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Morphological Changes in the Skin and Wool Fibres of Merino Sheep Infused with Mouse Epidermal Growth Factor

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Abstract

Intravenous infusion of 4·5–4·7 mg of mouse epidermal growth factor (mEGF) into nine castrated male Merino sheep for 26 h resulted in complete casting of the fleeces 6–8 days later. The morphological changes which occurred in the skin were studied in skin samples taken before infusion and at intervals between 1 h and 42 days after the infusion had begun. Wool fibres from the shed fleeces were examined with the scanning electron microscope.

Increased cell proliferation occurred in the epidermis and sebaceous glands, whereas the wool follicles regressed. Transient dermal haemorrhages occurred during the first 3 h of infusion. The fibre and inner root sheath in the keratogenous zone of 30–40% of the follicles were partially disrupted within the first 6 h of mEGF infusion; catagen began in all follicle bulbs within 24 h. Fibre and inner root sheath production, although markedly reduced, continued in about 60% of follicles which had partially regressed, but production ceased in the remainder in which tapered ends formed on the fibres prior to shedding. Follicles began to regenerate asynchronously 4–8 days after the beginning of infusion and completed their development during the next 3 weeks.

The follicle regression and fleece casting induced by mEGF infusion, and subsequent follicle regeneration were completed more rapidly than observed previously with other depilatory agents, and, except for prolonged epidermal thickening, there was no lasting cutaneous abnormality.

Introduction

Treatment of mice from birth with an extract of the submaxillary glands of male mice induces precocious eruption of incisors and opening of eyelids, and inhibition of hair growth (Levi-Montalcini and Cohen 1960). A protein, epidermal growth factor (mEGF), isolated from such an extract was subsequently found to stimulate epidermal proliferation and keratinization in cultured chick embryo skin (Cohen 1965). A reduction in the growth of monotrichs has also been observed in the first hair coat of mice dosed with mEGF (Moore *et al.* 1981*a*). Subsequently, inhibition of wool growth and shedding of the entire fleece were induced in castrated male Merino sheep infused subcutaneously with mEGF (Moore *et al.* 1981*b*, 1981*c*). The potential of mEGF as a depilatory agent is presently being investigated as an alternative to the mechanical harvesting of wool.

The morphological changes which occur in the skin of adult sheep in response to mEGF infusion have only been briefly described (Moore *et al.* 1981*b*, 1981*c*), and include some observations on the wool follicle bulbs. The aim of the present study was to examine more fully, with light and scanning electron microscopy, the changes induced by a depilatory amount of mEGF in sheep skin and in wool fibres and follicles.

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Materials and Methods

Adult castrated male Merino sheep were housed indoors in individual cages and offered a ration of 600 g of a pelleted mixture of 60% chopped lucerne hay and 40% oat grain as one meal daily. Drinking water was provided *ad libitum*. Liveweights were found not to alter significantly during the experiment.

mEGF was prepared from submaxillary glands of adult male mice using the method of Savage and Cohen (1972) outlined by Moore *et al.* (1982), and tested for biological activity as described by Moore *et al.* (1981*b*). Nine sheep (liveweight 34–38 kg) each received an infusion, via a jugular vein catheter, of 4·5–4·7 mg of mEGF dissolved in *c.* 53 ml sterile saline (0·9% w/v). Three similar sheep were infused with sterile saline as controls. Infusions were maintained at a steady rate for 26 h using a Harvard pump.

Skin samples (1 cm diameter) were taken from the lateral trunk region of each sheep prior to infusion and at intervals ranging from 1 h to 42 days after the commencement of infusion (see Fig. 1). The samples were fixed in Serra's fixative for 4–5 h and processed for paraffin embedding. Serial sections (8 μm thick) were cut both longitudinal to the follicles (vertical sections) and parallel to the skin surface (transverse sections), stained with haematoxylin, eosin and picric acid and examined with the light miscroscope.

Measurements of interfollicular epidermal thickness were obtained from vertical sections using a microprojector at a magnification of $\times 400$. Six regions of epidermis, each 0·5 mm long, were traced on cards of uniform thickness. These tracings were cut out and weighed and the mean epidermal thickness was obtained by the method of Lyne and Hollis (1968). Measurements of wool follicle depth were also obtained from vertical sections at a magnification of $\times 400$. Estimates of the proportion of follicles exhibiting various morphological features were obtained from transverse serial sections.

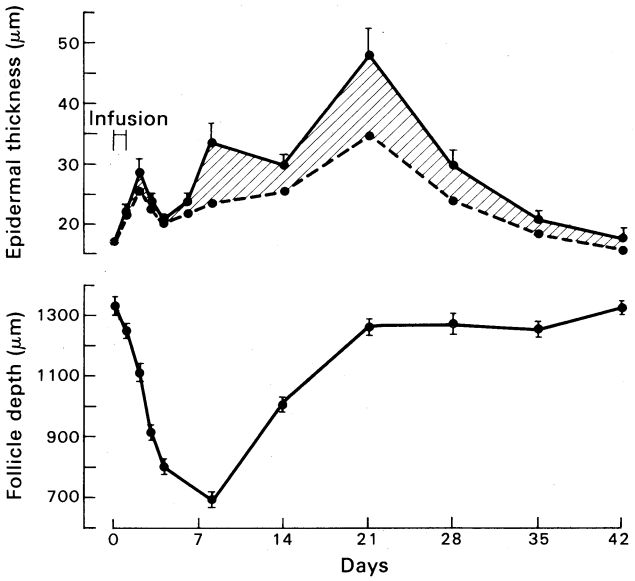
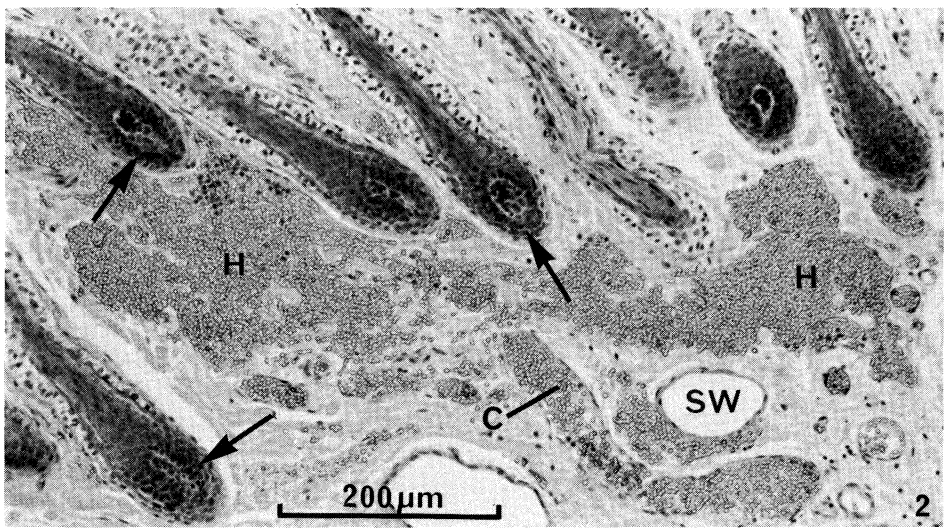
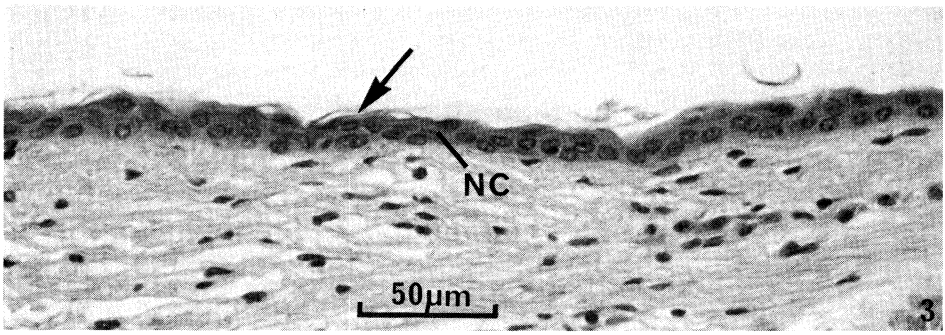


Fig. 1. Mean (±s.e.m.) epidermal thickness and depth of wool follicles of nine castrated male Merino sheep at intervals after the beginning of mEGF infusion. The thickness of the stratum corneum is indicated by the shaded area.

Wool fibres were collected from shed fleeces and cleaned with Shell X4 solvent. The basal portions were mounted on scanning electron microscope stubs (3 cm diameter) with strips of double-sided adhesive tape, sputter coated with gold and examined with an I.S.I. Super IIIA scanning electron microscope.





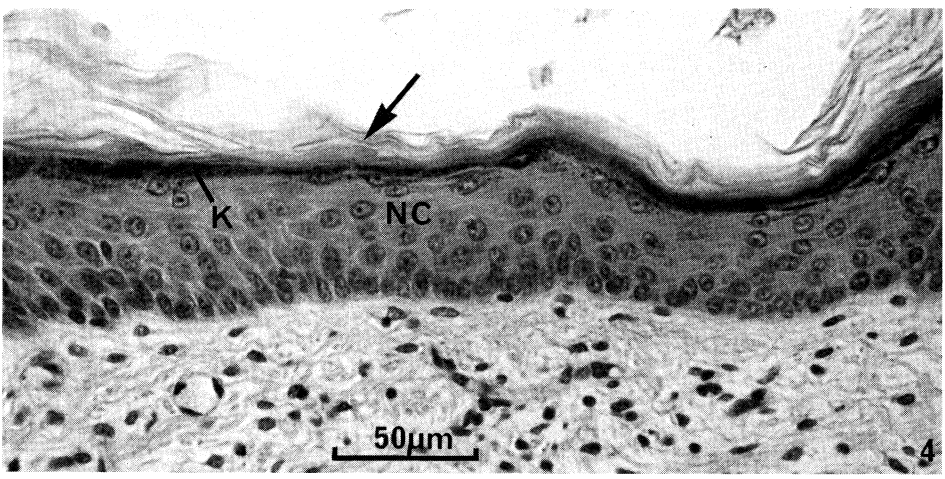


Fig. 2. Vertical section of skin 3 h after the beginning of mEGF infusion showing dermal haemorrhage (*H*) at follicle bulb (arrow) level. *C*, ruptured capillary; *SW*, sweat gland.

Fig. 3. Vertical section of epidermis prior to the commencement of mEGF infusion showing 2–3 layers of nucleated cells (*NC*), covered by a very thin cornified layer (arrow).

Fig. 4. Vertical section of epidermis taken from an mEGF infused sheep on day 21 showing greatly thickened nucleated cell layer (*NC*) and cornified layer (arrow) and increased presence of keratohyalin (*K*).

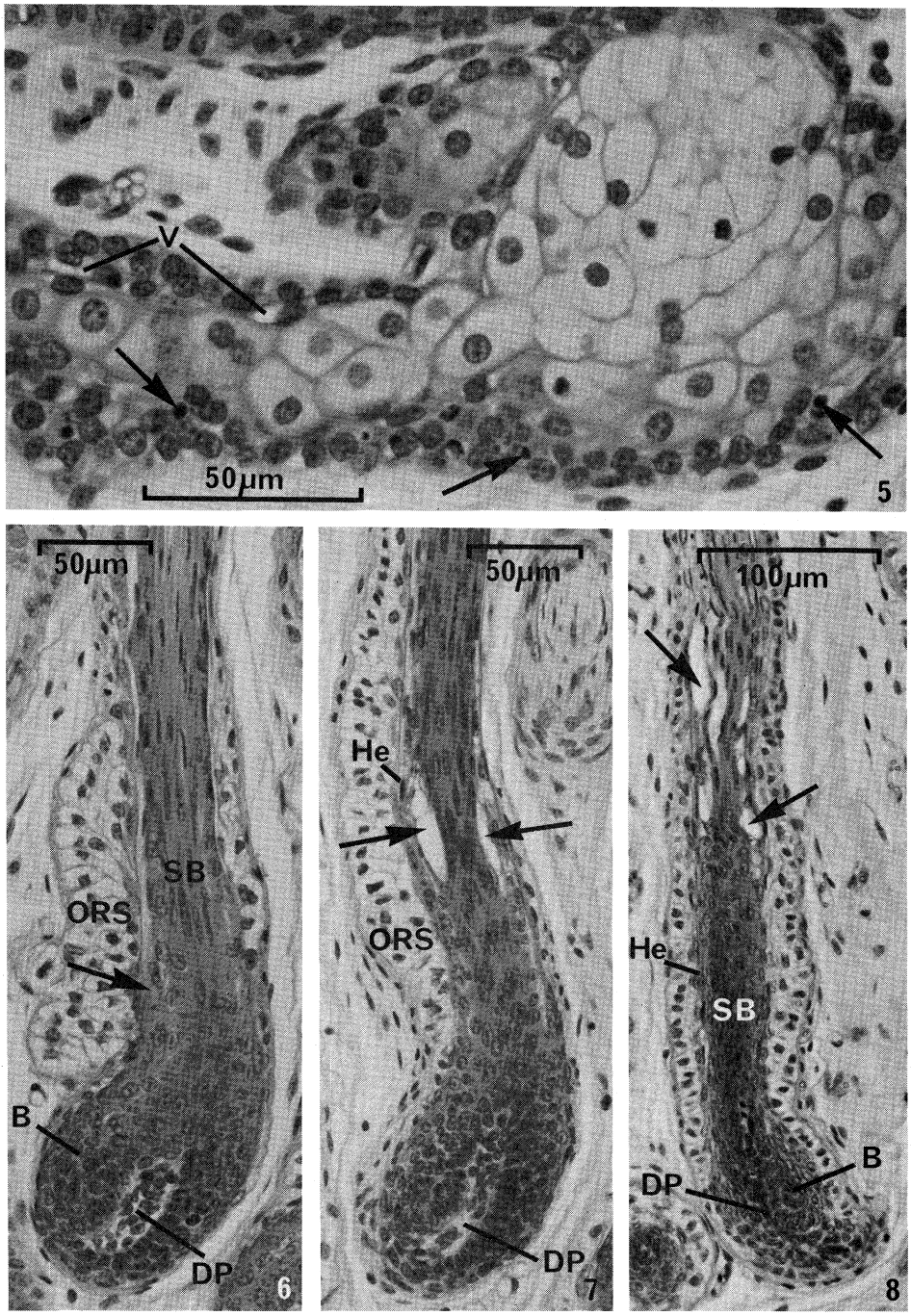


Fig. 5. Vertical section through a sebaceous gland 48 h after the commencement of mEGF infusion showing a prominent basal layer containing vacuoles (*V*) and darkly stained bodies (arrows).

Fig. 6. Longitudinal section of a normal anagen follicle prior to mEGF infusion. The arrow indicates the level of hardening of Henle's layer of the inner root sheath. *B*, bulb; *DP*, dermal papilla; *ORS*, outer root sheath; *SB*, elongation of fibre cortical cells in suprabulbar region.

Results

The infusions of mEGF resulted in complete casting of the fleeces 6–8 days later. Morphological responses to mEGF infusion were observed in the dermal blood vessels, epidermis, sebaceous glands and wool follicles and are described at designated times after the beginning of infusion.

Dermal Blood Vessels

These vessels in the 1-h and 3-h skin samples were greatly dilated and some of those at follicle bulb level had ruptured, releasing blood into the surrounding connective tissue (Fig. 2). These haemorrhages were less extensive at 6 and 12 h, and by 24 h little extravasated blood remained.

Epidermis

In samples taken prior to infusion the epidermis was approximately 17 μm thick (Fig. 1) and consisted of 2–3 layers of nucleated cells covered by a very thin cornified layer (Fig. 3). An initial proliferative response to mEGF was observed in the epidermis during the first 2 days. This was followed from day 4 by a continual increase in thickness (Fig. 1) to about 50 μm at 21 days and featured thickened keratohyalin and cornified layers (Fig. 4). Epidermal thickness subsequently decreased to normal values by 42 days. No comparable changes were observed in the epidermis of sheep infused with saline.

Sebaceous Glands

The basal layer of cells in the sebaceous glands was thicker and more darkly stained at 24 h than prior to treatment. Mitoses were more frequent at this time and also at 2 days, when small darkly stained cytoplasmic bodies and vacuoles were also common in the basal layer (Fig. 5) and there was a transient increase in gland size. By 4 days the sebaceous glands, in general, were normal in appearance and remained so throughout the remainder of the experiment.

Wool Follicle Depth

Follicle depth decreased during follicle regression from more than 1300 μm to a minimum of about 700 μm when fleece casting occurred, then subsequently increased during follicle regeneration to near normal depth by 21 days (Fig. 1).

Wool Follicle Regression

Following treatment with mEGF the follicles regressed from anagen (Fig. 6) to a catagen phase which lasted for 4–8 days.

Fig. 7. Longitudinal section of the lower part of a wool follicle in early catagen 6 h after the beginning of mEGF infusion showing the disruption of fibre and IRS cells in the keratogenous zone (arrows), below the level of retarded hardening of Henle's layer (*He*); *DP*, dermal papilla; *ORS*, outer root sheath.

Fig. 8. Longitudinal section of the lower half of an early catagen follicle 24 h after the beginning of mEGF infusion showing the shrinkage of the bulb (*B*) and dermal papilla (*DP*), the absence of cortical cell elongation and lateral enlargement of the unhardened fibre in the suprabulbar region (*SB*), and the disruption of fibre and IRS cells (arrows) now above the level of hardening of Henles' layer (*He*) in the keratogenous zone.

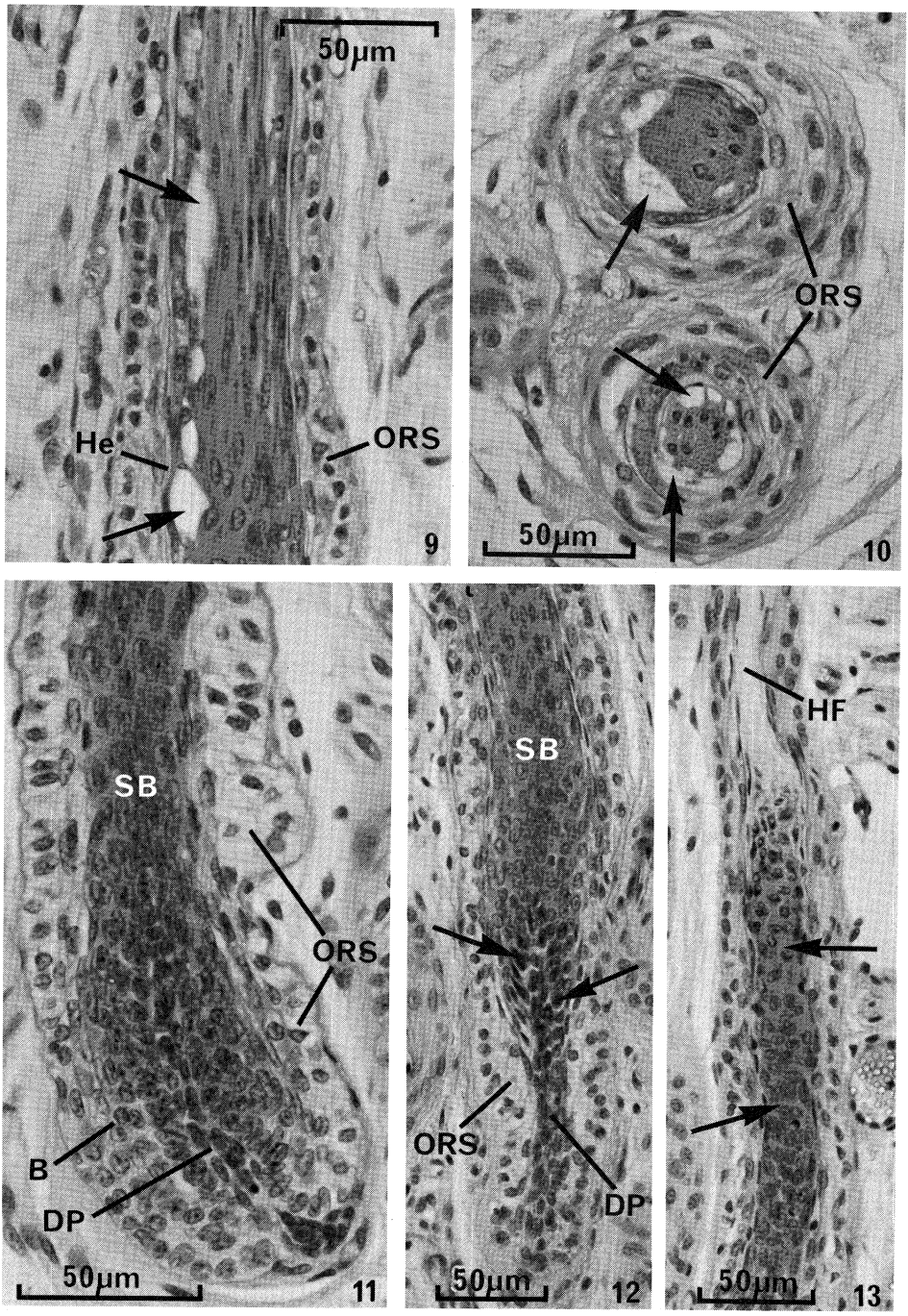


Fig. 9. Longitudinal section of the keratogenous zone of an early catagen follicle 24 h after the beginning of infusion with mEGF showing the disruption of the outer cortical cells (arrows), fibre and IRS cuticles and Huxley's layer of the IRS. Henle's layer (*He*) is hardened at this level and the ORS appears to be normal.

Fig. 10. Transverse section through the keratogenous zone of two early catagen follicles 24 h after the beginning of mEGF infusion showing the disruption of fibre and IRS cells (arrows). *ORS*, outer root sheath.

1–24 h

The earliest changes were detected in the keratogenous zone of 30–40% of the follicles. Fibre and inner root sheath (IRS) cells were partially disrupted proximal to the level of hardening of Henle's layer after 6 h of mEGF infusion (Fig. 7). This feature was also observed in the 12-h and 24-h (Fig. 8) samples, although the site of disruption was more distally located. The disruption was confined to the outer fibre cortical cells, the fibre and IRS cuticles and Huxley's layer of the IRS (Fig. 9). The lateral extent of disruption was considerable in some follicles while in others, the cells on only one side were affected (Fig. 10). The hardening of Henle's layer of the IRS appeared to be delayed (cf. Figs 6 and 7), while the outer root sheath (ORS) cells were unaltered.

In all of the follicles in the 24-h samples the bulbs were somewhat shrunken, the dermal papillae were reduced to thin strands and the ORS layer was thickened around the bulbs (Fig. 11). Nevertheless a few mitotic cells were observed in the bulbs. Elongation of the fibre cells, which normally commences in the suprabulbar region of anagen follicles (Fig. 6) was absent at this level (Figs 8 and 11). Lateral (radial) enlargement of the unhardened fibre in this region, associated with retarded hardening of Henle's layer of the IRS, was also observed (Fig. 8).

48 h

The bulbs of the most regressed follicles were further shrunken and the bulb cells, which were pyknotic, had withdrawn from around the lower part of the indistinct elongated dermal papillae (Fig. 12). Mitotic figures were still evident in some follicle bulbs. Lateral enlargement of the fibre in the suprabulbar region extended more distally than in the 24-h samples (Fig. 13). The regions of fibre/IRS disruption had also moved further up the follicles. These now hardened portions of the fibres were markedly deformed and reduced in diameter (Figs 13 and 14).

3–4 days

Mitotic activity had virtually ceased in the follicle bulbs by this time and the dermal papilla formed into a rounded mass. Regression ceased at 4 days in some follicles in which there were signs of regeneration. The follicles fell into two categories depending upon the degree of regression reached when regeneration began:

1. *Follicles in which fibre and IRS production was continuous.* Although markedly reduced, fibre and IRS production continued in approximately 60% of the

Fig. 11. Longitudinal section of the lower part of an early catagen follicle 24 h after the beginning of mEGF infusion showing the shrunken bulb (*B*), the thin strand of dermal papilla cells (*DP*), the absence of cortical cell elongation in the suprabulbar region (*SB*) and the thickened outer root sheath (*ORS*).

Fig. 12. Longitudinal section of the lower part of a catagen follicle 48 h after the beginning of mEGF infusion showing the withdrawal of pyknotic bulb cells (arrows) from around the elongated dermal papilla (*DP*) and their replacement by outer root sheath (*ORS*) cells. *SB*, lateral enlargement of unhardened fibre in suprabulbar region.

Fig. 13. Longitudinal section through the mid-region of a catagen follicle 48 h after the beginning of mEGF infusion showing the absence of cortical cell elongation, lateral enlargement of the unhardened fibre (arrows), and the thin, deformed, hardened region of the fibre (*HF*).

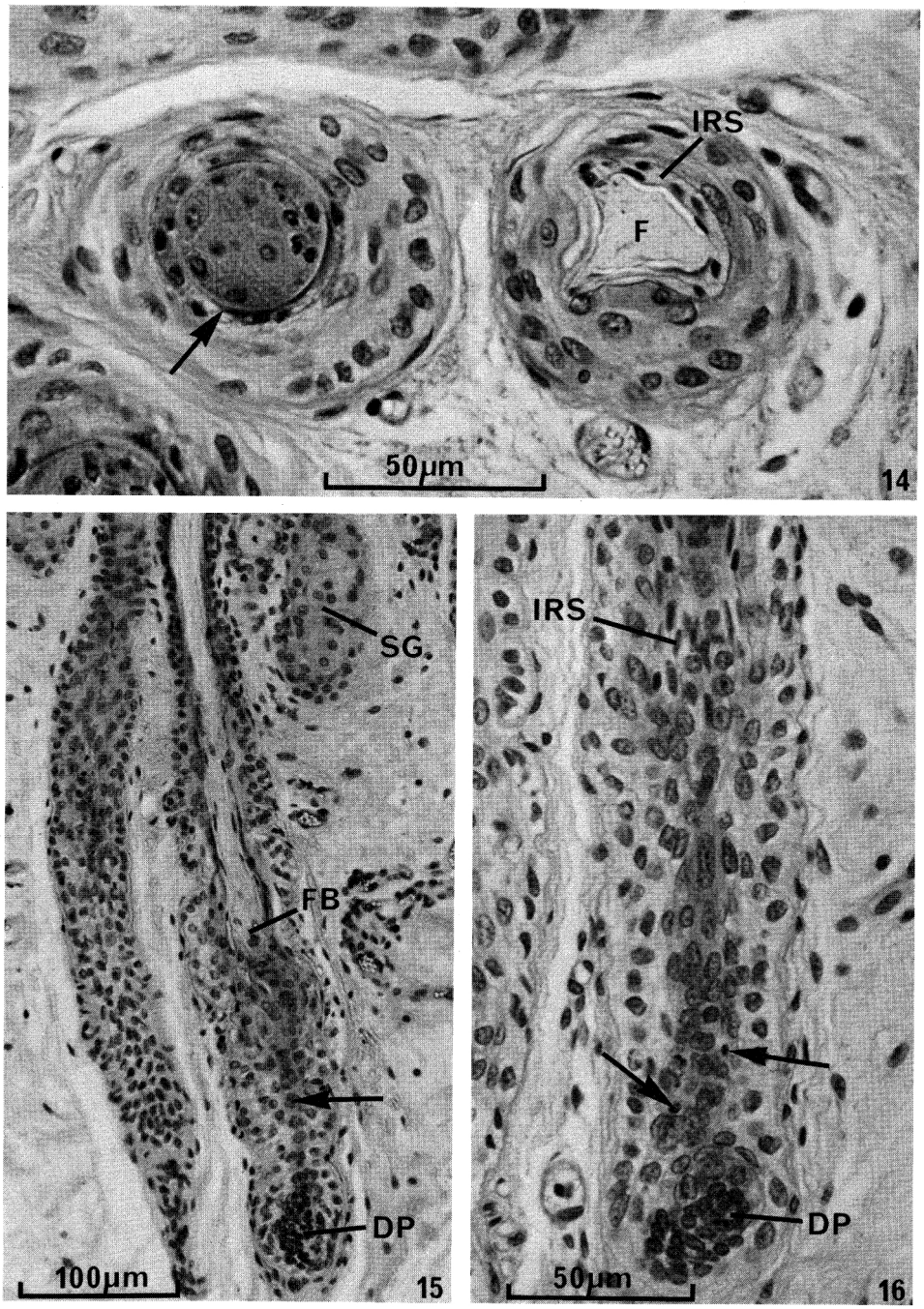


Fig. 14. Transverse section through the mid-region of two catagen follicles 48 h after the beginning of mEGF infusion. The hardened fibre (*F*) and inner root sheath (*IRS*) in one of the follicles are deformed and reduced in diameter whereas the fibre and IRS in the other follicle (arrow) appear normal.

Fig. 15. Longitudinal section of the lower part of a regressed catagen follicle 4 days after the beginning of mEGF infusion showing a thin column of darkly stained non-ORS cells (arrow) connecting the base of the hardened fibre (*FB*) with the dermal papilla (*DP*). *SG*, sebaceous glands.

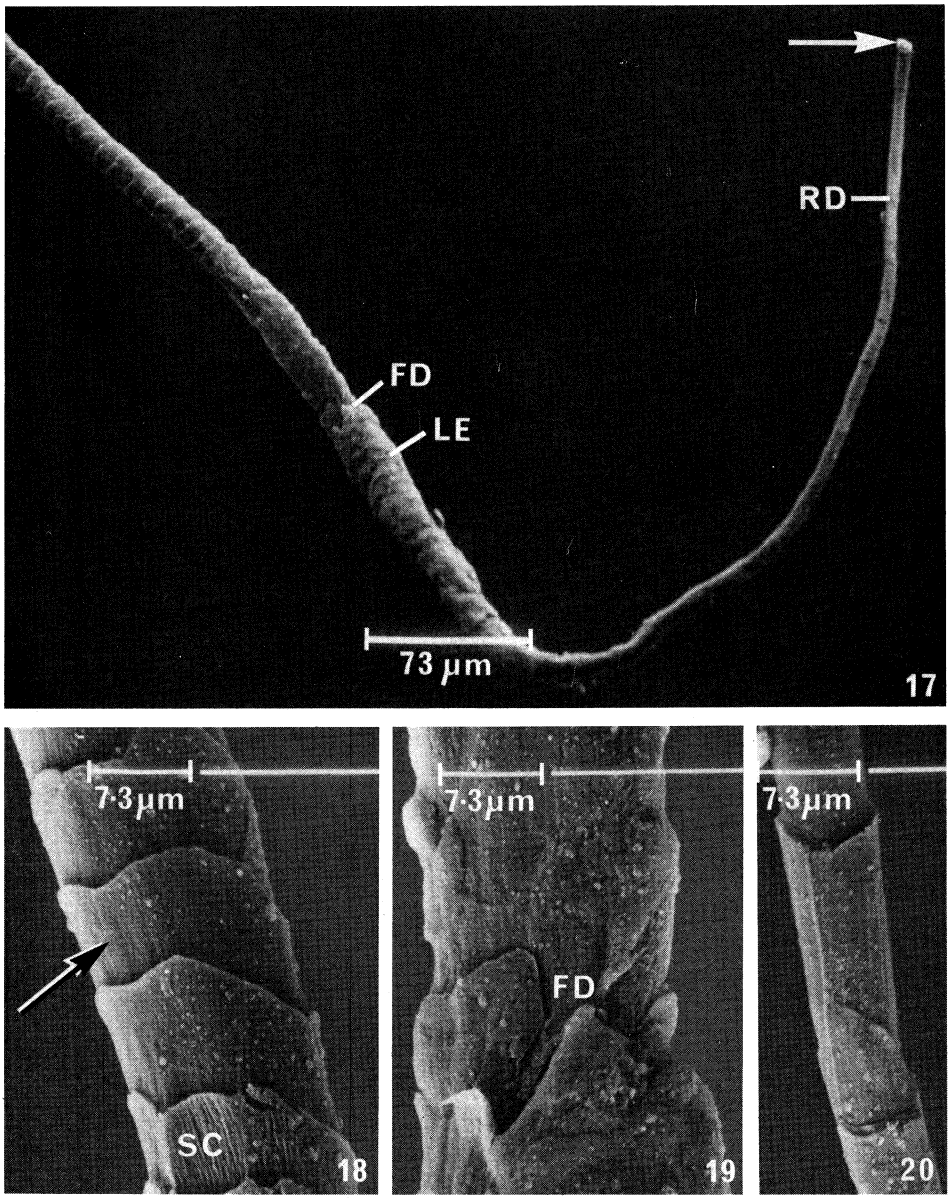


Fig. 17. Scanning electron micrograph of the proximal end of a wool fibre from a follicle which continuously produced fibre and IRS, collected from the fleece shed by a sheep 6 days after the beginning of mEGF infusion. Regions of fibre disruption (*FD*), lateral enlargement (*LE*) and reduced diameter (*RD*) are evident. Arrow indicates point of fibre break.

Fig. 18–20. Scanning electron micrographs of regions of the fibre shown in Fig. 17. Fig. 18 shows the normal cuticular pattern (arrow) prior to mEGF infusion and a striated cuticle cell (*SC*) formed at the beginning of infusion, Fig. 19 the abnormal cuticular pattern in the region of fibre disruption (*FD*), and Fig. 20 the region of reduced diameter which sometimes, as in this fibre, appears to have a normal cuticular pattern.

Fig. 16. Longitudinal section of the lower part of a regressed catagen follicle 3 days after the beginning of infusion with mEGF showing small, darkly stained bodies (arrows) in the non-ORS cells above the rounded dermal papilla (*DP*). *IRS*, hardening inner root sheath cells.

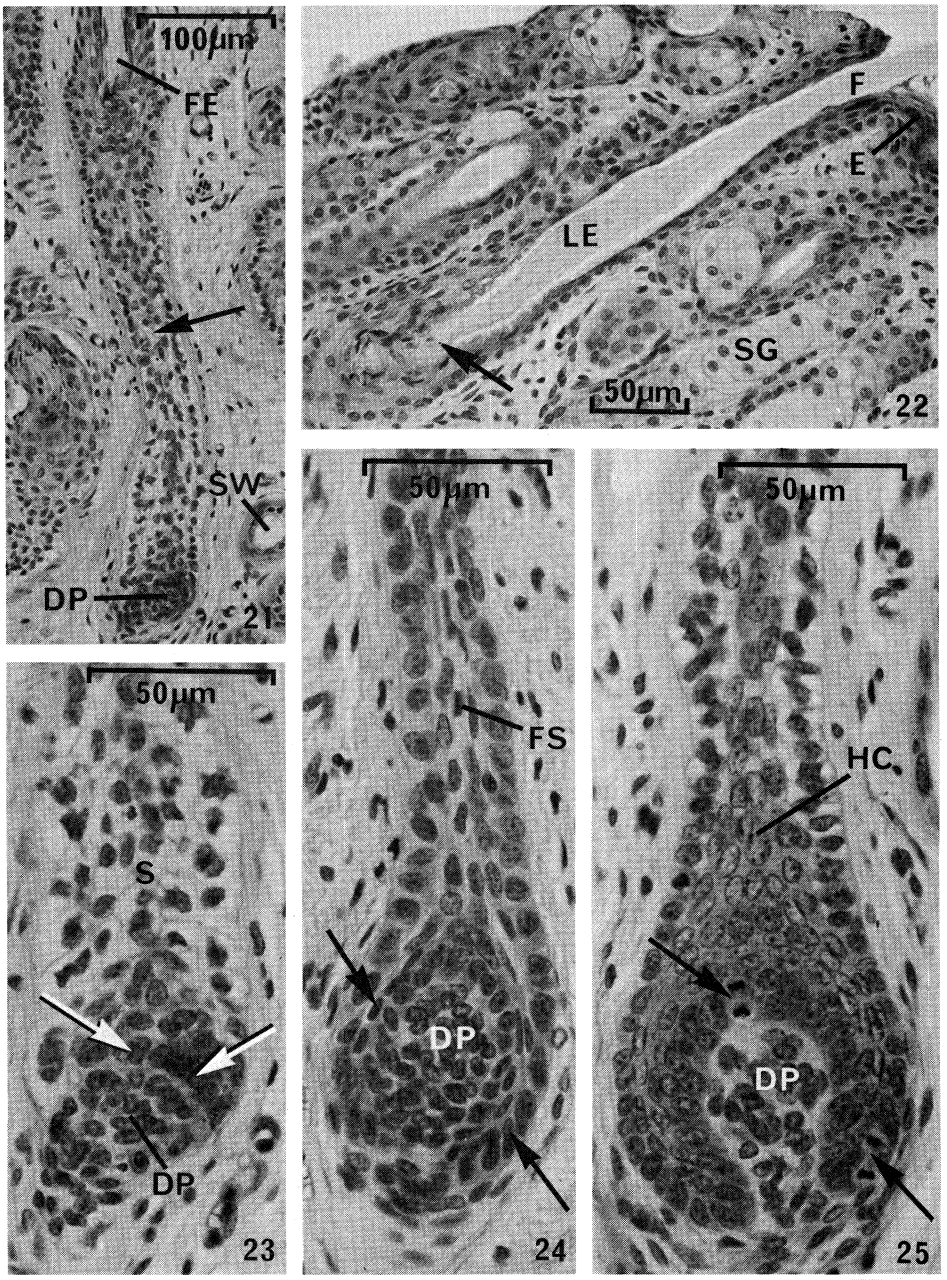


Fig. 21. Longitudinal section of the lower part of a fully regressed follicle 4 days after the beginning of mEGF infusion showing the long stalk of ORS cells (arrow) separating the hardened fibre end (*FE*) from the rounded dermal papilla (*DP*). *SW*, sweat gland.

Fig. 22. Longitudinal section of the proximal end of a wool fibre (*F*) in the upper part of a fully regressed follicle 4 days after the beginning of mEGF infusion. Arrow indicates tapered end. *E*, epidermis; *LE*, region of lateral enlargement of fibre; *SG*, sebaceous gland.

Fig. 23. Longitudinal section of the lower part of a regenerating follicle at the pre-papilla stage 4 days after the beginning of infusion with mEGF showing the reorganization of differentiating ORS cells (arrows) capping the dermal papilla cells (*DP*). *S*, stalk of ORS cells.

follicle population. In these follicles a thin column of relatively darkly stained non-ORS cells connected the hardened region of the fibre to the dermal papilla (Figs 15 and 16). Small darkly stained bodies resembling autophagic vacuoles were sometimes present in these cells close to the dermal papilla cells (Fig. 16). Scanning electron microscopy of wool fibres grown by these follicles revealed clearly the regions of fibre disruption, lateral enlargement and reduced diameter (Figs 17–20). Formation of the normal cuticular scale pattern was inhibited during mEGF infusion but resumed in some fibres in the region of reduced diameter (Fig. 20).

2. *Follicles in which fibre and IRS production ceased.* In these follicles, which accounted for the remaining 40% there was no column of darkly stained non-ORS cells and a fibre root end formed which was separated from the dermal papilla by a long stalk of ORS cells (Fig. 21). Tapered ends formed on these fibres and were located at about the depth of the sebaceous glands at 4 days (Fig. 22). Subsequently these ends came free of the follicle canals during fleece casting.

Wool Follicle Regeneration

Regeneration commenced in some follicles as early as 4 days after infusion and in all follicles by 8 days. The last to regenerate were mostly small secondary follicles.

Pre-papilla/papilla stages

The ORS cells capping the rounded, regressed dermal papilla were packed closely together into a distinct layer (Fig. 23). Subsequently the differentiating follicle cells began to enclose the dermal papilla cells (Fig. 24), and the ORS cells above the bulb became orientated (Fig. 24) in preparation for the advancing hair cone. No dividing cells were observed in these early stages of regeneration.

Hair cone stage

Follicle length and bulb size increased and dividing bulb cells were numerous (Fig. 25). The dermal papilla was now almost fully enclosed and a hair cone of IRS cells (Fig. 25) advanced along the central axis of the follicle towards the skin surface. Its outermost layer (Henle's layer) became hardened upon reaching a level mid-way between the bulb and the sebaceous glands (Fig. 26). The unhardened innermost layers (Huxley's layer and cuticle) and the developing fibre were present within the hair cone at this stage (Fig. 27).

Fig. 24. Longitudinal section of the lower part of a regenerating follicle at the enclosed papilla stage in a skin sample taken 14 days after the beginning of mEGF infusion showing the dermal papilla cells (*DP*) partly enclosed by the differentiating follicle cells (arrows) and the orientation of the ORS cells in the follicle stem (*FS*).

Fig. 25. Longitudinal section of the lower part of a regenerating follicle at the hair cone stage in a skin sample taken 8 days after the beginning of infusion with mEGF showing the almost fully enclosed dermal papilla (*DP*) and the regenerating hair cone (*HC*). Dividing bulb cells (arrows) were common and bulb enlargement occurred during this stage.

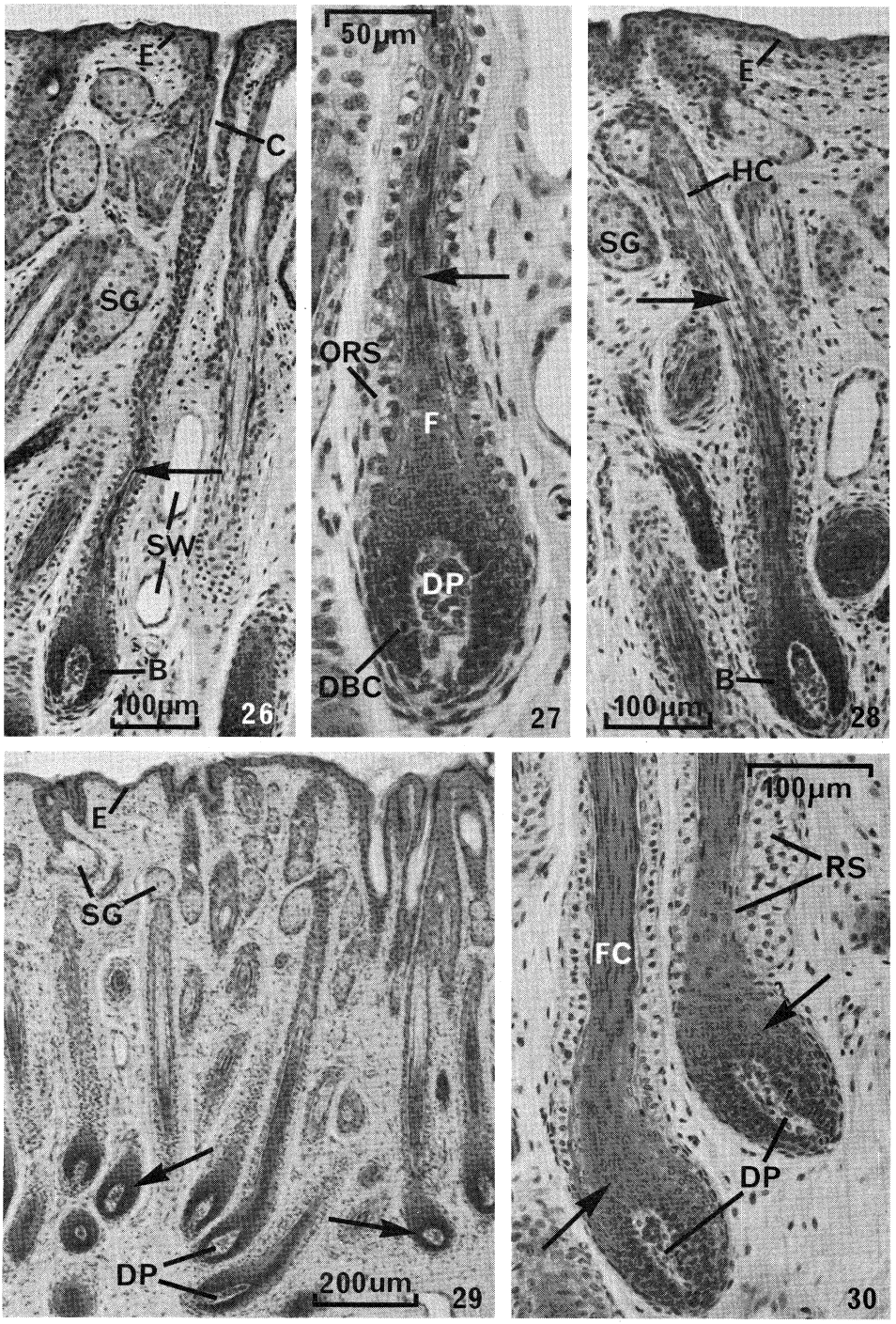


Fig. 26. Longitudinal section of a regenerating follicle at the hair cone stage in a skin sample taken 14 days after the beginning of mEGF infusion showing the hardened outermost layer (Henle's layer) of the hair cone (arrow) mid way betwen the bulb (*B*) and the level of the sebaceous glands (*SG*). The hair canal (*C*) has collapsed somewhat as a result of fibre shedding. *E*, epidermis; *SW*, sweat gland.

Fibre formation and emergence stages

As the hair cone moved up the follicle it became fully hardened and by the time it reached the level of the sebaceous glands, the enclosed fibre tip was keratinized (Fig. 28). Subsequently the dermal papillae of the follicles were greatly enlarged, and the bulb cells were darkly stained (Fig. 29) and mitotically active. Fibre growth and keratinization continued and the tip penetrated the hair cone and epidermis and emerged from the skin surface.

A few follicles had completely regenerated by 14 days, and most by 21 days, at which time follicle bulbs (Fig. 30) and depth (Fig. 1) had returned to normal. By 28 days all follicles had completely regenerated and the appearance of the skin had returned to normal, except for the thickened epidermis which subsequently decreased to pretreatment thickness by 42 days (Fig. 1).

Discussion

The first morphological change observed in the skin in response to mEGF was the haemorrhage of dilated dermal capillaries 1 and 3 h after the beginning of infusion. The effect was transient, the extravasated blood having virtually cleared from the connective tissue by 24 h. Similar haemorrhages have been described (Reis *et al.* 1975) in sheep infused with depilatory doses of mimosine. However, these haemorrhages persisted for the duration of the infusion and subsequently cleared.

mEGF infusion initiated a regression of the wool follicles which was more rapid than that induced by other depilatory compounds such as mimosine, isomimosine, cyclophosphamide, 1-*p*-aminophenoxy-5-phthalimidopentane(*N*-[5-(4-aminophenoxy)-pentyl]phthalimide), 1-*p*-aminophenoxy-5-phthalimidinopentane (2-[5-(4-aminophenoxy)pentyl]isoindole-1(3H)-one) (Chapman 1980) or dexamethasone trimethylacetate (Chapman *et al.* 1982). This regression proceeded through stages which resembled some of those described in mammalian catagen hair follicles (Montagna and Parakkal 1974; Straile *et al.* 1961). However, the disruption of fibre and IRS which occurred during mEGF infusion does not normally occur during the catagen phase, and the formation of the regressed, ball-shaped dermal papilla occurred more gradually than in normal catagen hair follicles (Straile *et al.* 1961). Furthermore, the follicles in which fibre and IRS production was continuous regressed no further than the mid-catagen stage described by Straile *et al.* (1961) (stage C V), and the follicles which

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Fig. 27. Higher magnification of the lower part of the follicle shown in Fig. 26. The unhardened inner layers of the hair cone (arrow) and the developing fibre cells (*F*) can be recognized. *DBC*, dividing bulb cell; *DP*, dermal papilla; *ORS*, outer root sheath.

Fig. 28. Longitudinal section of a regenerating follicle at the fibre formation stage in a skin sample taken 14 days after the beginning of mEGF infusion showing the keratinized fibre tip (arrow) within the fully hardened hair cone (*HC*) at the sebaceous gland (*SG*) level. *B*, bulb; *E*, epidermis.

Fig. 29. Vertical section of skin 14 days after the beginning of infusion with mEGF showing several regenerating follicles near the fibre emergence stage. The bulb cells are darkly stained (arrows) and the dermal papillae (*DP*) are enlarged. *E*, epidermis; *SG*, sebaceous glands.

Fig. 30. Longitudinal section of the lower parts of two fully regenerated follicles 21 days after the beginning of mEGF infusion. The dermal papillae (*DP*), bulb cells (arrows), elongating fibre cells (*FC*) and the root sheaths (*RS*) appear normal.

ceased fibre and IRS production advanced only one stage further (stage C VI). The structure of these regressed follicles differed in several respects from that of the typical telogen (resting) hair follicles. The dermal papilla did not retract to a position just beneath the fibre end, as is usual in telogen follicles (Straile *et al.* 1961), and was separated from it by a long column of ORS cells. Furthermore, IRS cells which are normally absent in telogen hair follicles remained in regressed follicles of mEGF treated sheep and, instead of the typical telogen 'club' or 'brush end' on the fibre, a tapered end was produced. This indicates that the telogen state was not reached in affected follicles due to the rapidity with which they began to regenerate and shed their fibres.

Disruption of fibre and IRS, which occurred during mEGF infusion, has not been observed with any other compounds examined except for *p*-aminophenol (A. M. Downes, personal communication). However, fewer follicles (<10%) were affected by *p*-aminophenol, elongation of fibre cortical cells was not disrupted, and the follicles did not enter into the catagen phase. Cytoplasmic bodies resembling autophagic vacuoles (Montagna and Parakkal 1974) were fewer in the catagen follicles after mEGF infusion than in those following treatment with the depilatory agents mentioned previously.

Inhibition of mitotic activity in the follicle bulbs in response to mEGF treatment occurred during the first 2–3 days. This was more gradual than that which occurred after administration of depilatory doses of *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Chapman and Rigby 1980), cyclophosphamide (Reis and Chapman 1974) or mimosine (Reis *et al.* 1975), which inhibited mitotic activity within the first day, but was faster than after dosing with dexamethasone trimethylacetate which caused cessation in about 5 days (Chapman *et al.* 1982).

It has been shown in the present study that complete fleece casting occurred even though only approximately 40% of the follicles stopped growing fibres and the remainder produced fibres of greatly reduced diameter. This is in contrast to the effect of cortisol which induced cessation of fibre growth in some follicles but did not cause reduction in diameter of the fibres in the remainder, in which fibre growth continued (Chapman and Bassett 1970).

The increase in epidermal thickness which occurred in response to mEGF infusion was more pronounced and usually more prolonged than with other compounds previously tested. These latter responses ranged from a moderate and fairly prolonged thickening with *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Chapman and Rigby 1980) and *p*-aminophenol (A. M. Downes, personal communication), to an inital decrease followed by a moderate increase with cyclophosphamide (Reis and Chapman 1974) and cortisol (Chapman and Bassett 1970). No changes in epidermal thickness were observed in response to dexamethasone trimethylacetate (Chapman *et al.* 1982) or mimosine (Reis *et al.* 1975).

Changes in sebaceous gland size similar to those which occurred in response to mEGF have also been reported in sheep foetuses treated with this protein (Thorburn *et al.* 1981) and in adult sheep treated with other depilatory compounds such as *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Chapman and Rigby 1980), cyclophosphamide (Reis and Chapman 1974) and mimosine (Reis *et al.* 1975). A reduction in sebaceous gland size was observed by Chapman and Bassett (1970) in sheep treated with cortisol. The location of the darkly stained bodies in the basal layer of the sebaceous glands 48 h after the beginning of mEGF infusion differs from that of

similar structures in the differentiated sebaceous cells in normal human and rat skin which are thought to be responsible for degradation of sebaceous cell cytoplasm (Mesquita-Guimarães *et al.* 1979).

The present study indicates that during the early stages of the regeneration of the follicles affected by mEGF treatment, the initial reformation of the bulbs occurred in the absence of mitotic divisions. Presumptive bulb cells therefore appear to have the ability to differentiate without the aid of division. This is in direct contrast to the subsequent abundance of mitoses during later IRS and fibre production. It is possible that a critical cell mass must be attained before cell division can recommence.

Follicles began to regenerate within a week following mEGF treatment and proceeded asynchronously over about a 3-week period. Whereas the regression of the follicles differed in several aspects from those described for catagen in other mammals (Montagna and Parakkal 1974; Straile *et al.* 1961), the sequence of regenerative stages observed during bulb and fibre regeneration closely resembled stages F2 to F8 described in developing wool follicles in foetal sheep (Hardy and Lyne 1956).

The present study has revealed various effects of infusion of a depilatory amount of mEGF on the structure of the skin of Merino sheep, namely dermal haemorrhage, proliferative changes in the epidermis and sebaceous glands, and successive regression and regeneration of the wool follicles. The regressive effects on the follicles were dual in nature and operated in two distinct anatomical regions at different times. These were a lytic effect on unhardened fibre and IRS cells in the keratogenous zone during the course of the infusion, and gradual inhibition of mitotic activity in the bulb cells over a period of 2–3 days with resultant catagen lasting 4–8 days.

Acknowledgments

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