The phenogenetics of hair mutants in the house mouse: Opossum and Ragged*†

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1. INTRODUCTION

The phenogenetics of hair and skin mutants is particularly amenable for analysis because many developmental and physiological processes, as well as associated pleiotropic effects, can be observed in late-foetal, post-natal, or even in adult animals. As developmental and physiological phenomena become better known through observations of variations attributable to mutant genes and teratogens, the biochemical steps underlying these phenomena will become more accessible for study (Chase & Mann, 1960).

The hair mutants investigated in the present study are Ragged (*Ra*) and Opossum (*Op*). Ragged, a semi-dominant gene, was first described by Carter & Phillips (1954) and later by Slee (1957 *a*, *b*). Their work shows that the pelage of heterozygous Ragged mice (*Ra +*) is more sparse than that of normal (*+ +*) mice. Although adjacent follicles grow asynchronously in *Ra +* skin, interaction of *Ra +* and *N +* (Naked) shows that indistinct hair waves occur in *Ra +* mice. Heterozygous Ragged mice are usually viable at birth, but approximately 40% of homozygous Ragged (*RaRa*) mice die as embryos; many *RaRa* embryos exhibit varying grades of oedema. Adult *RaRa* mice are almost naked, and the few hairs that are present are morphologically abnormal. In both *Ra +* and *RaRa* mice, the growth of vibrissae and pelage hairs is retarded, and many of the hair follicles in *Ra +* mice and most of the hair follicles in *RaRa* mice are incompletely developed. The semi-dominant lethal Opossum (*Op*) mutant was described by Green & Mann (1961). Attempts to produce living homozygous Opossum mice were not successful. At birth many of the heterozygous Opossum mice are dead or dying. They are cyanotic and oedematous, and lack vibrissae. Non-oedematous Opossum newborn mice appear normal except for the lack of vibrissae, but many of them die before weaning. Adult Opossum mice lack a full complement of vibrissae and have a sparse coat of hair; all the under-fur and some of the overhairs are missing.

B

Linkage studies (Green & Mann, 1961) reveal that Op and a (non-agouti) are in linkage group V. Ragged is also located in linkage group V (Carter & Phillips, 1954). The recombination between a and Op is $26 \cdot 8 \pm 2 \cdot 1 \%$ and is similar to that of a and Ra ($24 \cdot 2 \pm 2 \cdot 5 \%$). Furthermore, a three-point test cross with a, pa (pallid) and Op shows that the linkage order is Op–a–pa. This linkage order is the same as that of Ra (Ra–a–pa). These results suggest that Ra and Op are alleles. However, a direct test of allelism of Op and Ra requires the recovery of the Ra–Op compound, and attempts to produce the Ra–Op compound have not been successful. As suggested by Green & Mann (1961), it is unlikely that the compound is viable because $Op+$ mice have a reduced viability, $OpOp$ mice are inviable, and $RaRa$ mice rarely survive after birth.

This investigation describes and compares the phenogenetics of the mutants Ragged and Opossum. On the basis of developmental and morphological similarities, the results support the linkage studies, which indicate that Ra and Op are alleles. Throughout this investigation Op will be designated as a superscript to Ra (Ra^{op}).

2. MATERIALS AND METHODS

Normal, $Ra^{op}+$, and $Ra+$ mice, maintained on heterozygous genetic backgrounds, were mated in all combinations. Heterozygous Ragged and $Ra^{op}+$ mice were observed from birth to 360 days of age. Records were maintained on births, preweaning mortality, and the growth of the first and subsequent hair coats in the mutant mice. At various ages hair samples from the mutant mice were plucked, dry mounted and examined.

Timed embryos from various matings were examined at various stages from 12 to 19 days *in utero*. Each male was placed in a pen with three non-lactating females at 9 p.m. The females were checked before 9 a.m. for the presence of vaginal plugs. Thus, the age of the embryos varied by not more than half a day. Females pregnant from 12 to 19 days were killed by cervical dislocation. The embryos were removed and examined under a low-power binocular microscope. Living embryos between 17 and 19 days *in utero* were classified as to sex and genotype. The age of retarded or dead embryos was estimated according to the methods outlined by Grüneberg (1943). Embryos which were badly decomposed were recorded as moles and divided into two classes; those which lacked a pupillary ring of pigment surrounding the eye were classified as having died prior to 11 days *in utero*, and those with pigment were classified as having died 11 days or later *in utero*.

Specimens of skin were removed from various areas of the body of $Ra^{op}+$, $Ra+$ and $RaRa$ mice at different ages *in utero* and after birth. Some of these tissues were fixed in either Bouin's or Zenker's fixative and were embedded in paraffin. Serial sections, 5 to 7 μ in thickness, were stained with Delafield's hematoxylin and with eosin. The remaining tissues were fixed in formic acid, impregnated with gold chloride, and embedded in paraffin (Conn & Darrow, 1946). These tissues were serially sectioned at 5 to 7 μ.

3. OBSERVATIONS

(i) *Natal and post-natal observations*

It has been reported that there is a deficiency of $Ra^{op}+$ mice from $++ \times Ra^{op}+$ matings when examined within 10 hours after birth (Green & Mann, 1961). However, since a large number of $Ra^{op}+$ young are dead or dying at birth, some of these may have been eaten before being classified. Therefore, embryos were examined to determine if there were actually a deficiency of $Ra^{op}+$ young. $Ra^{op}+$ embryos can be distinguished from either $++$ or $Ra+$ embryos as early as 17 days *in utero* because of an incomplete complement of mystacial vibrissae on the upper lip and because of the absence or reduced size of the post-orbital vibrissa.

The classification of embryos from first parity $++ \times Ra^{op}+$ matings is given in Table 1. The number of $Ra^{op}+$ embryos found was slightly below the expected 50%, but the deficiency is not significant ($\chi_1^2 = 2\cdot13$). Furthermore, the proportion of dead embryos (4·5%) was not in excess of that found in nine control matings (7·6%). These matings, therefore, gave no conclusive evidence of the pre-natal loss of $Ra^{op}+$ embryos.

Table 1. *The classification of embryos between* 17 *and* 19 *days in utero from* 33 $++\female \times Ra^{op}+\male$ *matings*

Days *in utero*	$++$	$Ra^{op}+$	Moles and dead embryos	Total
17	11	11	2	24
18	95	72	7	174
19	21	23	2	46
Total:				
Observed	127	106	11	244
Expected	122	122	0	244

The classification of first parity embryos from $Ra^{op}+ \times Ra^{op}+$ and from $Ra+ \times Ra^{op}+$ matings is given in Table 2. The expected numbers shown in the table are based on the supposition that the moles and dead embryos represent the homozygous Opossum and the Ragged/Opossum compound genotypes. The moles and dead embryos in both types of mating were in excess of the expected numbers, and in both cases there were fewer $Ra^{op}+$ embryos than expected. These matings, therefore, support the supposition that $Ra^{op}Ra^{op}$ and $RaRa^{op}$ embryos die before birth, but they indicate also a considerable mortality among $Ra^{op}+$ embryos. Classification of the dead embryos according to whether they had died before or after 11 days (Table 3) showed that the majority had died before 11 days. In the $Ra+ \times Ra^{op}+$ matings the number of embryos dying after 11 days is sufficient to account for the deficiency of $Ra^{op}+$ embryos. But in the $Ra^{op}+ \times Ra^{op}+$ matings there is evidence that some $Ra^{op}+$ embryos died before 11 days. If those dying after 11 days are added to the $Ra^{op}+$ embryos, the ratio of

Table 2. *The classification of embryos at* 18 *days in utero from* 19 *litters of* $Ra^{op}+♀ \times Ra^{op}+♂$ *and from* 20 *litters of* $Ra+♀ \times Ra^{op}+♂$ *matings*

Mating ♀ × ♂	With vibrissae ($Ra+$ and/or $++$)	Without vibrissae ($Ra^{op}+$)	Living but unknown genotype	Moles and dead embryos	Total
$Ra^{op}+ \times Ra^{op}+$					
Observed	25	28	5	57	115
Expected	28·75	57·5	0	28·75	115
$Ra+ \times Ra^{op}+$					
Observed	81	28	1	53	163
Expected	81·5	40·75	0	40·75	163

$Ra^{op}+$ to $Ra^{op}Ra^{op}$ embryos becomes 42:43, and this still differs significantly from the expected 2:1 ratio ($\chi_1^2 = 11.39$).

The most noticeable feature of all the matings was the high frequency of oedematous $Ra^{op}+$ embryos (Table 3). The severity of oedema varies from mild swelling affecting only the pectoral region to severe swelling of the entire embryo. In the $++ \times Ra^{op}+$ matings, the frequency of oedema increased as the $Ra^{op}+$ embryos became older (Table 3).

Table 3. *Incidence of oedema and pre-natal death in Opossum and Ragged matings*

Mating	Age *in utero*	Percentage of oedematous living $Ra^{op}+$	Percentage of dead embryos	Percentage of dead embryos dying prior to 11 days
$Ra^{op}+ \times Ra^{op}+$	18	17·9 (5/28)	49·6 (57/115)	75·4 (43/57)
$Ra+ \times Ra^{op}+$	18	39·3 (11/28)	32·5 (53/163)	68·0 (36/53)
$++ \times Ra^{op}+$	17	18·2 (2/11)	8·3 (2/ 24)	—
$++ \times Ra^{op}+$	18	29·2 (21/72)	4·0 (7/174)	—
$++ \times Ra^{op}+$	19	52·2 (12/23)	4·3 (2/ 46)	—
$++ \times ++$	18	—	7·6 (5/ 66)	—

As previously reported, oedematous $Ra^{op}+$ newborn mice die shortly after birth, and many of the remaining $Ra^{op}+$ mice die before weaning (Green & Mann, 1961). Some of the $Ra^{op}+$ mice which die before weaning can be recognized shortly after birth. The abdomens of these mice are distinctly swollen and the peritoneal cavity appears to be filled with a thin, white liquid, which can be seen through the skin surface. By 15 days after birth, these mice are runted, lack almost all hair, have distended abdomens, and appear moribund. None of them has lived for more than 30 days after birth.

(ii) *Initiation and differentiation of pelage hair follicles*

The under-fur of the mouse consists of zigzags; the overhairs are auchenes, awls, and monotrichs (Dry, 1926). In the skin of several mammals there is a similar type of hair follicle which appears to be highly specialized for a sensory function; these are called *tylotrich* follicles (Straile, 1960). In the mouse the terms tylotrich and

monotrich are synonymous. Tylotrich follicles in the mouse embryo are initiated on the shoulders at about 13 days *in utero*, followed by the awl follicles at 16 days, and finally by the auchene and zigzag follicles at 18 days (Mann, 1962). The formation of each follicle type begins on the shoulders, and subsequent follicle initiation extends as a wave caudally, anteriorly, ventrally and dorsally.

The pattern of hair formation in Ragged and Opossum embryos is the same as in normal embryos. The time of follicle formation in $Ra+$, $Ra^{op}+$ and $RaRa$ embryos, however, is distinctly different from that of their $++$ litter mates. In the mutant embryos there is a delay in the initiation of each follicle type. For

Table 4. *The anagen stages of the various hair follicles on the caudal dorsum of newborn mutant and normal mice.*

	Hair follicle types		
Genotype	Tylotrichs	Awls	Auchenes and zigzags
$++$	late IV	late III	late I and early I
$Ra+$	early IV	early III	early I
$Ra^{op}+$ (non-oedematous)	late III	late I and early II	early I
$Ra^{op}+$ (oedematous)	late I and early II	early I	—
$RaRa$	late I and early II	early I	—

example, the auchene and zigzag follicles on the caudal dorsum of $++$ embryos begin to be initiated at about 18 days, whereas in non-oedematous $Ra^{op}+$ embryos they do not begin to be initiated until 19 days. Follicle initiation in $Ra+$ embryos is about 12 hours later than in $++$ embryos.

As a result of the delayed initiation of hair follicles in the mutant embryos, there remains throughout the period of differentiation of each follicle type a comparable delay in the development of the follicle and in the growth of the hair. A comparison of the anagen sub-stages of the follicles found at birth in the mutant and normal embryos is indicated in Table 4. (For a complete description of the anagen sub-stages, see Chase *et al.*, 1951.) Although auchene and zigzag follicles in the $Ra^{op}+$ embryos are present at birth, these follicles generally do not complete differentiation. Some undifferentiated follicles consist of as few as 6–8 cells. The $Ra+$ embryo differs from the $Ra^{op}+$ embryo in having a differentiation of some of the zigzag follicles; these follicles produce hair. (For a comparison of $++$, $Ra^{op}+$ and $Ra+$ skin at 24 hours after birth, see Plate I, Figs. 1, 2 and 3.)

Although there is a delay in the time of initiation of $Ra^{op}+$ and $Ra+$ hair follicles and a comparable delay in the eruption of hairs through the skin surface, the newly initiated follicles pass through the same sequence of development as normal follicles. Furthermore, the length of time from the beginning of follicle initiation to the time the hair reaches the surface of the skin is 7–8 days, the same as for normal follicles.

In $RaRa$ and oedemic $Ra^{op}+$ embryos, follicle formation is delayed even more than in $Ra+$ or non-oedemic $Ra^{op}+$ embryos. At 19 days and at birth, the skin of oedemic $Ra^{op}+$ and $RaRa$ embryos is very similar (Table 4). Some of the larger follicles, presumably tylotrichs, are in late anagen I and early anagen II, whereas the remaining follicles, presumably awls, are all in early anagen I. (See Figs. 4 and 5.)

(iii) *The distribution of tylotrich follicles*

One of the many distinguishing characteristics of the tylotrich follicle is the presence of a thick pad of epidermis, which acentrically surrounds the follicular orifice; this structure is called the *Haarscheibe* (Pinkus, 1905). *Haarscheiben* can be

Table 5. *Comparisons in* Haarscheiben *distribution between* Ra +, Ra^{op} +, *and their normal litter mates* (+ +) *at 18 days in utero.*

Skin Area	Genotype	Number of animals	Mean number of *Haarscheiben* within a 2 mm^2 area of skin	S.E.	t	P^*
Cephalic dorsum	$Ra+$	14	12·14	± 0·53	1·42	> 0·05
	+ +	14	11·00	± 0·60		
Caudal dorsum	$Ra+$	14	20·21	± 1·06	2·39	> 0·01 < 0·05
	+ +	14	16·36	± 1·22		
Venter	$Ra+$	14	11·36	± 0·72	1·82	> 0·05
	+ +	14	9·71	± 0·54		
Cephalic dorsum	$Ra^{op}+$	20	13·35	± 0·63	0·65	> 0·05
	+ +	20	12·55	± 0·60		
Caudal dorsum	$Ra^{op}+$	20	19·40	± 0·91	0·51	> 0·05
	+ +	20	18·50	± 0·86		
Venter	$Ra^{op}+$	20	11·40	± 0·54	1·09	> 0·05
	+ +	20	10·35	± 0·41		

* Probability that the difference between the means is due to chance alone.

clearly seen as small, rounded elevations on the skin surface of 18-day-old mouse embryos. The sizes of *Haarscheiben* differ on various parts of the embryo; i.e. they are larger on the medial and ventral surfaces than on the dorsum. Furthermore, the concentration of tylotrich follicles is greater on the caudal dorsum than on either the cephalic dorsum or the venter (Mann, 1962).

Haarscheiben are present on the skin of 18-day-old $Ra+$ and $Ra^{op}+$ embryos. Although some of the mutant embryos are slightly smaller than their + + litter mates, the size of individual *Haarscheiben* is the same on the mutant embryos as on + + embryos. As indicated in Table 5, the concentration and distribution of *Haarscheiben* on $Ra+$ and $Ra^{op}+$ embryos is similar to that of their + + litter mates. Thus, the mutant mice have a full complement of tylotrich follicles.
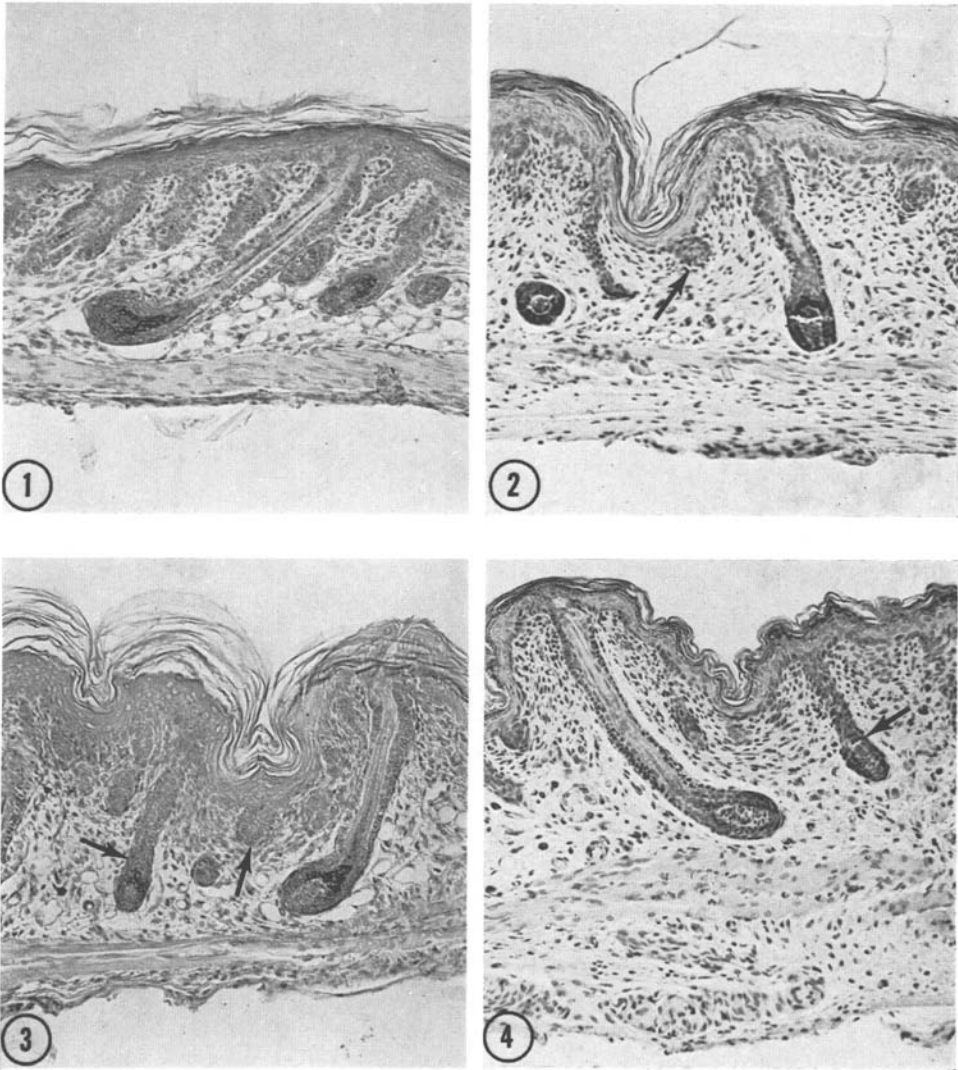
Fig. 1. A photomicrograph of a longitudinal section of normal skin from the rear dorsum 24 hours after birth. The large tylotrich follicle is in anagen V. Section stained with H. and E. × 140.

Fig. 2. A longitudinal section of Ra^{op}+ skin from the rear dorsum 24 hours after birth. The tylotrich follicle is an anagen IV. Notice at the base of the epidermis the auchene and zigzag follicles which have failed to proliferate. One of these structures is marked with an arrow. Compare with Fig. 1. Section stained with H. and E. × 140.

Fig. 3. A longitudinal section of skin from the posterior dorsum of a 24-hour-old Ra+ mouse. The large tylotrich follicle is in late anagen IV, and the smaller awl follicle, marked by the left arrow, is in anagen III. The arrow on the right marks an auchene or zigzag follicle which has failed to proliferate. Compare with Figs. 1 and 2. Sections stained with H. and E. × 140.

Fig. 4. A longitudinal section of skin from the rear dorsum of a + + mouse at birth. The large tylotrich follicle is in anagen IV and the awl follicle, marked with an arrow, is in early anagen III. Section stained with H. and E. × 140.

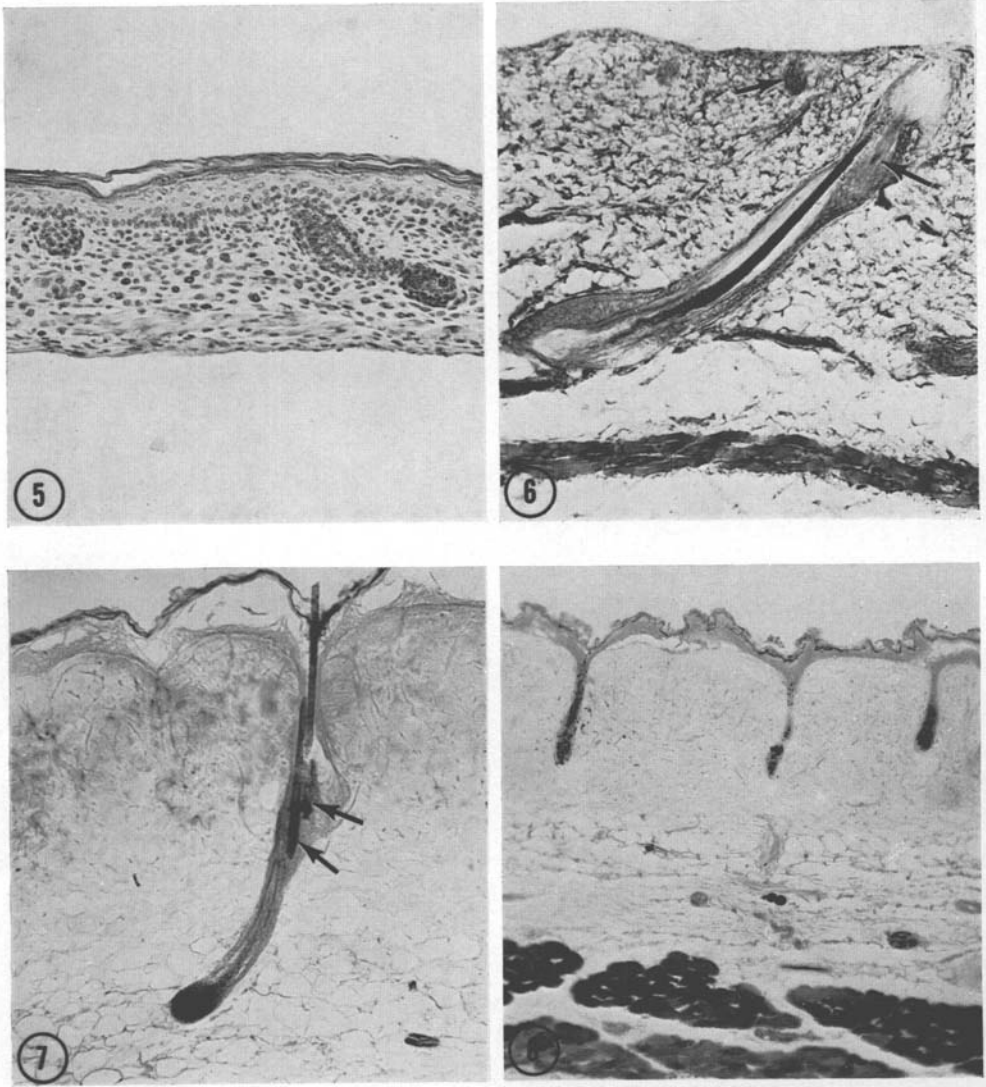STANLEY J. MANN   *(Facing p.* 6)

Fig. 5. A posterior dorsal longitudinal section of skin from a severely oedemic Ra^{op} + mouse at birth. The large follicle, presumably a tylotrich follicle, is in anagen II and the remaining follicles, awls, are in anagen I. The condition seen here is the same as in the oedemic $RaRa$ mouse at birth. Compare with Fig. 4. Section stained with H. and E. × 140.

Fig. 6. An active tylotrich follicle from the posterior dorsum of a 28-day-old Ra^{op} + mouse. Notice the club hair, marked with the arrow, indicating that this is the second hair growth cycle. Notice the undifferentiated auchene or zigzag follicle which is marked by the upper arrow. Section stained with gold chloride. × 140.

Fig. 7. An active tylotrich follicle in a 287-day-old Ra^{op} + mouse. The arrows mark two club hairs. Section stained with gold chloride. × 100.

Fig. 8. Three partially differentiated auchene and zigzag follicles in a 287-day-old Ra^{op} + mouse. Notice the heavy concentration of pigment granules in and around the incomplete follicles. Section stained with H. and E. × 65.

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The morphology of juvenile and adult skin

In + + mice 24–26 days of age the follicles on the cephalic and caudal dorsum are in the quiescent phase (telogen) of the hair growth cycle. When these hairs are plucked, new hairs erupt through the surface of the skin in approximately 8 days. On the other hand, when dorsal hairs are plucked from 24–26-day-old Ra^{op} + and Ra + mice, new hairs are already erupting through the skin surface, and new hairs continue to emerge each day after plucking (Fig. 6). As seen in histological sections, adjacent follicles in 24–26-day-old Ra + and Ra^{op} + skin are in different phases of growth whereas adjacent follicles in + + mice are in the telogen phase. Thus, hairs in Ra^{op} + and Ra + mice grow asynchronously; this condition continues throughout life.

The skin of Ra + and Ra^{op} + mice normally contains tylotrich and awls, but no hairs morphologically characteristic of the auchene have been found. Ra + mice have a reduced complement of zigzags, but some zigzags are always present. From a total of eighty Ra^{op} + mice, only six were observed with zigzags. On these mice, with one exception, the hairs were found in clumps, 5–10 mm. in diameter, on either the left or right shoulder.

There are two distinct types of zigzags in the Ra^{op} + mouse: A, morphologically normal hairs with three or four constrictions and one row of pigmented medulla cells; B, abnormal zigzags which are narrow, curled, lack constrictions, and contain small medulla cells which often lack pigment. Five of the six Ra^{op} + mice with isolated clumps of zigzags on the shoulders had either B or A and B type zigzags. The sixth Ra^{op} + mouse additionally had clumps of zigzags on other areas of the body. Most of the zigzags on the shoulders of this mouse were characteristic of type A, whereas the neck, caudal dorsum and venter contained either B or A and B type zigzags. No zigzags were found on the middle of the dorsum.

Most of the follicles in Ra^{op} + and Ra + mice from 14 to 265 days of age are actively growing; the absence of follicles in the quiescent stage is particularly noticeable in Ra^{op} + mice. Moreover, many of the follicles in these mice are morphologically abnormal because the bulbs are twisted and curved. Frequently, tylotrich club hairs are found within an active growing follicle; this is not found in + + mice (Fig. 7).

Small pigment spots are found on the skin of some adult Ra^{op} + mice. The distribution of these spots varies; generally they occur on the shoulders and head, but they have not been found on the middle of the back or on the caudal dorsum. These pigment spots are partially differentiated auchene and zigzag follicles. Although these structures do not produce a hair, they appear to be actively producing pigment which passes through the 'hair canal' to the surface of the skin (Fig. 8).

(v) *The distribution of vibrissae*

About forty mystacial vibrissa follicles are found on each side of the upper lip of normal mice. They are arranged in orderly horizontal and vertical rows on the snout. The most posterior vertical row, just in front of the eyes, contains four

follicles, and the next vertical row contains five follicles (cf. Danforth, 1925; Dun, 1958). Anterior to these two rows the hairs and follicles gradually decrease in size. In $Ra^{op}+$ mice, the most posterior vertical row of mystacial vibrissae is usually present. However, anterior to this row, most of the hairs are missing. Histological studies reveal that the mystacial follicles which produce hairs are morphologically normal, but the anterior follicles, which do not produce hairs, are not fully developed.

In addition to the mystacial vibrissae on the upper lip of normal mice, there are two supra-orbital, one post-orbital, and three postoral vibrissae on each side of the head. Also, there are three inter-ramal vibrissae on the chin (Dun, 1958). Examination of seventeen $Ra^{op}+$ mice revealed no inter-ramal and only one small postoral vibrissa. However, 76·5% (26/34) of the post-orbital hairs were present. All the anterior supra-orbital vibrissae were missing but 85·3% (29/34) of the adjacent posterior supra-orbital vibrissae were present.

4. DISCUSSION

(i) *Hair follicle formation and the distribution of hair types*

In $Ra+$, $Ra^{op}+$ and $++$ 18-day-old embryos tylotrich follicles are more densely distributed on the caudal dorsum than on the cephalic dorsum or the venter. The concentration of these follicles is the same in both mutant and normal embryos. These observations are in partial agreement with the findings of Slee (1957a). He reported that there are more guard hairs (tylotrichs) per surface area on the rear dorsum than on the neck of adult $++$ mice. Also, he observed that more tylotrichs are found on the neck and caudal dorsum of $Ra+$ than on $++$ mice. Slee based his criterion on the relationship between the frequency of pelage hair in a given area and the number of follicles found in longitudinal histological sections. In addition to counting complete follicles in $Ra+$ sections, Slee counted incomplete follicles, i.e. follicles not producing a hair. The present study shows that these incompletely formed follicles are primarily auchene and zigzag follicles. Therefore, Slee's results indicate a spuriously high ratio of tylotrich and awl follicles in the $Ra+$ mouse. No evidence was obtained in the present study to support the idea that $Ra+$ mice have a higher density of any hair type than do $++$ mice. There is, however, sufficient evidence to show that the density of some hair types is lower in $Ra+$ than in $++$ mice.

The distribution of vibrissae is the same in $Ra+$ mice as in $++$ mice (Slee, 1957b; Dun, 1959). In the $RaRa$ mouse, however, Dun reported that the postoral, inter-ramal, anterior supra-orbital, and anterior mystacial vibrissae were missing, but that 90·6% of the posterior supra-orbital and 75% of the post-orbital hairs were present. These findings for $RaRa$ mice are in close agreement with those for $Ra^{op}+$ mice. In $Ra^{op}+$ mice, the inter-ramal, postoral, anterior supra-orbital and anterior mystacial vibrissae are missing, but 85·3% of the posterior supra-orbital and 76·5% of the post-orbital hairs are present. These results indicate that the defects of vibrissae development are similar in the $Ra^{op}+$ and the $RaRa$ mutants.

(ii) *Pre-natal death*

Pre-natal mortality of mutant embryos is common in some types of Ragged and Opossum matings. In $+ + \times Ra +$ matings, pre-natal death is about the same as in control matings (Slee, 1957b). Similar results are found in $+ + \times Ra^{op} +$ matings (Table 3). In intercross matings, however, there is a rapid increase in pre-natal mortality. In $Ra + \times Ra +$ matings, 20·4% (64/313) of the $RaRa$ embryos die (Slee, 1957b). In $Ra + \times Ra^{op} +$ matings the frequency of death increases to 32·5% and in $Ra^{op} + \times Ra^{op} +$ matings to 49·6% (Table 3). Furthermore, as the rate of pre-natal mortality increases in the intercross matings, offspring of more than one genotype are affected. In $Ra + \times Ra +$ matings some $RaRa$ genotypes survive, whereas in $Ra + \times Ra^{op} +$ and in $Ra^{op} + \times Ra^{op} +$ matings all the homozygous compounds and some of the $Ra^{op} +$ embryos die *in utero*.

(iii) *Allelism*

The findings of the present study on the morphological and developmental similarities between Opossum and Ragged support the linkage data which suggest that Opossum and Ragged are alleles (Green & Mann, 1961). As indicated in Table 6, the phenotypic effects of the two genes are similar. There is a delay in the embryonic initiation of hair follicles and a reduction or absence of some hair types. The distribution and number of vibrissae is approximately the same in $Ra^{op} +$ and $RaRa$ mice. There are also similarities in pre-natal and post-natal mortality, and in the presence of oedema. And, finally, the compound $RaRa^{op}$ is inviable, like the $Ra^{op} Ra^{op}$ homozygote. The degree of gene action is in the following order of increased severity: $Ra +$, $Ra^{op} +$ (semi-lethal), $RaRa$ (semi-lethal), $Ra^{op} Ra$ (lethal) and $Ra^{op} Ra^{op}$ (lethal).

Table 6. *An organization scheme showing how the phenotype is affected by the* Ra *and* Ra^{op} *genes.*

Effect on phenotype	Genotype
All embryos die *in utero*	$RaRa^{op}$, $Ra^{op} Ra^{op}$
Some embryos die *in utero*	$RaRa$, $Ra^{op} +$ (depending on the type of mating)
Some oedemic dead and dying at birth	$RaRa$, $Ra^{op} +$
Delay in follicle formation	$RaRa$, $Ra^{op} +$, $Ra +$
Reduction in the density of vibrissae	$RaRa$, $Ra^{op} +$
Reduction in the density of awls	$RaRa$, $Ra^{op} +$, $Ra +$ (possibly)
Reduction in the density of auchenes and zigzags	$RaRa$, $Ra^{op} +$, $Ra +$

5. SUMMARY

1. The development and morphology of the semi-dominant mutations Ragged and Opossum are compared. The results of the present study support evidence of previous linkage studies which suggest that Ragged (Ra) and Opossum (Ra^{op}) are alleles.

2. Examination of embryos from 18-day-old first parity matings of $+ + \times Ra^{op} +$ reveals that there is no deficiency of $Ra^{op} +$ embryos. However, in $Ra + \times Ra^{op} +$ and $Ra^{op} + \times Ra^{op} +$ matings some $Ra^{op} +$ embryos die *in utero* in addition to the pre-natal death of the $RaRa^{op}$ and $Ra^{op} Ra^{op}$ embryos.

3. In all matings of Ragged and Opossum, there is a high frequency of oedematous $Ra^{op} +$ embryos. In $+ + \times Ra^{op} +$ matings, the frequency of oedema increases with increased age of the embryos.

4. Although the pattern of hair follicle formation is the same in both the mutant and normal embryos, follicles begin initiation later in the mutant embryos. The delay in follicle initiation is increased in the following order: $Ra +$, $Ra^{op} +$ (non-oedemic), $RaRa$ and $Ra^{op} +$ (oedemic).

5. Heterozygous Opossum and $Ra +$ mice have a full complement of pelage follicle primordia, but some follicles fail to differentiate. The differentiating follicles pass through the same developmental stages and require the same time for development as follicles in $+ +$ mice.

6. Heterozygous Ragged and $Ra^{op} +$ mice have a full complement of tylotrichs, whereas the concentration of awls is variable. In $Ra +$ mice there is a reduction in zigzags, and $Ra^{op} +$ mice usually lack zigzags. No auchenes are observed in the mutant mice.

7. Hair growth is asynchronous in $Ra +$ and $Ra^{op} +$ mice, with the exception of the first pelage coat. The quiescent phase of the hair growth cycle is short or even missing.

8. The degree of Ra and Ra^{op} gene activity is in the following order of increased severity: $Ra +$, $Ra^{op} +$ (semi-lethal), $RaRa$ (semi-lethal), $RaRa^{op}$ (lethal) and $Ra^{op} Ra^{op}$ (lethal).

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