STUDIES IN THE SECRETORY FUNCTION OF THE SKIN OF RUMINANTS

With special reference to the sebaceous glands of cattle

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for the Degree of Doctor of Philosophy in the Faculty of Science

by

MIRANDA EVELYN SMITH

The Hannah Research Institute

Ayr, Scotland KA6 5HL.

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SUMMARY

1. The work described in this thesis was initiated to investigate the physiology of bovine sebaceous glands, to endeavour to elucidate some of the factors which affect their output and mode of activity and hence to establish the function of bovine sebum. A method to determine sebum output in the thoracico-lumbar region of Ayrshire calves was developed. Sebum was collected from pre-cleaned areas of skin after a given period of time, usually 3 h, using a specially designed cup in conjunction with the solvent methanol. The collected sebum was then extracted from the methanol and weighed on a micro-balance.

2. No evidence was found to support the hypothesis that sebum flows from areas of high to low concentration on the skin of cattle indicating that the integrity of the surface lipid layer is dependent on local sebum production.

3. After cleaning the skin with methanol, the rate of sebum output decreased with time and after 24 h there was no appreciable increase in the weight of sebum on the skin surface. Thus the sebum layer when removed experimentally takes over 24 h to be completely replaced.

4. Sebum output measured over a period of 3 h appeared to be lower in winter than throughout the rest of the year but this reduction just failed to be significant.

5. Sebum output over a period of 3 h was not statistically different between animals of different ages, but tended to be higher in castrated males than in females.

6. Short-term changes in the environment had no consistent effect on sebum output. However, on prolonged exposure to a warm environment there was a significant increase in sebum output.

7. The composition of bovine sebum was similar to that of other species in that it contained phospholipids, free cholesterol, unesterified fatty acids, triglycerides, diester waxes, cholesteryl esters and squalene. However, more triglyceride was present than was found in a previous study on cow skin surface lipid (Nicolaides et al, 1968). Most of the linoleic acid present in cattle sebum was found in the triglyceride fraction.

8. Short-term exposure to different air temperatures and humidities had no appreciable effect on the fatty acid composition of bovine sebum. At a low humidity, however, the cutaneous output of palmitic acid was higher. On prolonged exposure to a warm environment, a higher output of sebum occurred together with an increase in the percentage of linoleic acid in the sebum.

9. Although the precise function of bovine sebum is unknown it is concluded that it has similar properties to that from other species. Thus the role of bovine sebum is to form a natural barrier - layer which aids in the thermoregulation of the animal, the prevention of water loss from the epidermis and acts as a deterrent against skin disease. The bovine sebaceous glands, unlike the sweat glands, are not of major importance in the regulation of body temperature in the heat.

10. Sebum output and sebaceous gland volume and cell number per m^2 of skin were measured after stimulation of the glands by cleaning the skin with methanol and compared with estimates of glandular mitotic activity. None of these quantities was altered by a single or by repeated stimulation even though the number of cells necessary to produce the sebum by a holocrine mechanism greatly exceeded the estimated level of cell production. Sebum output was not associated with the DNA content of skin washings but sweat output was; the DNA was probably derived from sweat. It was concluded that the mode of secretion of cattle sebaceous glands is unlikely to be holocrine. It is suggested that sebum secretion may be a more complex mechanism than previously supposed. Sebum may not be solely a product of cell degeneration but could conceivably be produced by a process involving lipogenesis and secretion from live cells.

CHAPTER I

GENERAL INTRODUCTION

In mammals the outer layer of the body, the skin, has 3 main functions; the protection of the animal against harmful environmental effects, the maintenance of homeostasis and the acquisition of information about the environment. Thus the integument must be capable of withstanding dehydration, ultraviolet radiation and the action of bacteria and fungi. To maintain homeostasis mammalian skin must be resistant to the influx of water and substances in solution from the environment and it also prevents undue loss of body fluids. However, the skin is not impervious to all substances as certain chemicals can be absorbed through it into the blood stream (Ham, 1965; Press, Hartop & Prottey, 1974). Similarly, the skin can facilitate the loss of heat. Heat loss is achieved not only by radiation, convection and conduction but also by evaporation. Heat lost through mammalian skin can be modified by the cutaneous blood capillaries, changes in the insulative properties of the hair coat, the presence of an adipose tissue layer in the dermis and by the production of sweat. In man (Rothman, 1955) and the cow (Jenkinson, 1972) an important function of the sweat gland is the maintenance of homeothermy. Mammalian skin also contains nerve endings which are sensitive to many different types of stimuli such as pressure, heat, cold and pain. Thus information about the environment is conveyed to the animal.

The skin of cattle, as in other mammals, is not uniform in appearance. Specialised areas devoid of hair, as for example the muzzle and the teat, contrast markedly with the skin of the general body surface. The latter, which has been loosely termed the "hairy skin" is characterised by an actively growing coat and extends over most of the animal's body. Microscopical examination of the "hairy skin" of cattle reveals that, as in human skin, it is composed of 2 main parts; the superficial epidermis and the subjacent dermis.

The epidermis is made up of 3 layers, the stratum germinativum, stratum granulosum and the stratum corneum. In bovine skin, unlike human skin, there is no stratum lucidum and the stratum corneum is relatively thin. The boundary between the epidermis and the underlying dermis is smooth but it may show slight elevations and a few small folds. Epidermal pegging, a characteristic of human skin is not found in the haired skin of cattle but is present in skin from the muzzle (Jenkinson, 1965).

The dermis of cattle, unlike that of man, does not contain adipose tissue. It can be divided, as in man, into 2 main layers, the stratum papillare and stratum reticulare (Jenkinson, 1965). The junction between these layers is often ill-defined but appears to be at approximately the level of the sebaceous glands. The stratum papillare, the narrower of the 2 layers, is composed of fine collagenous fibres interwoven with strands of elastic and reticular tissue.

Within the dermis, characteristic "hair follicle units" (Yamane &

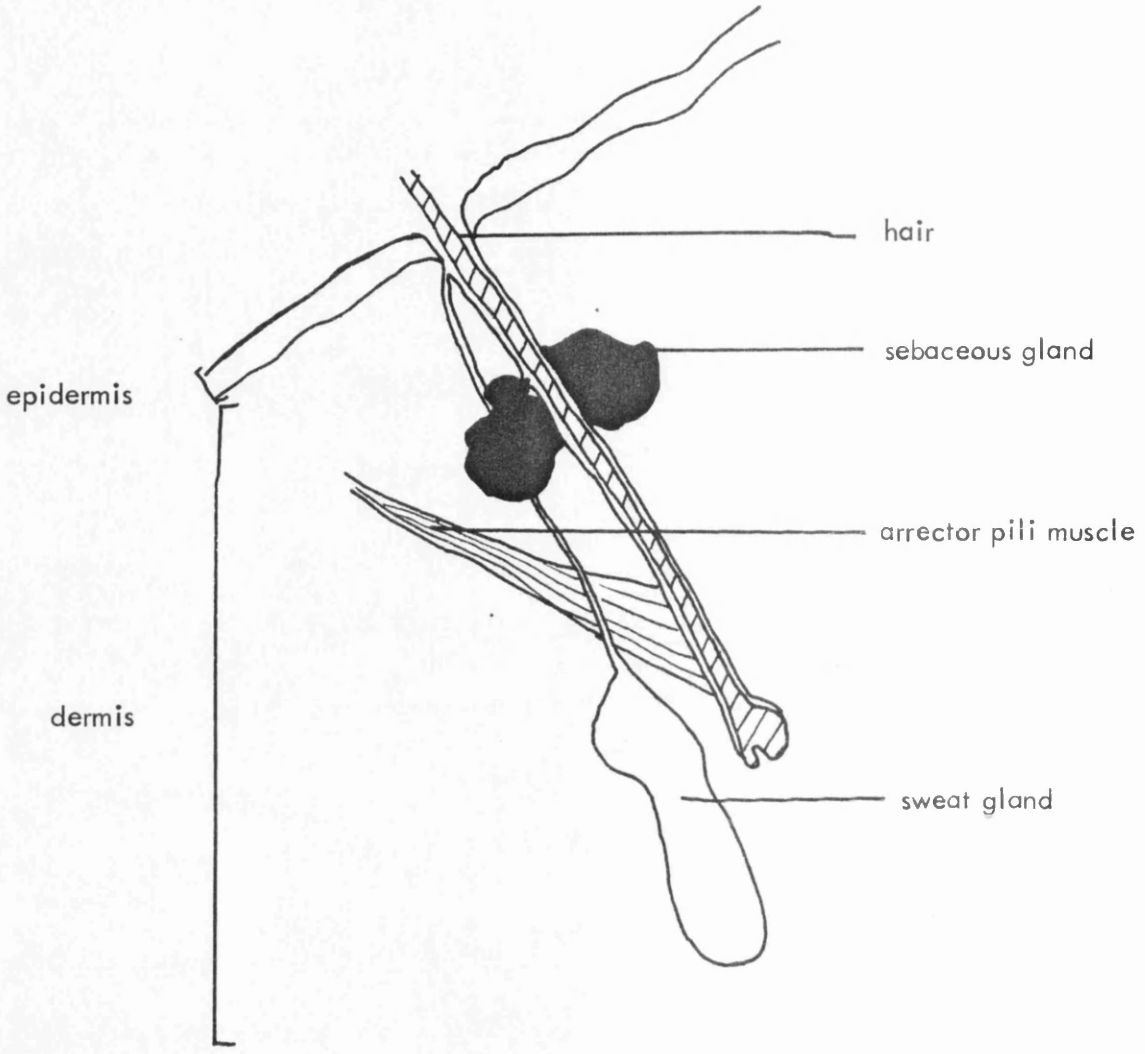

Fig 1.1 Diagram of a bovine hair follicle unit

Ono, 1936; Findlay & Yang, 1950; Carter & Dowling, 1954; Nay, 1959; Jenkinson & Nay, 1972, 1973) are found. Each hair follicle unit consists of a hair follicle, sebaceous gland, sweat gland and arrector pili muscle as shown in Fig. 1.1. These organs are always found together in all breeds of cattle no matter what region of haired skin is examined. It is now accepted that in cattle skin a sebaceous gland is always situated around and a sweat gland proximate to a hair follicle (Findlay & Yang, 1950; Dowling, 1955; Nay, 1959; Walker, 1960; Jenkinson & Nay, 1972, 1973). Both glands open into the hair follicle canal (Fig. 1.1) and consequently their secretions, sweat and sebum, emerge as a mixture on the skin surface. The hairs have a mean angle of slope of 69° 53' (Jenkinson & Nay, 1972, 1973). The hair follicle units, like those in horse and buffalo skin, are apparently situated at random and therefore exhibit no obvious pattern of distribution (Jenkinson, 1965).

The arrector pili muscle, a band of smooth muscle, is responsible for the erection of the hair and thus contributes to the protective function of the coat (Jenkinson, 1965). The mechanism which controls this muscle has not been completely elucidated. However, it seems to have a sympathetic nerve supply since pilo-erection in cattle occurs after intradermal injection of noradrenaline but not after intradermal injection of adrenaline and acetylcholine. Moreover, pilo-erection does not occur in areas of sympathectomized bovine skin (Findlay & Robertshaw, 1965) and the nerve supply to the muscle in such skin is not visible histochemically (Jenkinson,

Sengupta & Blackburn, 1966).

The sweat gland is a tubular gland with a long straight duct. The fundus of this gland is a simple sac or serpentine tube and is lined with an inner secretory epithelium and outer myoepithelium (Jenkinson, 1965). It is now known that sweat contributes about 50% of the animal's heat loss at an environment of 35°C low humidity (McLean & Calvert, 1972). The sweat glands of cattle are, therefore, important thermoregulatory organs (Jenkinson, 1972). The glands not only respond to heat but are reactive to intradermal and intravenous injection of adrenaline and to intradermal injection of noradrenaline, the former being the more potent activator (Findlay & Robertshaw, 1965). High concentrations of adrenaline (10^{-3} g/ml) administered intradermally, however, cause intense vasoconstriction and subsequent glandular degeneration (Findlay & Jenkinson, 1964). Atropine has no effect on the response to adrenaline (Jenkinson, 1969). The glands, however, do not respond to acetylcholine, parasympathiomimetic agents or their inhibitors (Findlay & Robertshaw, 1965). Thermal sweating in cattle is controlled by an adrenergic mechanism requiring an intact sympathetic nerve supply and it seems that adrenal medullary secretion does not contribute to this mechanism under conditions of mild heat stress (Findlay & Robertshaw, 1965). However, on the other hand histological evidence leads to the conclusion that bovine sweat glands are not innervated (Jenkinson et al, 1966). There would appear, therefore, to be a peripheral component in the activating mechanism, the nature of which is still unknown.

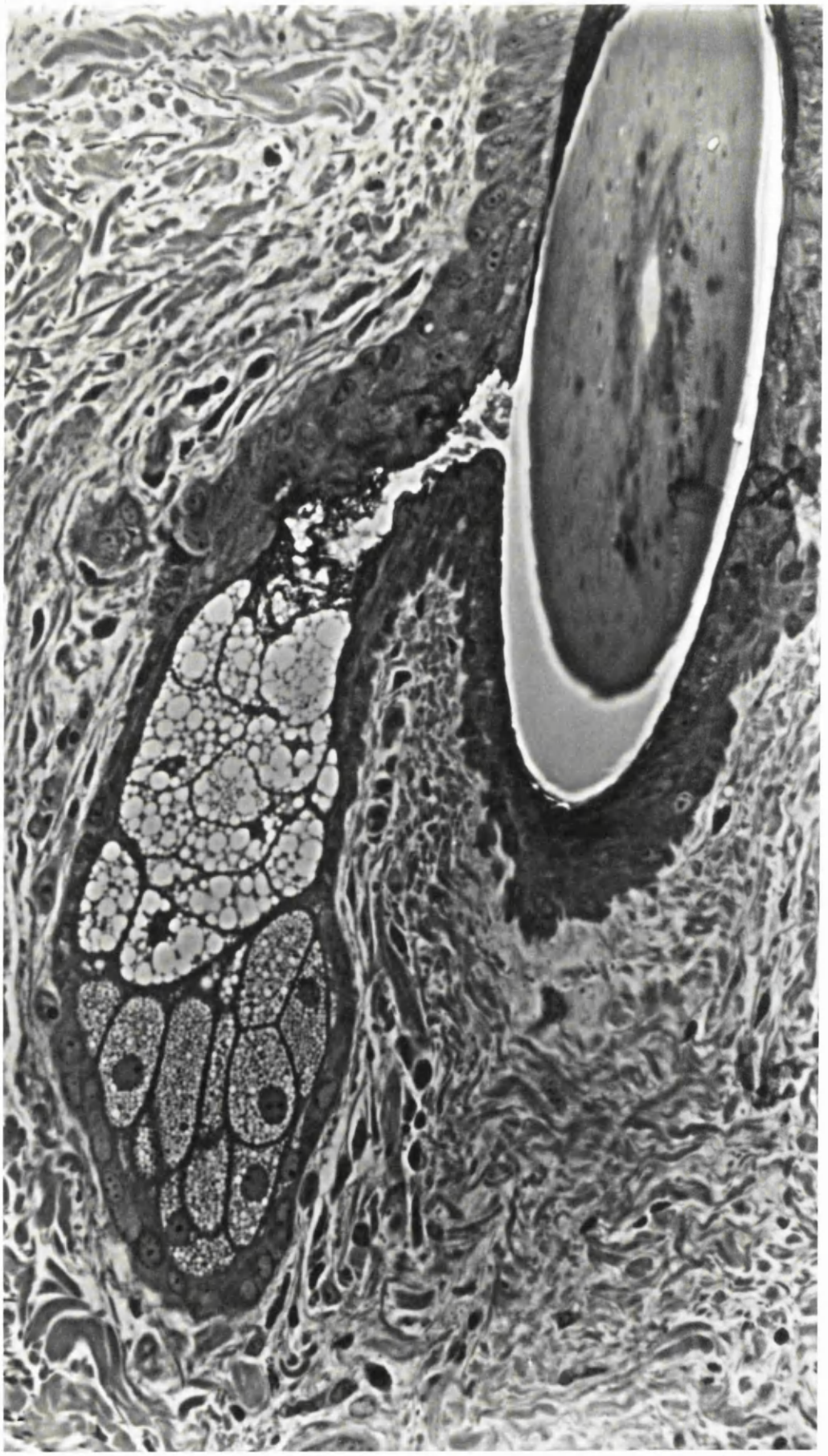

Fig 1.2 Photomicrograph illustrating the structure of sebaceous glands from the skin of the Ayrshire calf

phase contrast, X 3,000

The sebaceous gland is a typical compound acinar gland and consists of 2 pear shaped lobes situated on either side of the hair follicle. The glandular duct opens into the hair follicle canal at a point about one third of the distance from the skin surface to the root of the hair follicle (Yang, 1948). Fig. 1.2 illustrates the histological appearance of bovine sebaceous glands and shows the different stages of cell development which take place within each gland. Each lobe is surrounded by a basement membrane and is covered on its outer surface by a sheath of connective tissue. The inner side of the basement membrane, the stratum germinativum, or basal layer of the gland, is composed of thin flat epithelial cells. These cells progress inwards as they develop and become larger, more rounded and contain lipid droplets. In the skin of cattle, the glands have a copious blood supply which originates from the second of the three plexuses of blood vessels present in cattle skin (Goodall & Yang, 1954) and are innervated mainly by sympathetic fibres (Jenkinson et al, 1966).

In man the sebaceous glands are considered to function as holocrine glands (Rothman, 1955; Strauss & Ebling, 1969). By definition, a holocrine gland is one in which the cells propagate at the periphery by mitosis, develop as they progress inwards, then die and disintegrate to form the secretion (Ranvier, 1887). Since this hypothesis was first postulated it has never been re-investigated. Schiefferdecker (1917, 1922), who classified the sweat glands as being either apocrine or eccrine in function, produced no further evidence to substantiate the holocrine theory; the

original theory has been perpetuated. Schiefferdecker's apocrine and eccrine theory for bovine sweat gland secretion has been since disproved (Jenkinson, 1967). It has been suggested that the potentiality of the sebaceous gland cells to form sebum and the replacement of the cells are independent processes (Rothman, 1955; Ebling, 1963), as in the rat administration of oestradiol decreases the volume of the gland and sebum output but does not appreciably influence the mitotic activity (Ebling & Skinner, 1967); it was suggested that perhaps a mechanism other than mitosis is responsible for producing the sebum. However, the sebaceous gland is still believed to function as a holocrine gland (Strauss & Ebling, 1969; Strauss & Pochi, 1970). No study has been carried out on the mode of secretion of bovine sebaceous glands and hence investigation of their mode of secretion is required.

Although the role of the sebaceous glands in cattle skin is unknown, there is a great deal of information on human and rat sebaceous glands. Much of the work on human sebaceous glands has been concerned with important skin diseases and has been carried out from a clinical point of view.

In man the function of the sebaceous glands is to provide a layer of sebum on the skin surface. The role of the sebum is to form a natural barrier-layer which protects the epidermis against disease, which aids in the thermoregulation of the animal and in the prevention of water loss from the epidermis (Ham, 1965). Sebum was considered to have bacteriological and antifungal function (Rothman, 1955) but these properties have been

refuted (Kligman, 1963). It now seems that in certain instances human sebum may be bacteriocidal to certain strains of organsisms (Aly, Maibach, Shinefield & Strauss, 1972). Moreover, Hellgren & Vincent (1972) considered that in previous studies on human sebum the lipid collected had undergone auto-oxidation thereby destroying its antifungal properties. Further in vitro studies have shown that the substances contained in human sebum are bacteriocidal (Nieman, 1954) and antifungal (Kligman, 1963). In sheep, it is considered that an intact surface film is essential for the protection of the animal against the bacterium Dermatophilus congolensis (Roberts, 1963). In man the skin surface lipid also has an emollient effect (Hsia, 1971) and provides a natural protective barrier-layer which aids in the prevention of undue evaporation from the stratum corneum in cold weather, thus conserving body heat. In hot weather the stratum corneum is oiled by the lipid layer and hence the skin surface is prevented from becoming chapped and cracked when sweat evaporates from it (Ham, 1965). It was originally considered that the skin surface lipid together with the epidermis formed a barrier to water. However, the major barrier to water loss was later found to be the uppermost layers of the stratum corneum and not the lipid film on the skin surface (Blank, 1952). In a recent study the rate of diffusion of tritiated water through skin specimens from hairless mice and man was measured before and after the lipid in the skin specimens was extracted with various lipid solvents (Sweeney & Downing, 1970). Although there was an increase in the permeability of the skin to water

after extraction, there was no direct relationship between the increased permeability and the quantity or type of lipid removed. Instead the increased permeability was dependent on the length of time of exposure of the skin to the solvent and on the solvent used in the experiment. The solvents chloroform and dimethyl sulphoxide were found particularly effective in lowering the barrier to water. It was suggested, therefore, that these solvents altered the complex inter-relationship of water, lipid and mucopolysaccharides between the lipid film and underlying epidermis.

In man the most conspicuous feature of sebum production is its excretion immediately upon removal of the surface lipids. The sebum is secreted from the gland into the hair follicle canal, a possible reservoir, and then excreted upon the skin surface. The process of excretion is initially rapid but progressively slows down as more surface sebum accumulates, finally reaching a minimal rate when the lipid layer has been completely replaced. This means that the curve of replacement rises sharply at first and then reaches a plateau. Therefore if the lipid layer is removed from the skin surface in consecutive short periods of time, the sum of these lipid weights always is much larger than when the accumulating lipids are left untouched on the surface for the same total period of time (Schur & Goldfarb, 1927; Rothman, 1955; Kligman & Shelley, 1958).

The classical theory explaining this sebum curve was first put forward by Schur & Goldfarb (1927) and was supported and elaborated by Emmanuel (1936) and Butcher & Parnell (1947, 1948). It states that the

surface layer of sebum finally reaches an amount sufficient to counteract the excretory force of the gland thus causing it to stop excreting lipid. The gland would, therefore, function periodically in proportion to the amount of sebum lost from the skin surface. In support of this theory it has been claimed that the application of 20% cholesterol in olive oil to the skin surface did to some extent suppress sebum excretion (Kvorning, 1949b). On the other hand, no correlation between sebum viscosity and sebum excretion rate has been found (Burton, 1970). An alternative theory refuting the classical interpretation has been put forward by Kligman & Shelley (1958). This theory postulates that the sebaceous gland secretes continually and the excess sebum is stored in a reservoir, the sebaceous gland duct. The alternative theory is supported by evidence for continual secretion of the sebaceous glands (Kligman & Shelley, 1958), but there is no evidence to show that the excretion rate is unaltered by the surface lipid. Kligman & Shelley (1958) envisage the stratum corneum as a wick which attracts sebum from the follicular reservoir by capillary action. Thus the more frequently the sebum in the capillary reservoir of the stratum corneum is removed, the greater the amount of sebum which will be attracted from the follicular orifice. This hypothetical mechanism is itself a type of feed back control. It is felt (Burton, 1972) that the classical theory should not be too lightly dismissed because of its important cosmetic implications in man.

It is not known in cattle whether the amount of sebum excreted

upon the skin surface forms a curve of replacement similar to that found in man. If there is a similar curve, the time taken to replenish the lipid layer is unknown. The rate of bovine sebum excretion has also not been determined.

Whereas the sweat glands of cattle skin are affected by changes in climate (Findlay & Jenkinson, 1964; Findlay & Robertshaw, 1965), the effect of climate on the sebaceous glands is unknown. In man a greater amount of sebum is present on the skin surface if the air temperature is increased (Dünner, 1946). Moreover, local heating of the human forehead also causes an increase in the amount of sebum collected as well as an increased skin temperature (Cunliffe, Burton & Shuster, 1970; Williams, Cunliffe, Williamson, Forster, Cotterill & Edwards, 1973). It is probable, therefore, that climate might also affect the sebaceous glands in cattle skin. As temperature changes during the year, the different seasons might also affect the sebaceous glands of cattle. It is known that more lipid is present on the skin surface of cattle in winter than in summer (Pan, 1970; Shafie & Abou El-Khair, 1970). As the hair coat in cattle is shed twice yearly in autumn and spring, the greatest hair follicle activity therefore takes place during these two seasons (Dowling & Nay, 1960). The effect of hair follicle activity on bovine sebaceous glands is also unknown.

In the male goat the sebaceous glands of the general body surface increase in size at the beginning of the rutting season (Jenkinson, Blackburn & Proudfoot, 1967). In man, while testosterone and androgens stimulate

sebum production, oestradiol suppresses sebum excretion (Strauss, Kligman & Pochi, 1962). Administration of testosterone to a castrated male rat significantly increases the rate of sebum excretion whereas oestradiol, on the other hand, inhibits sebaceous gland excretion in the castrated male rat (Ebling & Skinner, 1967). Since the sex hormones in other species influence sebum production, it is probable that in cattle they might also affect sebum production.

In man and laboratory animals the activity of the sebaceous gland appears to be controlled by the endocrine system but the relative importance of the individual hormones has not been completely elucidated (Ebling, 1963; Strauss & Pochi, 1963; Strauss & Ebling, 1969; Ebling, 1970a,b; Burton, 1972). In man, sebum excretion increases with age reaching a maximum level at 26 years. In women sebum excretion also increases with age. Before puberty girls have a higher sebum output than boys, but men have a greater output than women (Cotterill, Cunliffe, Williamson & Bulusu, 1972). Hence it is probable that age might have some effect on bovine sebaceous glands.

The lipid composition of the skin surface secretions in man has been investigated both histochemically and biochemically. The histochemical evidence (Nasr, 1965) has been confirmed by biochemical results (Nicolaides, Fu & Rice, 1968) which show that human skin surface lipids contain free fatty acids, triglycerides, sterol esters, saturated hydrocarbons and squalene. The skin surface lipids of cattle seem to contain the same compounds (Nicolaides et al, 1968). Linoleic acid, one of the few essential

fatty acids which is not manufactured by the body, is found in human sebum (Rothman, 1955; Peter, Schröpl, Lipross & Weiss, 1970; Peter, Ritter, Schröpl & Peter, 1971). It is surprising that linoleic acid should be present on the skin surface but since pure linoleic acid is known to possess antibacterial activity (Nieman, 1954) perhaps its presence in sebum is protective. However, the antibacterial properties of sebum have been questioned (Kligman, 1963). Since no detailed study of the sebum of cattle has been undertaken the composition of bovine sebum merits further study. It is also probable that if temperature affects sebum output it might also influence its composition. The viscosity of human sebum is known to decrease with increasing temperature (Burton, 1970).

The present work was, therefore, initiated to investigate the physiology of the sebaceous glands of cattle and to endeavour to elucidate some of the factors which affect their output and mode of activity.

CHAPTER 2

EXPERIMENTAL TECHNIQUES

A. METHODS FOR THE COLLECTION OF SEBUM FROM THE SKIN SURFACE AND THE EXTRACTION OF THE COLLECTED SEBUM

INTRODUCTION

In cattle the sebaceous and sweat gland ducts open into the same pilosebaceous orifice. Sebum and sweat are therefore secreted as a mixture upon the skin surface. The angles at which both gland ducts open into the hair follicles prevent collection of pure sebum or sweat by cannulation. Histochemical evidence has shown that the sebaceous gland and its duct contain lipid unlike the sweat gland and its duct (Yang, 1952) indicating that the sebaceous gland is the main source of the lipid present on the skin surface of cattle. Other potential sources of lipid on the skin surface are the hair, bacteria and the epidermis. The hair can be removed by shaving thus eliminating this possible lipid source. The lipid from the number of bacteria multiplying on the skin surface over a short time interval is most probably insignificant. Finally the amount of lipid derived from epidermal sources is likely to be negligible if the sebum is collected over a short period of time (Emmanuel, 1936, 1938). Hence the amount of sebum produced over a specified time interval may be found by collecting the lipid on the skin surface.

COLLECTION OF SEBUM FROM THE SKIN SURFACE

A number of different techniques for collecting the lipid present on the skin surface have been described for man and laboratory animals. These fall into five main categories:-

1. Immersion of the whole body. The lipid is removed by submerging the whole body of a laboratory animal in a lipid solvent such as diethyl ether and ethanol (50:50 v/v) (Archibald & Shuster, 1970) or acetone (Nikkari, 1965).

2. Extraction of hair lipid. The hair is completely removed from an animal by clipping and is weighed. The lipid on the hair is extracted with diethyl ether and weighed (Ebling & Skinner, 1967). The amount of lipid obtained is expressed as milligrams of lipid per gram of hair. To estimate the amount of sebum produced on the skin surface over a specified time interval, the hair lipid initially present must be removed. This has been achieved by shampooing the animal with sodium lauryl sulphate (Ebling & Skinner, 1967).

3. Scrubbing method. The scrubbing method is based upon the assumption that if a defined area of skin is rubbed with a swab of lipid-free cotton wool or gauze soaked in a lipid solvent all the lipid present on the skin will be removed by the swab. The lipid is subsequently extracted from the swab with the same solvent (Schur & Goldfarb, 1927).

4. Cup method. The cup technique depends on the ability of lipid solvents to absorb the lipid present on the skin surface. A small container

usually made of glass is used. This is placed on the skin and the chosen lipid solvent can then be held in contact with the skin in the container, for a known length of time. The solvents which have been utilised are diethyl ether or a mixture of equal parts of diethyl ether and ethanol (Emmanuel, 1936; Butcher & Parnell, 1948; Jones, Spencer & Sanchez, 1951).

5. Absorption method. The premise of this technique is the capacity of certain papers, for example filter and cigarette papers, to absorb lipid from the skin surface. The paper is applied to the skin surface for a known length of time and the lipid is subsequently extracted from it using a suitable solvent such as diethyl ether (Strauss & Pochi, 1961; Cunliffe & Shuster, 1969).

All the above mentioned methods have disadvantages. The first four techniques use lipid solvents on the skin surface where their effect on the uppermost layers of the epidermis is unknown. In addition, whilst the methods for collecting sebum by immersion of the whole body and the extraction of hair lipid may be suitable for use with laboratory animals, they are unsatisfactory for use with large animalls such as cattle. The scrubbing method requires the initial purification of the cotton to remove inherent lipid and standardisation of the scrubbing action of the swab on the skin. These are difficult practical problems to overcome. The cup technique, on the other hand, has relatively few disadvantages. The solvent used in conjunction with the cup must be kept in a definite area

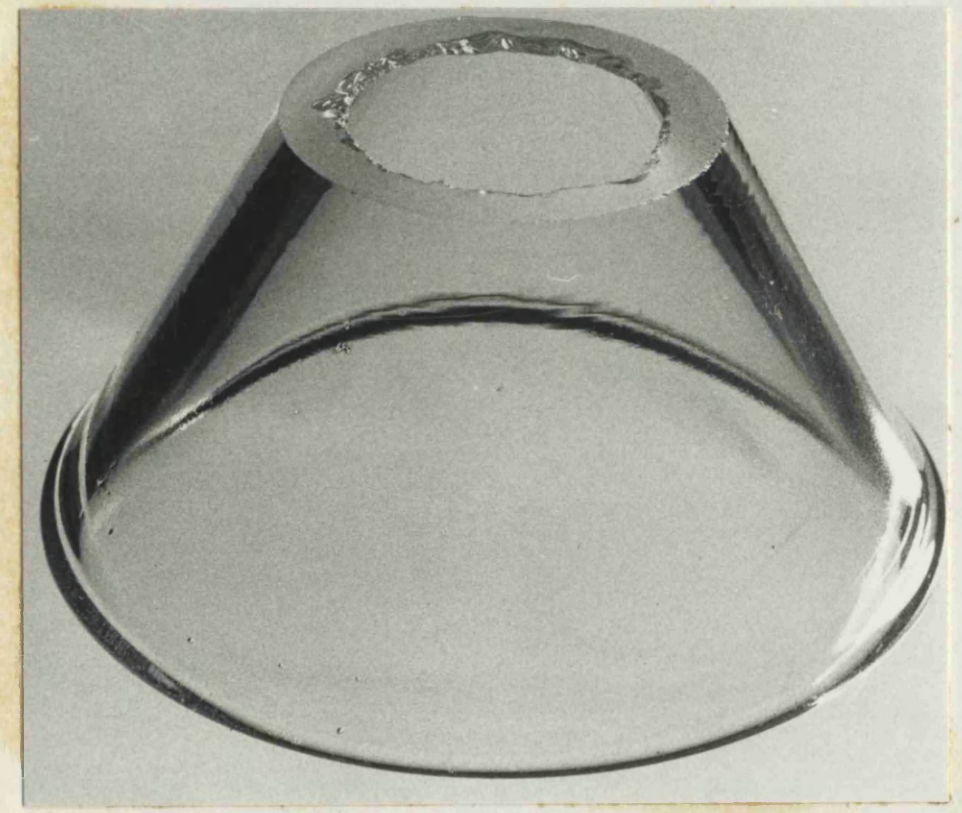

Fig 2.1 The cup used for collecting sebum from the skin surface of cattle with the cup technique.

without loss during application and recovery. In addition the solvent must be thoroughly agitated and thus brought into intimate contact with the skin otherwise all the lipid will not be removed. This technique could be used with ease on cattle skin for the collection of sebum after these technical problems had been overcome.

The limitations of the absorption method are the ability and efficiency of the paper to absorb lipid. For the paper to absorb the lipid, it must be in close contact with the skin surface. This is normally achieved by exerting pressure on the paper. For the total collection of sebum it seems that the pressure exerted is crucial (see p 25). However, unlike the other methods it has the advantage of not requiring the application of lipid solvents to the skin. Further the method could be developed for the collection of sebum from all areas of bovine skin.

Initially two methods were selected from those described above and adapted for the collection of sebum from the skin surface of cattle. These were the cup and absorption method.

The Cup Technique

A specially designed cup 30 sq cm in area was used in conjunction with a solvent (for the solvent selected see pp 20-21). The cup (Fig 2.1) was made from a glass funnel with a 4 mm rim which gave good contact with the skin and prevented loss of the solvent.

Procedure:-

1. At least 24 h before experimentation, an area of skin was shaved with soap and water to exclude the possible collection of hair lipid.

2. On the day of an experiment, the animal was restrained by placing its head between 2 vertical bars and thus contamination of the experimental area by licking was prevented.

3. The shaved area was cleaned by thoroughly washing with the solvent to remove the sebum present on the skin surface.

4. The sebum produced after a given period of time following the cleaning of the skin (usually 3 h) was collected by placing the cup on the pre-cleaned area of skin.

5. 10 ml of solvent were placed in the cup, which was gently agitated for 30 sec.

6. The lipid/solvent mixture was transferred to a glass stoppered sample bottle by means of a Pasteur pipette and stored at 4°C pending extraction of the lipid.

Selection of a Suitable Solvent

The lipid solvents acetone, diethyl ether and chloroform have been used for the collection of sebum on other animals (Nikkarj, 1965; Emmanuel, 1936; Jones et al, 1951; Marchionini, Manz & Huss, 1938). These solvents were found to be unsuitable for use on the skin of cattle since they caused marked visible skin dehydration and erythema. The solvent

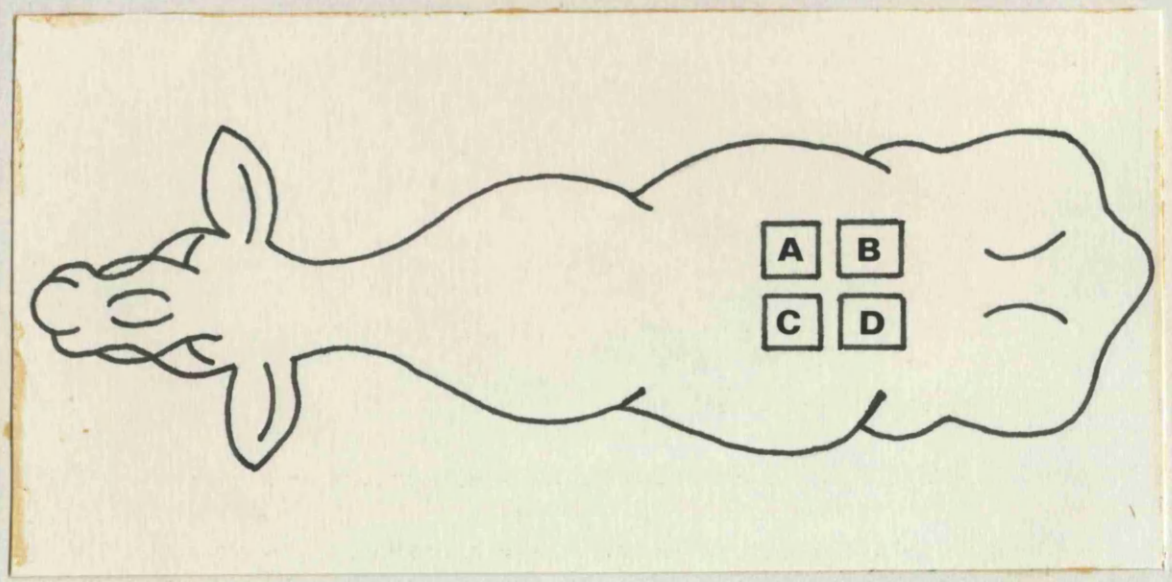

Fig 2.2 Diagram to show the positions of the sample areas on the back of the Ayrshire calf

Table 2.1

A comparison of the solvents methanol, ethanol and butanol (collection taken in each instance after 3 h) on their ability to absorb sebum from the skin surface

Animal	No of Areas	Solvent	Collection	Residue/mg.m^{-2}.h^{-1}.		
				Day 1	Day 2	Day 3
1 & 2	4	Methanol	1	144.28	105.97	139.11
	4		2	108.89	117.89	109.47
3 & 4	4	Methanol	1	129.64	111.78	142.17
	4		2	94.03	137.47	126.97
1 & 2	4	Ethanol	1	338.97	231.45	145.69
	4		2	226.25	277.42	211.17
3 & 4	4	Butanol	1	274.08	146.50	207.70
	4		2	223.36	230.25	163.03

(For original results see Appendix 1 Table 2)

methanol, on the other hand, showed neither of these effects when applied to cattle skin. Furthermore, similar amounts of sebum were obtained (see Appendix I, Table I), from twice daily collections using methanol from 8 areas in the same body region of a calf on 3 consecutive days. This experiment indicated that it was unsuitable to collect sebum from 8 areas of skin owing to the anatomy of the animal. It was, therefore, decided to collect sebum from a maximum of 4 areas in all future experiments. Since methanol is not a good lipid solvent ethanol and n-butanol were also tried, a comparison between these 3 solvents on their ability to absorb sebum from the skin surface was carried out as follows:

Sebum was collected twice per day at 1200 h (1st collection) and 1500 h (2nd collection) for 3 consecutive days from areas A, B, C and D (see Fig 2.2) on four animals 1, 2, 3 and 4 of the same age. Sebum was collected from areas A and B of all the animals using methanol, from areas C and D of animals 1 and 2 using ethanol and from areas C and D of animals 3 and 4 using n-butanol. The sebum was collected and extracted and weighed as described below (p 27).

The mean weights of sebum collected with each solvent are given in Table 2.1. A paired Students 't' test showed that there was no significant difference between the first and second collections except that in one instance (between the residues obtained with methanol from animals 1 and 2) the difference between collections was marginally significant ($P < 0.05$). Although methanol had been used in previous experiments, this was the

only time a significant difference between repeated collections had occurred. The difference was therefore attributed to random error and was assumed to have no biological significance. A 2-way analysis of variance demonstrated no difference between the weight of the sebum collected with methanol over the 3 days, but there was a significant decrease in the amount collected with the solvents ethanol and n-butanol ($P < 0.01$). This suggests that ethanol and n-butanol caused a progressive effect on the skin surface. N-butanol also caused visible dehydration of the skin surface.

Since there was no difference in the amount of sebum collected on each day using methanol and it had no visible ill effects on the skin surface, methanol was selected as being the most suitable solvent to use with the cup for collecting sebum from the skin surface. Methanol was used thereafter.

The effect of the solvent methanol on the skin surface of cattle is unknown. However, it does not cause visible dehydration or marked erythema. The amount of lipid collected from the skin surface of cattle with the solvent methanol in addition to the sebum is also unknown. Emmanuel (1936, 1938) using a similar technique for collecting sebum from human skin, claimed that the amount of lipid derived from epidermal sources would be negligible if collected over a short period of time. Although the validity of this assumption may be questioned, it is probable that as previously discussed most of the lipid collected from the skin of

cattle would be sebum and not epidermal or hair lipid since the hair, the other major lipid source, has been removed by shaving and the skin has been thoroughly cleaned to remove all initial lipid present.

The Absorption Method

The absorption method was adapted from a cigarette paper technique for the collection of sebum from the forehead of man (Strauss & Pochi, 1961).

Procedure:-

1. An area of skin was shaved with soap and water at least 24 h before experimentation.

2. On the day of an experiment the animal was restrained as described earlier.

3. An area of shaved skin was delineated by the attachment of a square of unbleached calico, to the skin with either Dunlop floor tile cement or Eastman 210 adhesive (Kodak Limited). The piece of unbleached calico had an area of 10 sq cm removed from its centre.

4. Removal of the sebum initially present on the delineated 10 cm^2 area of skin was achieved using 2 different sets of 3 diethyl ether washed cigarette papers (cleaning papers) each applied to the skin surface for 30 min and covered in as described in 6 below.

5. Three cigarette papers (measuring papers) which had been pre-washed in diethyl ether were applied to the delineated area of skin for a

known period of time, usually 3h.

6. The papers were covered with a gauze swab and held in position in intimate contact with the skin by two pieces of 4" wide zinc oxide tape.

7. The lipid was subsequently extracted from the measuring papers using diethyl ether.

Comparison of the Absorption and Cup Methods

The methods were compared by collecting sebum from areas of skin on the dorsal surface in the thoracico-lumbar region of an animal using both techniques. Negligible amounts of sebum were collected by the absorption method whereas appreciable amounts (138.1 $mg.h^{-1}.m^{-2}$) were collected by the cup technique. This indicated that either the sebum on the skin surface was not collected by the absorption method or the solvent, methanol, used in the cup technique was stimulating the output of sebum on the skin surface.

To test the latter possibility an experiment was done in which both techniques were used in succession. Sebum was collected from 4 areas on each of 4 calves of similar age using the cup technique. A second collection of sebum was then made using both techniques, i.e. papers were immediately applied for 3 h to 2 of the areas, and the full cup technique including the 3 h time period (after cleaning the skin) was repeated on the other 2 areas. The mean weight of sebum obtained with the first collection using the cup technique was 146.1 $mg.h^{-1}.m^{-2}$ with the second collection using the same

technique 128.3 $mg.h^{-1}.m^{-2}$ and with the absorption method 0.0 $mg.h^{-1}m^{-2}$. There was no significant difference between the first and second collections taken with the cup technique but there was a highly significant difference between the first collection and the absorption method of collection ($P < 0.001$). It can be concluded that the absorption method was not collecting the sebum on the skin surface. Moreover, as similar results were obtained when the experiment was repeated during 3 different seasons, the difference in the amount of sebum collected between the methods was not due to a seasonal effect on sebum output or composition.

The reason for the failure of the absorption method to take up lipid from the skin surface of cattle is unknown. In the original method (Strauss & Pochi, 1961) which was designed for the collection of sebum in man, absorption of the sebum by the paper seems to depend upon intimate contact of the paper with the skin surface. This is achieved by exerting a pressure on the paper by the use of a rubber bandage. In cattle it was impractical to use a rubber bandage and therefore two strips of 4" wide plaster were used instead to provide the necessary pressure. Although every effort was made to achieve close contact between the paper and the skin, it is possible that the contact achieved was insufficient since similar quantities of sebum were obtained after repeated collection from the skin and during the different seasons using the cup technique. The cup method was adopted for the collection of sebum from cattle skin.

Whereas the absorption method would have enabled the collection

of sebum from all areas of the animal, with the selection of the cup technique, sebum could only be reliably collected from the dorsal surface. An investigation to determine if there was a variation in sebum output over the body surface was, therefore, delayed.

EXTRACTION OF THE COLLECTED SEBUM

Since the amount of sebum collected from the skin surface of cattle using the cup technique was small, micromethods were required for its quantitation. The methods previously used by other workers fall into 3 main categories.

1. Micro-titration of free fatty acids. The amount of sebum collected is quantitated by estimating the amount of free fatty acid present in the sebum using a micro potentiometric titration technique (Strauss, Pochi, Masucci & Maithery, 1964). The free fatty acid is only one of the many different lipids in human sebum (Nicolaides et al, 1968). Since all the constituents of sebum are not necessarily produced from the gland at the same rate, this method may not give a reliable estimation of the total sebum output.

2. Oxidation with chromic acid mixtures. The lipid collected is oxidised with chromic acid. The amount of carbon dioxide released is measured on a Van Slyke apparatus thus giving an estimation of the total weight of sebum collected (Kvorning, 1949b). This technique is accurate but time consuming.

3. Gravimetric estimations. Lipid is extracted and weighed on a micro-balance (Strauss & Pochi, 1961; Nikkari, 1965; Ebling & Skinner, 1967; Cunliffe & Shuster, 1969; Archibald & Shuster, 1970). This method gives a rapid, accurate determination of total lipid collected and is simple to execute. Moreover, unlike the other methods described, detailed chemical analysis can be readily performed on the lipid after weighing. This method was therefore adopted for the quantitation of the sebum collected from the skin surface of cattle.

The Extraction Method

Procedure:-

1. The 10 ml of methanol, containing the sebum collected from the skin surface were passed through a filter (triacetate, Metricel Type GA membrane, Gelman Ltd.), pore size 1.2 μm. Debris such as epidermal squames was thus removed.

2. The filtrate was taken to dryness using a rotary-film evaporator.

3. The lipid in the residue was taken up in 3 x 2 ml washings of diethyl ether (Pronalys, May & Baker Ltd.) and transferred to a tared glass weighing bottle.

4. The ether was subsequently evaporated by placing the weighing bottle on a heated block (Tecan Driblock, DBI) at 50°C.

5. Thirty min after removal from the block, the sebum was weighed on an electronic balance (Beckman LMN 800).

Table 2.2

The weights of cholesterol determined by the method of direct weighing and by the test kit

Sample	Weight of residues (mg)	
	From direct weighing	From cholesterol determination
1	1.522	1.415
2	1.499	1.576
3	1.620	1.500
4	1.483	1.576
mean	1.531	1.518

NS Difference between direct weighing and cholesterol determination

Table 2.3

The percentage recovery of the extraction method

Sample	Weight of cholesterol obtained (mg)	% recovery
1	0.879	86
2	1.030	101
3	1.000	98
4	0.895	88
5	1.025	100
mean	0.966	95

It was important to establish that during extraction there was no contamination of the small amounts of sebum collected. This was achieved by undertaking trial extractions using cholesterol which is known to be present in human sebum and bovine sebaceous glands (MacKenna, Wheatley & Wormall, 1952; Yang, 1952). Known volumes of a cholesterol solution were extracted as described and the resulting residues were first weighed and then analysed for their cholesterol content using a test-kit (Boehringer, Mannheim, Germany).

The amounts determined by direct weighing and by the test-kit are shown in Table 2.2. A Students 't' test revealed no significant difference between the two sets which indicated that the residues obtained were solely cholesterol and therefore no contamination had occurred during the extraction.

The percentage recovery of cholesterol using this technique was determined by taking 1 ml aliquots of a cholesterol solution containing 1.022 mg per ml and extracting them by the method described. The weights and percentage of cholesterol recovered are shown in Table 2.3. An acceptable mean recovery of 95% was obtained.

Uniformity of Sebum Production

One of the few body regions which is free from contamination by urine and faeces is the dorsal surface on either side of the midline in the thoracico-lumbar region of the back. The animal can be prevented from

Table 2.4

Weights of sebum ($mg.m^{-2}.h^{-1}$.) obtained from four areas sampled over 3 hr to determine the uniformity of sebum production on the thoracico-lumbar region of the back

Animal	Areas			
	A	B	C	D
1	119.89	139.44	125.22	130.44
2	136.11	121.89	122.22	115.22
3	132.22	122.44	121.11	141.00
4	123.11	144.44	124.11	129.78
5	129.11	144.78	124.67	134.67
6	158.44	201.44	157.00	116.89
7	146.67	125.22	121.11	132.78
8	135.56	130.00	131.89	130.00
mean	135.14	141.21	128.42	128.85

licking this region by placing its head between 2 vertical bars. Furthermore, this is the most practical body region to use for the collection of sebum with the selected method of collection, the cup technique. It was, therefore, important to know whether this site could be regarded as a homogenous area with regard to sebum output. To test this, 8 Ayrshire calves aged between 6 and 10 months were used. Four areas were delineated within an experimental site, 30 x 24 cm, situated in the thoracico-lumbar region of the back on each animal (see Fig 2.2). Sebum was collected from the individual areas on each animal.

The amount of sebum obtained from each area is shown in Table 2.4. An analysis of variance carried out on the weights showed that there was no significant difference in sebum output between area or between animals indicating that sebum output is similar over the experimental site. One collection of sebum taken from within the experimental site could, therefore, be considered as representative of the sebum output from that body region.

The Flow of Sebum on the Skin of Cattle

In man, sebum is considered to flow across the skin surface with extreme facility (Kligman & Shelley, 1958) from areas of high to low secretion (Jones et al, 1951). It is claimed (Jones et al, 1951) that lipid spreads easily over moist skin but not so readily over dry skin. This finding is supported by Herrmann & Prose (1951) who stated that sweat acts as a lipid emulsifier thus aiding the spread of sebum. However, Dvorkin,

Table 2.5

Weights of sebum ($mg.m^{-2}.h^{-1}$.) obtained over a 3 h period to determine whether sebum flows across the skin of cattle

Environment	Animal	Area	
		Taped	Untaped
15°C	1	149.44 128.89	111.00 102.44
	2	99.33 76.33	107.22 98.89
	3	111.11 164.11	134.44 89.11
	mean	121.54	107.18
40°C	1	194.44 148.89	147.78 207.33
	2	164.00 149.44	167.33 184.78
	3	135.00 192.22	137.56 193.11
	mean	164.00	172.98

Maggiora & Jodassohn (1966) found that sebum does not flow from a region of high to one of low sebum concentration. This was true even when the site tested was not isolated. In view of the conflicting evidence a short study was carried out in 2 different environments to see if sebum flows across the skin of cattle.

Sebum was collected from 4 areas A, B, C and D (see Fig 2.2) on 3 animals 6-9 months old at 2 temperatures; 15°C and 40°C. Areas A and B were bounded by zinc oxide tape to prevent sebum flowing in from surrounding unwashed areas which have a relatively high sebum concentration and areas C and D were untaped. The animals were allowed to equilibrate at 15°C for 3 h and then 3 h later sebum was collected from each of the 4 areas. The procedure was repeated at 40°C at a later date. At this temperature calves sweat.

There was no significant difference in the weights of sebum obtained between taped and untaped areas in either environment (Table 2.5). The presence of sweat did not appear to enhance sebum flow on the skin of cattle since sebum did not travel rapidly from an area of high to one of low concentration even when the skin was visibly damp. The present results, therefore, differ from those of Jones et al (1951), Herrmann & Prose (1951) and Kligman & Shelley (1958) but are in agreement with the finding of Dvorkin et al (1966). It was thus considered unnecessary to collect sebum from delineated areas of bovine skin. The results also show that more sebum was collected from the animals in the hotter environment.

SUMMARY

The collection method using a specially designed cup in conjunction with the solvent methanol proved to be satisfactory for collecting sebum from the skin surface of cattle. Although methanol is not a good lipid solvent it was found to be the one most suitable for the collection of sebum from cattle skin. The sebum collected was quantitated by the method of direct weighing and was free from contamination. The method of direct weighing gave a 95% recovery. Since the thoracico-lumbar region of the back was homogeneous with regard to sebum output, it was considered acceptable to collect sebum from undelineated areas within this body region. Moreover, in cattle there was no evidence of a flow of sebum across the skin within the experimental site. Furthermore sebum output seemed to increase with rising temperature.

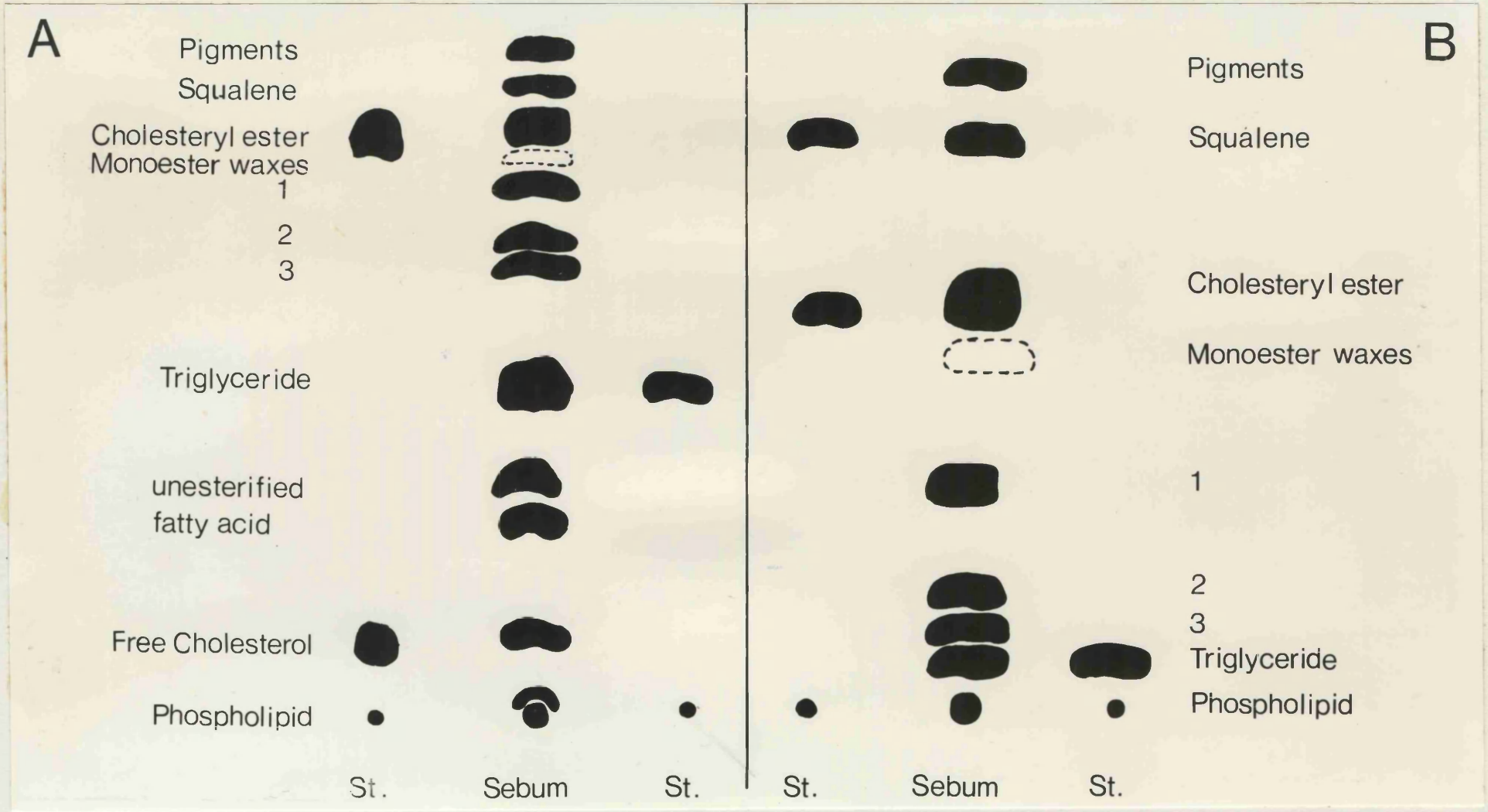

Fig 2.3 The position of the different lipid fractions of bovine sebum on T.L.C. plates. A - using the 3-solvent system of Nicolaides et al (1968); B - using the solvent system of Nikkari & Valaavara (1970). St - known standard.

B. METHODS FOR THE DETERMINATION OF SEBUM COMPOSITION

1. LIPID COMPOSITION OF TOTAL SEBUM

A combined sample of sebum collected from 7 month old Ayrshire cattle was dried under nitrogen on a rotary-film evaporator and dissolved in 5 ml of chloroform/methanol (9:1 v/v) for chemical analysis.

Analysis by Thin Layer Chromatography

The different lipid groups in the sebum were separated by fractionation on thin layer chromatoplates of silica gel G (E. Merk AG, Darmstadt, Germany) using the 3-solvent system of Nicolaides et al (1968) and the single solvent system of Nikkari & Valavaara (1970). The 3-solvent system of Nicolaides et al involves developing the chromatoplates to half plate in a hexane, diethyl ether, acetic acid system (80:20:1 v/v); to full plate in a hexane, ether system (95:5 v/v) and finally in a pure hexane system to full plate. The single solvent system of Nikkari & Valavaara uses benzene and hexane (45:55 v/v). The plates were sprayed with a solution of dichlorofluorescin (0.1 g/100 ml) in a methanol/water mixture (95:5 v/v) and the various major lipid fractions were located and identified under ultraviolet light by reference to standards. The lipid fractions obtained using both systems are shown in Fig 2.3 a and b.

Analysis by Gas-Liquid Chromatography

After identification of the lipid fractions obtained with the 3-

solvent system of Nicolaides et al (see Fig 2.3a) on the chromatoplates, the different bands of silica gel were placed in individual sintered glass funnels and all fractions other than the phospholipids were eluted with 20 ml of diethyl ether; phospholipids were eluted with 20 ml of methanol. Each fraction was taken to dryness under nitrogen and 5 ml of chloroform/ methanol (9:1 v/v) were added to the residue together with an internal standard of heptadecanoic acid. The mixture was again dried under nitrogen and the methyl esters of the fatty acids were prepared by transmethylation using 4 ml of 5% hydrochloric acid in dry methanol + 0.5 ml of benzene (Stoffel, Chu & Ahrens, 1959). Ten ml of hexane and of distilled water were added to the mixture and the hexane layer which contained the methyl esters was removed. The hexane layer was dried over a few grams of drying agent (4:1 Na_2SO_4 / $NaHCO_3$) for at least 1 h. It was then removed and concentrated before injection into a gas-liquid chromatograph (G.L.C.) for analysis of the individual fatty acids. The methyl esters were analysed on a Perkin-Elmer F11 gas chromatograph fitted with dual flame ionization detectors using 2 m glass columns filled with 15% EGSSX on 100 - 120 mesh Gas-chrom P (Perkin-Elmer) (polar columns). The G.L.C. was operating at 175°C and the flow of the carrier gas, nitrogen, was 41 ml/min. The fatty acid peaks; lauric, (12:0), myristic, (14:0), palmitic, (16:0), palmitoleic, (16:1), stearic, (18:0), oleic, (18:1) and linoleic, (18:2) were identified by reference to known standards. Their absolute concentrations were determined by reference to the heptadecanoic acid used as an

Table 2.6

The percentage composition of the individual fatty acids in the different bands obtained with the 3-solvent system when run on a polar and non polar column

Fatty acid	Cholesteryl ester		Band 1		Bands 2 & 3		Triglyceride		Unesterified fatty acid		Phospholipid	
	Polar column	Non Polar column	Polar column	Non Polar column	Polar column	Non Polar column	Polar column	Non Polar column	Polar column	Non Polar column	Polar column	Non Polar column
lauric 12:0	8.5	2.81	14.6	8.6	16.2	12.1	6.5	2.1	5.8	1.8	9.3	1.4
myristic 14:0	35.8	31.8	63.4	72.7	64.2	71.5	29.3	31.1	11.8	7.8	21.1	16.9
palmitic 16:0	28.1	26.7	6.3	8.2	8.7	8.7	33.1	37.3	35.7	39.3	31.2	37.1
palmitoleic 16:1	0.8	1.6	0.6	3.3	1.4	1.9	2.6	1.9	0.8	1.7	5.5	6.0
stearic 18:0	21.8	26.0	14.6	3.0	9.0	4.1	3.7	3.8	24.8	29.8	10.1	14.1
oleic 18:1	4.3	9.1	0.1	1.8	0.3	0.6	2.0	4.5	14.9	13.8	17.5	17.1
linoleic 18:2	0.8	2.1	0.4	3.4	0.2	0.9	22.9	19.4	6.5	6.0	5.3	6.2

internal standard for each fraction (Christie, Noble & Moore, 1970). For the latter purposes either an electronic integrator (Kent Instruments, Luton) or peak area calculation were used. To check the concentrations of the various fatty acids in the different lipid fractions, the procedure was repeated using columns of 10% apiezon L on 100 - 120 mesh Gas-chrom P (non polar columns). Solvents were distilled before use and 2-6-di-t-butyl-p-cresol was added to minimise auto-oxidation.

To determine the concentrations of the fatty acids in the lipid fractions obtained from the single solvent system of Nikkari & Valavaara the above procedure was repeated using columns of 15% EGSSX.

Table 2.6 shows the fatty acid composition of the individual bands separated by the 3-solvent system of Nicolaides et al obtained with the polar and non polar columns. There was in most instances excellent agreement between the two sets of results except for lauric acid. The consistently higher amounts of lauric acid obtained from the polar columns was most likely due to differences in the quantitation by electronic integration. In this instance only the integrator failed to re-set with the sloping base line obtained under the conditions used. In these circumstances the lauric acid would be over estimated. Quantitation of the lauric acid using peak area calculation was also inaccurate. Therefore, as lauric acid, although present in cattle sebum, was not readily quantitated accurately under the G.L.C. conditions used, its absolute concentration was not determined in future studies on cattle sebum.

Table 2.7

Mean percentage composition of the individual fatty acids in the different bands obtained with the one solvent system from a polar column

Fatty acid	Cholesteryl ester	Band 1	Band 2	Band 3	Triglyceride + Unesterified fatty acids + Phospholipid
lauric 12:0	4.3	10.3	18.9	15.3	4.1
myristic 14:0	39.2	61.6	64.3	60.7	21.6
palmitic 16:0	28.9	15.7	9.1	7.7	32.6
palmitoleic 16:1	-	1.3	1.4	1.1	3.2
stearic 18:0	19.3	7.6	4.9	14.5	12.6
oleic 18:1	6.1	2.5	0.33	-	8.6
linoleic 18:2	2.2	1.1	0.9	0.6	15.3

Table 2.7 shows the proportions of the individual fatty acids in the bands obtained from the one solvent system of Nikkari & Valavaara (1970). Bands 1, 2 and 3 had a similar fatty acid composition to each other and to those obtained from the 3-solvent system (Table 2.6).

As good agreement was obtained between the composition of the individual lipid fractions quantitated using different analytical procedures, the 3-solvent system of Nicolaides et al (1968) was chosen for separating the sebum lipid into its different fractions in all future work. The composition of the individual fractions was determined by the G.L.C. method using polar columns.

Purification of the Methyl Esters

As an additional check on the fatty acid composition of the individual bands obtained using the 3-solvent system, the methyl esters from the different bands were purified by fractionation on T.L.C. plates using a solvent system of hexane, ether, acetic acid (80:20:2 v/v). The plates were sprayed with dichlorofluorescin and the methyl esters were located under ultraviolet light at the solvent front. They were eluted from the silica gel with diethyl ether and their concentration in each lipid band was determined by the G.L.C. method described above using columns of 15% EGSSX. In general good agreement was obtained between the results for the purified and non purified methyl esters as shown by the comparison of Table 2.6 with Table 2.8. However, the percentage

Table 2.8

The percentage composition of the purified methyl esters

Fatty Acid	Cholesteryl ester	Band 1	Bands 2 & 3	Triglyceride	Unesterified fatty acid	Phospholipid
lauric 12:0	s a m p l e l o s t	22.34	3.79	7.39	6.03	4.30
myristic 14:0		60.79	56.58	29.61	10.70	13.52
palmitic 16:0		5.83	16.86	32.00	36.64	26.70
palmitoleic 16:1		0.53	2.51	1.70	0.00	5.64
stearic 18:0		6.01	15.58	4.20	24.65	15.36
oleic 18:1		4.50	8.08	1.99	14.85	18.39
linoleic 18:2		0.00	0.00	23.11	7.13	16.10

composition of the fatty acids in fractions I, 2 and 3 were marginally lower after purification. This was due to the removal of background contamination with purification. Purification of the methyl esters prior to analysis by the G.L.C. method was, therefore, considered unnecessary for routine analysis of sebum lipid as accurate results were obtained without purification.

Further Identification of the Triglyceride Fraction

Sebum samples were separated on silica gel chromatoplates in a solvent system of cyclohexane and chloroform (50:50 v/v). The lipid fractions were visualized with iodine vapour and a fraction having the same Rf value as a triglyceride standard was always present. This band was eluted with diethyl ether and the amount of glyceride glycerol present was determined by the technique described by Moore (1962). The glyceride glycerol present is saponified and the liberated glycerol is oxidised to formaldehyde. The chromogen resulting from the reaction of the formaldehyde with chromotopic acid is measured at 570 mμ. Glyceride glycerol was always present in the fraction indicating that it was triglyceride. The amount of triglyceride in the sebum of cattle measured with this method correlated well with the amount quantitated by the G.L.C. method.

Further Identification of Linoleic Acid

Confirmation of the presence of linoleic acid was obtained by running the methyl esters from (a) the whole sebum (b) the neutral lipid fraction and (c) pure sebum triglyceride against a pure sample of linoleic

on T.L.C. plates of silica gel G impregnated with silver nitrate (10% W/W) in a solvent system of diethyl ether, light petroleum and acetic acid (5:95:1 v/v) (Morris, 1966). The band corresponding to linoleic acid in each case was analysed by the G.L.C. method described above using both polar and non polar columns (EGSSX and 10% apiezon L) and the correct retention time for linoleic acid was obtained in each instance. This confirms that linoleic acid is found in the sebum of cattle and demonstrates its presence in the sebum triglyceride.

2. FATTY ACID COMPOSITION OF TOTAL SEBUM

This estimation was carried out on two combined sebum collections. The methyl esters of the fatty acids in the sebum were prepared by trans-methylation of the total sebum as described above for the different lipid fractions. Analysis of the methyl esters was carried out using the G.L.C. fitted with polar columns (EGSSX). The concentrations of myristic, palmitic, palmitoleic, oleic, stearic and linoleic acids were determined by reference to an internal standard of heptadecanoic acid (Christie et al, 1970).

3. ESTIMATION OF THE DEOXYRIBONUCLEIC ACID (DNA) IN BOVINE SKIN WASHINGS

The amount of DNA on the skin surface was determined by the method of Mabon (1974) which is a modification of the technique described

by Kissane & Robins (1958) as adapted by Hinegardner (1971). An estimation of DNA was carried out on a combined sample of two skin washings each taken with 10 ml of 50% methanol from 28 sq cm of shaved cattle skin. The fluorescence produced when 3,5-diaminobenzoic acid reacts with the deoxyribose exposed after removal of the purine bases by hot acid hydrolysis was measured, thus quantitating the amount of DNA present in the sample.

SUMMARY

The 3-stage development system of hexane:diethyl ether:acetic acid (80:20:1 v/v) to half plate, then development with hexane:diethyl ether (95:5 v/v) followed by full development with pure hexane as outlined by Nicolaides et al (1968) was found to be satisfactory for the separation of bovine sebum into its major lipid classes. A G.L.C. method using non purified methyl esters and a polar column was found to be suitable for estimating the composition of the different lipid classes and for determining the amounts of the free fatty acids, myristic, palmitic, palmitoleic, stearic, oleic and linoleic in the total sebum.

Estimations of the triglyceride fraction using the method of Moore (1962) correlated well with the G.L.C. method thus substantiating its presence in the sebum of cattle. The presence of linoleic acid in bovine sebum was confirmed by specialised lipid analysis. The method selected

for estimating DNA on the skin surface of cattle had a recovery of almost 90% and was deemed accurate for the measurement of micro-quantities of DNA (Mabon, 1974).

C. HISTOLOGICAL METHODS

Skin Sampling and Processing Techniques

Skin specimens, 0.183 sq cm in area, were taken from all the Ayrshire calves using the high speed biopsy punch technique of Findlay & Jenkinson (1960). The skin specimens were fixed in Bouin's fluid for at least 24 h, dehydrated, cleared and embedded in paraffin wax. Bouin's fluid, which gave clear cytological detail of the sebaceous glands after staining, was superior to neutral formalin and Carnoy fixatives. Serial sections 8 μm thick were cut perpendicular to the skin surface and stained with Erhlich's haematoxylin and eosin. However, specimens taken for determination of shrinkage due to processing and of sebaceous gland density were cut parallel to the skin surface.

Skin Measurements

The number of sebaceous glands per m^2 was determined from four skin specimens taken from each of 4 Ayrshire calves by counting the total number of hair follicles in each instance. In cattle there is one sebaceous gland associated with each hair (Findlay & Yang, 1950).

Additional skin specimens were taken for the estimation of the dimensions of the hair follicles. The measurements were taken from a population of 50 hair follicles. The parameters measured were the mean depth of the glandular duct from the skin surface, the mean hair diameter at the point where the sebaceous duct enters the hair follicle, and the

mean hair follicle width which was calculated from the measurements taken at 3 different levels between the skin surface and the sebaceous gland ducts.

Sebaceous Gland Volume, Cell Number and Cell Volume

The total area of sebaceous gland in every 7th section throughout the entire skin specimen was traced on the screen of a projection microscope (Projectina Ltd., Skelmorlie) at a magnification of X 280 and subsequently measured by planimetry. A measure of section shrinkage due to fixation and processing was similarly obtained by tracing and measuring the areas of 12 sections from 4 skin specimens obtained from 4 similarly aged Ayrshire calves. The mean section area after processing was 0.144 sq cm and since the specimens were of known area (0.183 sq cm) the mean percentage shrinkage was therefore 21.3%. The volume of sebaceous gland/m^2 of skin was calculated from the areas of sebaceous gland by integration of the results using a programme on an Olivetti desk computer which allowed for shrinkage due to histological processing. The total number of sebaceous gland cells/m^2 of skin was similarly determined from counts of the total number of sebaceous gland cells in every 7th section excluding those cells in the germinative layer at the basement membrane. Mean cell volume was calculated from sebaceous gland volume/m^2 and cell number/m^2.

A comparison was made of the glandular volume and cell number calculated from every section of 5 different blocks of serial sections with

Table 2.9

Volume of sebaceous gland/m^2 of skin obtained with every section and every 7th section

Block	Volume (ml/m^2 skin)		% difference
	Every Section	Every 7th Section	
1	3.90	3.92	+ 0.5
2	27.25	26.67	- 2.1
3	12.43	12.36	- 0.6
4	0.85	0.97	+ 14.1
5	4.07	3.88	- 4.7
mean	9.70	9.56	- 0.1

those determined from using every 7th section in the 5 blocks. The values for glandular volume are shown in Table 2.9 and those for sebaceous cell number in Table 2.10. The discrepancy between using each section and every 7th section was on average 0.1% for glandular volume and 0.0% for cell number. Duplicate measurements were made of cell number and glandular volume using every 7th section of a skin specimen at different times. As shown by the results in Table 2.11 estimations of glandular volume and cell number were within 3 and 2% respectively of each other.

The technique of examining every 7th section for the estimation of sebaceous gland volume and cell number was, therefore, considered an accurate method for showing changes larger than 10% and used in all subsequent determinations.

The Mitotic Activity of the Sebaceous Gland

Two methods for measuring mitotic activity were considered, one was an *in vitro* technique and the other was an *in vivo* method. Both techniques used colchicine which inhibits the mitotic process at the metaphase stage. The *in vitro* technique involved taking a skin specimen by biopsy. The biopsy was incubated at 37°C, in aerated Kreb's solution containing colchicine, for a known time period, normally 4 h. Although varying concentrations of colchicine were used together with different incubation times no colchicine arrested metaphases were observed in the resulting sections cut from the biopsy. An *in vivo*

Table 2.10

Number of cells in the sebaceous glands/m^2 of skin estimated from every section and every 7th section

Block	Number of cells/m^2 of skin		% difference
	Every Section	Every 7th Section	
1	3.7×10^9	3.8×10^9	+ 2.7
2	3.2×10^9	2.9×10^9	- 9.4
3	1.3×10^9	1.3×10^9	0.0
4	3.3×10^9	3.5×10^9	+ 6.1
5	4.2×10^9	4.0×10^9	- 0.5
mean	3.1×10^9	3.1×10^9	0.0

Table 2.11

Repeatability study for the measurements of gland volume and cell number using every 7th section

	1st Estimation	2nd Estimation	% difference
Volume ml/m^2 of skin	3.21×10^4	3.13×10^4	2.6
Cell number/ m^2 of skin	8.8×10^9	9.0×10^9	2.1

method was therefore adopted.

Glandular mitotic activity was determined at a specific time by first injecting 1 ml of colchicine solution in saline intradermally. Two hours later a second injection was administered in the same area and 4 hours after the initial injection a skin specimen was removed from the injection site by biopsy. The second injection was given to ensure that the sebaceous gland cells were affected by colchicine for the entire 4 hours and the volume used (1 ml) ensured that the colchicine permeated an area of skin larger than that of the specimen removed. The biopsy sample was processed for histological examination and the total number of arrested metaphase configurations in all the glands in every section was counted. These were summated and mitotic activity was expressed as the total number of metaphases/m^2 of skin.

Different concentrations of colchicine were tried on Ayrshire calves kept under standard conditions to determine the optimum dose, i.e. the one which would give a maximum number of metaphases. Preliminary investigations showed that using a colchicine concentration of 6 mg/ml more metaphases were present in the gland than with 4 mg/ml. A short experiment was carried out on three 4-month old animals kept under standard conditions to determine the optimum dose of colchicine. In the rat, the mitotic activity of the epidermis and sebaceous glands exhibit a circadian rhythm (Bullough, 1946 and 1948). In an attempt to standardise any possible circadian rhythm in bovine sebaceous glands the animals were fed

Table 2.12

The number of metaphases in the sebaceous glands per m^2 of skin using different levels of colchicine

Animal	Concentration mg/ml	No metaphases/m^2 of skin
1	6	5.57×10^6
2	8	11.75×10^6
3	10	6.28×10^6

at exactly the same time of day for 14 days before the start of the experiment and during it. Each animal was given a different concentration of colchicine in the manner described. The concentrations used were 6 mg/ml; 8 mg/ml; 10 mg/ml. Table 2.12 shows the number of metaphases/ m^2 of skin for each concentration used. Although the concentration of 8 mg/ml gave the highest count, the results provide no evidence to suggest that any of the doses administered was insufficient. The concentration adopted was 8 mg/ml.

Since the in vivo technique involved the use of large doses of colchicine, only one estimation of mitotic activity was possible during each experiment on an animal. Hence control values had to be either obtained from other animals or after a suitable recovery period which was considered to be 14 days.

SUMMARY

Skin specimens taken by biopsy were fixed in Bouin's fluid, cleared, embedded in wax, cut at 8 μ and stained with Erhlich's haematoxylin and eosin for histological examination. Estimations of sebaceous gland volume and cell number using every 7th section were found to be suitable for showing differences larger than 10%. An in vivo technique using two successive 1 ml intradermal injections of colchicine proved to be satisfactory for the determination of the mitotic activity of bovine sebaceous glands.

CHAPTER 3

THE OUTPUT OF SEBUM

INTRODUCTION

In cattle, a film of lipid covers the epidermis (Yang, 1952). In other species the lipid in the film is known to have an emollient effect which prevents scaliness (Hsia, 1971) and can provide an effective barrier against disease (Roberts, 1963). The maintenance of this barrier and thus its effectiveness must depend upon the production of lipid. In cattle, the skin surface lipid (Pan, 1970; Shafie & Abou El-Khair, 1970) if collected over a short period of time is mainly derived from sebum (Chapter 2). The rate of sebum production in cattle and the factors affecting it are, however, unknown.

In man the most conspicuous feature of the process of sebum production is the excretion of sebum immediately upon removal of the surface lipid. The process is at first rapid but progressively slows down as more sebum accumulates and finally reaches a standstill or minimal rate when the lipid layer has been completely replaced (Schur & Goldfarb, 1927). In cattle the time taken for replacement of the sebum layer after its removal is unknown.

In the male goat, the sebaceous glands increase in volume during autumn which suggests a rise in sebum output (Jenkinson et al, 1967). The output of sebum in cattle could be affected by season since more lipid is

found on the skin surface in winter than in summer (Pan, 1970; Shafie & Abou El-Khair, 1970). Both these authors attributed the difference in the quantity of skin surface lipid to a change in the rate of sebum excretion and a consequent change in sebaceous gland activity. Pan (1970) and Shafie & Abou El-Khair (1970), however, did not give rates of sebum output but only cited the quantity of lipid obtained from a given area of the skin surface. As they did not state the duration of the collection period, sebum output, per unit time, cannot be determined from their results and the widely different amounts of sebum found by Pan (4 $g.m^{-2}$) and Shafie & Abou El-Khair (0.03 $g.m^{-2}$) could well be due to differences in the period of collection. The effect of season on sebum output in cattle is therefore unknown.

It is probable that short term exposure to different temperatures might also affect sebum output in cattle since in the experiment on the flow of sebum on the skin surface (Chapter 2) it was found that sebum output was higher at 40°C than at 15°C air temperature. In man sebaceous gland activity is increased at higher air temperatures (Dunner, 1946). Moreover, local heating of the human forehead seems to cause not only a raised skin temperature but also an increase in the amount of sebum collected (Cunliffe et al, 1970; Williams et al, 1973).

Sebum output in man increases with age and reaches a maximum level at 26 years. After puberty the male has a higher sebum output than the female (Cotterill et al, 1972). However, in cattle the effect of age

and sex on the output of sebum is unknown. Pan (1970) found no difference in the amount of lipid obtained from the skin surface when sampling the same Sahiwal and Jersey cattle after an interval of 1 year and concluded that increasing age had no effect on the amount of lipid obtained from the skin surface. Shafie & Abou El-Khair (1970) found that the amount of sebum present on the skin of cattle was not significantly altered by age.

The object of the present work was to determine the time taken for replacement of the skin surface lipid and to investigate the effects of season, climate, age and sex on the rate of sebum excretion of cattle.

METHODS AND RESULTS

Castrated male Ayrshire calves were used in all the experiments. In the experiments on the effect of age and sex (Experiment 5) on sebum output female calves were also utilised. The animals were normally maintained on a standard diet with water ad lib and were housed at a temperature between 18.8°C (Max) and 15.5°C (Min). However, for the experiment on the effect of season on sebum output (Experiment 2), the animals were kept in an unheated cowshed. Sebum was collected from the dorsal surface of the thoracico-lumbar region of each animal, extracted and weighed as described in Chapter 2.

A. Sebum Output With Time (Experiment 1)

Over a period of 3 weeks sebum was collected from 2 areas on each

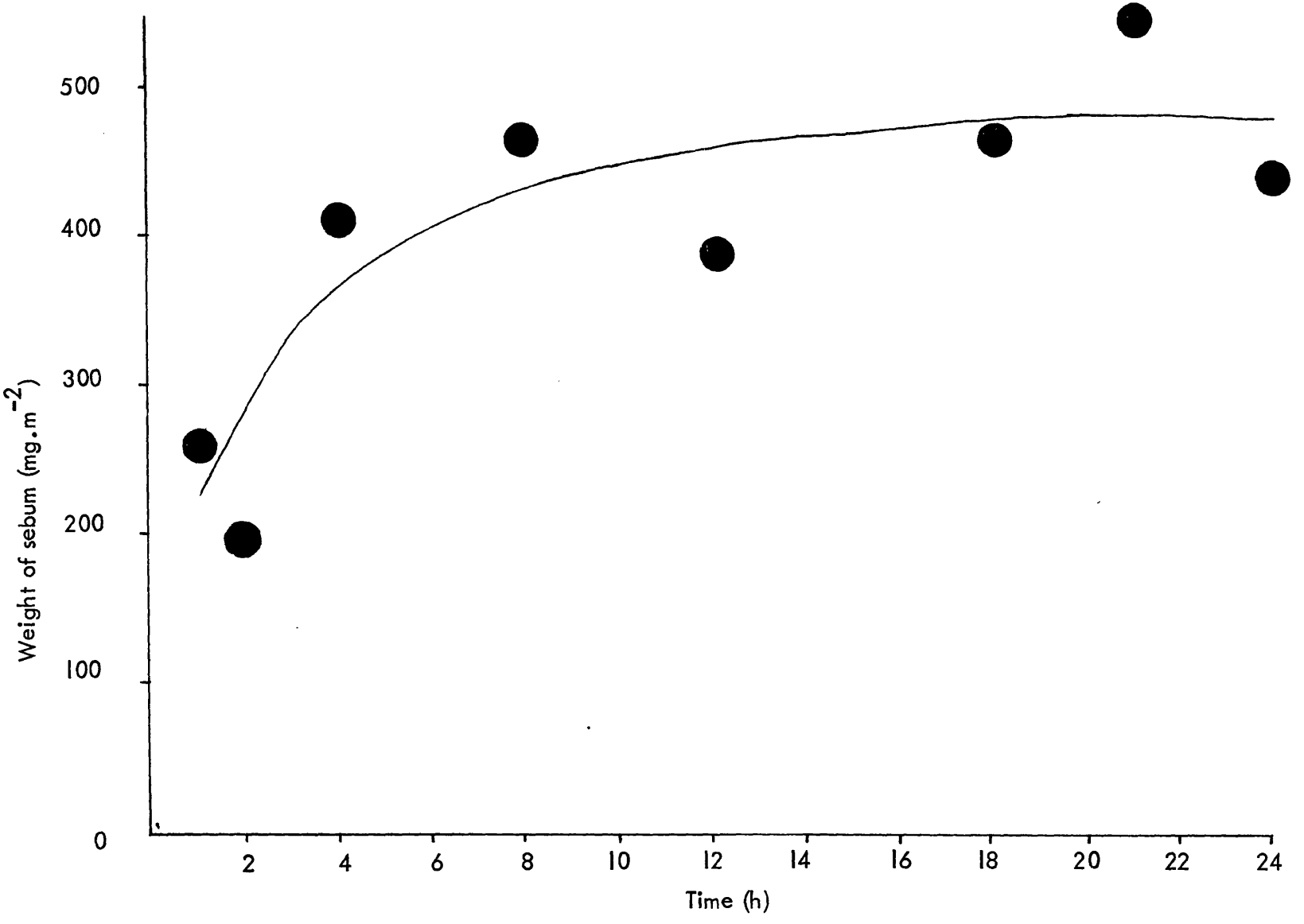

Fig 3.1 The amount of sebum collected in relation to the time after cleaning the skin (Experiment 1). For original results see Appendix 2 Table 1.

of four 8-month old animals. The collections were made at 1, 2, 4, 8, 12, 18, 21 and 24 h after cleaning the skin, in a random sequence. A regression curve was fitted to the results using the mathematical model $y = a + bx + c \log_{10} x$ (Snedecor & Cochran, 1967) where y = the sebum weight and x = time.

The calculated regression (Fig 3.1) over the range of values studied was $y = 228.68 - 4.64x + 264.40 \log_{10} x$ where y = sebum weight ($mg.m^{-2}.h^{-1}$) and x = time (h). The regression was highly significant ($P < 0.001$) and the coefficient of determination (R^2) was 0.61. The exact time of zero gradient, i.e. when no change in the rate of sebum replacement occurs, was calculated by setting the first differential to zero. The predicted value was 24.7 h. It should be noted that interpolation from this model is only valid over the range studied.

B. The Effect of Season on Sebum Output (Experiment 2)

Sebum samples were collected from 4 areas on each of sixteen 4-6 month old calves, in an environment of 20°C dry bulb (DB), 15.3°C wet bulb (WB). Four of the animals were sampled in February (winter), 4 in April (spring), 4 in July (summer) and 4 in October (autumn).

Table 3.1 gives the mean output of sebum found in each season. Sebum output appeared to be substantially lower in February but a nested analysis of variance (Table 3.2) showed that the difference just failed to reach statistical significance at $P < 0.05$.

Table 3.1

The mean outputs of sebum collected over 3 hr ($mg.m^{-2}.h^{-1}$.) at 4 different times during one year (Experiment 2)

Weight of sebum	February (Winter)	April (Spring)	July (Summer)	October (Autumn)
	117.30	150.69	149.06	142.09
S.E. of mean				±16.92

Table 3.2

Analysis of variance of data summarised in Table 3.1

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean squares (MS)	F	P
Seasons	3	11453.71	3817.90	3.33	NS
Animals within seasons	12	13749.09	1145.76	2.15	< 0.05
Residual	48	25558.05	532.46		

NS not significant

(For original results see Appendix 2 Table 2)

Table 3.3

Environments used in Experiment 3

(The Effect of Short-Term Exposure to Different Environments on Sebum Output)

Dry Bulb °C	Wet Bulb °C	Relative Humidity %
15	8.5	40 (Low)
	10.8	60 (Medium)
	13.0	80 (High)
25	16.3	40 (Low)
	19.5	60 (Medium)
	22.4	80 (High)
35	24.0	40 (Low)
	28.2	60 (Medium)
	31.8	80 (High)

C. The Effect of Ambient Temperature and Humidities on Sebum Output

1. Short-term Exposure (Experiment 3)

Eight calves aged between 7 and 13 months were exposed in random order to the environments shown in Table 3.3. Low, medium and high relative humidities were selected at each temperature. The animals were placed in an experimental chamber (Findlay, McLean & Bennet, 1959) and were exposed to each environment for 6 hours. After 3 hours exposure the collection areas were washed with methanol to remove the skin surface lipid and 3 hours later the animals were transferred to an environment of 18.8 ± 2.3°C DB, 14.5 ± 1.7°C WB, to standardise the collection technique. Fifteen minutes later sebum was collected from 4 areas on each animal. The skin temperaturesof the animals were measured at the end of the 6 hours exposure in each environment and again at the time of the sebum collection in the standard environment. A dependatherm electronic thermometer (Kane - May Ltd., Welwyn Garden City) was used.

The mean weights of sebum collected in the different environments are shown in Table 3.4. A 3-way analysis of variance showed that humidity had no effect on the quantity of sebum produced over the $3^{1}/_{4}$ h period. On the other hand, temperature did have a statistically significant effect on sebum output ($P < 0.01$) but this was due solely to an increased output at a particular environment (25°C Medium Humidity).

The same animals were also subjected to 2 further environments (15°C and 40°C, both at 90 - 92% relative humidity). The same procedure

Table 3.4

Mean weights of sebum collected over a $3\,^{1}/_{4}$ h period during short-term exposure to different environments (Experiment 3)

Temperature DB °C	Humidity	Sebum $mg.m^{-2}.h^{-1}$.
15	Low	124.79
	Medium	120.87
	High	100.98
25	Low	109.11
	Medium	145.97
	High	128.59
35	Low	124.67
	Medium	102.45
	High	121.94
S.E. of mean		± 3.80

(For original results see Appendix 2 Table 3)

as described above was adopted for the 15°C environment. The 40°C environment was too extreme for the animals to tolerate a 6 h exposure and a different procedure had to be adopted. The skin was cleaned immediately on entry into the environment and the animals were removed to the standard environment when their rectal temperatures reached 41.9°C or after 3 h exposure. Skin washings were taken after 15 min in the standard environment. Animals 1, 2, 3 and 4 had to be removed from the 40°C environment before expiry of the full 3 h. Sebum output recorded from these animals (see Appendix 2, Table 3) was adjusted by the appropriate time factor. Table 3.5 gives the mean weight of sebum obtained at these 2 environments. A paired Students 't' test demonstrated that there was no appreciable difference between the two sets of results.

The mean skin temperatures of the animals measured at the end of each experimental exposure and under the standard conditions are given in Table 3.6. As indicated by a paired Students 't' test, skin temperature was significantly higher ($P < 0.001$) at the end of 6 h exposure to 25, 35 and 40°C than in the standard conditions 15 min later, with one exception (25°C DB, 19.5°C WB). There was no appreciable change in skin temperature during the 15 min following exposure to 15°C.

In a later experiment (Experiment 7) 4 further animals were also subjected to an identical pattern of low, medium and high humidity environments at 15, 25 and 35°C. This experiment which was designed to investigate environmental effects on sebum composition is described

Table 3.5

Mean weights of sebum collected over a 3 1/4 h period at 2 environments of high humidity (Experiment 3)

Temperature DB °C	Humidity WB °C	Relative Humidity %	Sebum $mg.m^{-2}.h^{-1}$.
15	14.0	90	117.99
40	38.0	92	120.75
S.E. of mean			± 12.19

(For original results see Appendix 2 Table 3)

Table 3.6

Mean skin temperature of animals during short-term exposure to different test environments and 15 min later in the standard environment (Experiment 3)

Test Environment			Skin Temperature °C	
DB °C	WB °C		Test Environment	Standard Environment
15	8.5	(Low)	27.8	28.6
	10.8	(Medium)	28.0	29.4*
	13.0	(High)	28.9	28.6
25	16.4	(Low)	32.3	30.9***
	19.5	(Medium)	31.4	31.1
	22.4	(High)	32.4	30.7***
35	24.0	(Low)	34.0	30.0***
	28.2	(Medium)	35.5	29.3***
	31.8	(High)	35.1	30.4***
15	14.0		28.0	27.9
40	38.6		34.5	32.3***

* $P < 0.05$

*** $P < 0.001$

(For original results see Appendix 2 Table 4)

Table 3.7

Mean weights of sebum collected over a 3 1/4 h period ($mg.m^{-2}.h^{-1}$.) after short-term exposure to different environments

The results are from Experiment 7 which is detailed in Chapter 4

Temperature DB °C	Humidity	Sebum
15	Low	152.77
	Medium	136.61
	High	112.10
25	Low	149.16
	Medium	130.41
	High	124.50
35	Low	139.98
	Medium	104.13
	High	98.15
S.E. of mean		± 4.54

(For original results see Appendix 3 Table 1)

fully in Chapter 4. So far as sebum output is concerned the procedure was identical to that used in Experiment 3 except that skin washings for the collection of sebum were made with the animals still in the test environment. Skin temperatures were not measured. The results of Experiment 7 are summarised in Table 3.7. A 3-way analysis of variance showed that temperature and humidity had a significant effect on sebum output ($P < 0.001$); sebum output was less at 35°C than at the other 2 temperatures and also at the lowest humidity in each instance.

2. Long Term Exposure (Experiment 4)

The four oldest calves used in the previous experiment were kept in an environment of 22°C DB, 12.5°C WB for 3 days followed by 9 days at 35°C DB, 24°C WB. Sebum was collected each day from 4 areas on each animal $3^{1}/_{4}$ h after cleaning the skin.

The mean outputs of sebum (with standard errors) collected over a period of $3^{1}/_{4}$ h are illustrated in Fig 3.2 by closed circles. As demonstrated by an analysis of variance (Table 3.8) sebum output in the control environment was significantly lower than in the warm condition ($P < 0.01$). Sebum output did not vary appreciably during days 1 - 3 but varied significantly within the period at the warmer environment. The outputs were significantly higher on days 6 and 9.

The results obtained on day 4 were compared with those obtained from the same animals (Nos 5, 6, 7 and 8) after the short term exposure to the same environment (Experiment 3) since, in that experiment sebum

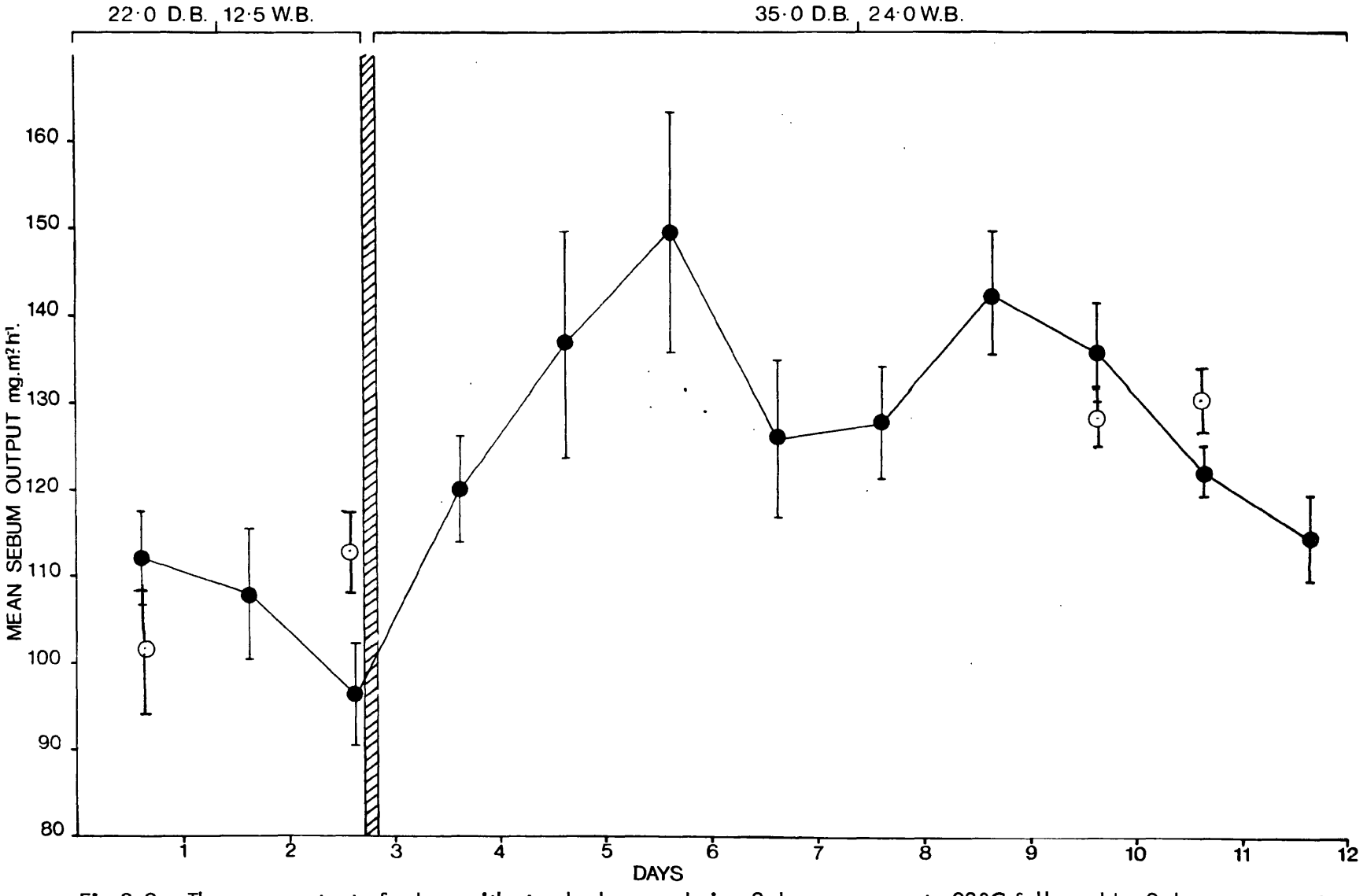

Fig 3.2 The mean output of sebum with standard errors during 3 days exposure to 22°C followed by 9 days exposure to 35°C air temperature. ● Results of Experiment 4. ○ Results of Experiment 8 (detailed in chapter 4). (For original results see Appendix 2 Table 5 and Appendix 3 Table 9).

Table 3.8

The results of an analysis of variance of the sebum outputs illustrated in Fig 3.2

	df	SS	MS	F
Animals	3	498.46	166.15	32.90***
Days	11	380.88	34.62	6.86***
(1 to 3) v. (4 to 12)	1	208.35	208.35	41.26***
within 1 to 3	2	23.63	11.98	2.37 NS
within 4 to 12	8	149.01	18.57	3.68***
Animals x Days	33	225.71	6.81	1.35 NS
Error	144	742.97	5.05	
Total	191	1848.02		

NS not significant; *** $P < 0.001$

(For original results see Appendix 2 Table 5)

collections were taken over the same period of exposure but after the animals had been returned to a cooler environment. A Students 't' test showed that there was no significant difference between the two sets of results even though there was a difference in the collecting environment.

In a later experiment (Experiment 8), 4 different animals were exposed to the same 2 environments as described above for a similar length of time. This experiment which was designed to investigate the effect of prolonged exposure to a warmer environment on sebum composition is described fully in Chapter 4. So far as sebum output is concerned the procedure was the same except that skin washings for the collection of sebum were taken on days 1 and 3 and days 10 and 11. The mean weights of sebum (with standard errors) in the 2 environments are illustrated in Fig 3.2 as open circles. As shown by a 2-way analysis of variance there was a significantly higher sebum output after prolonged exposure to the warm environment ($P < 0.001$) than in the control environment.

D. The Effect of Age and Sex on Sebum Output (Experiment 5)

Sebum was collected from 4 areas on each of 16 castrated male calves. The animals were grouped in fours according to age as follows:- Group 1, 4 months; Group 2, 7 months; Group 3, 14 months and Group 4, 18 months. In the same season 4 areas were similarly sampled on 16 intact female calves which were grouped in fours into the equivalent age groups. At the same time 2 further 18 month old castrated male calves were sampled.

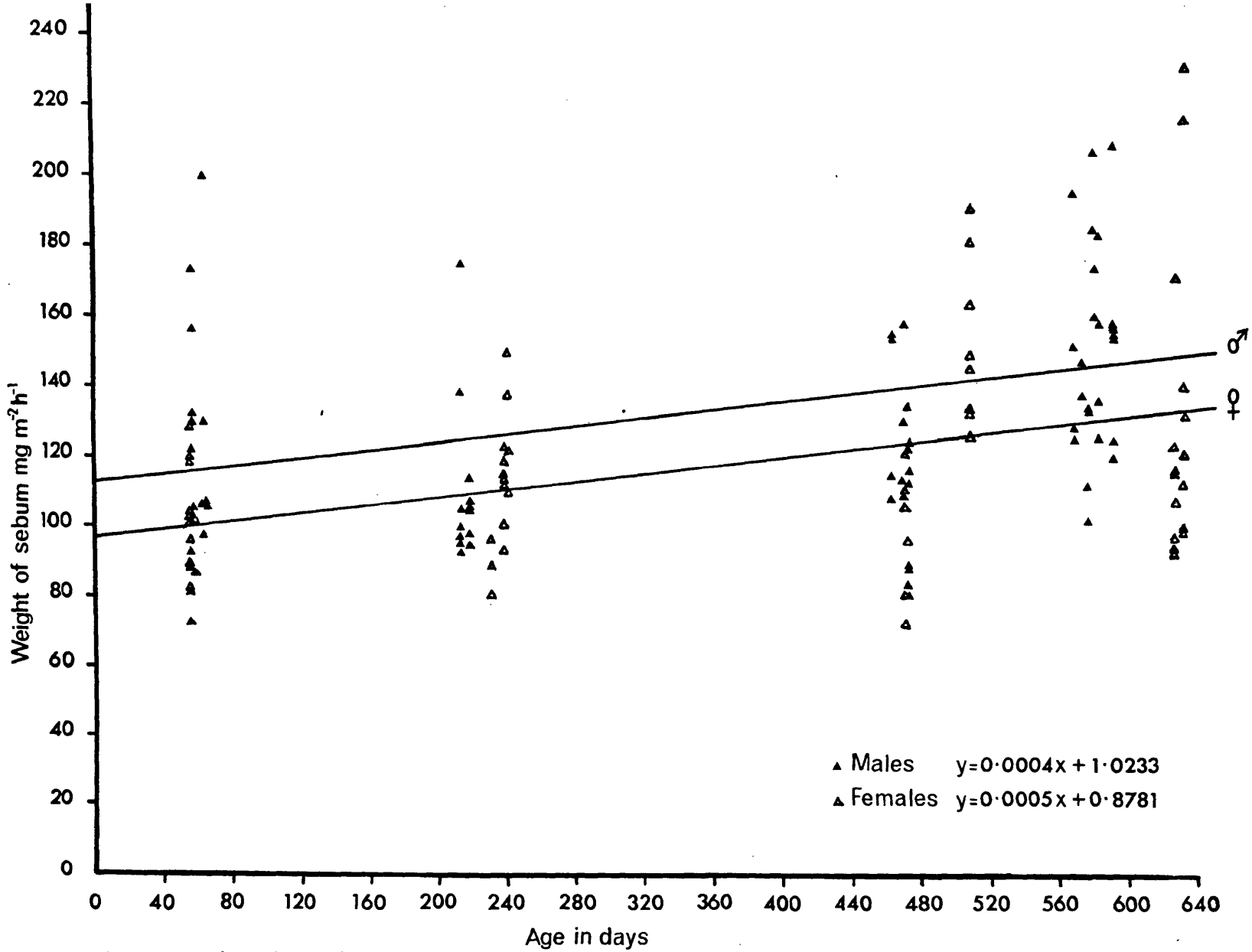

Fig 3.3 The relationship of sebum output measured over a 3 h period with age - Results of Experiment 5 (For original results see Appendix 2 Tables 6 and 7).

The relationships between sebum output and age in both castrated male and female calves are illustrated in Fig 3.3. F-tests showed that neither slope was significantly different from zero, i.e. that there was no evidence of a relationship between the 3 h output of sebum and age. The correlation coefficient between age and sebum weight was 0.33 for castrated males and 0.42 for females. The variation in the weight of sebum collected was, however, high for both sexes. A Students 't' test indicated that the output of sebum was higher in the castrates than in the females ($P < 0.05$).

The calves used in Experiment 3 were of 2 age groups, 7 months and 13 months. The mean weights of sebum obtained from the animals of different age groups in Experiment 3 are shown in Table 3.9. There was a tendency for the older group to have a higher sebum output but the difference was not statistically significant.

DISCUSSION

The relationship of sebum replacement with time after removal of the sebum from the skin surface was similar in general pattern to that found in man (Rothman, 1955). This suggests that the mode of secretion of the sebaceous gland is the same in both species. The amount of sebum on the skin surface increased markedly during the first 8 h. However, although sebum weight continued to increase until 24.7 h, there was very little increase after 18 h. The skin, therefore, should always be prepared at

Table 3.9

Mean weight of sebum collected over a $3\frac{1}{4}$ h period from each animal in Experiment 3

Animal	Weight of Sebum ($mg.m^{-2}.h^{-1}$.)	Age (Months)
1	120.99	7
2	108.24	
3	102.64	
4	116.37	
mean	112.06	
5	109.78	13
6	134.62	
7	139.56	
8	113.08	
mean	124.26	
S.E. of difference ±24.11		

(For original results see Appendix 2 Table 3)

least 18 h before making estimations of sebum output to allow time for complete replacement of the lipid layer. The output at zero time could not be measured in practice or be predicted accurately from the regression equation since the error of weighing the small amounts of sebum obtained immediately after cleaning the skin was too high.

The output of sebum from calves appeared to be reduced in winter but this reduction just failed to be significant. A reduction in output might be expected due to the influence of the winter temperatures as it has been found in man that low external temperatures reduce glandular activity (Dunner, 1946). The peaks of hair follicle growth in cattle which occur in spring and autumn (Dowling & Nay, 1960), apparently had no effect on sebum output. Significantly higher amounts of lipid have been measured on the skin of cattle during the winter (Pan, 1970; Shafie & Abou El-Khair, 1970). However, this increased amount of sebum could have resulted from greater retention of sebum on the skin surface and these results, therefore, do not necessarily conflict with the current findings on sebum output.

The results from the experiments on the effect of short term exposure to warm environments on sebum output are difficult to interpret. In the experiments of Chapter 2, sebum output was higher at warm temperatures than at low temperatures. This might have been due either to a real difference in sebum output at the two temperatures or to a difference resultings solely from the collection procedure being performed at 2 different temperatures. To eliminate the latter possibility, Experiment 3,

described in this Chapter was designed with the collection of sebum from the skin surface in a standard environment. This experiment showed no effect on sebum output due to temperature or humidity except for a higher sebum output at the intermediate temperature used. Skin temperature in the test environment increased with rising temperature but not with humidity. However, skin temperatures measured later in the standard environment where collections of sebum were made, had returned more nearly to a uniform level. This might be taken as evidence suggesting that skin temperature is an important factor in determining the amount of sebum collected from the skin. On the other hand, there was no difference between the sebum output measured on the first day at 35°C (low humidity) of long term exposure (Experiment 4) and that measured in the standard environment when skin temperatures had fallen after exposure to 35°C (low humidity) (Experiment 3). This is the only comparison available between measurements made on the same animals subjected to the same environment with only the collection conditions altered. Finally, in the experiment to test short term temperature and humidity effects on sebum composition (Experiment 7, described in Chapter 4) where collections of sebum were made in the test environment, it was found that both temperature and humidity appeared to exhibit significant effects on sebum output. Sebum output was reduced at high temperatures and also at high humidities, which was contrary to the findings of the experiment in Chapter 2 and to Experiment 3. It may, therefore, be concluded that

short term exposure to warm environments seems to have no definite effect on sebum output. Similarly, it can also be concluded that skin temperature seems to have no effect on the amount of sebum collected using the technique described in Chapter 2. In man a 1°C rise in skin temperature caused a 10% increase in sebum output (Cunliffe et al, 1970; Williams et al, 1973). However, in both instances, the increase in sebum output could be attributed to a change in the viscosity of the different sebum components and not necessarily to an actual rise in sebum output since the method of collection used was found to be temperature dependent (Cunliffe et al, 1970). The viscosity of human sebum decreases with increasing temperature (Burton, 1970). It could, therefore, be concluded that in man skin temperature has little effect on sebum output. Thus the finding in man could be considered to agree with that found in cattle.

The present investigations clearly show that prolonged exposure to a warmer environment (Experiment 4) in contrast to short term exposure to warm environments (Experiment 3), increased the output of sebum. The sebum outputs on the third and sixth day of exposure to the heat were significantly different from the other sebum weights obtained in the same environment. The reason for the higher outputs on the third and sixth days is unknown but it is unlikely that they have any biological significance as they seem to be randomly distributed. The result of Experiment 4 was confirmed by a similar rise in sebum output in Experiment

8, the effect of prolonged exposure to a warmer environment on sebum composition. The increase in sebum output was in agreement with the trend shown by the results from Experiment 2, the effect of season on sebum output, and with evidence on sebum output in man (Dunner, 1946).

In man and the rat testosterone potentiates sebum production (Strauss et al, 1962; Ebling & Skinner, 1967) whereas it has been shown in the rat that oestradiol suppresses sebum output (Ebling & Skinner, 1967). In the present study castrated male calves had a higher sebum output than females although generalisations could not be made from this finding since the variation between animals in the small samples was high. The reason for the lower output in the female calves compared with the castrated calves was unknown but it may have been a result of the higher blood oestrogen level in the female. Sebum excretion rate is greater in men than in women (Cotterill et al, 1972). An even bigger difference in sebum output might exist between intact male calves and females in view of the effect which testosterone has on sebum production in other species.

There was no significant increase in sebum output with age in either sex. This finding was in agreement with the work on the quantity of the skin surface lipid of Egyptian, Shorthorn and Friesian cattle (Shafie & Abou El-Khair, 1970) and Jersey and Sahiwal cattle (Pan, 1970). It was also confirmed by the results of Experiment 3, which indicated no significant difference between the sebum outputs for 7 month and 13 month old castrated male Ayrshire calves. It was, however, contrary to evidence

from man where sebum output increased with age reaching a maximum level at 26 years (Cotterill et al, 1972). In women, sebum output is higher after the menarch (Cotterill et al, 1972) and it was surprising, therefore, that there was no significant effect of age on the output from the female calves since the oldest were sexually mature.

In conclusion, therefore, the sebum layer when removed experimentally takes over 24 h to be replaced. A lower sebum output was observed in winter than throughout the rest of the year but the difference was not significant. Although short term changes in the environment had no consistent effect on sebum output, the output was significantly raised on prolonged exposure to a warmer environment. Sebum output was influenced by sex as castrated male Ayrshire calves had a higher output than female calves but it was unaffected by age.

CHAPTER 4

THE LIPID COMPOSITION OF SEBUM

INTRODUCTION

In sheep an intact lipid film on the surface of the skin is important in providing a barrier against infection (Roberts, 1963). This film is a complex material (Rothman, 1955) and in man it is reputed to have antibacterial and antifungal properties (Kligman, 1963). Although these properties have not yet been fully substantiated (Kligman, 1963), linoleic acid, an essential fatty acid with antibacterial properties (Nieman, 1954), is found in human sebum (Rothman, 1955; Peter et al, 1970). It is not known if linoleic acid is present in the skin surface lipid of cattle even though the composition of cow skin surface lipids have been investigated (Nicolaides et al, 1968). The lipid in skin washings collected from cattle over a short period of time may be considered to be mainly derived from sebum since, apart from potential contribution from the epidermis, hair and bacteria which are likely to be small, the sebaceous glands are the only source of the lipid (Yang, 1952). Furthermore, it has been shown histochemically in man that apart from the presence of free cholesterol on the skin, the composition of the skin surface lipid is very similar to that of sebum in the sebaceous gland (Nasr, 1965).

Various factors are known to affect the composition of sebum.

Season affects the composition of some of the skin surface lipids in the male goat (Jenkinson et al, 1967). Age, sex and hypophysectomy influence the sebum composition of the rat (Nikkari & Valavaara, 1970). Although sebum output in cattle increased on prolonged exposure to a warmer environment (Chapter 3), the effect of environment on the integrity and composition of the lipid film on the skin surface is unknown. In this chapter, therefore, an attempt has been made:-

(1) to study the lipid composition of sebum collected from the skin surface.

(2) to determine the effects of environmental temperature and humidity on the composition of bovine sebum.

METHODS AND RESULTS

Eight Ayrshire steers aged 7 - 10 months were housed at ambient temperatures between 15 and 20°C (Max/Min). Each animal received a standard complete diet and water was available ad libitum. Sebum was collected, extracted and weighed by the standard method described in Chapter 2.

A. Sebum Composition (Experiment 6)

The major lipid classes in combined sebum collections from four 7-month old Ayrshire steers were separated on thin layer chromatoplates

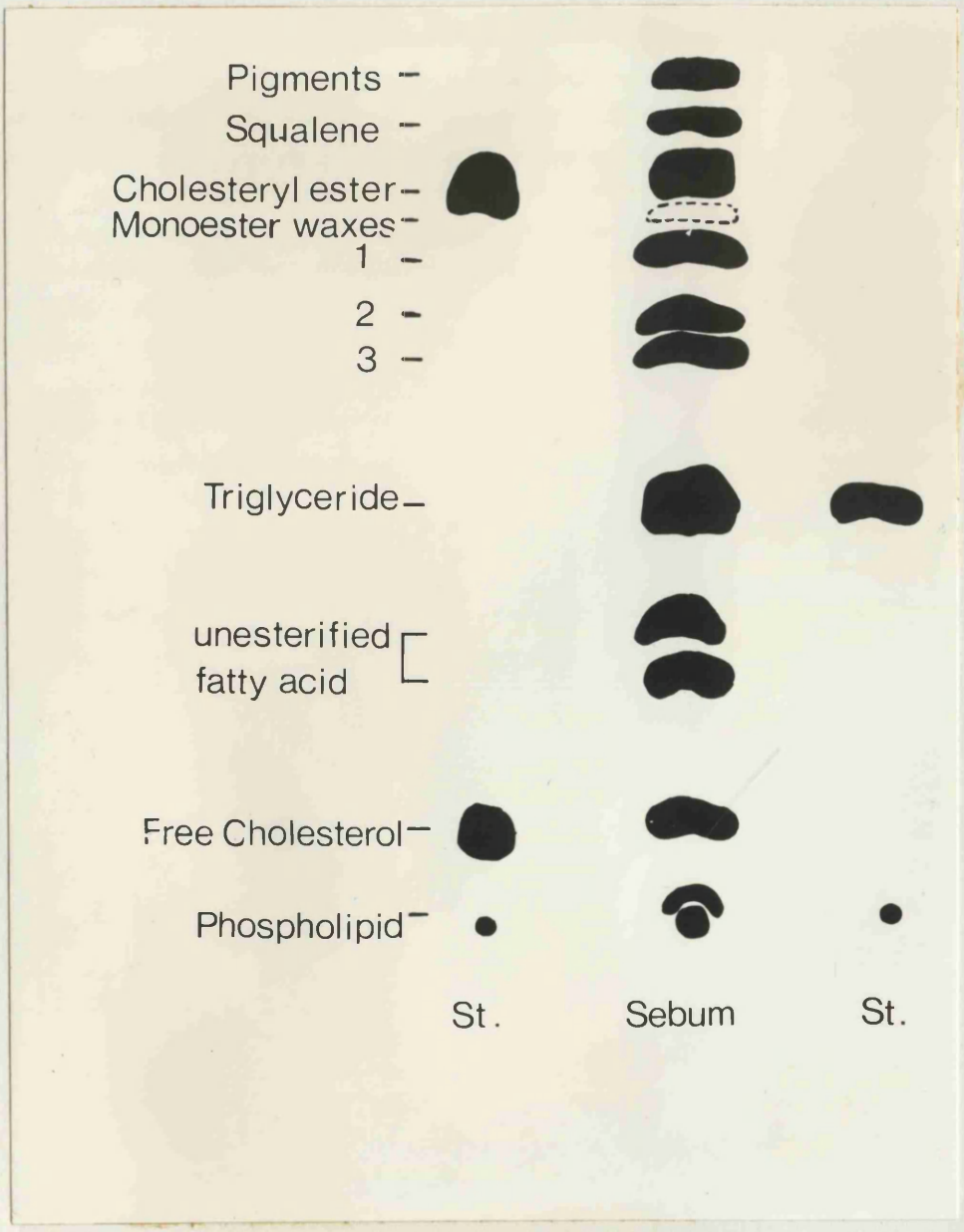

Fig 4.1 The distribution of the major lipid components of bovine sebum on a T.L.C. plate as shown by the 3-solvent system of Nicolaides et al (1968).

St - known standard

using the 3-solvent system of Nicolaides et al (1968). Each class was identified by reference to a standard and its composition determined by a gas liquid chromatographic method (Chapter 2).

The major lipid components of bovine sebum are shown in Fig 4.1. Sebum contains squalene, cholesteryl esters, triglyceride, unesterified fatty acids, free cholesterol and phospholipid as well as 3 other compounds, bands 1, 2 and 3, which were not specifically identified. Using heptadecanoic as an internal standard the percentage of each of the lipid fractions in the total sebum (expressed as percentages of the total fatty acid concentration) was: cholesteryl ester 5.9; band 1, 9.6; bands 2 and 3, 25.3; triglycerides, 27.4; unesterified fatty acids, 24.5; phospholipid, 7.3. The fatty acid composition of these fractions is shown in Table 4.1. Most of the linoleic acid in the sebum was found in the triglyceride fraction.

B. The Effects of Temperature and Humidity on Sebum Composition

1. Short-term Exposure (Experiment 7)

Four 10 month old Ayrshire steers were exposed in random order to the same 9 environmental conditions used in the preceding chapter for the determination of the effect of short term exposure of temperature and humidity on sebum output (Experiment 4) (see Table 3.3). Three hours after entering each environment the surface of the shaved skin was washed with methanol and $3^{1}/_{4}$ h later sebum was collected from 4 areas

Table 4.1

The percentage composition of the individual fatty acids in the different lipid bands of bovine sebum obtained with the 3-solvent system (Experiment 6)

Fatty acid	Cholesteryl ester	Band 1	Bands 2 & 3	Triglyceride	Unesterified fatty acid	Phospholipid
Lauric 12:0	8.5	14.6	16.2	6.5	5.8	9.3
Myristic 14:0	35.8	63.4	64.2	29.3	11.5	21.1
Palmitic 16:0	28.1	6.3	8.7	33.1	35.7	31.2
Palmitoleic 16:1	0.8	0.6	1.4	2.6	0.8	5.5
Stearic 18:0	21.8	14.6	9.0	3.7	24.8	10.1
Oleic 18:1	4.3	0.1	0.3	2.0	14.9	17.5
Linoleic 18:2	0.8	0.4	0.2	22.9	6.5	5.3

on each animal. The sebum collections were taken in the experimental environment and skin temperature was not measured. The sebum extracted from each collection was weighed and the lipid obtained from 2 collections was combined to give 2 lipid samples per animal. The amounts of myristic, palmitic, palmitoleic, stearic, oleic, linoleic acids in each sebum sample were estimated by the gas liquid chromatographic method (Chapter 2).

The results for sebum output (Table 3.7) are given and discussed in Chapter 3. The mean weights of the different fatty acids in the sebum obtained over a $3^{1}/_{4}$ h period at each of the environments are given in Table 4.2a. A 3-way analysis of variance demonstrated that the output of the individual fatty acids studied showed little change with either temperature or humidity. The only change with temperature was in the output of linoleic acid ($P < 0.01$) which was significantly higher at 25°C than at 15°C or 35°C. This trend was confirmed by a similar rise in the mean percentage of linoleic acid (Table 4.2b). The output of palmitic acid was significantly affected by humidity ($P < 0.01$). In general there was a greater output of the acid at the lowest humidity than at the medium and high humidities. The mean percentage of palmitic acid (Table 4.2b) indicated a similar trend.

2. Long-term Exposure (Experiment 8)

Two months later, the same four steers, used in the previous experimental study (Experiment 7), were placed in an environment of

Table 4.2a

Mean weights of the individual fatty acids in the sebum collected over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.) at different air temperatures and humidities (Experiment 7)

Temperature DB °C	Humidity	Myristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
15	Low	14.54	10.02	0.42	2.20	1.49	3.47
	Medium	11.14	6.30	0.45	1.94	1.22	3.44
	High	14.09	8.68	0.60	2.39	1.54	2.82
25	Low	13.61	8.50	0.47	1.66	0.73	3.59
	Medium	12.40	10.50	0.88	3.34	3.62	4.59
	High	13.00	6.19	0.62	1.64	1.15	3.66
35	Low	14.91	11.34	0.66	2.35	2.66	3.87
	Medium	10.59	6.60	0.48	1.84	1.07	2.11
	High	10.45	6.62	0.65	1.94	2.26	3.62
S.E. of mean		± 0.05	± 0.03	± 0.01	± 0.01	± 0.01	± 0.01
Significance level for Temperature		NS	NS	NS	NS	NS	**
Significance level for humidity		NS	**	NS	NS	NS	NS

NS not significant ** $P < 0.01$ (For original results see Appendix 3 Tables 2-7)

Table 4.2b

Mean percentage of the different fatty acids expressed as weight percentages of the total fatty acid measured, determined over a 3 1/4 h period after short-term exposure to different air temperatures and humidities

Temperature DB	Humidity	Myristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
15	Low	45.2	31.2	1.3	6.8	4.6	10.8
	Medium	45.6	25.8	1.8	7.9	5.0	14.1
	High	46.8	28.8	2.0	7.9	5.1	9.4
25	Low	47.6	29.4	1.6	5.8	2.6	12.6
	Medium	35.1	29.7	2.5	9.5	10.2	13.0
	High	49.5	23.6	2.4	6.2	4.4	13.9
35	Low	41.7	31.7	1.8	6.6	7.4	10.8
	Medium	46.7	29.1	2.1	8.1	4.7	9.3
	High	40.9	25.9	2.5	7.6	8.8	14.2

(calculated from Table 4.2a and Appendix 3 Table 8)

22°C DB, 12.5°C WB for 3 days followed by 9 days at 35°C DB, 24.0°C WB (35°C low humidity). On days 1, 3, 10 and 11 sebum was collected from 4 areas on each animal $3^{1}/_{4}$ h after cleaning the skin. The sebum collected from each area was weighed and the amounts of myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids were determined from 2 combined sebum collections per animal.

The results for sebum output (Fig 3.2) are given and discussed in Chapter 3. The mean weights of the fatty acids obtained from the $3^{1}/_{4}$ h collections on days 1 and 3 and 10 and 11 in the two environments are shown in Table 4.3a. A Students 't' test demonstrated that the outputs of linoleic ($P < 0.001$), palmitoleic ($P < 0.01$) and oleic ($P < 0.05$) were all significantly increased by prolonged exposure to heat. This was confirmed by a rise in the percentages of these fatty acids (Table 4.3b) at the warmer environment. Although the mean percentage of stearic acid was decreased at the warmer environment (Table 4.3b) there was no significant change in its output (Table 4.3a). This was most probably due to the within animal variation in the results for the output of stearic acid (Appendix 3, Table 5).

DISCUSSION

Bovine sebum contains a diversity of lipid components similar to those described in other species (Nicolaides et al, 1968; Nikkari & Valavaara, 1970; Peters et al, 1970). These compounds have been

Table 4.3a

Mean weights of the fatty acids quantitated from the sebum collected over a 3 1/4 h period ($mg.m^{-2}.h^{-1}$.) in a control environment and after 7 days exposure to a warmer environment (Experiment 8)

Fatty acid	Environment		S.E. of difference	Significance level
	22°C DB 12.5°C WB	35°C DB 24.0°C WB		
Myristic	14.44	16.19	6.08	NS
Palmitic	8.35	8.95	2.13	NS
Palmitoleic	0.20	0.90	0.69	**
Stearic	3.06	2.91	2.78	NS
Oleic	2.63	3.76	1.70	*
Linoleic	2.83	4.06	1.15	***

NS - Not significant

* - $P < 0.01$

** - $P < 0.001$

*** - $P < 0.001$

(For original results see Appendix 3 Tables 10-15)

Table 4.3b

Mean percentage of the fatty acids at the 2 environments expressed as weight percentages of the total fatty acid measured

Acid	Environment	
	22°C DB, 12.5°C WB	35°C DB, 24.0°C WB
Myristic 14:0	45.8	43.9
Palmitic 16:0	26.5	24.3
Palmitoleic 16:1	0.6	2.4
Stearic 18:0	9.7	7.9
Oleic 18:1	8.3	10.2
Linoleic 18:2	9.0	11.0

(calculated from Table 4.3a and Appendix 3 Table 16)

identified as phospholipid, cholesterol, unesterified fatty acids, triglyceride, cholesteryl esters and squalene. In other species there are generally 1-3 major lipid bands which have Rf values between the cholesteryl ester and triglyceride fractions (Nicolaides et al, 1968; Nicolaides, Fu & Ansari, 1970; Nikkari & Valavaara, 1970). These have been identified as monoester waxes and Type 1 and Type 2 diester waxes (Nicolaides et al, 1970). In previous work on the lipid composition of cow sebum (Nicolaides et al, 1968, 1970), the two fractions have been designated as monoester waxes and Type 1 diester waxes. In the present work 3 major lipid bands were consistently present between the cholesteryl esters and triglyceride fractions. Investigation into the thin-layer chromatographic behaviour of these 3 fractions in various solvent systems showed that they had similar Rf values to Type 1 and Type 2 diester waxes. Although the presence of monoester waxes in the sebum from cattle could be detected, their concentration was very much less than that of the 'diester' band. The presence of a Type 2 diester wax has not been previously found in the sebum of cattle. The fact that in the present work 3 fractions could be identified with Rf values in the 'diester' region is a further interesting feature of bovine sebum compared to that of other species. In view of the presence of a relatively high proportion of shorter chain fatty acids in these diester bands, the possibility exists that some further separation of the diester bands has occurred due to molecular species differences.

In the present work it has been found that the triglyceride fraction of bovine sebum may constitute up to 27% of the total lipid present. This finding represents a much higher proportion of the sebum lipids than found by Nicolaides et al (1968, 1970). Evidence for a substantial triglyceride fraction in cattle sebum comes from several different experiments.

(1) A fraction with the same Rf value as a triglyceride standard is present on T.L.C. separations of bovine sebum involving solvent systems for the separation of neutral lipids.

(2) Glyceride glycerol is present in this fraction.

(3) There is excellent agreement between the quantitative analysis of the triglyceride fraction by the glyceride glycerol and fatty acid methods.

Human sebum is termed a 'glyceride' type of sebum, i.e. one which contains primarily triglycerides and their breakdown products (Nicolaides et al, 1968) since it contains 60% triglyceride. Therefore, although bovine sebum cannot be considered a 'glyceride' sebum it does contain appreciable quantities of trglyceride. The reason for these conflicting results in the sebum composition of the cow is unknown, however, they may be a result of differences in age and sex of the animals sampled or perhaps a reflection of a seasonal change in sebum composition.

This study shows that most of the linoleic acid is found in the triglyceride fraction. This is contrary to the work on human sebum where the triglyceride fractions contain negligible quantities of linoleic

acid (Peter et al, 1970). In bovine sebum it was confirmed as linoleic acid and it was most unlikely that contamination of the sample had occurred during the collection procedure or during the stages of sample preparation. Since pure linoleic acid, an essential fatty acid, is known to possess antibacterial activity (Nieman, 1954) and as hydrolysis of the triglyceride fraction would give rise to free linoleic acid, it could influence the survival of bacteria on the skin surface.

Preliminary investigations using the technique of Wybenga, Pileggi, Dirstine & Di Giorgio (1970) have shown that free cholesterol accounts for approximately 8% of the total lipid isolated from the skin surface of cattle. There is some histochemical evidence to show that small amounts of free cholesterol are present in the sebaceous glands of cattle (Yang, 1952). Therefore, it would seem that some of the free cholesterol in the skin surface secretions of cattle is directly derived from the sebaceous glands. In man cholesterol is present in the skin surface lipid (Nicolaides et al, 1968) and has been found in isolated sebaceous glands (Peter et al, 1970, 1971).

Short term exposure to heat caused no effective change in the fatty acid composition of the sebum. There was some evidence, however, to suggest that a low humidity results in a lower output of palmitic acid from the skin. The most marked alteration in bovine sebum composition after prolonged exposure to an elevated ambient temperature was an increased output of linoleic acid. There is some evidence to suggest that in man linoleic acid may play a part in regulating the loss of moisture through the

skin (Holman, 1965). As cattle lose on average approximately 50% of their excess heat through sweating in an environment of 35°C low humidity (McLean & Calvert, 1972), linoleic acid might have some bearing on this heat loss mechanism.

CHAPTER 5

THE MODE OF SECRETION OF THE SEBACEOUS GLANDS

INTRODUCTION

Sebaceous glands are believed to function as holocrine glands. By definition a holocrine gland is one whose cells arise by mitosis at the periphery, develop as they progress inwards, then die and disintegrate to form the secretion (Ranvier, 1887). It has been suggested that the potentiality of the cells to form sebum and the replacement of the cells are independent phenomena (Rothman, 1955; Ebling, 1963). In the rat, administration of oestradiol decreases the volume of the gland and sebum output, but does not appreciably influence the mitotic activity (Ebling & Skinner, 1967) suggesting that a mechanism other than mitosis is responsible for producing the sebum.

The rat sebaceous gland has a regeneration time of about 8 days (Bertalanffy, 1957). In man the time taken for the renewal of the glandular cells appears to vary from 7 to more than 14 days (Epstein & Epstein, 1966; Plewig, Christophers & Braun-Falco, 1971) which suggests that regeneration is slow. In addition the lipid-producing cells in the glandular fundus, show slow cell movement with a replacement time of more than 14 days (Plewig et al, 1971). In man if sebum is removed from the skin surface it is always replaced immediately by freshly

secreted sebum (Rothman, 1955). These findings and the comparison of the life time of a human sebaceous gland cell with estimates of the sebaceous gland tissue per square centimetre of skin (6.94 µg; Johnsen & Kirk, 1952) and the sebum output of the hand (0.54 µg $cm^{-2}h^{-1}$; Johnsen, 1952), cast doubt on the hypothesis that the mode of secretion of the sebaceous gland is holocrine.

In cattle although the glands are known to have a good capillary blood supply (Goodall & Yang, 1954) and are innervated (Jenkinson et al, 1966) little is known about their mode of secretion; even the size of the gland is unknown. The object of the present work was to investigate the mode of secretion of the sebaceous glands of Ayrshire cattle and to test the hypothesis that they are holocrine in nature.

METHODS AND RESULTS

SEBUM OUTPUT AND SEBACEOUS GLAND HISTOLOGY

In two experiments the volume of, and number of cells in the sebaceous glands/unit area of skin and the output of sebum were measured simultaneously after stimulation of the glands. Stimulation was initiated by removing the skin surface lipid with the solvent methanol. The values obtained were compared with estimates of glandular mitotic activity after colchicine injections. Eleven, 4-month old castrated male Ayrshire calves were housed in a cowshed, which was

kept at a temperature between 16.5 and 13.5°C (Max/Min). The animals were allowed water ad libitum and were fed a complete diet twice daily at 0700 h and 1700 h for at least 14 days before the start of the experiments and during them. Sebum was collected from the experimental area situated either side of the midline in the thoracico-lumbar region of the body. As this body region is homogenous with regard to sebum output (Chapter 2) the left side of each animal was allocated for sebum collection and the right side for estimation of the dimensions of the glands and their corresponding mitotic activities.

Experiment 9

The aim of this experiment was to determine the effect of a single stimulation on the volume of and number of cells in the sebaceous glands per unit area of skin and on the mitotic activity of the gland. In addition the volume of the hair follicle canal and the depth of the sebaceous gland duct from the skin surface were also quantitated.

The experimental region on each of 4 animals was shaved and the following day a control estimate of mitotic activity, as described in Chapter 2, was made at 0900 h on the right side of each animal. After 14 days the experimental region was reshaved. At 0900 h on the following day the region was thoroughly cleaned with methanol and at 1200 h two sebum collections were made from the left side of each animal for estimation of sebum output. Just before the initial cleaning of the skin, at 0900 h and again at 1030 h and 1200 h, skin specimens were

Table 5.1

Mean sebaceous gland measurements before and after one stimulation of the glands - The results of Experiment 9

Time (h)	Seb. G. Vol. (ml/m^2)	Cell No x 10^9 (per m^2)	Cell Vol. ($mm^3 \times 10^{-6}$)	No of metaphases x 10^6 (per m^2)
0	29.48	8.13	3.66	12.03 +
1.5	30.87	9.17	3.36	*
3	27.51	8.67	3.16	11.60
S.E. of mean	±3.30	±0.66	± 0.24	± 2.8

* not measured

\+ measured 14 days earlier

(For original results see Appendix 4 Table 1)

removed from the right side of each animal, using the high speed biopsy punch technique of Findlay & Jenkinson (1960), for the determination of sebaceous gland volume and cell number/m^2 of skin. At 1200 h the mitotic activity of the glands was measured. A further control estimation of mitotic activity was made at the same time of day, i.e. at 1200 h on 4 different castrated male Ayrshire calves of the same age. Several weeks later skin specimens were taken by biopsy from the right side of the original four animals for determination of the number/m^2 and depth of the sebaceous glands and the width of the hair follicles (for details of the methods used see Chapter 2).

The mean values, with their standard errors of sebaceous gland volume per unit area of skin, the number of sebaceous cells per unit area of skin and the calculated mean individual cell volume before and after a single stimulation of the gland are given in Table 5.1. Two way analyses of variance of the glandular dimensions demonstrated that there were no significant differences in these parameters due to glandular stimulation although they all just varied significantly between animals ($P < 0.05$). Moreover, as shown by a Students 't' test, the number of metaphases found after stimulation (Table 5.1) did not vary significantly from the control value obtained at 0900 h 2 weeks earlier or from that obtained from 4 animals of the same age sampled at the same time of day ($7.05 \times 10^6 \pm 1.6 \times 10^6$). The mean output of sebum over the 3 h collection period was 0.429 $g.m^{-2}$ (or 0.143 $g.m^{-2}.h^{-1}$.). Using the

specific gravity of human sebum, 0.91 (Rothman, 1955) this output can be expressed as 0.471 ml.m^{-2} which corresponds to 1.61% of the mean glandular volume found and to 13.93 x 10^7 sebaceous cells per m^2 (Table 5.1). The specific gravity of human sebum was used as no such information exists for cattle sebum.

The mean number of sebaceous glands/m^2 of skin was 12.46 x 10^6 ± 0.2 x 10^6 (S.E.) and the mean depth of the sebaceous gland was 300.7 ± 8.6 μm. Mean hair follicle width was found to be 36.8 ± 1.4 μm and mean hair diameter 25.71 ± 1.8 μm (see Appendix 4, Table 3 for original results). Using these measurements and assuming a hair and the hair follicle canal to be circular and the latter to be completely full of sebum, the maximum amount of sebum which could be stored in the hair follicle canal above the sebaceous gland in a square centimetre of skin was estimated. This amounted to 2.04 ml per m^2. It would seem, therefore, that under the present experimental conditions (3 hourly washings) if sebum accumulates in the hair follicle at the initial 3 h rate it could take as long as 13 h for this stored sebum to be completely replaced.

Experiment 10

This experiment was designed to remove the accumulated sebum in the hair follicle canal. On the basis of calculations in the preceding section, it would require 5, 3-hourly washings for removal of this sebum. The experimental region on each of 3 animals was shaved and the following day was cleaned with methanol every 3 h for 5 consecutive

Table 5.2

Mean amounts of sebum and standard errors obtained at the end of each 3 h period - Results of Experiment 10

Period	Weight of sebum g^{-2}
1	0.423
2	0.337
3	0.309
4	0.299
5	0.329
S.E. of mean	± 0.085

(For original data see Appendix 4 Table 4)

periods starting at 2100 h. Sebum was collected from 2 areas within the experimental region on the left side of each animal at the end of every 3 h period just before re-cleaning the skin, i.e. at 2400, 0300, 0600, 0900 and 1200 h. The volume of sebaceous gland/m^2 and cell number/m^2 were determined on skin specimens taken from the right side of each animal at the start and the end of the 5th period, i.e. at 0900 and 1200 h. On the same side of the animal the mitotic activity of the glands was estimated at 1200 h. This timetable was chosen so that the 5th period coincided with the time of day of the preceding experiment thus eliminating any circadian effect on the mitotic activity of the sebaceous glands. Control values for all the sebaceous gland measurements were obtained from the same animals 14 days later at the corresponding times of day. In this instance the skin was not shaved or washed beforehand. This experiment was carried out during the summer whereas the preceding experiment was performed in the winter.

The mean sebum output obtained from all 3 animals at each of the 3 h collection periods is given in Table 5.2. A 2-way analysis of variance demonstrated that sebum output differed significantly between animals ($P < 0.01$) and there tended to be more sebum produced during the first period ($P < 0.05$) than during the remainder. The mean values for sebaceous gland volume/m^2, cell volume and cell number/m^2 are given in Table 5.3, with their standard errors. As indicated by a 2-way analysis of variance there was no significant difference in these parameters

Table 5.3

Mean sebaceous gland measurements at 12 and 15 h after washing the skin every 3 h - Results of Experiment 10

Time (h)	Seb. G. Vol. (ml/m^2)		Cell no x 10^9 (per m^2)		Cell Vol ($mm^3 \times 10^{-6}$)		No of metaphases x 10^6 (per m^2)	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
12	57.92	37.93	13.95	9.36	4.13	4.03	*	*
15	41.16	32.57	11.61	8.14	3.45	4.02	9.85	8.21
S.E. of mean	± 8.94		± 0.17		± 0.39		± 2.6	

(For original results see Appendix 4 Table 5)

between the two sampling times and the experimental and the control values although cell number and sebaceous gland volume tended to be higher after repeated washing of the skin. A Students 't' test showed that there was no significant difference between the experimental and control values (Table 5.3) obtained for the number of metaphases/m^2.

SEBUM OUTPUT AND THE DNA CONTENT OF SKIN WASHINGS

If the sebaceous glands were holocrine a relationship would be expected between sebum output and the quantity of sebaceous gland cell nuclei on the skin surface. This relationship was therefore sought using the amount of DNA on the skin surface as an indirect measure of cell degredation.

A total of eight 8-month old castrated male Ayrshire calves in 2 experiments were housed in separate stalls in a shelter at a temperature between 18.5 and 14.9°C (Max/Min) and fed a complete diet with water ad libitum. Sebum was collected and extracted from the experimental region as previously described (Chapter 2) and the amount of DNA on the skin surface was measured by the method of Mabon (1974) on 2 combined skin washings each taken with 50% methanol in the manner described in Chapter 2.

Experiment II

Sebum was collected from 2 areas within the experimental region, one from either side of each of 4 animals at 0, 1, 2, 4, 8, 12, 17, 21 and

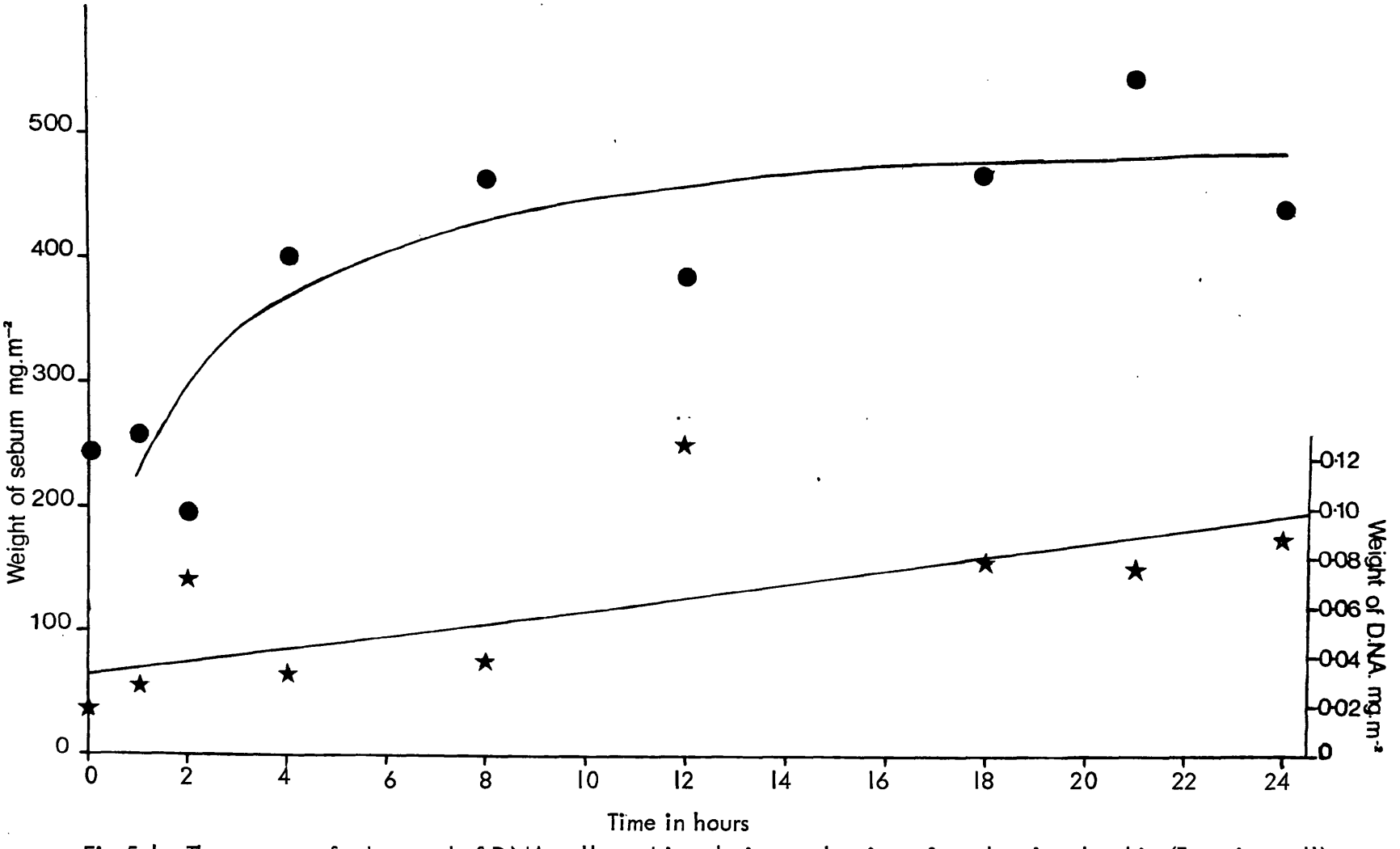

Fig 5.1 The amount of sebum and of DNA collected in relation to the time after cleaning the skin (Experiment II)

● sebum output ★ DNA output

(For original results see Appendix 4 Table 6)

24 h after cleaning the skin. The sebum collections were taken at random time intervals over a period of 3 weeks. At the same time 4 skin washings, 2 from each side of the animal, were taken with 50% methanol in the experimental region and 2 estimations of the amount of DNA on the skin surface of each animal were obtained by combining the skin washings from the same side.

Fig 5.1 shows the distribution of the mean values of both sebum and DNA output with time. The sebum values exhibit a curve similar to that found in Chapter 3 demonstrating that the sebaceous glands under the applied conditions are functioning normally but the DNA results fit a straight line ($y = 0.002x + 0.0329$). The relationship of DNA output with sebum output is shown in Fig 5.2. An 'F' test (Table 5.4) demonstrated that the correlation between the two outputs ($r = 0.244$) was not significant.

Experiment 12

To further investigate the source of DNA on the skin surface, four more calves were prepared for collection of DNA and were exposed for 6 h in random order to environments of 10, 20, 30 and 40°C DB all at 20% relative humidity. The skin of each animal was cleaned at 0900 h and 4 skin washings were taken at 1500 h for estimations of the amount of DNA on the skin surface. As in Experiment 11, the two combined skin washings were analysed. During the entire exposure to each climatic

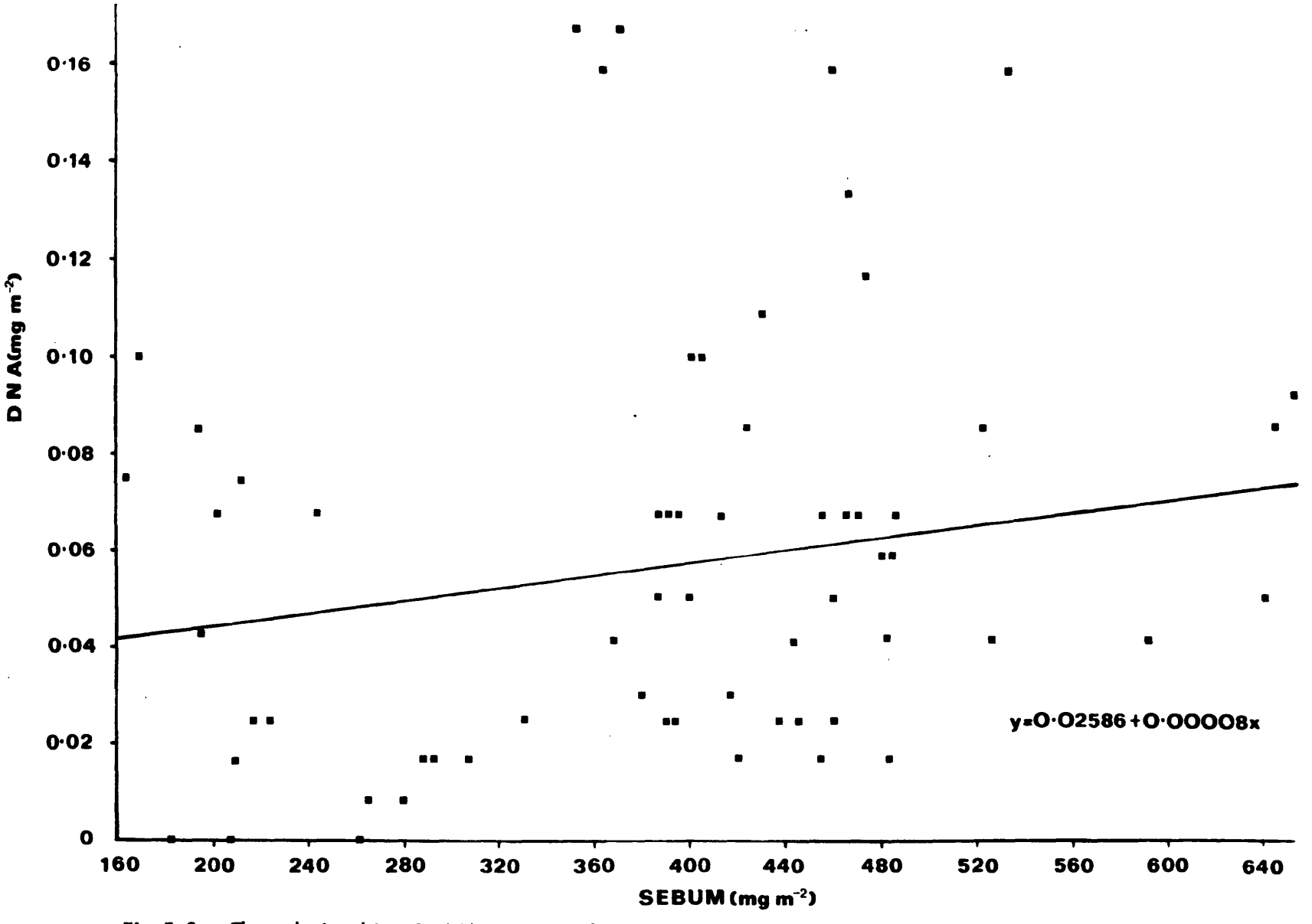

Fig 5.2 The relationship of DNA output with sebum output, determined over different periods of time after cleaning the skin (Experiment II)
(For original results see Appendix 4 Table 6)

Table 5.4

F test on DNA output and sebum output determined over different periods of time after cleaning the skin (Experiment II)

	df	SS	MS	F
Regression	1	0.00688	0.00688	3.82 NS
Residual	60	0.10835	0.00180	
Total	61	0.11523	0.00188	

NS not significant

(For original results see Appendix 4 Table 6)

condition the sweating rate of each animal was determined by the method of McLean (1963) and the mean sweat output over the 6 h period was calculated.

The relationship of DNA output with the sweat rate is shown in Fig 5.3 and as demonstrated by an 'F' test (Table 5.5) was highly significant ($P < 0.001$). The correlation coefficient ($r = 0.59$) was also highly significant ($P < 0.001$).

DISCUSSION

As determined from the measurements made in the first experiment (Experiment 9), the volume of sebaceous gland tissue/m^2 of skin in Ayrshire calves was about 30-50% of that of the sweat gland in animals kept under similar conditions (Findlay & Jenkinson, 1964) and is larger than expected from visual examination of histological sections. Consequently, the sebum output, estimated in Experiment 9, represented a smaller proportion of the potential sebum in the skin than had been expected. The output of sebum over the 3 h following stimulation of the glands by cleaning the skin represented only about 1.61% of the sebaceous gland volume in an equivalent area and consequentially no change in sebaceous gland volume or cell number was detected. However, on the basis of the overall mean measurements made under the present experimental conditions it can be calculated that if the glands were truly holocrine about 13.93×10^7 sebaceous cells/m^2 would have to be

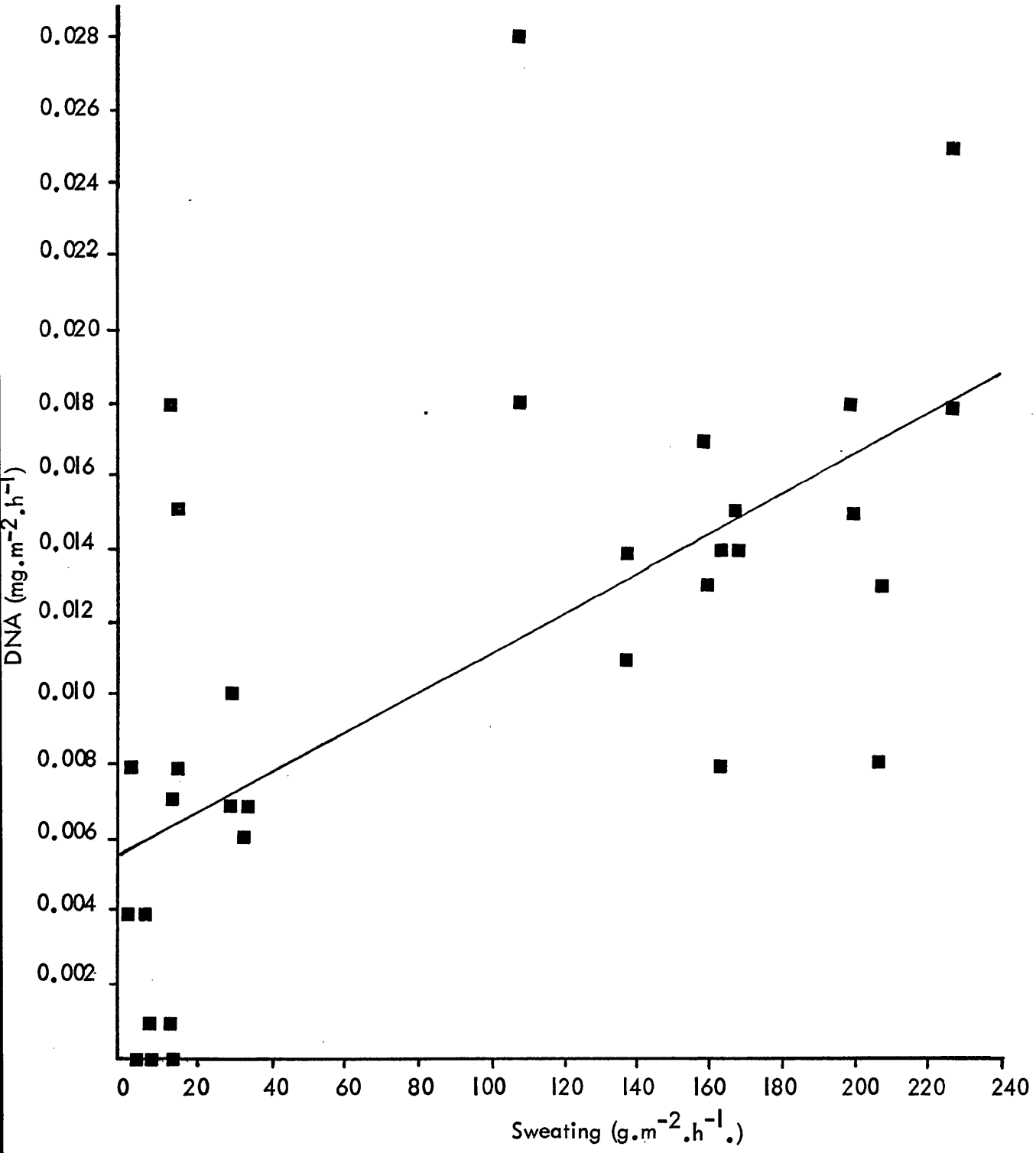

Fig 5.3 The relationship of DNA output with sweating rate determined after 6 h exposure to different environments (Experiment 12). (For original results see Appendix 4 Table 7).

Table 5.5

Results of an 'F' test on DNA output against sweating shown in Fig 5.3

	df	SS	MS	F
Regression	1	0.00064	0.00064	21.33 ***
Residual	30	0.00092	0.00003	
Total	31	0.00156	0.00005	

*** $P < 0.001$

(For original results see Appendix 4 Table 7)

lost in 3 h to provide the quantity of sebum found on the skin surface. There was no evidence of a correspondingly high rate of cell production as indicated by an increase in mitotic rate during the 3 h experimental period. This suggests that either the gland was not holocrine in nature or that the sebum produced was derived from a reservoir such as the hair follicle which could be replenished by subsequent glandular activity.

In samples taken after repeated washings of the skin over a period sufficiently long to have enabled complete replacement of the accumulated sebum in the hair follicle canal, there was again no significant change in the dimensions of the glands. However, there was in this instance a tendency for the glands to have larger volumes and more cells in samples from untreated skin. Once more there was no evidence of increased mitotic activity and the number of cells required to produce the sebum by a holocrine mechanism greatly exceeded the level of potential cell production. It would seem therefore that the sebaceous gland did not function in a holocrine manner. Consequently, the bovine sebaceous gland is, under conditions of repeated stimulation, not a holocrine gland.

If the sebaceous glands were holocrine, according to the definition given by Ranvier (1887) sebum should be composed of cellular components. Since DNA is an indirect measure of cell degredation, there should be a definite relationship between the amount of DNA on the skin surface and the sebum output. No such relationship was found, again suggesting that the sebaceous gland is not a holocrine gland. It would seem therefore

that the bovine sebaceous gland is not holocrine but functions in a more complex manner than was originally believed. There could be several different inter-related potential processes, as for example, cell production, cell growth and sebum formation whose modes of control may all differ. Sebum could be produced by a lipogenic process combined with secretion from live cells as opposed to a holocrine mechanism which involves total degredation of the cell.

Although similar quantities of sebum were collected after repeated stimulation at the end of each 3 h period,there was as expected from previous work a high between animal variation in the amount of sebum produced. The difference in the amount of sebum collected at the end of the first 3 h could be partially ascribed to a circadian rhythm as it exists in the human sebaceous gland (Burton, Cunliffe & Shuster, 1970) and also to the collection of sebum with the solvent methanol from the same area of skin. Even though methanol, the lipid solvent chosen for the collection of cattle sebum, does not seem to have visible detrimental effect on the skin surface (Chapter 2), it may remove more than just the freshly extracted lipid on the skin surface.

If sebaceous gland size is to be used as a measure of sebaceous gland activity (Ebling, 1963), it must be remembered that gland size per se may not be an accurate estimation of glandular activity as it is dependent on the turnover time of the cells and their size (Ebling, 1963).

Since the first two experiments were performed at different times of the year, the first in winter and the second in summer, and as there is some evidence to suggest that season might affect sebum output (Chapter 3), it is not unexpected that there is a difference in the sebaceous gland volume/m^2 estimated from control specimens between the experiments (Tables 5.1 and 5.3). The sebaceous gland volume was larger in the second experiment suggesting that the gland was more active in the summer, thus producing more sebum. This agrees with the finding from Chapter 3 where sebum output seemed to be greater in summer than winter.

The source of DNA found on the skin surface of cattle is unknown. Some of it may have arisen from cell death in the epidermis, but the significant relationship with sweat output suggests that most of the DNA is derived from sweat. Mitoses are common in the intradermal portion of the sweat gland ducts of mouse foot pads (Bullough & Deol, 1972) and if a similar process occurs in cattle it could explain the presence of DNA in sweat. The sebaceous glands are unlikely to be a major source of the DNA, since sebum output does not increase during short-term exposure to heat (Chapter 3) and is not related to the DNA content of the skin surface after stimulation of the glands by cleaning the skin.

CHAPTER 6

GENERAL DISCUSSION

The role of sebum in other species is to form a natural barrier-layer which assists in the thermoregulation of the animal, in the prevention of water loss from the epidermis and acts as a deterrent against skin disease, (Ham, 1965; Hsia, 1971; Roberts, 1963). Therefore, even though the function of bovine sebum is unknown, it is likely to fulfil the same role as that of sebum from other animals. However, it is known that the sebum of cattle, as in other species, provides a layer of lipid on the skin surface (Yang, 1952). Since in the ox, sebum and sweat emerge on the skin surface as a mixture, they probably exist as an emulsion and together form the natural barrier-layer. Nevertheless, the composition and integrity of the lipid layer on the bovine skin surface is clearly of major importance to the fulfilment of the barrier-layer function. The current studies into the physiology of sebaceous glands of Ayrshire calves have demonstrated some of the factors which affect the integrity and composition of the sebum layer and also its rate of production.

In cattle the thickness and integrity of the sebum layer would appear to depend largely on local sebum production, as no evidence was found to suggest that sebum flows across the skin surface even when it is damp (Chapter 2). This finding correlates with recent work in man by

Dvorkin et al (1966) but is contrary to earlier findings in man (Herrmann & Prose, 1951; Jones et al, 1951; Kligman & Shelley, 1958). It is not known if a layer of sebum exists in areas of cattle skin devoid of sebaceous glands, as for example the teat. Hence it would be interesting to study the distribution of lipid on the skin surface in such regions of the animal.

It takes a day for the replacement of the lipid film on the skin surface (Chapter 3), this could be of consequence in the protection of cattle against disease. Although the time taken for the lipid to be completely replaced is a day (Chapter 3), the majority of the lipid seems to be replaced as in man (Rothman, 1955), during the first 12 h in thermoneutral conditions. Although the time taken to replace the sebum layer under warmer environmental conditions still has to be determined, it is likely to be of importance in view of the probable role the sebum of cattle plays in the conservation of water on the skin surface to protect the skin from dehydration and subsequent damage. Support for this function of bovine sebum comes from the increased sebum output and linoleic acid content of sebum on prolonged exposure to a warmer environment (Chapters 3 and 4) on account of the potential involvement of linoleic acid in the regulation of cutaneous moisture loss (Holman, 1968) and the function of human sebum (Ham, 1965).

It is clear from the present results that the sebaceous glands of cattle are not of major importance in the regulation of body temperature

in the heat. Sebum output compared with the sweat output (McLean & Calvert, 1972) is small and is not increased by short-term exposure to warm conditions (Chapter 3). Even on long-term exposure to a warm environment the observed increase in output was unlikely to have had an appreciable effect on the regulation of body temperature (Chapter 4). These findings agree with work on human sebaceous glands where short-term exposure to temperature is considered to have no effect on sebum output (Cunliffe et al, 1970; Williams et al, 1973; Chapter 3). The effects of adversely low temperatures on the sebum output of cattle have not been studied in depth, but preliminary work indicates that no change occurs in cooler environments. Nevertheless, bovine sebum may well aid body insulation in the cold and influence the evaporation of sweat from the skin in the heat as in man (Ham, 1965).

The integrity of the sebum layer and changes in physical characteristics of sebum with temperature probably have an important role to play in the body defence against foreign organisms. Indeed this may well be the most important function of bovine sebum.

The sebum of cattle contains not only potential nutrients for bacterial and fungal growth but also possible inhibitors. The study of bovine sebum illustrated the presence of squalene, wax esters, triglycerides, unesterified fatty acids, cholesterol and phospholipids (Chapter 4) which as nutrients could easily influence the bacterial population on the skin surface. On the other hand, however, other compounds such as myristic, palmitic, oleic and linoleic acid, which

are present in the sebum of cattle (Chapter 4) can be bacteriostatic and even bacteriocidal (Nieman, 1954; Adams & Richards, 1963). During the warmer months of the year when more skin parasites abound, there is an increase in the output of sebum (Chapter 3). Hence bovine sebum could also be a deterrent against the invasion of foreign bodies and thus assist in the preservation of an intact stratum corneum. Since as already mentioned, it is probable that sweat and sebum exist as an emulsion on the skin, they could both contribute to the effectiveness of the barrier-layer. It is already known that the sweat of cattle contains protein (Jenkinson, Mabon & Manson, 1974) which may well be antigenic to certain foreign bodies. Linoleic acid in the sebum of cattle is of particular interest as it is an essential fatty acid which in vitro studies have shown, has bacteriocidal properties (Nieman, 1954). Unlike the other fatty acids, linoleic acid not only increases in output but is present in the sebum in relatively larger amounts on long-term exposure to heat (Chapter 4). It is interesting and perhaps significant that the linoleic acid in the sebum of cattle is found largely in the triglyceride fraction. This is different from man (Peter et al, 1970) and may have some relevance to species differences in body defence systems against foreign organisms.

Human sebum is unique in that its chemical composition is quite different from subcutaneous lipid and other lipid deposits in the body (Rothman, 1955; Nicolaides, 1974). The fatty acid composition of

Table 6.1

Percentage fatty acid compositions of the triglycerides from various tissues of the ox

Fatty Acid	Liver+	Kidney +	Heart+	Plasma+	Lung+	Muscle+	Intestine+	Adipose+	Milk+	Skin surface
Lauric	0.6	0.8	0.6	0.0	2.3	0.3	0.4	0.5	6.0	7.4
Myristic	3.9	4.5	2.0	tr	2.0	2.5	4.6	3.9	15.1	29.6
Palmitic	34.1	29.7	19.1	35.0	24.1	20.0	29.7	24.2	38.7	32.0
Palmitoleic	8.3	4.4	3.7	1.8	3.9	7.0	4.4	11.7	4.2	1.7
Stearic	5.3	19.7	19.2	23.8	18.4	10.1	20.5	4.9	2.9	4.2
Oleic	40.5	40.3	52.0	31.3	47.1	58.5	38.7	53.3	11.9	2.0
Linoleic	4.6	0.6	3.3	6.0	2.1	1.7	1.5	1.7	1.0	23.1
Linolenic	2.8	0.0	0.2	2.2	0.0	0.0	0.3	0.2	tr	tr

+ results from Noble, Crouchman & Moore (1974)

tr - trace

bovine sebum triglyceride (Table 6.1) is quite different from the triglycerides of bovine tissues. In this respect the sebum of cattle is similar to human sebum. Bovine sebum triglyceride when compared with the triglyceride from other bovine tissues is quite distinctive in not only containing higher proportions of lauric and myristic acids but considerably more linoleic acid. It has also a low proportion of oleic acid. The reason for the higher amounts of lauric, myristic and linoleic acid in the sebum may well be to protect the animal against disease since the "lactosebum" found in the keratin of the bovine teat canal contains myristic and linoleic acids which are bacteriocidal to Str. agalactiae, the responsible agent in most chronic mastitis (Adams & Richards, 1963).

Milk like sebum is the secretory product of a skin gland; the mammary gland like the sebaceous gland is derived from ectoderm. Thus it is interesting (Table 6.1) that the composition of milk triglyceride like sebum is different from the triglycerides of the other bovine tissues. There are similarities between the two secretions (Table 6.1), as like sebum triglyceride, milk triglyceride contains higher proportions of lauric and myristic acids and lower amounts of oleic acid than the triglycerides of other bovine tissues. Furthermore there is some evidence to suggest that the mammary gland is a modified sebaceous gland (Carlisle, 1954).

Age seems to have little effect on the production of the protective layer of sebum in Ayrshire cattle (Chapter 3). The effect of age on sebum composition was not investigated. However, in neonatal

Ayrshire calves the composition of the skin surface lipid alters after parturition (Noble, Crouchman, Jenkinson & Moore, 1975). At birth the saturated fatty acids decrease and there is a concomitant increase in the proportion of oleic acid. Some 3-4 weeks after birth the proportion of linoleic acid in the skin surface triglyceride increases to approximately 14% and there is a decrease in the proportion of oleic acid. In view of the antibacterial properties of linoleic acid, this change in the composition of the skin surface lipid suggests that perhaps the potential protective function of the bovine lipid film is not fully developed until after the first few weeks of life. Hence the neonatal calf could be more susceptible to skin disease.

Sex only had a slight effect on the sebum output of Ayrshire cattle; castrated male calves had a higher output than females (Chapter 3). This slight difference could be attributed simply to a general increase in all the constituents in the male sebum. Alternatively, it could be due to heterogenity in the composition which was not determined and could thus be a difference in the defence system on male skin. In the male goat, the volatile fatty acid fraction of the sebum is known to increase during the rutting season (Jenkinson et al, 1967). Since human sebum is reputed to contain pheromones (Nicolaides, 1974), it is possible that the volatile fatty acids in goat sebum could have pheromonic properties. Preliminary investigations have shown

that the sebum of cattle does contain small quantities of volatile fatty acids but further research is required to establish if they are pheromones.

The mode of secretion of the sebaceous gland is considered to be holocrine (Ranvier, 1887; Rothman, 1955; Strauss & Ebling, 1969). In this thesis the results indicate that the mechanism of secretion is not holocrine in cattle at least not under experimental conditions of continual secretion (Chapter 5). The mode of secretion in the human gland is probably the same as that of the bovine gland since the curves of sebum replacement are similar for both species (Chapter 3). Moreover, the immediate replacement of sebum after its removal from the skin surface and the evidence of Johnsen & Kirk (1952) and Johnsen (1952) in man, as previously discussed (Chapter 5), casts doubt on the validity of the holocrine theory of secretion and suggests that a truly holocrine mechanism does not exist in sebaceous glands. It seems probable therefore that in cattle and perhaps also in other species, the secretion of sebum is more complex than originally envisaged. It could conceivably involve three inter-related mechanisms, cell production, cell growth and the production of sebum by a lipogenic process. As indicated in the rat by the results of Ebling & Skinner (1967) the dividing cells at the periphery of the sebaceous gland are not necessarily under the same hormonal control as the secretory process. Thus the function of these cells in the sebaceous glands of cattle is perhaps merely to replace those lost by death. In the rat, the mitotic activity of the cells, round the

periphery of the gland is controlled by a negative feedback mechanism in which a tissue specific antimitotic messenger molecule called a chalone plays a crucial role (Bullough & Laurence, 1970).

Sebum may be not only a product of cell degeneration but could conceivably also be produced by a process involving lipogenesis and secretion from live cells. Although it is already known that squalene synthesis from cholesterol takes place in rat skin and rat preputial gland (Wilson, 1963), it has not been established whether sebum is secreted from either live or dead cells. The process of lipogenesis in the sebaceous glands of cattle could be controlled by a hormonal process since in cattle the glands have a copious blood supply (Goodall & Yang, 1954) and in the rat it is known that steroids can alter the production of sebum (Ebling, 1970b). On the other hand, the lipogenic process could be sympathetically controlled as the nerve supply to the sebaceous glands of cattle is mainly of sympathetic origin (Jenkinson et al, 1966).

The manner in which the secreted bovine sebum is conveyed to the skin surface is unknown. It could be expelled by means of a contractile sheath as in the bat wing (Cortese & Nicoll, 1970), by capillarity as suggested for human sebum by Kligman & Shelley (1958) or perhaps even by contraction of the arrector pili muscle as speculated by earlier workers (Starling, 1936). In the skin of cattle it is not known if a contractile sheath is present. Further the arrector pili muscle controls the movement of the hair (Jenkinson, 1965) and on contraction alters the

size of the hair follicle canal and may thus aid expulsion of sebum onto the skin surface from the hair follicle canal. Further work is required to elucidate this aspect of sebum production.

It is likely that in bovine skin the sebum expelled upon the surface is initially derived from accumulated sebum in the hair follicle as in man (Kligman & Shelley, 1958). The hair follicle canal in the skin of cattle can, therefore, be considered a potential reservoir for the storage of sebum. Although an estimation of the amount of sebum "stored" in bovine skin has been made (Chapter 5) it must be remembered that in the present work the hair follicle canal is presumed to be completely packed with sebum. This presumption has been made because histochemical staining of sections of bovine skin suggest there is a continuous stream of lipid from the gland to the skin surface. There is no information on the quantity of sebum "stored" for other species.

Although the mode of controlling sebum expulsion is unknown, it may well be independent of the secretory process. However, like the secretory process sebum expulsion could be under hormonal control or under sympathetic control. On the other hand, it might be controlled by a simple physical feedback mechanism stimulated into action after removal of the lipid from the skin surface.

A more sensitive method of measuring bovine sebaceous gland activity is being developed by using the lipid constituents in the sebum.

Autoradiographical techniques will be used to study the incorporation of compounds into the glands and the transfer of sebum to the skin surface. It is envisaged that these methods will enable further more detailed study of the mode of control and secretion of the sebaceous glands of cattle.

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APPENDIX I

RESULTS OF EXPERIMENTS IN CHAPTER 2

Table I

Sebum output ($mg.m^{-2}.h^{-1}$.) from 8 areas of an Ayrshire calf on 3 consecutive days using methanol

Area	Day 1		Day 2		Day 3	
	Collection 1	Collection 2	Collection 1	Collection 2	Collection 1	Collection 2
1	Lost	129.11	146.67	135.56	139.89	115.78
2	201.44	144.78	125.22	130.00	158.00	133.33
3	157.00	124.67	121.11	Lost	165.44	110.22
4	116.89	134.67	132.78	130.00	Lost	127.67
5	91.44	123.56	Lost	123.00	131.67	Lost
6	89.44	100.89	111.33	110.00	172.44	113.78
7	Lost	100.89	Lost	103.00	Lost	Lost
8	127.56	146.67	149.44	Lost	140.00	Lost

Table 2

Sebum output ($mg.m^{-2}.h^{-1}$.) from 4 animals for the comparison of the solvents, methanol, ethanol and butanol on their ability to absorb sebum from the skin surface

(Refer to Table 2.1)

Animal	Solvent	Area	Collection	Sebum $mg.m^{-2}.h^{-1}$.		
				Day 1	Day 2	Day 3
1	Methanol	A	1	132.56	105.11	138.00
			2	80.44	115.44	123.22
		B	1	146.67	108.56	138.22
			1	98.67	115.22	114.11
2	Methanol	A	1	134.78	103.33	143.00
			2	106.33	127.11	96.11
		B	1	163.11	106.89	137.22
			2	150.11	137.78	104.44
3	Methanol	A	1	105.67	107.22	143.89
			2	76.44	138.00	122.78
		B	1	106.56	92.78	150.00
			2	66.56	116.33	137.22
4	Methanol	A	1	139.78	116.33	109.33
			2	98.11	157.22	130.67*
		B	1	166.56	130.78	165.44
			2	135.00	138.33	117.22
1	Ethanol	C	1	298.33	171.11	125.33
			2	233.89	256.44	186.44
		D	1	293.00	154.89	153.11
			2	212.22	253.33	183.00
2	Ethanol	C	1	352.78	216.67	136.89
			2	210.00	400.78	233.56
		D	1	411.78	383.11	167.44
			2	248.89	199.11	241.67
3	Butanol	C	1	341.33	216.56*	207.78
			2	205.00	213.22	222.44
		D	1	331.44	171.11	246.00
			2	284.11	265.44	176.00
4	Butanol	C	1	216.33	93.89	204.89
			2	224.00	243.22	157.67*
		D	1	207.22	104.44	172.11
			2	180.33	199.11	96.00

* sample lost, mean value used for analysis

APPENDIX 2

RESULTS OF EXPERIMENTS IN CHAPTER 3

Table 1 - Results of Experiment 1
The relationship of sebum output with time
(Original data for Fig 3.1)

Time (h)	Sebum output (mg/m^2)	Time (h)	Sebum output (mg/m^2)
	306.37		
	331.94		429.14
	279.92		387.48
	265.81		371.17
1	182.28	12	353.10
	217.55		364.56
	191.76		405.55
	261.63		
	mean 254.66		mean 385.17
	212.26		460.22
	202.12		522.81
	195.06		528.54
	244.21		480.27
2	169.28	18	391.01
	167.51		414.59
	mean 198.41		mean 466.24
	400.27		640.07
	438.62		644.04
	372.49		654.84
	393.65		592.90
4	379.99	21	473.00
	415.57		467.93
	443.47		389.24
	455.37		487.11
	mean 412.43		mean 543.64
	460.66		401.59
	483.58		424.07
	460.88		469.03
	420.98		394.98
8	482.26	24	387.26
	460.00		533.83
	446.99		465.73
	484.90		
	mean 462.53		mean 439.50

Table 2 - Results for Experiment 2

Sebum outputs collected over 3 h period ($mg.m^{-2}.h^{-1}$.) in different seasons

(Refers to Tables 3.1 and 3.2)

Season	Animal	Area				Mean
		A	B	C	D	
February (Winter)	1	130.89	120.00	141.11	145.33	117.30
	2	123.11	144.44	124.11	129.78	
	3	72.00	96.11	115.78	100.56	
	4	94.67	114.78	128.89	95.22	
April (Spring)	5	133.00	94.11	144.22	159.44	150.69
	6	142.00	141.44	167.44	111.67	
	7	181.89	151.44	119.44	131.44	
	8	192.44	165.89	198.00	174.22	
July (Summer)	9	193.22	136.67	122.44	121.00	149.06
	10	142.78	156.11	144.33	147.78	
	11	184.67	131.89	120.44	156.00	
	12	109.89	153.33	164.11	200.22	
October (Autumn)	13	144.44	145.33	175.56	171.67	142.09
	14	155.56	134.44	116.56	152.11	
	15	105.56	149.89	105.00	116.33	
	16	150.22	110.56	151.67	188.56	

Tables 3 and 4 The results of experiment 3

Table 3

Sebum output over a 3 1/4 h period ($mg.m^{-2}.h^{-1}$.) after exposure to different environment (Refers to Tables 3.4 and 3.5)

Animal	15°C			25°C			35°C			15	40
	L	M	H	L	M	H	L	M	H		
1	96.70	148.79	104.07	142.86	262.97	164.62	188.46	90.44	129.67	141.76	129.98 +
	100.11	120.77	103.19	115.60	206.15	148.35	149.67	95.60	117.03	143.19	124.16 +
	119.23	159.01	137.58	108.79	200.00	117.58	148.57	106.70	116.70	105.49	124.16 +
	112.97	127.91	109.34	107.58	125.38	155.60	166.70	115.38	139.78	132.97	123.24 +
2	72.53	110.11	103.85	97.36	156.15	130.88	151.87	64.87	107.36	107.69	125.69 +
	76.15	108.24	123.52	81.54	152.31	94.29	184.07	91.32	101.54	63.19	118.79 +
	76.48	107.14	129.56	93.30	116.59	127.03	139.34	77.25	102.75	126.92	119.56 +
	68.24	109.01	131.87	100.55	116.81	100.33	94.29	82.42	115.93	82.97	121.70 +
3	137.69	109.01	100.00	120.33	99.78	156.70	90.88	113.96	86.15	115.93	87.20 +
	68.02	128.79	97.80	95.49	101.10	117.69	79.89	101.32	105.38	83.30	90.48 +
	86.92	101.87	89.56	106.81	132.75	105.60	109.56	87.03	98.24	99.01	154.47 +
	82.20	92.53	98.35	88.35	122.86	89.34	89.78	94.62	109.89	105.82	264.89 +
4	177.58	130.77	104.07	151.10	120.88	134.73	140.66	81.32	174.62	135.27	78.38 +
	81.98	121.40	117.80	108.68	137.14	110.22	135.93	64.29	96.70	96.68	95.37 +
	93.36	119.78	106.59	76.81	178.02	111.65	131.32	88.57	100.99	100.33	90.16 +
	114.29	139.45	101.65	100.55	125.38	97.91	105.27	113.52	93.41	103.85	114.23 +

5	154.94	109.56	90.99	82.42	115.93	135.05	89.78	98.35	86.81	164.84	51.65
	162.64	58.46	78.35	78.68	127.03	116.48	88.90	73.30	76.37	129.45	65.71
	143.30	92.53	81.43	119.01	150.11	144.51	100.99	109.45	117.91	151.65	62.86
	134.84	120.77	86.26	106.70	178.57	128.24	89.23	112.20	110.99	129.45	60.44
6	208.79	134.62	107.47	144.29	177.58	180.11	157.25	172.75	132.53	129.45	66.48
	168.68	120.55	88.46	126.70	147.25	144.94	153.85	113.30	146.81	177.69	89.78
	218.35	120.88	86.26	111 .54	165.71	140.88	112.09	72.75	102.31	178.02	66.48
	187.36	114.00	107.25	101.10	152.53	94.40	120.88	85.27	124.84	153.08	133.85
7	118.68	163.74	109.45	170.66	183.52	118.13	131.54	189.45	151.65	122.53	196.70
	131.43	146.37	89.67	113.74	132.20	159.01	140.66	141.43	176.48	135.49	220.55
	152.97	125.82	89.01	86.92	182.20	168.13	133.08	133.74	221.98	100.55	272.31
	122.75	116.48	95.71	119.56	147.36	145.49	126.04	117.36	170.44	103.08	229.85*
8	113.63	118.57	106.92	90.66	137.69	145.49	136.26	84.51	121.98	84.29	76.59
	134.84	137.91	92.64	86.81	97.58	132.97	108.57	92.09	125.60	75.27	130.99
	158.79	123.85	83.63	132.53	116.37	107.36	103.19	120.99	131.98	100.22	84.84
	116.37	129.01	78.90	114.62	105.05	91.21	90.99	93.30	107.25	94.29	92.31
mean	124.79	120.87	100.98	109.11	145.97	128.59	124.67	102.45	121.97	117.99	120.75

* sample lost, mean value used for analysis

+ adjusted results (see page 50)

L = Low M = Medium H = High

Table 4

Skin temperatures of animals during short term exposure to different test environments and 15 min later in the standard environment

(Refer to Table 3.6)

Environment		Animal	Skin Temperatures °C	
Dry Bulb °C	Wet Bulb °C		Test Environment	Standard Environment
15	8.5	1	25.5	28.5
		2	25.5	29.0
		3	26.0	29.0
		4	26.0	27.5
		5	29.0	27.5
		6	29.0	28.5
		7	30.0	29.0
		8	31.0	30.0
15	10.8	1	27.0	29.0
		2	28.0	31.0
		3	28.0	30.5
		4	29.0	30.0
		5	28.0	29.0
		6	28.0	29.0
		7	27.5	28.5
		8	28.5	28.0
15	13.0	1	28.5	28.0
		2	28.5	29.0
		3	29.0	29.0
		4	28.5	28.0
		5	29.0	28.5
		6	29.5	29.0
		7	29.5	29.0
		8	29.5	28.5
25	16.4	1	32.0	30.0
		2	32.5	31.0
		3	32.0	30.5
		4	33.0	31.0
		5	31.0	30.0
		6	32.0	30.5
		7	32.5	32.0
		8	33.5	32.0

(Table 4 continued)

Environment		Animal	Skin Temperatures °C	
Dry Bulb °C	Wet Bulb °C		Test Environment	Standard Environment
25	19.5	1	31.5	30.5
		2	32.0	31.0
		3	32.0	31.0
		4	32.0	32.5
		5	30.0	30.0
		6	31.0	32.0
		7	31.5	32.0
		8	31.5	29.5
25	22.4	1	31.5	30.0
		2	32.0	31.0
		3	32.5	31.0
		4	34.0	32.0
		5	32.0	29.5
		6	32.0	31.5
		7	33.0	30.0
		8	32.5	30.8
35	20.0	1	34.0	31.5
		2	34.0	30.5
		3	33.0	30.0
		4	33.0	31.0
		5	35.0	28.5
		6	34.5	28.5
		7	34.5	30.0
		8	34.0	29.0
35	28.2	1	36.0	29.0
		2	35.0	30.0
		3	36.0	31.0
		4	37.0	31.0
		5	35.0	26.5
		6	35.0	28.0
		7	35.0	30.0
		8	35.0	28.5

(Table 4 continued)

Environment		Animal	Skin Temperatures °C	
Dry Bulb °C	Wet Bulb °C		Test Environment	Standard Environment
35	31.8	1	35.0	30.0
		2	35.0	30.0
		3	35.0	29.5
		4	36.0	34.5
		5	35.0	28.5
		6	35.0	30.0
		7	35.0	30.0
		8	35.0	31.0
15	14	1	27.0	27.0
		2	28.0	29.0
		3	30.0	30.0
		4	31.0	29.0
		5	27.0	27.0
		6	27.0	27.0
		7	27.0	27.0
		8	27.0	27.0
40	28.2	1	36.0	32.0
		2	36.0	32.5
		3	35.5	33.0
		4	35.5	33.0
		5	32.0	31.0
		6	32.0	31.0
		7	35.0	33.0
		8	34.0	32.5

Table 5 - The results of Experiment 4

Sebum output over a $3\,^{1}/_{4}$ h period ($mg^{-2}.h^{-1}$.) for long-term exposure experiment

Original date for Fig 3.2 and Table 3.8

Animal	22°C DB 12.5°C WB			35°C DB 24°C WB								
	Days											
	1	2	3	4	5	6	7	8	9	10	11	12
5	106.92	108.02	74.73	80.55	77.36	123.63	83.52	109.34	84.84	113.74	114.84	91.76
	81.54	87.91	76.15	97.47	90.88	103.30	80.22	120.66	92.53	119.12	110.99	105.28
	106.37	57.69	60.77	114.84	102.97	113.74	113.19	139.34	138.13	142.31	107.14	83.19
	108.24	90.66	70.55*	123.96	125.82	100.33	79.78	129.23	137.14	109.89	115.39	102.97
6	126.70	101.17*	113.52	108.57	105.39	162.42	117.80	108.35	190.66	126.26	109.01	116.70
	94.51	79.45	112.31	128.57	102.64	139.56	115.39	140.77	145.93	131.32	120.66	102.75
	92.31	117.25	73.63	82.75	226.04	255.17	184.73	150.11	153.08	155.93	128.24	110.22
	93.19	106.81	83.19	145.38	255.38	202.20	139.12	186.37	151.10	132.31	143.41	103.85
7	111.87	138.79	112.09	141.76	156.26	150.22	121.10	131.98	171.43	153.30	143.19	135.17
	128.79	154.07	120.11	165.93	144.18	109.01	130.33	130.22	154.07	148.79	121.65	128.57
	160.11	134.40	119.56	117.36	151.32	188.24	179.12	132.42	158.02	169.12	136.48	125.60
	154.18	145.06	133.85	159.89	200.77	276.70	199.56	150.11	155.17	184.07	105.49	132.20
8	110.99	112.86	95.82	126.15	123.85	128.57	136.48	70.44	121.65	124.73	121.98	115.17
	107.36	112.09	108.02	108.24	82.42	103.08	94.29	124.51	156.26	145.60	115.60	102.42
	97.47	60.09	67.25	110.99	102.64	129.34	119.01	116.59	112.64	108.24	130.77	114.51
	112.86	116.48	94.73	110.99	142.31	110.66	124.40	106.65	161.21	112.53	134.07	166.48
mean	112.09	107.68	89.43	120.21	136.89	149.76	126.13	127.94	142.74	136.08	122.43	114.80

* sample lost, mean value used for analysis

Tables 6 and 7 - Results of Experiment 5

Table 6

Age of male animals and amount of sebum collected over a period of 3 h.

Refer to Fig 3.3

Age (days)	Wt Sebum ($mg.m^{-2}.h^{-1}$.)	Age (days)	Wt Sebum ($mg.m^{-2}.h^{-1}$.)
56	155.66 173.21 92.62 104.88	56	121.55 131.48 129.48
63	190.99 129.83 97.59 106.20	65	106.98 106.64
213	92.29 104.88 99.47 94.19	213	96.82 137.67 174.32
218	97.37 104.99 107.20 94.83	218	113.82 106.98
462	114.81 152.79 108.19 154.00	469	108.08 156.76 113.38 129.05
472	88.32 83.68 80.70	472	123.87 115.81 112.16 87.99
567	124.20 194.30 127.62 150.80	571	145.61 136.78
575	101.57 111.61 132.59 132.15	579	205.89 183.92 158.97 172.55
582	181.93 134.57 124.64 156.76	590	155.33 119.01 153.67 123.98
590	207.66 152.46 156.32 156.65		

Table 7

Age of female animals and amount of sebum collected over a period of 3 h.

Refer to Fig 3.3

Age (days)	Wt Sebum ($mg.m^{-2}.h^{-1}$.)	Age (days)	Wt Sebum ($mg.m^{-2}.h^{-1}$.)
55	100.13 128.06 119.45 102.43	55	81.58 103.99 88.65 88.32
55	72.75 118.24 82.03 95.94	58	86.73 101.79
230	88.98 96.16 80.26	237	114.92 93.29 100.79
238	111.61 113.16 118.79 122.76	240	149.04 109.62 137.00 121.33
470	105.76 80.04 120.33 110.07	471	72.53 95.71 121.55 133.47
507	132.92 162.62 189.66 180.61	507	131.37 124.97 147.82 144.62
626	107.53 115.70 96.93 94.50	626	115.25 170.12 93.29 122.43
631	214.94 230.29 139.10 100.46	631	120.22 112.16 99.14 131.81

APPENDIX 3
RESULTS OF EXPERIMENTS IN CHAPTER 4

Tables 1-8 The results of Experiment 7

Table 1

Sebum output measured over a 3 1/4 h period ($mg.m^{-2}.h^{-1}$.)
(Refer to Table 3.8)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	155.56	139.21	138.94*	165.54	108.51	123.42	158.77	95.66	107.56
	147.28	153.78	136.48	158.91	141.54	117.54	156.51	111.66	110.43
	157.95	119.04	162.05	184.07	133.61	139.21	158.97	102.29	113.98
	156.44	157.47	118.29	181.47	143.59	119.86	143.79	99.49	125.06
2	119.66	147.76	90.32	136.14	120.96	130.80	173.68	99.15	74.26
	128.34	145.16	95.04	146.94	118.70	145.44	95.18	71.59	108.92
	122.40	141.20	96.07	161.50	140.51	118.91	137.03	86.77	90.44
	146.87	144.68	102.83	120.62	135.52	131.08	71.73	110.91	78.29
3	184.07	162.80	105.64	91.69	133.61	114.12*	153.85*	101.06	78.70
	165.88	142.56	108.99	105.03	126.36	117.95	157.13	98.53	90.73
	183.04	127.66	123.62	158.50	134.29	107.56	150.56	93.40	85.68
	157.74	118.36	140.17	173.40	169.76	116.92	153.85*	109.68	112.62
4	158.22	96.14	68.99	125.40	140.85	127.32	132.72	105.91	99.49
	163.28	117.13	104.82	160.62	132.85	118.63	131.69	128.75	97.09
	113.37	139.28	109.06	140.79	127.79	127.52	150.43	107.76	98.32*
	184.27	117.54*	92.24	175.93	108.17	135.73	113.78	143.52	98.32*
mean	152.77	135.61	112.10	149.16	130.41	124.50	139.98	104.13	98.15

* sample lost, mean value used for analysis

Table 2

Myristic acid output measured over a $3\ ^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.2a and b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	16.48	11.15*	7.30	19.86	15.73	14.63	12.10	18.46	11.25
	21.33	11.15*	11.86	14.77	10.05	15.79	12.82	14.04	10.39
2	12.99	14.94	9.20	13.98	14.53	11.79	18.12	10.68	9.13
	10.94	6.84	9.49	16.96	16.21	7.52	12.99	5.29	10.43*
3	12.17	11.11	12.27	8.24	8.99	13.50	13.61	8.27	10.05
	13.47	12.17	12.88	5.71	18.50	11.25	20.55	2.80	10.94
4	13.61	12.10	39.50	14.26	4.14	12.17	15.93	13.47	10.97
	15.35	9.64	10.22	15.11	11.04	17.33	13.16	11.73	10.43*
mean	14.54	11.14	14.09	13.61	12.40	13.00	14.91	10.59	10.45

* sample lost, mean value used for analysis

Table 3

Palmitic acid output measured over a 3 1/4 h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.2a and b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	11.49	6.29*	6.18	11.79	10.77	8.07	8.96	11.28	8.24
	11.83	6.29*	7.53	10.39	8.17	1.13	9.03	6.26	7.15
2	8.68	8.72	6.15	9.23	9.37	8.14	11.18	6.05	6.12
	6.84	4.72	6.55	10.29	12.31	4.72	8.38	2.94	6.63*
3	9.50	5.61	7.70	3.86	12.24	5.78	10.05	5.74	5.74
	10.63	6.53	9.43	4.07	14.05	6.02	24.72	3.86	5.78
4	10.94	6.94	17.15	8.68	9.37	6.97	9.81	8.92	6.67
	10.26	5.26	8.72	9.68	7.73	8.65	8.58	7.73	6.63*
mean	10.02	6.30	8.68	8.50	10.50	6.19	11.34	6.60	6.62

* sample lost, mean value used for analysis

Table 4

Palmitoleic acid output measured over a $3\ ^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.2a and b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	0.00	0.44*	0.79	0.31	0.00	0.24	1.09	0.24	0.92
	0.00	0.44*	1.03	0.58	0.00	1.57	0.14	0.14	1.09
2	0.00	0.34	0.15	0.51	0.96	1.20	0.85	0.72	0.99
	0.00	0.14	0.75	0.89	0.10	0.03	0.21	0.00	0.65*
3	1.03	0.21	1.03	0.21	1.64	0.27	0.79	1.03	0.21
	1.23	1.16	0.00	0.14	1.57	0.96	0.00	0.00	0.31
4	1.13	0.82	0.00	0.38	1.85	0.21	1.33	0.82	0.41
	0.00	0.07	1.02	0.75	0.89	0.51	0.85	0.89	0.65*
mean	0.42	0.45	0.60	0.47	0.88	0.62	0.66	0.48	0.65

* sample lost, mean value used for analysis

Table 5

Stearic acid output measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.2a and b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	2.39	2.02*	2.44	1.95	2.53	1.50	3.15	2.43	2.22
	1.91	2.02*	2.41	2.12	1.88	2.02	1.98	1.33	2.39
2	1.85	1.95	1.49	1.88	2.74	2.12	1.71	1.50	1.78
	1.50	1.26	2.20	1.64	3.97	1.16	1.91	0.58	1.95*
3	2.19	1.54	2.37	0.92	5.44	1.26	2.36	2.36	1.54
	3.79	3.04	3.62	1.09	2.74	1.64	2.67	1.54	1.64
4	2.32	2.36	1.17	1.50	5.74	1.47	2.74	2.56	2.02
	1.68	1.30	3.45	2.15	1.71	1.95	2.29	2.39	1.95*
mean	2.20	1.94	2.39	1.66	3.34	1.64	2.35	1.84	1.94

* sample lost, mean value used for analysis

Table 6

Oleic acid output measured over a $3\ ^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.2a and b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	1.98	1.23*	1.88	0.55	1.09	0.55	0.79	1.24	1.47
	1.37	1.23*	1.84	1.47	1.20	1.57	1.30	1.01	2.97
2	0.89	1.64	1.05	0.85	0.89	2.39	1.13	0.51	2.12
	0.75	0.75	1.45	0.48	1.88	1.30	1.37	0.38	2.26*
3	1.44	1.26	1.87	0.44	8.75	0.99	1.44	1.75	2.39
	2.63	1.81	0.90	0.89	2.70	0.65	11.90	0.66	2.19
4	2.05	1.20	0.45	0.51	10.56	0.89	1.88	1.39	2.46
	0.79	0.62	2.87	0.68	1.91	0.82	1.47	1.61	2.26*
mean	1.49	1.22	1.54	0.73	3.62	1.15	2.66	1.07	2.26

* sample lost, mean value used for analysis

Table 7

Linoleic acid output measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.2a and b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	4.10	3.45	1.74	3.79	3.76	3.11	2.70	4.41	3.83
	3.76	3.45*	2.70	4.79	3.32	4.82	3.49	2.09	3.93
2	3.01	5.03	3.04	4.58	3.01	3.86	3.93	2.12	2.22
	2.80	3.52	2.51	4.24	4.44	3.59	4.75	0.96	3.62*
3	3.62	3.32	3.71	1.98	6.94	3.11	4.03	1.37	4.38
	3.28	3.79	3.72	2.15	4.07	2.19	4.79	0.65	3.90
4	3.73	2.39	2.31	3.49	7.59	3.90	3.56	2.80	3.49
	3.42	2.56	2.81	3.66	3.62	4.68	3.73	2.46	3.62*
mean	3.47	3.44	2.82	3.59	4.59	3.66	3.87	2.11	3.62

* sample lost, mean value used for analysis

Table 8

Total weight of fatty acids measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(The basis of Table 4.2b)

Animal	15°C			25°C			35°C		
	L	M	H	L	M	H	L	M	H
1	36.44	24.46*	20.34	38.28	33.91	28.10	28.77	38.08	27.90
	40.21	24.46*	27.36	34.12	24.61	26.94	28.80	24.87	27.92
2	27.42	32.60	21.09	31.09	31.50	29.49	36.94	21.56	22.37
	22.84	17.22	22.96	34.55	38.94	18.29	29.60	10.13	25.54*
3	29.96	23.06	28.95	15.67	44.00	24.90	32.24	20.55	24.29
	35.07	28.52	30.56	14.04	43.64	22.70	64.59	9.50	24.74
4	33.72	25.77	60.57	28.83	39.24	25.63	35.28	29.98	26.01
	31.50	19.48	29.09	31.99	26.90	33.97	30.06	26.84	25.54*
mean	32.15	24.45	30.12	28.57	35.34	26.25	35.79	22.69	25.54

* sample lost, mean value used for analysis

Tables 9-16 The results of Experiment 8

The effect of long-term exposure of a warmer environment on sebum output and the individual fatty acids in the sebum

Table 9

Sebum output measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Fig 3.2)

Animal	Day			
	1	2	3	4
1	152.51 129.74 144.41 150.87	91.28 156.10 123.38 125.74	129.33 126.36 126.67 122.15	138.77 142.56 143.90 135.28
2	78.67 85.44 89.33 102.56	109.54 112.31 73.85 88.41	118.87 138.47 129.95 145.23	130.36 117.64 116.82 114.36
3	102.87 81.23 71.08 108.82	118.87 115.90 103.38 110.15	120.72 126.36 95.69 130.05	102.46 112.51 115.18 157.74
4	80.41 64.31 100.10 76.92	153.44 103.28 113.23 109.85	154.87 130.56 138.26 129.44	142.87 133.85 138.46 157.85
mean	101.20	113.04	128.94	131.29

Table 10

Output of myristic acid measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.4a and b)

Animal	Day			
	1	3	10	11
1	18.96 10.82	1.65 16.30	12.09 12.22	18.04 Lost
2	12.96 19.62	19.02 14.70	13.37 18.84	11.76 Lost
3	11.76 16.42	12.30 12.81	13.41 24.06	Lost 16.25
4	Lost 14.86	16.39 17.97	20.11 17.92	Lost Lost
mean	14.44		16.19	

Table II

Output of palmitic acid measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.4a and b)

Animal	Day			
	1	3	10	11
1	12.24 7.24	8.00 8.23	8.96 8.93	8.38 Lost
2	8.92 9.72	8.04 7.33	8.12 11.58	7.74 Lost
3	6.25 8.98	6.69 5.53	8.39 10.22	Lost 7.52
4	Lost 8.99	8.56 10.58	8.96 9.68	Lost Lost
mean	8.35		8.95	

Table 12

Output of palmitoleic acid measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.4a and b)

Animal	Day			
	1	3	10	11
1	0.00 0.00	0.00 0.00	0.31 0.95	0.00 Lost
2	0.00 0.00	0.15 0.15	1.24 0.28	1.28 Lost
3	0.00 0.00	1.29 0.00	1.35 1.44	Lost 1.31
4	Lost 0.09	1.44 0.00	0.56 1.17	Lost Lost
mean	0.20		0.90	

Table 13

Output of stearic acid measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.4a and b)

Animal	Day			
	1	3	10	11
1	4.68 2.56	1.77 1.54	4.66 3.25	1.71 Lost
2	1.73 1.95	2.04 1.38	2.78 4.02	2.79 Lost
3	11.07 3.00	2.89 0.95	3.20 2.11	Lost 1.31
4	Lost 4.93	3.10 2.33	2.78 3.39	Lost Lost
mean	3.06		2.91	

Table 14

Output of oleic acid measured over a $3\,^{1}/_{4}$ h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.4a and b)

Animal	Day			
	1	3	10	11
1	2.94 1.45	1.98 1.57	3.67 3.39	2.24 Lost
2	3.64 4.68	1.74 1.46	3.08 6.96	3.00 Lost
3	2.80 4.36	3.19 0.76	3.76 3.03	Lost 3.35
4	Lost 3.48	3.37 2.07	4.85 4.06	Lost Lost
mean	2.63		3.76	

Table 15

Output of linoleic acid measured over a 3 1/4 h period ($mg.m^{-2}.h^{-1}$.)

(Refer to Tables 4.4a and b)

Animal	Day			
	1	3	10	11
1	3.86 2.53	2.89 2.94	4.71 5.21	2.66 Lost
2	4.38 3.79	2.53 2.48	2.89 4.05	5.44 Lost
3	2.24 1.76	2.05 1.93	3.50 4.04	Lost 4.19
4	Lost 3.53	2.48 3.10	3.68 4.25	Lost Lost
mean	2.83		4.06	

Table 16

Total weight of fatty acid measured ($mg.m^{-2}.h^{-1}$.)

(The basis of Table 4.4b)

Animal	Day			
	1	3	10	11
1	42.67 24.61	16.29 30.58	34.39 33.75	33.04 Lost
2	31.63 39.78	33.52 27.49	31.50 45.73	32.01 Lost
3	34.12 34.52	28.40 21.97	33.62 44.89	Lost 35.54
4	Lost 35.87	35.33 36.05	40.94 40.49	Lost Lost
mean	31.52		36.9	

APPENDIX 4

RESULTS OF EXPERIMENTS IN CHAPTER 5

Tables 1-3 Results for Experiment 9

Table 1

Sebaceous gland measurements after one stimulation of the glands
(Refer to Table 5)

Time (h)	Animal	Sebaceous gland volume (ml/m^2)	Cell Number x 10^9 (per m^2)	Cell Volume ($mm^3 \times 10^{-6}$)	No of metaphases x 10^6 (per m^2)
0	1	29.37	6.94	4.23	15.36
	2	30.26	7.39	4.09	10.93
	3	36.30	10.46	3.47	15.85
	4	21.97	7.72	2.85	5.96
	mean	29.48	8.13	3.66	12.03
1.5	1	27.34	8.00	3.42	*
	2	39.20	9.98	3.93	*
	3	29.50	9.92	2.97	*
	4	27.42	8.76	3.13	*
	mean	30.87	9.17	3.36	
3	1	22.42	7.17	3.13	13.55
	2	35.38	9.73	3.64	19.67
	3	29.38	9.80	3.04	8.31
	4	22.40	7.97	2.81	4.86
	mean	27.51	8.67	3.16	11.60

* not measured

Control values for the number of metaphases in the sebaceous glands/m^2 of skin at 1200 h.

Results taken from 4 different animals

	5.52×10^6
	6.12×10^6
	9.13×10^6
	7.43×10^6
Mean	7.05×10^6
S.E.	$\pm 1.60 \times 10^6$

Table 2

Amounts of sebum obtained after one stimulation of gland from each of the 4 experimental animals

Animal	Weight of sebum (g/m^2)
1	Lost 0.371
2	0.373 0.426
3	0.666 0.444
4	0.387 0.337
mean	0.429

Table 3

Skin measurements for the mean values given in Experiment 9

A: Number of hairs per skin specimen: an estimate of the number of sebaceous glands per skin specimen

Skin Specimen

1	2	3	4
269	233	245	226
224	220	255	235
272	226	256	215
265	199	253	206
242	232	231	203
234	228	268	247
200	219	259	221
246	214	237	189
236	214	199	179
256	216		221
231			205
211			210
			208
			212

Mean number of hairs per skin specimen 228 ± 3.3 (S.E.)

Number of hairs per m^2 of skin = $12.46 \times 10^6 \pm 0.18 \times 10^6$

Table 3 continued

B Skin Dimensions

Width of hair follicle μm			Width of hair μm	Depth of sebaceous gland from skin surface μm
35.71	17.86	19.64	14.29	357.14
42.86	28.57	42.36	32.14	321.43
53.57	57.14	64.29	32.14	350.00
64.29	57.14	42.86	35.71	267.86
25.00	21.43	32.14	25.00	335.71
28.57	25.00	57.14	17.86	357.14
17.86	25.00	35.71	14.29	285.71
57.14	71.43	60.71	21.43	392.86
14.29	28.57	32.14	14.29	232.14
14.29	14.29	25.00	10.71	232.14
53.57	64.29	60.71	28.57	357.14
28.57	28.57	32.14	14.29	250.00
57.14	42.86	50.00	35.71	350.00
25.00	21.43	28.57	28.57	314.29
35.71	35.71	28.57	14.29	232.14
60.71	60.71	57.14	14.29	214.29
28.57	35.71	35.71	28.57	314.29
17.86	32.14	39.29	25.00	350.00
39.29	35.71	25.00	14.29	214.29
25.00	35.71	35.71	21.43	250.00
25.00	17.86	14.29	14.29	292.86
14.29	14.29	17.86	10.71	214.29
25.00	32.14	28.57	21.43	339.29
71.43	53.57	64.29	39.29	535.71
89.29	71.43	78.57	50.00	264.29
21.43	14.29	10.71	14.29	267.86
14.29	14.29	14.29	10.71	285.71
46.43	50.00	42.86	28.57	339.29

Table 3 continued

B Skin Dimensions continued

Width of hair follicle μm			Width of hair μm	Depth of sebaceous gland from skin surface μm
25.00	42.86	42.86	28.57	303.57
17.86	17.86	32.14	28.57	285.71
17.86	32.14	28.57	17.86	357.14
32.14	35.71	28.57	30.36	275.00
42.86	39.26	28.57	35.71	246.43
14.29	25.00	35.71	32.14	321.43
28.57	17.86	28.57	10.71	267.86
21.43	21.43	32.14	25.00	285.71
14.29	28.57	25.00	32.14	310.71
28.57	32.14	42.86	35.71	339.29
32.14	25.00	25.00	35.71	371.43
53.57	64.29	67.86	42.86	357.14
14.29	10.71	14.29	7.14	214.29
50.00	50.00	39.29	28.57	357.14
28.57	32.14	28.57	14.29	250.00
28.57	21.43	28.57	14.29	260.71
39.29	53.57	50.00	46.43	321.43
64.29	71.43	67.86	35.71	321.43
42.86	39.29	46.43	14.29	242.86
		125.00	78.57	357.14
		92.86	39.29	278.57
		21.43	17.86	200.00
mean	36.8		25.71	300.7
S.E.	± 1.4		± 1.8	± 8.6

Tables 4 and 5 Results for Experiment 10

Table 4

Amounts of sebum obtained at the end of each 3 h period (g/m^2)

(Refer to Table 5.2)

Animal	Periods				
	1	2	3	4	5
1	0.484 0.438	0.288 0.479	0.501 0.477	0.492 0.371	0.355 0.375
2	0.417 0.341	0.424 0.248	0.235 0.181	0.205 0.188	0.323 0.270
3	0.432 0.423*	0.244 0.338	0.210 0.252	0.249 0.291	0.298 0.346
mean	0.423	0.337	0.309	0.299	0.329

* sample lost, mean value used for analysis

Table 5

Sebaceous gland measurements at 12 and 15 h after washing the skin every 3 h

(Refer to Table 5.3)

Time	Animal	Seb. G. Vol. (ml/m^2)		Cell Number x 10^9 (per m^2)		Cell Volume ($mm^3 \times 10^{-6}$)		No of metaphases x 10^6 (per m^2)	
		Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
12	1	66.96	28.25	14.42	7.92	4.65	3.57	*	*
	2	37.17	43.92	9.97	9.70	3.77	4.53	*	*
	3	69.64	41.63	17.55	10.64	3.97	3.98	*	*
	mean	57.92	37.93	13.95	9.36	4.13	4.03		
15	1	56.97	28.34	15.58	8.82	3.66	3.21	14.81	8.85
	2	45.05	30.03	11.15	7.22	4.04	4.16	11.20	10.60
	3	21.47	39.32	8.10	8.38	2.65	4.69	3.55	5.19
	mean	41.16	32.57	11.61	8.14	3.45	4.02	9.85	8.21

* not measured

Table 6 The results of Experiment II

The correlation between sebum output and DNA output

(Original results for Fig 5.1, 5.2 and Table 5.4)

Time	x ($mg.m^{-2}$) Sebum output	y ($mg.m^{-2}$) DNA output
0 hr	206.524	0.0000
	292.264	0.0167
	287.635	0.0167
	210.271	0.0167
	223.716	0.0251
mean	244.082	0.0150
1 hr	306.370	0.0167
	331.937	0.0251
	279.921	0.0084
	265.814	0.0084
	182.279	0.0000
	217.545	0.0251
	191.757	0.0835
	261.627	0.0000
mean	254.656	0.0209
2 hr	212.255	0.0752
	202.116	0.0668
	195.063	0.0418
	244.214	0.0668
	169.275	0.1002
	167.512	0.0752
mean	198.406	0.0710
4 hr	400.265	0.0501
	438.616	0.0251
	372.493	0.0418
	393.652	0.0251
	379.987	0.0334
	415.473	0.0334
	443.465	0.0418
	455.367	0.0167
mean	412.415	0.0334

Table 6 continued

Time	x ($mg.m^{-2}$) Sebum output	y ($mg.m^{-2}$) DNA output
8 hr	460.657	0.0251
	483.580	0.0167
	460.877	0.0501
	420.983	0.0167
	482.257	0.0418
	459.996	0.0668
	446.991	0.0251
	484.902	0.0585
mean	462.530	0.0376
12 hr	429.138	0.1086
	387.481	0.0501
	371.170	0.1670
	353.097	0.1670
	364.558	0.1587
	405.554	0.1002
mean	385.166	0.1253
18 hr	460.216	0.1587
	522.813	0.0835
	528.543	0.0418
	480.273	0.0585
	391.007	0.0668
	414.591	0.0668
mean	466.241	0.0794
21 hr	640.071	0.0501
	644.038	0.0835
	654.838	0.0919
	592.903	0.0418
	473.000	0.1169
	467.930	0.1336
	389.244	0.0251
	487.106	0.0668
mean	543.641	0.0762

Table 6 continued

Time	x ($mg.m^{-2}$) Sebum output	y ($mg.m^{-2}$) DNA output
	401.587	0.1002
	424.069	0.0835
	469.032	0.0668
24 hr	394.975	0.0668
	387.260	0.0668
	533.833	0.1587
	465.726	0.0668
mean	439.497	0.0871

Table 7 The results of Experiment 12

The correlation of DNA against sweating

(Original results for Fig 5.3 and Table 5.5)

Temperature	X sweating $g/m^2/hr$	Y DNA mg/m^2
10°C	13.176	0.001
	13.176	0.007
	12.504	0.018
	12.504	0.000
	6.664	0.001
	6.664	0.000
	1.125	0.004
	1.125	0.008
mean	8.367	0.005
20°C	33.313	0.007
	33.313	0.006
	29.556	0.007
	29.556	0.010
	6.158	0.004
	6.158	0.000
	14.778	0.015
	14.778	0.008
mean	20.951	0.007
30°C	206.240	0.008
	206.240	0.013
	135.760	0.014
	135.760	0.011
	106.979	0.018
	106.979	0.028
	157.262	0.013
	157.262	0.017
mean	151.560	0.015
40°C	162.020	0.008
	162.020	0.014
	166.970	0.014
	166.970	0.015
	197.940	0.015
	197.940	0.018
	225.890	0.018
	225.890	0.025
mean	188.205	0.016

APPENDIX V

PUBLICATIONS BY THE AUTHOR

SMITH, Miranda E. and JENKINSON, D. McEwan (1975). The effect of age, sex and season on sebum output of Ayrshire calves. J. agric. Sci., Camb., 84, 57-60.

SMITH, Miranda E., NOBLE, R.C. and JENKINSON, D. McEwan (1975). The Effect of Environment on Sebum Output and Composition in Cattle. Res. vet. Sci., 19, 253-258.

SMITH, Miranda E. and JENKINSON, D. McEwan (1975). The Mode of Secretion of the Sebaceous Glands of Cattle. Br. vet. J., 131, 610-618.

STUDIES IN THE SECRETORY FUNCTION OF THE SKIN OF RUMINANTS

By

MIRANDA EVELYN SMITH B.Sc.

Sebum forms a natural barrier-layer on the outside of the animal which is believed to assist in thermoregulation, affect water loss from the epidermis and acts as a deterrent against skin pathogens. In some species such as the ox, sebum and sweat emerge as a mixture and the barrier-layer may be in the form of an emulsion. A knowledge of the factors which affect the integrity of this layer is essential to a full appreciation of its function. The current studies were therefore initiated to investigate in cattle the effects of various factors including environment on the mode and the rate of production of the skin surface lipid and its composition.

A method to determine sebum output in the thoracico-lumbar region of Ayrshire calves was developed. Sebum was collected from pre-cleaned areas of skin after a given period of time, usually 3 h, using a specially designed cup in conjunction with the solvent methanol. The collected sebum was then extracted from the methanol and weighed on a micro-balance.

Complete replacement of the skin surface lipid film in thermoneutral

conditions, after cleaning the skin with methanol, took over 24 h although most of it was replaced during the first 12 h. Sebum apparently did not flow across the skin surface even when it was damp illustrating that the thickness and integrity of the surface layer is largely dependent on local sebum production.

Short-term changes in the environment had no consistent effect on sebum extraction. However, sebum output tended to be lower in winter than throughout the rest of the year and on prolonged exposure to a warm environment there was a small but significant increase in sebum output. This was unlikely to have had an appreciable effect on evaporative heat loss and hence it was concluded that bovine sebaceous glands, unlike the sweat glands, are not of major importance to the regulation of body temperature in the heat.

Age had little effect on sebum output in either castrated male or intact female calves but the output in castrated males tended to be greater than in females.

The composition of bovine sebum was similar to that of other species in that it contained phospholipid, free cholesterol, unesterified fatty acids, triglycerides, diester waxes, cholesteryl esters and squalene. However, more triglyceride was present than was found in a previous study on cow skin surface lipid. Lauric, palmitic, palmitoleic, oleic, stearic and linoleic acids were found to be present in the sebum in varying concentrations. On prolonged exposure to a warm environment there was an increase in sebum

output and in the percentage of linoleic acid which was found mainly in the triglyceride fraction. The increase in linoleic acid output is of particular interest since this essential fatty acid has been shown to have bacteriocidal properties and is involved in water loss through the skin surface. Short-term exposure to different air temperatures and humidities had no appreciable effect on the fatty acid composition of bovine sebum.

The mode of secretion of the sebaceous glands was investigated by measuring sebum output and sebaceous gland volume and cell number per m^2 of skin after stimulation of the glands by cleaning the skin with methanol. These measurements were compared with estimates of glandular mitotic activity. None of these quantities was altered by a single or by repeated stimulation and the number of cells necessary to produce the sebum by a holocrine mechanism greatly exceeded the estimated level cell production. Sebum output was not associated with the DNA content of skin washings but sweat output was; the DNA was probably derived from sweat. It was concluded that the mode of secretion of cattle sebaceous glands was unlikely to be holocrine. It was suggested that the secretion of sebum may be a more complex mechanism than previously supposed. Sebum may not be solely a product of cell degeneration but could conceivably result from a process involving lipogenesis and secretion from live cells.

The studies have illustrated the importance of local sebum production to the integrity of the surface lipid layer and have demonstrated that sebum

formation is more complex than previously supposed. They have shown that short-term changes in temperature and humidity have little effect on the rate of production of the surface lipid or its composition but that appreciable alterations occur in both output and composition on prolonged heat exposure and with season.

J. agric. Sci., Camb., 1975, 84, 57-60.

Res. vet. Sci., 1975, 19, 253-258.

Br. vet. J., 1975, 131, 610-618.