The effect of plucking hairs during different phases of the follicular cycle

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WITH TWO PLATES

Hair growth is intermittent; periods of activity when the follicle is producing the hair alternating with periods of rest when the dead hair is retained. In the rat, hair growth occurs in a series of waves which start ventrally and pass over the flanks to the back (Dry, 1926; Butcher, 1934; Johnson, 1958).

It has long been known (Collins, 1918; David, 1934) that if hairs are pulled out of resting follicles activity is induced. To explain this phenomenon, Chase (1955) put forward the view that an inhibitor accumulates in the hair follicle during active growth and is dissipated during the resting stage. Plucking during the resting stage removes this inhibitor along with the club hair, so that activity recommences.

Preliminary observations on rats with plucked follicles indicated that the response to plucking varied at different stages of the hair cycle. A detailed study seemed desirable, therefore, in order to test more closely than hitherto the validity of the inhibitor hypothesis.

METHODS

Animals

All the observations were made on albino Wistar rats from a small randomly mated colony.

Design of experiment

In all the experiments, hairs were plucked with blunt-ended forceps from a strip about 2 cm. wide along the right flank, extending from just behind the ear to the hind limb.

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Observations on eruption of hair after plucking

Studies on intact rats (Johnson, 1958) had shown that hairs of the first wave erupt along the flank at about 5 weeks of age, grow for about 14 days, then enter a resting period of about 14 days until growth of the second wave begins.

Groups of rats were plucked during the growing period of the first wave and at 2-day intervals throughout the succeeding resting period. After plucking, the rats were shaved and dyed black with a commercial hair dye, ‘Inecto’ (Rapidol Ltd.), and thereafter observed daily for eruption of new hairs on the plucked flank and the opposite control flank.

Investigation of the histological changes after plucking

1. Resting hairs were plucked from rats at 55 days of age. Groups of four rats were killed at 2, 3, 4, 6, 7 and 10 days after plucking.
2. Hairs were plucked from follicles in early anagen, that is, in the period of follicular activity before eruption of new hair, when each club hair has an actively dividing matrix at its base. Previous observations (Johnson, 1958) had shown that this phase lasts for about 5 days. Since the timing of this stage could be estimated only approximately, a small biopsy sample was removed from the control flank at the time of plucking in order to determine the exact stage of growth. Rats which had been plucked at the appropriate time were then killed at intervals.
3. Newly erupted growing hairs of the first wave, together with their adjacent club hairs, were plucked as soon as it was possible to grasp them with forceps (2 or 3 days after eruption). Four rats were killed on the same day and at 2, 4, 6, 7, 8 and 11 days after plucking.

Histological methods

Five hours before being killed, the rats were injected intraperitoneally with 0·1 mg. colchicine in water/100 g. body weight at 10 a.m. in order to arrest developing mitoses in metaphase. For a discussion of the dosage chosen see Ebling (1954).

The rats were killed with chloroform and the whole skin was removed and fixed in aqueous Bouin for 24 hr. Samples taken from the middle of the plucked strip and from an opposite control site were embedded in ester wax, sectioned at 8μ and stained in Erhlich’s haematoxylin and alcoholic eosin. Biopsy samples were similarly treated.

RESULTS

Eruption of hairs

The exact age at eruption of hairs on the control flank varies from rat to rat. For analysis, the rats are grouped according to the time of plucking, expressed
as days before eruption of hairs on the control flank. The results are shown in Table 1 and the data is presented diagrammatically in Text-figs. 1 and 2.

### Table 1

**Effect of plucking on eruption of hairs of the second wave**

<table>
<thead>
<tr>
<th>Time of plucking in days before eruption on control side</th>
<th>Mean advancement (days)</th>
<th>n</th>
<th>Mean delay (days)</th>
<th>State of the follicles when plucked</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>4.75</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>3.0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>7.6</td>
<td>3</td>
<td></td>
<td>Growing, with erupted hairs of the first wave</td>
</tr>
<tr>
<td>25</td>
<td>3.0</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3.0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>5.5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>2.3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3.8</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean advancement by plucking during growing period: $4.16 \pm 0.89$

| 20                                                       | 8.8                     | 2 |                   |                                   |
| 19                                                       | 6.0                     | 2 |                   |                                   |
| 18                                                       | 5.0                     | 3 |                   |                                   |
| 17                                                       | 4.5                     | 2 |                   |                                   |
| 16                                                       | 3.0                     | 5 |                   |                                   |
| 15                                                       | 3.8                     | 7 |                   |                                   |
| 14                                                       | 3.5                     | 2 |                   |                                   |
| 12                                                       | 0                      | 4 |                   |                                   |
| 11                                                       | 0.5                     | 2 |                   |                                   |
| 9                                                        | 3                      | 2.3 |                |                                   |
| 8                                                        | 1                      | 4.0 |                |                                   |
| 7                                                        | 4                      | 4.5 |                |                                   |
| 6                                                        | 0                      | 3 | 0 | Active matrix at the base of a club hair |
| 3                                                        | 0                      | 3 | 0 |                                   |

Hairs of the second wave erupted about 9 days earlier than on the control flank from follicles plucked at 20 days before expected eruption (Text-fig. 1); at this time activity of the first wave had just ceased. Hairs erupted from these follicles after an interval of 11 days after plucking.

When follicles were plucked during the resting period, between 20 and 12 days before expected eruption, hairs continued to erupt about 11 days later (Text-fig. 2). Thus, the amount by which activity in the follicles was advanced by plucking progressively decreased over this period, until at 12 days before eruption plucking had no effect (Text-fig. 2).

A delay in eruption of hairs was achieved by plucking resting follicles between 9 and 6 days before expected eruption (Text-fig. 1). The interval between plucking and eruption remained the same at about 11 days (Text-fig. 2).
TEXT-FIG. 1. Advancement or delay of eruption of new hairs along the flank induced by plucking at different intervals before eruption of hairs on the control flank. The state of the follicles when plucked is shown below the graph; the growing phase with erupted hairs is indicated by light shading, the resting phase (telogen) is unshaded, and activity before eruption is shown by dark shading.
Effect of plucking on the follicular cycle

TEXT-FIG. 2. The same data as in Text-fig. 1 arranged to show the interval between plucking of hairs and the eruption of new hairs.

Days before eruption on control flank

Interval (days)

28 26 24 22 20 18 16 14 12 10 8 6 4 2 0
No effect was produced by plucking hairs at 6 or 3 days before their expected eruption.

Plucking while hairs of the first wave were growing, between 31 and 22 days before expected eruption of the second wave, caused a mean advancement of this eruption of 4.16 days (Table 1).

**Histological changes**

Follicles from which the resting club hairs were plucked at 55 days of age, showed mitotic activity in the hair germ 3 days later (Plate 1, Fig. B), at which time follicles from the opposite control flank were still resting (Plate 1, Fig. A). Mitotic activity was induced even when the club hairs were not removed at their base, as can be seen in Plate 1, Fig. B, and mitoses were abundant in the epithelial sac and the associated sebaceous gland—a condition not usually found in normal follicles at the commencement of activity. By 7 days after plucking an active follicle was established with a new hair growing up through the skin (Plate 1, Fig. C). At this time, follicles from the control flank were still resting (Plate 1, Fig. D). The new hairs from the plucked follicles erupted at the skin surface some 10 or 11 days after plucking. Thus, once mitotic activity has begun, the organization of the follicle and its upgrowth through the skin takes about 7 days in a plucked follicle compared with about 5 days in normal follicles (Johnson, 1958).

Follicles in the stage of early anagen showed no disruption of their activity when the club hair lying above the dividing matrix was plucked. Such a follicle, from which the club hair was plucked 3 days earlier, is shown in Plate 1, Fig. E. This skin from this site differed from that of the control site only in the increased thickness of the epidermis and in the numbers of mitoses observed, both in the epidermis, and in the upper region of the epithelial sac from which the club had been removed. Such plucked follicles erupted their new hairs at the normal time. This explains why no effect was produced by plucking hairs at 6 or 3 days before their expected eruption; at this time activity of the matrix below the club hair would have begun.

When newly erupted growing hairs were plucked, the follicle was broken at the point where the expanded base narrowed into the hair channel (Plate 2, Fig. F). Two days later mitoses were still to be seen in the base of the follicle (Plate 2, Fig. G), and mitotic activity continued up to 6 days after plucking. By this time the new hairs had almost reached the skin surface. By 7 or 8 days after plucking, mitotic activity had ceased as in early catagen, but the plucked follicles appeared abnormal (Plate 2, Fig. H). At this time, follicles from the control flank were still active (Plate 2, Fig. I). Although no true catagen stage was observed, the plucked follicles had entered a clearly recognizable telogen stage by 11 days after plucking (Plate 2, Fig. J). The control follicles at this time were just entering the catagen stage (Plate 2, Fig. K). It is clear, therefore, that plucking of a newly erupted hair shortens its growing period by 3 to 4 days.
Figs. A–D are sagittal sections of skin, taken from the plucked flank and opposite control flank, of rats at intervals after plucking resting hairs at 55 days of age.

Fig. A. Three days after plucking resting hairs; follicles from the control flank are still in the resting stage.

Fig. B. Three days after plucking resting hairs; the plucked follicles show mitotic activity in the hair germ, and in the associated sebaceous glands and epithelial sacs. Note that the base of the club hair has not been removed.

Fig. C. Seven days after plucking resting hairs; the plucked follicles are actively producing a new hair, which is growing up through the skin.

Fig. D. Seven days after plucking resting hairs; follicles from the control flank are still resting.

Fig. E. Sagittal section of skin from which club hairs were plucked from follicles in early anagen, i.e. when mitotic activity had begun in the hair germ below the club. Sample taken 3 days after plucking shows that activity of such follicles continues.

(The magnification for each figure is shown by a line representing 0.1 mm.)

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(Facing page 470)
Sagittal sections of skin, taken from the plucked flank and opposite control flank, of rats at intervals after plucking newly erupted hairs.

**FIG. F.** Immediately after plucking newly erupted hairs; the plucked follicles are broken at the point where the expanded base narrows into the hair channel (indicated by the arrow).

**FIG. G.** Two days after plucking newly erupted hairs; mitotic activity is continuing in the base of the plucked follicles.

**FIG. H.** Seven days after plucking newly erupted hairs; the plucked follicles have an abnormal appearance and there is no mitotic activity.

**FIG. I.** Seven days after plucking newly erupted hairs; follicles from the control flank are still active.

**FIG. J.** Eleven days after plucking newly erupted hairs; the plucked follicles are now in the telogen stage.

**FIG. K.** Eleven days after plucking newly erupted hairs; follicles from the control flank are in the catagen stage.

The magnification for each figure is shown by a line representing 0.1 mm.
DISCUSSION

Our experiments show that plucking does not have a consistent effect on the hair follicle at every stage of its cycle. When newly erupted hairs are plucked, the ensuing eruption is advanced by about 4 days, occurring after an interval of 26 days. As growth proceeds (between 31 and 20 days before expected eruption) the degree of advancement remains more or less constant and the interval falls (Text-figs. 1, 2). At the end of activity plucking induces a maximum degree of advancement and minimum interval. Throughout the resting period the interval remains the same (about 12 days) (Text-fig. 2). Thus, it can be seen (Text-fig. 1) that the degree of advancement steadily falls until day 12 and thereafter retardation is achieved. Once mitotic activity has begun, plucking of club hairs has no effect.

Plucking of hairs, which seems to cause mitosis in the associated superficial epidermis, epithelial sac and sebaceous glands in addition to any effect on the hair bulb, is clearly analogous to wounding, and other authors have reported that wounding itself brings about initiation of follicular activity (Argyris, 1956, 1962; Argyris & Argyris, 1962; Ghadially, 1958). Many authors (see review by Abercrombie, 1957) have suggested that the stimulant to mitosis after wounding is an increase in local concentration of some product of the damaged cells, usually called a wound hormone. Thus, Swann (1958) proposes that cells are normally quiescent, requiring an inductive stimulus for their division. On the other hand, Bullough & Laurence (1960) have presented evidence in support of the hypothesis that wounding reduces the concentration of an inhibitor, thus suggesting that a mechanism of inhibition is an essential feature controlling normal growth.

Chase (1954) has suggested that an inhibitor controls the inherent rhythm of activity in the hair follicle. The hair follicle differs from the epidermis and its other derivatives in that activity of its cells is intermittent and more or less synchronous; during the resting phase no mitoses are observed. Thus it is proposed that an inhibitor accumulates in the follicle during active growth and disperses during the resting period.

If there is a build up of such an inhibitor during active growth, we might, unless the rate of growth is greatly altered, expect that plucking of a growing hair will always be followed by a period of growth of the same duration as the normal period of activity. If an inhibitor is removed or dispersed by plucking during the resting phase we should expect a constant interval before eruption of a new hair. How far is our evidence consistent with these expectations?

Plucking of a growing hair, in fact, shortens the growing period by about 4 days, which is the opposite to what we would expect on the inhibitor hypothesis. Moreover, we might postulate that during such a shortened growing phase less inhibitor would accumulate so that the ensuing resting phase should be shortened. It remains constant; the ensuing eruption is advanced by exactly the period by which the previous active phase was shortened.
The fact that, between 20 and 12 days before expected eruption, plucking induces activity after a constant interval is consistent with the inhibitor hypothesis. This result can, however, equally well be explained by postulating that an inductive stimulus is produced.

The interval between plucking of a resting hair and eruption of the new hair is 12 days. It is reasonable to assume, therefore, that normally, 12 days before eruption, any inhibitor is at a low level similar to that produced by plucking, and that the subsequent pattern of events in the follicle becomes determined. Thus, on the inhibitor hypothesis, we might expect that plucking at this time, or at any subsequent stage until eruption of the hair, would have no effect. In fact, although it is true that plucking at 12 days has no effect, plucking at 9, 8 or 7 days before eruption delayed the event, which continued to occur 12 days after plucking. If this delay were due to damage, from which the follicle might take time to recover, we need to explain why plucking of club hairs after mitotic activity has begun, i.e. at 6 or 3 days before eruption, has no such delaying effect. It is possible that once mitosis has begun the club becomes loosened and may thus be removed without damaging the follicle. On the other hand, these results might be explained by postulating that a sequence of inductive events are set in train at about 12 days before eruption of the hair, and that plucking after 12 days, but before mitosis begins, interferes with this but at the same time initiates a further sequence.

Our evidence is insufficient to resolve the conflicting hypotheses of inhibitor and inducer mechanisms. We need, for example, to know whether hair grows abnormally fast from plucked growing follicles. Nevertheless, it appears difficult to explain all our experimental results by the inhibitor hypothesis, whereas an inductor hypothesis could reasonably account for most of them.

**SUMMARY**

1. Using rats, hairs were plucked at intervals throughout the follicular cycle.
2. Plucking of a growing hair shortens its period of activity by 4 days, and advances the ensuing eruption of new hair by about 4 days; the length of the resting period thus remains constant.
3. Plucking at the end of activity advances the subsequent eruption of hairs, which occurs after an interval of 12 days. Throughout the resting period the interval between plucking and eruption remains the same. Thus, the degree of advancement of eruption falls until there is no effect at 12 days before eruption, and plucking at 9, 8 or 7 days before expected eruption causes it to be delayed.
4. Once mitotic activity has begun in the hair germ, at about 6 days before eruption, plucking of the club has no effect.
5. The hypothesis that an inhibitor builds up during active growth and is dispersed during the resting phase or by plucking does not easily account for all of these results. The hypothesis that an inductive stimulus is released by plucking could more readily account for most of them.
Effect of plucking on the follicular cycle

RÉSUMÉ

L'effet de l'arrachage des poils pendant les phases différentes du cycle du follicule

1. Chez le rat, des poils sont arrachés à des intervalles déterminés par le cycle du follicule.

2. Si on arrache un poil pendant sa croissance, la période d'activité du follicule est raccourcie de 4 jours ; l'apparition du nouveau poil est avancée d'environ 4 jours. Ainsi la phase de repos reste constante.

3. Si on arrache un poil à la fin de la période d'activité du follicule d'éruption du poil suivant, qui a lieu après un intervalle de 12 jours, est avancé. La période de repos qui correspond à l'intervalle entre l'arrachage et la repousse reste la même. Si on arrache un poil plus de 12 jours avant la date prévue de l'éruption, celle-ci est avancée. 12 jours avant il n'y a pas de changement. 9, 8 ou 7 jours avant, la repousse est retardée.

4. Une fois que l'activité mitotique a commencé dans le germe du poil, environ 6 jours avant l'éruption, l'arrachage n'a plus d'effet.

5. L'hypothèse selon laquelle un inhibiteur serait formé pendant la phase active de croissance et dispersé pendant la phase de repos ou pendant l'arrachage ne concorde pas facilement avec ces résultats. La plupart des résultats semblerait plutôt en accord avec l'hypothèse d'un stimulus inducteur libéré par l'arrachage.

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REFERENCES


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