Development 114, 887-897 (1992)
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# Cellular and extracellular involvement in the regeneration of the rat lower vibrissa follicle

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## Summary

**The sequence of events leading to the reconstruction of a fibre-producing hair follicle, after microsurgical amputation of the lower follicle bulb, has been detailed by immunohistology and electron microscopy. The initial response was essentially found to be a wound reaction, in that hyperproliferative follicle epidermis quickly spread to below the level of amputation - associated with downward movement of mesenchymal (or dermal) sheath cells. Fibronectin was prominent in both dermis and epidermis at this stage and, as in wound repair, preceded laminin and type IV collagen in covering the lower dermal-epidermal junction. Once a new basal line of epidermis and a complete basement membrane were established, laminin and type IV collagen were detected below this junction and within the prospective papilla-forming mesenchyme. This coincided with ultrastructural observations of profuse sub-basement membrane extracellular material in the region of new papilla formation. The glassy membrane displayed extensive ultrastructural modifications at its lower level, and these corresponded with localized variations in staining intensities for all three antibodies over time. The membrane hung below the level of the epidermis, and was crossed by migrating cells from the mesenchymal dermal sheath of the follicle - it acted to segregate the inner group of follicular dermal cells from wound fibroblasts. Extracellular matrix may be a mediator of the dermal-epidermal interactions associated with this hair follicle regeneration phenomenon.**

Key words: vibrissa, hair follicle, regeneration, wound healing, dermal sheath, basement membrane, fibronectin, laminin, type IV collagen.

## Introduction

Regenerative activity is relatively rare in adult mammalian biology, therefore it is intriguing that the hair follicle, which is one of the distinguishing features of mammalia, should display regenerative phenomena under normal and experimental conditions. The shedding of hair fibres gives external notice that hair growth is not a continuous process, and the lower, fibre-producing regions of follicles, undergo profound organizational changes during the transition from the active (anagen) to the inactive (telogen) state, and back again. In this respect, vibrissa follicles show less follicle shortening than most other follicle types, but nevertheless undergo modifications to both dermal and epidermal components (Young and Oliver, 1976; Jahoda et al., 1991a).

The remarkable recupertive powers of the hair follicle after experimental ablation were first demonstrated by Oliver (1966a,b). Using the rat vibrissa follicle as a model system, he showed that microsurgical removal of up to one third of active lower follicles, with elimination of the major elements involved in hair growth (the dermal papilla and the germinative epidermal matrix), only temporarily curtailed fibre production. A new papilla is formed, apparently from the mesenchymal sheath cells which run immediately exterior to the follicular epidermis. In turn, the epidermis is organized to form a new, fibre-producing, germinative epidermal matrix (Oliver, 1966b). The same process has been described in mouse whisker follicles (Ibrahim and Wright, 1982). Further evidence that the elements required for regeneration are contained within a region of the lower follicle comes from experiments where sections of follicle tube are isolated and transplanted ectopically. Under these circumstances, the observed construction of an active hair-forming bulb must come about through interactions between the dermal sheath and epidermal outer root sheath from the isolated bits of follicle (Oliver, 1967a;

Kobayashi and Nishimura, 1989). This regeneration phenomenon is not universal among mature skin appendages. For example, Lillie and Wang (1941, 1944) and Wang (1943) showed that, although the domestic hen feather papilla has the capacity to induce feather growth, a feather papilla will not be regenerated if removed from the base of a follicle by amputation.

Following lower vibrissa follicle amputation or dermal papilla removal, the essential events in vibrissa follicle regeneration are: (a) formation of a solid core by the remaining epidermis: (b) aggregation of dermal sheath cells at the proximal or basal end of the epidermis (first indenting into the epidermis then enlarging to form a dermal papilla) and (c) induction of a new epidermal matrix and restoration of hair growth, Oliver (1966a). However, it is not known whether the formation of a new dermal papilla by dermal sheath cells is a result of their release from some inhibitory influence, or an example of adult dermal cells responding to inductive influences from epidermis (Sengel, 1976, 1986), or both. It is also not clear exactly when sheath-to-papilla cell transition takes place. One follicle component that has a role in the process is the glassy membrane - a thick and specialized basement membrane unique to the hair follicle. Cells of the dermal sheath cross through this membrane to become part of the new papilla (Oliver, 1966b), but structural and compositional changes to the membrane have not been studied.

If the bases of vibrissa follicles are wounded with sharp needles, they recover to produce normal hair-growing structures, and many of the reconstructive events are similar to those seen in follicle regeneration (Jahoda and Oliver, 1984b). Scar tissue does not appear in the papillae of the restored structures, and this raises the question of whether regenerated papillae have the highly specific extracellular matrix of undamaged follicles (Couchman, 1986; Jahoda et al., 1991), and what role extracellular matrix plays in regenerative events?

Extracellular materials have been implicated in a wide range of developmental activities, including the adult hair growth cycle (Couchman and Gibson, 1985; Couchman, 1986; Couchman et al., 1990), and laminin and type IV collagen are two universal basement membrane constituents which are synthesised by cultured follicle dermal papilla cells (Couchman, 1986; Messenger, 1989). In the present study, after ablation of lower vibrissa follicle bases, the regeneration process was re-examined with the aid of electron microscopy and indirect immunohistochemistry, using antibodies to fibronectin, laminin and type IV collagen.

## Materials and methods

### *Operational procedures*

Adult albino rats of both sexes, aged between 3 and 15 months, were anaesthetized by intraperitoneal injection of Sagatal, 0.055 ml 100 g body weight. All operations were performed on the largest whisker follicles contained within the three most posterior rows of the left mystacial pad. Follicles to be manipulated were chosen from those in mid-growing phase (anagen), recognizable by the fact that they had a growing fibre between one and two thirds of the length of a terminal or club hair. These follicle positions were noted according to the identification system of Oliver (1965), then both growing and club fibres were plucked. Follicles were exposed for operation as previously described (Oliver, 1966a; Jahoda and Oliver, 1984a). Briefly, an incision was made behind and parallel to the most posterior row of follicles, and continued at right angles parallel to the ventral row. The skin flap was subsequently reflected to reveal the follicle bases, which were cleaned of surrounding connective tissue. Sections of lower follicle were then amputated at the same level by a transverse cut. In each case the so-called bulb region, containing the dermal papilla and the epidermal germinative component, was removed. Following surgery, the skin flap was sutured back into place.

Animals were killed and their experimental follicles biopsied for immunohistochemical and ultrastructural observation at intervals from 2 to 30 days postoperatively. One animal, which possessed five amputated follicles, was kept under observation for six months to confirm that the operational protocol would produce external hair fibre regeneration, as previously described (Oliver, 1966a).

In total 15 rats incorporating 73 experimental follicles were employed in this study.

### *Electron microscopy*

Biopsied specimens were processed for electron microscopy as described elsewhere (Jahoda et al., 1991a). Longitudinal sections of 1 $\mu$m thickness were cut from epon resin blocks and stained with toluidine blue for light microscopic observation. Ultrathin sections were cut longitudinally through the lower region of the follicles, and stained with uranyl acetate followed by lead citrate. Thin sections were examined with a Zeiss EM 109 electron microscope.

### *Antibodies*

Antibodies to fibronectin, laminin and type IV collagen were raised and characterized as previously described (Mauger et al., 1982, 1983).

### *Immunofluorescent staining*

Specimens were immersed in Tissue-Tek water-based embedding fluid (Miles Scientific) in aluminim foil boats, and orientated for cutting. The blocks were then frozen after flotation in liquid nitrogen and sections cut at 6 $\mu$m (mainly longitudinally) on a cryostat at $-20$°C, before being air dried and immunolabelled at room temperature by the indirect method. Sections were then immersed for 30 minutes in the primary antibody solution (1:20 dilution in phosphate-buffered saline (PBS) at pH 7.4 for anti-fibronectin and anti-type IV collagen antibody; 1:40 dilution for anti-laminin antibody), rinsed in PBS and immersed for another 30 minutes in fluorescein-isothiocyanate (FITC)-labelled goat anti-rabbit Ig globulin (Institut Pasteur, Paris) solution (5:67 dilution) containing 70 $\mu$g/ml of Evans blue background counterstain. Control procedures included treatment of sections with preimmune sera, or buffered saline, before incubation with the second conjugated antibody. All controls showed little or no fluorescence.

Sections were mounted in buffered glycerin and observed with a Leitz Ortholux II fluorescence microscope equipped with epi-illumination and $I_2$ filter combination (excitation band 450-490 nm, stop 510 nm).

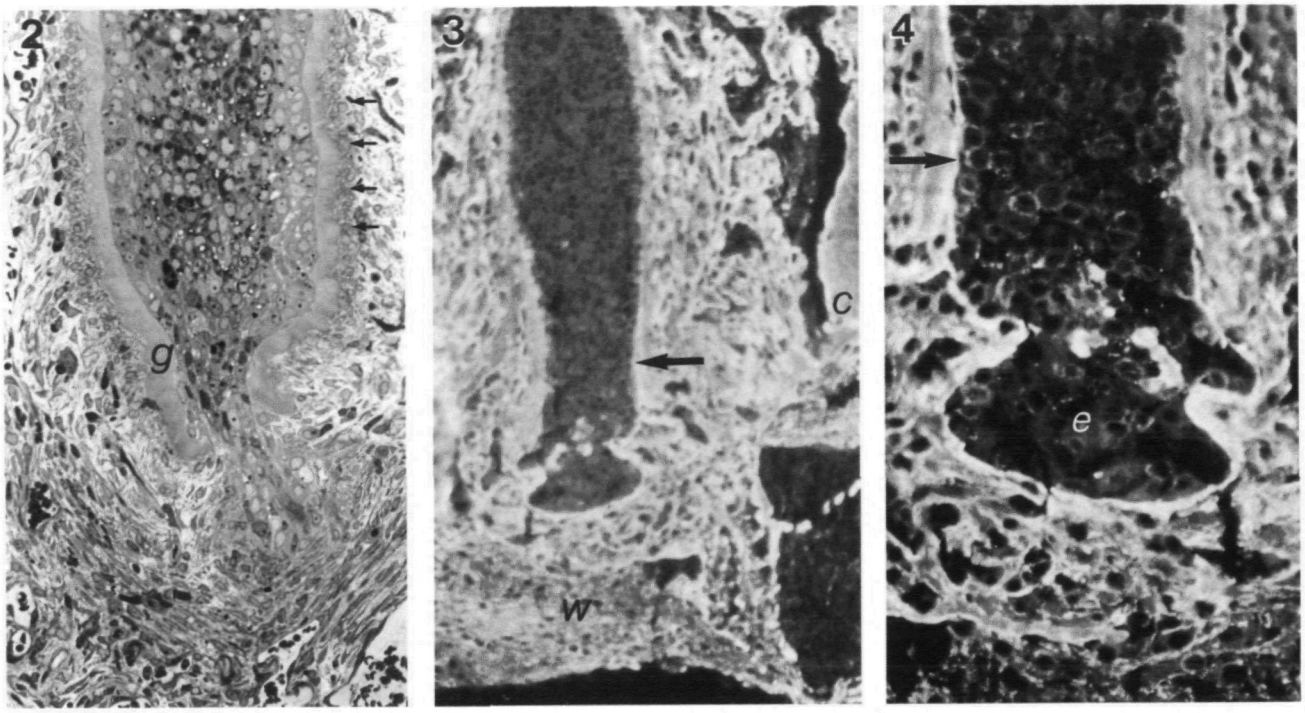



**Fig. 2.** Semi-thin longitudinal section through a regenerating follicle after 5 days. The epidermal cells in the centre of the follicle tube appear darker than those that run along the highly thickened glassy membrane (g), and the epidermis projects down to contact horizontally aligned cells which appear to form part of the dermal wound healing response. A dense line of rounded dermal sheath cell nuclei (arrowed) is visible just outside the glassy membrane. Toluidine blue stained. × 230.
**Figs 3 and 4.** Immunohistochemical staining of a regenerating vibrissa follicle in longitudinal section with antibody to fibronectin at 5 days.
**Fig. 3.** Low magnification shows strong labelling of all non-epidermal elements, including the lowest region (w) formed as part of the wound healing response. The cut end of the outer collagenous capsule (c) is visible. × 120.
**Fig. 4.** Higher magnification shows marking of the dermal-epidermal junction all around the irregular epidermal "tongue" (e), perinuclear labelling in cells of the lower epidermis, and strong staining of the inner glassy membrane (arrowed in 3 and 4) × 305.

## Results

The five control follicle positions that were observed for six months postoperatively all produced emergent whisker fibres measuring between 25 and 45% of the terminal lengths shown by their respective club hairs prior to the operation. In spite of the attempt to standardize responses by operating on follicles in the same growth phase, some variation in the timing of subsequent events was observed. Nevertheless, the continuum of regeneration activities could be conveniently divided into four phases whose main events and approximate timings are indicated in Fig. 1.

*STAGE 1*

Semi-thin sections showed that the epidermal cells that had influxed to fill the centre of the plucked hair canal differed in appearance from the one- to two-cell layer lining its side. The former had light-coloured nuclei with uniform background, while the latter a heterogeneous population with many darker cells (Fig. 2) extended below the cut level of the thick glassy membrane, and beneath the level of amputation - as established from the level of the solid outer collagenous capsule. Surrounding the glassy membrane, the mesenchymal or dermal sheath was seen as a compact layer of cells, delineated by the almost uniformly rounded shape and density of their nuclei. Dermal sheath cells also moved down (Fig. 2), thus remaining in continuous close association with the epidermis. Other fibroblasts in horizontal parallel alignment, could be observed in granulation-like tissue across the wound gap.

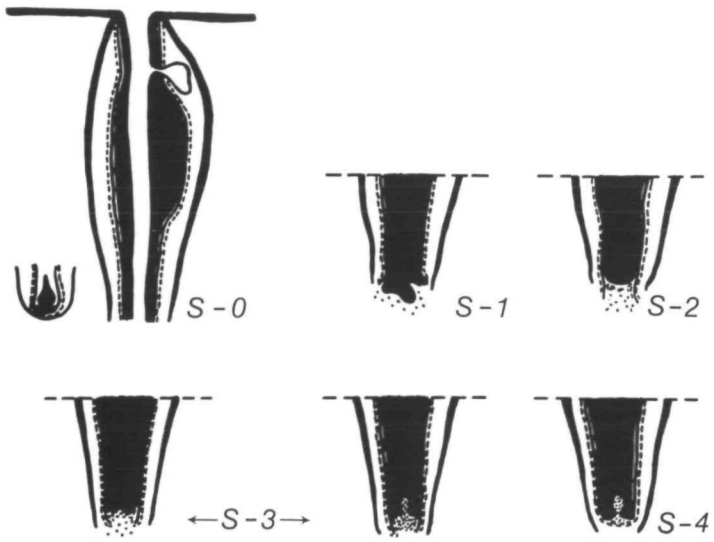



**Fig. 1.** Principal events in lower follicle regeneration. Stage 0 (Immediately after plucking and amputation): S - 0: With the whole bulb structure and hair shaft removed, the remaining follicular epithelium is left as a hollow cylinder, still separated from encircling lower mesenchyme (dermal sheath) cells by the glassy membrane. The level of each cut is clearly delineated by the line of the thick outer collagen capsule. Stage 1 (up to 6 days): S - 1: Epidermal cells fill up the space inside the hair shaft forming a solid column of cells whose base is irregularly shaped and which pushes out to lie below the end of the glassy membrane and beneath the original level of amputation. Dermal sheath cells become active, and move to surround the base of the follicle. Stage 2 (7 to 10 days): S - 2: The follicle epidermis moves up and regularises, so that its base forms a level surface within the confines of the glassy membrane, which now extends below the bottom of the epidermis as an overhanging "skirt" stucture. Dermal sheath cells accumulate beneath the epidermis. Stage 3 (11 to 16 days): S - 3: Papilla formation takes place with progessive indentation of the epidermis by dermal cells still enclosed within the membranous skirt. Stage 4 (17 to 30 days): S - 4: The new dermal papilla increases in size, as hair differentiation becomes apparent and a fibre is produced. The extension of glassy membrane beneath the new fibre-producing follicle bulb gradually disappears.

Fibronectin was present in all non-epidermal elements of the follicle, including the wound tissue (Table 1; Fig. 3). The glassy membrane appeared comparatively weakly stained, except on the epidermal side, near to its base. Fibronectin extended around the junction between the basal outgrowth of epidermis and the dermis (Fig. 4) and perinuclear labelling was consistently seen within epidermal cells near the base of the epidermal column (Fig. 4).

Laminin and type IV collagen which displayed similar patterns of marking (Table 1) richly stained the blood vessels, the trabeculae, and the glassy membrane of the follicle cavities. New tissue which had massed beneath the level of amputation level was initially unmarked (Fig. 4), but over time a few blood vessels appeared. The glassy membrane, sometimes separated from the lower outer root sheath, was always more brightly fluorescent on the inside than the outside. The projecting downgrowth of epidermis was not completely labelled at the dermal-epidermal junction (Figs 6,7; Table 1). This corresponded ultrastructurally with the absence of a distinct basement membrane structure, although large streaky lines of underlying extracellular materials were abundant (Fig. 8). Normally the glassy membrane appears double-layered with an internal, almost amorphous, electron-dense layer, incorporating a single well-defined basal lamina and an outer less-dense stratum, containing a fibrous collagen element (Jahoda et al., 1991a). After amputation, the lower structure thickened and became highly structured. The inner layer displayed multiple basement membrane-like lamellar loops, in a series of irregularly sized ridges. This was bordered by a semi-structured line containing patches of dark material which merged with a lighter, more variable and diffuse substratum, which was streaked with fibrous collagen (Fig. 9).

*STAGE 2*

In the epidermis, which had moved up to be completely enclosed by a hanging glassy membrane, a lightly stained layer of cells was visible at the sides and basally, although darker stained cells were still present at the core of the structure (Fig. 10). Fibronectin was particularly prominent on the inside of the thick and folded lower glassy membrane, and relatively uniformly

**Table 1.** *Staining by fibronectin (FN) and laminin (LAM) type IV collagen (C IV) antibodies in amputated rat vibrissa follicles*

| | STAGE 1 | | STAGE 2 | | STAGE 3 | | STAGE 4 | |
|---|---|---|---|---|---|---|---|---|
| | FN | LAM/C IV | FN | LAM/C IV | FN | LAM/C IV | FN | LAM/C IV |
| Dermis | Profuse staining all regions | Vasculature above level of amputation | Wound region strongly marked | Vasculature including revascularization in wound region | Pre-papilla cells, and those enclosed by lower glassy membrane, well marked | All vasculature | New dermal papilla and dermal sheath well marked | All vasculature including capillaries in new dermal papilla |
| Epidermis | Perinuclear labelling of cells in lower follicle | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lower epidermal-dermal junction | Uninterrupted marking | Incomplete marking | Complete labelling | Complete labelling. Sub-basement membrane region strong patches of label | Complete labelling | Complete labelling. Sub-basement membrane region strong patches of label | Papilla-epidermal junction feebly stained | Papilla-epidermal junction well marked |
| Glassy membrane | Strong on inner line only and near follicle base | Strongest on inner line of membrane near follicle base | Strongest label inner membrane | Strongest label inner membrane | Intensely marked | Intensely marked | Lost from unattached lower membrane | Lost from unattached lower membrane |

0 = marking absent.

distributed in the mesenchyme, inside and outside of the glassy membrane skirt (Fig. 11). The basal or proximal epidermal-dermal junction was also well marked, with additional downward jutting projections of labelled material. Laminin and type IV collagen consistently stained capillaries in new tissue beneath the level of amputation. The density and distribution of these increased progressively over time. With both antibodies, the inner glassy membrane was highly fluorescent and the basal dermal-epidermal junction was strongly marked. The streaks of material which jutted out from the base of the epidermis were also well stained and their irregular outline and consistency, indicated that they were not all blood vessels (Fig. 12).

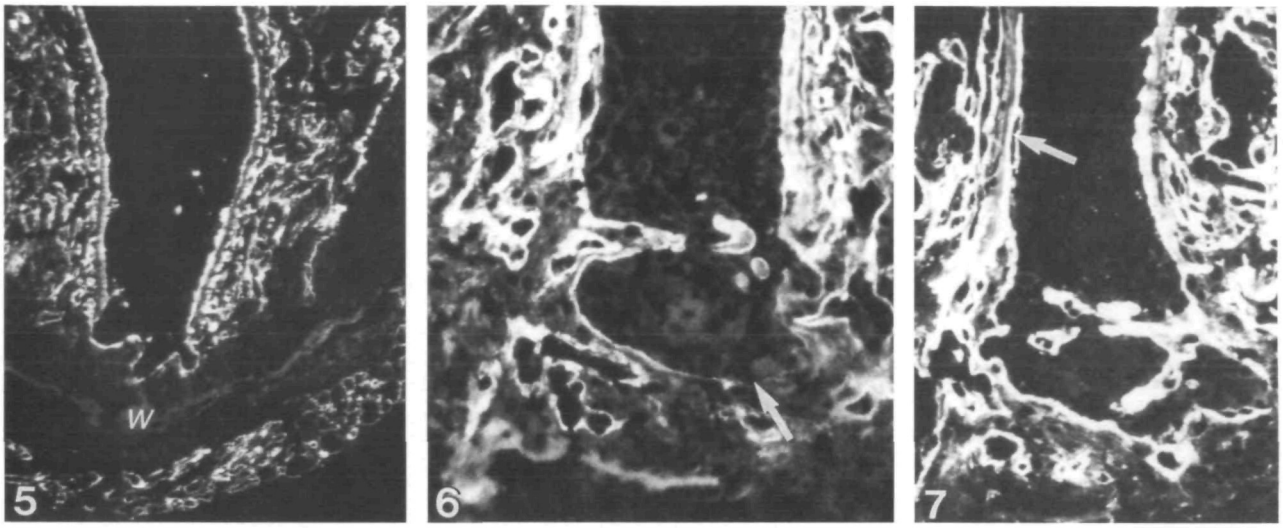
During and after the upward epidermal movement, separation of the glassy membrane from the sides of the lower epidermal column occurred. This was mostly a relatively clean split, which left the bulk of the glassy membrane structurally intact, and a residual basement membrane as a barrier between mesenchymal cells that had penetrated into the spaces, and follicular epidermis (Fig. 13). Whole dermal sheath cells (or cell elements) seen within, or just adjacent to, gaps in the glassy membrane were presumed to be in the process of passing through it (Fig. 14). Below the level of the epidermis, the trailing glassy membrane lost facets of its complex structure to become more uniform, with fewer laminations (Fig. 15). It remained broad, and mesenchymal cells were again visible within it, or apparently in the process of penetrating it. There was some evidence that mesenchymal cells, which were on the inside of the membranous curtain, differed from those on the exterior, in that their nuclei were more uniformly rounded or ovoid compared with the more elongated appearance and indented nuclei of outer cells.

Where separation of the glassy membrane from the epidermis did not take place, but dermal sheath cells had infiltrated the membrane, they caused widespread disruption and destruction to it (Fig. 16). The profuse amounts of extracellular material visible below the base of the epidermis, (Fig. 17) corresponded to laminin and type IV collagen marking seen by immunofluorescence (Fig. 12).

### *STAGE 3*

Further development involved changes to the shape of the basal epidermis, with the gradual formation of an upward indentation by mesenchymal cells (Fig. 18). Throughout this period extensive marking of all three extracellular materials including non-vascular laminin and type IV collagen continued, within the glassy membrane skirt and the developing dermal papilla (Fig. 19). By this time, new blood vessels had pervaded the whole of the dermal tissue at the site of amputation (Fig. 19).

The initial onset of epidermal pre-fibre differentiation, coincided with cell nuclei within the newly developed dermal papilla assuming a typical rounded appearance (Fig. 20). Labelled glassy membrane was still observed trailing below the new hair bulb, although from this point it became progressively less solid.




**Figs 5-7.** Immunohistochemical staining of regenerating vibrissa follicles with antibodies to laminin and type IV collagen all showing strong marking on the inner glassy membrane.
**Fig. 5.** At 6 days, labelling of the dermal-epidermal junction extends down to surround the lower projection of wound epidermis. The small blood vessels between the epidermis and the collagen capsule are stained; however there are no labelled blood vessels in the wound tissue (w) beneath the epidermis. × 95.
**Fig. 6.** Type IV collagen marking of another follicle shows gaps in staining at the junction between the epidermal wound projection and the underlying dermis (arrow). × 200.
**Fig. 7.** In this follicle stained with laminin antibody, the lower wound epidermis is indented with lines of labelled material. Evidence of separation of the glassy membrane from the epidermis can be seen (arrowed) and in Fig. 6. × 170.

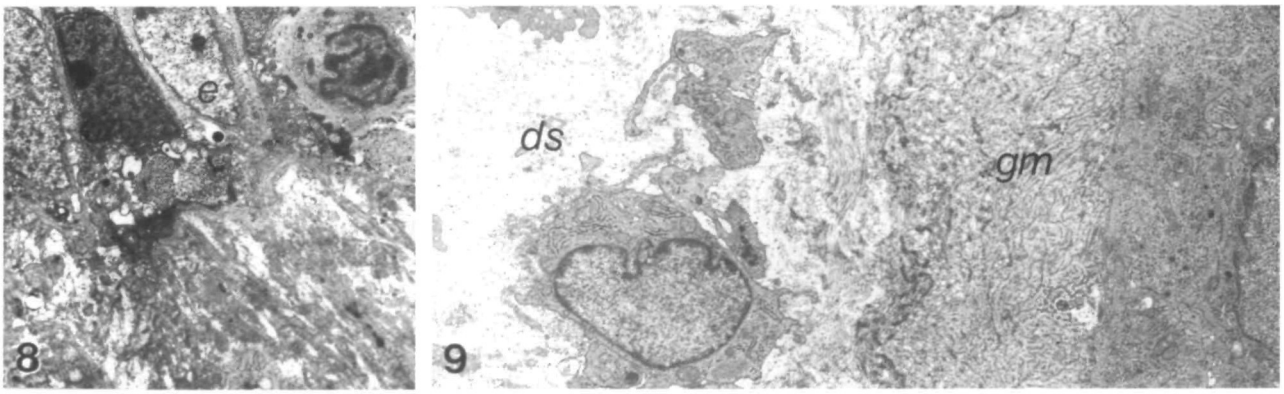



**Fig. 8.** Massive amounts of extracellular material around the base of the epidermis (e). This corresponded with strong labelling for laminin and type IV collagen in this locality. × 2000.
**Fig. 9.** Electron micrograph of the glassy membrane (gm) in a regenerating follicle at day 8. Nearest the follicle epidermis the structure consists of loosely organised laminae; the centre is generally more amorphous with lines of electron-dense material; the region adjacent to dermal sheath cells (ds) contains bundles of fibrillar collagen. × 3900.

Strong marking with anti-fibronectin antibody (Fig. 21), delineated the new dermal papilla and follicular dermis within the glassy membrane enclosure as a single common entity. Here, laminin was also present in granular, non-vascular form, although it principally stained the inner dermal papilla-epidermal junction (Fig. 22). By contrast, fibronectin was not uniformly visible around the papilla-epidermal interface (Fig. 21).

Cells in the enclosed region directly below the papilla displayed rounded papilla-like nuclei, and were surrounded by electron-dense extracellular material (23). This contrasted sharply with the shape and organization of the fibroblast-like cells in horizontal alignment within the wound tissue, not far below them (Fig. 24).

*STAGE 4*

As hair differentiation proceeded, the hanging glassy membrane gradually disappeared and the new dermal papillae became enlarged and rounded (Fig. 25). Most papillae lacked the long apex typical of vibrissa follicles, a few were doubled. Fibronectin was seen within the dermal papilla, and marking the lower papilla-epidermal junction (Fig. 26). Laminin and type IV collagen distribution now became strongest in the new papilla

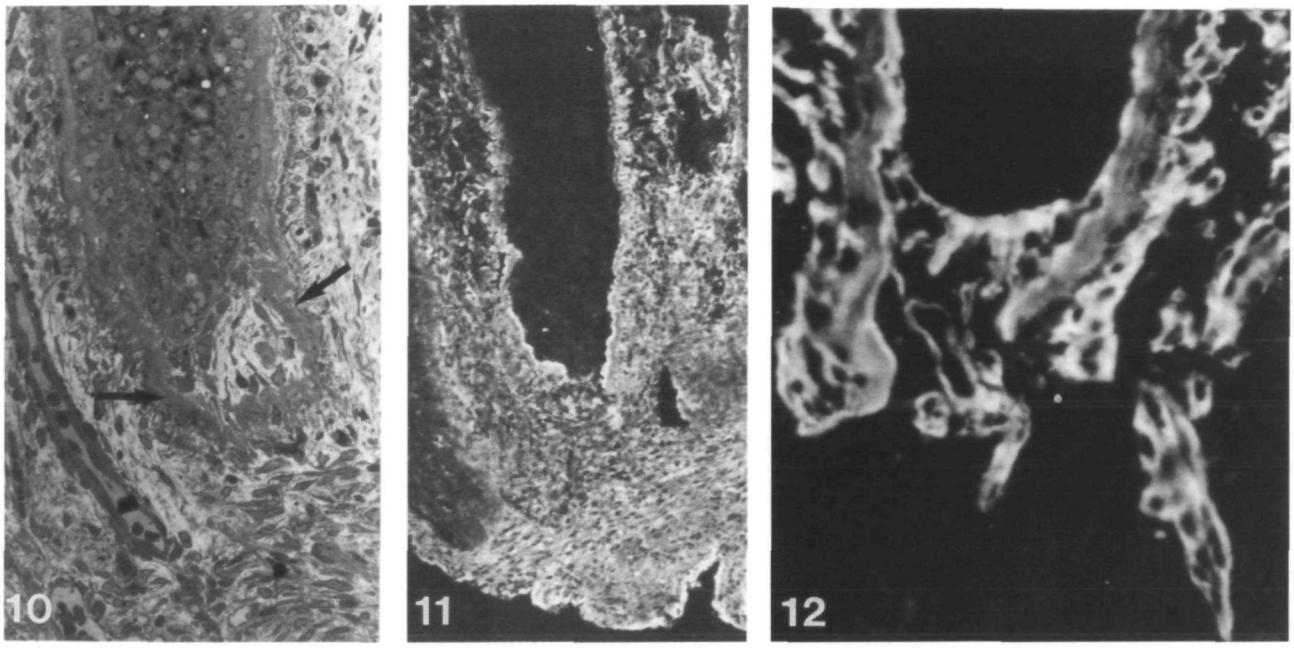



**Fig. 10.** Toluidine-blue-stained semi-thin section showing the follicle epidermis at a higher level, and the glassy membrane now trailing below it (arrowed). A line of dermal cells lies close to the epidermis, enclosed by the membrane × 260.
**Fig. 11.** At eight days, fibronectin marking is less intense, but still relatively uniform above and below the level of amputation. The inner side of the glassy membrane is still intensely stained low down. × 95.
**Fig. 12.** Laminin marking establishes that blood vessels have infiltrated the new tissue below the level of amputation at 8 days. The glassy membrane is strongly stained, and streaks of marking are still present beneath the basal epidermis. × 305.

blood vessel supply and basement membrane zone, with only traces of loose granular labelling within the papilla (Fig. 27). Papilla cells, which were initially closely associated (Fig. 28), became increasingly spaced apart - surrounded by electron-light extracellular spaces. They displayed a multitude of processes, many of which extended towards a thickened and periodically multilayered papilla-epidermal basement membrane (Fig. 29).

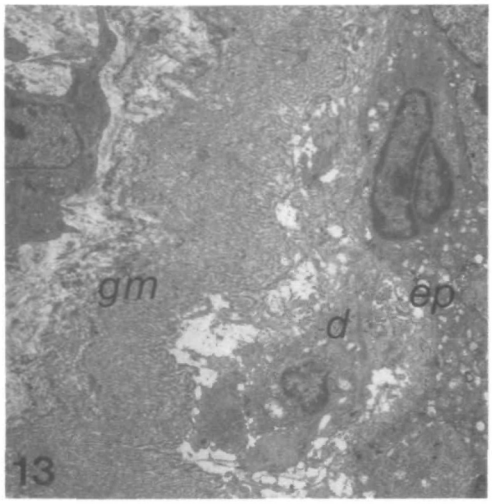
## Discussion

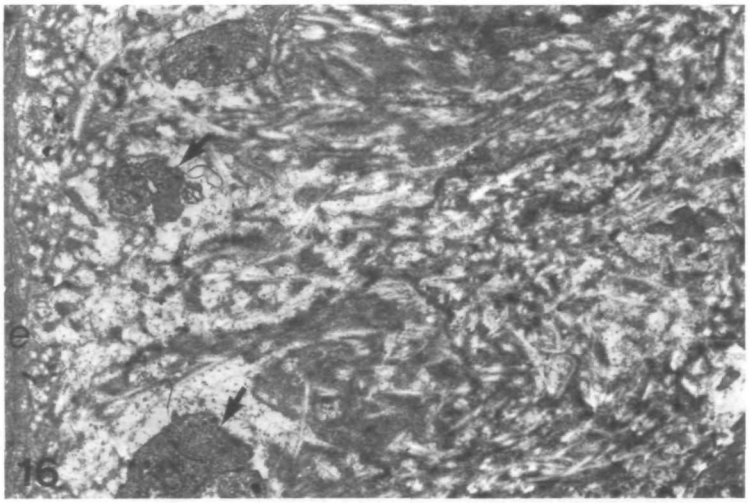
The progressive stages of lower whisker follicle regeneration seen in the present work largely conform to histological observations of this phenomenon by Oliver (1966a,b). However, this study has shown that extracellular matrix components are present in profuse quantities at the site of mesenchymal cell aggregation and papilla formation, and as such may be implicated in these events. It has also demonstrated changes in glassy membrane structure and composition, and it has emphasized the migratory and penetrative powers of the dermal sheath cells. An initial point of interest is by way of interpretation, since our observations suggest that the early post-amputation events can be looked on as paralleling general skin wounding responses.

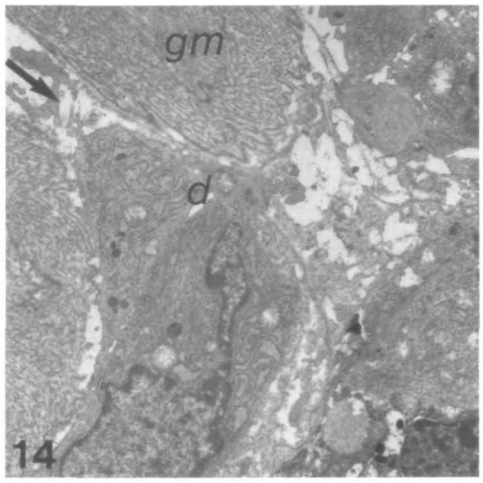
Plucking of the hair shaft after removal of follicle bases was successful as a way of standardizing the time scale of subsequent events, and consistently produced the interesting epidermal "wounding" response, in which the core of follicular epidermis spread to below the edge of the cut. This epidermal overshoot was not seen to any extent in pilot experiments where plucking was omitted. Oliver (1966b) describes pronounced epidermal downgrowth following papilla removal, but not after lower follicle excision. It appears that early loss of the hair shaft enhances this effect, perhaps by stimulating the outer root sheath cells which line the follicle cavity to divide rapidly and fill in the space. At the same time, early downward movement of dermal sheath cells ensured continuing intimate contact between responding epidermal and dermal cells. Although dermal sheath cell division was not investigated, these cells may undergo replication during the anagen phase of the hair growth cycle (Uno, 1989) and probably did so here. However, the point is that both dermal and epidermal cells initially acted as though they were participating in a skin wound healing response. Furthermore, the observation that fibronectin preceded the basement membrane components laminin and type IV collagen at the basal dermal-epidermal interface is consistent with skin replacement where fibronectin is laid down first to provide a basis for basement membrane restoration (Clark et al., 1982; Woodley and Briggaman, 1988). Therefore, for the current protocol, papilla formation did not appear to start until a basement membrane had been restored, and a morphologically distinct basal epidermis established.

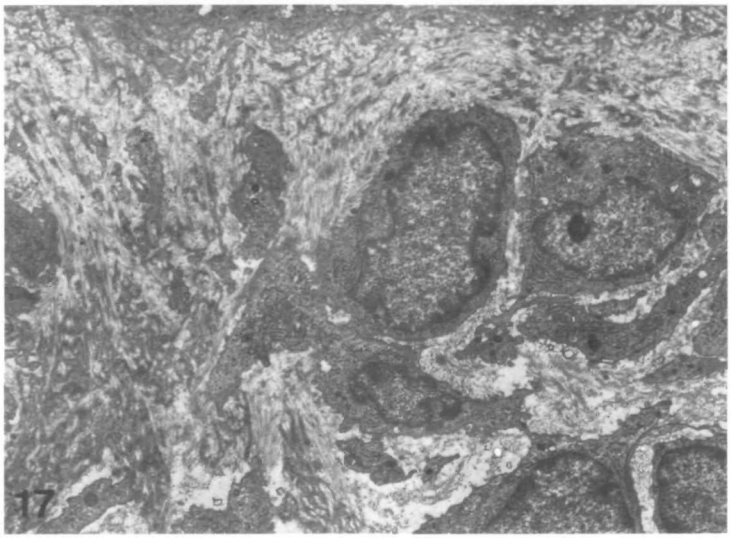
### *Mechanism of dermal papilla reconstitution*

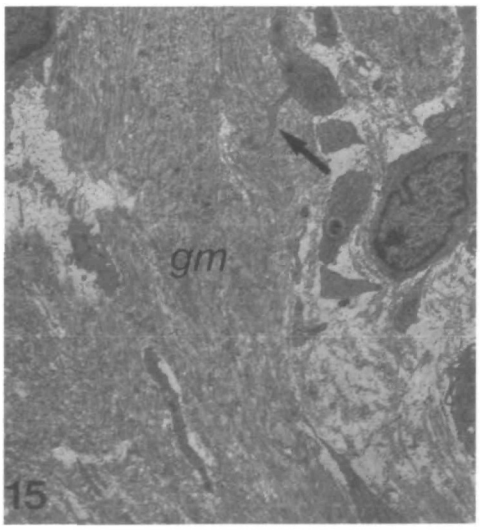
The adult hair follicle dermal papilla ultimately derives




**Figs 13-17.** Transmission electron microscopy of the dermal-epidermal junction.

**Fig. 13.** Dermal sheath cell (d) in the space where glassy membrane (gm) has detached from the epidermis (ep). A basal lamina and additional material remain attached to the epidermal side. × 2500.

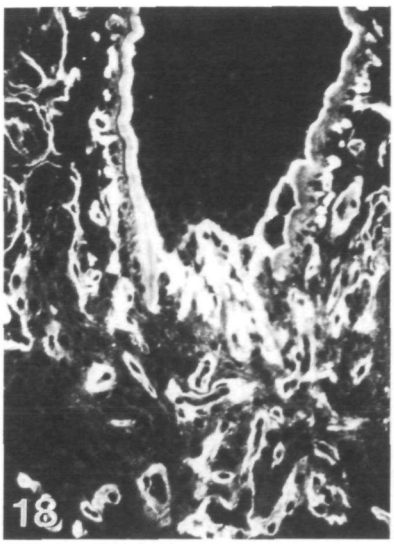
**Fig. 14.** A dermal sheath cell (d) which has apparently just moved through a gap (arrowed) in the glassy membrane (gm). × 4000.

**Fig. 15.** Glassy membrane (gm) hanging beneath the level of the epidermis - structurally it appears relatively uniform. One dermal sheath cell has a projecting process into the membrane (arrowed), and bits of other cells are visible lower down. ×1600.

**Fig. 16.** Lower glassy membrane which has not separated from the epidermis showing disruption to its inner layer associated with the presence of dermal sheath cells (arrowed) in close proximity to the epidermal junction (e) × 6250.

**Fig. 17.** Large patches of extracellular material interspersed between cells at the base of the epidermis. × 3900.

from an embryonic mesenchymal cell condensation which underlies an epidermal placodal thickening (Davidson and Hardy, 1952; Wessells and Roessner, 1965). While the mechanism of mesenchymal cell aggregation is still open to question (Bard, 1990), in the embryonic process, the cells involved do not have to move great distances or traverse extracellular barriers. By contrast, dermal papilla regeneration, as observed here, and elsewhere (Horne, 1987), involved a different and fairly complex series of events. First the dermal sheath cells at the side of the follicle became activated, altered in morphology and alignment, and began the



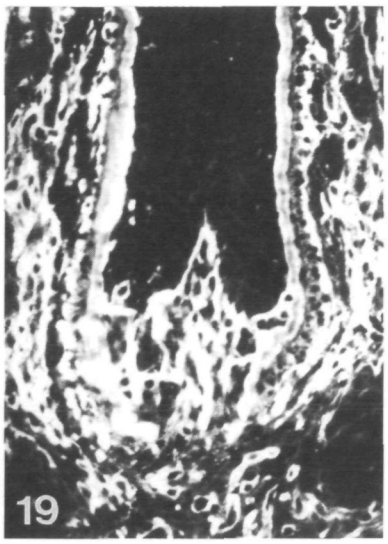
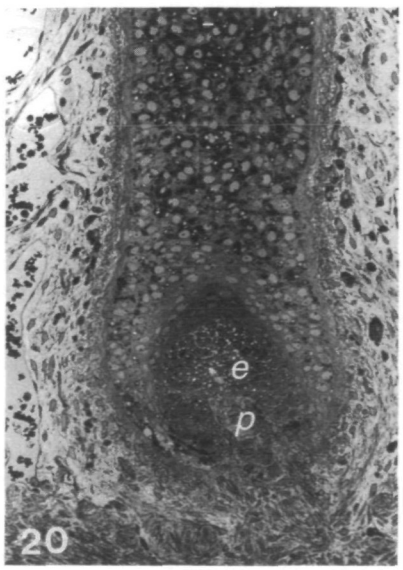




**Fig. 18.** Type IV collagen distributed beneath the epidermis and showing the presence of capillaries that have infiltrated the region below the level of amputation. A small indentation is visible in the epidermis. × 305.

**Fig. 19.** Staining with antibody to laminin shows strong marking all around the basement membrane and between the cells of the newly forming dermal papilla, which cuts into the base of the epidermis. The glassy membrane remains well marked, and blood vessels are present throughout the restored tissue below. × 305.

above mentioned migration. The fact that early dermal sheath cell movement coincided with localized loss of fibronectin in the adjacent glassy membrane, was an indication of reduced fibronectin by the sheath cells. Loss of fibronectin is a feature of migratory cells, such as embryonic neural crest (ffrench-Constant and Hynes, 1988). After withdrawal of epidermis to within the glassy membrane, the continued movement of dermal sheath cells, and in particular their passage across the glassy membrane and recruitment into the papilla-forming region (Oliver, 1966b) is a rare phenomenon in normal adult tissues. Given the considerable physical obstacle constituted by the membrane, it suggests that dermal sheath cells must employ extracellular protease activity in order to pass through it. This idea was supported by evidence of massive destruction and organizational disruption of those lower glassy membranes in which dermal sheath cells had remained trapped. Sheath cell migratory behaviour was also an indicaton that some chemotactic stimulus was specifically attracting the sheath cells to this region. Fibronectin, although a chemoattractant, was not considered a likely candidate, because at the time of most sheath cell movements it was relatively uniformly distributed. The effect could be due to the massive amounts of extracellular type IV collagen and laminin streaked through the mesenchyme in the region of papilla formation. Production of large amounts of basement membrane-type extracellular matrix is a feature of hair follicle dermis, and both laminin and type IV collagen are synthesised by dermal papilla, and lower dermal sheath cells in culture (Couchman, 1986; Messenger, 1989; Jahoda et al., 1991b). Growth factors, including PDGF and FGF, are present during wound healing phenomena (reviews Huang et al., 1988; Fox, 1988), and one or both of these could be involved, perhaps sequestered by the extracellular matrix. In this context, PDGF is a recognized chemoattractant (Huang et al., 1988) and FGF has been found in basement membranes and the dermal sheath region of developing hair follicles (Gonzalez et al., 1990).

While cell movements account for dermal sheath cells being in the zone of papilla renewal, a crucial question is what causes their transition to dermal papilla cells?



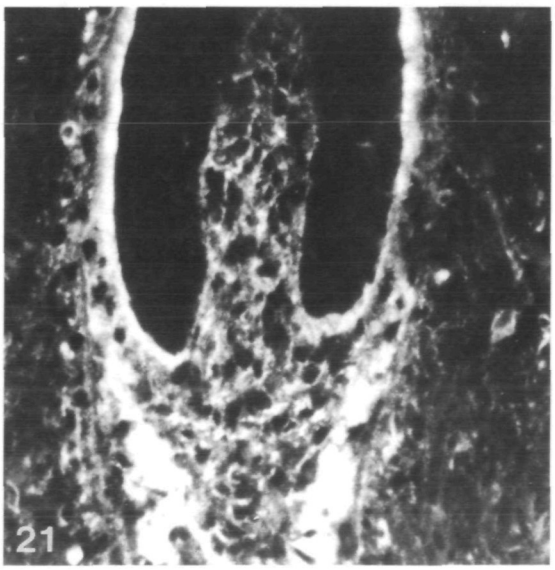




**Fig. 20.** Toluidine-blue-stained semi-thin section showing evidence of epidermal differentiation (e) around a forming dermal papilla (p). × 175.

**Fig. 21.** Fibronectin marks a newly forming papilla, and a group of sub-papillary cells enclosed by the glassy membrane, more strongly than surrounding stroma. The papilla-epidermal junction is poorly labelled compared with the glassy membrane, which shows signs of disintegration lower down. × 205.

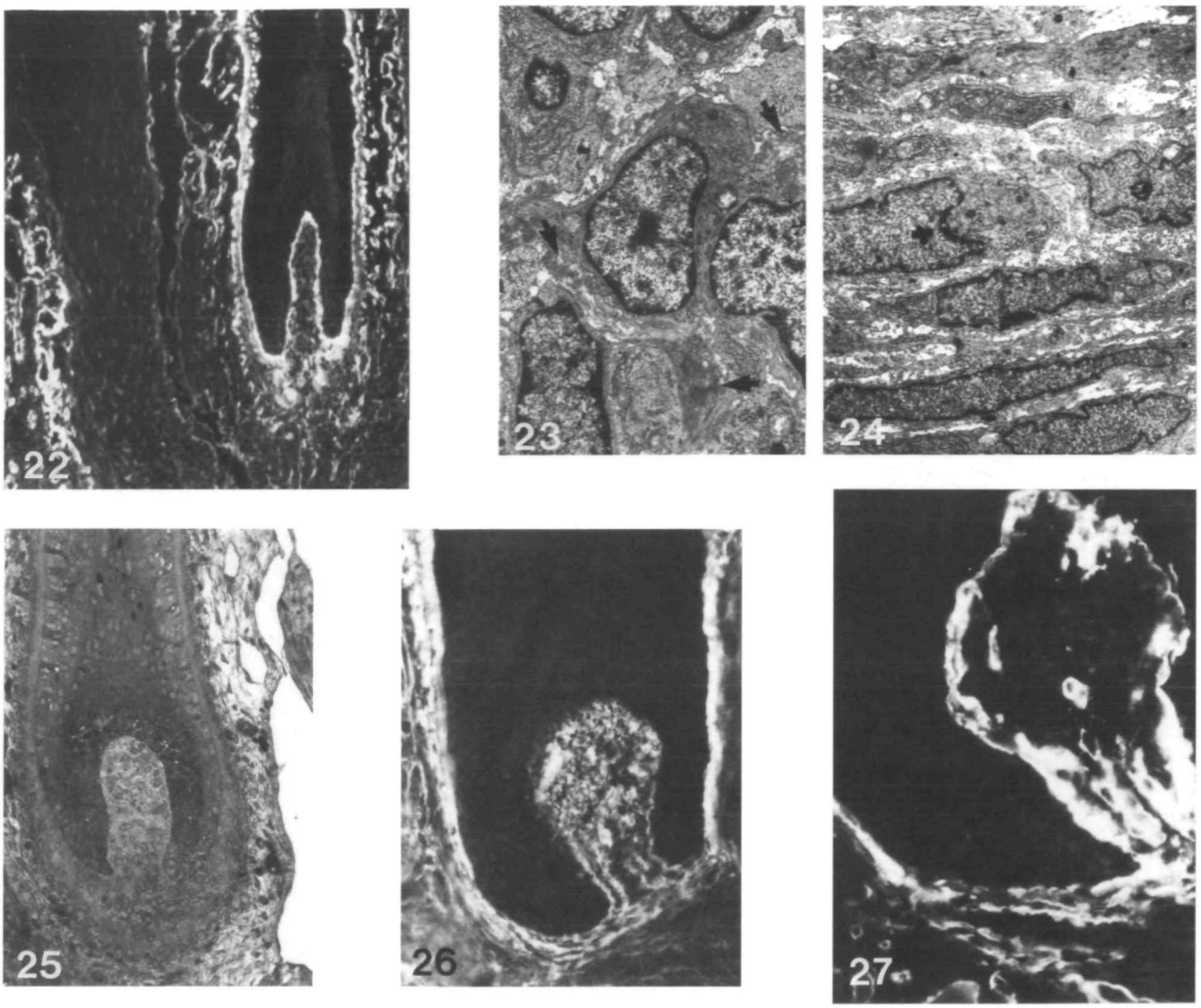



**Fig. 22.** Laminin marking highlighting the dermal-epidermal junction all around an elongated papillary structure at 12 days. Some hanging glassy membrane is still visible. × 95.
**Fig. 23.** Transmission electron micrograph of cells at the base of a newly reconstituted papilla. The cells have rounded nuclei typical of those found in papillae, and patches of dense extracellular material (arrowed) are present. × 4000.
**Fig. 24.** Cells in the wound repair tissue beneath the papilla have flattened nuclei, and a clear horizontal alignment. × 4800.
**Fig. 25.** Toluidine-blue-stained follicle showing the formation of a more clearly defined follicle bulb structure, with papilla and differentiating epidermis. the trailing glassy membrane has all but disappeared. × 130.
**Fig. 26.** At 21 days, strong fibronectin labelling is restricted to the papilla, and does not extend to any depth below it. Once more the glassy membrane is well marked, while the inner papilla epidermal junction labelling appears to be limited to the lower half of the papilla. ×135.
**Fig. 27.** Laminin staining of a new, more typically shaped papilla at 27 days, clearly shows elements of new vasculature. × 270.

The idea that the lower dermis is self regulatory, and that removal of the dermal papilla releases some inhibitory influence on the sheath cells is countered by the observation that taking sheath cells from local environmental controls (by culturing) does not automatically imbue them with inductive properties. Adult papilla cells experimentally induce hair formation (Jahoda et al., 1984; Horne et al., 1986; Reynolds and Jahoda, 1991), cultured lower follicle dermal sheath cells do not (Horne et al., 1986). Similarly, the effect of passing through the glassy membrane cannot be the crucial influence, as, following dermal papilla removal alone (Oliver, 1966b), papilla regeneration can occur without this phenomenon. More likely, the epidermis at the base of the regenerating follicle acts on the dermal sheath cells to produce a dermal sheath to papilla cell change (Sengel, 1976). This would mirror hair follicle embryogenesis, which involves a sequence of dermal-epidermal interactions in which the pre-papillary mesenchymal cell aggregation is induced by the epidermis

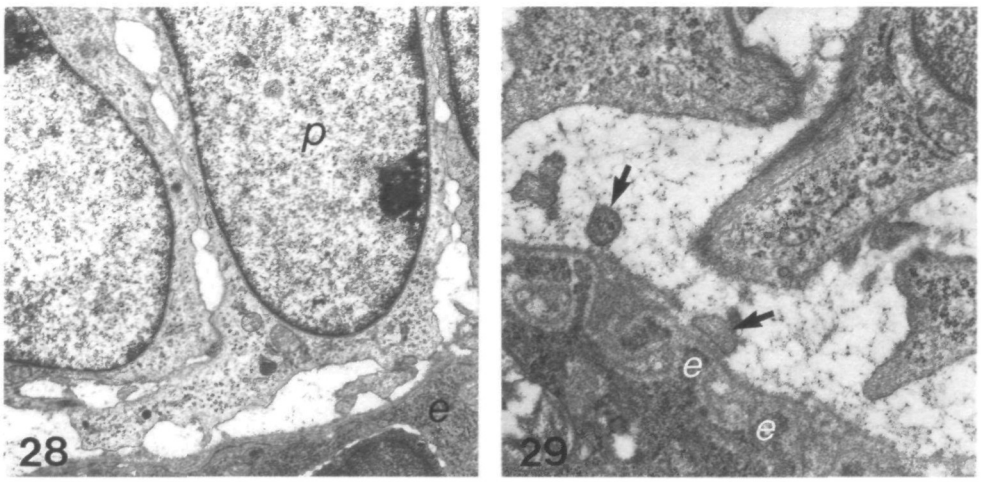



**Fig. 28.** Transmission electron microscopy of papilla cells (p) in close association with each other and separated from the epidermis (e) by basement membrane. The extracellular spaces around the papilla cells are no longer filled with electron-dense materials. × 4000.

**Fig. 29.** More detailed examination of the basement membrane region, showing finger-like projections of epidermal cells (e), and dermal cell elements (arrowed) close to a fairly elaborate lamina structure. × 16000.

(Sengel, 1986). Whether or not this activity is modulated by the large amounts of basement membrane extracellular matrix found in this zone is unclear. What is certain is that subsequent wound responses and the formation of scar tissue is suppressed, a phenomenon also observed after lower follicle wounding (Jahoda and Oliver, 1984b).

*Glassy membrane and the extracellular matrix*

In the regenerative process, the glassy membrane has been shown: (a) to act in the control of epidermal cell structuring after the initial epidermal wounding response; (b) to be one route for dermal sheath migration and (c) to form an enclosing curtain for dermal sheath cells prior to papilla formation. The action of 'loose' glassy membrane in providing an enclosure for dermal cells, and perhaps assisting in aggregation phenomena, has no obvious parallel in follicle development, or the growth cycle. It appears to be an unusual example of a basement membrane acting as a physical aid to morphogenetic activity.

In its size and complexity the glassy membrane can be compared with other specialized basement membranes such as Reichert's membrane. The latter has been proposed as a model system for the study of basement membrane assembly (Hogan et al., 1984) and has been used for isolation of extracellular matrix components (McCarthy et al., 1989). The glassy membrane is interesting because of the many intermediate forms of structural organization it displays. Recent work has shown that mixtures of basement membrane components will self assemble into a variety of structural forms when incubated together *in vitro* (Grant et al., 1989), and after the amputation process the glassy membrane showed profound structural changes, presumably as a result of altered synthetic activity by contributing cells. Although the normal function of the glassy membrane is still not clear, what is certain is that both dermal and epidermal cells invest considerable metabolic activity in its formation and maintenance.

While one previous study using mouse vibrissa follicles has suggested that regenerated papillae do not contain blood vessels (Ibrahim and Wright, 1982), immunomarking clearly reinforced the original observations of Oliver (1966) that, in rat follicles, the new papillae contained blood vessels.

The perinuclear fibronectin staining of lower follicle epidermal cells in the early wounding response mirrors a similar phenomenon seen in epithelial cells in basal cell carcinomas (Peltonen et al., 1988). This is relevant because of increasing suggestions that basal cell carcinomas may have their origin in the outer root sheath cells of hair follicles (Asada et al., 1990; Markey et al., 1990). The present results infer that fibronectin production may be initiated as a general response in hyperproliferative outer root sheath cells, whether transformed or not. Once a definite papilla-like structure was visible, the delineation of papilla and subpapillary dermal sheath cells from surrounding mesenchyme by strong fibronectin marking suggested that it was acting in an aggregative capacity. However, the paucity of fibronectin at the dermal-epidermal junction of early regenerating papillae has been seen at the earliest stages of each growth phase in mature follicles (Jahoda et al., 1991a).

Hair follicle regeneration remains an useful model for wounding and morphogenesis studies. The key process of dermal sheath to dermal papilla cell transition, is the subject of current investigation.

We thank Yolande Bouvat and Bruce Pert for photographic assistance, and Dr. A. Reynolds for critical reading of the manuscript. C.A.B.J. held an SERC (NATO) Fellowship.

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*(Accepted 12 December 1991)*