STUDIES IN DEPILATION

II.* STRUCTURAL CHANGES IN THE WOOL FOLLICLE DURING BACTERIAL WOOL LOOSENING ("SWEATING")

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Summary

The changes in the various structural components of the wool follicle during the "sweating" process were followed by histological staining of sections prepared from the skin at appropriate intervals. Tissue breakdown starts in the lower part of the outer root sheath, progresses up the sheath, and ultimately involves the epidermis. The epidermis usually separates from the underlying dermis at a certain stage in the depilation process before the epidermal cells start to disintegrate. The gradual breakdown of the cells of the wool root bulb is an integral part of the wool-loosening process. The inner root sheath, the elastic tissue, and the fat cells are all broken down during depilation, but this is incidental to, and not the cause of, the loosening of the wool fibre. Sulphated mucopolysaccharides are gradually removed from the skin during depilation.

It is established that bacteria actually penetrate the skin tissues during the process, but their distribution within the skin appears to be random.

I. INTRODUCTION

A knowledge of the structural changes which take place in the wool follicle during depilation would shed some light on the nature of the chemical processes involved, and materially assist in the development of new depilatory systems. Several studies have been made of the changes that take place during wool or hair loosening on various types of skins. Wilson and Daub (1921) suggested that destruction of the malphigian layer was principally responsible for wool loosening, but the work of Ellis (1945) indicated that degradation of the cells of the wool root bulb is more likely to be responsible. Giffee *et al.* (1965) found that after enzyme treatment the first noticeable changes were seen in the basal cell area of the epidermis, and changes occurring in the basement membrane area have been held primarily responsible by other workers for wool loosening (Bose, Madhava Krishna, and Das 1955; Burton, Reed, and Flint 1955; Everett and Cordon 1958; Lindroth 1961; Stirtz 1965). This theory is supported by the work of Delcamp (1963) and Giffee *et al.* (1965) who show photomicrographs of sheets of intact epidermis separating from the dermis. Whatever the initial stages in the loosening process, there is little doubt that the hair or wool only becomes free as a result of total degeneration of the follicle structure (Dempsey 1940; Ellis 1945; Whiteley 1960; Lindroth 1961).

The work of Maxwell (1945) and the Mazamet Chamber of Commerce Laboratories (1961) has shown that a large number of bacterial strains develop during the "sweating" of sheepskins. Only a few of these, however, actively contribute to

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wool loosening. Maxwell (1945) and Green (1955) state that bacterial penetration in the follicle is observed during the last stages of sweating and Dempsey (1940), states that in the putrefaction of ox-hide, the bacteria had completely penetrated the skin after 48 hr, and the follicles were lined with bacteria. Whiteley (1960) concludes that the bacteria penetrate from the flesh side of the skin and proliferate around the follicles. Millet (1963), however, states that bacterial penetration in the follicle was not seen at the end of the loosening process, the cultures developing only on the surface of the skin.

The present paper reports the changes that take place in the follicle tissues of sheepskins as a result of which wool loosening occurs. An attempt is also made to establish the necessity for penetration and the site of multiplication of the bacteria responsible.

II. Materials and Methods

The sheepskins used in the experiments were selected to be as free from fat as possible.

(*a*) *Design of Experiments*

Samples of skin 3 × 3 in. were taken from position 2/(P_2) (Yates 1965) on a number of green skins. These were "sweated" at 28°C and the depilation loads* taken at appropriate intervals as described previously (Yates 1964). In order to get a more gradual decline in the depilation load, sweating was effectively stopped overnight by placing the sweating jars in the cold room. As each depilation load was taken, squares of sides 0·5 in. were taken from the centre of the sample. The small squares were divided into two and the fibres were removed from one piece. For histological staining and analysis a sample was chosen which gave a steady gradation in depilation load, in order that the gradual changes in the follicle structure could be seen clearly. After the histological processing, longitudinal and transverse sections of each block (with and without wool) were cut and stained by the methods outlined below. The root ends of the detached fibres were also stained. Serial transverse sections were made so that the structural changes that normally occur within the follicle should not be allowed to confuse the results.

Bacterial counts were made through the depth of the skin sections, in 200 randomly chosen positions at each stage of depilation. A graticule used in conjunction with the oil-immersion objective was moved stepwise over the skin section from the flesh side, and the number of bacteria in each square was counted. In this way an idea of the distribution of the bacteria in the skin was obtained.

(*b*) *Histological Techniques*

For staining glycogen, fat, and elastic tissue, blocks were fixed with the appropriate fixative and sectioned in the frozen state. For the other staining techniques, the blocks were placed immediately in 10% formalin in 0·1M phosphate buffer at pH 6·5, and fixed for 48 hr. After this time, they were wax-embedded, and subsequently sectioned and stained. The staining techniques used were as follows:

(1) A modification of Auber's (1950–51) SACPIC method suggested by Clarke (personal communication, 1964) was used for general structural staining. This technique gives excellent differentiation between the various structural components of the follicle, which stain as follows: nuclei, black; epithelium, pale green; inner root sheath, red; prekeratinized cortex, red; keratinized cortex, yellow; stratum corneum, green; and collagen, sharp blue. The root ends of the fibres were stained by the same procedure.

* The depilation load is a measure of the force required to remove the fibre from the follicle, and is defined as the force in grams weight required to detach a staple of which 1·5 cm weighs 1 mg.

(2) Periodic acid–Schiff (PAS) technique was used for polysaccharides (Hotchkiss 1948).

(3) Alcian blue technique was used for mucopolysaccharides (Carlo 1963).

(4) Kirkpatrick method was used for bacteria in sections (Browning and Mackie 1949).

(5) For glycogen staining, blocks were fixed in Lison and Vokaer fixative and sections stained with Best's carmine stain (Pearse 1960).

(6) For elastic tissue staining, blocks were fixed in formalin and sections stained with Verkoeff's stain (Pearse 1960).

(7) For fat staining, blocks were fixed in formalin and sections stained by the method of Kay and Whitehead (Pearse 1960).

All sections were cut at a thickness of 10 μ except those containing glycogen, elastic tissue, and fat. These sections were cut at 40 μ.

III. Results and Discussion

(*a*) *Disappearance of Glycogen from the Outer Root Sheath Cells*

The presence of glycogen in the cells of the outer root sheath (ORS) is well known (Chase 1954), and in fact many cells of the ORS contain so much glycogen that there is little or no cytoplasm left (Montagna, Chase, and Hamilton 1951). In sections of sheepskins fixed and stained immediately after the death of the animal, dense deposits of glycogen are seen in the cells of the ORS immediately above the bulb. The disappearance of glycogen from these cells is the first sign of any degenerative or destructive activity taking place in the skin, and the glycogen disappears long before structural changes take place in any component parts of the follicle or the epidermis. This observation supports those made by the SACPIC method of staining (see later), which suggest that the first site of any degenerative activity leading to depilation occurs in the cells of the ORS in the level immediately above the bulb.

Nevertheless the disappearance of the glycogen may be due to autolytic activity, and not therefore a part of the depilation process in other than a coincidental manner. This possibility was tested by taking samples of skin straight from the slaughtered animal and placing them in 0·2% merthiolate (Yates 1964) which would stop bacterial activity very quickly. The skin samples were then given a 5 Mrad dose of γ-radiation which would give complete sterilization. If the glycogen still disappeared after this treatment, it would be as a result of autolytic activity and not of bacterial action. In every experiment, the glycogen deposits were quite intact after a 5-day period (i.e. longer than the time required for complete "sweating") following the sterilization pretreatment, showing that the disappearance of glycogen from the normal skin is an indication of bacterial and therefore depilatory activity. In a separate series of experiments autolytic processes have been shown to play a negligible part in the overall wool-loosening process.

It is unlikely that the breakdown of the glycogen is an integral part of the depilation process, and it is probably simply an indication of the commencement of the production of extracellular enzymes by the bacteria, some of which ultimately are active in the wool-loosening process.

(*b*) *Structural Changes in the Epidermis and the Outer Root Sheath for Various Depilation Loads*

Figure 1 shows the structural details of the lower half of an intact wool follicle before depilation starts. Unless otherwise stated, the following comments apply to the sections before removal of the fibre. Changes in the sebaceous gland structures are not referred to in detail, but the cellular degeneration and disappearance of nuclear staining closely follows that of the ORS at that level. A depilation load of 2 is taken to be a commercially acceptable level of wool looseness. The times quoted are actual sweating times and do not include times spent in refrigeration.

(i) *Depilation Load 81·9 (i.e. fresh control skin)*

The cells of the ORS and the follicle bulb are all clearly distinguishable, and have distinct well-defined nuclei. It is of interest that the sections with the wool removed show that the removal of the fibre at this stage does not cause any damage to the ORS or the epidermis.

(ii) *Depilation Load 58·5 (17 hr)*

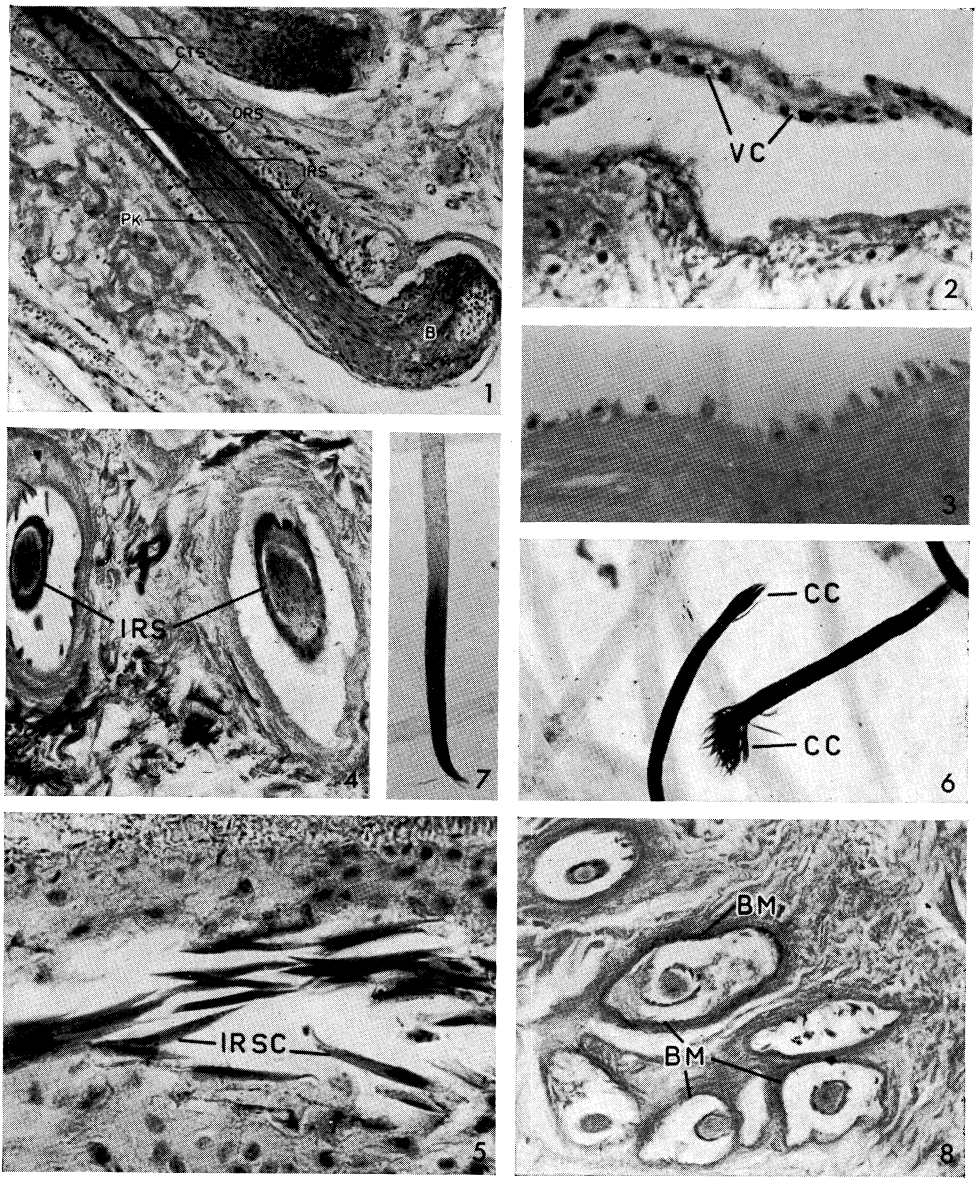
The epidermis and the upper part of the ORS are both intact and quite clearly nucleated, but the nuclei of the cells in the fibre bulb and the lower portion of the ORS are slightly less intensely stained than at the previous depilation load, and the cells of the ORS in the immediate vicinity of the bulb show signs of disintegration. There is some evidence of cell death in a few cases in the basal layer of the ORS in the lower part of the follicle as indicated by the pycnotic nuclei and the appearance of large vacuoles in the cell cytoplasm.

(iii) *Depilation Load 30·9 (30 hr)*

The epidermis is still quite intact and clearly nucleated, but the nuclei of the ORS cells do not stain as heavily at this stage and are less regular and more pycnotic. This effect increases markedly from the top to the bottom of the follicle and the cells in the inner region of the ORS at the level of and immediately above the bulb, are quite disintegrated. The cells of the bulb also show distinct signs of disintegration. Some of the columnar basal cells of the ORS have large vacuoles and it is at these points that separation of the ORS from the connective tissue layer is occurring. In sections with the wool removed it can be seen that at this stage, too, fibre removal takes place without any damage to the upper ORS and epidermal structures. Giffee *et al.* (1965) find that after enzyme treatment of cow-hide, the first noticeable changes are seen in the basal cell areas of the epidermis. The present results do not confirm that similar changes take place in sheepskin when wool loosening is brought about by bacterial processes.

(iv) *Depilation Load 21·0 (36 hr)*

The epidermis is still essentially intact and clearly nucleated but a number of the cells are beginning to take on an irregular structure and some have pycnotic



Figs. 1–8.—Showing changes in structural components of the wool follicle during the depilation process. *1*, Lower half of intact wool follicle. *CTS*, connective tissue sheath; *PK*, prekeratinous zone; *IRS*, inner root sheath; *ORS*, outer root sheath; *B*, wool root bulb. *2*, Separation of the epidermis from the dermis. *VC*, vacuolating cells. *3*, Intact basal cells still adhering to the basement membrane after most of the epidermis has separated. *4*, Inner root sheath (IRS) left in position around the fibre after complete breakdown of the outer root sheath structure. *5*, Disintegration of the IRS into spindle-shaped cells (IRSC). *6*, Filamentous cortical cells (*CC*) at end of fibre. *7*, Characteristic tapered appearance of root end at the completion of wool loosening. *8*, Basement membrane (*BM*) still intact at the end of the depilation process.

nuclei. There are a few instances of separation of the epidermis from the grain layer and these occur in areas where the basal cells are becoming vacuolated and have pycnotic nuclei (Fig. 2). Stirtz (1965) comments that the cohesion between the epidermal cells is dissolved later than the adhesion of the bases of the basal cells to the basement membrane, and the present work confirms this.

However, marked changes have occurred in the ORS. In the lower half of the follicle it has completely lost its nuclear staining and is quite amorphous, while in a number of cases it has completely degenerated leaving the fibre surrounded only by the inner root sheath (IRS). The cells of the follicle bulb are also at an advanced stage of degeneration. In the top part of the follicle above the level of the IRS, the ORS is quite intact and for the most part clearly nucleated, although a few cells have pycnotic nuclei. The upper ORS and the epidermis are not damaged by the removal of the fibre even at this stage.

(v) *Depilation Load 9·4 (55 hr)*

The epidermis is seen in a number of stages of breakdown. In some sections it is quite intact and clearly nucleated, while in others it is apparently intact but no nuclei can be seen. Elsewhere it is flaking off in either a nucleated or denucleated condition, and in some sections has disappeared altogether. The points where the intact epidermis is flaking off are again those where vacuolation of the basal cells has occurred. In a few cases where the partially denucleated epidermis is flaking off, some intact basal cells are left attached to the grain layer (Fig. 3).

In the majority of follicles the lower half of the ORS has gone completely, and only the faint outlines of the denucleated cells of the follicle bulb can be seen. Where there is some ORS left in the lower half of the follicle, it is detached from both the connective tissue sheath and the IRS. The upper half of the follicle is seen with an intact ring of ORS material with or without nuclei. Dempsey (1940) finds that with ox-hide after 10 days putrefaction the fibre is still surrounded by the top of the root sheath. In every follicle it is clearly seen that the lower half of the ORS is much more disintegrated and less nucleated than the upper half. Transverse sections with wool in show the fibre still, in most cases, with an intact ring of IRS separated from a partially or completely denucleated ORS. Removal of the fibre at this stage does not disturb either the epidermis or the upper half of the ORS, irrespective of the stage of disintegration. Whiteley (1960), Giffee *et al.* (1965), and Stirtz (1965), the latter two working with enzyme depilation, all state that at some stage of the depilation process the entire follicle structures can be removed with the fibre. This phenomenon was not observed at any stage of this work, and in no cases were ORS and epidermal material withdrawn with the fibres.

(vi) *Depilation Load 4·5 (80 hr)*

The epidermis is missing altogether in many places while in others it is flaking off as a denucleated whole. The ORS is also completely denucleated but an amorphous mass can still be seen in many places, especially in the upper part of the follicle. The fibre bulb maintains its shape at this stage but the cell outlines are

very faint and the cell nuclei have disappeared. Transverse sections with the wool removed show that the wool can be removed even at this stage without disturbing what is left of the ORS.

(vii) *Depilation Load 2·0 (94 hr)*

The epidermis has completely flaked off and many of the follicles contain no trace of any ORS material at all, while the fibre bulb is a mass of amorphous material. Occasionally there is a ring of completely amorphous material around the top of the follicle. In no cases are there any nuclei present at this stage. After fibre removal at this depilation level the follicles contain only an amorphous mass of cell debris.

(c) *Structural Changes in the Inner Root Sheath*

(i) *Depilation Load 81·9*

IRS can be seen clearly in both transverse and longitudinal sections, and the cell nuclei are quite distinct. Sections with the wool removed show that the IRS is still in position and mostly intact, indicating that the IRS does not normally come out with the fibre. On some of the fibres, however, a small section from the top of the IRS is sometimes withdrawn with the fibre.

(ii) *Depilation Load 58·8*

Sections at this stage show essentially the same features as at the previous stage but there is a suggestion in a few follicles that the IRS is becoming separated from the ORS. Sections with the fibres removed also show a slight incidence of fragmentation of the IRS into spindle-shaped cells.

(iii) *Depilation Load 30·9*

The incidence of fragmentation is more frequent and there is a definite, though small number of examples of separation of the IRS from the ORS. In the sections with the fibres removed the vast majority of follicles still have an intact IRS, but removal of the fibres results in an increased incidence of fragmentation. This fragmentation is no doubt due to the disruptive effect of the fibre removal, but the substances cementing the IRS cells together appear to be broken down as one of the incidental processes of depilation. In a few cases the IRS is splitting longitudinally into its two components, the Henle and the Huxley layers—the Huxley layer being removed with the removal of the fibre, but this is by no means a general phenomenon. Transverse sections at this stage again indicate that the ORS is being separated from the IRS.

(iv) *Depilation Load 21·0*

In many cases the IRS has become completely detached from the ORS in the lower half of the follicle, but is still for the most part intact around the fibre (Fig. 4).

The IRS is showing signs of disintegration into spindle-shaped components in an increasing number of follicles.

In the sections with the wool removed many of the follicles are free of IRS material completely, while others have IRS debris either in the form of fragmented spindle-shaped cells or a distorted ring. Dempsey (1940) also finds that with ox-hide, bacterial putrefaction results in the breakdown of the IRS to threads which subsequently break down into shorter segments.

(v) *Depilation Load 9·4*

Separation of the ORS and IRS is in most cases complete, and a large number of the IRS show some degree of fragmentation.

Sections with the fibres removed show the lower part of most of the follicles to be completely free of IRS material, while near the top of the follicle there is a residue of disintegrated IRS in the form of spindle-shaped cells which are still nucleated.

(vi) *Depilation Load 4·5*

The IRS is still largely intact but in a number of sections is separating away in the intact state from the ORS. Transverse sections show that most of the fibres still have a ring of IRS material in various stages of disintegration surrounding them.

Sections with the wool removed show that many of the follicles are free of IRS material, while others still have a thin ring or remnants of a ring in the middle of the follicle. This indicates that even at this stage of the wool loosening process the fibre can be pulled out independently of the IRS. After wool removal some follicles contain the intact remains of the IRS, while others contain the completely disintegrated IRS in the form of the spindle-shaped cells.

(vii) *Depilation Load 2·0*

Most of the IRS remnants clinging to the fibre have fragmented into the spindle-shaped cells, but a few IRS's are quite intact around the fibre. Sections with the fibres removed show that all the IRS material is disintegrated into the characteristic cells (Fig. 5). It is interesting that Kuntzel and Stirtz (1958) find that such fragmentation occurs in cow-hide only after chemical depilation and does not occur with enzyme depilation. Rogers (1964) states that IRS cells are dissolved by the action of trypsin, but it is apparent that the proteolytic enzymes involved in depilation only separate the cells, and do not digest the cells themselves.

(*d*) *Appearance of the Root End of the Pulled Fibre*

(i) *Fibres Removed from Fresh "Green" Skins*

The appearance of the root ends of the fibres after removal from the skin can give valuable information concerning the components of the follicle which are

responsible for anchoring the fibre. The breakage point of the fibres and their points of detachment from the follicle are found to vary in a complex manner with the type of skin and the manner of fibre removal.

Examination of the root ends of the fibres following depilation load measurements (i.e. fibres slowly withdrawn vertically from the skin), showed that irrespective of the skin type, it was exceptional for bulb material to be removed with the fibre, and skin sections stained with SACPIC showed intact bulbs still in position, and also that the fibre breakage point was usually just above the bulb level. These observations differ from those of Ellis (1945) who found that fibres removed from the "green" skin usually had the inverted ORS and IRS and the major part of the bulb with them. This was never observed in the present work.

The manner of removal of the fibres affects the appearance of the root ends. Fibres were removed from a medium crossbred skin in three different ways, slowly in a vertical direction, slowly in a lateral direction, and rapidly by snatching in a vertical direction. The fibres removed slowly in both the lateral and vertical directions have the same appearance. In less than 10% of the fibres in these groups is the IRS removed with the fibre. In all cases the fibre breaks off just above the neck of the follicle bulb. At the root end of each fibre there are seen filamentous cortical cells (Fig. 6) which stain blue, below the level of the keratogenous zone.

However, fibres that have been snatched out quickly in a vertical direction have a totally different appearance. In the vast majority of cases the IRS is completely removed together with a small amount of bulb material. As a consequence of this none of the blue-staining cortical filamentous cells are seen. This observation was independent of skin type.

It appears, therefore, that there are two possible planes of cleavage, depending on the manner of removal of the fibre. With a slow movement it seems that the interlocking scales on the fibre and the IRS can deform sufficiently to permit fibre removal without causing structural damage to the follicle. On the other hand a rapid movement does not permit the scales to unlock and the plane of cleavage is then between the ORS and the IRS.

(ii) *Fibres Removed at Various Stages of the Depilation Process*

Whatever the state of the fibre immediately after removal from the fresh skin, the depilation process produces fibres which show progressively less follicular material with time. Towards the end of depilation, the fibres show the characteristic tapered appearance (Fig. 7).

(e) *Changes in the Elastic Tissue Structure*

The elastic tissue fibres are seen only in the top half of the follicle area, and in the control section are extended and attached at both ends. As the depilation load gets lower an increasingly large number of elastic fibres are detached at one end and have contracted. However, even at a load of 4·5, the majority of the elastic fibres are still intact. Only after 45 hr and a load less than 2 does the elastic tissue

become disorganized, and some fragmentation into short pieces occur, but even at this stage it is still present and none of it seems to be removed during depilation. These observations are in agreement with those of Giffee *et al.* (1965), but differ from those of Lindroth (1961) who finds that there is no elastic tissue remaining in the epidermal area after depilation by enzyme treatment. It is interesting that neither Cordon *et al.* (1961) nor Morihara, Oka, and Tsuzuki (1965) found any correlation between the elastase activity of enzyme preparations and their ability to depilate.

It is clear that any changes in the elastic tissue only take place some time after most of the cell destruction required for wool loosening has occurred, and it is concluded that any such changes in the elastic tissue are incidental to and not responsible for wool loosening.

(*f*) *Changes in the Fatty Components of the Tissue*

Before a depilation load of 21 is reached there is no evidence of any breakdown in the cellular structure of the fatty areas, and these are still clearly defined in globules. The glands at the top of the follicles are still quite intact, and there is no evidence of any spreading of the fat. At a load of 9·4 there are indications that the fat globules at the base of the follicles are beginning to disperse, but only at lower loads do the fat cells in the gland areas break up. It seems, therefore, that the fat storage cells are not broken up until the very late stages of the depilation process.

(*g*) *Changes in the Mucopolysaccharide Components of the Skin*

The Alcian blue technique stains chondroitin sulphuric acid a blue-green colour. There is a slight overall take up of Alcian blue throughout the connective tissue, and particularly in the connective tissue sheath surrounding the follicles. This highly positive zone spreads throughout the entire width of the connective tissue sheath and there is no particular localization just outside the ORS, nor under the epidermis. As depilation proceeds there is a gradual reduction in the general staining intensity with Alcian blue which suggests that the positively staining mucopolysaccharides are slowly disappearing from the tissues. In particular, the intensity of staining in the collagen sheath is reduced, and towards the end of the depilation process, it is equal to that of the rest of the connective tissue. Everett and Cordon (1958) also find that sulphated mucopolysaccharides are removed from the skin during depilation by enzyme treatment. The theoretical and practical implications of the disappearance of chondroitin sulphate from the tissues during wool loosening are open to speculation.

The role played by the hyaluronic acid, one of the constituents of the ground substance of the skin, in depilation will be the subject of further investigation.

(*h*) *Changes in the Basement Membrane*

In the control sections there is a thin narrow band of PAS-positive material situated immediately below the epidermis and continuous around the entire follicle between the connective tissue sheath and the ORS which completely separates the

follicle and the epidermal components of the skin from the collagenous parts. In the present work it is difficult to distinguish any details of the structure of this PAS-positive layer, and with the histological techniques used it cannot be decided whether it is a structurally distinct membrane or simply a concentration of some polysaccharide type material at the dermo-epidermal junction. However, in longitudinal sections of some follicles, this layer appears to be in the form of a network, and for convenience it will be referred to as the basement membrane. (Glycogen deposits can, of course, also be detected by this technique, but this has been dealt with previously.)

The existence of an intensely PAS-positive zone under the epidermis and around the ORS has been observed previously by a number of workers (e.g. Stoughton and Wells 1950; Everett and Cordon 1958). Leblond (1951) suggests a relationship between the thickness of the basement membrane and the intensity of the metabolic exchanges across such a layer from the subadjacent connective tissue, areas of high metabolic activity, such as the base of the follicle bulb, having a very thin membrane. It is thought that most basement membranes either contain fibres with a carbohydrate matrix or consist of some form of a carbohydrate complex (Gersh and Catchpole 1960).

Throughout depilation there is no overall decrease in the amount or change in the distribution of PAS-positive material and the staining is still as intense and sharply defined at the end of the process as it is in the control sections. In the subepidermal areas, where the epidermis is still present, the membrane can be seen easily, but where the epidermis has flaked off it is more difficult to detect. However, it can be clearly seen in transverse sections of the follicles at the end of the process (Fig. 8). It is highly significant that the entire degenerative processes resulting in depilation have taken place outside the basement membrane and have not produced any detectable changes in the membrane itself. Giffee *et al.* (1965) comment that the structures at the epidermal basal cell junction with the underlying dermis are of importance in the mechanism of depilation, and that there is a need to elucidate the nature of the molecular substances which provide the natural adhesion of the epidermis to the dermis. It is also relevant that Stirtz (1965) comments that "It seems that the loosening of hair or epidermis by enzymes is based on the decomposition of the dermal membrane, and possibly of the overlying layer between dermal membrane and cell membrane of the basal cells", and Mukerjee, Sri Ram, and Pierce (1965) state that the basement membrane is dissolved only by reagents which degrade it. The present work, however, shows that the destruction of the basement membrane is not a necessary part of the wool-loosening process, and gives weight to the suggestion of Giffee *et al.* (1965) that the dissolution of the cementing material between basal epidermal cells and basement membrane is the key step in the depilation process.

(*i*) *Location and Direction of Penetration of Bacteria in the Skin during Depilation*

There are two possible alternative theories for the activities of the bacteria during depilation. Millett (1963) and the work of the Mazamet Chamber of Commerce Laboratories (1962) suggest that there is no actual bacterial penetration in the skin, and that the bacteria multiply only on the skin surface and secrete enzymes which diffuse to the site of action, while other workers (Dempsey 1940; Maxwell 1945;

Haines 1953; Green 1955) favour the alternative theory which postulates that bacteria actively migrate through the skin, multiply *in situ*, and secrete enzymes which then perform their function in a more localized area.

The results of the bacterial counts show unequivocally that while there is an expected build up of bacteria on the flesh surface as depilation proceeds, large numbers of bacteria actually penetrate the skin tissues and become distributed evenly throughout the entire skin thickness. The penetration appears to be quite random in nature and in the vast majority of cases no localized build-up of bacteria is observed in the immediate vicinity of the follicles. The results also show that penetration is entirely from the flesh side, and penetration from the wool side or down the follicle plays no part in the wool-loosening process. It has been suggested (Whiteley 1960) that bacteria penetrate from the flesh side and multiply in the immediate vicinity of the wool follicle. This might be expected because degradation of the follicle tissues is occurring, and bacterial activity would probably centre around the areas where food is plentiful, but this was not observed in the present work.

It is not possible to say whether the actual penetration of the tissues is primarily responsible for depilation, or whether this is merely incidental to the major effect of diffusion of extracellular enzymes produced by enormous numbers of bacteria on the flesh surface.

There are two factors which would play some part in influencing the movement of bacteria through the skin tissues. These are the natural motility of the particular bacterial species, and its ability to depolymerize the viscous ground substance of the skin of which hyaluronic acid is one of the main components. Indeed the work of the Mazamet Chamber of Commerce Laboratories (1961) concludes that the bacterial strains which cause the most rapid depilation are those which multiply rapidly, are very motile, and possess the ability to hydrolyse protein and "polyglucides". If the depilatory action of the bacteria is due to their penetration into the skin, then it is obvious that, all other things being equal, the species which are most highly motile will give the most rapid depilation. It would be impossible to decide unequivocally by experiment whether or not penetration is primarily responsible for depilation but in an experiment on sterile skin (5 Mrad dose of γ-radiation), inoculated with pure cultures of *Serratia marcescens* and a strain of *Pseudomonas*, histological examination showed that after 24 hr *Pseudomonas* had completely penetrated the skin and become evenly dispersed throughout, while *S. marcescens* had penetrated only to about the level of the follicle bulbs. (Here again there was no localization of either species around the follicles). The depilation loads of both samples were equal after 24 hr. This points to motility not being of prime importance, but it is not possible to be dogmatic about this because there are many other unknown factors, e.g. the composition of the enzyme mixture secreted by both species.

All the bacteria that have been shown to be associated with the "sweating" process (Chambard and Azemar 1932; Maxwell 1950; Green 1955; Toyoda and Futami 1957; Mazamet Chamber of Commerce Laboratories 1961) are motile or, if the particular species is not identified, belongs to a genus, the majority of whose members are motile.

IV. Conclusions

The degradative processes necessary for the loosening of the wool fibre in the follicle during the "sweating" process have been established. It is apparent that for the fibres to be loosened the cell structure of the ORS and the fibre bulb must be destroyed. The epidermis is separated from the dermis and is usually completely degraded in the depilation process, but whether this is necessary for depilation to occur is not possible to determine from the present work. The IRS is not necessarily disintegrated during the process and it is possible in some cases to remove the fibre with an intact IRS at the conclusion of the process. In other cases, the IRS is fragmented into spindle-shaped cells. However, it seems that breakdown of the IRS is not an integral part of the fibre-loosening process. The elastic tissues of the skin are also broken down as an incidental process to wool loosening. The entire degradative processes associated with wool loosening take place outside the basement membrane, which is still clearly defined at the end of the process.

There is no doubt that bacteria penetrate the skin tissues during wool loosening but they do not gather selectively in the immediate vicinity of the follicles and their distribution in the skin appears to be quite random. It is not possible to separate the parts played by the diffusion of extracellular enzymes and actual invasion of the skin by the bacteria in the loosening process.

V. Acknowledgment

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