Histological studies of the effects of wounding vibrissa follicles in the hooded rat

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SUMMARY

The effects of wounding the lower region of rat vibrissa follicles with a sharp tungsten needle were examined histologically, both shortly after injury and up to one year postoperatively. Following cell damage in the dermal papilla component hair growth ceased, and resumption of fibre production was always preceded by dermal papilla reformation. This papilla healing and regeneration was not associated with the production of scar tissue. In follicles undergoing no cell displacement during wounding (an effect associated with the growth of longer than normal hairs) dermal papillae were reformed from the residual papilla cell population, with recruitment of cells from surrounding mesenchyme. Follicles plucked just prior to wounding revealed little or no original epidermal matrix three days later, confirming that dermal components were primarily affected. Papilla cell counts performed on follicles which had consistently produced longer hairs gave no indication of increased papilla cell numbers. Follicles which underwent displacement of cellular material and displayed distortion of normal follicle morphology shortly after wounding (effects associated with the production of shorter than normal hairs) also revealed abnormalities at long-term biopsy. Moreover these follicles often had a history of altered fibre characteristics from one postoperative generation to the next. It is concluded that gross morphological disruption of the normal cellular relationships in the lower follicle results in a series of reorganizational difficulties with each recurring phase of the hair cycle.

INTRODUCTION

The cyclic growth of hair is an important aspect of hair follicle behaviour which is capable of being modified through injury. Argyris (1969) reported that resting follicles could be stimulated to undergo premature hair growth in a number of ways, including wounding of nearby skin. Plucking can also initiate growth in quiescent follicles, and Silver, Chase & Arsenault (1969) emphasized that plucking is a direct method of follicle wounding. Furthermore it has been shown that following removal of the dermal papilla, or up to one third of the follicle base, vibrissa follicles are capable of regenerating a new papilla from the mesenchymal sheath, after which hair growth is resumed (Oliver, 1966*b*; Ibrahim & Wright, 1982).

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It has been shown that following physical damage to the whisker follicle base with a tungsten needle point, hair growth is restored, and under certain experimental conditions longer than normal fibres are produced (Jahoda & Oliver, 1984*b*). These longer hairs are produced through a prolongation of the vibrissa hair cycle, and are often associated with a reduction in fibre growth rate.

One aim of this paper, therefore, was to examine histologically the events leading to the restoration of follicle activity and follicle morphology in the period immediately following wounding. Moreover, since it was found that a comparatively minor alteration in operational technique caused a relatively major shift in both hair length measurements and consistency of response (Jahoda & Oliver, 1984*b*), an attempt has been made to explain this phenomenon. Histological observations were also performed on wounded follicles with recorded growth characteristics in an attempt to relate follicle morphology to postoperative follicle behaviour. Finally, since there was a possibility that wounding the dermal papilla might stimulate cell division, and thus increase papilla cell numbers, papilla cell counts were performed on follicles which had persistently produced longer fibres, and these results compared with their controls.

MATERIALS AND METHODS

Operations were performed on vibrissa follicles on the upper lip of 6-month-old inbred hooded rats. Anaesthesia was induced by the intraperitoneal injection of Sagatal, 0·055 ml/100 g body weight. Details of vibrissa follicle exposure, and the wounding procedures employed have been previously described (Jahoda & Oliver, 1984*b*). Experimental follicles and their contralateral counterparts were kept under regular observation for up to 10 months postoperatively, and then biopsied. Most of these follicles and their controls underwent histological processing. In addition, for the three wounding techniques (horizontal introduction of the tungsten needle into the bulb region, method I; distoproximal introduction of the needle into the bulb, method II; and plucking, followed by the method II procedure, method III) wounded follicles were biopsied up to 21 days after injury and processed histologically to determine the immediate effects of wounding on the follicles. The total number of experimental follicles processed was as follows:

Method I : 59
Method II : 35
Method III : 16

Histology

Follicles were fixed in formol saline, processed and serially sectioned at 8 μm, parallel to the long axis of the follicle. Sections were stained with Ehrlich's haematoxylin and eosin, or in a combination of Weigert's haematoxylin, alcian blue and Curtis's Ponceau S.

Papilla cell counts

From wounding methods II and III six follicles which had consistently produced longer than expected fibres, together with their controls, were used for comparative papilla cell counts. Cell numbers were estimated by direct counting of dermal papilla cell nuclei in serial sections, using a method based on the one employed by Van Scott & Ekel (1958).

For each follicle every fifth section was photographed. Large prints of each section were then gridded into 1 cm² areas, and each square containing papilla cell nuclei counted three times. The mean figures for each area were then added together and multiplied by five to give a final estimate for each papilla.

RESULTS

*Observations on the immediate responses to wounding*

Methods II and III

25 follicles were operated on in the same manner as those which produced regular hair length increases (Jahoda & Oliver, 1984b). In no case was loss of follicular material from the bulb observed during the wounding procedure. Follicles were biopsied at the following intervals: 24 h – (6); 3 days – (5); 6 days – (5); 10 days – (5); 14 days – (4).

24 hours: A high proportion of dermal papilla cells had been destroyed, and pyknotic nuclei and cellular debris were scattered throughout the papillary milieu (Fig. 1). Some lengths of capillary appeared to have retained their integrity, and blood was clotted around the papilla base. In the upper matrix the distinctive cell layering was becoming obscure and melanocytes were in a regressed state. Epidermal cells in the lower matrix had assumed the appearance of outer root sheath. While the extent of papilla damage varied from specimen to specimen, a feature common to all follicles was the absence of gross morphological disruption of the bulb region.

3 days: Undamaged papilla cells had assumed a more condensed configuration, occasionally interspersed with acellular patches. Scattered pigment was visible around the upper papilla. The mesenchymal layer was noticeably broadened and the glassy membrane extended to the base of the papilla. Importantly, in method III follicles, the ring of basophilic germinative matrix which would have been visible after plucking alone, was no longer in evidence (Fig. 2).

6 days: Dermal papilla cells were confluent with surrounding mesenchyme at the broad base of the matrix. Mesenchymal cells were also seen in, and apparently migrating through, the glassy membrane. A round cell infiltrate was intermixed with the dermal tissue. The highly basophilic matrix displayed some mitotic activity (Fig. 3).

10 days: The dermal papilla had formed a more discrete, often rounded, structure, while papilla cells remained tightly packed with little intercellular
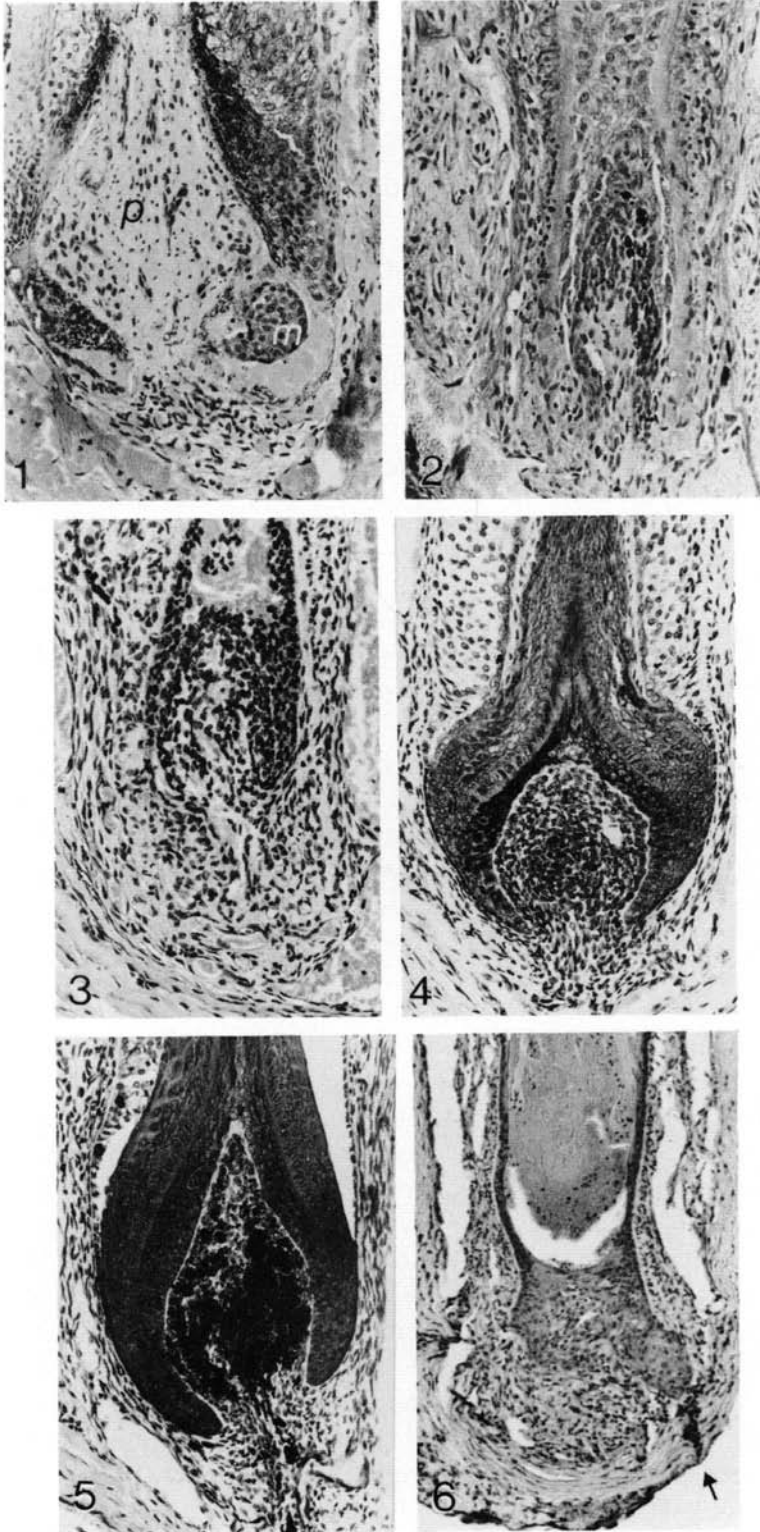
Figs 1–6

space. At the base of the papilla the mesenchyme was clearly defined and separated from the papilla by a basal stalk. The matrix had differentiated into its various cell layers, and fibre production had commenced.

14 days: By 14 days external hairs were usually visible. The cells of the dermal papilla were less condensed and more alcian-blue-stained intercellular material was visible. An apex was present in some, though not all reconstituted papillae (Fig. 5), and a capillary system was in evidence.

The results of wounding methods II and III were consistent. Following papilla cell destruction mitotic division was not observed and the new papilla appeared to be reconstituted from regrouped residual cells with recruitment of additional cells from the surrounding mesenchyme. There were no signs of scar tissue development in the papilla region.

Method I

The numbers of follicles processed for immediate examination were: 3 h – (3); 2 days – (3); 4 days – (6); 6 days – (3); 7 days – (3); 10 days – (3); 14 days – (3). Loss of follicular material through the capsule was a usual occurrence. This was reflected in the histological findings where the initial damage represented a combination of morphological disruption, cell injury, and displacement of cellular material outside the follicle capsule. As a result the observations have been subdivided to illustrate the major variations in effect.

One group of follicles displayed a reaction similar to that described for methods II and III.

In another group in which follicles suffered total loss of papillary material by 4 days the proximal outer root sheath had coalesced basally to produce a solid, though not always symmetrical structure (Fig. 6). No recognizable papilla-like organization was discernible though mesenchymal cells were seen invading the base of the epidermal column.

Figs 1–6. All figures are of longitudinal sections stained with alcian blue, Weigert's haematoxylin and Curtis's Ponceau S. unless otherwise indicated.

Fig. 1. Method II follicle showing widespread damage to the papilla (*p*) and matrix (*m*) 24 h after injury. The lower matrix is reverting to an outer root sheath-like appearance. ×140.

Fig. 2. Method III follicle at 3 days. The dermal papilla cells have regressed. Only a few epithelial cells lie between the dermal papilla and the glassy membrane. ×180.

Fig. 3. Method II follicle at 6 days. The mesenchyme is highly active and openly confluent with the base of the papilla. ×140.

Fig. 4. Method II follicle at 10 days. The papilla cells remain tightly clustered. ×140.

Fig. 5. Method II follicle at 14 days. Normal bulbar structure has been restored and a new papilla apex is being formed. Note the incorporation of pigment into the dermal papilla. Haematoxylin and eosin. ×140.

Fig. 6. Method I follicle at 4 days. A dermal papilla is no longer present and only the lower ectoderm is solid. Damage to the lower capsule wall is arrowed. ×70.
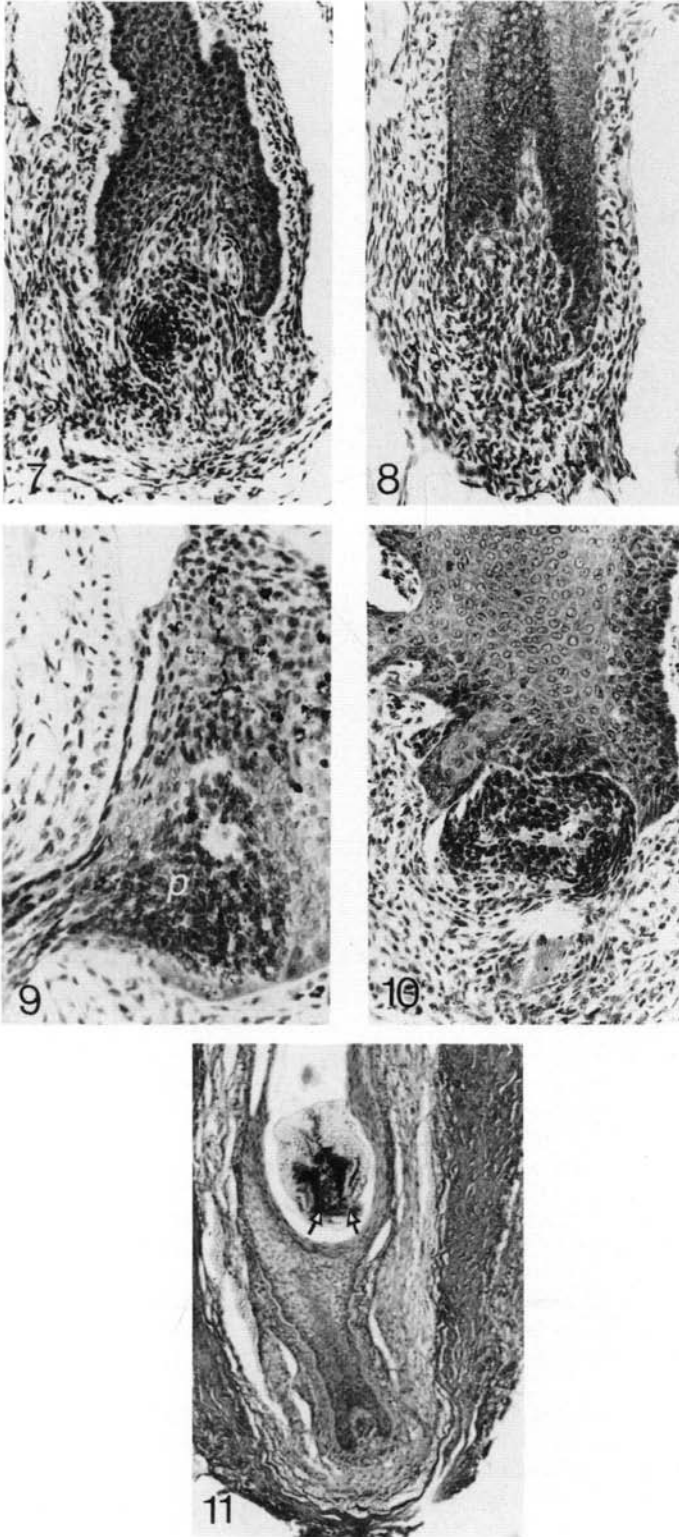
Figs 7–11

A regenerating papilla was visible at 7 days within the follicle as a dense round aggregate of cells, surrounded by less-closely associated mesenchyme which was apparently in the process of being recruited into the presumptive papilla (Fig. 7). The dermal elements formed a large indentation in the base of the epidermal column which displayed mitotic activity, and was encased by a thickened glassy membrane.

By 10 days a restored dermal papilla, with a developing apical spire but not yet constricted at its base, and a developing matrix were present (Fig. 8).

Other follicles displayed various degrees of distortion, and partial cell displacement or loss. For example, in a 4-day biopsy (Fig. 9), both papilla and matrix cells were extruded through the follicle capsule wall. The remaining papilla cells were packed into a closely knit cluster, with no affinity for alcian blue stain. Delay in the restoration of normal follicle morphology was often observed (Fig. 10). This 10-day specimen shows a papilla cell aggregate proximal to a mitotically active but distorted epidermal rod. Another response was seen at 6 days post-operatively (Fig. 11). Here the remainder of the original dermal papilla had moved distally up the hair shaft attached to the former matrix, while a new and perfectly formed papilla and matrix were developing at the base of the follicle.

Observations on the extended effects of follicle wounding

Methods II and III

Few morphological abnormalities were seen in the 26 method II and III follicles examined. Papilla cell number counts were made in three pairs of follicles from each of the two procedures in which the experimental follicles had grown 'long' vibrissae. The results (Table 1) produced no evidence of increased papilla cell numbers in follicles producing longer experimental hairs.

Figs 7–11. All figures are of longitudinal sections stained with alcian blue, Weigert's haematoxylin and Curtis's Ponceau S.

Fig. 7. Method I follicle at 6 days. The regenerating dermal papilla is visible as a rounded cell cluster whose peripheral cells are invading the ectoderm distally. The glassy membrane extends below the proximal level of the ectoderm. ×140.

Fig. 8. Method I follicle at 10 days. The regenerating dermal papilla is still openly confluent with the thickened mesenchyme proximally. Matrix differentiation is under way. ×140.

Fig. 9. Disrupted method I follicle at 4 days. A cluster of dermal papilla cells (*p*) is surrounded by outer root sheath-like cells. Extruded material is visible to the left of picture. ×180.

Fig. 10. Method I follicle at 10 days. A dermal papilla cell aggregate is partially invested by the epidermis. However, while the right side glassy membrane is complete, the left side is discontinuous. ×140.

Fig. 11. Method I follicle at 6 days. The original dermal papilla has been carried upwards with the matrix (arrows) and papilla and matrix regeneration is under way proximally. ×70.

Table 1. *Dermal papilla cell counts, wounding methods II and III*

Rat	Follicle	Cell Numbers in Papilla	
		Experimental	Control
2/C	3a	4,215	4,645
2/D	4a	5,730	8,230
2/H	4a	8,140	5,770
3/A	3a	6,970	8,925
3/A	4b	5,930	9,005
3/B	3a	5,650	8,925

Method I

35 follicles were examined and compared with control follicles. Abnormalities were recorded and particular attention was paid to the size and appearance of the dermal papilla.

An important finding was that in no specimen had damage to the papilla resulted in replacement by scar tissue during the healing process. However, in 12 follicles, recognizable aggregates of papilla cells, often accompanied by pigment, were seen near the functional papilla located either in the surrounding
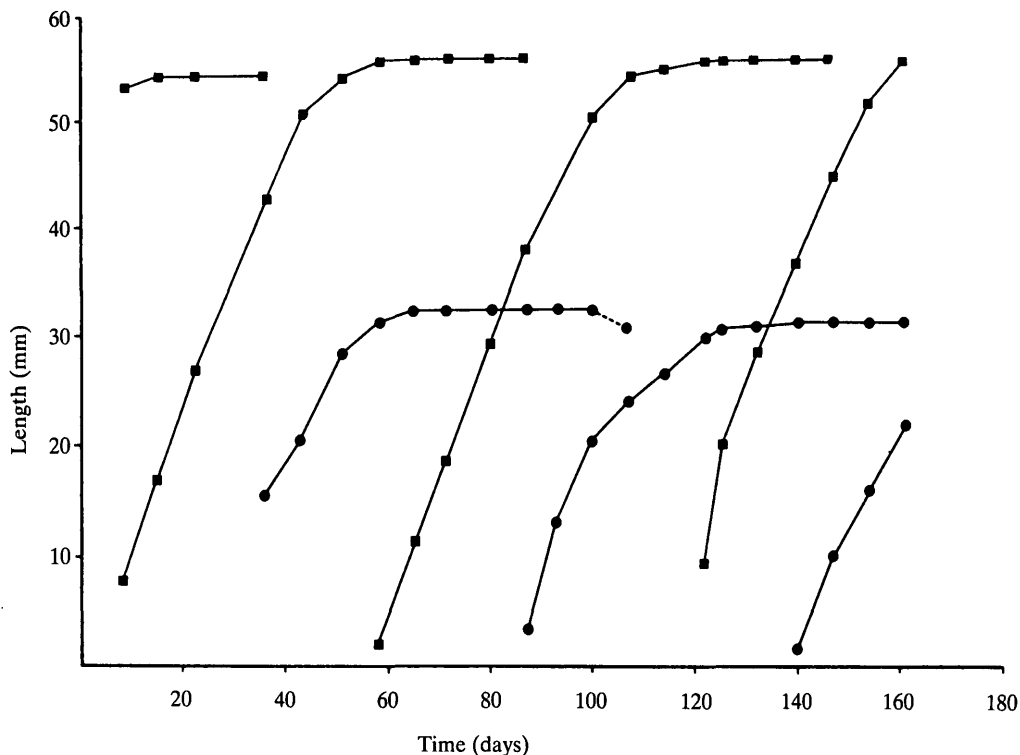

Fig. 12. The growth curves of the small whiskers produced by the wounded follicle in Fig. 15 ● and of its control counterpart■.
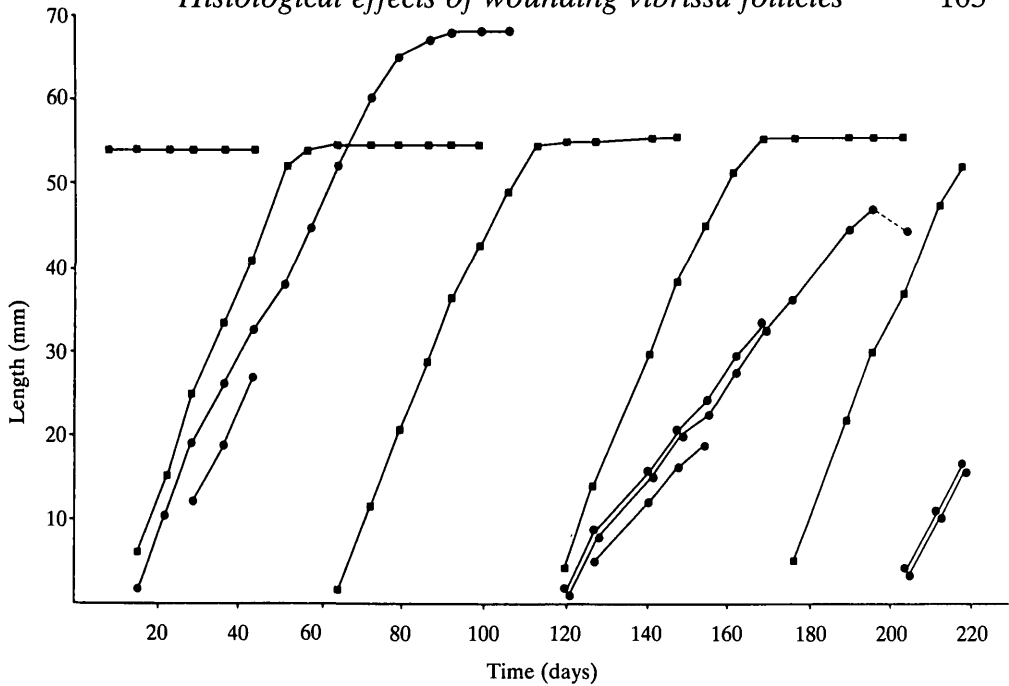
Fig. 13. Growth curves showing multiple fibre production by an experimental follicle ●. Note the altered characteristics of the G2 response, with the appearance of a third fibre, a reduced growth rate and shorter terminal length, to be compared with the consistent growth of the control follicle hairs ■.

mesenchyme or in the collagenous capsule wall. In one example (Fig. 14), papilla material had remained extruded through the capsule wall over a period of 9 months.

While it was difficult to relate papilla size to fibre characteristics, five follicles with manifestly small papillae and which had regularly produced shorter whiskers revealed abnormally thin hairs at biopsy. One of these follicles (Fig. 15), whose postoperative growth is shown graphically (Fig. 12), revealed a small functional papilla distal to its normal position, with the bulk of displaced papilla cells below it.

Morphological abnormalities were common in the bulb region of specimens whose postoperative growth curves had revealed alterations in normal follicle behaviour, such as abrupt cessation of fibre production; non-retention of club hairs; and an increased delay between termination of one cycle and the start of the next one. In a single follicle with a history of irregular growth, and which did not contain an emergent hair at biopsy, the upper follicle was distorted with a convoluted glassy membrane and outer root sheath column isolated from a group of matrix cells at the base of the follicle (Fig. 16). The mesenchymal cells in contact with the matrix were not organized into recognizable papilla. One follicle which had failed to produce an emergent hair was found to have continued fibre production inside of its capsule, which was distended and packed with twisted hair.
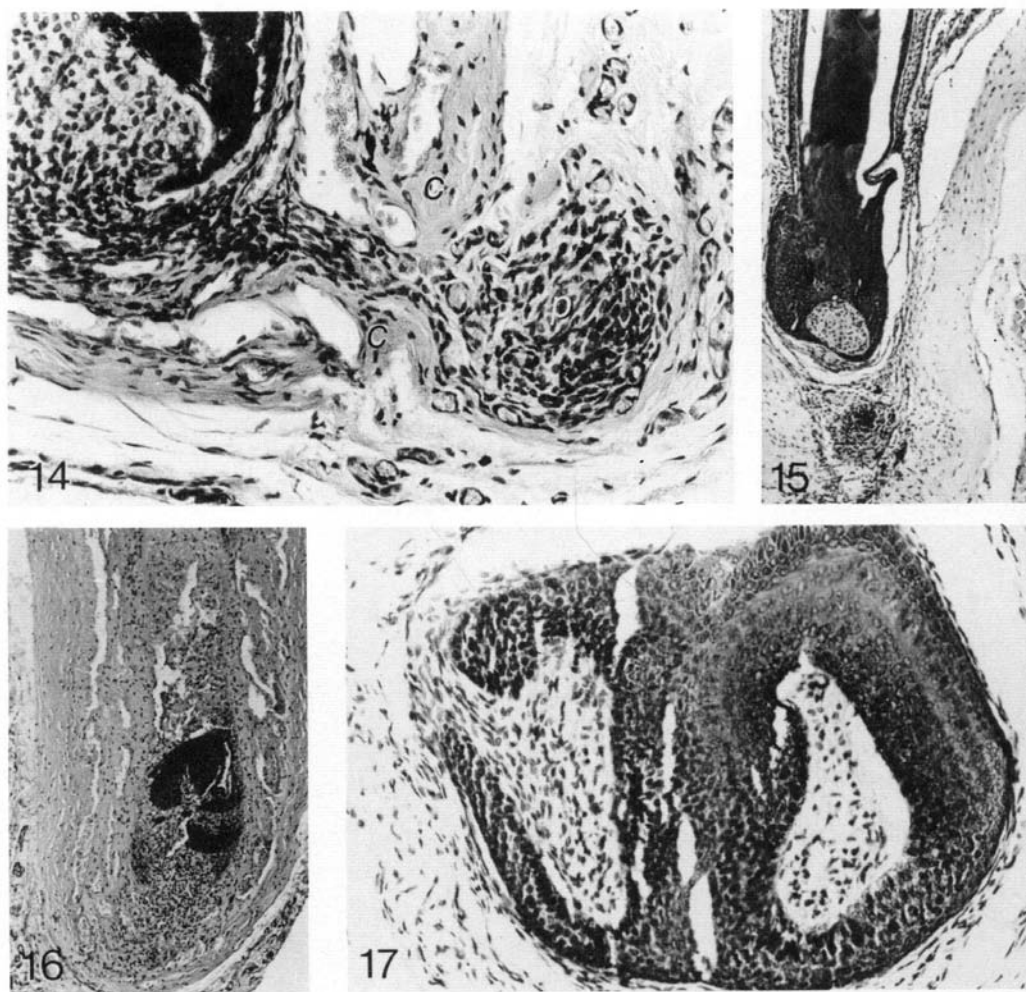
Figs 14–17. All figures are of longitudinal sections stained with alcian blue, Weigert's haematoxylin and Curtis's Ponceau S. unless otherwise indicated.

Fig. 14. Section displaying a ball of dermal papilla cells (*p*) connected to the functional papilla through the unsealed capsule wall (*c*) nine months after wounding. ×180.

Fig. 15. Follicle showing a small functional papilla and surrounding matrix, with the bulk of papillary material proximal to it. ×55.

Fig. 16. Section through a follicle not producing a fibre at biopsy. Note the absence of a recognizable dermal papilla and the lack of a continuous outer root sheath above the dark section of matrix. ×55.

Fig. 17. Split dermal papilla and matrix showing the connection between one papilla half and the lateral, rather than proximal, mesenchyme. ×175.

Seven follicles had produced multiple fibres at some point post-operatively. Those in which the secondary fibre grew at the same rate, and in close association with the primary hair, revealed a dermal papilla with a subsidiary apical region. Other follicles showed a completely divided papilla (Fig. 17) and this

phenomenon was associated graphically with a minor vibrissa growing more independently of a main hair (Fig. 13). Note that the altered response to wounding shown by this follicle in the second postoperative generation was, typically, preceded by a long delay between growth cycles.

DISCUSSION

Following the demonstration of dermal papilla regeneration in the rat vibrissa follicle (Oliver, 1966*a,b*) considerable significance was attached to the discovery that the original papilla was dispensable, and could be replaced by cells from the lower third of the mesenchymal sheath. In contrast to the conclusions of Butcher (1965), Oliver (1966*b*) emphasized that following dermal papilla or root end removal, fibre production did not recommence until a new dermal papilla had been formed. Similarly in the present experiments renewed hair growth was always dependent on the presence of a dermal papilla, regardless of how it was formed.

Following initial papilla cell destruction and loss of matrix organization in method II and III follicles, a new papilla was reconstituted from regrouped surviving papilla cells with recruitment of cells from the local, lower follicle, mesenchyme. Interestingly, while division of papilla cells was not observed (a feature characteristic of intact adult hair follicles, Pierard & de la Brassinne, 1975), papilla cells do divide in culture (Jahoda & Oliver, 1984*a*). Subsequent organization of a matrix and hair growth took place in a similar manner to complete papilla regeneration (Oliver, 1966*b*).

It is most probable that the above responses were due to damage to dermal elements rather than to the epidermal matrix. This view is supported by the observation that in method III follicles, where growing hairs were plucked prior to injury, the ring of germinative matrix reverted to outer root sheath, and did not immediately generate a new basophilic matrix. Furthermore plucking (damage to the matrix alone) has little effect on subsequent whisker characteristics (Ibrahim & Wright, 1978; Jahoda & Oliver, 1984*b*).

After lateral penetration of the bulb (method I) some follicles responded as described above. In others, total papilla displacement was associated with complete papilla regeneration. However the major feature was alteration of normal bulb morphology coupled with cell displacement.

This gross anatomical disruption could be responsible for many of the aberrant growth characteristics shown by method I injured follicles (Jahoda & Oliver, 1984*b*). Accordingly it could be envisaged that distortion of both dermal and epidermal elements would be accommodated, though not rectified, during the initial reconstruction process, and a first postoperative fibre manufactured. However, similar or possibly greater problems of morphogenesis would be presented at the termination of the first and subsequent cycles, when papilla regression is followed by the construction of a new hair bulb complex. Such an interpretation would account for the apparently random fluctuations in

behaviour displayed by many follicles over successive generations, and conforms to the histological evidence. The period between the termination of fibre growth and emergence of a new whisker represents both the telogen and early anagen periods of the vibrissa cycle, and is normally very brief, in the order of 3 days (Ibrahim & Wright, 1975; Young & Oliver, 1976). Thus the regular extension of this period in follicles producing consistently abnormal hairs was probable due to a prolonged reorganizational process during early anagen.

A consistent feature of the study was the behaviour of displaced dermal papilla cells, irrespective of their location. These cells were easily recognized because they had retained a typically condensed and rounded collective organization (Jahoda & Oliver, 1984a). Where papilla cells had been extruded through the capsule wall and had maintained contact with the follicle mesenchyme the capsule had not sealed as occurred with other follicles. In this respect, and by undergoing healing without the development of scar tissue, cells of the dermal papilla and those of the associated lower mesenchyme behaved differently from the closely related fibroblasts of the collagenous capsule wall.

Relationship between dermal papilla and hair fibre characteristics

After the removal of vibrissa follicle root ends Oliver (1966b) noted a possible link between dermal papilla size and hair length in that small regenerated papillae were responsible for the production of short hairs. Similarly in the present studies, those follicles which had manifestly small dermal papillae at biopsy had all grown short whiskers postoperatively. However, the length increases which occurred in many follicles after operation cannot be attributed to increases in papilla cell numbers, perhaps associated with faster growth rates. Cell counts in follicles which had produced long hairs confirmed that papilla cell numbers were not increased and graphical analysis of length measurements demonstrated that growth rates, when altered, were reduced (Jahoda & Oliver, 1984b). Indeed the link between papilla size and hair growth rate is only indirect. Thus while there is a relationship between papilla and germinative matrix volumes in human scalp hair (Van Scott & Ekel, 1958) and in vibrissae (Ibrahim & Wright, 1982), the size of the germinative cell population is only one of a number of factors affecting hair growth rates (Malkinson & Keane, 1978).

A more straightforward relationship is the positive one between papilla volume and fibre volume which has been demonstrated in normal vibrissa follicles, and those which have undergone lower follicle regeneration (Ibrahim & Wright, 1982). Intriguingly, vibrissa hair diameter continually increases throughout the growth of the fibre, and histological observations show a corresponding increase in papilla size due to greater cell cytoplasmic volume, more extracellular material and, most probably, increased vascularization (Young & Oliver, 1976). This underlines the point that papilla cell number cannot simply be equated with papilla volume, as the latter varies during the hair cycle whereas cell number does not.

One problem in relation to hair growth is to determine which component, dermal or epidermal, controls the size of the other. During embryonic development it appears that in hair follicles, as in other skin appendages, it is the dermal component which determines this characteristic (Sengel, 1976). Following the transplantation of isolated adult vibrissa dermal papillae into rat ear dermis, Cohen (1965) postulated that local dermis acts via local epidermis which in turn associates with the papillae to produce a diminution of papilla size and follicles of a local type. While there is some doubt as to whether isolated dermal papillae can interact with local epidermis unless they are in contact or close association with it (Oliver, 1970; Jahoda & Oliver, 1984a), all authors agree that papillae which fail to make contact with epidermis seem to be able to retain cell numbers and morphological characteristics without epidermal influence.

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