THE RESPONSE OF SHEEP SKIN TO TOPICALLY APPLIED SUBSTANCES*

By R. E. CHAPMAN† and B. F. SHORT†

Gross hyperplasia of follicle outer root sheaths is associated with the severe crimp abnormality in doggy wool (Chapman, Short, and Hyland 1960; Chapman and Short 1965). The fleece first shows crimp abnormality on the dorsum, the region of highest wax production (Chapman 1962), and the incidence of doggy wool increases with age (Chapman and Short 1964). Larger proportions of "rough fleece" are produced in areas of higher rainfall (Chapman 1964), presumably resulting from the cumulative effects of many factors, including a possible effect on sheep skin of moisture and of solutions applied to sheep for the control of external parasites in the areas of higher rainfall.

Such solutions contain surface-active compounds to facilitate wetting of the fleece and skin. Epidermal hyperplasia occurs in varying degrees in mice to which surface-active compounds based on long-chain fatty acids have been applied; Tween 60 (polyoxyethylene sorbitan monostearate) is one of the most potent (Setälä 1956; Dammert 1957; Setälä *et al.* 1959*a*, 1959*b*).

The acute and latent effects of moisture and of substances likely to be applied to, or present on, the skin of sheep have therefore been studied with particular reference to follicle outer root sheaths.

Experimental Procedure

Tween 60 (Chemical Materials Ltd., Sydney), in concentrations of 1·25, 2·5, 5, 10, 20% (w/v) in deionized water, was applied at the rate of 1 ml per 20 cm² five times a week to randomly allotted positions amongst eight sites along the back of each of three adult medium-wool Merino ewes housed indoors. Two other sites per sheep were similarly treated with 10% (w/v) filtered solutions of an ether extract and a water extract of a grossly doggy fleece; this represented 10 times the combined excretion rates of wax and suint for Merino sheep (Daly and Carter 1955). Precautions were taken to avoid deterioration of the water extract due to fungal or bacterial contamination. The remaining site on each sheep was similarly treated with deionized water.

Skin samples were taken from each sheep prior to treatment, after 5 and 20 applications, and from the treated sites and adjacent untreated skin two years after cessation of treatment. Histological sections, 25 μ thick, were cut longitudinal to the follicles, and stained with haematoxylin, eosin, and picric acid. 100–200 follicle outer root sheaths per sample were graded and counted as normal, irregular but not

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† Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

enlarged, enlarged, or cystic. The thicknesses of the interfollicular epidermis and dermis were measured, and the number of cell layers in the epidermis beneath the stratum corneum was counted for each sample.

Results

Prior to treatment less than 8% of follicle outer root sheaths were irregular, less than 1% were enlarged, and no follicles had cystic outer root sheaths.

After five applications the percentage of irregular outer root sheaths in all treated sites increased by 6–30%. The percentage of enlarged outer root sheaths increased in only one sheep by 2–9% with all treatments. Two sheep had 0·5–1·5% of cystic outer root sheaths after five treatments with the ether and water extracts, whereas prior to treatment no outer root sheaths were cystic.

After 20 applications the percentages of irregular and of enlarged outer root sheaths had not further increased. In one sheep 0·5–1·5% of cystic outer root sheaths were present in all sites, whereas previously after five applications only the site treated with the ether extract and the site treated with the water extract had cystic outer root sheaths. The other two animals showed no additional change in the percentages of cystic outer root sheaths.

During the next two years the percentages of irregular outer root sheaths of one sheep and the percentages of irregular, enlarged, and cystic outer root sheaths of the other two sheep increased in the untreated skin, presumably reflecting the normal age trend (Chapman and Short 1964). At the end of this period the difference between treated and untreated skin in the percentage of irregular outer root sheaths was slightly smaller for all sheep than after 20 applications. By contrast, the differences between treated and untreated skin in the percentages of enlarged and of cystic outer root sheaths persisted and increased slightly in the sheep that showed earlier responses.

All sites treated with Tween 60 developed marked hyperplasia and some hypertrophy of the interfollicular epidermis within 5 days, which continued during treatment but subsequently retrogressed; the dermal thickness did not alter. The ether and water extracts and water alone had little effect on the interfollicular epidermal and dermal thicknesses, although after 20 applications some thickening of the distal lumen walls of the follicles was observed.

The responses of the epidermis and of the outer root sheaths to Tween 60 did not increase with concentration.

An inflammatory reaction occurred in the dermis at all treated sites during treatment.

Discussion

The differences between sheep in their responses to these treatments are not unexpected, but no physiological basis can be determined without more extensive data.

The continuing differences between treated and untreated skin in the percentages of enlarged and of cystic follicle outer root sheaths indicate that the expected age trend was exacerbated by the treatments in the susceptible sheep.

The rapid and marked thickening of the interfollicular epidermis induced by Tween 60 is similar to its effect on the skin of mice (Dammert 1957; Setälä *et al.* 1959*a*). But, in contrast to the hyperplasia which persisted in follicle outer root sheaths, the epidermal hyperplasia retrogressed after cessation of treatment.

A feature common to all treated sites was an inflammatory reaction. Perhaps the enlargement of follicle outer root sheaths is a permanent side effect of this reaction, mediated, for instance, through substances either present or applied. Due to the continuous presence of natural products on the skin, it is tentatively concluded that some component of these products plays a greater role in inducing the outer root sheath hyperplasia associated with the growth of doggy wool than substances which are applied intermittently.

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