GROWTH OF THE MOUSE COAT

II. Effect of Sex and Pregnancy

By A. S. Fraser* and T. Nay*

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Summary

Cycles of hair growth on Naked mice are described. Effects of sex differences are small. Patterns of hair growth are markedly affected by pregnancy and lactation.

I. Introduction

Growth of the mouse coat is a cyclic phenomenon; during the growth phase the skin follicles form hairs which, when completed, are retained in the follicles. At the next growth phase the follicles form a new set of complete hairs. Hairs formed during previous growth phases may be shed during a growth phase, but Dry (1926) has shown that it is usual for a follicle to retain several completed hairs; as several hairs are retained by one follicle it is difficult to study growth in normal mice. The Naked gene has a dominant effect which results in the hairs breaking off at skin level just before, or soon after, the end of the growth phase (cf. Grüneberg 1951), so that mice carrying this gene are covered with hair only in parts of the body which are in the growth phase. Naked mice should, therefore, be very useful for studies of the characteristics of hair growth cycles in general, if it can be assumed that the Naked gene has no effect on the pattern of the hair growth cycles, since in them the presence of hair on the skin marks the passage of a growth cycle. In a later paper we will describe the results of comparisons made to check the assumption that Naked does not interrupt the normal growth cycle. In this paper some preliminary observations of the effect of sex and pregnancy on the growth cycles of Naked mice are given.

II. Material

The mice used in these studies are all from the NA stock, which was formed at the Genetics Laboratory, Animal Breeding and Genetics Research Organization, Edinburgh, by Dr. D. S. Falconer and A.S.F., assisted by Mrs. S. Sobey. The stock was formed by back-crossing Naked mice to the Strong A line of inbred mice for five generations. Thereafter the stock has been maintained by sib matings, extending over 7-10 generations, and can be considered to be very uniform genetically.

* Animal Genetics Section, C.S.I.R.O., University of Sydney.

III. Methods

Drawings of the mice were made once or twice a week, initially as shown in Figure 1*A*, *B*, later by the simpler method shown in Figure 1*C*, *D*. Drawings were made on a standard printed silhouette, and no check was made of body

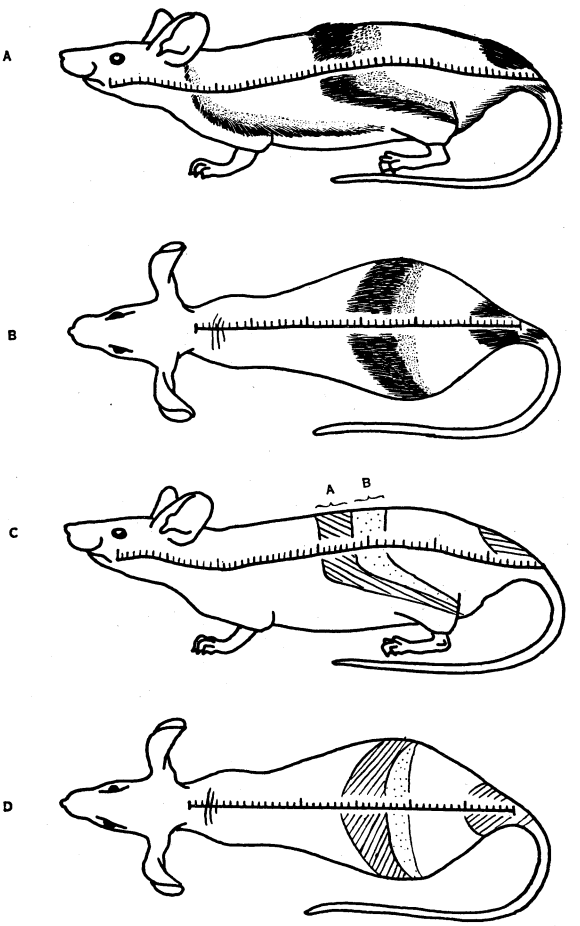

Fig. 1.—Standard silhouettes over which drawings were made. The original method of drawing, in which a copy of the structure of the hair bands was attempted, is shown in *A* and *B*. A schematized method of drawing was used later, involving the separation into a hair band (*A*) and a band of newly initiated hair growth (*B*). This is shown in *C* and *D*.

size. In order to make any statistical studies of the movement of hair bands it is necessary to transform these drawings onto a numerical scale. The scales are shown superimposed on the standard silhouette in Figure 1.

The transformations were effected by laying a celluloid sheet, on which the silhouette and scale were drawn, over each of the drawings; the positions of the front and rear edges of the hair bands were then read. The accuracy of the method was investigated, as described in Appendix I; the conclusion from these investigations was that the errors were sufficiently low to make the technique of value for the study of time changes in the bands, though the accuracy of plotting varied on different parts of the body.

IV. Results

Naked mice typically carry two or three bands of hair spaced over the body. Drawings of a few mice showed that the bands of hair growth commence on the head and neck, move along the neck and over the head to form a band across the shoulders, which then moves along the back, terminating at the root of the tail. The drawings in Figure 2 are all of a single mouse, drawn at weekly intervals. A faint shadow extends beyond the caudal edge of the hair bands. This shadow is caused by the activity of hair follicles, which have only just begun their growth phase, and is the advancing edge of the hair band, which is moving from head to tail.

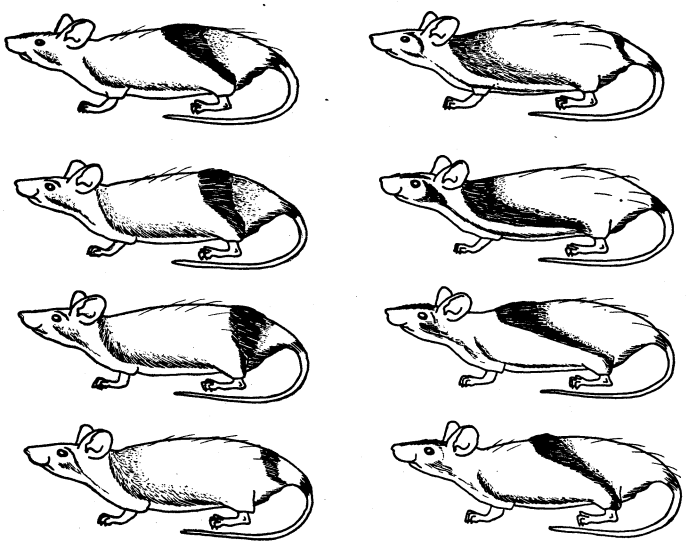

Fig. 2.—Drawings of an adult mouse, made at weekly intervals, demonstrating the pattern of movement of the hair bands.

(*a*) *Rate of Movement of the Hair Bands*

The rate of movement and width of a hair band change during the path from head to tail. The band moves faster along the back and sides than it does on the neck, shoulders, and near the tail. The bands on the neck, shoulders, and near the tail, are narrower than along the back. These points are illustrated in Figure 3, and in Table 1. The differences of width of the hair bands can be

explained as an outcome both of the differences of speed of movement, and of differences of the length of the period during which a follicle is in the growth

TABLE 1

RELATION OF WIDTH OF BAND TO ITS LOCATION

Location of Cranial Edge of Band	Mean Width of Band	Nos. of Bands
0-10	3·4	56
10-20	4·8	38
20-30	8·1	19
30-40	7·1	9
40-50	8·9	27
50-60	7·1	35
60-64	3·4	15

phase. The graphs of positions of hair bands plotted against time, shown in Figure 3, indicate that differences of length of the growth phase are small.

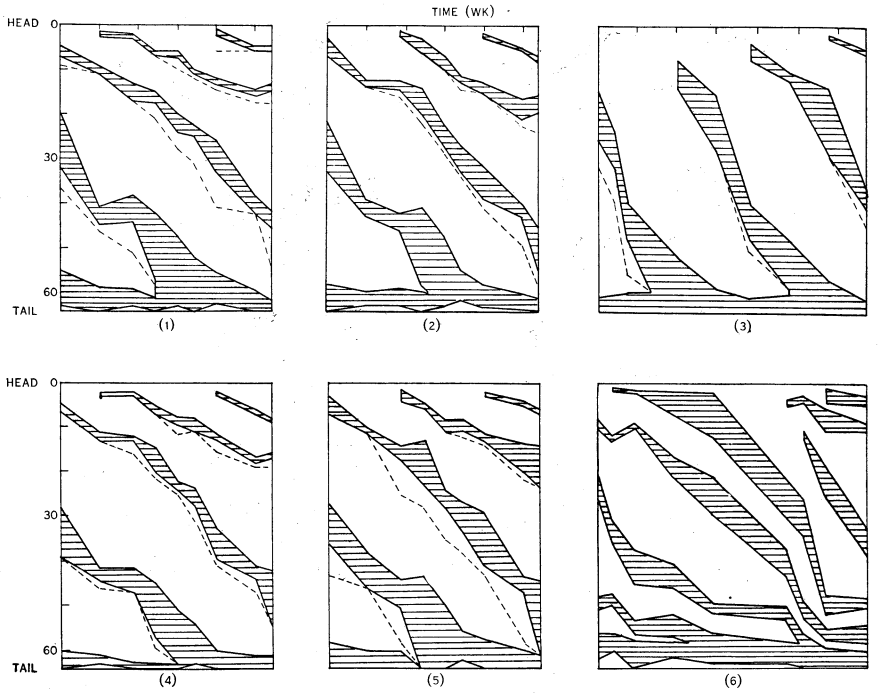

Fig. 3.—The positions of hair bands plotted against age, to show the pattern of movement and initiation of waves of hair growth. Each square is of a single mouse, (1) and (4), (2) and (5) are sister pairs. (1) and (2) were ovariectomized just before the beginning of observations. (3) and (6) are two unrelated, older females.

(*b*) *Similarity between Litter Mates*

The positions of hair bands in litter mates are very closely similar, which is not surprising, considering the genetic uniformity of the stock of mice used. In Figure 4 the similarity of sib pairs can be seen by comparing the graphs of hair band position. Divergence between sibs does occur, but at a very slow rate.

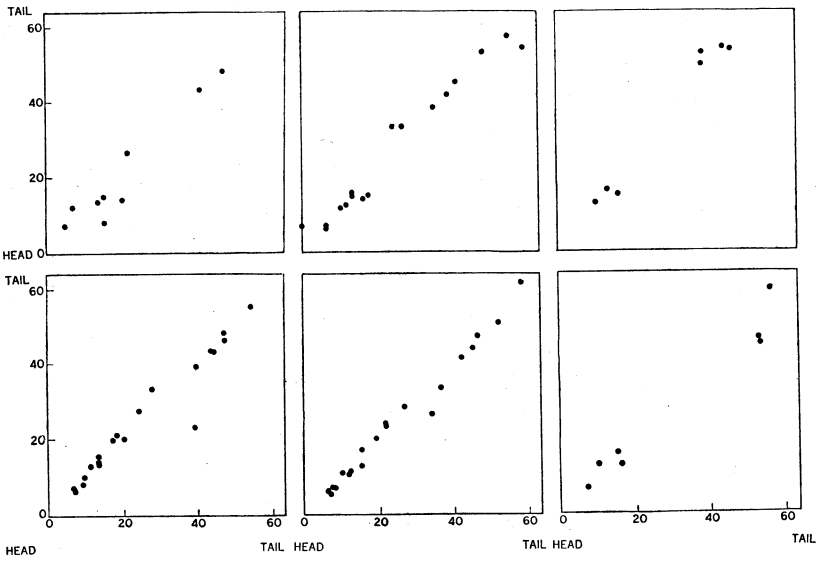

Fig. 4.—Positions of the front, or rear edges of hair bands are shown, for six pairs of sibs, plotted as a regression diagram, to illustrate the similarities between sibs.

(*c*) *Differences between Sexes*

Three types of comparisons were made to see if the hair cycle was the same in both sexes; between male and female litter mates (two pairs), castrated and entire males (one pair), ovariectomized and entire females (three pairs). No clear differences were detected between any of these pairs over the 4-5 wk during which drawings were made. A month after detailed observations were stopped the mice were examined and it was found that the ovariectomized females had wider hair bands than their controls, indicating that the rate of movement of the hair bands was increased by ovariectomy. The effect of sex, however, is quite small.

(*d*) *Effect of Pregnancy*

The first indication that pregnancy had an effect on hair growth was seen in a mouse which, although mated, did not become pregnant until it was 6-7 months old; prior to this the mouse had shown normal hair cycles. However, at 203 days it was found to be both pregnant and almost completely naked. Three days later a faint shadow appeared all over the body, except in the region where the preceding hair band had been located. Eight days later the

TABLE 2

HISTORY OF SECOND GROUP

	Days Drawings Made (day No.)																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
A								H											F			L				N			
B	L							H											F					L				N	
C	L															R	R	R											

F = fading of hair growth. H = reappearance of hair all over body. L = litter. N = completely naked. R = reappearance of hair growth as "reversed" band.

TABLE 3

HISTORY OF THE THIRD GROUP

	Days Drawings Made (day No.)																																		
Mouse	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
A	V.P.															F				L*				N									H						
B	V.P.															F					L*				N			H										
C	V.P.																	F			L				N													
D†	V.P.																																					

F = fading of hair cover. H = reappearance of hair all over the back. L = litter. N = completely naked. V.P. = vaginal plug. * = Killed. †No litter. Normal hair cycles.

whole body, except for the same narrow band, was covered with hair. On the ninth day the hair started to thin out, and 15 days after the appearance of the shadow a litter was born, and the mouse was again almost completely naked. This pattern of hair growth was so different from all our other observations that three groups of mice were observed to see if the aberrant pattern was correlated with pregnancy.

(i) *First Group.*—Drawings were made of six female litter mates; one was mated, the others being left unmated as controls. The controls showed normal hair cycles. Observations were not begun until some time after the female had been mated, and by the beginning of observations, she already had a litter. She differed strikingly from the other five mice, being naked except for a very narrow strip of hair. About 12 days after the birth of her next litter, a coat of dense, short hair appeared all over the back, except in the place previously occupied by the last hair band, which had now disappeared completely.

(ii) *Second Group.*—Three female litter mates were all mated, and had had 2-3 litters before detailed observations were begun. Their history is shown in Table 2.

Two (*A, B*) showed hair growth spreading all over the body 14 and 17 days before parturition, with subsequent fading until they became completely naked four days after parturition. The other mouse (*C*) was suckling a litter at the beginning of observations. This mouse was naked, and remained naked for 15-17 days after the birth of her litter. The animal then developed rather rudimentary bands, in which the new short hair appeared on the rear (cranial) edge of the band, instead of on the front (caudal) edge, as is usual. This phenomenon of "reversed" hair growth has been seen in other pregnant mice, and it will be studied in more detail.

(iii) *Third Group.*—Four virgin females (*A, B, C, D*) from different litters, and of different ages, were all mated to a single male, and then examined for vaginal plugs. All four showed plugs, but one (*D*) did not become pregnant. Their history is shown in Table 3. In the three pregnant females, the hair bands began to disappear 4, 5, and 3 days before the birth of the litter, and by 4 days after parturition they were completely naked. The fourth mouse (*D*) did not differ from other non-mated females in its pattern of hair growth. The females were separated from the male immediately after vaginal plugs were observed, and two of the litters (*A, B*) were killed shortly after birth. Thirteen and 7 days after parturition these two females showed hair growth spreading all over the back, except in one small region on one mouse. This was located where the previous hair band had disappeared. The skin was paper thin and transparent during the last days of pregnancy. A few days after parturition it became velvet-like, thick, and opaque, except in the region mentioned above. The skin of this region remained thin, and consequently was reduced to a deep furrow. The third female *C*, whose litter was left alive, remained naked until the end of observations; 26 days after parturition. These four mice are illustrated in Figure 5.

We can conclude that pregnancy has a distinct effect on hair growth. Summarizing, the hair bands become stationary as pregnancy progresses, and they begin to disappear approximately 5 days before the birth of a litter, resulting in the mouse becoming completely naked about 5 days after parturition. The renewal of hair growth after parturition followed one of two patterns:

(1) A new coat was formed simultaneously all over the body, except in regions which had been occupied by hair bands at the onset of the "pregnancy" effect.

(2) Approximately 2 wk after parturition rather rudimentary bands of hair were formed, which remained stationary and then disappeared. As noted above, the hair within these bands appeared to be initiated in a reverse order to normal (cranial to caudal instead of caudal to cranial).

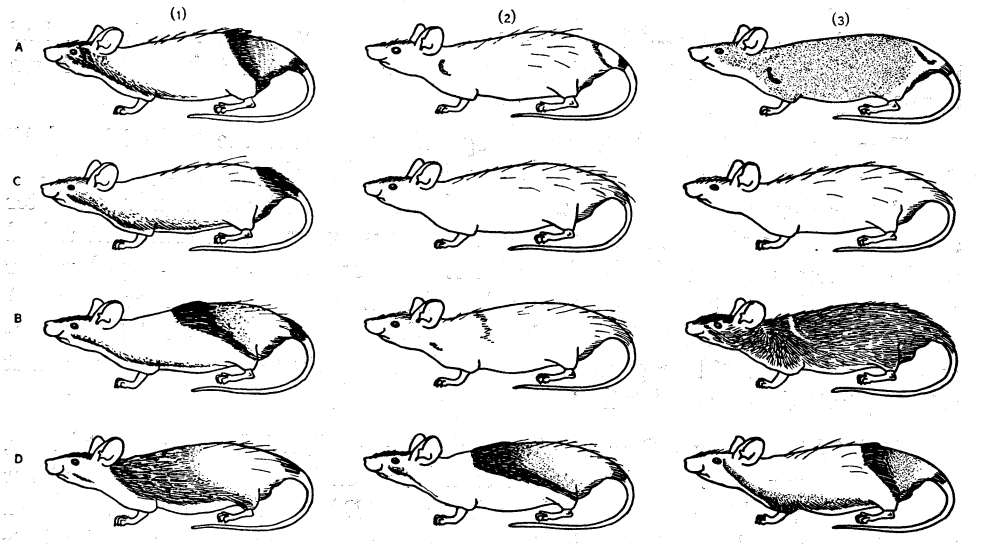

Fig. 5.—Effect of pregnancy on the third group (see text). The first column shows the four females before mating. The second column shows the mice 24-28 days later. The third column shows the mice 6-10 days later, to illustrate the resumption of hair growth.

V. Discussion

The density of hair cover in a mouse depends on (*a*) length of individual hairs, (*b*) diameter of the individual hairs, (*c*) number of hair follicles, (*d*) retention of completed hairs by the follicles, (*e*) frequency of waves of hair growth, and (*f*) the speed with which a wave of hair growth moves over the body. The Naked gene by stopping (*d*) allows us to observe (*e*) and (*f*) with comparative ease. We have shown that our methods allow fairly accurate estimates of these factors, and provided the statistical problems are solved, it is possible, using Naked mice, accurately to evaluate the effects of hormones etc. on the various determinants.

The cycling of hair growth is an intriguing example of a "biological" clock, and it is as such that we are primarily interested in its study. Durward and Rudall (1949) have shown, in the rat, using *iso*alloxazine to locate the waves of hair growth, that the timing of these cycles is regionally autonomous, and it is very probable that this regional autonomy also occurs in mice. Transplantation experiments are in progress to test this. The effects of ovariectomy and pregnancy indicate that some endocrine system has a regulating power over the timing of hair growth cycles.

Emmens (1942) in the rat, showed that "administration of androgens did not affect hair growth but oestrogens slowed it, more strongly in males than in females, in which more endogenous oestrogen is presumably already at work. Oestrogens also tend to cause bare patches to persist on the shaved area." These results suggest an explanation of the effects of ovariectomy and pregnancy. In our ovariectomized mice, the speed of movement of the hair band is faster than in the control females, and this is in accordance with Emmens' findings, since ovariectomized females will have a lower oestrogen level. In pregnant females the oestrogen level rises sharply towards the end of pregnancy and is paralleled by an inhibition of hair growth at about the same time. It is, however, difficult to reconcile the two types of resumption of hair growth with a simple removal of the inhibition.

VI. References

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Durward, A., and Rudall, K. (1949).—*J. Anat.* **83**: 325-35.
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Appendix I

Statistical Treatment

By Helen Newton Turner

To check the accuracy of plotting hair bands on standardized mouse silhouettes (left side and top), each of 10 mice was plotted three times, each diagram then being measured once. The number of bands present and their location on the mouse were studied on these diagrams, and because it seemed reasonable to expect that the accuracy of plotting might vary according to the location of the band, five arbitrary positions were defined:

(I) In front of ears;
(II) Commencing immediately behind ears;
(III) Commencing near centre of body;
(IV) Commencing above hind legs;
(V) Commencing at root of tail.

For the side view, measurements were made from a point near the tip of the nose, and on a line 1 cm from the back. For the top, measurements com-

menced just behind the ears; consequently bands at position I are missing from the top analyses. For each position, two or three measurements were available. The mean of these was taken as indicating the *location* of the band, and differences between consecutive pairs of measurements gave the *widths* of the two parts of the band, if present—the "full" band (A), and the "shadow" band (B). If in one drawing of a set of three, one band was omitted, as occasionally happened, the location measurement was omitted, but the width was entered as zero.

Standard deviations between repeated observations on the same mouse at the same position were then calculated, and are given in Table 4. The corresponding mean values are shown in Table 5.

TABLE 4

STANDARD DEVIATIONS OF BAND MEASUREMENTS (UNITS OF 1/16 IN.)

Position	Side			Top		
	Location	Width		Location	Width	
		A	*B*		*A*	*B*
I	1·33	2·05	—	—	—	—
	(10)*	(12)				
II	1·85	1·31	1·50	1·04	1·90	—
	(17)	(18)	(16)	(5)	(6)	
III	2·14	0·80	1·21	1·94	0·76	1·10
	(10)	(10)	(8)	(10)	(10)	(8)
IV	1·14	1·34	—	1·34	1·21	—
	(14)	(14)		(14)	(14)	
V	0·78	1·20	—	0·58	0·92	—
	(18)	(20)		(15)	(16)	

* Numbers in brackets indicate the numbers of degrees of freedom on which each standard deviation was based.

The mean values for the locations of positions I-V on the side were 5.1, 15.4, 32.7, 52.3, and 60.8 units respectively. With the order of accuracy shown in Table 4, there would be clear discrimination even for positions intermediate between those chosen. The error of estimation of band width on position I is high, owing to the fact that this position was missed once in three drawings on each of two mice, although in one the width was considerable. For position II on the side, the standard deviation is also high in relation to the mean. The high value arises mainly from the contribution from one mouse, where the band on position II was missed. For the other positions, the standard deviations were sufficiently low to render the method valuable for the study of time changes in the bands. On the evidence of the present data, the side view gives

more information than the top view; the standard deviations of measurements for top and side are similar, while not all bands present on the side are present on top. Table 5, for example, shows that only three of the 10 mice observed had bands in position II on the top view, whereas a band in this position on the side was present in nine out of the 10 mice. If the fact that a band fails to continue from the side over the top is important in any set of observations, then both side and top views should be included. Bands in positions III, IV, and V in the present series all continued across the top, so that a side view plus a top view from the shoulders forward should give a complete picture.

TABLE 5

MEAN VALUES OF BAND MEASUREMENTS (UNITS OF 1/16 IN.)

Position	Side			Top		
	Location	Width		Location	Width	
		A	*B*		*A*	*B*
I	5·1 (6)*	3·0 (6)	—	—	—	—
II	15·4† (9)	1·5 (9)	3·7 (8)	4·4 (3)	2·9 (3)	—
III	32·7 (5)	3·8 (5)	4·5 (4)	18·0 (5)	3·9 (5)	3·3 (4)
IV	52·3 (7)	5·2 (7)	—	37·5 (7)	5·2 (7)	—
V	60·8 (10)	3·7 (10)	—	44·9 (8)	4·2 (8)	—

* Numbers in brackets indicate the numbers of mice on which each mean is based.

† The scale for top measurements began behind the ears, that for the side near the nose; hence the differences in the location measurements between side and top.

Before concluding that the left-hand side view, as drawn, gives a satisfactory picture of the location and width of the bands, it was necessary to check the symmetry of the two sides. Ten further mice were included in this test, one drawing being made of each side. For positions II-V the between-side standard deviations were of the same order as those given in Table 4 for differences between repeated measurements on the same side. In one of the 10 mice only was there any serious discrepancy between the two sides; a "shadow" band (*B*) in position II was here shown as running over the back for the left-hand side drawing, but not for the right-hand side. A wide band extending over both positions III and IV in the same mouse was so poorly defined as to give bad agreement between the sides.

For position I, agreement between the two sides was poor. Only in four of the 10 mice were there bands of the same pattern and in the same location

on both sides of the head in front of the ears. In three mice, bands were present on both sides but were different in pattern, while in the other three a band was shown on one side but not on the other.

The main use of the technique will be for studies in time changes of bands on the same mouse. The degree of asymmetry observed between left and right sides is not sufficiently great to invalidate the technique if used for such a purpose, but is sufficient to make it necessary for the observer to decide on one side and record it constantly.

The question of the optimum time interval between drawings for a study of time changes in the bands is now being investigated.