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Close linkage between genes which cause hairlessness in the mouse

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1. INTRODUCTION

New genes which affect the mouse coat are of interest, (*a*) because they expand the chromosome map and reveal new linkage relationships, and (*b*) because they may render additional knowledge on coat development. The two genes patch (*Ph*) and dominant spotting (W^v) are very closely linked and have similar phenotypic effects (Grüneberg & Truslove, 1960). Genes such as crinkled (Falconer, Fraser & King, 1951), hairless (Fraser, 1946) and naked (David, 1932) modify coat development so that the differentiation of certain types of follicles is suppressed or the hairs are abnormally keratinized.

A new gene which causes hairlessness and which has distinctive genetical properties is described in this paper. The gene is semi-dominant in a characteristic way. Homozygotes never grow a first coat and the adults grow a few short hairs only. Heterozygotes, however, grow a full coat which is greasy and they resemble rough (*ro*) mice. Evidence will be given to show that the new gene is closely linked to another gene which causes hairlessness but no satisfactory explanation for this phenomenon can be offered. Three-point linkage tests designed to determine the location of the new gene proved very troublesome to analyse but nevertheless they enabled some conclusions to be established. The skin histology was examined in order to see whether the two adjacent loci mimic one another in the mechanisms by which the hairlessness arises or whether they cause hairlessness by different development processes.

2. GENETICS

(i) *Origin*

The hairless mutant was found by Dr K. E. Kirkham and Dr E. T. Bell, M.R.C. Clinical Endocrinology Research Unit, Edinburgh, and it was given to the Genetics Department for investigation. The hairless animal was outcrossed to

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normal mice and all the F_1 animals had a fully grown coat but they were noticeably greasy. Intercrossing the F_1 mice gave three phenotypic classes: hairless, greasy and normal mice. It was supposed that the gene is semi-dominant, this supposition being later confirmed by the segregation data. The mutant is called shaven, symbol *Sha*. For convenience, homozygous shaven mice will be referred to as *Sha Sha*, heterozygotes as *Sha*+, and normals as ++.

(ii) *Segregation data*

When the original *Sha Sha* mouse was outcrossed to an unrelated laboratory strain, a stock heterozygous for the shaven gene was established and the gene was kept segregating by means of intercross matings. The *Sha Sha* mice can be classified at birth by the paucity and waviness of the vibrissae but *Sha*+ mice cannot be recognized until 13–16 days of age when the greasy coat becomes noticeable. The segregation of shaven from different types of matings is given in Table 1. The

Table 1. *Segregation of* Sha

Type of mating	No. of matings	Phenotypes of progeny			Total	χ^2_1	P
		Sha Sha	*Sha*+	++			
Sha+ × *Sha*+	33	230	482	214	926	2·11	0·15
Sha Sha × *Sha*+	62	821	822	—	1653	0·05	0·80
Sha+ × ++	46	—	558	593	1151	1·06	0·30

progeny of intercross matings *Sha*+ × *Sha*+ were always classifiable into the three phenotypes, hairless, greasy and normal. The results of the intercross matings are in agreement with expectation and verify that shaven is a semi-dominant gene. Backcross data from *Sha Sha* × *Sha*+ matings are close to the expected 1:1 ratio. In the other backcross, *Sha*+ × ++, there is an excess of ++ mice because of normal overlapping. When *Sha*+ mice were outcrossed to unrelated ++ mice the greasiness of the coat was diminished and some *Sha*+ animals appeared phenotypically normal.

(iii) *Linkage tests*

(*a*) *Two-point tests*

In order to identify its linkage group the shaven gene was tested against the five multiple linkage testing stocks described by Carter & Falconer (1951), which had been slightly modified, and two additional ones. The stocks were as follows:

Stock No.	Markers present
I	*Va Sd Ra*
II	*Wh T N*
III	*p se fz v*
IV	*s wa–2 ln b a*
V	*ru je f* c^e
VI	*Re* W^v
VII	*Os So Sl*

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The tests required two generations in all cases. The first generation consisted of outcrossing a shaven homozygote to an animal from each stock. The multiple heterozygote, whose gametic constitution was tested for evidence of linkage, was recovered from the F_1 generation. The second generation was obtained by backcrossing the multiple heterozygote of the F_1 to the multiple recessive. The offspring of this backcross were classified and the deviation of shaven from independent segregation with all markers was estimated.

It was soon evident that shaven was either allelic or closely linked to naked (*N*) in linkage group VI. Difficulty in recognizing possible cross-overs in the backcross *Sha* +/+ *N* × + +/+ + was foreseen because the greasy coat of shaven heterozygotes cannot be easily recognized in the presence of naked. Hence, all semi-hairless

Table 2. *Results of two-point tests between* Sha *and* N *from matings of* Sha +/+ N × + +/+ N. (*All semi-hairless progeny were tested for the presence of* N)

Genotypes and numbers of progeny				
Sha+	+ *N*	*Sha N*	++	Total
40	40	1	0	81

progeny were tested for the presence of shaven by mating with *Sha* +/*Sha* + animals. One semi-hairless mouse so tested produced seventeen offspring, seven of which were completely hairless. This mouse was thus proved to carry the shaven gene and its genotype was *Sha N*/++. The results of the two-point test are given in Table 2.

Two further types of backcross were also made but in these the shaven gene was conveniently regarded as recessive. The backcrosses were:

Sha +/+ *N* × *Sha* +/*Sha* +
Sha N/+ + × *Sha* +/*Sha* +

Again it was not possible to distinguish between shaven naked heterozygotes and non-shaven naked heterozygotes so that only three progeny groups were recognizable. The results are given in Table 3. Only non-naked segregants can be used to estimate recombination frequency. There were 5+3=8 recombinants out of 543+3+480+5=1031 mice. Combining these data with Table 2 gives 8+1=9

Table 3. *Results of two-point tests between* Sha *and* N *from backcross matings.* (*The* Sha + *and* Sha N *progeny were not test-mated and could not be distinguished phenotypically*)

Type of mating	Phenotypes and number of progeny		
	+ *N*	*Sha* + *Sha N*	++
Sha +/+ *N* × *Sha* +/*Sha* +	543	460	5
Sha N/+ + × *Sha* +/*Sha* +	3	402	480

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recombinants out of a total of $1031 + 81 = 1112$ mice, giving a percentage recombination of 0·8%. The 5% confidence limits are 0·37% and 1·54% (Fisher & Yates, 1948, Table VIII). There was no sex difference in the frequency of recombination.

(b) Three-point tests

For the purpose of determining linear order three-point backcross tests were made with naked and caracul, shaven being treated as a dominant. A total of 2030 progeny were bred, of which 1080 were fully classified. The only recombinants recovered were exchanges of naked, of which there were 12. Consequently, *N* cannot lie between *Sha* and *Ca* and the order must be either *N–Sha–Ca* or *N–Ca–Sha*. Since no recombinants between *Sha* and *Ca* were obtained it is not possible to discriminate between these two orders.

The classification of the phenotypes segregating in the three-point tests proved to be very difficult. As mentioned before, shaven heterozygotes could not be recognized in the presence of naked and, with shaven segregating, the classification of caracul was not thought to be reliable. Consequently, the naked phenotypes were not fully classified and only non-naked segregants can be used to estimate the recombination frequencies. Among the non-naked phenotypes there was some doubt whether *Sha Ca* could be distinguished from + *Ca*, and it was found that *Sha*+ animals were occasionally misclassified as ++. (Fully wild-type animals would have been recombinants and four animals so classified were proven by test to be shaven.) In view of these difficulties two types of three-point tests were made so that the different types of exchange would be represented by different phenotypes. The two types of test, one of which included belted (*bt*) in some matings, were as follows:

A. $\dfrac{N\ Sha\ +}{+\ +\ Ca}$ $\qquad$ $\dfrac{+\ +\ +}{+\ +\ +}$

B. $\dfrac{+\ Sha\ +\ +}{N\ +\ Ca\ bt}$ $\qquad$ $\dfrac{+\ +\ +\ bt}{+\ +\ +\ bt}$

The recognizable non-naked phenotypes and the exchanges they represent are shown in Table 4, with the numbers observed. The two types of mating were con-

Table 4. *Reliably classified phenotypes from three-point tests. (The two recombinants from B-matings were both proved* + + Ca *by test matings)*

Type of mating	Sex of segregating parent		Exchange			
			None	*N*	*Sha*	*Ca*
A		(Naked)	+ + *Ca*	+ *Sha* +	+ *Sha Ca*	+ + +
	♀	(371)	395	7	0	0
	♂	(357)	407	3	0	0
B		(Naked)	+ *Sha* +	+ + *Ca*	+ + +	+ *Sha Ca*
	♀	(72)	86	0	0	0
	♂	(150)	180	2	0	0

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sistent in giving only naked exchanges, so that there cannot be any error from misclassification in establishing the two possible orders.

The recombination frequencies between *N* and *Sha* or *Ca*, from the two types of mating combined, are 1·4% in females and 0·8% in males. The sexes are not significantly different and the joint estimate is 1·1%, with 5% confidence limits of 0·6% and 1·9%. Thus, the recombination between *N* and *Sha* in the three-point tests agrees with the value found in the two-point tests. The upper confidence limit for recombination between *Sha* and *Ca*, with no recombination in 1080 progeny, is 0·34%. Thus, *Sha* must lie closer to *Ca* than to *N*.

Table 5. *Segregation of* Ca *and* bt *from matings of* Ca bt/+ + × + bt/+ bt. (*These data are included with those under Type B matings in Table* 4)

Sex of segregating parent	Progeny			
	Ca bt	*Ca* +	+ *bt*	+ +
♀	34	5	4	48
♂	125	19	14	150

The inclusion of belted in some of the matings does not help to establish the position of *Sha* in relation to *N* and *Ca*, but it is useful for comparison of the recombination frequencies with those obtained from previous data. Previous estimates of recombination in the *N–Ca* and the *Ca–bt* segments have shown higher values in males than in females (Murray & Snell, 1945; Mallyon, 1951). The present data are given in Table 5, and the recombination frequencies, with those of Mallyon for comparison, are given in Table 6. The estimates obtained from the present data may perhaps be in error through lack of phase-balance, though the sex difference observed is in fact more consistent with that generally found in other mouse linkage groups.

Table 6. *Recombination frequencies* (%), *with* 5% *confidence limits calculated from Fisher and Yates* (6*th ed.*, 1963)

Segment	Sex	Mallyon (1951)	Present data*
N–Ca	♀	1·5 (0·6–3·3)	1·4 (0·6–2·9)
	♂	2·7 (1·6–4·3)	0·8 (0·3–2·0)
Ca–bt	♀	3·8 (2·1–6·2)	9·9 (4·6–17·9)
	♂	11·1 (8·7–13·8)	10·7 (7·4–14·8)

* *N–Ca* recombination from data in Table 4. *Ca–bt* recombination from data in Table 5.

3. MORPHOLOGY

(i) Sha Sha *mice*

(*a*) *External appearance*

The criteria for the classification of *Sha Sha* mice are the paucity and waviness of the vibrissae at birth and the non-appearance of the first coat during the preweaning period. Although there is no delay in the thickening or pigmentation of

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the skin up to 14 days of age, only a few short hairs are noticeable on the head and feet, the main body area remaining hairless. During the third week skin thickness is reduced considerably and this reduction corresponds to the catagen stage of normal mouse skin. Hair growth on the main body area of *Sha Sha* mice is first noticeable between 28 and 34 days of age. The skin thickens again and sparse fuzzy hairs about $\frac{1}{8}$ in. in length appear on the head and anterior dorsum. As the fibres appear on the posterior regions those of the anterior regions are shed. All *Sha Sha* adults undergo cyclic regeneration and loss of short fuzzy hair and short wavy vibrissae. The toe-nails are generally normal in shape and remain so with increasing age but in a few cases malformations were observed.

(*b*) *Mortality and fertility*

The mortality of *Sha Sha* mice does not exceed that of normals except during the suckling period when *Sha Sha* animals have difficulty in competing with fully coated sibs for food, causing emaciation in some cases. The adults are fully fertile and the mothering ability of *Sha Sha* females is satisfactory.

(*c*) *Body growth*

Body growth of *Sha Sha* mice is reduced in comparison with normal littermates. Table 7 gives the mean weights of *Sha Sha* and normal mice at birth, 3 weeks and

Table 7. *The mean weights and standard errors of* Sha Sha *and normal mice at birth, 3 weeks and 6 weeks of age*

		Sha Sha		Normal	
		♀♀	♂♂	♀♀	♂♂
Birth:	No. of mice	73	85	92	77
	Mean ± s.e. (g)	1·6 ± 0·01	1·6 ± 0·01	1·6 ± 0·04	1·6 ± 0·05
3 weeks:	No. of mice	36	54	74	71
	Mean ± s.e.	6·1 ± 0·42	6·3 ± 0·36	10·9 ± 0·57	10·9 ± 0·62
6 weeks:	No. of mice	21	35	41	37
	Mean ± s.e.	18·7 ± 0·69	18·8 ± 0·92	24·0 ± 0·46	27·1 ± 0·82

6 weeks of age. There was no difference between the birthweights of *Sha Sha* mice and normals but at 3 weeks normals were nearly double the weight of *Sha Sha* animals. At 6 weeks the weight differences were not as great but they were nevertheless significant at the 1% level.

(*d*) *Hair morphology*

The short hairs of *Sha Sha* adult mice vary from straight awl-like fibres to twisted types resembling zig-zags. The internal morphology of normal and *Sha Sha* fibres, as observed in whole fibre mounts, is illustrated in Text-fig. 1. Fibre diameter of *Sha Sha* hairs varies along the length of the fibre and the cortex is thin and frequently non-existent. In the medulla the ladder-like arrangement of septa and air-cells, as seen in normal hairs, is absent. The septa appear very thick and the air spaces are reduced in size. Most of the air spaces are clear and glassy when viewed by reflected light. This defect will be referred to again later.

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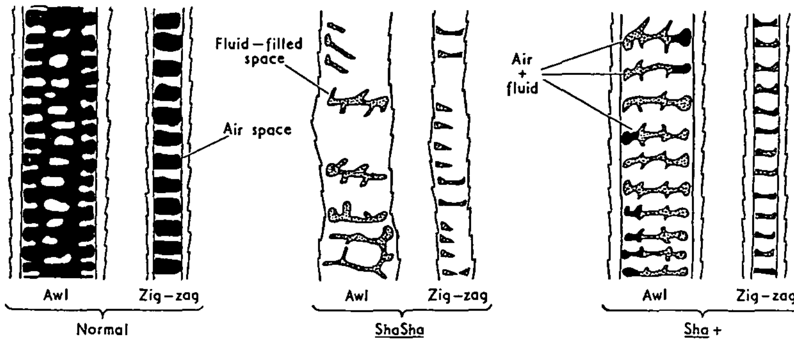

Text-fig. 1. Camera lucida drawings of hairs from normal, *Sha Sha* and *Sha*+ mice to illustrate the internal morphology of the different types. The air spaces are black and the fluid-filled spaces are stippled.

(ii) Sha+ *mice*

(*a*) *External appearance*

Heterozygous shaven mice are entirely different in appearance from shaven homozygotes. There is no delay in hair eruption and at 2 weeks of age the coat is fully established but it is greasy. Hair-loss never occurs. The hairs tend to stick together in bundles so that the coat looks ruffled. The greasiness of the coat is variable. It was found that outcrossing *Sha*+ mice to unrelated strains, e.g. the linkage stocks, reduced the expression of the shaven gene so that coat greasiness was diminished. In these cases classification was sometimes difficult. Most *Sha*+ animals exhibit variable degrees of vibrissal waviness.

(*b*) *Hair morphology*

Although the hairs of *Sha*+ mice are of normal dimensions the internal morphology is abnormal. Diagrams of *Sha*+ hairs are shown in Text-fig. 1. The air spaces in the medulla are reduced in size and they have a glassy appearance. The greasy coat suggested that the glassy spaces in the medullae might contain a fatty fluid similar to that found in the hairs of rough mice by Falconer & Snell (1952). The suggestion was supported by observations when hair samples from *Sha*+ and normal mice were stained with Herxheimer's solution (Sudan *III* and *IV* in equal parts of 70% alcohol and acetone) and washed briefly in 70% alcohol. The medullary spaces of *Sha*+ hairs were sudanophilic where normal hairs reacted negatively. No sudanophilic material was observed on the external surfaces of either *Sha*+ or normal hairs. Thus, the greasiness of the *Sha*+ coat can be explained by the presence of a sudanophilic fluid in the hair medullae.

Attention was then focused on the short hairs of *Sha Sha* adult mice to see if these also were sudanophilic. A Sudan positive reaction in the hair medullae was obtained after staining with Herxheimer's solution. Thus, microscopic examination revealed one defect common to both *Sha Sha* and *Sha*+ phenotypes, namely, the occurrence of a sudanophilic fluid in the fibres.

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4. HISTOLOGY

(i) Sha Sha *mice*

The skin histology is similar to that of naked mice. Skin samples were taken from normal and *Sha Sha* mice at birth, 3, 6 and 9 days of age. Bouin-fixed, paraffin-wax sections were stained with haematoxylin and eosin. Histological abnormalities first become visible in *Sha Sha* skin at about 3 days of age. The follicles are initiated in the normal manner and grow downwards through the dermis but as the hair tips reach the dermal-epidermal junction they bend and coil and fail to penetrate the epidermis. Sections of 6-day-old normal and *Sha Sha* mice are shown in Plate I*a* and *b*. The hairs of normal skin have erupted through the epidermis but the hairs of *Sha Sha* mice are thin and they become entangled in the cells of the upper dermis. The *Sha Sha* hairs show poor affinity for picric acid, indicating that they are abnormally keratinized. The cortex is very thin and the hairs consist mostly of medullary substance. In contrast to the hairs the stratum corneum appears to be normally keratinized.

The poor affinity for picric acid shown by *Sha Sha* fibres can be traced, by using the high power of the microscope, to a deficiency of cortical elements in the mid-follicle region. In this region of the normal follicle the cells migrating upwards from the bulb undergo dehydration and fibrillation. The cells attain a high degree of elongation and become rigidly fused together to form the hair. The nuclei of these cells are highly condensed elongated rods. In *Sha Sha* follicles the cortex is thin and lacks fibrils in the mid-follicle region. The cell nuclei are vesicular and hydrated instead of being rod-like and condensed. These observations explain the poor rigidity of the fibre in the upper follicle region. No histological abnormality was apparent in the follicle bulb. The sebaceous glands, outer root sheath and dermal papilla are fully differentiated. Thus, hair development in *Sha Sha* mice proceeds normally through the process of follicle initiation and differentiation up to a certain stage after which the differentiation of the hair fails to reach completion.

The deficiency of fibrils and rod-like nuclei in the mid-follicle region was found to be associated with a deficiency of sulphydryl groups (—SH). In normal skin these are concentrated in the mid-follicle region and are oxidized to form the disulphide

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EXPLANATION OF PLATE

a. Normal mouse skin at 6 days showing follicles with fully keratinized hairs. H. and E. stain, ×85.

b. *Sha Sha* skin at 6 days showing follicles with poorly keratinized hairs. H. and E. stain, ×85.

c. The mid-region of a normal hair follicle showing intense sulphydryl reaction as the hair cells are being keratinized. At the distal end of this region the fully keratinized hair is unreactive because the sulphydryl groups are oxidized to disulphide bonds. Bennett's sulphydryl reagent stain, ×450.

d. The mid-follicle region of *Sha Sha* skin showing weak sulphydryl reaction of two hairs undergoing keratinization. The hairs consist mostly of medullary substance. Bennett's sulphydryl reagent stain, ×450.

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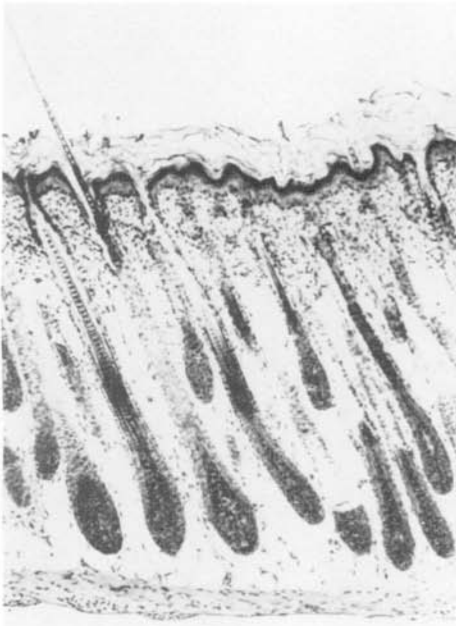

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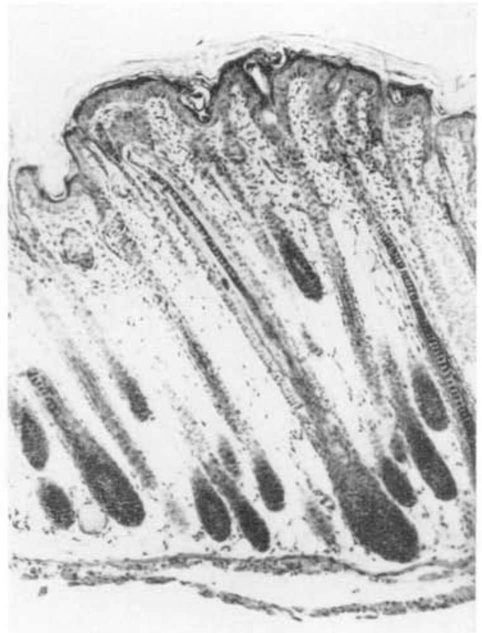

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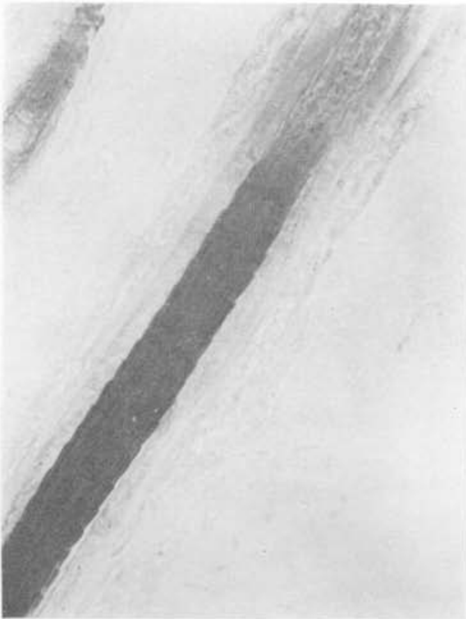

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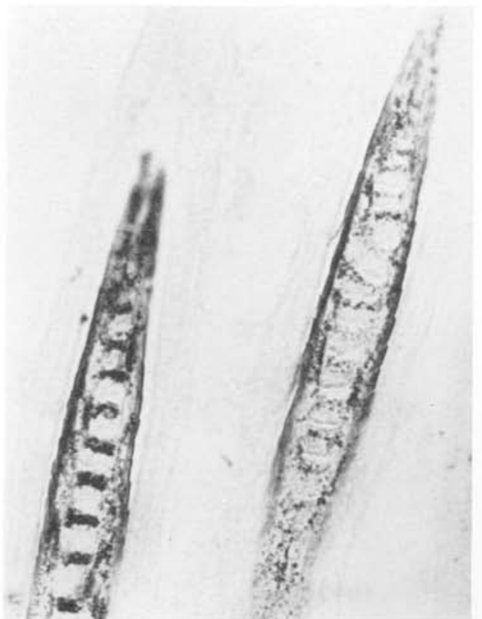

d

S. P. FLANAGAN AND J. H. ISAACSON

(*Facing p.* 106)

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bonds (—S—S—) of hair keratin. Normal and *Sha Sha* skin follicles were examined for the distribution of sulphydryl groups by the method of Bennett (1951) and both types of follicles are shown in Plate I *c* and *d*. Normal follicles reacted intensely with sulphydryl reagent in the mid-follicle region indicating localization of sulphydryl groups. At the distal end of this region the reaction ended suddenly and the fully keratinized hair in the upper follicle was unreactive, the sulphydryl groups having been oxidized to disulphide bonds. There was no intense sulphydryl reaction in the follicles of *Sha Sha* mice. Many follicles reacted weakly and some reacted negatively. The deficiency of sulphydryl reactive fibrils was very noticeable.

(ii) Sha+ *mice*

The presence of a sudanophilic fluid in the hairs of *Sha*+ mice led to an examination of the skin histology to see if the sebaceous glands are morphologically abnormal or if lipid substances are deposited in the follicles. Formalin-fixed paraffin-wax skin sections from 14-, 17- and 20-day-old normal and *Sha*+ mice were stained with haematoxylin and eosin; frozen sections were stained with Herxheimer's solution and Sudan Black. The results were negative. There was no difference between normal and *Sha*+ mice in the size and shape of the sebaceous glands and no excess lipid material was found either in the sebaceous glands or in the follicles of *Sha*+ mice. Thus, the origin of the sudanophilic fluid in the hairs of the coat was not traced.

5. DISCUSSION

Homozygous shaven mice are phenotypically similar to homozygous naked mice and the two loci *Sha* and *N* are closely linked. This is the third example in the mouse of a phenomenon in which adjacent loci have similar phenotypic effects. The other cases are: (*a*) patch *Ph* and dominant spotting with macrocytic anæmia W, W^v in linkage group III; (*b*) the Brachyury (*T*), fused (*Fu*) and anury (*t*) series of mutations in linkage group *IX* (Dunn & Caspari, 1942). The questions which arise again are whether shaven and naked are mutations at repeated loci similar to the doublets in *Drosophila*, whether they are different mutational sites within one gene, or whether the close proximity of the *Sha* and *N* is merely a matter of chance.

To prove that the shaven and naked mutations represent the replication of a chromosomal segment requires both genetical and cytological evidence. The genetical evidence is consistent with the repeat hypothesis. The two genes are similar in function and they are separable by crossing-over. Mutations at repeated loci are expected to act in similar ways during development and this expectation is realized in the case of *Sha Sha* and *N N* mice. Cytological evidence for adjacent gene repetitions in *Drosophila* is plentiful and the doublet structures of salivary gland chromosomes have been correlated with similarity of effect on the phenotype. Similar evidence is not available in the mouse. The second possibility, that *Sha* and *N* form a complex gene with different mutational sites, must be considered in the context of traditional views regarding gene structure in the mouse. The fact

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that *Sha* and *N* are readily separable by crossing-over proves that they are non-allelic. Hence, it is more appropriate to regard *Sha* and *N* as two distinct loci which are closely linked. As regards the third possibility, the increasing frequency of such cases as *Sha* and *N*, *Ph* and W^v etc., suggest that close linkage and similarity of effect are not a matter of chance alone. It is likely that further examples in the mouse will be demonstrated as more loci are discovered.

The absence of the coat in shaven homozygotes is caused by weak keratinization of the hairs. As the young hairs are about to emerge from the follicles they bend at the dermal-epidermal junction. The hairs are thin and lack a rigid cortex. These defects can be traced to the mid-follicle region which is deficient in fibrils and rod-like nuclei. The cell layers in the lower follicle regions are morphologically normal but as the cells migrate upwards from the follicle bulb they fail to differentiate into fully keratinized hair. In seeking an underlying physiological explanation for the incomplete hair differentiation, the sulphydryl content of the follicles was examined and found to be abnormally low. It appears that the shaven gene interferes with the synthesis of adequate amounts of sulphydryl-containing compounds. A deficiency of sulphydryl groups was also noted in the hair follicles of nude (*nu*) mice (Flanagan, 1966). In studying the sulphur metabolism of skin follicles Bern, Harkness & Blair (1955) and Ryder (1958) injected mice with cystine labelled with S-35 and noted the sulphur concentrations at different levels of the follicles. Radioisotope techniques were not used in studying shaven mice but such procedures would very likely render useful information on the mobilization of sulphur in the follicles.

The greasiness of the coat of shaven heterozygotes was found to be associated with the presence of sudanophilic fluid in the hair medullae. The skin histology showed no morphological abnormalities in the follicles or sebaceous glands which might account for the fluid in the hairs. Two other conditions of coat greasiness have previously been reported, namely, rough (*ro*) in the mouse (Falconer & Snell, 1952) and 'sticky' in the guinea pig (Herbertson, Skinner & Tatchell, 1959). The skin histology of 'sticky' animals was found to be normal but the hairs were Sudan positive and the greasiness of the coat was attributed to abnormal sebaceous secretions. Although the origin of the sudanophilic substance in *Sha*+ hairs was not traced it is probably derived from the follicle bulb rather than from the sebaceous glands. The glands open into the hair canal at the distal end of the follicle and the fatty substances are applied to the outer surface of the hair. Any fatty substance present in the medulla must ultimately be derived either from the plasma of the follicle bulb or by penetration of the gland secretions through the fully keratinized hair cortex. But since the cortex is a rigid structure it may be considered impenetrable by large fatty molecules. Thus, the possibility that the sudanophilic fluid of the hair medullae originates from the lower follicle regions seems more plausible.

Another unusual feature of the shaven gene is its dominance over the normal allele. Previously it has been found that semi-dominant genes causing hairlessness exhibit dosage effects, the lack of hair being more extreme in homozygotes than in

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heterozygotes, e.g. naked and ragged. It is difficult to explain how the shaven gene in double dose causes almost complete hairlessness while in single dose it has no effect on the growth of the coat but causes greasy hairs. It is worth noting, however, that microscopic examination of the hairs from both phenotypes showed a common defect, namely, a sudanophilic substance in the fibre medullae. Thus, the shaven gene in double dose may be considered to cause a specific physiological process, such as synthesis of inadequate amounts of keratin precursor, to exceed a certain threshold so that the hairs are weakly keratinized. In addition, sudanophilic material is deposited in the short hairs as a result of abnormal differentiation of the medullae. In the heterozygote one normal allele ensures the synthesis of hair keratin but the shaven allele may have a residual effect on the differentiation of the hair medullae so that a sudanophilic substance is deposited as a by-product. In this manner the *Sha*+ and *Sha Sha* phenotypes may be physiologically related.

SUMMARY

1. A new semi-dominant gene called shaven (*Sha*) causes hairlessness in the mouse and it is closely linked to naked. Homozygous shaven mice never grow a first coat and adults grow a few short hairs only. Shaven heterozygotes grow a full coat which is greasy.

2. The percentage recombination between shaven and naked was found to be 0·8%. Three-point tests with naked and caracul showed that the order of the three loci is either *N–Sha–Ca* or *N–Ca–Sha*.

3. The failure of homozygous shaven mice to grow a coat is due to abnormal keratinization of the hairs in the follicles. The follicles were found to be deficient in sulphydryl groups.

4. The greasiness of the *Sha*+ coat is due to the presence of a sudanophilic fluid in the hair medullae.

5. The close linkage between the two genes with similar phenotypic effects is discussed.

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