from the inner surface with a freezing microtome and stained with 0.5% toluidine blue. The images of the sections were projected on white paper using a field of 2.19 sq.mm. Ten counts were made of the number of hair follicles at a level near the surface of the skin in the various sections, and the results averaged.

As a measure of the milk production of the cows, it was decided to use the milk yields for the first

Table 7. *The milk yields and number of sweat glands per sq.cm. of skin from the pinna of the right ear of cows from herd A (Exp. 1)*

Cow	Year of birth	Av. 180-day milk yield of the first three lactations (lb.)	Av. 180-day* milk yield of the two lactations in 1946 and 1947 (lb.)	No. of sweat glands per sq.cm. of skin in pinna
Dora	1941	6098	7065	1348
Misty Morn	1940	5382	6325	1238
Jennifer I	1941	5964	5485	1680
Gadfly	1938	4029	5125	1294
Griselda	1940	5694	4581	763
Gertrude	1940	4422	5108	918
Giddy	1943	6976	7645	986
Eve	1943	6113	8565	1308
Effie	1943	6032	9005	1152
Trixie	1943	5754	6805	1165
Dorothy	1943	5516	6870	781
Dinky	1943	5247	5800	1442
Dewdrop II	1943	5229	6140	1341
Joyce	1942	5159	5500	1023

(1) Correlation between the number of sweat glands per sq.cm. of skin and the average 180-day milk yield of the first three lactations:

$r = 0{\cdot}030$, not significant.

(2) Correlation between the number of sweat glands per sq.cm. of skin and the average 180-day milk yield of the two lactations in 1946 and 1947 (with corrections made for age):

$r = 0{\cdot}072$, not significant.

* Corrections made for age.

Table 8. *The milk yields and number of sweat glands in the pinna of the right ear of cows from herd B (Exp. 1)*

Cow no.	Age	180-day yield for one lactation (lb.)	Sweat glands per sq.cm. of skin in the pinna
52	6	5,166	1,290
72	6	6,078	1,174
28	6	8,331	1,174
21	6	8,058	1,506
3	14	8,996	1,476
34	6	8,341	1,700
25	3	4,795	1,484
11	8	10,994	1,073
58	5	4,427	1,223
62	6	4,828	1,415
84	5	3,945	1,370
16	3	4,013	1,141
60	5	4,854	1,732
22	6	8,929	1,200
5	8	9,281	1,392
80	6	5,687	1,302

Correlation between the number of sweat glands and the milk yield:

$r = -0{\cdot}111$, not significant.

180 days of lactation, since it is well established that there is a high correlation of around 0.9 between the 180-day yield and the yield for the whole lactation (Tuff, 1931; Zorn & Funke, 1935; Johansson & Hansson, 1940). The Russian workers whose publications have been cited, e.g. Burcev (1937), adopted the 300-day yields, but few of the cows used in the present work had a lactation period extending to 300 days.

The results for herd A are given in Table 7. From the records of the milk yields of the cows the average 180-day milk yields for the first three lactations were obtained. The correlation coefficient between the number of sweat glands in the ear and the milk yield was found to be 0.030 and was not significant. The 180-day yield for the same cows for the period 1946-7 was next taken, corrections being made for the age of the cows, using the age-correction factors of the Dairy Herd Improvement Association as published by Rice (1942) and averages for each cow for the 2 years were calculated. The correlation coefficient between these yields and the number of sweat glands in the ear was 0.072 and again was not significant.

The results for herd B are given in Table 8. The correlation coefficient between the milk yield and the number of sweat glands in the ear was -0.111 and was not significant.

From the very inadequate description which Burcev (1937) gives of his methods it is not possible

to be sure that his procedure was repeated in every detail. He appeared to make counts on both the inner and outer surfaces of the ear at random and averaged the counts. In an attempt to follow this procedure the experiments which have just been described were repeated with the same skin samples, but counts were made not only at a level near the surface but on both sides of the cartilage in a field area of 20 sq.mm. There is no need to give detailed tables of the results. It will suffice to record that the correlation coefficients obtained in this second experiment for herds A and B corresponding to the three correlation coefficients in Tables 7 and 8 for the first experiment were 0.091, 0.134 and -0.238 and none of them was significant.

It appeared therefore from the statistical analysis of the data that the number of sweat glands per unit area of the ear obtained by the methods which have just been described was not an indication of milk-producing capacity in the Ayrshire cow, and it is of interest to observe that in herd A (Table 7) one cow gave an average of nearly 7000 lb. of milk in the first three lactations and had a sweat-gland count of 986 per sq.cm., whereas another cow giving a considerably lower yield of just under 6000 lb. had a count of 1680. Similarly, in herd B (Table 8), the highest yielder (10,994 lb.) had the lowest count (1073) while the lowest yielder (3945 lb.) had a fairly high count (1370).

Experiment 2. In Exp. 1 the object was to

Table 9. *The number of sweat glands per sq.cm. in an area of skin measuring approximately 5 sq.cm. which was taken from the outer surface of the left ears of three cows at a position shown by the arrow in Diagram* 1

Cow ...	A				B				C			
Row ...	2	3	4	5	2	3	4	5	2	3	4	5
Col. 1	257	202	182	187	380	311	262	247	380	251	207	256
2	242	182	222	197	331	261	286	320	297	272	251	277
3	271	281	242	252	469	301	271	242	282	247	272	251
4	301	227	227	242	306	247	271	232	307	242	292	297
5	276	264	192	232	326	321	286	242	336	276	301	282
Average	233·9				295·6				278·8			
S.E.	±7·7				±12·4				±8·2			

repeat Burcev's methods as far as possible, and like him to pay no particular attention to obtaining the pieces of ear from precisely the same region for all the cows. It was possible, therefore, that if a region of the ear could be chosen in which the sweat-gland count was reasonably constant, a higher correlation between that count and the milk yield might be obtained than was obtained in Exp. 1. Inspection of Table 2 and Diagram 1 suggested that in the region AC of the lower margin of the outer surface the sweat-gland counts were relatively close in rows 2-4. This suggested that, provided a piece of skin were taken from the ear in this region of 'relative constancy' and counts were avoided close to the margin, i.e. in row 1, any tendency which might exist for the sweat-gland count and the milk yield to be highly correlated would not be masked by the high sweat-gland counts in the extreme margin of the ear or by other large variations in count which occur from place to place in the ear. This region of 'relative constancy' had an area of about 5 sq.cm. Before proceeding with the experiment, however, it was necessary to establish whether this region exhibited this 'relative constancy' in other ears. Three left ears were therefore obtained from the slaughterhouse and region AC was cut out, shaved and direct counts of the hairs made in the rows which in Diagram 1 are designated nos. 2-5.

The results are shown in Table 9, together with the means and standard error of the means. It will be seen that for these three ears, which were chosen at

Table 10. *The relation between the sweat-gland count in a carefully chosen area of the ear and the milk yield (Exp. 2)*

Cow no.	Age in years	No. of sweat glands/sq.cm.	Milk yield (lb.) Average of two lactations	Milk yield (lb.) Average of three lactations
10	5	258	6281	—
2	5	280	4984	—
5	5	220	7032	—
28	5	289	4816	—
4	5	251	5705	—
19	5	248	7224	—
11	5	290	4890	—
26	5	201	7220	—
16	6	364	6215	6757
12	6	174	6199	6669
8	6	216	5367	6317
22	6	289	4766	5872
14	6	282	5775	6188
17	6	284	6438	6133
13	6	217	4555	5479
9	6	256	3408	4247
18	6	238	6053	6715
3	7	206	5099	6656
15	7	347	5848	6085
27	7	370	6365	6471
20	8	238	6365	6471
24	8	289	4929	5308
6	8	311	5995	6027
25	8	317	5896	6000
21	8	294	6835	6758
7	9	237	6353	6789
23	9	367	5737	5949
1	11	258	5748	6360

Correlations between sweat-gland count and milk yield

Age group (years)	No. of lactations considered	n	r
All ages	2	28	−0·17
All ages	3	20	−0·03
5	2	8	−0·91**
6	2	9	+0·09
6	3	9	+0·07
8	2	5	−0·20
8	3	5	−0·29

** Highly significant, $P < 0{\cdot}01$.

random, the standard error was much the same in this region. This being so, the following experiment was performed on the Ayrshire herd of the West of Scotland Agricultural College.

Immediately after the evening milking, pieces of skin were cut from the left ears of twenty-eight cows using the technique described on p. 28, but with the following extra precautions. The pieces of ear were always taken from the region AC of Diagram 1 which was identified each time by reference to the nearest cartilaginous ridge, and the branding tool was used in that region in such a way that the piece cut was certain to include rows 2-4. A streak of paint on the tool made a mark on the skin and ensured that the position of rows 2-4 in the cut piece could be readily identified. The pieces were fixed and the follicle counts made as previously described on pp.22& 23. The results are given in Table 10, which also shows the average milk yield of the animals for two lactations, and for three lactations where a third one had been completed. The results are arranged according to the age of the cows, which varied from 5 to 11 years. It is clear that none of the correlation coefficients between milk yield and sweat-gland count was significant except for the 5-year-old group where a high negative correlation was obtained.

It will be noted that the counts in Table 10 are considerably less than those given in Tables 7 and 8 for Exp. 1. This is due to the fact that in Exp. 1 the pieces of skin were taken near the margin of each

ear, whereas in making the counts in Exp. 2 the area for counting was carefully chosen so as to exclude the margin in which, as is shown in Table 2, the counts are particularly high.

Conclusion

The main conclusion to be drawn from these experiments is that the close correlation which was found by Burcev and others to exist between the number of sweat glands per unit area of skin in the ear and the milk yield in Red German cattle does not exist in Ayrshire cattle. It is clear from the present results for Ayrshires that the sweat-gland count cannot possibly be used as an indication of milk-producing capacity.

The results of the present work also show that in Ayrshires, the number of sweat glands per unit area in the skin of the ear varies very greatly from one part of the ear to another and even from one field to another in any particular part, whereas Burcev claimed that for the Red German breed any portion of the ear could be used to obtain a reliable count and that the count so obtained would be representative of the ear as a whole. The present investigation shows also that in the Ayrshire breed the ear of the calf has more sweat glands per unit area than that of the fully grown cow. This again is a conclusion which is completely at variance with the claims of Burcev for the Red German breed.

PART II

Studies in the vascularisation of bovine skin

Chapter 4

The arrangement of blood vessels in the skin of Ayrshire calves and embryos

Introduction

It has already been observed (see page 4) that very little attention has been paid to the arrangement of blood vessels in bovine skin. Findlay and Yang (1948 and 1950) noticed that there was a very poor blood supply to the apocrine sweat glands in Ayrshire cattle skin but that just below the epidermis there seemed to be a rich network of blood capillaries encircling the hair follicles. Hafez, Badreldin and Shafei (1955) described much the same arrangement in the skin of Egyptian cattle and buffaloes. Since the amount of heat lost by radiation, conduction and convection and by the vaporisation of insensible perspiration is dependent on the blood supply of the skin a study has been made of the arrangement of blood vessels in the skin of calves and embryos.

Materials and Methods

The skins of two 3-months-old Ayrshire calves and of three embryos, each about 15 in. long were injected with Indian ink in the following way. A calf was given an intravenous injection of 5 g. amyl nitrite to induce maximum vaso-dilatation of the blood vessels. After a period of 5 min. the calf was killed with a humane killing gun. The thoracic and abdominal cavities of the animal were opened, and the

anterior and posterior ends of the dorsal aorta and its main visceral branches were ligatured. Thus only the intercostal arteries were left in free communication with the aorta. A glass cannula, connected by rubber tubing to a reservoir of Indian ink (Winsor and Newton's Mandarin Black), was introduced into the dorsal aorta near its anterior end. Preliminary perfusion with isotonic saline or Ringer's fluid was omitted, as it had been found that precipitation of the ink particles occurred readily on contact with either fluid. It was feared that this might cause blockage of the larger blood vessels and so prevent complete perfusion. The ink was gradually pumped into the aorta by applying gentle pressure with a hand bulb to the reservoir. This was continued for about 3 hr. to ensure complete perfusion. The capillaries of the intercostal muscles were soon filled with ink. Eventually some of the ink found its way into the cutaneous blood vessels by the small vessels which connect the skin with the intercostal muscles. Massage was employed on the skin to promote an even flow of ink in the cutaneous blood vessels. Blood and perfused ink seeped out of the intercostal muscles at the cut edges of the thorax, and after a time the vena hemiazygos, into which the dorsal intercostal veins drain and which opens into the great cardiac vein, became distended.

The embryos were injected in the same way immediately after they were obtained from the slaughter-house, but, of course, no preliminary

Fig.15. A section of calf skin perfused with Indian ink. The complete courses of the blood vessels have been traced. Arteries are stippled in white.

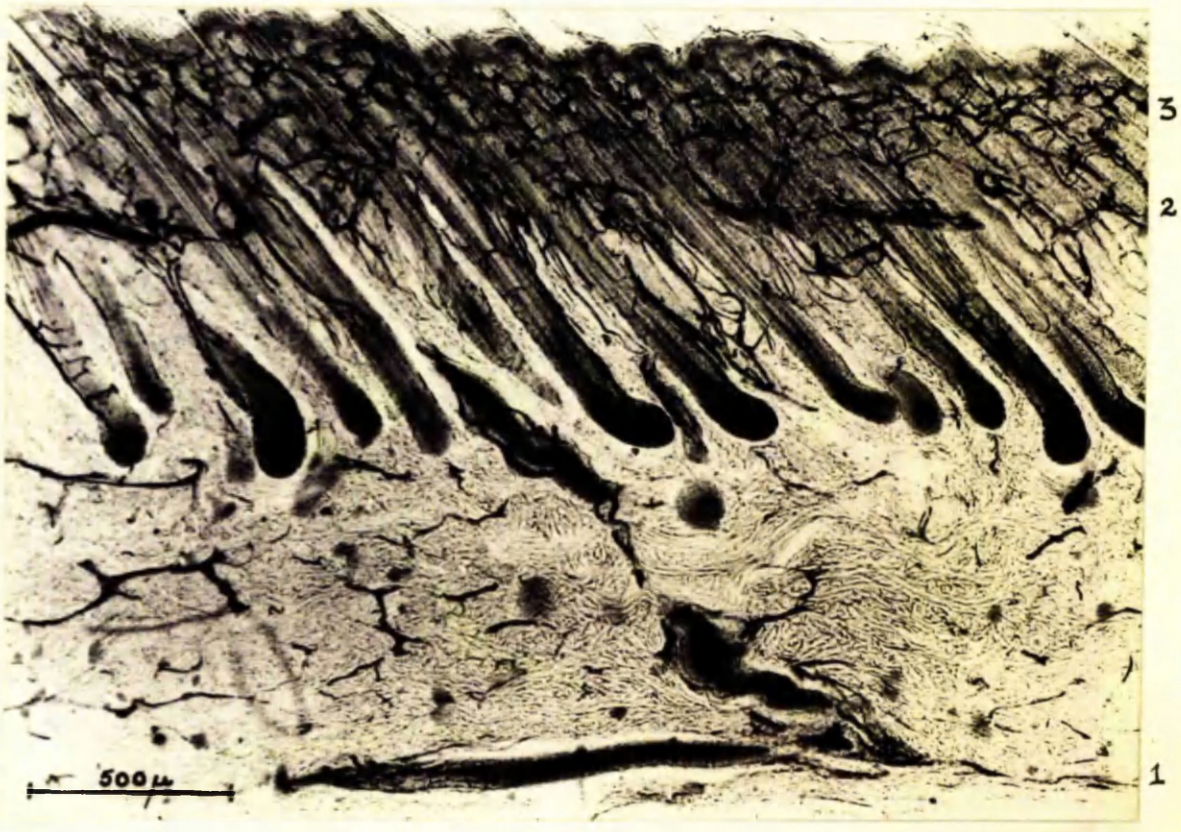
injection of amyl nitrite could be given.

The injected skin was removed from the backs and flank regions of the animals and fixed in 10% formalin. A few drops of glacial acetic acid were added to the formalin to hasten the precipitation of the ink particles in the blood vessels. After fixation, blocks of the skin were embedded in paraffin and serial sections, 50μ thick, were cut both parallel and perpendicular to the skin surface. They were stained lightly with haematoxylin and eosin.

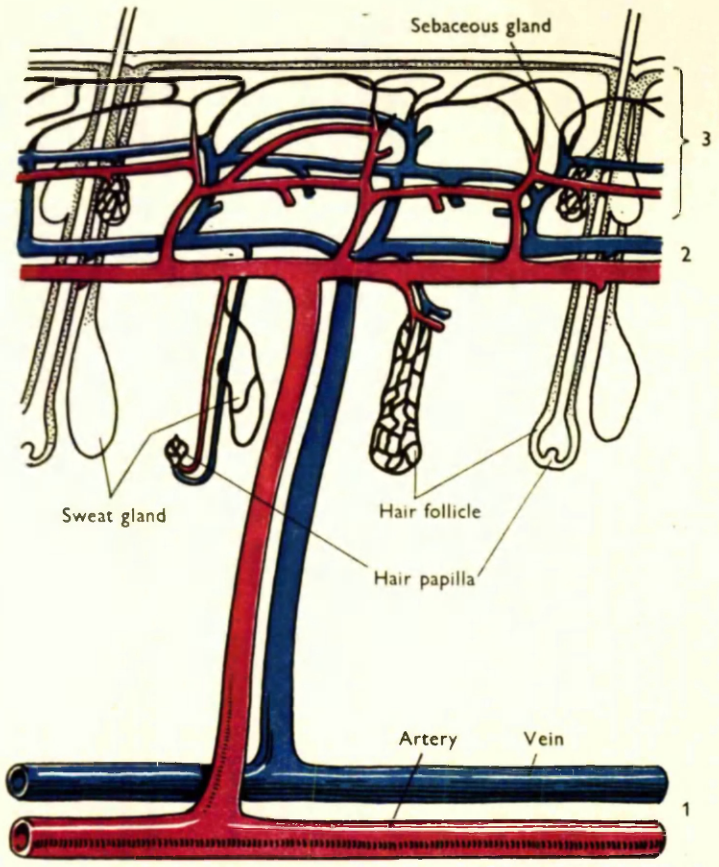
In such thick sections it was sometimes difficult to ascertain whether a certain vessel was an artery or a vein so the following method of examining the sections was used. A section showing clearly a large artery or vein was photographed under low power magnification. A light print of the plate was then made on matt paper. The section was subsequently examined under the microscope. Branches of the large artery or vein were followed and their courses traced in Indian ink onto the print where their outline already showed faintly. To distinguish arteries from veins and capillaries the arteries were dotted in white and the veins and capillaries left black. A typical tracing is shown in Fig. 15. Reference had to be made to adjacent sections in the series in order to follow the course of vessels which passed into the adjacent section and then returned to the original.

Results

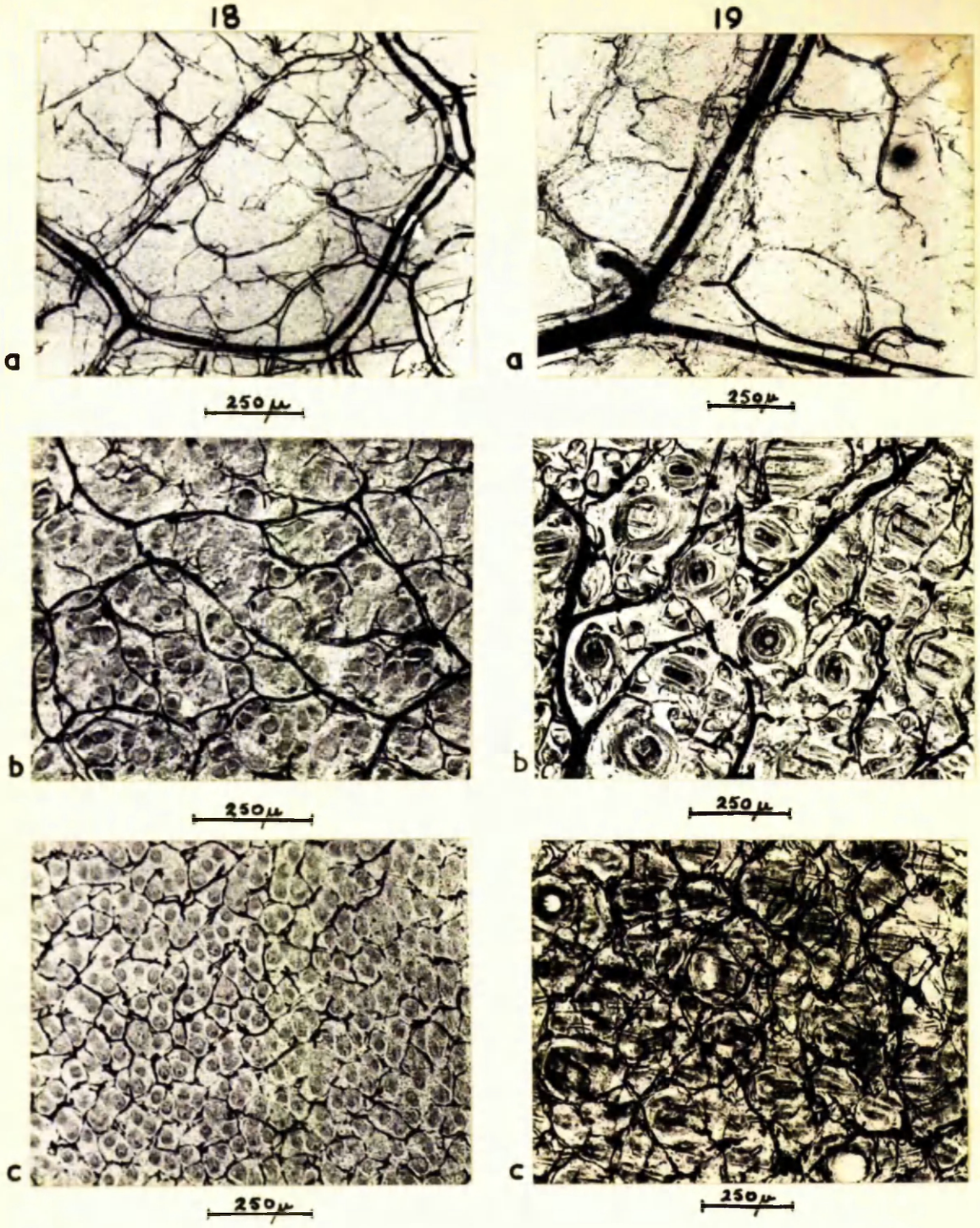
Examination of the sections of the injected



Figs.16 and 17. Sections of embryo (Fig.16) and calf skin (Fig.17), perfused with Indian ink, cut perpendicular to the skin surface. Three vascular plexuses can be identified at levels 1, 2 and 3.



Coloured Diagram 1. A diagram of the blood supply in the skin of a calf. The three main vascular plexuses can be identified at levels 1, 2 and 3. The blood supply to the hair follicle and its appendages can be seen.



Figs.18 and 19. Sections of embryo skin and calf skin parallel to the skin surface. (a) At level 1 (Figs.15 and 16) showing large vessels with accompanying smaller vessels and a network of finer vessels. (b) At level 2 showing vessels sometimes in pairs surrounding groups of hair follicles. (c) At level 3 showing hair follicles surrounded by meshes of capillaries.

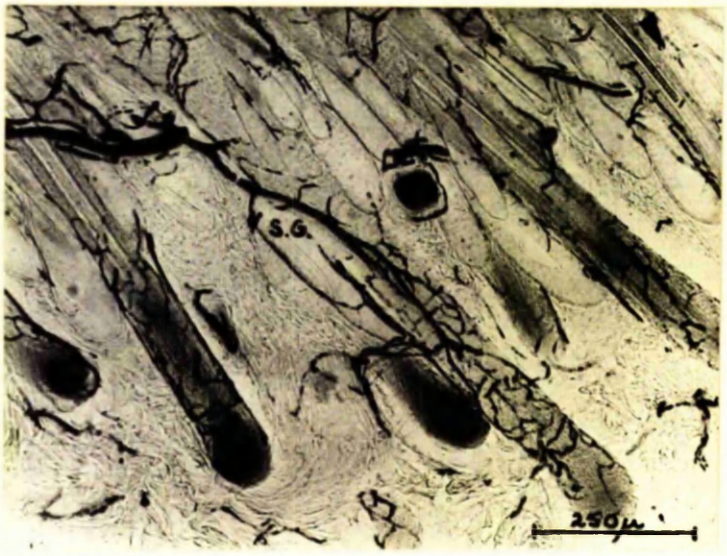


Fig.20. The blood supply to a sweat gland (S.G.) and to a hair follicle.

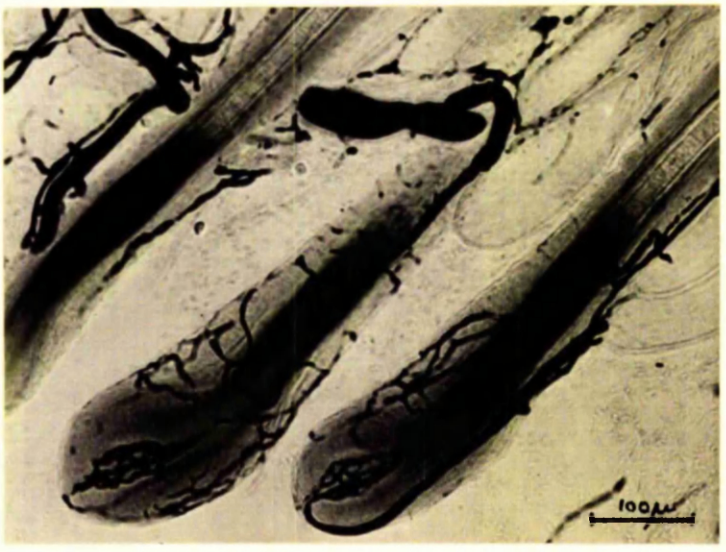


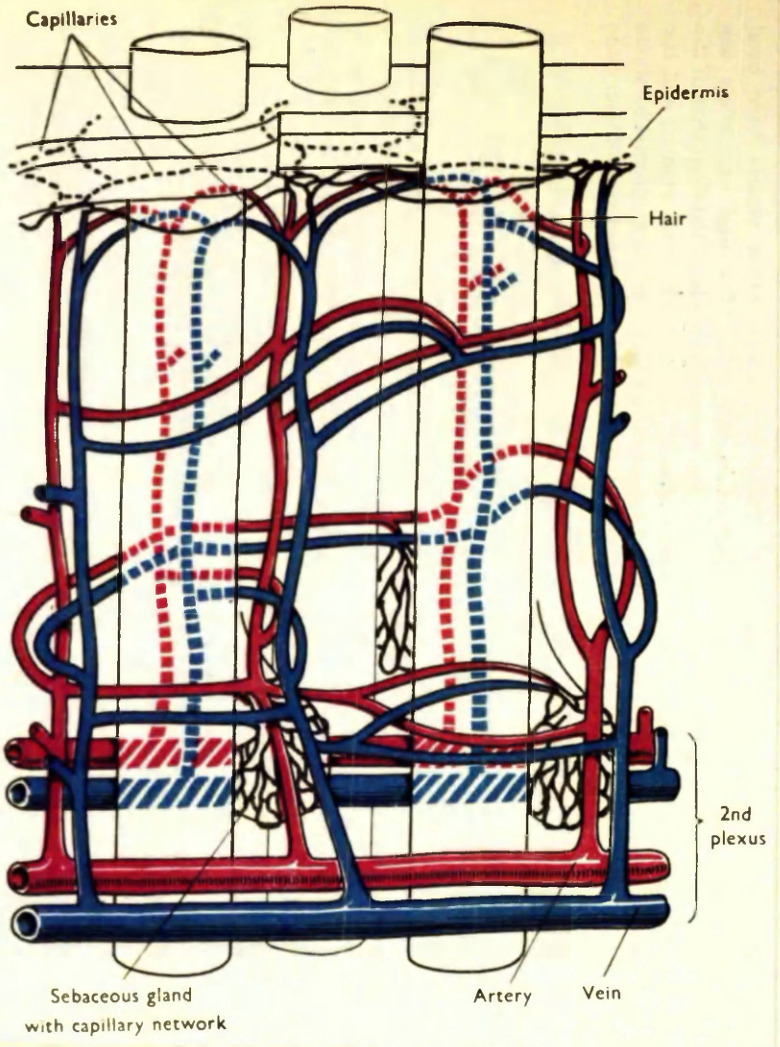
Fig.21. The blood supply to the papillae of two hair follicles.

skins of both embryos and calves showed that three definite vascular plexuses exist. These occur parallel to the skin surface at the following levels: (1) at the base of the corium, (2) between the sebaceous gland level and the sweat gland level, and (3) below the epidermis. They can be seen at 1, 2 and 3 in Figs. 16 and 17 and in coloured Diagram 1.

In the first plexus arteries of comparatively large dimensions send horizontal branches in all directions to form a network of fine vessels and capillaries (Figs. 18a and 19a). Most larger arteries appear to be associated with two parallel veins. Smaller arterial branches have only one accompanying vein.

The second plexus is connected with the first by a few large vessels (Figs. 16 and 17, and coloured Diagram 1). This plexus consists of a coarse network of arteries and veins running horizontally and encircling groups of several hair follicles (Figs. 18b and 19b). From these vessels branches go vertically downwards to supply the hair follicles and the sweat glands (Fig. 20 and coloured Diagram 1). From Fig. 20 and coloured Diagram 1, it can be seen that the blood supply of the sweat gland is much poorer than that of the hair follicle. Fig. 21 and coloured Diagram 1, show the rich capillary supply in the papilla of the hair follicle.

The third plexus is a horizontal plexus of fine vessels. These originate from vertical arterial and venous branches of the second plexus. These vertical



Coloured Diagram 2. A diagram to show the blood supply in calf skin at the level between the 2nd plexus and the skin surface, i.e. the 3rd plexus. It should be noted how horizontal branches of vertical branches of the 2nd plexus anastomose with each other, encircling the hair follicles at various levels.

Fig.22. The blood supply to a sebaceous gland (S) which is lying between a hair follicle (H) and its arrector pili muscle (M).

branches give off horizontal branches at different levels (Fig. 15 and coloured Diagrams 1 and 2), so that the plexus extends over a considerable depth beneath the epidermis and above the second plexus. The horizontal branches supply the sebaceous glands (Fig. 22 and coloured Diagrams 1 and 2). They also anastomose with horizontal branches from other vertical branches of the second plexus, and so encircle the individual hair follicles (coloured Diagram 2). Finally, the arterial and venous branches join through a system of fine capillaries lying just beneath the epidermis. These capillaries also encircle the individual hair follicles anastomosing with capillaries originating from other vertical branches of the second plexus (Figs. 18c and 19c, and coloured Diagram 2). Findlay & Yang (1948), after examination of horizontal sections of uninjected bovine skin from the subepidermal region, claimed that each hair was surrounded by a single capillary loop. The present work modifies this claim in that it can be seen that individual hairs are encircled at different levels by blood vessels varying in calibre and number.

Discussion

The three vascular plexuses found in cattle skin are comparable with those in human skin described by Spalteholz (1893). However, Spalteholz described the 2nd and 3rd venous plexuses as lying at different levels from the 2nd or subpapillary arteriolar plexus. He depicted the larger arteries of the cutaneous

arteriolar plexus and the veins of the 4th venous plexus as running independently of each other. This does not agree with the distribution of arteries and veins in the corresponding plexuses in bovine skin, where they always appear to run together. In fact, in the 1st plexus in bovine skin many of the larger arteries are accompanied by two veins (Figs. 18a and 19a). As was first recognized by Bernard (1876), and further stressed by Bazett (1949), the interchange of heat between an artery and its accompanying vein or veins (venae comites) plays an important part in the conservation of heat in the body. The warm blood in the artery going towards the periphery is cooled by the venous blood returning from the skin and so the temperature gradient at the skin surface is reduced and less heat is lost to the environment. In the human being these venae comites appear to be present only in conjunction with the large arteries of the limbs, i.e. the femoral, radial, etc. In the Ayrshire calf and embryo they appear to be widely distributed throughout the skin. Thus it would appear that the Ayrshire calf and presumably the adult Ayrshire animal, too, are extremely well adapted to a cold environment. Although the rich blood supply to the skin especially in the subepidermal region seems to indicate that the skin may be an effective organ of heat dissipation it seems doubtful if it is, when one takes into consideration the widespread distribution of venae comites.

It has been shown that the third plexus is an

intricate horizontal network of arterioles, venules and capillaries, in which the individual hair follicles are encircled at different levels by a varying number of blood vessels. Therefore as the number of hairs per unit area of skin surface increases there will be a corresponding increase in capillary surface area per unit area of skim surface. When considering the dissipation of heat, a paradox seems to exist since the advantage of having a larger capillary surface area for heat dissipation may be overcome by having more hairs for heat conservation. The compromise would be for the animal to have a large number of fine short hairs in order to obtain the advantage of more capillary surface and of a thin coat. A comparative study of the number, length and thickness of hairs of both temperate and tropical breeds of cattle might lead to better understanding of this paradox.

Since an injection of amyl nitrite was administered to the calf prior to the perfusion with Indian ink, it can be assumed that maximum dilatation and filling of the minute vessels of the skin was obtained. This is not the true physiological picture, as it is known that under normal environmental conditions all of the vessels in the skin are not open at one time (Lewis, 1927), but local and general application of heat bring about complete vasodilatation.

A large area of skin on the back and flanks of the calf was perfused when the intercostal arteries were injected with Indian ink. This means that

certainly most of the blood reaching that area of skin passes through the intercostal muscles, and presumably the venous return to the heart and thence to the lungs is by a similar path. This passage through the muscles of blood going to the skin was first noted by Spalteholz (1893). Bazett (1927), in considering the dissipation of heat during heavy exercise, points out that the skin over active muscles shows an increase in temperature long before there is an increase in rectal temperature. So the interchange of heat between the intercostal muscles and the blood in both the arteries and veins must play an important part in the heat regulating system of the animal. This will be especially important when the animal is panting as a result of excessive heat strain.

From the injected specimens it can be seen that the sweat glands have a very poor blood supply as compared with the hair follicles. It is known that human eccrine sweat glands have a rich blood supply (Maximow & Bloom, 1948). If blood supply is regarded as an index of activity in a gland, then the sweat glands of Ayrshire cattle may not be very active. It should be noted too that the blood supply of the sweat gland compares unfavourably with that of the sebaceous gland.

Chapter 5

Arterio-venous anastomoses in the skin of certain body regions of the calf

Introduction

Arterio-venous anastomoses are known to play an important part in controlling the amount of heat lost from the periphery (Grant, 1930). During the examination of the injected skin in the investigation reported in the previous chapter, particular heed was paid to vessels which linked the arterial and venous systems to see if arterio-venous anastomoses existed in bovine skin. However in the thick sections used it was difficult to discern clearly the structure of the walls of the linking vessels and impossible to be certain whether all of those vessels were capillaries. The following study was undertaken in an attempt to ascertain if there were any arterio-venous anastomoses in the skin of various body regions of the calf.

Materials and Methods

The skin of the trunk of a calf which has been killed by excess pentothal sodium was perfused with Ehrlich's acid haematoxylin following the method of Grant (1930), the procedure detailed in Chapter 4 being employed. Frozen sections, 100μ thick, of the perfused skin were cut in series both parallel and perpendicular to the skin surface. They were differentiated in 1% HCl in 70% ethanol, dehydrated, cleared and mounted in Depex.

The heads of about a dozen Ayrshire bull calves that had been killed by excess chloroform anaesthesia or pentothal sodium were also perfused with Ehrlich's acid haematoxylin following the method of Grant (1930). Attempts at perfusion of individual excised ears were not satisfactory and better results were obtained from perfusing the whole heads through one or both of the carotid arteries. One of the jugular veins was ligated and the other cannulated to allow drainage of the perfused fluids. The ears and pieces of skin from the forehead, cheek and neck were removed.

To trace the blood supply to the ear the heads of two calves were perfused with a synthetic rubber latex (Hycar) coloured with gentian violet. The heads were then fixed in 10% formalin and subsequently dissected to show the blood supply to the ears.

The ears were cut into four pieces and the skin of both the outer and inner surfaces was dissected leaving the perichondrium with the main blood vessels attached to the cartilage. The perichondrium was then dissected from the cartilage. Each piece of perichondrium was differentiated in acid alcohol if necessary and blued in Scott's tap-water substitute. The piece of perichondrium was then pinned on a wooden block and immersed in increasing concentrations of ethanol to dehydrate it. Mounting on the wooden block prevented it from curling. It was cleared in xylol and mounted in DePeX on a 3¼ x 3¼ in. lantern slide cover glass. As the perichondrium is fairly thick it was found necessary to build up the corners

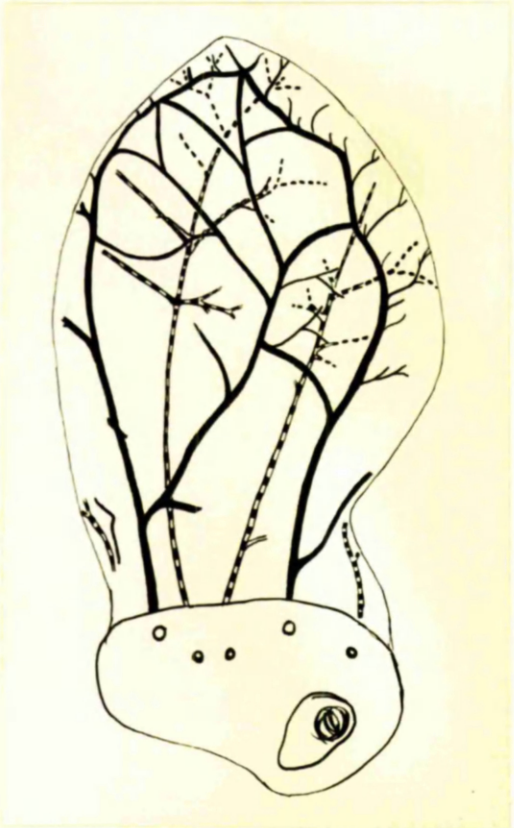


Diagram 3. The main blood vessels in the perichondrium of the outer surface of the calf's ear. The arteries are dotted and the veins black.

of the slides with pieces of microslides before covering the mounts with 3¼ x 3¼ in. no. 1 micro cover-glasses, otherwise the cover glass did not remain flat and air-bubbles penetrated the DePeX.

The skin from the ears and the head was further fixed in 10% formalin and frozen sections in series parallel to the skin surface were cut at 100 and 200 μ. The sections were differentiated, blued, cleared and mounted in DePeX.

Blocks were cut from several ears obtained from the abattoir. They were fixed in 10% formol saline, embedded in paraffin and serial sections were cut at 10μ. The sections were stained with acid haematoxylin and eosin but in some series alternate slides were stained with haematoxylin and eosin, and orcein and polychrome methylene blue.

Results

Examination of the sections of skin from the general body surface revealed no arterio-venous anastomoses in any of the plexuses of blood vessels. However, examination of the skin of the heads and ears proved more rewarding.

The blood supply to the helix of the ear is through the posterior auricular artery. From the injected specimens it was observed that in the helix there are four main arteries which lie in the perichondrium of the outer surface of the ear (Diagram 3). Branches from them supply the skin of the outer surface and also penetrate through the

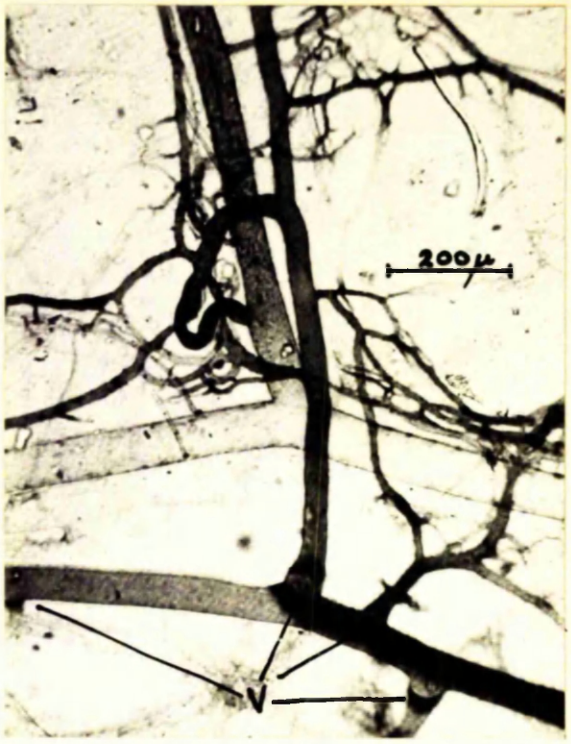


Fig.23. An arterio-venous anastomosis in the perichondrium of a calf's ear. Valves (V) can be seen in the veins.

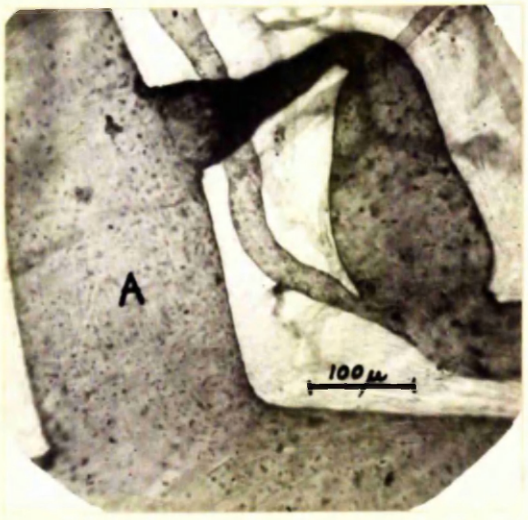


Fig.24. A contracted arterio-venous anastomosis in the perichondrium, linking a large artery (A) with a smaller vein.

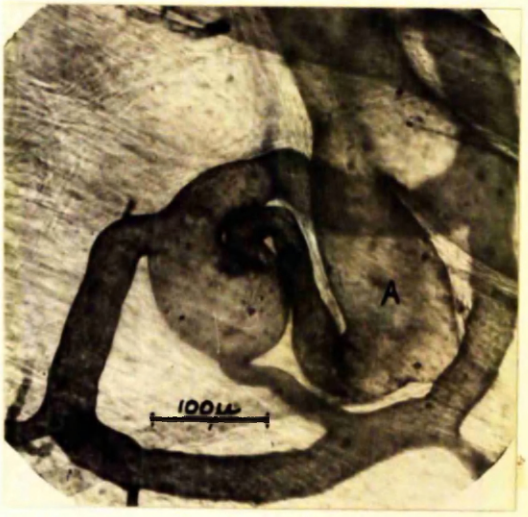


Fig.25. A convoluted arterio-venous anastomosis in the perichondrium, arising from an artery (A) and opening into a wide sack-like vessel drained by three veins which finally join.

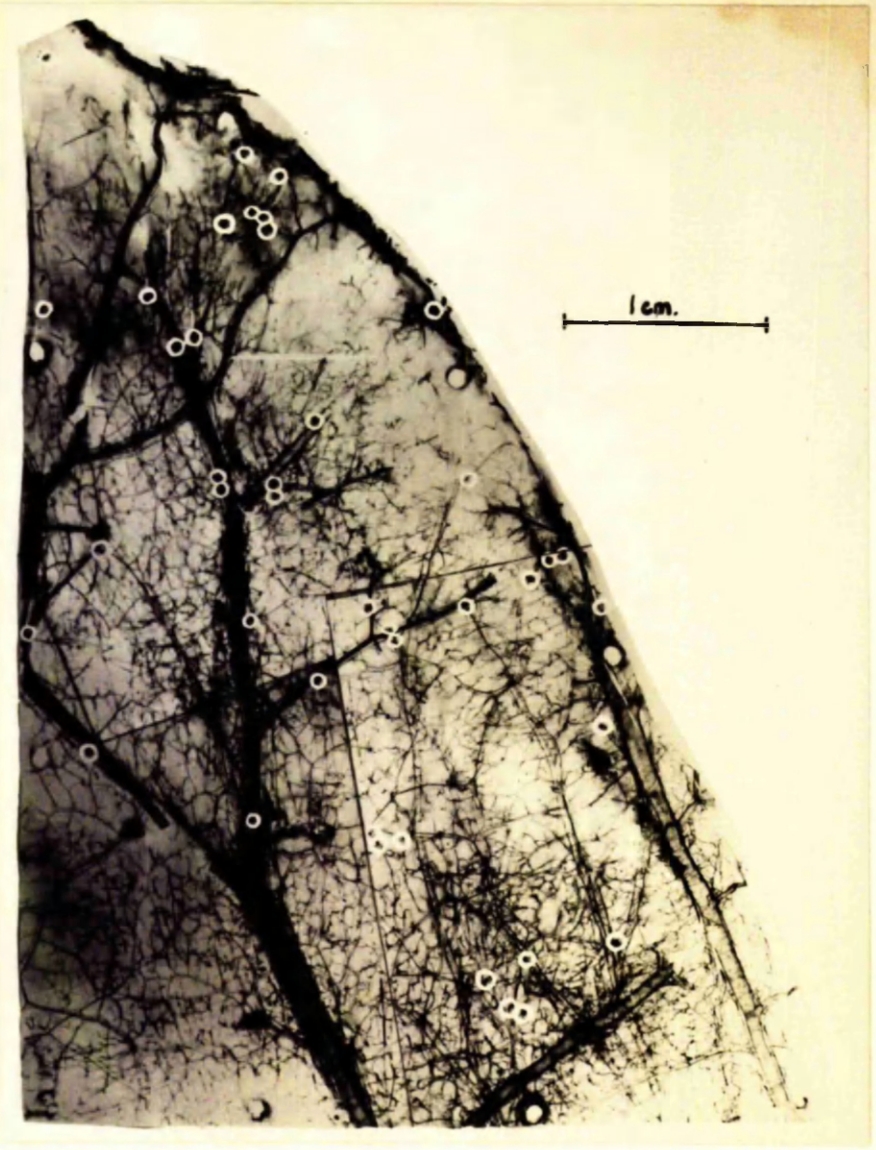


Fig.26. Part of the perichondrium of a calf's ear showing the distribution of arterio-venous anastomoses (circled in white).

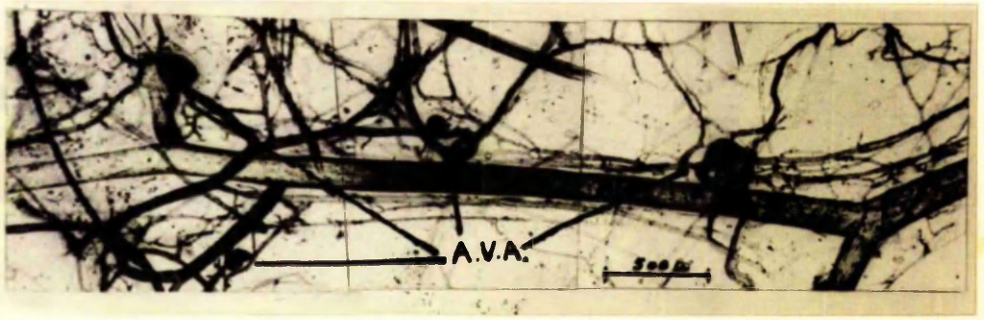


Fig.27. A group of arterio-venous anastomoses (A.V.A.) originating from the same artery in the perichondrium.

cartilage to anastomose in the perichondrium of the inner surface and then supply the skin of the inner surface. The ear is drained by a system of venous arches culminating in two main veins.

Low power microscopic examination of the perichondrium, whose blood vessels can be regarded as vessels of the first vascular plexus of bovine skin (Goodall & Yang, 1954), showed that there were many arterio-venous anastomoses present. They were sometimes short direct shunts between an artery and vein (Figs. 23 and 24) and sometimes were more coiled, dividing into several branches which after a short course joined together to form a common larger vein (Fig. 25), resembling the glomus bodies in the human finger described by Masson (1935) and Popoff (1935).

They were found on both the inner and outer surface of the ear but were more numerous on the outer surface. Maps were made to show the distribution of arterio-venous anastomoses in the perichondrium. It was found that they were more numerous along the margin of the ear than in the centre and more numerous towards the tip than at the base. Fig. 26 shows such a map. Generally only about forty arterio-venous anastomoses were present in any one quarter of the perichondrium. They varied in calibre, their outer diameters ranging from 15 to 90 μ. They usually occurred in groups along first and second branches of one of the main arteries (Fig. 27).

Examination of the frozen sections of the skin from the ear, forehead and cheek revealed arterio-

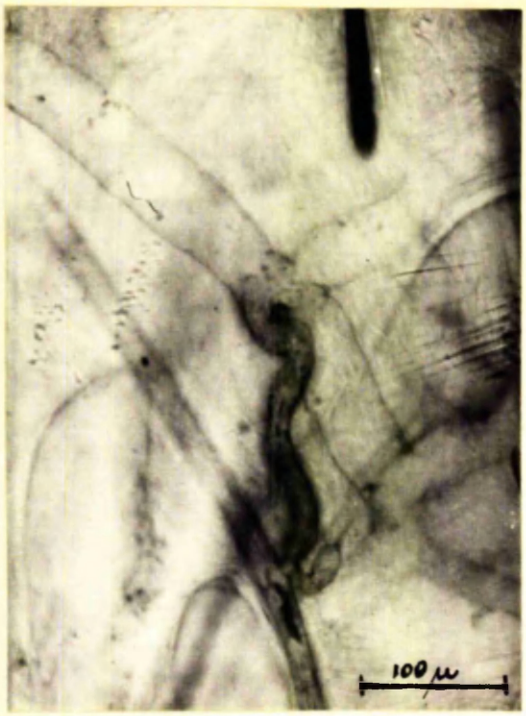


Fig.28. An arterio-venous anastomosis in the skin of a calf's ear.

Fig.29. An arterio-venous anastomosis in the skin of a calf's forehead.

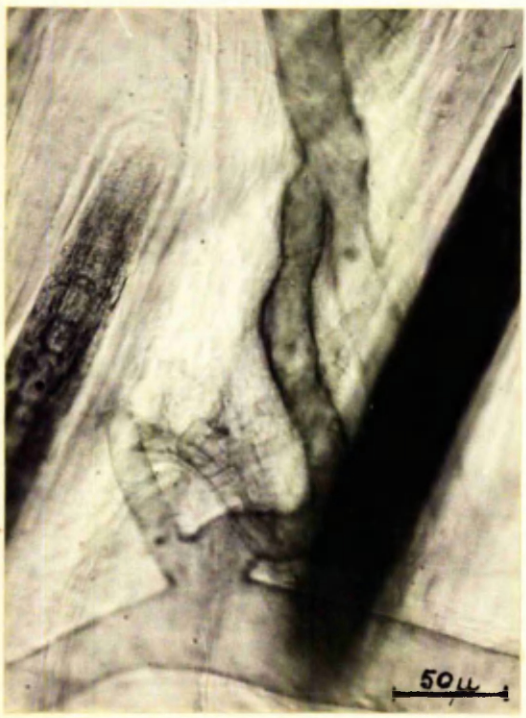


Fig.30. An arterio-venous anastomosis in the skin of a calf's cheek.

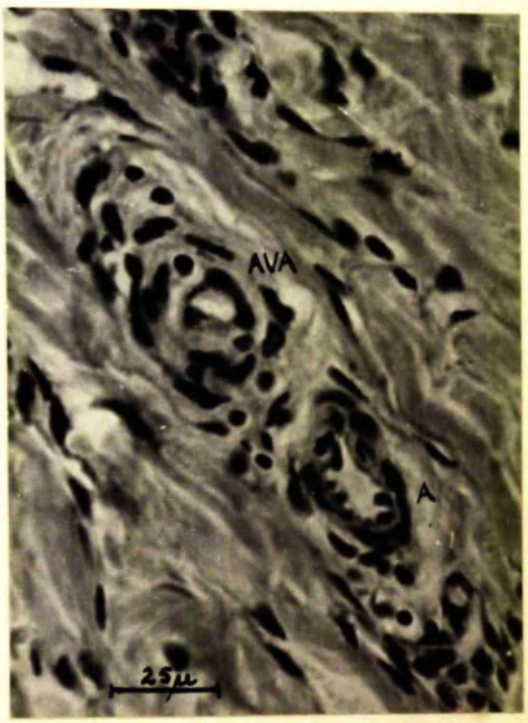


Fig.31. Cross-sections of a small artery (A) and an arterio-venous anastomosis (AVA) in the skin of a calf's ear.

venous anastomoses in the second vascular plexus of the skin (Goodall & Yang, 1954). They were more numerous than those in the perichondrium but were of finer calibre (Figs. 28-30), and numbered about 10-20/sq.cm.

In the paraffin sections arterio-venous anastomoses in transverse section could be recognized by the thickness of their walls and the presence of epithelioid cells in the media. Fig. 31 shows an arterio-venous anastomosis alongside a small artery. Paraffin sections stained with orcein showed no internal elastic lamina in the arterio-venous anastomoses.

The tongues from several of the perfused heads were removed and frozen sections at 100μ were cut in series to determine whether any arterio-venous anastomoses were present, but none were seen.

Discussion

Although as a rule capillaries are regarded as being the only communications between arteries and veins the presence of arterio-venous anastomoses in certain organs has for long been known. They were first described by Lealis-Lealis (1707) in the male genital organs. Sucquet (1862) described them in the skin of man in certain body regions, viz. the lips, cheeks, nose and ears and the ends of the fingers and toes. Hyrtl (1862, 1864) described direct communications between the radial artery and the marginal vein in the bat's wing and also in the matrix

of the hoof of the horse and of ruminants. Two early workers, Grosser (1902) and Vastarini-Cresi (1902) described the histological structure of the walls of arterio-venous anastomoses. Schumacher (1915) and Clara (1927) showed that such a vessel could be identified by its thick wall and the 'epithelioid' modification of its smooth muscle cells. The muscle cells instead of being spindle-shaped and flat were swollen and possessed intercellular bridges like epithelial cells. The vessels had no internal elastic lamina. The anastomotic vessels which are described above appear to have the same structure.

An article by Clark (1938) and a monograph by Clara (1939) give excellent reviews of the literature.

Bennet & Kilham (1940), Nonidez (1942) and Prichard & Daniel (1953) have found arterio-venous anastomoses in several organs, using histological methods. Other workers have perfused organs with glass beads of various diameters and have estimated the size of the largest arterio-venous bridges by measuring the largest beads in the venous outflow (Prinzmetal, Ornitz, Simkin & Bergman, 1948; Tobin & Zariquiey, 1950; Walder, 1952). The more recent work of Gordon, Flasher & Drury (1953) tends to refute the findings of workers using the glass-bead method although Walder, using that method, confirmed findings made previously by micro-dissection (Barlow, Bentley & Walder, 1951).

The work of Clark & Clark (1930, 1932, 1934a,b) and of Grant (1930) has shown that arterio-venous

anastomoses are important in the control of blood flow in the rabbit's ear, changing calibre spontaneously and independently of the arteries. By remaining open and so allowing an increase in blood flow through the ear, they maintain local temperatures, preventing frost-bite when environmental temperature is low, and aid in the dissipation of heat when environmental temperature is high.

Grant & Bland (1931) found that during prolonged cooling of the fingers the rise in skin temperature following an initial fall and the rate of this rise are proportional to the number of arterio-venous anastomoses in various skin regions. It may be assumed that the arterio-venous anastomoses as found in the skin of the ears, forehead and cheek of the Ayrshire calf have a similar function. Recent work on the surface temperature of the pinna of the ear of the Ayrshire calf (Beakley & Findlay, 1955a) has shown that at an environmental temperature of 10-15°C. the ear temperature undulates around 18°C. If the environmental temperature is raised to 18°C. the temperature of the ear rises quickly to 35°C. and remains at that level. This seems to indicate a great increase in blood flow in the ear at an environmental temperature of 18°C. and it is possible that the arterio-venous anastomoses may play an important part in the mechanism controlling this increase.

Grant (1930) found as many as 100 arterio-venous anastomoses/sq.cm. in the perichondrium of the rabbit's ear. There are not nearly so many in the

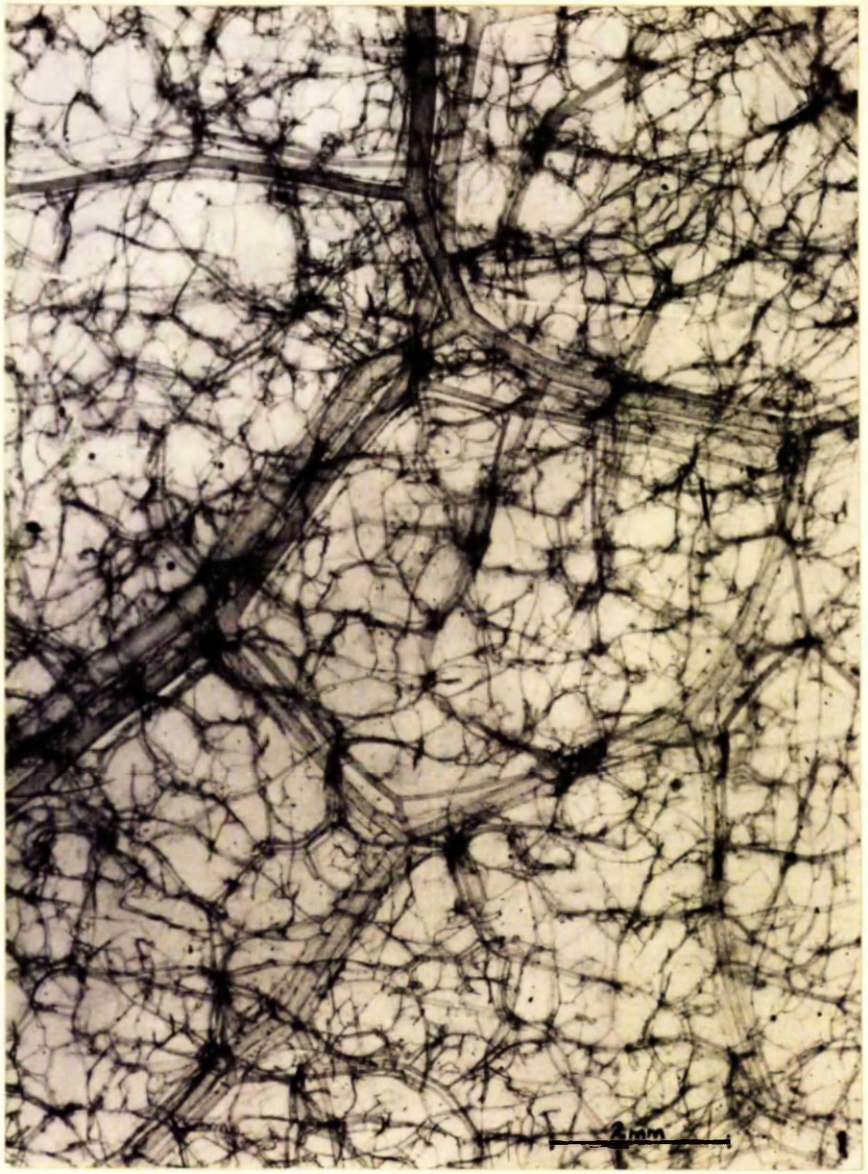


Fig.32. Blood vessels in the perichondrium of the ear of a calf. Notice that all the arteries and fine arterial branches are accompanied by veins (venae comites).

perichondrium of the calf's ear. This may, however, be explained by the work of Grant & Bland (1931) who found that the number of arterio-venous anastomoses in the skin of the human finger increased from birth to maturity. Thus it is not surprising to find that there are few arterio-venous anastomoses/unit area in the perichondrium of the young calf.

Arterio-venous anastomoses have been found in the tongue of the dog by Brown (1937) and Prichard & Daniel (1953) and also in the tongue of the sheep and the goat by Prichard & Daniel (1954). It has been suggested that they may play some part in the elimination of heat from the animal especially when panting results from excessive heat load. Nevertheless the perfusion method used in the present study has not revealed any anastomoses in the tongue of the Ayrshire calf, which employs polypnoea as a major means of heat dissipation.

It can be seen that arterio-venous anastomoses have never been found in the skin of the general body surface of any species but have been found in peripheral regions. Consequently it is not surprising to find that no arterio-venous anastomoses are present in the skin of the trunk of the calf.

Venae comites were found throughout the perichondrium, which can be regarded as the 1st vascular plexus of the skin of the ear. Fig. 32 illustrates this finding, the importance of which has been discussed in the previous chapter.

PART III

Studies in the structure of the bovine muzzle (planum nasolabialis)

Fig.33. The muzzle of an Ayrshire calf. Note how the hairless surface is divided by furrows into polygonal areas. Small beads of secretion from the nasolabial glands can be seen.

Chapter 6

The general structure, blood vessel arrangement and the structure and histochemistry of the glands of the bovine muzzle (planum nasolabialis)

Introduction

The muzzle is the only hairless part of the integument of cattle. In further contrast to the rest of the body surface it is almost continually moistened by a clear watery secretion, the product, it is assumed, of glands whose pores can be seen on the surface of the muzzle. The muzzle consists of the central portion of the upper lip and the extension of this upwards between the nostrils as may be seen in Fig. 33. It has already been mentioned that the bovine sweat glands do not appear to be particularly active and that they are always associated with hair follicles. It would appear, therefore, that the glands producing the secretion seen on the surface of the muzzle must differ from ordinary sweat glands in these two respects. Again, it has been shown that the blood vessels in bovine skin are arranged in relation to the hair follicles and so it would seem reasonable to assume that their arrangement in the muzzle is different. As the presence of arterio-venous anastomoses in the skin of several regions of the head of the calf has also been demonstrated it would seem likely that they may also occur in the muzzle. The following study of the muzzle has

therefore been undertaken with special reference to the type of glands found there and to the arrangement and types of its blood vessels.

Materials and Methods

General structure. To study the general structure of the muzzle blocks of Bouin-fixed tissue from four cows and four calves were embedded in paraffin and serial sections, 7-10μ thick, were cut both perpendicular and parallel to the skin surface. They were stained with Delafield's haematoxylin and eosin, and by Masson's trichrome method.

Muscular elements. Lendrum's lissamine-tartrazine method (1947) was employed as a stain for muscle and myoepithelium. The phosphotungstic acid haematoxylin (P.T.A.H.) of Mallory (1940) was also used.

Blood supply. The blood supply was studied in the haematoxylin and eosin and Masson stained sections and also in frozen sections, 50-100μ thick, from blocks of muzzle obtained from the heads of calves perfused with haematoxylin or with Indian ink. Further investigation of the blood supply was made by examination of a cast of the blood vessels made by perfusing the head of a calf with synthetic rubber latex (Hycar) under a pressure of 400-500 mm. Hg and macerating the tissue in concentrated hydrochloric acid. The cast, after washing, was examined and dissected under a binocular stereoscopic microscope.

Histochemical tests. The periodic acid-Schiff test (P.A.S.) of Hotchkiss (1948) was used to test for

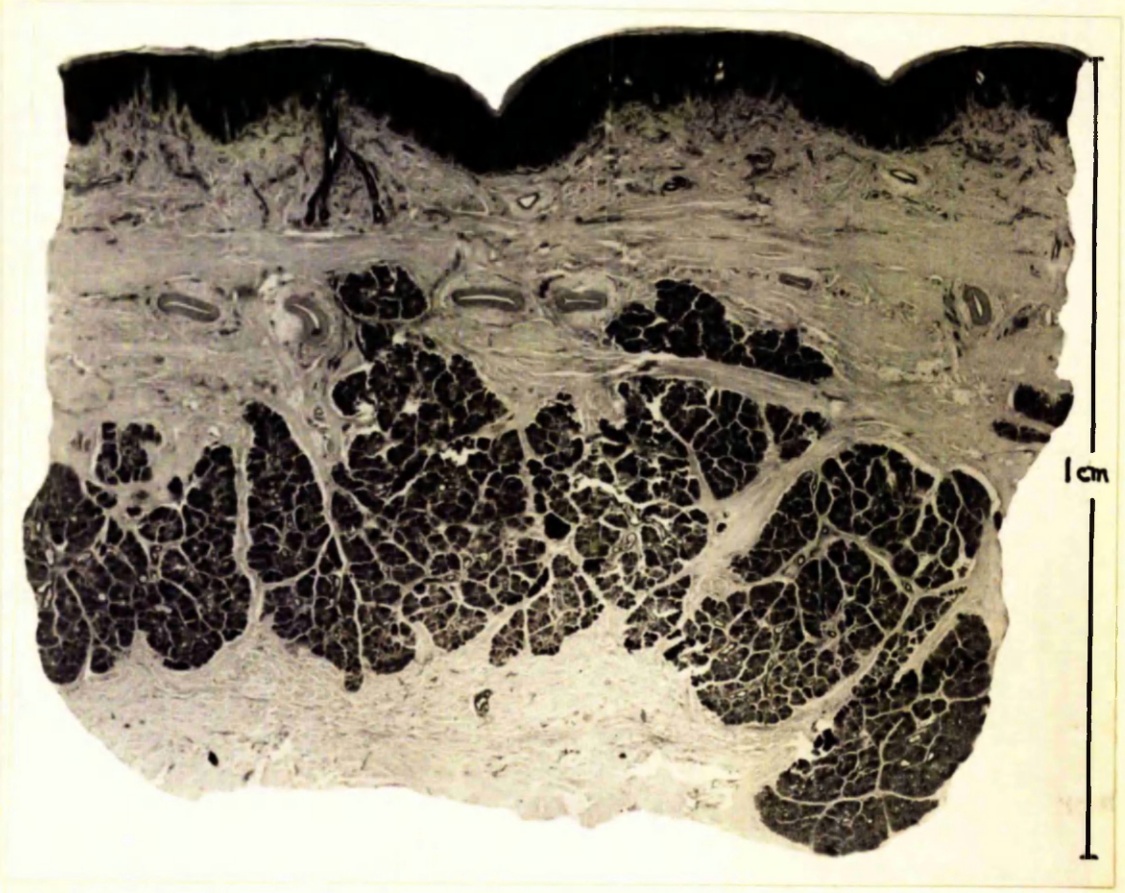


Fig.34. A section of the muzzle of a cow. Many long dermal papillae extend far up into the thick epidermis. The general structure of the nasolabial glands and the joining of three interlobular ducts to form one pore can be seen. Note also the veins in cross-section lying below the furrows, and the arteries in cross-section lying deeper in the dermis.

mucin in the glands, the nuclear stain being celestine blue and Mayer's haemalum (Lendrum & McFarlane, 1940) with orange G as a counterstain. Control sections of nasolabial glands were stained with Schiff's reagent without previous treatment with periodic acid. Control sections of sub-lingual glands, Brunner's glands (duodenum) and umbilical cord were also used in all the tests.

Frozen sections were stained in 1% toluidine blue for 10 min. to test for metachromasia. The sections were examined in water and in glycerine jelly.

To test for glycogen, sections were stained by the P.A.S. test after treatment with saliva for 10 min. at room temperature.

Frozen sections were stained with Sudan black B in propylene glycol (Chiffelle & Putt, 1951) to test for glycolipids.

Results

General structure. The structure of the muzzle can be seen from Fig. 34 which is a photograph of a section of muzzle perpendicular to the skin surface. It consists of two layers, the epidermis and dermis or corium. The naso-labial glands lie in the dermis embedded in its fibrous tissue.

Epidermis. The surface of the muzzle has a very characteristic appearance. It is divided by furrows into polygonal areas visible to the naked eye (Fig. 33). If the larger areas are examined with a

magnifying glass it can be seen that they are divided by shallower furrows into smaller polygonal areas. In the centre of each of these latter areas and in the centre of the smaller of the areas visible to the naked eye a pore of the naso-labial glands opens. In sections perpendicular to the surface numerous dermal papillae can be seen. They penetrate almost to the stratum corneum. These are not found in the skin of the general body surface of the cow. In sections parallel to the skin surface it can be seen that within each polygonal area these papillae are arranged in concentric circles around the glandular pore.

The epidermis is 1-1.5mm. thick. It is divided clearly into four layers, the stratum germinativum, the stratum mucosum, the stratum granulosum and the stratum corneum. The cells of the stratum mucosum are large and polyhedral, and intercellular bridging is well marked. The epidermis stratum mucosum and stratum corneum are all much thicker in the muzzle than they are in the rest of the skin of the cow.

The muzzle is usually pigmented and histological examination shows numerous melanin granules, mainly in the stratum germinativum. Even apparently unpigmented muzzles contain large numbers of melanin granules. Dendritic cells are present in the stratum germinativum: the presence of these cells is of particular interest since they cannot be found in the skin of the general body surface. They are found in greater numbers around the ducts of naso-labial glands: this is reminiscent of the location of

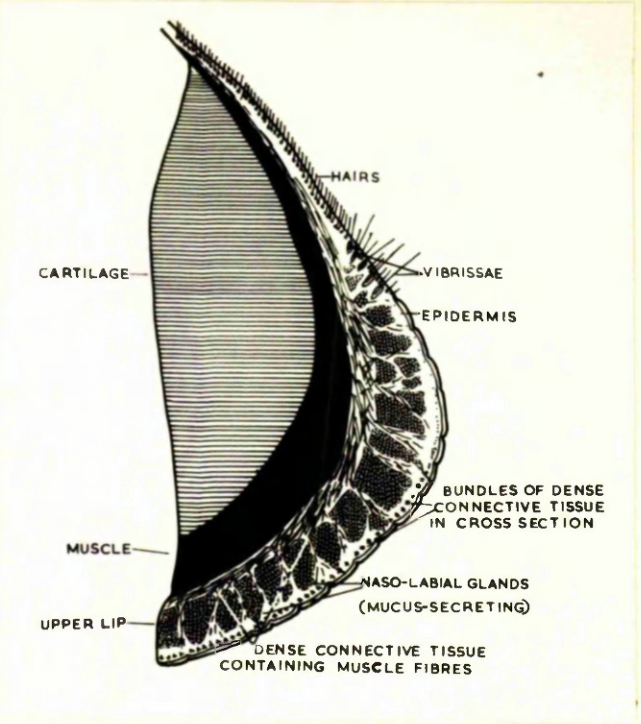
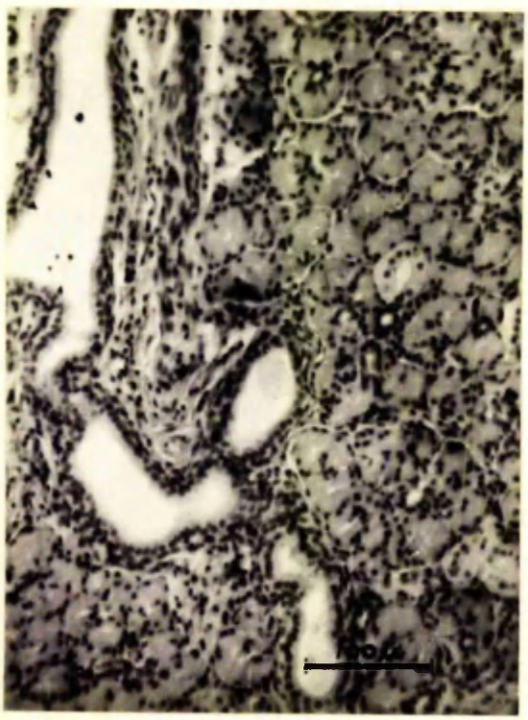
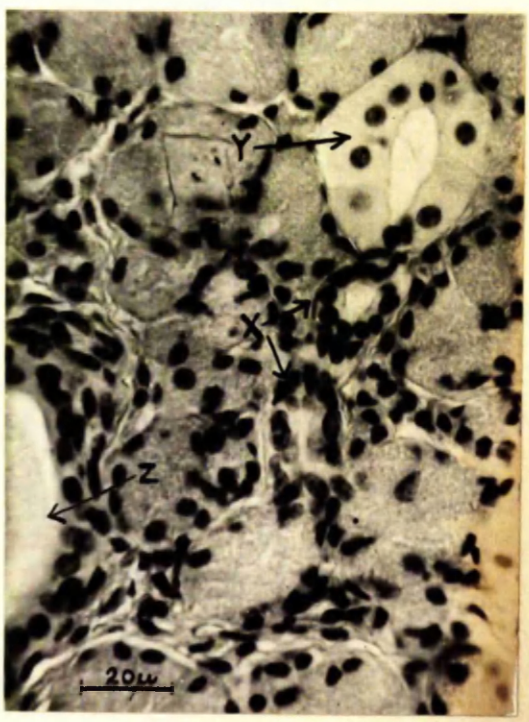


Diagram 4. A sagittal section of the bovine muzzle.





Figs.35 and 36. A section of a calf's muzzle photographed at different magnifications. In Fig.36 numerous acini, an intercalated duct (X), an intralobular duct (Y) and an interlobular duct (Z) can be seen.

melanin in the human nipple.

Dermis. The connective tissue of the dermis can be described in two parts, (1) a softer connective tissue in the papillae and the sub-papillary region, and (2) a firmer connective tissue surrounding the nasolabial glands, from which the interglandular and interlobular septa are derived. Above the glands this connective tissue lies in bands which criss-cross to form a lattice work through the spaces of which the terminal parts of the gland ducts, the nerves and blood vessels pass towards the epidermis. The connective tissue of these bands is so dense that in a sagittal section of the muzzle, such as is shown in Diagram 4, the bands cut in cross-sections can be seen clearly by the naked eye.

Nasolabial glands. As can be seen from Fig. 34 the nasolabial glands form a layer in the dermis about 5 mm. deep in parts. Histologically they may be classified as multilobular tubulo-acinar glands similar in structure to the salivary glands. They have the same characteristic features. They are made up of secretory acini linked by intercalated ducts leading into intra-lobular striated ducts, which in turn open into interlobular ducts. These latter travel towards the epidermis joining with others on the way and finally open on the surface of the muzzle in the centre of one of the numerous polygonal areas. Figs. 35 and 36 show the general structure of the glands.

The acinar cells are basophilic and their

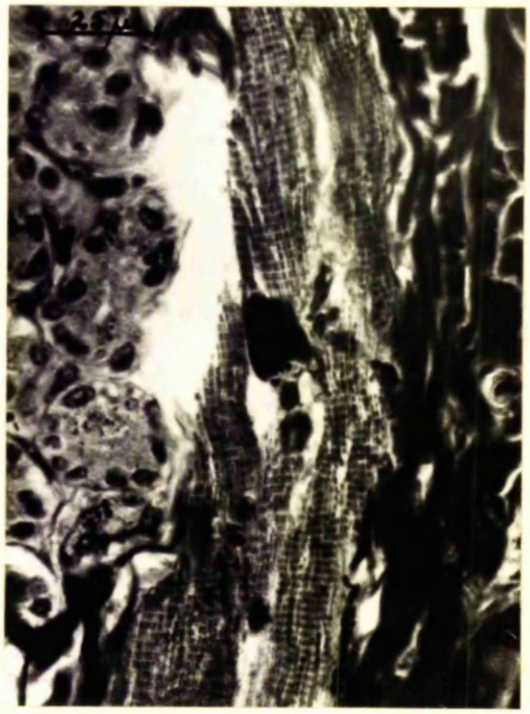


Fig.37. A band of striped muscle in an interglandular septum of a muzzle.

nuclei lie at the base of the cells. The lumina of the acini are very narrow.

The walls of the intercalated ducts are thin. The nuclei are oval in shape and the cytoplasm of the cells is very clear. The lumina of these ducts are quite narrow.

The interlobular striated ducts have wider lumina in which the secretion of the glands can often be seen. The walls consist of a layer of cubical epithelial cells with large round nuclei placed centrally. The cytoplasm of the cells contains striae of eosinophilic granules perpendicular to the free border.

The interlobular secretory ducts in their initial portions are lined with a cubical epithelium. As the ducts pass towards the skin surface the epithelium becomes more columnar and in the terminal portions there is usually a double layer of cells which changes into stratified epithelium resembling the epidermis, as the duct reaches the dermal-epidermal junction.

Muscular elements. Bands of striped muscle originating from one of the facial muscles, probably the dilator naris apicalis, are found in the interglandular septa in some regions (see Fig. 37). They seem to be attached to the bands of connective tissue between the glands and the epidermis.

Myoepithelial cells occur in the acini and in the ducts. They lie between the epithelial cells and the basement membrane. They can be recognised by

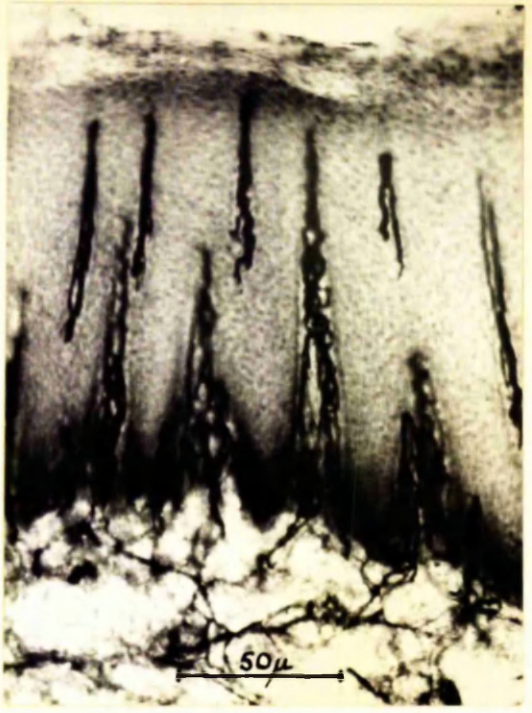


Fig.38. Capillaries in the dermal papillae of a muzzle perfused with Indian ink.

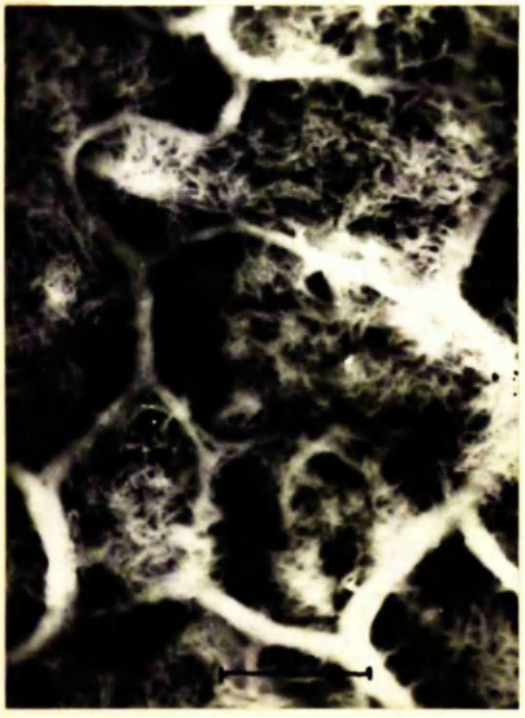


Fig.39. Part of a cast of the blood vessels of a calf's muzzle showing the plexus of veins which lies underneath the furrows on the surface, and the tufts of capillaries in the dermal papillae.

Fig.40. Part of a cast of the blood vessels of a calf's muzzle. The superficial vessels have been dissected away to show how the arteries spread out fanwise from the under side of the lip up to the nostril.

their strongly eosinophilic cytoplasm and their spindle-shaped nuclei. Those occurring in the acini are probably similar to the typical basket cells of the salivary glands.

Blood supply. According to Sisson (1930) the blood supply to the muzzle originates from three arteries, the superior labial, the lateral nasal continuation of the infra-orbital and the dorsal nasal continuation of the malar. The arteries supplying the naso-labial glands are derived from arteries lying between the glands and the epidermis. Several of these arteries can be seen in cross-section in Fig. 34. The blood supply to the dermal papillae which is very rich (Fig. 38) is also derived from these arteries. Between the arteries in the corium and the epidermis lies a plexus of veins into which the vessels from the papillae and the glands drain. The veins can be seen in cross-section in Fig. 34. Examination of the cast of the blood vessels shows that the veins form a pattern exactly similar to that of the overlying furrows dividing the surface of the muzzle into polygonal areas. Fig. 34 shows how the veins lie just under the furrows, and Fig. 39 is a photograph of part of the cast showing the pattern formed by the veins, and the capillaries of the dermal papillae as tufts within the polygonal areas outlined by the veins. The main arterial supply to the muzzle runs along under the upper lip; branches then run upwards towards the nostril, spreading out fanwise (Fig. 40).

Many of the small arteries arising from the main

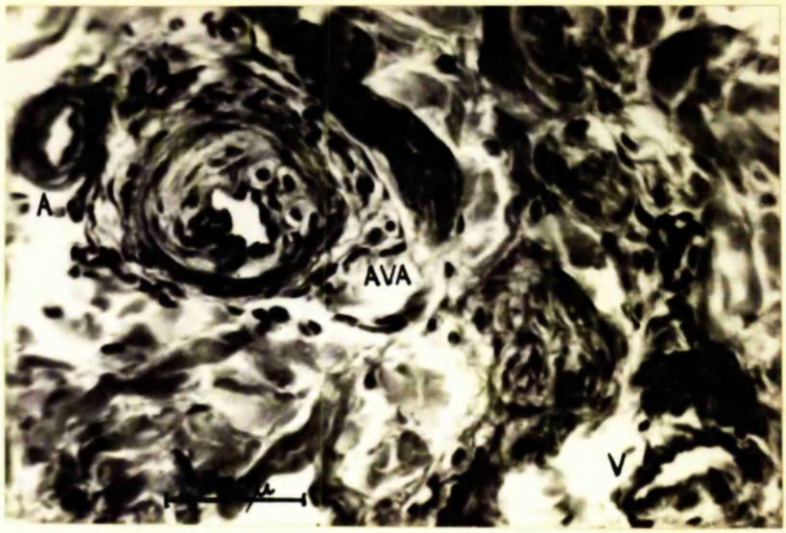


Fig.41. Cross-sections of an arterio-venous anastomosis (AVA) in the dermis of a muzzle and the artery (A) and vein (V) which it links.

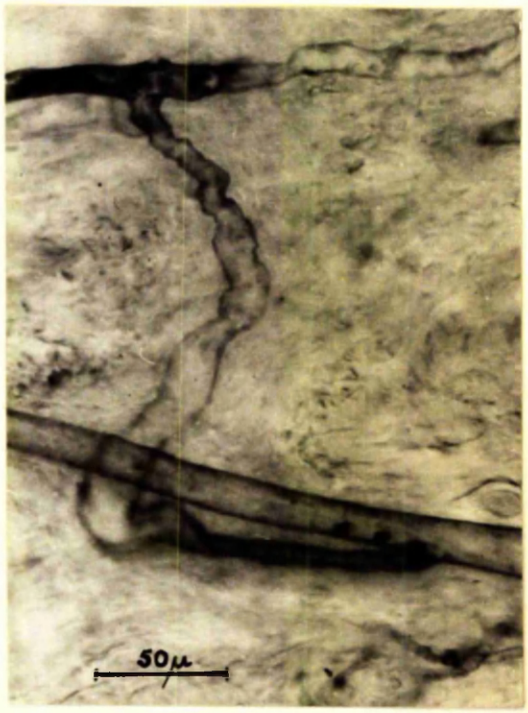


Fig.42. An arterio-venous anastomosis in the dermis of a muzzle perfused with haematoxylin.

arteries in the dermis and supplying the papillae and sub-epidermal plexus give rise to arterio-venous anastomoses. Fig. 41 is a photomicrograph of a section showing an arterio-venous anastomosis with the artery from which it arises and the vein into which it drains. The arterio-venous anastomosis has the typical thick wall containing epithelioid cells in its media and can be distinguished easily from either the artery or the vein. It is interesting to note that although the external diameter of the arterio-venous anastomosis is much larger than that of the artery or the vein its lumen is about the same size. Arterio-venous anastomoses can also be recognised in haematoxylin perfused tissue. They are always located in the same area in the dermis. One can be seen in Fig. 42.

Histochemical tests. The cytoplasm of the acinar cells stained positively with P.A.S. It did not lose this property after treatment with diastase. It showed no metachromasia with toluidin blue nor did it stain positively with Sudan black B. It therefore may be concluded that the naso-labial glands secrete either a mucoprotein or a mucopolysaccharide which is not strongly acidic.

Discussion

Brief accounts of the muzzle have been published by Ellenberger and Baum (1926) and Trautmann and Fiebiger (1949) but they are very incomplete. Another account by Ellenberger (1911) gives more detail

describing the epidermis and glands quite fully but his findings are not completely in agreement with the results detailed above. For instance, he described smooth muscle bands in the connective tissue between the glands whereas in the present work striped muscle has been found in that location. Another point of difference is that Ellenberger thought that the secretion of the nasolabial glands was serous while it has now been shown to be mucous.

It can be seen that the muzzle differs in many respects from the rest of the bovine integument. Not only is it hairless and continually kept moist but the type of gland producing the watery secretion differs markedly from the sweat glands, and the arrangement of the blood vessels is completely different from that in haired skin.

The secretion of the apocrine sweat glands of cattle has been shown by Lennox, Pearse and Symmers (1952) to contain mucin. In their discussion they list many accepted sweat gland homologues and suggest that the salivary glands may be further homologues, since it is possible that all are derived from the mucous glands of the skin of amphibians. The fact that the naso-labial glands are mucus-secreting skin glands structurally similar to the salivary glands indicates that their hypothesis is correct. A further point of interest is that Lennox et al. found that the glands of the muzzle of the dog are structurally sweat glands but that they secrete a watery mucin-containing fluid. The glands of the

cow, although structurally different, secrete the same type of watery secretion.

In the preceding chapter the possible function of the arterio-venous anastomoses found in the skin of the ears, forehead and cheeks of the calf has been discussed. Presumably their function will be the same in the muzzle. Judging from their position they may control the amount of blood flowing in the dermal papillae and in the sub-papillary plexus. In this respect they resemble the arterio-venous anastomoses in the tongue of the dog (Prichard & Daniel, 1953) and of the sheep and the goat (Prichard & Daniel, 1954).

Chapter 7

Nerve endings in the bovine muzzle

Introduction

The location of the muzzle leads one to imagine that it may play a part in the selection of fodder when the animal is grazing. If this is so it seems reasonable to assume that there will be many sensory nerve endings in the muzzle. In the investigation reported in the previous chapter several unusual structures were seen in some of the sections stained with Masson's trichrome method. They occurred in the dermis just under the epidermis, and consisted of several lamellae of connective tissue enclosing a fuchsinophilic fibre and were usually oval in shape. It was thought that these might be organised nerve endings and so the following investigation was undertaken to determine if specialised nerve endings occurred in the bovine muzzle.

Since this work was begun the method used to demonstrate the nerve fibres and endings has met with severe criticism (Weddell, Pallie & Palmer, 1954; Weddell & Pallie, 1954; Weddell, Palmer & Pallie, 1955). These authors maintain that unless tissue which is to be stained with silver to demonstrate nervous structures within it, is infiltrated with hyaluronidase previous to fixation, great distortion of the nervous tissue results, and that many of the sensory nerve endings described by workers in the past are merely artefacts. On the basis of their findings

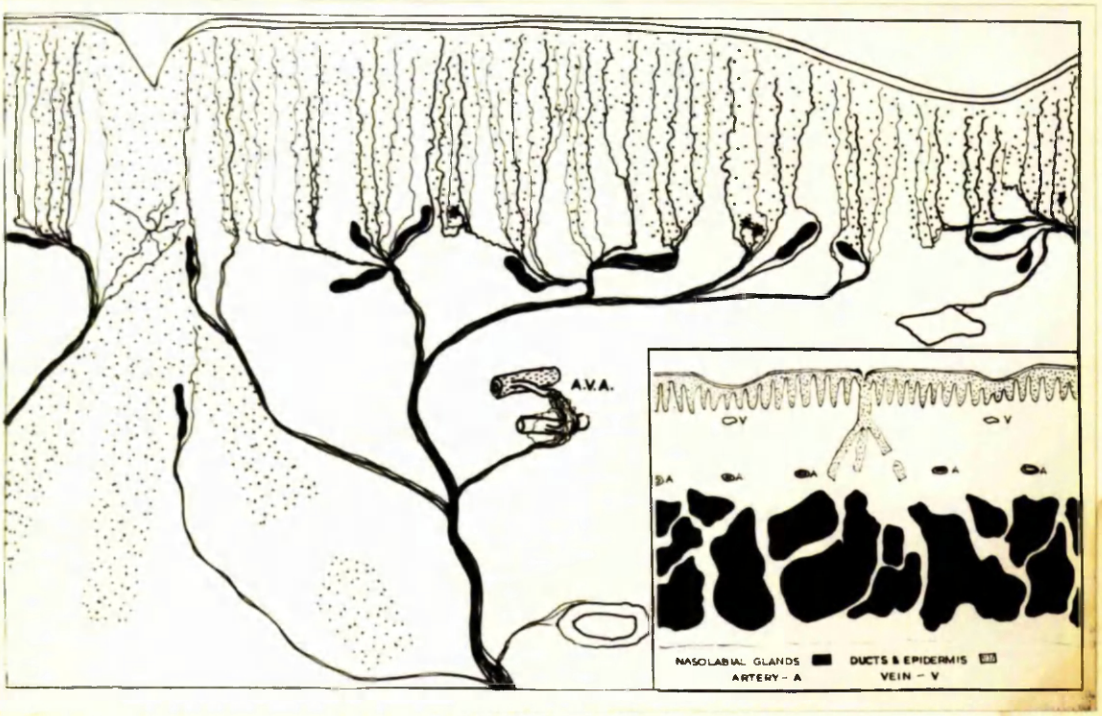


Diagram 5. The locations of nerve fibres and endings in the bovine muzzle. The inset shows the general structure of the muzzle. In the larger drawing an arterio-venous anastomosis (A.V.A.) is shown in the position in which they are usually found in the muzzle.

in the cornea this seems justifiable, but it is difficult to be completely in agreement with them. In the last of their articles (Weddell et al., 1955) they show a drawing of a myelinated nerve fibre in the dermis which after losing its myelin sheath divides and enters the epidermis where its branches end freely. They regard this as the true and undistorted picture. Yet Gairns (1955) using a method which they consider to be unsatisfactory has published a photomicrograph of a similar fibre. The two pictures are almost indistinguishable. Thus the present author is not prepared to admit that the preparations of muzzle made in this investigation show only artefacts but the investigation is to be repeated using material treated with hyaluronidase prior to fixation, and the results of the two methods compared.

Materials and Methods

Muzzles were obtained from two cows and six calves of the Ayrshire breed, and were fixed in either 2% pyridine in 10% formalin or 12% neutral formalin according to which method of silver impregnation was subsequently to be used. Frozen sections, 15-20μ thick, were stained by either Aitken's (1950) or Garven and Gairns' (1952) modification of the Bielschowsky-gros method.

Results

Diagram 5 shows the general structure of the muzzle and the location of its nerve bundles and

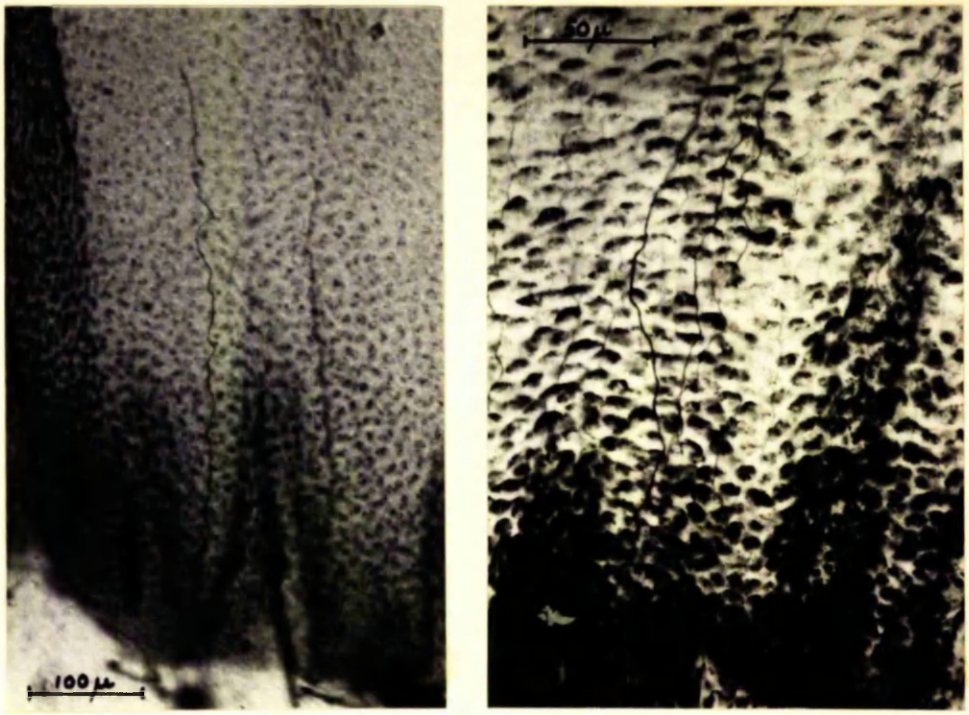


Fig.43a and b. Fine freely-ending nerve fibrils in the epidermis of the muzzles of a cow and a calf.

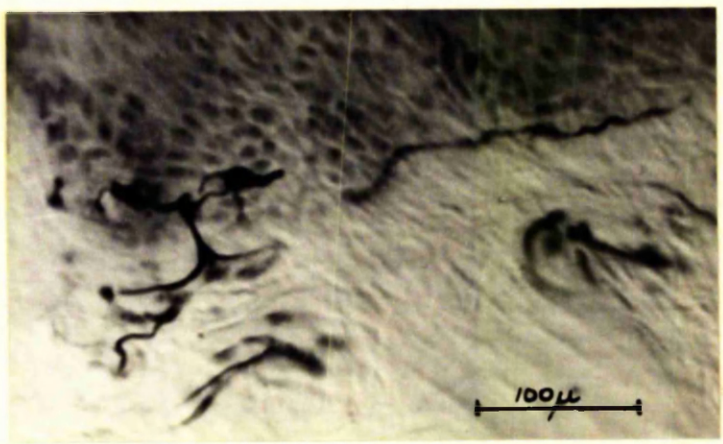


Fig.44. Menisci in the basal cells of the epidermis of the muzzle of a cow.

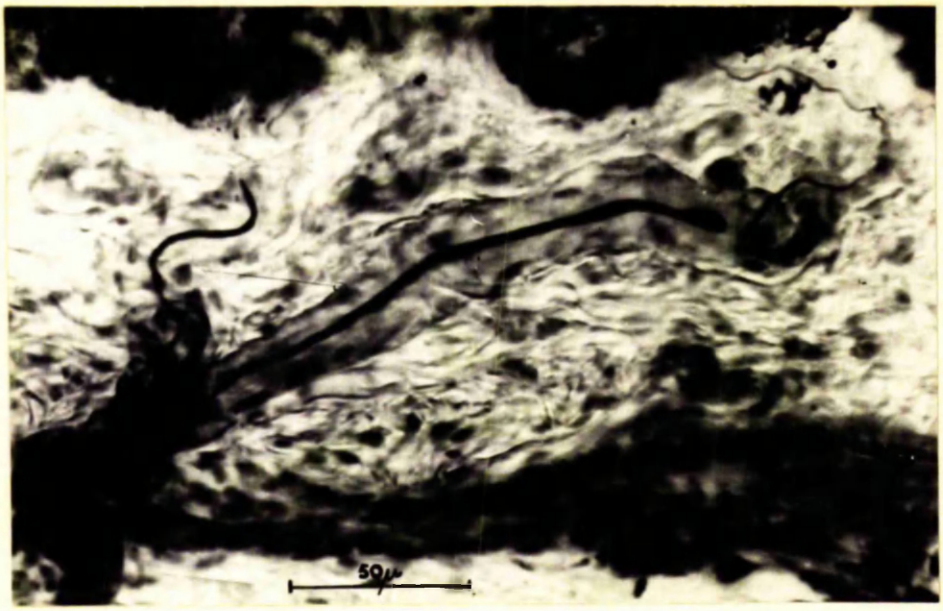


Fig.45. A complete end-bulb in the dermis of the muzzle of a calf. A fine accessory fibril by-passes it and enters the epidermis.

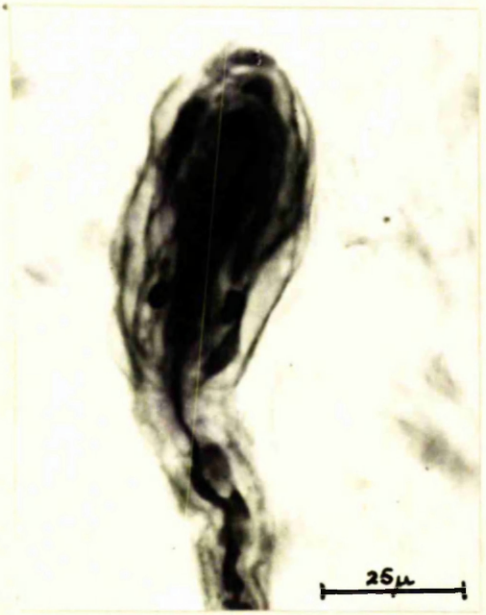


Fig.46. A small complete end-bulb in the dermis showing clearly the lamellae in its capsule.

endings. The nerve bundles pass between the naso-labial glands and then fan out above the level of the glands to form (a) free endings in either the epidermis or in the dermis and (b) organised endings in the subpapillary region of the dermis.

Endings in the epidermis. Throughout the epidermis there are many long fine nerve fibrils which run a wavy but generally direct course, without branching, from the basal layers to the stratum corneum where they end freely. The thickness of these fibres varies but this may be due to slight differences in the degree of impregnation of silver. Several of these fibrils may be seen in Figs. 43 a & b.

In the basal layers of the epidermis menisci occur which resemble Merkel's discs in the snout of the pig. Two of them are shown in Fig. 44.

Endings in the dermis. There are many organised endings in the subpapillary region of the dermis. The central nerve fibre is thick and neurofibrillar and appears to end in a knob-like thickening. It is encapsulated in several concentric lamellae giving the complete structure a cucumber-like shape. Fig. 45 shows one of these end-bulbs. The structures seen in the Masson stained sections are obviously oblique sections of such end-bulbs. An accessory fibre from the bundle supplying the organised ending can usually be seen by-passing the ending and entering the epidermis where it presumably ends in one of the forms described already. A smaller end-bulb showing clearly the nuclei of the cells of the lamellae can be

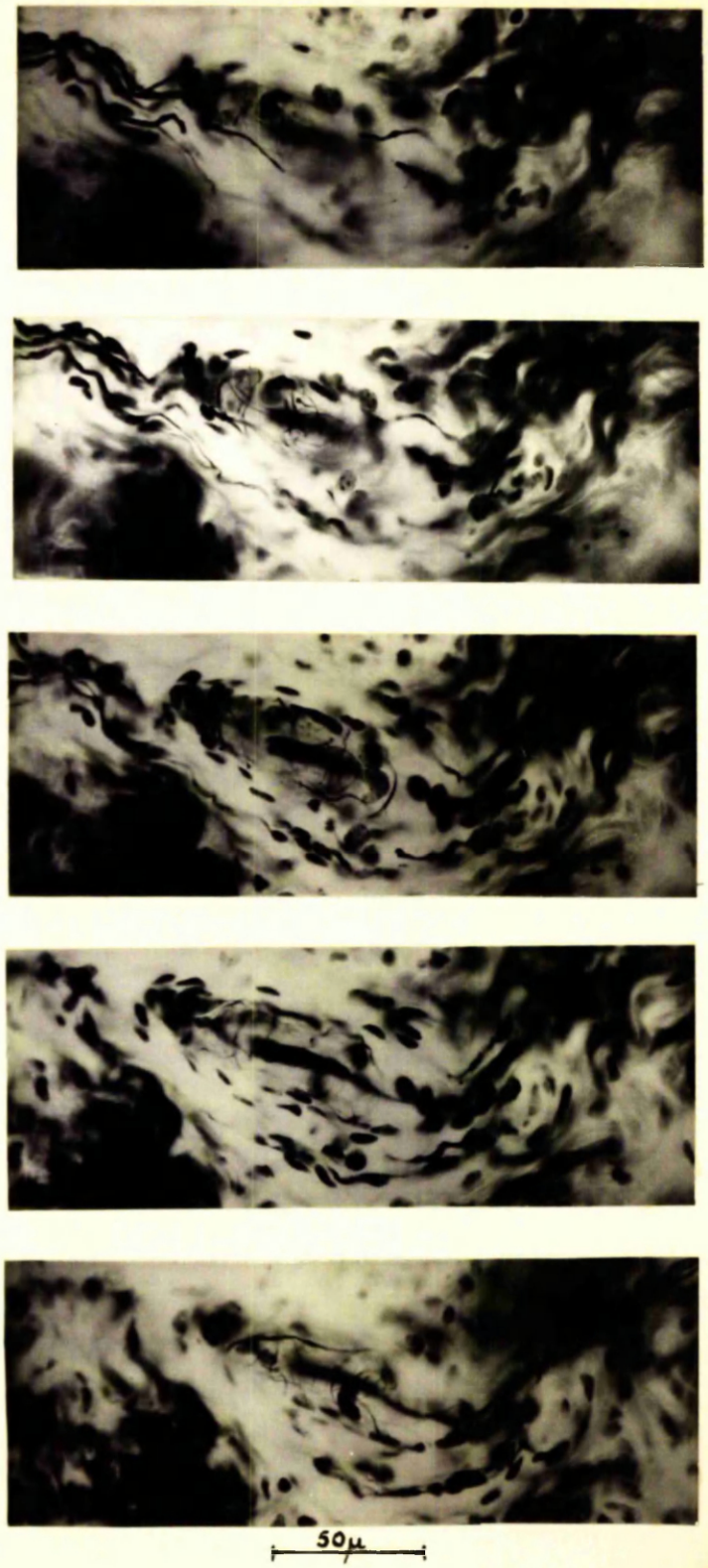


Fig.47. A series of photomicrographs, taken at different focal planes, of an end-bulb which has a network of fine beaded fibrils forming a lattice-work sheath in its capsule. Notice the neurofibrillar structure of the central fibre.

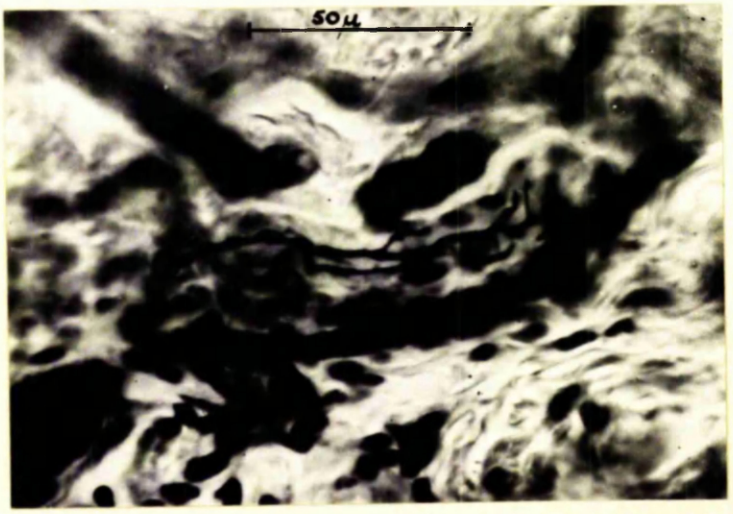


Fig.48. An end-bulb in which the central fibre branches several times all along its length.

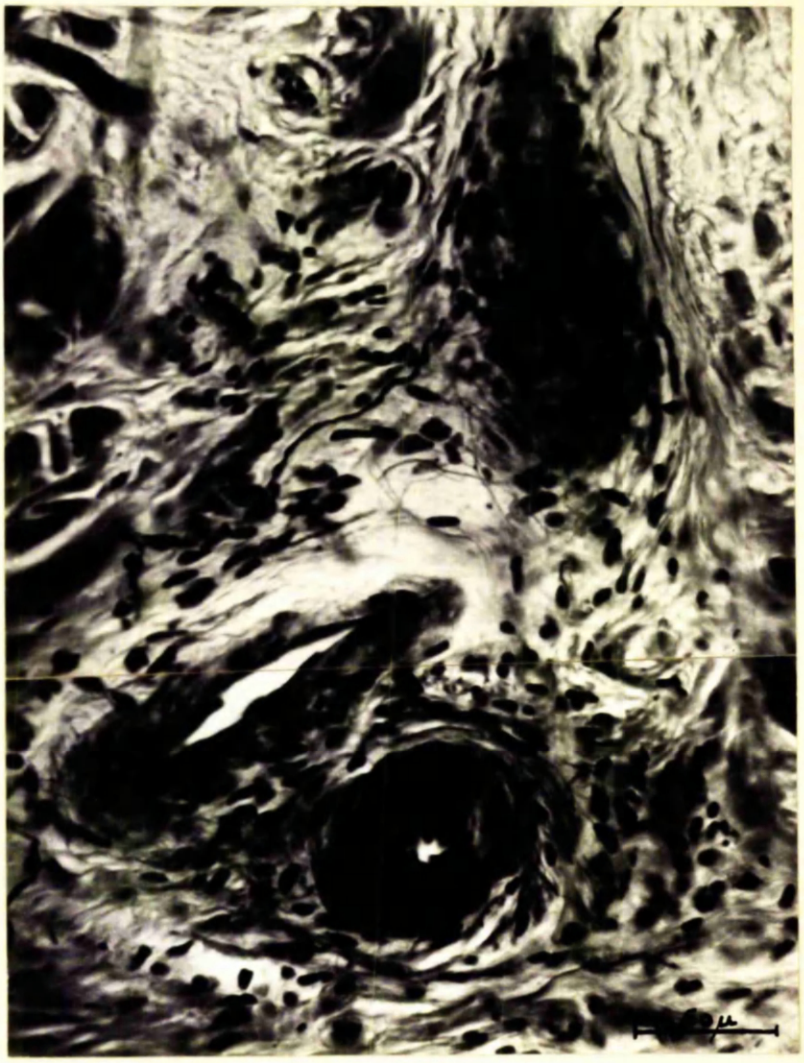


Fig.49. A photomicrograph showing, at the bottom right, an arterio-venous anastomosis in cross section. Above it, to the right, the wall of the arterio-venous anastomosis has been cut longitudinally, and, at the left, a vein has been cut obliquely. Many fine nerve fibres can be seen in and around the walls of the arterio-venous anastomosis.

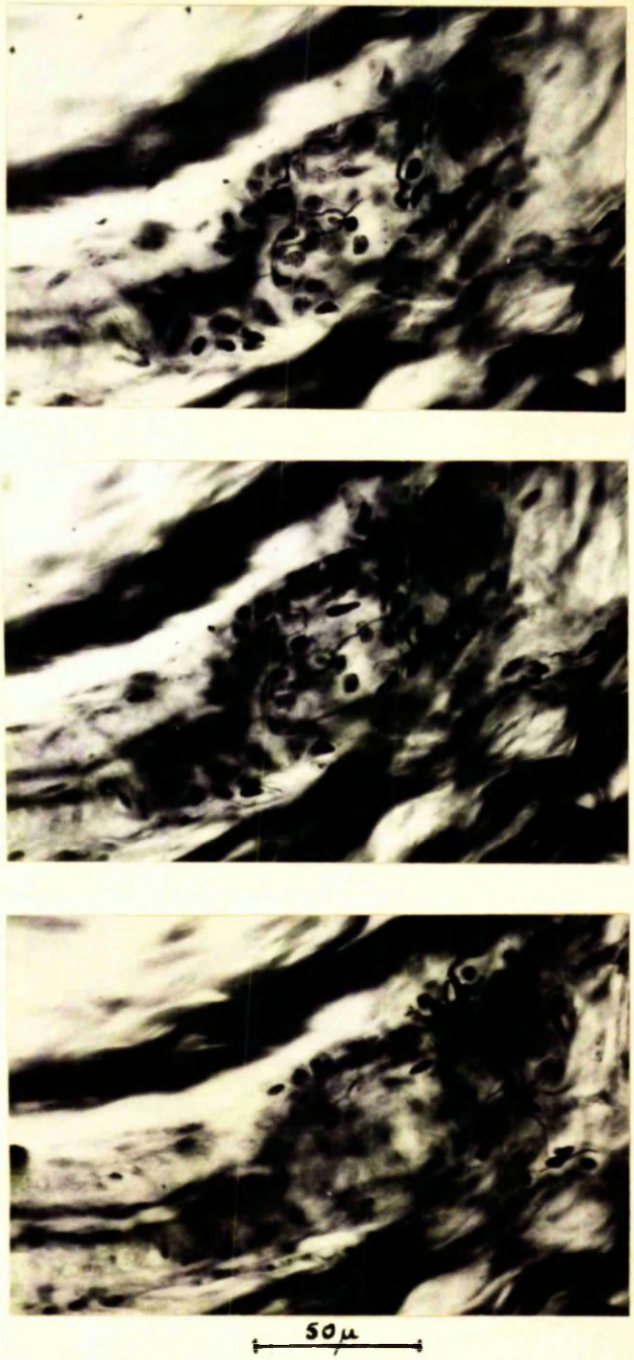


Fig.50. An arterio-venous anastomosis photographed at three different focal planes in order to show the large number of nerve fibres in its wall.

seen in Fig. 46. One or two end-bulbs have been seen possessing accessory fibres which having divided into fine neurofibrils wind themselves round the end-bulb thus forming a sort of lattice-work sheath within the capsule. Photomicrographs of one of these end-bulbs have been taken at several focal planes to show the extraordinary number of neurofibrils in the sheath. (Fig. 47). The end-bulbs often occur in groups of two, three or even four and occasionally the central fibre branches within the capsule (Fig. 48). The fibres supplying the end-bulbs are myelinated but appear to lose their myelin sheath before entering the capsule.

Fine unmyelinated fibres can be seen on the walls of the ducts of the naso-labial glands and in the interlobular septae of the glands. Ganglia do not occur in the glands.

The walls of the arterio-venous anastomoses contain a large number of nerve fibres. In Fig. 49 an arterio-venous anastomosis in cross section lies beside a vein cut obliquely while above them the wall of another part of the anastomosis has been cut longitudinally. Both portions of the anastomosis have many fine nerve fibres in and around their walls. Fig. 50 shows another arterio-venous anastomosis cut obliquely which has a large number of fine nerve fibres in its walls and also several thicker myelinated fibres around it.

Discussion

Sinclair, Weddell and Zander (1954) and Hagen, Knoche, Sinclair and Weddell (1953) have found that the sensitivity of an area of skin is not dependent on the number of organised nerve endings in it and that the long-established concept of one particular type of ending being associated with a specific modality of sensation is not valid. So it seems rash to postulate what may be the function of the various endings found in the muzzle. However, the cucumber-shaped end-bulbs in the dermis closely resemble the "sausage-shaped" end-bulbs found in the knee-joint of the cat by Boyd (1954). He found these endings in pieces of tissue still attached to nerve fibres from which he had picked up potentials when the area had been stimulated by the local application of pressure. This seems irrefutable evidence that this type of ending is pressure sensitive.

Gairns and Aitchison (1950) and Gairns (1955) found fine fibrils in the epidermis of the human gum and hard palate which were very similar to those just described in the epidermis of the bovine muzzle. There are, however, two differences between the bovine and human fibrils: those of the bovine muzzle have no specialised ending and arise from a subepidermal plexus of nerves or from the accessory fibres which bypass the dermal organised endings, while those in the human tissues end in a small bead and arise from within the capsule of organised endings in the papillae.

The unusually large number of nerve elements in and around the walls of arterio-venous anastomoses was noticed by Masson in 1937. He stated that both sensory and motor endings occurred in the walls of the vessels. In the vessels shown in Figs. 49 & 50 it was impossible even under high magnification to trace the fibres to any kind of ending. Masson postulated that arterio-venous anastomoses control tissue fluid pressure, that the sensory endings in their walls are pressure sensitive and that when they are stimulated by an increase in tissue fluid pressure they bring about a reflex dilatation of the vessels. This dilatation will cause the pressure in the capillaries to drop which will, in turn, bring about a drop in tissue fluid pressure with a subsequent cessation of stimulation of the pressure sensitive endings. This will then bring about constriction of the arterio-venous anastomoses and the whole cycle will be repeated. Since this hypothesis was made it has been shown by Grant (1930) that the arterio-venous anastomoses play an important part in the control of body temperature. Possibly the calibre of the vessels is controlled by reflexes whose motor endings are located in the vessel walls and whose sensory endings are located either peripherally or centrally where they are activated respectively by changes in skin or blood temperature. It seems possible that the arterio-venous anastomoses combine both these functions.

GENERAL DISCUSSION AND SUMMARY

General Discussion

From the results of the investigation into the vascularisation of bovine skin it can be seen that the skin is well supplied with blood vessels. This fact naturally leads one to assume that this rich blood supply will enable a great deal of heat to be lost from the skin but certain factors in the distribution of the blood vessels within the skin tend to limit the loss of heat. As already explained the venae comites allow an interchange of heat between the arterial and venous systems, precooling the arterial blood before it reaches the skin surface and so lessening the temperature gradient at the skin surface. This, however, only holds when the environmental temperature is lower than the deep body temperature, for if the environmental temperature is higher than the deep body temperature the arterial blood will be heated on its way to the skin surface. Under these conditions the difference in temperature between the environment and the skin will be reduced with a resultant decrease in the amount of heat absorbed by the body from the environment. So it seems that venae comites are beneficial in extreme conditions when the environmental temperature is higher than the body temperature in that they reduce the amount of heat that would otherwise be absorbed and also ~~that~~ at environmental temperatures lower than the body temperature when they are instrumental in conserving heat.

The poor blood supply to the sweat glands of Ayrshire cattle appears to be another factor which limits the amount of heat lost from the skin for not only does it compare unfavourably with that of

human sweat glands but the work of Ferguson and Dowling (1955) indicates that it may be much poorer than that of more heat tolerant breeds. They found that the sweating response of a Zebu x Jersey heifer was much greater than that of an Ayrshire heifer, each animal having about the same number of sweat glands per unit area in its skin. Thus the difference can only be due to a greater activity of the sweat glands of the Zebu x Jersey animal. As the glands of both breeds are so similar histologically this greater activity is probably the result of a better blood supply to the sweat glands of the Zebu x Jersey animal, but of course until injected skin from an animal of a heat tolerant breed can be obtained and examined this can only be assumed.

Another interesting point in relation to the blood supply in bovine skin is that Dowling (1955b) found that the papillary layer of Zebu skin (the layer containing the hair follicles and their appendages) is thinner than that of Shorthorn skin. Bearing in mind the results of the investigation into the blood supply in bovine skin it seems possible that part of the Zebu's superior ability to dissipate heat may be due to the fact that it has a thin papillary layer with a large number of hair follicles. As a result the large number of blood vessels attendant on the high hair density will be concentrated in a shallow layer under the epidermis. This will enable more heat to be lost through radiation, conduction and convection and possibly through insensible perspiration than would be possible if the blood vessels were spread

over a deeper layer.

Although it has been shown by Ferguson and Dowling (1955) that the secretion of the bovine sweat glands produces some cooling when its fluid content evaporates, it is possible that the solids contained in the secretion may also have a function. The connection between the secretion of the sweat glands and the ceruminous glands has been pointed out (p.17). The apocrine sweat glands of the human axilla are usually regarded as being responsible for the characteristic odour which emanates from the axilla and they undergo changes in relation to the oestrus cycle. In mammals there are quite a few examples of apocrine glands which produce strongly smelling substances, e.g., the glands of the anal sac of the dog. The bovine animal too possesses a characteristic odour which is, most likely, produced by the secretion of the sweat glands whose activity, like that of the apocrine sweat glands of the human axilla, may be related to the oestrus cycle.

Until appropriate physiological experiments are carried out, it may be assumed that the function of the arterio-venous anastomoses in the skin of cattle is the same as the function described for those in human skin by Grant and Bland (1931). It is proposed to insert transparent chambers into the ears of cows in order to observe changes in the calibre of the arterio-venous anastomoses with changes in environmental temperature. Preliminary experiments using calves have proved unsuccessful because the chamber always became

occluded by the growth of cartilage. This is probably peculiar to the young growing animal and may not occur in the adult animal.

The function of the muzzle and the nasolabial glands is still obscure. Obviously the constant evaporation of the secretion of the glands from the surface of the muzzle must play an important part in local heat loss but the area is so small that it can have little effect on general body temperature. Perhaps the function of the secretion of the glands is simply to wash the surface of the muzzle for, as has been suggested, it may play some part in the selection of fodder and, from the large number of nerve endings found in the muzzle, it appears that it is highly sensitive and well equipped to undertake such a function. However, the striking difference between the muzzle and the rest of the integument seems to indicate a much more specific function for the specialised structure. It has been shown that the organised nerve endings in the muzzle are most likely pressure sensitive, but the function of the fine fibrils which occur in such profusion throughout the epidermis is unknown. They and others like them in the human gum and hard palate occur in the epidermis of areas where the secretion of the mucous glands of the under-lying dermis keeps the epidermal surface moist. Perhaps they can detect when the surface dries up and can bring into play a reflex which will activate the mucous glands to secrete. If this is so they are therefore, indirectly, humidity

receptors. Results from experiments carried out in the psychrometric chamber at the Hannah Institute (Beakley & Findlay, 1955b) suggest that in either the skin or the respiratory tract there must be receptors which can sense a variation in the humidity of the environment. Unpublished observations at the Hannah Institute (Bligh, 1956) suggest that there are peripheral receptors which can detect a change in environmental temperature and initiate panting. It may be that these receptors are located in the muzzle and respiratory tract. In view of this evidence it is proposed to investigate the innervation of various areas of the respiratory tract, viz., the turbinates, the pharynx, and probably the tongue, hard palate, soft palate and gums to see if the same long fine fibrils occur in the epidermis of these areas.

The information gained from these studies of the micro-anatomy and histology of bovine skin, is contributing towards the understanding of the physiological function of the organ and its appendages, especially with respect to its ability to dissipate heat.

Summary

1. The myoepithelial coat of the bovine sweat glands has been studied and has been found to envelope completely the dermal surface of the gland. The myoepithelial cells are spindle-shaped, contain myofibrils and possess spindle-shaped nuclei.

2. The sweat glands and ceruminous glands of cattle have been compared with human ceruminous glands. Both types of bovine glands are histochemically identical but the ceruminous glands are larger and more coiled than the sweat glands. The human ceruminous glands, although structurally similar to the bovine ceruminous glands differ from them histochemically. Lipids and other sudanophilic material are found in the cytoplasm of the secretory epithelium of the human glands but are absent from that of the bovine glands.
3. An investigation has been made into the relation between the milk yield of cattle and the number of sweat glands in the pinna of the ear in order to verify claims made by several Russian workers that a highly significant correlation exists between the two factors. No such correlation was found for Ayrshire cattle.
4. Three plexuses of blood vessels have been found to exist in bovine skin. In all plexuses there is a widespread occurrence of venae comites. The blood supply to the sweat glands of Ayrshire cattle is very poor. The blood supply to the greater part of the skin of the trunk is through branches of the intercostal arteries which before reaching the skin pass through the heat-generating intercostal muscles.
5. Arterio-venous anastomoses have been found in the perichondrium of the ears and in the skin of the ears, forehead and cheeks of Ayrshire calves.

They do not occur in the skin of the trunk.

6. The structure of the bovine muzzle has been investigated. The secretion which usually keeps its hairless surface moist is produced by multilobular tubulo-acinar glands which lie in the dermis. Bundles of striped muscle occur in the interlobular and interglandular septa. The arrangement of the blood vessels differs from that found in the rest of the body surface and arterio-venous anastomoses occur in the dermis between the glands and the epidermis. The nasolabial glands are mucus-secreting.

7. Large numbers of different types of nerve endings have been found in the dermis and epidermis of the bovine muzzle. In the epidermis some fibres end freely and others in menisci, while in the dermis some end in organised endings and others in relation to blood vessels. The dermal organised endings are cucumber-shaped lamellated structures resembling the pressure sensitive endings in the knee-joint of the cat. The arterio-venous anastomoses possess a large number of nerve fibres in and around their walls.

8. The possible importance of all the findings listed above in the function of the skin are discussed, with special reference to the ability of the skin to dissipate heat.

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