STUDIES IN DEPILATION

IV.* STRUCTURAL CHANGES IN THE WOOL FOLLICLE DURING DEPILATION WITH ALKALI AND ALKALINE REDUCING SYSTEMS

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Summary

Structural changes in the wool follicle during depilation with sodium sulphide and with ammonia are described. Sodium sulphide exerts its principal action on the prekeratinous zone of the wool fibre, but also dissolves the lower part of the outer root sheath (ORS). The fibre breaks off in the prekeratinous zone and is easily removed with virtually no disturbance of the upper ORS or the epidermis, leaving a degraded bulb still in position. Ammonia solution (1M) causes a severe disruption of the cell structure of the ORS and some disorganization of the prekeratinous zone, but does not cause sufficient protein dissolution to permit depilation to go to completion.

I. Introduction

Sodium sulphide is probably the oldest established and undoubtedly the most widely used chemical for depilating sheepskins. It has the advantage of speed compared to the bacterial "sweating" method, but it suffers from the disadvantage that up to 4% of the wool is lost in the process and, in addition, the disposal of the waste liquors creates severe problems.

Alkaline non-reducing systems have long been known to cause depilation of hides and skins, and Lennox (1945) discusses the rapid depilatory effect of ammonia on sheepskins. Unfortunately, however, this effect stops short of the commercially acceptable level of wool looseness. Two patents have subsequently been taken out covering the depilatory effect of ammonia in the presence of certain additives (Strandine, Connick, and Oldenburg 1958; Pelissier 1966), but neither of these are widely used commercially. Other amine compounds are also known to have a depilatory effect on hides and skins (Moore 1933; Lennox 1945), and dimethylamine in conjunction with sodium hydroxide is used at present in the United States of America in a commercial unhairing process (Somerville 1966).

A knowledge of the chemical and structural changes involved in wool loosening in the alkaline system would be of value in designing a more efficient depilatory agent. It is desirable to establish the reasons why wool loosening stops short of a satisfactory level with the ammonia system, and also to attempt to devise some method which will take advantage of its rapid partial loosening effect and complete the loosening quickly.

The present paper describes the structural changes that take place in the wool follicle during depilation with sodium sulphide and with ammonia.

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II. Materials and Methods

The sheepskins were selected to be as free from fat as possible.

Samples of skin 3 by 3 in. were taken from a position centrally situated on the backline (position P_2, Yates 1965) on the "green" skins. Preliminary work had shown that there was no positional variation in the structural changes taking place. In the case of sodium sulphide depilation, a paste of 10% sodium sulphide in a saturated lime suspension (pH 12·5) was applied to the flesh side of the skins, and the samples were incubated in sealed jars at 28°C. Depilation loads* were taken at appropriate intervals as described previously (Yates 1964).

As each depilation load reading was taken, half-inch squares were removed from the centre of the sample, processed, and prepared for histological examination following wax embedding as described previously (Yates 1968*a*).

In the case of the ammonia depilation, the skin samples were soaked in 1M ammonia solution at 28°C and depilation loads taken at 30 min, 8 hr, and 24 hr. Small samples were removed and processed as in the case of the sulphide-painted samples.

The histological techniques used for demonstrating the structural features of the follicle were the modified SACPIC method for general structural staining, and the periodic acid–Schiff (PAS) technique for polysaccharides. The details of these techniques have been described previously (Yates 1968*a*).

III. Results

(*a*) *Structural Changes in the Wool Follicle after Treatment with Ammonia*

Treatment of sheepskins with dilute ammonia (i.e. 0·1–1·0M) is well known to cause a rapid loosening of the wool. The structural changes in the follicle which cause this wool loosening occur rapidly and are substantially complete in half an hour. The depilation load after this time drops to *c.* 5–10 and prolonged exposure to ammonia solution does not cause any further reduction. The changes described here are those which have taken place over a 40-min exposure time during which the depilation load dropped to 5·7. Subsequent changes with increased exposure times, which do not contribute to wool loosening, will be mentioned briefly.

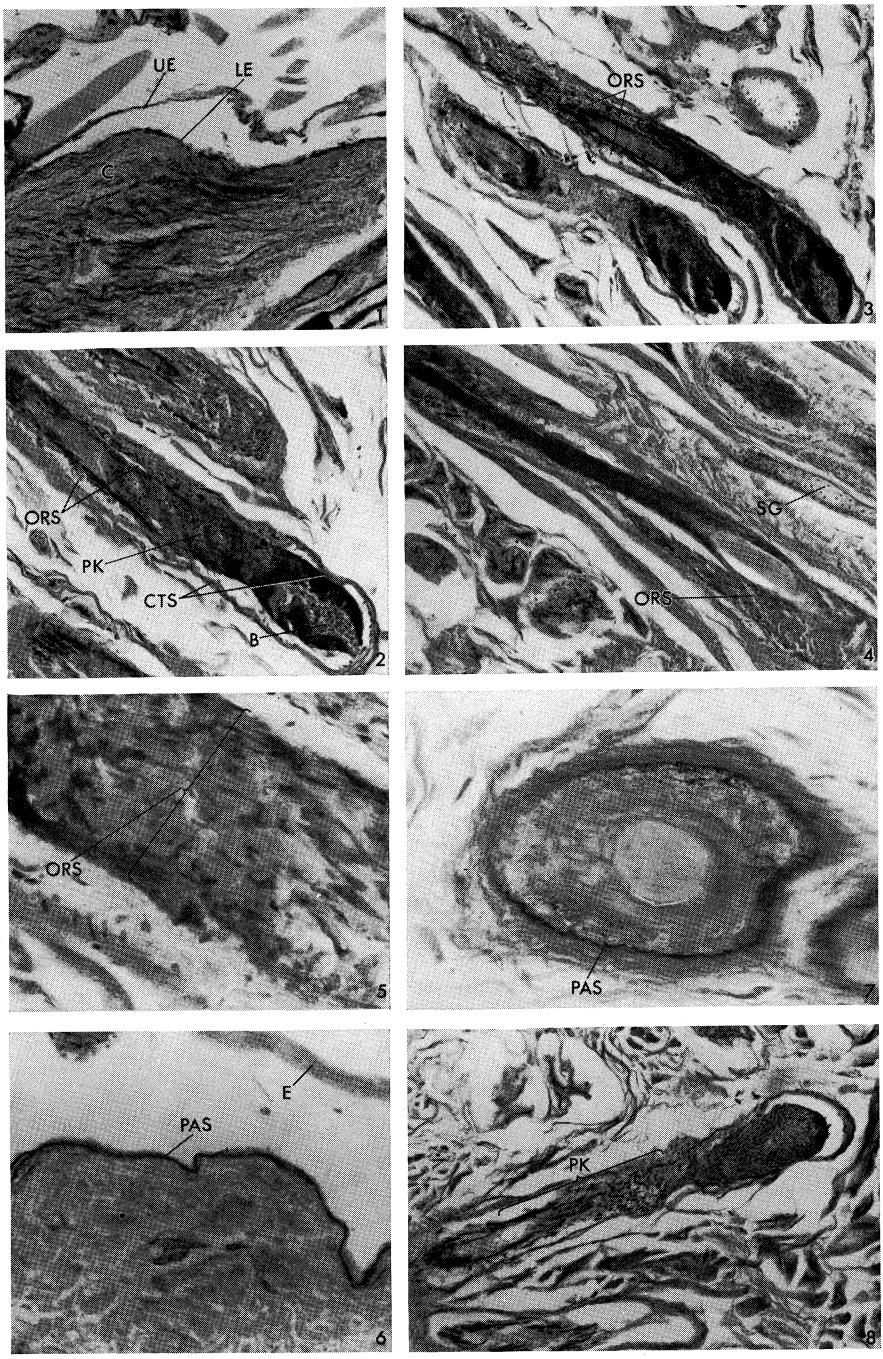
The epidermis appears compressed and is much thinner than in the control skins, but there is no reduction in staining intensity of the epidermal cells. In many places the epidermis has undergone a layerwise splitting with the upper layers of the

* 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. A depilation load of 2·0 is regarded as indicating completion of the wool loosening process, and a fresh green skin has a depilation load of *c.* 80.

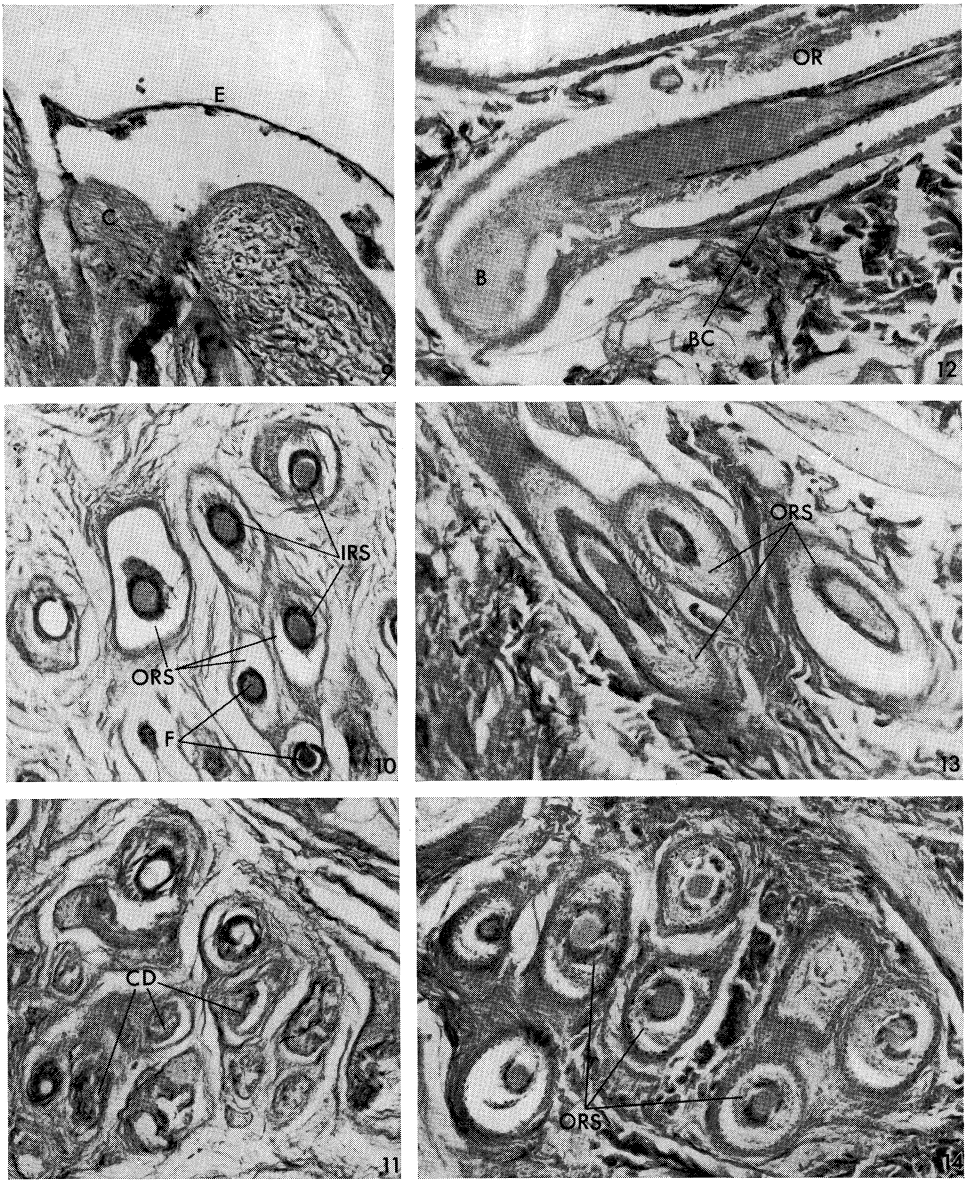
CTS, connective tissue sheath. Note also the disruption of the ORS and the prekeratinous zone. **3** and **4**, Destruction of the ORS with loss of detailed cell structure and nuclear integrity. Note intact cell structure of the glands in Figure 4. SG, sebaceous gland. **5**, High power view of ORS showing general loss of structure and in particular nuclear breakdown.

Figs. 6 and 7.—PAS-positive zone after prolonged (24 hr) treatment with ammonia. **6**, Zone under the epidermis. **7**, Zone shown in transverse section across follicle. E, epidermis; PAS, positive-staining zone.

Fig. 8.—Ammonia depilation, depilation load 5·5 (24 hr). General loss of structure of prekeratinous area. PK, prekeratinous zone.



Figs. 1–5.—Ammonia depilation, depilation load 5·7 (30 min). **1**, Epidermis splitting layerwise with the upper layer of cells flaking off in an intact sheet, and the lower layer of cells remaining firmly attached to the corium. UE, upper epidermis; LE, lower epidermis; C, corium. **2**, Separation of the follicle bulb from the connective tissue sheath. B, bulb; PK, prekeratinous zone;



Figs. 9–11.—Ammonia depilation, depilation load 5·5 (24 hr). **9**, Complete separation of the epidermis from the corium after longer exposure time. C, corium; E, epidermis. **10**, Transverse section showing intact IRS and destruction of ORS. F, fibre. **11**, Transverse section after fibre removal showing absence of IRS and disorganized ORS debris. CD, outer root sheath cell debris.

Figs. 12–14.—Sulphide depilation, depilation load 9·0 (6 hr). **12**, Longitudinal section of follicle showing destruction of ORS and loss of nuclear staining in the bulb. Note layer of basal cells adhering to CTS. B, bulb; BC, basal cells; OR, outer root sheath remains. **13** and **14**, Transverse section of lower part of follicle (**13**) showing loss of structure of ORS, and upper part of follicle (**14**) showing a much less degree of disintegration.

epidermis separating completely, leaving the basal cell layer still in position attached to the dermis (Fig. 1). This phenomenon has been observed previously with human skin (Baumberger, Suntzeff, and Cowdry 1942). Removal of the fibre at this stage does not cause any further disruption of the epidermis.

Considerable destructive changes have taken place in the follicle itself and the follicle bulb has in most cases become detached from the connective tissue sheath (CTS) (Fig. 2). The lower part of the follicle bulb is still quite intact and the nuclei are quite distinct and clearly stained, but the upper part is in very poor condition with much nuclear fragmentation. The cell outlines of the outer root sheath (ORS) have completely disappeared and the nuclei are no longer distinct (Figs. 3 and 4), but the cell remains of the ORS have not separated clearly from the CTS, and it appears that any separation is due to the general destruction of the ORS cells. The nuclear membrane appears to have broken, and the nuclear material, which still stains intensely, is spreading throughout the remains of the cell cytoplasm (Fig. 5). The cells of the sebaceous glands are quite intact and have their normal structure although a few of the nuclei are slightly pycnotic (Figs. 3 and 4).

The inner root sheath (IRS) is not affected by the ammonia and is usually removed in its intact state with the fibre on depilation. In the few cases where the IRS is left behind after fibre removal, it is usually fragmented in the process to characteristic spindle-shaped cells (Yates 1968*a*). It seems that the intercellular binding cement is destroyed by the ammonia while the cells themselves remain intact. The fact that the fibre can be completely withdrawn with an intact IRS is further evidence that breakdown of the IRS is not necessary for satisfactory wool loosening.

PAS staining clearly shows the positive-staining band in the control skin underneath the epidermis and surrounding the follicle. After treatment with ammonia the PAS-positive zone is still intact, and even prolonged treatment does not affect either the chemical or physical integrity of this zone (Figs. 6 and 7). As is the case with bacterial wool loosening (Yates 1968*a*), the entire depilatory process takes place outside this PAS-positive layer.

The root ends of the fibres removed from ammonia-treated skins have in every case a tapered appearance, and the fibre has broken off just above the level of the bulb at the lower end of the prekeratinous zone.

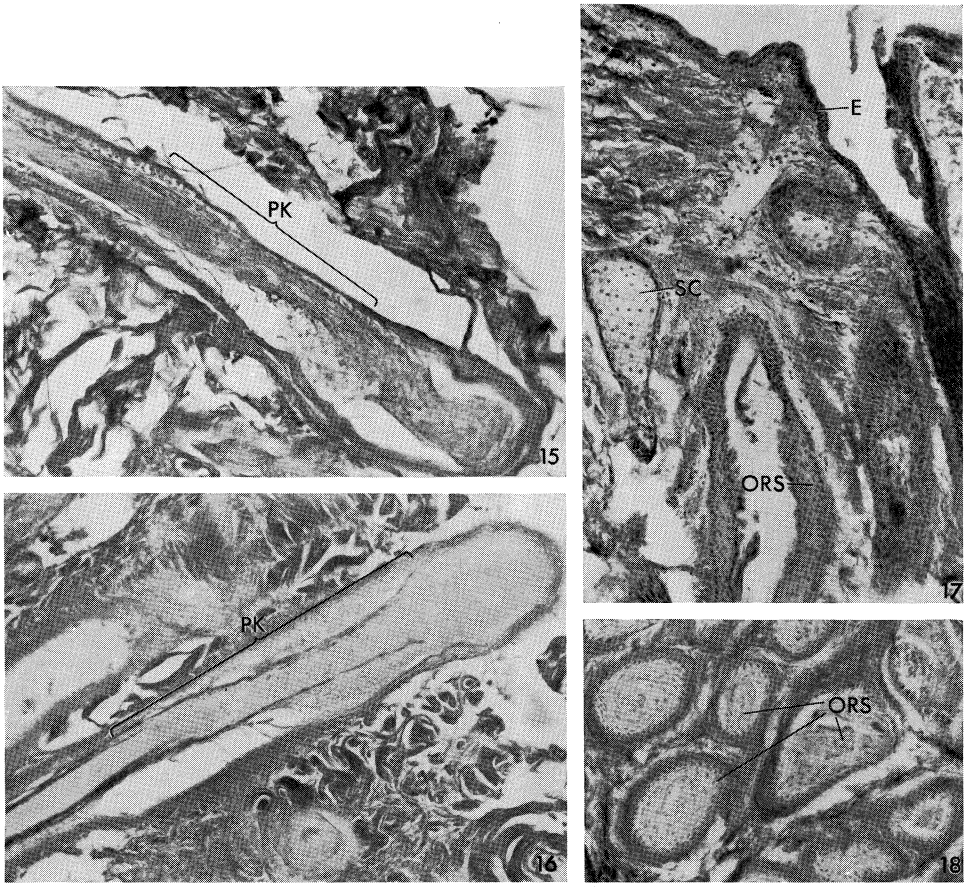
One of the most notable features of the ammonia treatment is that the cells in the prekeratinous zone are affected in the same way as those of the ORS. The nuclei and cell structure disappear and the area becomes amorphous and no longer takes up the red stain (Fig. 8).

After exposure for 8 hr to the ammonia solution the entire epidermis has completely separated from the dermis (Fig. 9). The cell structure of the ORS has completely disintegrated with hardly any trace of nuclear material remaining, but even after exposure for 24 hr the cellular integrity of the glands is intact and the nuclei are staining clearly. The nuclei of the bulbs are also staining quite clearly after 24 hr, but the bulb itself is contracted and has shrunken away completely from the CTS.

The IRS is essentially undamaged and is still clearly nucleated even after the long exposure times (Fig. 10). It is usually withdrawn with the fibre, leaving the

follicle full of green-staining amorphous ORS debris only (Fig. 11). The fibre bulb remains in position and is never withdrawn with the fibre, which always breaks off in the lower prekeratinous zone.

Two major degenerative changes occur in the follicle which are probably responsible for wool loosening. These are the degeneration and partial disintegration of the ORS cells and the softening and partial dissolution of the prekeratinous zone.



Figs. 15–18.—Sulphide depilation, depilation load 0·4 (24 hr). **15** and **16**, Longitudinal section of follicle showing destruction of the ORS and the deterioration of the prekeratinous areas. PK, prekeratinous zone. **17**, Intact epidermis and upper ORS. Note intact cells of the sebaceous gland SC. E, epidermis. **18**, Transverse section of follicle showing nucleated ORS debris.

Separation of the epidermis does not seem to be a major factor because the depilation load does not change with time, although the complete epidermis only flakes off on longer exposure times. The fibre invariably breaks off in the prekeratinous zone where weakening has occurred, and the resistance of fibre removal is due to the force required to break the fibre in the softened area, and that required to remove the fibre (and usually the IRS) against the physical resistance to the debris in the

follicle. In many of the follicles the ORS debris can be seen puckered up at the top of the follicle, and it is apparent that the sheer volume of debris will create a physical barrier to fibre removal. The damage caused in the follicle structure by ammonia is very rapid, but is not extensive enough to permit fibre removal, and prolonged exposure does not produce any further cell destruction.

(*b*) *Structural Changes in the Follicle after Treatment with Sodium Sulphide at pH 12·5*

In the first 2 hr following the application of the sulphide to the flesh side, there is no change in the upper part of the follicle structure. There are suggestions in many follicles that the bulb is shrinking away from the CTS and also that there is a slight drop in the staining intensity of the nuclei of the bulb. There are, however, definite indications of deterioration in the cells of the prekeratinous zone.

After treatment for 6 hr, at a depilation load of 9·0, the changes in follicle structure are much more profound. The ORS in the lower part of the follicle is completely digested away, but it is of interest that a single layer of basal cells is in most cases left in position where the ORS has disappeared (Fig. 12). Nuclear staining has nearly disappeared from the follicle bulb although the bulb maintains its overall integrity (Fig. 12). Transverse sections taken in the lower half of the follicle show the ORS in different stages of deterioration, and the accompanying loss of nuclear staining intensity (Fig. 13), while a transverse section taken about mid-follicle level shows much less disintegration and distinct nuclear staining (Fig. 14). The IRS is not affected by the treatment at this stage. The epidermis is still intact, shows no sign of separation, and is not dislodged by removal of the fibre. If the fibre is removed at this stage it breaks off in the prekeratinous zone, leaving the partly destroyed bulb in position, but usually removing the IRS with it. In many cases the ORS material is puckered up at the top of the follicle after fibre removal.

After 24 hr, at a depilation load of 0·4, there are marked changes in the lower half of the follicle structure but surprisingly little change in the epidermis and the upper part of the follicle. Nuclear staining has disappeared entirely from the bulb region, and the prekeratinous zone is completely amorphous, although both maintain their shape. The ORS has almost completely disappeared from the bottom half of the follicle (Figs. 15 and 16). Figure 17, however, shows the epidermis and the upper half of the follicle in a quite intact condition. The fibre at this stage breaks off in the prekeratinous zone and is completely removed with the IRS leaving the follicle full of nucleated ORS debris (Fig. 18). The epidermis can easily be removed in sheets at this stage in spite of the fact that histological examination does not show any separation. The PAS-positive zone is still quite intact at the end of depilation, and its chemical and structural integrity are not affected by prolonged treatment with alkaline sulphide. As in the case of ammonia depilation, and bacterial depilation (Yates 1968*a*), the entire depilation process takes place outside the PAS-positive layer.

Although in the experiments described the fibre separated at the prekeratinous zone at the completion of depilation, examination of a commercial sulphide process showed that the fibre is usually destroyed to approximately the level of the skin surface, indicating that there is a greater destruction of the wool fibre than is necessary for satisfactory wool loosening.

IV. Discussion

A comparison of the sulphide depilation process with the ammonia process shows a number of important differences. Although ammonia causes disruption of the cells of the ORS it does not appear to cause any cell dissolution and consequently the sheer volume of the ORS debris remaining contributes a large part of the physical resistance to fibre removal. Sulphide at pH 12·5 causes considerable dissolution of the ORS cells, but more important, however, is its effect on the prekeratinous zone, where its ability to disrupt the partially organized structure by its reducing effect gives an almost zero resistance to breaking in this area. This effect, coupled with a lowered resistance in the ORS, permits ready removal of the fibre. It is not possible to separate or evaluate these two factors independently because they occur concurrently, but it is likely that the weakening of the prekeratinous zone is the more important of the two, and that the fibre could be withdrawn with considerably less structural damage to the ORS than is actually seen. The ammonia by itself does not cause sufficient weakening of the fibre, and this, coupled with its inability to dissolve the cells of the ORS, prevents the depilation load from falling to an acceptably low level.

In dealing with these depilatory systems there are three major factors to be considered, namely, the splitting of the disulphide bonds by the reducing agents, the pH of the system, and the specific effect of the uncharged ammonia molecules. It is possible in some degree to isolate the effects of these factors, but the picture is complicated by the possibility that the various structural parts of the follicle may react concurrently in different ways to the different factors. In alkaline reducing systems there is little doubt that depilation is caused by a primary reduction of disulphide bonds followed by some degree of dissolution of the reduced proteins in the alkaline solution (see Merrill 1956). The effect of alkali itself at the same pH, i.e. a saturated lime suspension, does not cause any wool loosening on short term treatment (i.e. time comparable to that taken by sulphide depilation), although, of course, longer exposures do eventually cause wool loosening (cf. the old liming process of the tanning industry).

The depilatory effect of amines has long been known (see Moore 1933), and Lennox (1945) has investigated the optimum conditions for depilation with ammonia. One important point of difference between these systems and the reducing systems is that the amines are applied as a bath and not as a paint. Merrill (1956) states that the depilatory effects of ammonia and of other amines are not the same, but the actual mechanism of either depilatory effect is not known. The effect of dimethylamine on the depilation process has been stated to be simply catalytic, and it has been shown (McLaughlin, Highberger, and Moore 1927, 1928) that the amine concentration is effectively not changed during the depilation process. Lennox (1945) has shown, in the case of ammonia, that the depilatory effect is a specific one of the NH_3 species rather than of the NH_4^+ species, or being merely a pH effect.

The histological studies show that the major effect of the ammonia treatment is a partially destructive one on the ORS and the upper part of the follicle bulb, with some degree of degenerative changes in the prekeratinous zone of the fibre, similar to those occurring after treatment with sulphide. However, amines or

ammonia are not known to cause scission of disulphide bonds and it appears that after the treatment the structure is still sufficiently intact to prevent any extensive dissolution of protein material. Thus, in spite of the fact that the prekeratinous zone has become sufficiently structurally disorganized to permit a large drop in the depilation load, conditions do not permit any further reduction in the depilation load because the disulphide bond system cannot be destroyed. A combination of ammonia treatment and a reducing treatment, i.e. ammonia thioglycollate at pH 12·5, causes the depilation load to drop to an acceptable level. It is not possible to explain the different activities of the various amines and ammonia in terms of any of their known physical or chemical properties; rather it appears to be some specific effect on the cells themselves.

It has long been known that destruction of the epidermal structure is concomitant with depilation (Shaw and Lollar 1954), and before skins can be made into leather all the epidermal structures must be removed. In the commercial sulphide process the epidermis is usually removed, together with the fibre. The histological studies have shown, however, that with both sulphide and ammonia depilation the wool fibre can be removed without any destruction of the epidermis or the upper ORS.

TABLE 1

AMINO ACID ANALYSIS OF THE EPIDERMAL PREPARATION

Values are expressed in grams of component per 100 g of dry material

Amino Acid	Amount*	Other Values†	Amino Acid	Amount*	Other Values†
Nitrogen (total)	15·96	14·2–15·5	Threonine	4·76	3·4
Amide nitrogen	1·45	1·16	Tyrosine	3·97	3·4–5·7
Glycine	10·80	6·0–13·8	Aspartic acid	7·08	6·4–8·1
Alanine	3·57	—	Glutamic acid	15·05	9·1–15·4
Valine	4·47	4·2–5·6	Arginine	7·60	5·9–11·7
Leucine	6·72	8·3	Lysine	5·09	3·1–6·9
Isoleucine	2·97	6·8	Histidine	2·32	0·6–1·8
Phenylalanine	3·37	2·8	Tryptophan	0·95	0·5–1·8
Proline	4·05	3·2	½Cystine	4·57	2·3–3·8
Serine	11·44	16·5	Methionine	1·11	1·0–2·5

* Total amount recovered was 101·34 g amino acids per 100 g dry material.

† Typical amounts of amino acids found in other epidermal preparations (Ward and Lundgren 1954).

Epidermis, and therefore presumably the ORS, contains only about 25–50% as much sulphur as the wool fibre itself (Ward and Lundgren 1954) and this is predominantly in the disulphide form (Van Scott and Flesch 1954). Hence it may be expected that agents capable of splitting disulphide bonds would have a greater destructive effect on the ORS structure than agents which do not possess this power. This is quite clearly seen in the histological appearance of the ORS after sulphide and ammonia treatment respectively. ORS destruction proceeds simultaneously with the action on the prekeratinous zone and it is not therefore possible to say if this is an essential step in wool loosening with these systems, or is merely incidental. It seems likely, however, that some degree of softening of the ORS structure is essential for satisfactory wool loosening.

If the depilation process is stopped exactly at the point when the depilation load is 2·0, the epidermis can be removed from the corium as an intact sheet. If, however, depilation is allowed to proceed for a longer time, the epidermis is removed as a slime. It would obviously be most desirable commercially to cease the depilation process precisely at the point of commercial acceptability, but due to extensive skin variations, the process must be allowed to go on for longer times in order to give even looseness of the wool. At this point depilation in many follicles has gone further than necessary and actual destruction of the keratinous part of the fibre has occurred, resulting in a loss of wool.

In the depilation experiments described, at the point where the depilation load is exactly equal to 2·0, the epidermis was separated simply by pulling it off as a sheet. It was ground and thoroughly washed free of any sulphide. An amino acid analysis of this material is shown in Table 1 and it is seen to be comparable with epidermal preparations separated by other techniques. This epidermal material was used in separate experiments to assess the effect of depilatory enzymes and it was found that there was no correlation between the activity of the enzymes against this preparation, and their depilatory activity (Yates 1968*b*).

V. Acknowledgments

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