Systemic Influence on Activity of Hair Follicles in Skin Homografts

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INTRODUCTION

ACTIVITY of the hair follicle is cyclic, periods of hair-growth alternating with periods of quiescence during which the dead hair is retained as a 'club'. 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*a*).

The object of our work was to find out how far such activity is dependent upon factors within the follicle and how far it is subject to systemic control. When hair follicles are translocated, either by rotation of grafts in the mid-flank or by transposition of flaps in two stages, they continue to maintain the periodicity characteristic of their sites of origin (Ebling & Johnson, 1959). Vascularization of such follicles is always consequent upon follicular activity, even when this is out of phase with the normal waves of hair-growth on the adjacent body. This evidence suggests that follicular activity is inherent and that the hyperaemia which accompanies hair-growth is consequent upon an innate demand of follicles and is not itself the cause of their activity.

Nevertheless, the fact that, when the resting phase is prolonged by steroid hormones or shortened by gonadectomy or adrenalectomy (Johnson, 1958 *b*, *c*), the pattern of the hair-growth waves is never altered suggests that some form of systemic control is involved. The possibility of a systemic influence could be harmonized with the evidence of inherent properties by supposing that a systemic rhythm is superimposed on local differences of reactivity. If, for example, follicular activity in different regions of the body were determined by graded thresholds to a systemic stimulus, then translocated autografts would appear to behave selfwise. On the other hand, normally sited homografts might not do so and it seemed desirable to investigate their behaviour.

METHODS

Animals

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

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Design of experiments

Studies on intact rats (Johnson, 1958*a*) had shown that the first wave of hair-growth begins at about 5 weeks of age and is complete by 7 weeks; the second wave begins at 8–9 weeks and takes much longer for completion. We had some evidence to suggest that a third wave of growth begins at about 16 weeks of age.

Skin exchanges between rats of different ages were planned so that their expected hair-growth waves would be out of phase. Homografts from the left flank were exchanged between rats aged 28 and 49 days respectively. Thus hair-growth was due on the flank of the host in advance of the homograft in the younger rats and in retard of the homograft in the older rats. Since eruption of hair on grafts is a little in retard of that on the body (Ebling & Johnson, 1959), each rat also carried—for comparison with the homografts—an autograft on its right flank.

Two series of experiments were carried out; one was begun during May–June (summer series) and one during October–November (winter series). In the summer series all the younger rats when 1 day old were injected intraperitoneally with a suspension in sterile saline of spleen cells from the prospective older graft donor to induce immunological tolerance to the subsequent graft (Billingham, Brent, & Medawar, 1956; Woodruff, 1957). Most of the homografts from the older to the younger rats appeared to survive indefinitely, but so did those from the younger to the older rats. This might be explained on one of two hypotheses; either our colony was immunologically homogenous, or the injection of spleen cells had not only induced tolerance in the recipient but had altered its antigenic structure so as to render its skin innocuous to the spleen-cell donor. To find out which was true, in the winter series, for each younger rat injected with spleen from an older rat, an uninjected litter mate was also used for an exchange of homografts with a litter mate of the spleen donor. A high proportion of all grafts survived indefinitely; of those from younger to older rats there was no significant difference in survival between grafts from injected and grafts from uninjected donors. We concluded that our colony was immunologically homogenous. Of 41 successfully grafted pairs in the winter series, 63 per cent. of the total number of homografts survived until eruption of hairs of the wave following operation. Most of these rats were observed for the subsequent wave and some for a third wave after grafting.

Operative procedures

Strips of skin from the flank (about 4×2 cm.) extending from the belly to the back were used for the grafts, which were stitched into position with interrupted sutures of fine silk. Full details of the methods and dressings have been given previously (Ebling & Johnson, 1959).

RESULTS

In the summer series the hairs erupted on the autografts at about 6 weeks (first wave), 10 weeks (second wave), and 20 weeks (third wave). On the autografts of the winter series hairs of the third wave erupted earlier (14–16 weeks). There may be some seasonal variation involved and for this reason the results for the two series have been presented separately.

TABLE 1

Age in days at eruption on grafts of hairs of first and second waves (summer series)

	Older rat		*Age of younger donor at eruption on homograft*	*Younger rat*		*Age of older donor at eruption on homograft*
Pair	*Autograft second wave* (1)	*Homograft from younger rat* (2)	(3)	*Autograft first wave* (4)	*Homograft from older rat* (5)	(6)
Males						
a	69	64	43	42	54	75
b	79	65	44	46	—	—
c	80	67	46	44	47	68
d	72	—	—	42	47	68
e	68	63	42	41	48	69
f	74	67	46	42	47	68
g	71	—	—	46	48	69
h	77	67	46	45	55	76
Females						
i	68	66	45	52	—	—
j	73	67	46	44	53	74
k	76	63	42	42	—	—
l	72	—	—	44	55	76
m	75	59	38	44	—	—
n	70	—	—	44	42	63

Behaviour of homografts:
Mean difference from autografts of recipients (columns 2 and 1) = $-8{\cdot}10 \pm 1{\cdot}54$ ($t = 5{\cdot}26$; $P < 0{\cdot}001$).
Mean difference from autografts of donors (columns 3 and 4) = $-0{\cdot}44 \pm 0{\cdot}39$ ($t = 0{\cdot}39$; $P = 0{\cdot}8-0{\cdot}7$).

Behaviour of homografts:
Mean difference from autografts of recipients (columns 5 and 4) = $+6{\cdot}20 \pm 1{\cdot}41$ ($t = 4{\cdot}41$; $P < 0{\cdot}01$).
Mean difference from autografts of donors (columns 6 and 1) = $-2{\cdot}0 \pm 1{\cdot}71$; ($t = 1{\cdot}17$; $P = 0{\cdot}3-0{\cdot}2$).

The complete results for pairs of rats of the summer series, in which at least 3 out of 4 grafts survived are shown in Tables 1 and 2. Each analysis compares the behaviour of homografts with the autograft of the recipient, on the one hand, and with the autograft of the donor, on the other. Similar analyses are given for the winter series of experiments in Table 3. Table 4 summarizes all observations made, i.e. including pairs of rats in which only one or two grafts survived, and this data is presented diagrammatically in Text-figs. 1 and 2.

In the waves of growth immediately following grafting, i.e. the first and second waves respectively (Tables 1, 3, 4; Text-figs. 1, 2), it is clear that on all homografts eruption of hair occurred at the same time as on the donor's

autograft. In the older recipients this was significantly in advance and in the younger recipients significantly in retard of eruption of hair on their autografts.

TABLE 2

Age in days at eruption on grafts of hairs of second and third waves (summer series)

Pair	Older rat: Autograft third wave (1)	Older rat: Homograft from younger rat (2)	Age of younger donor at eruption on homograft (3)	Younger rat: Autograft second wave (4)	Younger rat: Homograft from older rat (5)	Age of older donor at eruption on homograft (6)
Males						
a	126	98	77	77	77	98
b	168	97	76	76	—	—
c	157	—	—	89	83	104
f	114	—	—	63	71	92
h	112	—	—	69	71	92
Females						
i	130	94	73	84	—	—
j	159	96	75	74	79	100
k	161	92	71	91	—	—

Behaviour of homografts:
Mean difference from autografts of recipients (columns 2 and 1) = $-53{\cdot}40 \pm 8{\cdot}93$ ($t = 5{\cdot}98$; $P < 0{\cdot}01$).
Mean difference from autografts of donors (columns 3 and 4) = $-8{\cdot}40 \pm 3{\cdot}96$ ($t = 2{\cdot}12$; $P = 0{\cdot}10-0{\cdot}05$).

Behaviour of homografts:
Mean difference from autografts of recipients (columns 5 and 4) = $+1{\cdot}80 \pm 2{\cdot}37$ ($t = 0{\cdot}76$; $P = 0{\cdot}50-0{\cdot}40$).
Mean difference from autografts of donors (columns 6 and 1) = $-36{\cdot}40 \pm 8{\cdot}17$ ($t = 4{\cdot}46$; $P < 0{\cdot}02$).

TABLE 3

Eruption of hairs on homografts and autografts compared (winter series)

Behaviour of homografts from younger to older rats

Wave on recipient	Number of pairs	Difference from autografts of recipients (days): Mean	s.e.	t	P	Difference from autografts of donors (days): Mean	s.e.	t	P
2	25	−4·04	0·93	4·37	< 0·001	+1·00	0·68	1·47	0·2–0·1
3	10	−8·00	1·93	4·15	< 0·01	+1·50	0·54	2·78	< 0·02
4	9	−25·00	6·57	3·80	< 0·01	+19·89	8·17	2·43	< 0·05

Behaviour of homografts from older to younger rats

Wave on recipient	Number of pairs	Difference from autografts of recipients (days): Mean	s.e.	t	P	Difference from autografts of donors (days): Mean	s.e.	t	P
1	18	+4·67	0·83	5·63	< 0·001	−1·22	0·73	1·67	0·2–0·1
2	8	+6·00	1·66	3·61	<0·01	−4·25	2·21	1·92	0·10–0·05
3	6	+5·67	2·74	2·07	0·10–0·05	−41·00	10·65	3·85	< 0·02

In the next wave of growth (second and third waves respectively, Tables 2, 3, 4; Text-figs. 1, 2) eruption of hair on homografts from the younger donors was

TABLE 4

Mean age in days at eruption of hairs on grafts based on all observations

Wave	Older rat: Autograft Mean±s.e.	n	Homograft from younger rat Mean±s.e.	n	Age of younger donor at eruption on homograft mean*	Wave	Younger rat: Autograft Mean±s.e.	n	Homograft from older rat Mean±s.e.	n	Age of older donor at eruption on homograft mean*
					Summer series						
2	73·11±0·81	19	65·23±0·74	13	44·23	1	44·72±0·82	18	49·67±1·15	12	70·67
3	143·11±7·44	9	95·33±0·88	6	74·33	2	80·67±3·44	9	79·17±3·52	6	100·17
					Winter series						
2	70·92±0·67	36	66·50±0·62	28	45·50	1	44·73±0·44	41	48·42±0·73	24	69·42
3	99·31±1·18	26	91·41±0·86	17	70·41	2	68·89±0·53	29	74·57±0·60	21	95·57
4	171·00±6·51	13	144·86±4·65	14	123·86	3	111·23±3·77	13	110·00±2·53	9	131·00

* Standard errors as in previous column.

again greatly in advance of the autograft of the older recipient and not greatly different from the autograft of the donor. On the other hand, eruption of hairs on homografts from the older donors occurred at the same time as on the autograft of the younger recipient (summer series, Table 2) and well in advance of the donor's autograft. In the winter series, also, eruption of hairs on homografts from the older donors was well in advance of their autograft, although still significantly in retard of the recipient's autograft (Table 3).

In the ultimate waves of growth studied (third and fourth waves, Tables 3, 4; Text-figs. 1, 2), homografts from the older donors were completely in line with the younger recipients, coming into activity on average some 41 days before the autografts of the donors. In this wave, eruption on the younger homograft was still significantly in advance of the autograft of the older recipient, although somewhat later than eruption on its donor's autograft.

DISCUSSION

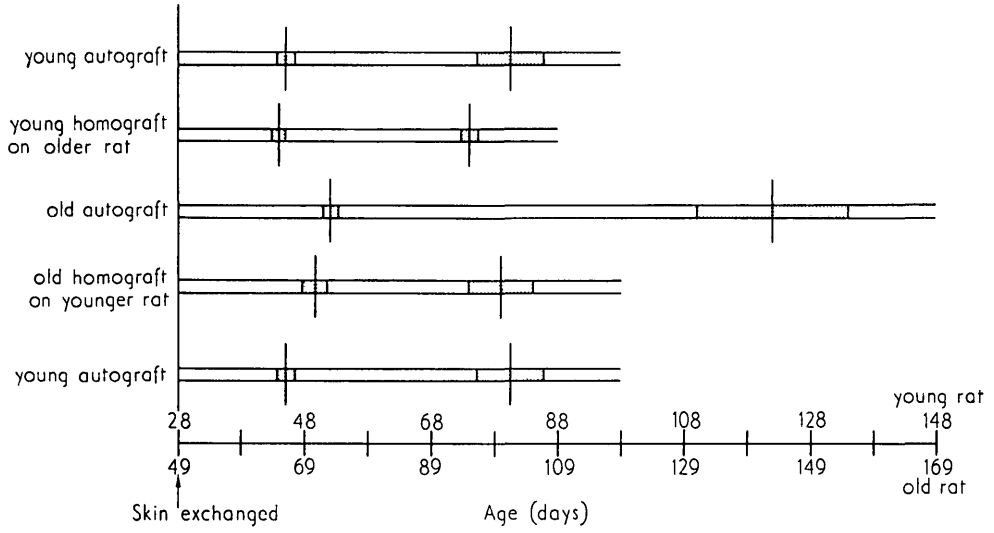
The results show clearly that both an inherent rhythm and a systemic influence are involved in follicular activity at a particular site.

An inherent rhythm appears to persist in homografts for one cycle after exchange, irrespective of whether follicular activity in the host precedes or succeeds that in the donor. Subsequently, if follicular activity in the recipient succeeds that in the donor, homografts continue their tendency to behave donor-wise, although by the third wave after grafting eruption of hair was significantly delayed.

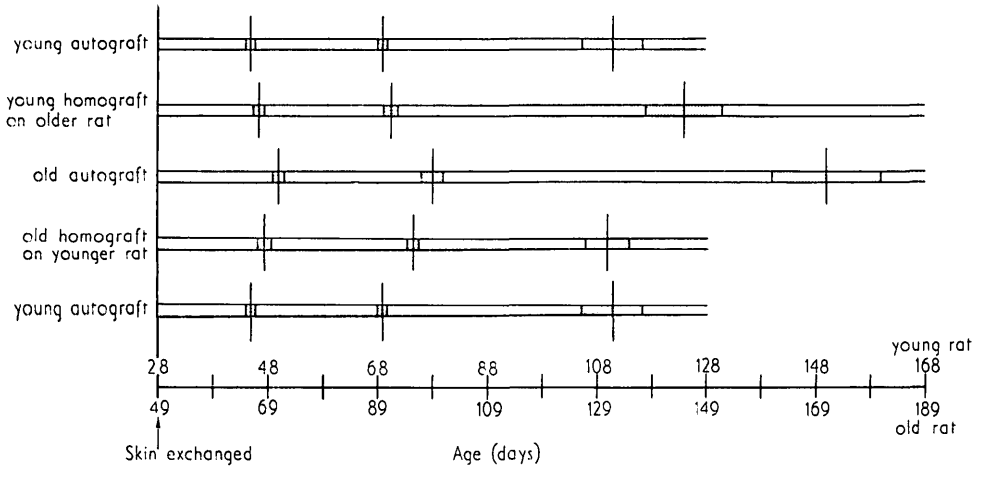
On the other hand, when follicular activity in the recipient precedes that in the donor, the time of eruption of hair on the homograft is advanced. By the second or third cycle after grafting the activity of such a homograft may be brought forward completely into step with its host. There must, therefore, be some systemic influence which stimulates follicular activity at a particular time. The question of whether this stimulus involves a lowered concentration of an inhibitor or an increased concentration of an accelerator has not yet been resolved. It is of interest that the young grafted follicles, after completing their donor-wise growth of the second and third waves in advance of the recipient follicles, did not redevelop activity in synchrony with the third and fourth waves of their host (Text-fig. 2). We must conclude, therefore, that follicles have a refractory period following active growth during which the systemic stimulus is unable to exert an effect.

The evidence we have presented does not resolve the hypothesis put forward by Chase (1955). He has suggested that cessation of follicular activity is caused by the accumulation of inhibiting substances within the follicle; activity recommences when the inhibitor has diffused away, broken down, or is otherwise destroyed as when the hair is plucked. Our systemic factor must be quite distinct from any such non-transmissible substances. It is possible, however, that any systemic influence could act by affecting the rate of dispersal of such a local inhibitor.

Various identified hormones are known to affect initiation of follicular activity; for example, gonadal and adrenocortical steroids retard initiation (Johnson, 1958 *b*, *c*) and thyroxine has been reported to have the opposite effect (Mohn, 1958). We do not know whether the systemic influence described here could be due to factors such as these, or whether some, as yet, unidentified substance is involved.



TEXT-FIG. 1. Mean ages of rats at eruption of hairs on grafts (summer series). The shaded limits represent plus or minus 1·5 times the standard errors. In comparing homografts with autografts, when the limits do not overlap the differences are significant with $P < 0{\cdot}05$.



TEXT-FIG. 2. Mean ages of rats at eruption of hairs on grafts (winter series). The shaded limits represent plus or minus 1·5 times the standard errors. In comparing homografts with autografts, when the limits do not overlap the differences are significant with $P < 0{\cdot}05$.

SUMMARY

1. Strips of skin from the flank were exchanged between rats aged 28 and 49 days respectively, so that their expected hair growth waves would be out of phase. Similarly sited autografts were used as controls.

2. In the wave of growth immediately following grafting eruption of hairs on the homografts coincided with that on the donor's autograft. In the older recipient this was significantly in advance and in the younger recipient significantly in retard of hair growth on their autografts. Thus, follicular activity has an inherent rhythm which continues for at least one cycle after homotopic grafting.

3. In the succeeding waves of growth, when follicular activity in the recipient preceded that in the donor, the time of eruption of hair on the homograft was advanced, and by the third wave after grafting was completely in line with its host. When follicular activity in the recipient succeeded that in the donor the homografts tended to behave donor-wise.

4. These results show clearly that both an inherent rhythm and a systemic stimulus are involved in follicular activity at a particular site.

RÉSUMÉ

Influence de l'organisme sur l'activité des follicules pileux dans les homogreffes de peau

1. Des lambeaux de peau du flanc ont été échangés entre des rats âgés respectivement de 28 et 49 jours, de sorte que soient déphasées les ondes, prévues, de croissance de leurs poils. Des autogreffes en position semblable ont servi de témoins.

2. Dans l'onde de croissance immédiatement consécutive à la greffe, l'éruption des poils sur les homogreffes a coïncidé avec celle de l'autogreffe du donneur. Chez l'hôte le plus âgé, elle était nettement en avance, et chez le plus jeune nettement en retard sur la croissance des poils portés par les autogreffes correspondantes. Ainsi, l'activité folliculaire possède un rythme propre qui se poursuit pendant un cycle au moins après une greffe homotopique.

3. Dans les ondes de croissance suivantes, quand l'activité folliculaire de l'hôte précède celle du donneur, l'éruption des poils sur l'homogreffe est avancée, et, à la troisième onde consécutive à la greffe, se trouve complètement alignée sur celle de l'hôte. Quand l'activité folliculaire de l'hôte a succédé à celle du donneur, les homogreffes ont tendu à se comporter comme le donneur.

4. Ces résultats mettent clairement en évidence qu'un rythme propre et une influence systémique tous deux prennent part dans l'activité des follicules à une position donnée.

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