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Changes in hair growth characteristics following the wounding of vibrissa follicles in the hooded rat

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SUMMARY

The effect on hair growth of wounding the lower region of whisker follicles, and in particular the dermal papilla, with sharply pointed tungsten needles was studied in adult hooded rats. Following injury hair growth ceased, but was subsequently resumed. While it might have been anticipated that follicle wounding would have a negative effect on whisker length, regular post-operative length measurements revealed that in follicles where cellular material was not displaced from the follicle by the original manipulation, 50 % of the subsequent hairs produced were longer than their counterparts on the opposite side of the face, with 25 % shorter and 25 % with their length unchanged. In every case increased hair length was achieved by a prolongation of the growing period of the hair. Growth rate, when altered, was reduced. These results suggest that the factors which control the duration of the hair cycle and fibre growth rate are independent in vibrissa follicles. Since removal of most of the epidermal component by plucking of the hair just prior to injury produced equivalent hair length increases, this implicated the proximal dermal components as being mainly responsible for the observed changes.

INTRODUCTION

Much of the experimental work on hair growth has involved direct or indirect interference with normal follicle activity. Where severe destructive agents have been employed on adult follicles (e.g. carcinogenic hydrocarbons, Wolbach, 1951; X-irradiation, Geary, 1952), recovery of fibre production appears to have depended on the degree of damage to the dermal papilla, and more recently it has been shown that the papilla can prove resistant to high doses of X-irradiation (Ibrahim & Wright, 1977).

Direct evidence of the necessity for a dermal papilla for growth and maintenance of adult follicles was first demonstrated in a series of experiments by Lillie & Wang (1941, 1944) and Wang (1943). They discovered that removal of the dermal papilla from feather follicles resulted in the permanent termination of feather production. However, reimplantation of a new papilla resulted in restoration of feather growth. Following the excision of rat pelage hair follicle bulbs, Butcher (1965) reported subsequent growth of fragile hairs in the absence

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of a dermal papilla. This conflicted with the findings of Oliver (1966*a*,*b*) who, using operational procedures pioneered by Cohen (1961), established that fibre production in the vibrissa follicle was terminated by removal of the dermal papilla, and was only restored after papilla regeneration from surrounding lower follicular mesenchymal cells which are continuous with the base of the papilla. Furthermore, it was discovered that a papilla could be reformed after removal of short lengths of follicle root, a finding confirmed by Ibrahim & Wright (1982). Similarly, the regrowth of human axillary hair following the removal of the bulb and follicle up to the level of the sebaceous gland has also been described (Inaba, Anthony & McKinstry, 1979).

One of the advantages of using vibrissa follicles, particularly for quantitative studies, is the ease with which individual fibres can be measured. Normal growth characteristics of vibrissae on the upper lip of the rat are therefore well documented (Oliver, 1965; Ibrahim & Wright, 1975; Young, 1977). In addition, each follicle has an exact counterpart on the opposite side which produces a fibre of similar length. This inbuilt 'control' system was exploited by Oliver (1966*a*, 1967) who looked at alterations in whisker length brought about by various microsurgical manipulations.

In the present study a comprehensive examination of the effects of wounding the lower region of vibrissa follicles with a tungsten needle was undertaken.

MATERIALS AND METHODS

All work was performed on animals from an inbred strain of hooded rat (colony Dundee University). Only the major vibrissa follicles of the mystacial region were used, and identification of individual follicle position and subsequent recording of information were carried out according to the annotation of Oliver (1966*a*). Observations of whiskers were performed under a dissecting microscope (magnification ×20). Fine-pointed watchmakers' forceps were used to locate and separate emergent growing hairs from the thicker, and generally larger, non-growing club vibrissae. Lengths were measured using transparent flexible polythene tubes of internal diameter 0·8 mm, graduated at intervals of 1 mm, and estimates were to the nearest 0·5 mm.

As a preliminary investigation the club whiskers of 17 male rats, 12 aged six months or more and 5 aged three months, were measured in order to obtain the characteristics of maximum fibre length for each follicle position, and length differences for paired positions on opposite sides of the face.

Wounding experiments

30 male rats of six months of age were utilized for all experiments. Anaesthesia was induced by intraperitoneal injection of Sagatal, 0·055 ml/100 g body weight. The method employed to expose follicles for operation was the same as that used by Cohen (1961) and Oliver (1966*a*). After being cleaned of surrounding

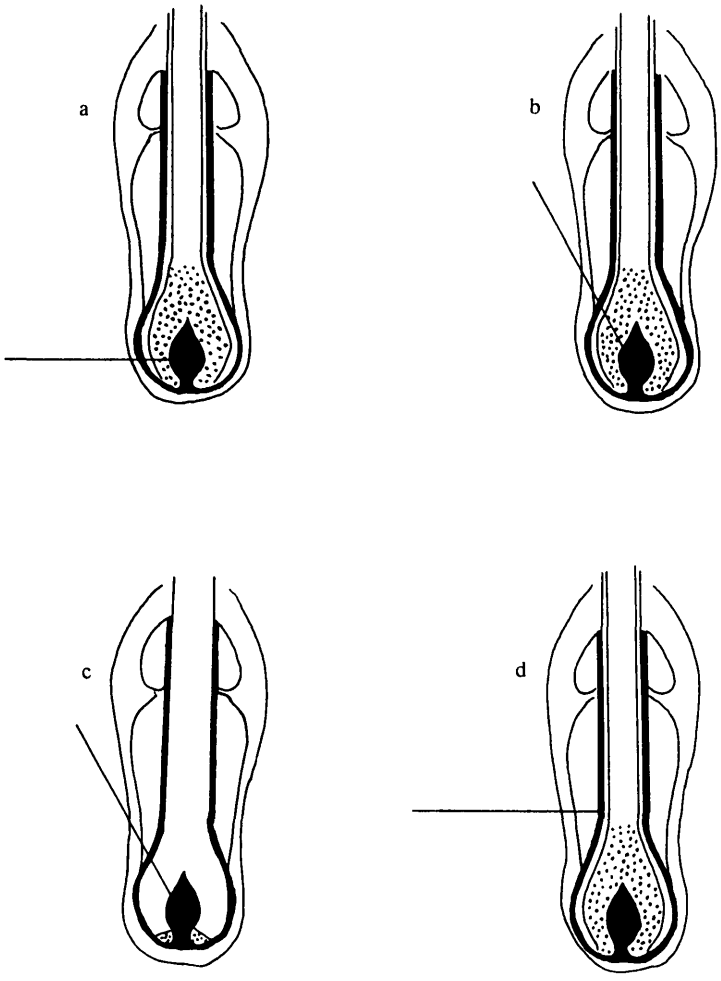

Fig. 1. Diagram of the four different methods used to injure the whisker follicles.

connective tissue, the positional identity of prospective experimental follicles was confirmed and recorded. For convenience, operations were performed on the left-hand side of the face. Damage was inflicted on follicles which were growing hairs (in anagen) using finely pointed tungsten needles mounted on glass rods. A diagrammatic representation of the four operational techniques is shown in Fig. 1.

Method I (1a): A needle was inserted horizontally into the bulb region of a follicle, and then rotated to disrupt the dermal papilla. 52 follicles operated on in this manner were kept under long term observation.

Method II (1b): With this procedure, the follicle capsule was initially penetrated distal to the bulb region, about one third of the way up the follicle. The needle was then carefully pushed into the middle of the papilla, and rotated as before. 18 follicles were kept under regular scrutiny.

Method III (1c): Follicles were damaged as for method II. However, in these specimens injury to the dermal papilla was preceded by plucking of the club and growing fibres prior to the introduction of the needle. 12 follicles damaged in this manner were used for extended observations. In addition vibrissae were plucked from 20 follicles in four animals to examine the effects of a single epilation on whisker length. 10 of these had emergent fibres extracted after removal of the club hair, while with the remaining 10 follicles only the growing hairs were plucked.

Method IV (1d): As a control, a further 19 follicles underwent penetration of the capsule and follicle wall at a level above the bulb and thus without subsequent injury to the proximal region or dermal papilla.

Compilation of experimental data

Length measurements

Following operational procedures experimental follicle positions were examined at regular intervals, normally not exceeding 7 days. Both growing and club hairs were measured as previously described and parallel observations were made on control follicles. Examination commenced 1 to 2 weeks after the initial intervention, was continued throughout the growing phase of the hair cycle, and frequently extended to a second or third postoperative whisker. The first hair produced was termed the first generation or G1 hair, and the second G2 and so on. Results were tabulated and displayed graphically as a basis for further analysis. The absence of vibrissae from follicular positions was always noted, as were irregularities such as multiple hairs, or abnormalities in fibre thickness or shape.

Growth rates

Growth rate estimates (mm/day) were obtained by dividing the increase in length (mm) between two points on the length measurement graphs, by the period of time in days between the two measurements. In general the first observation after fibre emergence was taken as the lower point, and high-point measurements were obtained from positions as similar as possible on the respective growth curves. Growth rates were only calculated where over half the total club length was available.

RESULTS

Maximum vibrissa length

With all follicles there was close agreement between left and right side club lengths as described by Ibrahim & Wright (1975). With the six month and older group of rats, of the 109 paired observations, 104 were within a 2 mm margin (i.e. 4 % of a hair measuring 50 mm). Of the remaining 5 pairs none displayed a

difference of more than 3 mm. Similar results were obtained with the 3-month-old animals, in which the average club lengths were up to 4 mm shorter than vibrissae from the same positions on older rats.

Excluding the top horizontal row (which was not used experimentally), for the older rats the range of measurements within vertical rows of follicles fell within discrete non-overlapping groups; i.e. passing from the most posterior row 'a' anteriorly 'a': 49·5–59 mm; 'b': 42–47·5 mm; 'c': 31–38 mm; 'd': 19–25·5 mm; 'e': 10–16·5 mm.

Wounding experiments

Method I

During this procedure it was observed that cellular material was being lost from the follicle capsule (Jahoda & Oliver, 1984). Omitted data represented occasions where information was inadequate to satisfy the criteria necessary to make growth rate estimates.

Following injury, all but one follicle terminated fibre production and lost their growing hairs. Subsequently, two of the experimental follicles failed to produce emergent whiskers. Six follicles produced new hairs less than 10 days after being damaged. Thirty-three vibrissae were visible between 10 and 20 days post-operatively and a further four new hairs were first seen at 24, 28, 43 and 49 days after wounding.

Length (Table 1)

Differences of 4 mm and greater between experimental and control vibrissae were regarded as significant. This value represented a margin of 2 mm above the differences in terminal length found in over 95 % of the previously described measurements of undamaged whisker pairs, and 1 mm above the maximum difference.

In the first generation following injury (G1) the majority of follicles produced whiskers within 4 mm of their controls. However, over 40 % of the postoperative

Table 1. *Effect of injury on vibrissa length, method I*

Terminal length of experimental fibres compared with control	Number of whiskers for each generation		
	G1	G2	G3
Within 4 mm	24 (1b)	24 (1b)	19
Shorter by ≥ 4 mm	15 (2b)	19 (2b)	8
Longer by ≥ 4 mm	5	3	6
Total	44	46	33

(x b) = number of vibrissae broken during growth.

Table 2. *Effect of injury on vibrissa growth rate, method I*

Growth rates of experimental fibres compared with control	Number of whiskers for each generation		
	G1	G2	G3
Within 0·1 mmm/day	16	17	18
Slower by ≥ 0·1 mm/day	18	18	15
Faster by ≥ 0·1 mm/day	0	1	1
Total	34	36	34

vibrissae were either 'longer' or 'shorter', with the latter constituting three-quarters of this figure. In G2 and G3 the combined number of hairs with altered length remained at over 40 % of the total.

Growth rate (Table 2)

A margin of 0·1 mm/day was chosen to distinguish differences in follicle behaviour between the experimental and control sides. While essentially an arbitrary figure, this represents a club length difference of 5 mm over a 50-day period of growth, and provides a convenient indication of variation in growth rate following injury.

In G1 just over half of the wounded follicles produced vibrissae at a growth rate below that of their controls. The percentage of slower growing hairs was raised slightly in G2, but fell to below 50 % in G3. Each of the latter two generations revealed a single faster growing hair.

It is of great interest that the distribution of reduced growth rate was almost uniform among experimental follicles producing whiskers of equivalent, longer or shorter length to their controls in G1. Thus it is clear that the five follicles which had manufactured longer hairs (at equivalent or reduced rate), had produced them over a prolonged growing period. Similarly, ten vibrissae which had not altered in length, had grown at a reduced rate and had been produced over a longer period of time.

In every case control follicles showed remarkable consistency in both length and growth rate over successive generations.

In addition to these quantitative observations, method I follicles displayed a number of abnormal phenomena including the production of multiple fibres; the abrupt cessation of growth and premature fibre loss; and an increase in the length of time between termination of hair growth and the emergence of a new whisker.

Method II

Wounding operations were successfully performed on 18 follicles in 10 male rats. In contrast to method I, there was no loss of follicular material from the

capsule. Wounded follicles and their controls were kept under observation for up to 170 days, by which time the majority of experimental follicles had produced two complete generations of whiskers.

Table 3. *Effect of injury on whisker length, methods II and III*

Terminal length of experimental vibrissae compared with controls	Number of whiskers for each generation		
	Method II		Method III
	G1	G2	G1
Within 4 mm	4	4 (1b)*	3
Shorter by ≥ 4 mm	4 (1b)	4*	2
Longer by ≥ 4 mm	8	8*	5*
Total	16	16	10

(xb) = number of hairs broken during growth.
* includes hair which had not fully attained terminal length.

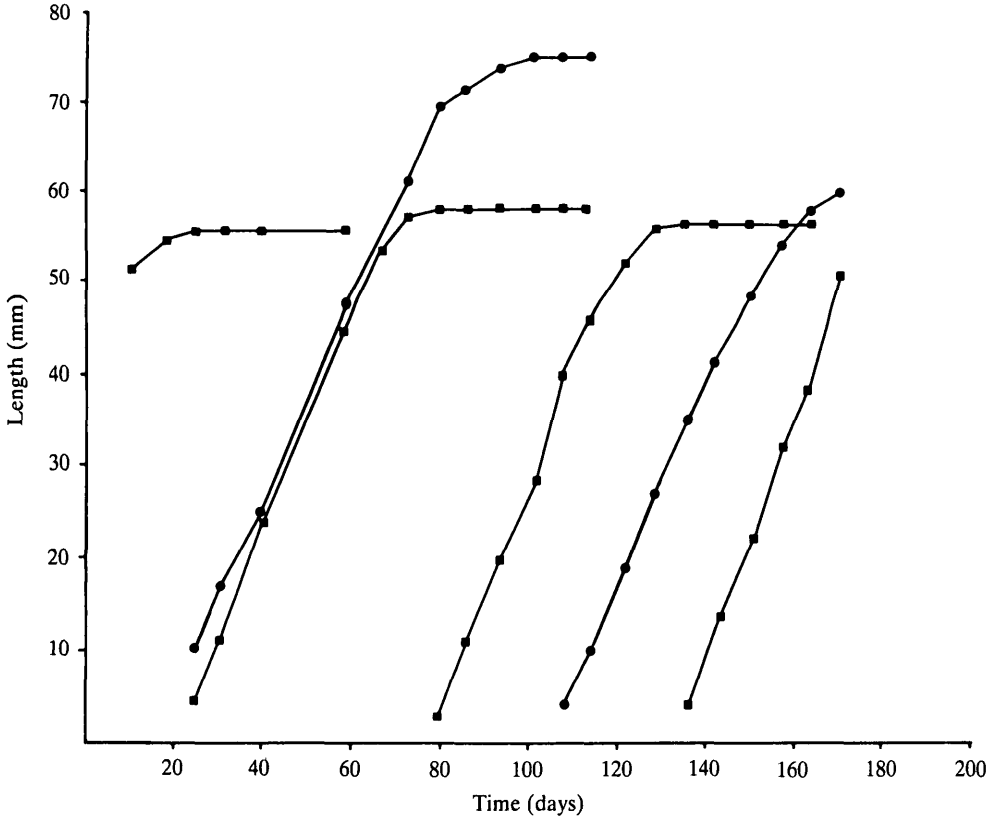

Fig. 2. Growth curves of the experimental follicle which manufactured the giant whisker in Fig. 3. ●, and of its control ■. The increased fibre length of the experimental whisker is clearly brought about through an extension of the growing period, and not by a difference in growth rate.

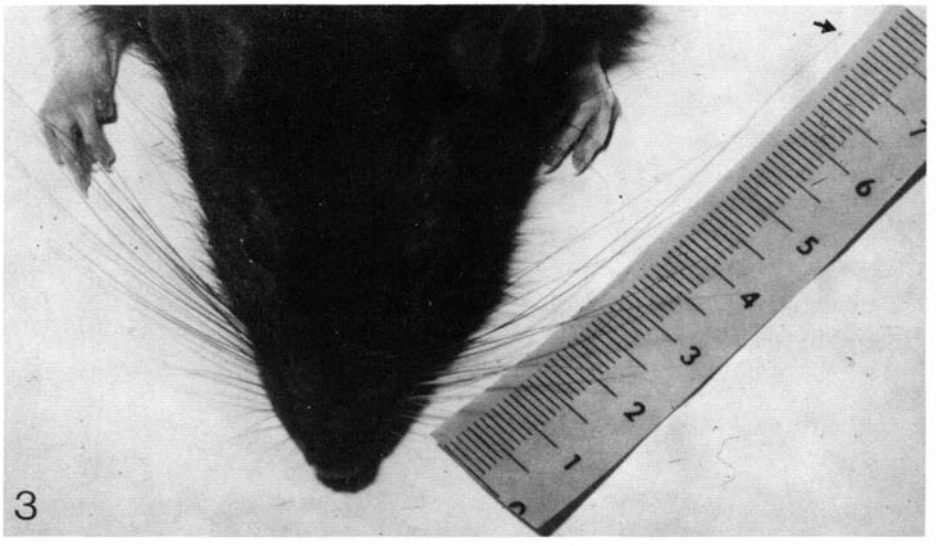

Fig. 3. Giant whisker (arrowed) produced after the method II wounding procedure.

Following injury all follicles ceased hair production and lost their growing fibres. Subsequently, two failed to display emergent vibrissae. Of the remaining sixteen follicles, fourteen produced hairs between 9 and 20 days post-operatively and two whiskers were first seen at 27 and 44 days respectively.

Terminal length measurements (Table 3) revealed that half of the follicles which produced vibrissae in G1 had hairs at least 4 mm longer than their controls. The longest of these (Figs 2, 3) measured 15 mm more, and was therefore 29 % longer, than its right side counterpart. A further four whiskers were of equivalent length to their controls, and four were shorter, though one of this latter group was a broken fibre.

More detailed information about the eleven follicles which produced at least one long hair postoperatively is displayed in Table 4. Five of these manufactured significantly longer hairs in both G1 and G2. Therefore, although three follicles switched to producing longer hairs in G2, three others went in the reverse direction. None of the latter group attained full terminal length, as one was broken, and the other two biopsied prematurely. Nevertheless, the mean percentage increase in length for G1 whiskers of 15·6 % fell by over 5 % in G2.

There was no increase in rates of growth; over 75 % of the follicles produced hairs at a reduced rate in both G1 and G2. Increases in whisker length were therefore associated with extended growing periods.

Method III

Both club and growing hairs were plucked just prior to wounding in 12 follicles in four rats. Two subsequently failed to produce emergent vibrissae. As shown

Table 4. *Table showing % increase in length of experimental hairs compared with controls, method II*

Rat	Follicle	% Increase in length of experimental hairs against control G1	G2
2/A	4a	(=)	12
2/B	3a	22	9
	4a	29	(=)*
2/C	3a	7	16
	5b	11	(=)b
2/D	4a	9	8b
2/G	3a	23	(−)*
	4a	(=)	7
2/H	4a	9	12
	5b	(−)	9
2/I	3a	15	9
	Mean %	15·6	10·3

G = Postoperative generation number.
(=) = Experimental hair equivalent in length to control
(−) = Experimental hair shorter in length than control
b = Whisker broken during growth phase
* = Terminal length not attained at biopsy.

in Table 3, of the remaining follicles, five produced longer hairs; three had fibres of equivalent length to their controls; and two manufactured shorter fibres. Therefore, in close agreement with method II, half of the damaged follicles had formed longer hairs in G1. Moreover, the mean length increase of 14·8 % was also near to that attained by G1 follicles in the previous method.

Effect of plucking alone

Following plucking, hair production was terminated in all twenty follicles. Sixteen new whiskers were seen between 7 and 17 days, and four between 27 and 55 days, but none of the plucked follicles produced G1 vibrissae longer than their controls.

Method IV

In three rats where 19 follicles were damaged above the bulb region, 3 follicles failed to produce emergent whiskers. The G1 fibre lengths for the remaining 16 follicles revealed no hairs of longer than control length. Four whiskers were shorter than their controls, while the remaining twelve were equivalent in length.

DISCUSSION

Normal whisker length

Vibrissa length measurements agreed with all previous investigations in that a distinct anteroposterior gradient of increasing fibre size was observed. Apart from the top horizontal row, the lengths could all be divided into discrete non-overlapping groups representing vertical rows. The range of length displayed within these groups was similar to that found on male Wistar rats by Ibrahim & Wright (1975).

Paired length measurements carried out to determine normal differences in club length between whiskers in identical positions on either side of the face revealed a margin rarely exceeding 2 mm, or about 4 % for the hairs of length normally used experimentally. Ibrahim & Wright (1975) also found that vibrissae grew synchronously within the same margin. These authors showed that for the first three postnatal cycles, whiskers underwent a stepwise increase in length before levelling off. Age-related length differences were confirmed in the current work, and it was for this reason that 6-month-old rats, whose whisker lengths had stabilized, were used for all experimental procedures.

Follicle wounding

As four operational procedures were performed, the reasoning behind the use of these different techniques will be briefly considered.

Using the method I procedure it was apparent that apart from inflicting injury *in situ*, cellular material was being lost from the bulb region of many follicles. To overcome any influence this loss of bulbar material might have on subsequent hair growth, method II was employed. As damage with these two methods was inflicted on both the epidermal matrix and the dermal papilla, method III was devised. It has already been shown that plucking of growing vibrissae removes a high proportion of epidermal matrix with no stimulatory effect on whisker length (Oliver, 1965; Ibrahim & Wright, 1978), and plucking procedures in this study confirmed these findings. Thus in method III, which acted as a quasi control, the removal of club and growing hairs prior to wounding meant that damage was essentially restricted to the dermal papilla alone (Jahoda & Oliver, 1984). The method IV control procedure was designed to show the effects on whisker growth of damage above the bulb region.

The present experiments confirmed the recuperative powers of vibrissa follicles following injury, with over 90 % of them manufacturing external fibres after being wounded. However, the most striking result was the confirmation of consistent increases in hair length (Oliver & Jahoda, 1981). This effect was manifested by some 10 % of follicles after method I operations, where a complex response included production of over 33 % short hairs. However, following methods II and III, in which no loss of cellular material from the capsule occurred, some 50 % of postoperative vibrissae were longer than expected. This

effect persisted into the second postoperative generation. In contrast, none of the follicles wounded distal to the bulb region produced longer than expected hairs. Recently, Ibrahim & Wright (1982) observed the formation of a single long vibrissa fibre following lower follicle regeneration. They have also shown whisker length increases of the same degree as in the present experiments following exposure of adult mice to high levels of testosterone (Ibrahim & Wright, 1983). What is intriguing about the present results is that they specifically implicate the dermal papilla in the production of longer hairs.

Hair length, which was employed as the primary measure of follicle activity, is the product of the rate of fibre growth, and the duration of fibre production. Where changes in these two parameters occurred they were consistent for all wounding methods. Growth rate when affected was reduced. Therefore longer whisker production was exclusively due to increases in the duration of growth. This effect was especially demonstrated in the results from methods II and III where more longer hairs were produced despite the increased number of follicles showing reduced growth rates. Interestingly, where experimental follicles produced hairs of normal length, this could either reflect the absence of wounding effects, or (as in ten method I follicles) be the result of increases in duration of growth and reductions in growth rate anulling each other.

Increased duration of hair growth has only rarely been demonstrated experimentally. Therefore it is noteworthy that when Hale & Ebling (1975) elicited this phenomenon through the action of propylthiouracil on rat pelage hairs, it was associated with a reduction in growth rate. At the same time treatment with thyroxine caused hairs to grow faster, while reducing the length of the growing period. Likewise in the present study a balance between the two altered parameters could negate any effect on fibre length. Indeed a major point to emerge from these findings is that factors which control growth rate appear to be independent of those which determine the duration of growth. Interestingly vibrissa length increases produced through the influence of testosterone involved longer growth periods, but unaffected growth rates (Ibrahim & Wright, 1983).

The hair growth cycle can be divided into three basic stages; anagen, the growing phase; catagen, the period of follicle regression; and telogen, when epidermal proliferation and hair growth is arrested. The mechanisms which control the repeated initiation and termination of the adult hair cycle represent the principal enigmas of hair growth. The timing of this cycle is astonishingly consistent for individual vibrissa follicles, and Oliver (1965) has noted the relationship between the order of hair replacement in adult follicles, and the sequence of vibrissa follicle development in embryos. The same author (Oliver, 1980) has proposed a control system based on an active mesenchymal element which would both initiate growth, and maintain it through a growing period. It was suggested that follicle behaviour might be governed by stimuli from the papilla, therefore reflecting some 'intrapapillary cycle of events'. This infers that the dermal papilla of each follicle starts off with an internal mechanism regulating

the timing of the cycle, perhaps derived from embryonic regulatory mechanisms. While the current results were in accordance with the above ideas to the extent that stimulation of the dermal papilla by wounding appeared to be primarily responsible for the production of longer fibres, the question as to why papilla injury should affect the duration of the growing period remains unresolved. Nevertheless, a histological study offers a reasonable explanation for some aspects of follicle behaviour after injury (Jahoda & Oliver, 1984).

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