The experimental induction
of whisker growth in the hooded rat by
implantation of dermal papillae

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Dermal papillae are regenerated and whiskers are produced after the experimental removal of up to the lower third of the vibrissa follicle, but if more of the follicle is removed neither event occurs (Oliver, 1966a, b). Similarly, when lengths of the lower third of the vibrissa follicle wall are transplanted into ear skin, whiskers are again produced, but not if lengths of wall are taken from within the upper two-thirds of the follicle (Oliver, 1967). From these experiments it was clear that the outer root sheath and the adherent mesenchymal layer, from which the new papillae are apparently derived, are the essential tissues in the regeneration process.

The failure of papilla regeneration in the upper two-thirds of the follicle could be explained in several ways. It is possible that the outer root sheath at this level is incapable of supporting whisker growth or of stimulating papilla formation. Alternatively, the cells of the mesenchymal layer at this level, unlike those in the lower third of the follicle, may be incompetent to form a dermal papilla.

In an attempt to analyse these factors, vibrissa dermal papillae were implanted into the bases of whisker follicles from which more than the lower third of the root had been removed.

MATERIALS AND METHODS

Operation

All operations were performed on the vibrissa follicles on the upper lip of animals 2½–3 months old from an inbred strain of hooded rat. Anaesthesia was induced by the intra-peritoneal injection of a 10×diluted solution of Nembutal (Abbott), 0·65 ml/100 g body weight. The whisker roots were exposed for operation by reflecting the whisker pad as previously described (Oliver, 1966a).

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The follicles selected for operation were identified and a record was made of the length of the whiskers present in the follicles. Lengths of follicle were removed with a transverse cut so as to leave half or less of the upper region of the follicle in situ. The cut whisker shafts were then plucked from these follicle remnants.

The removed lengths of follicle were placed in Hank's solution and the bulbar ends isolated, once again, with a transverse cut. The dermal papilla was dissected from each of these 'end bulbs' using a method similar to that described by Cohen (1961). Two opposing cuts were made in the length of the capsule to the base of the bulb with a fine pair of iridectomy scissors and the two resulting flaps of capsule reflected. Gentle pressure was exerted on the sides of the remaining part of the bulb to effect the separation of either the whole of the ectodermal component from the dermal papilla or, more usually, just the matrix leaving the dermal papilla within a tube of outer root sheath and the adherent mesenchymal layer. This tube was reflected in an identical manner to the capsule. The dermal papilla was then very carefully 'cleaned', by scraping quite firmly with a fine pair of watchmaker's forceps, under x 25 binocular vision, to ensure that no matrix cells were left adherent to the surface of the papilla. The dermal papilla was isolated from the rest of the bulb with a transverse cut across its base which in most instances precluded the removal of the basal stalk since it was not always possible to determine with certainty that all traces of the matrix had been dislodged from the stalk. The dermal papilla was then placed on to the cut surface of the follicle remnant and pushed gently into the mouth of the circular tube provided by plucking the growing whisker. It was found impossible to orientate the papillae in any particular way with certainty. In later operations the keratinized inner lining of the follicle was broken up and removed as completely as possible with a fine pair of forceps before implanting the dermal papilla. After implantation of the dermal papillae the lip flap was stitched back in position. At no time after operation did the animals have difficulty in feeding or drinking.

Experimental

Twenty-seven lengths of follicle were removed in a total of nine rats (three follicles in each rat). Dermal papillae were successfully dissected from nineteen of these lengths of follicle and implanted singly as autografts into nineteen of the follicle remnants leaving eight as controls. Three rats received single implants, two rats received two implants each and four animals received three implants each.

The approximate stages of the growth cycle of all the follicles operated on were determined by the lengths of the whiskers present in the follicles at operation and confirmed by the size and shape of the dermal papillae in those follicles from which they were removed. Thus dermal papillae from known follicles and at a known stage in the growth cycle were implanted into known follicle remnants also at a known stage in the growth cycle.
Induction of whisker growth

All the follicles, including those without papilla implants, were kept under occasional observation for at least 110 days after operation and where whiskers were produced their lengths were recorded. Four of the rats were then killed 112–141 days after operation and the operated follicles excised and prepared for histological examination.

Identical operations were also performed on a further four rats, implanting two or three dermal papillae in each animal, and these were killed at 1, 7, 14 and 21 days after operation for information on the histological changes which occur at early stages after implantation.

To determine whether, in fact, any matrix cells were left on the surface of the papillae prior to implantation several dermal papillae which had been removed routinely were prepared for histological examination.

Histology

Dermal papillae were fixed in Zenker's fixative and 5 μ serial sections were stained with Ehrlich's haematoxylin and eosin. Operated follicles were fixed in formol saline and serial sections 8μ in thickness were stained in a combination of Weigert's haematoxylin, alcian blue and Curtis's Ponceau S.

RESULTS

None of the eight control follicles, those without papilla implants, produced whiskers after operation. Of the nineteen follicles into which dermal papillae were implanted, fourteen produced generations of whiskers which first appeared above skin level between 18 and 25 days after operation.

The lengths of whiskers produced were extremely variable ranging from 14 to 73 % of the expected length for the various follicle positions. Although the majority of implants were from late anagen donor follicles into late anagen follicle remnants, the number of transplants made at other stages in the growth cycle of either donor or recipient follicles were insufficient to permit any deduction as to the effect of this factor on subsequent whisker growth or whisker length.

None of the papillae prepared for histological examination showed any evidence of contamination with ectodermal matrix cells (Plate 1, fig. A.)

Nine follicles which had received grafts were examined histologically at early intervals after operation. All nine of the implanted papillae had persisted and were identified at histological examination. The exact level of removal of the root ends was clearly indicated in all of the follicles by the cut edge of the capsule and the abrupt termination of the thick glassy membrane.

In both follicles examined after 24 h the implanted papillae were present as a discrete cell mass at the base of the follicle and isolated from the outer root sheath by the keratinized inner root sheath (Plate 1, fig. B). The cytoplasm of the papillae was stained blue-green indicating the presence of acid mucopoly-
saccharides, although the central region of one of the papillae, in which the nuclei were more densely arranged, was less intensely stained.

By 7 days in the three follicles studied no traces of the inner root sheath were present and the lower region of the outer root sheath had become a solid column of cells which extended to just below the termination of the glassy membrane. Dermal papillae were present as a distinct indentation at the most proximal end of the outer root sheath in two of the follicles (Plate 1, fig. C), presenting a slightly advanced stage in bulb development over the third follicle in which the papilla was present as a cell mass at the flattened proximal surface of the outer root sheath. Although in all three follicles the basal region of the outer root sheath was markedly basophilic and contained cells in division, none of the follicles had reached the stage of development of the hair cone. The nuclei of the papillae were heavily stained and were more densely arranged than one day after implantation showing a loss in overall papilla volume; none of the papillae had taken up the alcian blue stain. More lightly stained nuclei, however, were present in the most distal region of the papillae immediately adjacent to the outer root sheath. The papillae, in which erythrocytes were present, were encapsulated by a downgrowth from the cut edge of the mesenchymal layer.

![Plate 1](image)

Fig. A. Vertical section of a dermal papilla dissected from the base of an anagen whisker follicle. No epidermal matrix cells are attached to the surface of the papilla. Ehrlich’s haematoxylin and eosin. × 230.

Figs. B–F. Longitudinal sections of the proximal end of whisker follicles at various stages after implantation of dermal papillae into the bases of whisker follicle remnants after removal of half or more of the lower region of the follicle. The level of removal is indicated by the cut edge of the capsule.

Fig. B. Twenty-four hours after implantation of a dermal papilla (arrow). The papilla is isolated from the outer root sheath by the keratinized inner root sheath. Weigert’s haematoxylin, alcian blue and Curtis’s Ponceau S. × 70.

Fig. C. Seven days after implantation of a dermal papilla. The inner root sheath has been ejected distally and the lower region of the outer root sheath has become a solid column of cells. The most proximal region of the outer root sheath is present as a shallow inverted cup over the dermal papilla which has not taken up the alcian blue stain. Weigert’s haematoxylin, alcian blue, Curtis’s Ponceau S. × 55.

Fig. D. Fourteen days after implantation of a dermal papilla. The developing bulb projects below the cut edge of the capsule and a whisker is being produced. The dermal papilla has taken up the alcian blue stain. The abrupt termination of the thick glassy membrane indicates the level of the original cut across the follicle wall. Weigert’s haematoxylin, alcian blue and Curtis’s Ponceau S. × 60.

Fig. E. Twenty-one days after implantation of a dermal papilla. The follicle extends well below the cut edge of the capsule. Weigert’s haematoxylin, alcian blue and Curtis’s Ponceau S. × 60.

Fig. F. One hundred and twenty-six days after implantation of a dermal papilla. A dermal papilla is not present. Shown is a small sebaceous gland, whose duct opens into the follicle cavity in adjacent sections, which has developed at the base of the follicle. Weigert’s haematoxylin, alcian blue and Curtis’s Ponceau S. × 200.
By 14 days in both follicles examined the outer root sheath extended well below the original cut across the follicle. A typical early anagen bulb was present at the base of both follicles and whisker growth was in progress (Plate 1, fig. D). The nuclei of the papillae were large and lightly stained and the cytoplasm had taken up the alcian blue stain. A well developed capillary system was present in both papillae.

At 21 days one of the two follicles presented a very similar histological picture to that described for the 14 days follicle and was producing a fine whisker shaft (Plate 1, fig. E).

An extension of the follicle below the level of removal of the root end was not present in the second follicle. The dermal papilla, which was stained blue-green and contained capillaries, the matrix and the growing whisker shaft were not arranged axially with respect to the length of the follicle segment. This follicle was not producing a normal early anagen whisker fibre but a widely medullated, thick, curved shaft without a cuticle which was fully keratinized at an abnormally low level with respect to the bulb; peripheral to the apical region of the papilla was a layer of living ectodermal cells 2–3 cells deep, and external to this was the already keratinized shaft.

Four rats, in which three follicles in each rat had received papilla implants, were killed for histological examination 112–141 days after operation. Of these twelve follicles, four had not produced whiskers after operation and eight had produced several generations of whiskers 7–22 mm in length, 14–52% of the club lengths normally produced by these follicles at the time of operation.

Histological examination confirmed that lengths of the root had been removed in all of these follicles, as indicated by the cut across the capsule, from a level within the inferior enlargement, just below the ring sinus, up to the level of the ringwulst; that is, well within the region in which papillae are not regenerated after simple removal of lengths of the follicle (a diagram of the structure of the whisker follicle is presented in Oliver, 1966b). There was no obvious correlation between the level at which removal had occurred and the length of the whiskers subsequently produced or even whether whiskers were produced or not.

Seven of the eight follicles which had produced whiskers were in various stages of anagen at the time of biopsy and one was in early catagen. In all cases the follicle extended below the cut across the capsule. Seven of the eight follicles had apparently normal looking bulbar regions, although one was producing a whisker whose tip was spiralled within the follicle and had not penetrated the skin surface. A spiralled whisker shaft was also found in association with another follicle although at the time of biopsy the follicle was producing an apparently normal whisker which extended above the skin surface. The spiralled whisker had grown out of the side of the capsule into the surrounding connective tissue. The eighth follicle, which had produced at least three generations of doubled whiskers simultaneously, contained two bulbs from each of which was being
produced a single whisker. Both whiskers were contained within a common outer root sheath higher in the follicle.

In three of the four follicle segments which had no history of whisker production after operation, the follicle cavity extended down to a level just above the ringwulst and below this the outer root sheath was present as a solid column of cells terminating at or just above the cut across the capsule. There was no evidence of papilla cells or whisker production in any of the follicles. At the base of one of the follicles, however, was a small sebaceous gland with a sebaceous duct which opened into the bottom of the follicle cavity (Plate 1, fig. F); a sebaceous gland was also present in the normal position at the neck of the follicle.

The fourth follicle segment contained a remarkably distended thin walled (1–2 cells thick) ectodermal sac which extended below the cut across the capsule. Within the lumen of this sac were red-stained keratinized fragments and a yellow-stained keratinized body which did not resemble, however, a whisker shaft. In this follicle segment also there was no evidence of papilla cells or whisker production.

**DISCUSSION**

Although it is generally assumed that the dermal papilla of the hair follicle induces hair growth, conclusive evidence that the dermal papilla induces the growth of epidermal appendages is only available for the feather follicle (Lillie & Wang, 1941, 1944; Wang, 1943). They have shown that after removal of the 'whole papilla' (the dermal papilla and its ectodermal investment) from the base of the feather follicle there is no further feather production and no regeneration of a dermal papilla. However, if a dermal papilla is implanted into the basal half of a follicle, from which the whole papilla has been removed, generations of feathers are produced.

The present results clearly demonstrate that the dermal papilla is also the inductive agent in the formation of the whisker bulb and in whisker growth. Histological examination of the isolated dermal papillae has shown that the technique used for their removal reliably ensures that no ectodermal matrix cells from the donor follicle remain adherent to the papilla surface. None of the eight follicle segments without papilla implants produced whiskers, confirming the inability of the upper two-thirds of the follicle to regenerate papillae, and the five which had received implants but which subsequently did not produce whiskers showed no evidence of the presence of a papilla at biopsy—presumably in these five follicles either the papillae did not remain in position after implantation or did not become successfully incorporated into the follicle and degenerated. Since whiskers were produced after the randomly orientated implantation of papillae, often with the basal stalks absent, it seems possible that the implanted papilla interacts as a non-organized cell mass with the outer root sheath to trigger the sequence of events leading to whisker growth. Among these events
Induction of whisker growth

are the reorganization of the papilla cell mass in its spatial relationship with the ectodermal component of the follicle, the initial loss of staining for acid mucopolysaccharides and then the reappearance of acid mucopolysaccharides in the papilla in the later stages of bulb development, and the extension of the follicle below the level of root removal. The ejection of the inner root sheath as the outer root sheath becomes a solid column of cells also occurs after removal of the lower region of the vibrissa follicle prior to papilla regeneration (Oliver, 1966b), but in this case there is little or no eventual growth of the follicle below the level of root removal.

It is also clear that the non-regeneration of papillae and whiskers from the upper two-thirds of the follicle after simple removal of lengths of follicle arises from the inability of the mesenchymal layer to form a papilla at this level and is not due to the incompetence of the outer root sheath to become organized for hair production. Although it seems most likely that it is the dermal element of the follicle which initiates whisker growth it may be that it is the ectodermal element which is the prime mover both in initiating the regeneration of papillae and in the 'activation' of the resting papilla so that it induces renewed hair growth in the normal hair growth cycle. In other words, the signal for the mesenchymal layer to form a dermal papilla may still ultimately come from the outer root sheath and in the upper two-thirds of the follicle it is unable to give this signal.

The design of the present experiment does not allow a conclusive explanation for the variation in the lengths of whiskers produced. Lack of correlation between the stages of the growth cycle of the donor and host follicles at the time of operation and the lengths of whiskers produced does not provide any significant indications that this factor is important; however, a consistent length of root was not removed from each follicle. Although there did not appear to be a correlation between the level at which removal of the root occurred and the length of the whiskers subsequently produced, the length of follicle into which the papilla is implanted and the size (cell number) of the implanted papilla and the degree of successful incorporation of the papilla into the follicle may well be the most important factors in determining the length of whisker produced.

Wang (1943) has demonstrated that the feather dermal papilla cannot induce feather growth from the distal or superficial half of the feather follicle nor when implanted under extra-follicular epidermis. This contrasts with the vibrissa papilla which can induce whisker growth from the distal half of the vibrissa follicle. The question now arises as to whether the vibrissa papilla can also induce hair follicle formation and hair growth when placed in contact with extra-follicular epidermis, a possibility suggested by Billingham & Silvers (1963). Experiments are now being performed to test this hypothesis.
SUMMARY

1. A method is described for isolating dermal papillae from the vibrissa follicles on the upper lip of the hooded rat.
2. Dermal papillae have been implanted into the bases of the upper region of vibrissa follicles which do not otherwise regenerate papillae and whiskers.
3. Fourteen out of nineteen of the follicle segments which received papilla implants subsequently produced regenerations of whiskers. None of the eight control follicle segments (those without papilla implants) produced whiskers.
4. The vibrissa dermal papilla was thus conclusively shown to be the inductive agent in bulb formation and whisker growth.
5. The non-regeneration of papillae and whiskers from the upper two-thirds of the follicle therefore arises from the inability of the mesenchymal layer to form a papilla and is not due to the inability of the outer root sheath at that level to become organized for whisker growth.
6. The dermal papillae, which were randomly orientated at implantation, lose their initial organization by 7 days after implantation and then become incorporated in the bulb which develops at the base of the outer root sheath. The follicle then grows down below the level of the root removal.
7. A conclusive explanation could not be offered for the variation in length of the whiskers produced after implantation of dermal papillae.

RÉSUMÉ

Induction expérimentale de la croissance des vibrisses chez le rat mantelé par implantation de papilles dermiques

1. On décrit une méthode d'isolement des papilles dermiques à partir des follicules des vibrisses, sur la lèvre supérieure du rat mantelé.
2. On a implanté des papilles dermiques dans la base de la région supérieure de follicules de vibrisses qui ne régénèrent pas, d'autre part, de papilles ni de vibrisses.
3. Sur 19 segments folliculaires ayant reçu des implantations de papilles, 14 ont par la suite régénéré des vibrisses. Aucun des 8 segments folliculaires témoins (ceux qui n’avaient pas de papilles implantées) n’a produit de vibrisses.
4. On a donc montré de manière concluante que la papille dermique de la vibrisse est l’agent inducteur de la formation du bulbe et de la croissance de la vibrisse.
5. L’absence de régénération de papilles et de vibrisses à partir des deux tiers supérieurs du follicule provient donc de l’inaptitude de la couche mésenchymateuse à former une papille et n’est pas due à l’inaptitude de la gaine externe de la racine, à ce niveau, à s’organiser pour la croissance d’une vibrisse.
6. Les papilles dermiques, qui ont été orientées au hasard lors de l’implantation, perdent leur organisation initiale sept jours après cette dernière, puis
Induction of whisker growth

s’incorporent au bulbe qui se développe à la base de la gaine externe de la racine. Le follicule s’accroît alors au-dessous du niveau de l’exérèse de la racine.

7. On n’a pu trouver d’explication concluante pour la variation de longueur des vibrisses produites après implantation de papilles dermiques.

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REFERENCES


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