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The site of action of the asebia locus (*ab*) in the skin of the mouse

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Summary

In mice homozygous for the asebia mutation (ab^J/ab^J) the sebaceous glands were small and the sebaceous cells abnormal, the fully grown hair follicles were twice as long as those in wild-type (+/+) mice, the catagen and telogen follicles were abnormal and, because the active phase of the hair cycle was longer than in +/+ mice, the initiation of the second hair cycle was delayed. The abnormalities in the sebaceous glands and in catagen and telogen follicles were also present in ab^J/ab^J embryonic skin grown on a nude host but anagen of the second hair cycle commenced at about the same time in ab^J/ab^J and +/+ grafts. When recombinants incorporating mutant or wild-type epidermis and dermis were grown on a nude host, the abnormalities in the sebaceous glands and the catagen and telogen follicles were only observed in the recombinants incorporating ab^J/ab^J epidermis. It was concluded that mutant activity in the epidermis was responsible for the abnormalities in the sebaceous glands and in catagen and telogen follicles and that mutant activity at some site distant from the skin was responsible for the abnormalities in the timing of the hair cycles.

1. Introduction

The asebia mutation (*ab*) received its name because homozygous asebia animals from the colony in which the mutant first appeared were reported to be without sebaceous glands (Gates & Karasek, 1965). Subsequently, it was found that sebaceous gland development was not entirely suppressed in *ab*/*ab* mice. Nay (1972, unpublished data) observed immature sebaceous (seboid) cells in the outer root sheath of the hair follicles of *ab*/*ab* mice from Gates' colony and from a random-bred colony, and Josefowicz and Hardy (1978*c*) found both seboid outer root sheath cells and abnormal sebaceous glands in *ab*/*ab* mice of BALB/*c* strain derived from Gates' colony.

In their studies of *ab*/*ab*, +/+ and +/*ab* mice of several different ages, Josefowicz & Hardy (1978*a–c*) observed that, in mice of the same chronological age, differences between skins of *ab*/*ab* and the other genotypes were not confined to the sebaceous glands, but also occurred in the hair follicles, epidermis and dermis. It was also observed that *ab*/*ab* mice were older than +/+ mice and +/*ab* mice when the hair follicles passed into telogen of the first hair cycle and catagen and telogen of the second (Nay, unpublished data; Josefowicz & Hardy, 1978*b*). Differences in the timing of the hair cycles contributed to the differences in the morphology of the hair follicles, epidermis and dermis in the *ab*/*ab* and +/*ab* skin samples matched for age (Chase *et al.*, 1953; Parakkal, 1970).

The abnormalities in the epidermis and its derivatives and in the dermis could be due to the activity of the mutation in the epidermis or dermis, to its activity at a site remote from the skin or to its activity at more than one site. Of the mutations affecting hair and follicle morphology whose sites of activity have been investigated, eight are active in the epidermis (for review see Raphael & Pennycuik, 1980; Pennycuik & Raphael, 1984*b*), one is active in the dermis (Billingham & Silvers, 1973), one cannot be confined to either the epidermis or the dermis (Pennycuik & Raphael, 1984*a*) and one is active at a site remote from the skin (Fisher *et al.* 1984). Josefowicz & Hardy (1978*a–c*), who observed that the differences between *ab*/*ab* and +/*ab* mice in the cellularity of the dermis could be detected earlier than differences between *ab*/*ab* and +/*ab* epidermis, suggested that the mutation might

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act primarily in the dermis or at some site remote from the skin.

This paper reports the results of experiments designed to identify the site of action of the asebia mutation. Skins from mutant and wild-type foetuses or recombinants of mutant and wild-type epidermis and dermis were transferred to nude (*nu/nu*) mice, where they were allowed to develop to an age where the hair follicles in the wild-type were expected to have completed the first hair cycle (21 days post-natal; Chase *et al.*, 1953; Raphael & Pennycuik, 1980). The morphology of the hair follicles, sebaceous glands, epidermis, dermis and hypodermis in these skin grafts was then compared with that in skin from *ab/ab* and wild-type mice of comparable age, and also, in the case of asebia mice, at a comparable stage of follicle activity. The mutation used in these experiments was ab^J, which arose in the Jackson Laboratory, rather than the original *ab* of Gates, and the strain in which it was segregating was different from those used by Nay (1972) and Josefowicz & Hardy (1978*a–c*). Since there were differences between *ab/ab* and ab^J/ab^J mice in the appearance and distribution of the hair, it seemed advisable to examine the effects of ab^J on the timing of the hair cycles and on the histology of the skin and its appendages before the transplant and recombinant studies were undertaken. The results of these investigations are also reported briefly in this paper.

2. Materials and Methods

(i) *Mouse stocks*

Wild-type (+/+) and asebia (ab^J/ab^J) mice and embryos were obtained from a random-bred wild-type stock and a random-bred asebia^J stock respectively. The asebia^J stock was descended from two ab^J/ab^J males from the Jackson Laboratory, Maine, USA, and females from the wild-type stock. Both stocks were coloured. Heterozygous naked ($N/+$) mice, used to introduce the *N* mutation into the asebia^J stock, were obtained from a random-bred, coloured stock segregating for *N*. Homozygous nude (*nu/nu*) mice were obtained from a random-bred albino stock segregating for *nu*. These mice were used as hosts for grafts when they reached 4–6 weeks of age. All stocks were maintained under conventional conditions.

(ii) *Observations on hair generations*

The effect of the asebia mutation on age at eruption of the second generation (G2) of hairs and on the intervals between emergence of hairs of the second, third, fourth and fifth hair generations was measured by observing the mid-sides of ab^J/ab^J and wild-type mice heterozygous for naked (*N*) (Slee, 1957; Kindred, 1967). The mice used for this study were the male and female offspring of a $+/ab^J\ N/+ \times +/ab^J\ N/+$ cross. The phenotypically asebia^J, naked mice, therefore, were all of one genotype, $ab^J/ab^J\ N/+$, and the phenotypically non-asebia, naked mice ($+/-\ N/+$) were either $+/ab^J\ N/+$ or $+/+\ N/+$. Observations were made at 25 days and at two or three day intervals to about 120 days.

(iii) *Grafts, preparation and grafting procedures*

The preparation and grafting of transplants of embryonic skin and recombinants of mutant and wild-type epidermis and dermis were performed as described elsewhere (Raphael & Pennycuik, 1980; Pennycuik & Raphael, 1984*a*). The skin pieces used to prepare both transplants and recombinants were from ab^J/ab^J and +/+ embryos of 14 days gestational age. At this age the epidermis and dermis were undifferentiated and the hair follicles had just begun as epidermal downgrowths without any signs of sebaceous glands. Both transplants and recombinants were allowed to remain on their nude hosts until the grafts reached an age equivalent to 40 days post-conception (i.e. equivalent to that of 21-day-old mice), when they were removed for histological examination.

(iv) *Histology*

Mid-side skin samples were obtained from two 23-day-old +/+ mice and from one 20-, two 22- and one 31-day-old ab^J/ab^J mice. Successful skin grafts were removed from the *nu/nu* mice together with a border of about 3 mm of host skin. After fixation one skin sample from each mouse and all grafts were used to prepare whole mounts. These were stained with Oil Blue N (Nay, 1960) which facilitated observation of the outer root-sheath and the sebaceous glands. When the examination of the whole mounts was completed a second sample from each mouse and two grafts of each type were embedded in paraffin and sectioned (7–8 μm). Sections from each sample were stained with haematoxylin, eosin and picric acid (HEP; Carter & Clarke, 1957), aldehyde fuchsin–Halmi (Halmi & Davies, 1953) and Mallory's triple connective tissue stain (McManus & Mowry, 1965).

(v) *Classification of hair cycle stages*

The follicles in the skin samples and the grafts were classified on the basis of the hair cycle stage reached. Anagen of the first hair cycle was divided into stages 1–8 as described and illustrated by Hardy (1949, 1951, 1969), while in subsequent hair cycles the stages of anagen ranged from 2 to 8. Stage 8 was divided into three sub-stages: 8a, unmedullated tips of hairs emerging from skin; 8b, medullated portions of hairs emerging; 8c, medullated portions of emerging hairs equal to, or greater than, follicle depth. Catagen was divided into stages I–VIII as described and illustrated by Straile *et al.* (1961), Parakkal (1970) and Mantagna & Parakkal (1974).

(vi) *Thickness of skin layers and dermal cell densities*

The thickness of the dermis and the hypodermis (defined as the adipose layer between the lower border of the dermis and the panniculus carnosus) were measured with an eyepiece micrometer at × 63 magnification. Dermal cell densities were measured at × 400 magnification with the aid of an eyepiece graticule, at the mid-dermal level in samples with thick dermis and throughout the depth of the dermis in samples where this layer was thin.

3. Results

(i) *Effects of asebia* (ab^J/ab^J) *on the skin*

Timing of hair cycles

The development of second cycle (G2) hairs occurred later in ab^J/ab^J mice than in +/+ mice (Table 1). In the 23-day-old +/+ mice all follicles were in stages 7–8a of anagen of G2, but in ab^J/ab^J mice of 20 and 22 days the follicles were all in catagen or telogen of G1. Only in the 31-day-old ab^J/ab^J mouse were there some follicles in anagen stages of G2, the most advanced being in stage 8b.

Hairs of the second cycle erupted about 4 days later in ab^J/ab^J $N/+$ mice than in $+/-$ $N/+$ mice (Table 2). The differences between the two genotypes were significant for both sexes (males, $t = 9{\cdot}19$, $P < 0{\cdot}001$; females, $t = 4{\cdot}23$, $P < 0{\cdot}001$). The $asebia^J$ mutation also produced a slight, but non-significant, increase in the length of the interval between successive eruptions of the hairs of cycles G2 to G5; in +/+ mice this interval was about 19 days, in ab^J/ab^J it was about 20 days.

Modifications of hair follicles at different stages of the cycle

In both 20- and 22-day-old ab^J/ab^J skin most of the catagen and telogen follicles were about twice as long as the longest anagen follicles found in 23-day-old +/+ skin. This was apparently due to retardation of upward movement of the keratinized hair club in catagen VI and of the hair germ in catagen VII and VIII. The hair shafts were waved, the hair bases were frequently bent at an angle to the follicle axis, and the outer root sheath cells formed a two- or three-layered border of flattened cells around the hair base. The dermal papillae were occasionally displaced to the side of the follicle. In addition, the skin samples from these mice had an appreciable number of follicles in which the proximal outer root sheath appeared to be missing leaving the hair ends exposed in the hypodermis. These hair ends were surrounded by numerous small, dark, rounded cells and amorphous material. In the 31-day-old ab^J/ab^J mouse a few of the telogen follicles were still as long as those in the younger ab^J/ab^J mice, some were as short as those in 23-day-old +/+ mice while most were of intermediate length.

Sebaceous glands

In skin sections the sebaceous glands of all our ab^J/ab^J mice were similar in appearance to those in the *ab/ab* mice described by Josefowicz & Hardy (1978*c*). The glands, which were smaller than those in +/+ mice, were attached to the distal wall of the follicles throughout their length. Mature sebaceous cells were scattered throughout the gland and seboid cells were present among the outer root-sheath cells of the follicles.

Table 1. *The numbers of mouse skin samples, transplants and recombinants with wild-type (+/+) sebaceous glands and with follicles in phases of the first and second hair cycle*

Genotype Epidermis/ Dermis	Age (days)	No. samples	+/+ Sebaceous glands	1st Cycle		2nd Cycle
				Catagen	Telogen	Anagen
Mice						
+/+	23	2	2	—	—	2
ab^J/ab^J	20–22	3	—	3	3	—
ab^J/ab^J	31	1	—	—	1	1
Transplants						
+/+	21	7	7	2	7	5
ab^J/ab^J	21	10	—	10	10	5
Recombinants						
+/+/+/+	21	4	4	—	4	4
$+/+/ab^J/ab^J$	21	8	8	2	8	5
$ab^J/ab^J/+/+$	21	6	—	3	6	3
$ab^J/ab^J/ab^J/ab^J$	21	5	—	4	5	3

The data for the mice are from the sectioned samples. The data for the transplants and recombinants are from both grafts examined as whole mounts and from grafts examined after sectioning.

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Table 2. *Age (days ± S.E.) at eruption of the second hair cycle (G2) and duration of the between-eruption interval for cycles G2, G3, G4 and G5 in +/− N/+ and* ab^J/ab^J *N/+ mice*

Sex	Genotype	No. Mice	Age	Between-eruption interval G2–G3	G3–G4	G4–G5	Mean
M	+/− *N/+*	19	31·3±0·29	18·4±0·50	19·4±0·59	19·2±0·38	19·0±0·50
	ab^J/ab^J *N/+*	13	35·3±0·24	20·9±0·46	21·1±0·42	18·8±0·46	20·3±0·66
F	+/− *N/+*	14	32·4±0·55	19·4±0·40	18·9±0·25	19·0±0·31	19·1±0·31
	ab^J/ab^J *N/+*	12	35·8±0·52	20·3±0·74	20·3±0·51	20·4±0·42	20·3±0·70

Other modifications in skin

Table 3 summarizes observations made on the epidermis, dermis and hypodermis of 23-day-old +/+ mice and 20- to 22- and 31-day-old ab^J/ab^J mice. Corresponding differences observed by Josefowicz & Hardy (1978*a*) between BALB/c mice which were heterozygous and homozygous for the original *ab* mutation are indicated. Although the numbers of layers of living cells in the epidermis of ab^J/ab^J mice were similar to those of the *ab/ab* mice of Josefowicz & Hardy (1978*a*), the epidermis was thinner in ab^J/ab^J than in *ab/ab* mice because the epidermal cells were flatter in the former than in the latter. In ab^J/ab^J skin, unlike *ab/ab* skin, the dermis and the hypodermis were no thicker than the corresponding layers in +/+ skin and in the skin from the 20- to 22-day-old ab^J/ab^J mice the vascularity of these two skin layers was not noticeably increased. In the skin of the 31-day-old ab^J/ab^J mouse, on the other hand, the vascularity of the dermis and the hypodermis was greatly increased.

(ii) *Grafts – skin transplants*

Timing of hair cycles

Table 1 shows that five out of the seven +/+ transplants had reached anagen stages of the second hair cycle by 21 days. In two of these, G2 anagen stages 8b and 8c follicles were seen, so development was even further advanced than in 23-day-old +/+ skin *in situ*. The remaining two transplants had no follicles beyond telogen of G1, and some follicles were still in catagen of G1. It was concluded that most of the +/+ follicles were not retarded in their cycling by grafting to nude hosts.

In five of the ten ab^J/ab^J transplants also, a few follicles were more advanced than they would have been *in situ*, having reached anagen of G2. This suggested that the follicle cycles of asebia^J skin may have been speeded up by the grafting to nude skin.

Morphology of hair follicles

It can be seen from Table 1 that catagen and telogen follicles of the first hair cycle were present in many of the transplants. Those in the +/+ grafts were indistinguishable from the corresponding stages *in situ*. The catagen follicles in the ab^J/ab^J grafts were morphologically similar to those in ab^J/ab^J skin *in situ*, although none of them had the follicle base missing. A few of the telogen follicles were long, as in the ab^J/ab^J mice, but most of them were short, as in +/+ mice.

Sebaceous glands

All sebaceous glands in the whole mounts of +/+ transplants showed the normal wild-type morphology. All glands in ab^J/ab^J transplants showed the same abnormal morphology as in ab^J/ab^J skin *in situ* and

Table 3. *Some characteristics of wild-type (+/+) and asebia*^{J} (ab^J/ab^J) *mice*

Genotype	+/+	ab^J/ab^J	ab^J/ab^J
Age (days)	23	20,22	31
Hair cycle stage	G2 anagen	G1 telogen	G2 anagen
Mean epidermal thickness (μm)	14	14	12
No. of living cell layers, epidermis[a]	2–3	5–6	3–4
No. of cell layers with keratohyalin[a]	0–1	2–3	1–2
Mean dermal thickness (μm)	160	160	80
No. of dermal cells/1000 μm^2[a]	2–3	5–6	5
Mean hypodermal thickness (μm)	300	160	80

[a] Josefowicz & Hardy (1978*a*) reported similar effects of *ab/ab* on these characteristics.

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seboid cells were present in the outer root-sheath. Paraffin sections of asebia transplants showed that the sebaceous glands had the same irregular distribution of mature sebaceous cells characteristic of the donor tissue. The +/+ grafts showed normal sebaceous gland histology (Table 1).

Other skin features

Of the characteristics listed in Table 3, only dermal cell density was consistently different in ab^J/ab^J grafts, being higher (5–6/1000 μm^2) than the density in +/+ grafts (3–3·5/1000 μm^2). The epidermal characteristics were too variable within and between grafts to show consistent differences between the genotypes. The hypodermis was very thin in grafts of both genotypes and there were no differences between genotypes in the degree of vascularization.

(iii) *Grafts – recombinants*

Timing of hair cycles

In all recombinants of +/+ epidermis with +/+ dermis, some follicles had reached the early stages of G2 anagen (Table 1). In one of these a few follicles had reached stage 8c, indicating that in one graft, at least, splitting of layers and recombination did not retard follicle development. In each of the other three groups of recombinants, which contained asebia^J epidermis or dermis or both, at least half of the grafts contained G2 anagen follicles. Thus the splitting and recombination of tissues did not prevent the speeding up of the second cycle in asebia^J grafted skin which was reported for transplants. The number of recombinant grafts was too small to attach significance to differences between the three groups.

Morphology of hair follicles

In recombinants of +/+ epidermis with +/+ dermis all follicles had advanced beyond G1 catagen to G1 telogen or G2 anagen (Table 1). The telogen follicles were all shortened, as is normal for this genotype. All of the other recombinant groups had G1 catagen follicles in some grafts, but the proportion was slightly higher when ab^J/ab^J epidermis was included (3/6, 4/5) than when +/+ epidermis was included. Furthermore, in those grafts in which some follicles were still in G1 catagen, only those including asebia^J epidermis had some long late catagen and long telogen follicles like those in ab^J/ab^J mice. In grafts including wild-type epidermis all catagen and telogen follicles were indistinguishable from those in +/+ mice.

Sebaceous glands

All sebaceous glands in recombinants incorporating asebia^J epidermis had the distinctive features of glands in asebia and asebia^J mice. All sebaceous glands in recombinants incorporating wild-type epidermis were indistinguishable from glands in +/+ mice.

Other skin features

While the epidermis, dermis and hypodermis of the recombinants had features similar to those in the transplants, there were no consistent differences between the four groups.

4. Discussion

Both Nay (unpublished) and Josefowicz & Hardy (1978*b*) reported that the *ab* mutation of Gates increased the length of hair cycles. This study has shown that ab^J also increased the cycle length. The morphological differences in catagen and telogen follicles between our ab^J/ab^J and +/+ mice were in the main similar to those observed by Josefowicz & Hardy (1978*a–c*) between *ab/ab* and +/*ab* mice. The differences in sebaceous glands and seboid modification of outer root sheath cells were identical in ab^J and *ab* mutants, and present at all hair cycle stages. An increase in number of living cell layers in the epidermis was also a constant feature of ab^J/ab^J and *ab/ab* mice at all stages of the hair cycle. The density of dermal cells were increased in both mutants. It seems probable that *ab* and ab^J are either identical or very similar alleles, but we did not have the opportunity to do breeding tests to establish this. The quantitative differences reported in other features such as epidermal thickness may be due to the different strain backgrounds.

The transplantation experiments showed that the activity of the asebia^J mutation at some site distant from the skin may contribute to the abnormal timing of events in the first and second hair cycle. About half of the asebia^J transplants showed a near-normal rate of hair cycle progress, and the same was true of recombinants containing ab^J/ab^J epidermis or dermis. Since Josefowicz & Hardy (1974) and Josefowicz (1975) reported that *ab/ab* mice had alterations in the steroid secretory cells of the ovary and adrenal gland, and since steroid hormone levels modulate hair cycles (Mohn, 1958), it may be that hormone levels in the *nu/nu* hosts are responsible for the near-normal cycles in transplants and recombinants.

Elongated and abnormal catagen follicles were found in ab^J/ab^J skin after transplantation, but, among recombinant grafts, only in those in which the epidermis was from an ab^J/ab^J donor. This indicates that the activity of the mutation in the embryonic epidermis was responsible for the follicle abnormality. Sebaceous glands of asebia^J type and seboid cells in the outer root sheath were consistently found in all transplants and recombinants containing epidermis from ab^J/ab^J donor, but in no other grafts. Action of

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the asebia^J mutation in the embryonic epidermis is therefore responsible for the sebaceous gland abnormalities. The nature of this action has not been identified. One possibility is that the mutation disturbs lipid synthesis in the sebaceous glands and/or the epidermis, for the total lipid composition of *ab/ab* skin is abnormal (Wilkinson & Karasek, 1966). The abnormal appearance of the sebaceous and outer root-sheath cells in *ab/ab* mice may result from this abnormality in lipid synthesis. Disturbances in these cells in their turn could affect follicle morphology, for it has been postulated that the orderly emergence of the hair is due, in part, to the removal of the inner root-sheath cells at the follicle neck and that the removal of these cells is due to the action of lytic agents released from the sebaceous glands (Straile, 1965) and/or the outer root-sheath (Gemmell & Chapman, 1971).

The results of these experiments thus suggest that the mutation *ab*^{J}, probably equivalent to *ab*, has primary activity in more than one tissue. Other mutations which affect skin morphology may also act in several tissues; for example crinkled, (*cr*) which acts in the epidermis (Mayer *et al.* 1977), also causes myelin abnormalities (Theriault *et al.* 1977), tabby, (*Ta*) which acts in epidermis and dermis (Pennycuik & Raphael, 1984*a*), affects the development of a variety of structures which arise from other epithelia (glands, teeth) (Grüneberg, 1971), and both ichthyosis (*ic*) and naked (*N*), which act in the epidermis (Green *et al.* 1974; Raphael & Pennycuik, 1980), reduce viability and fertility in homozygotes.

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References

Billingham, R. E. & Silvers, W. K. (1973). Transplantation and cutaneous genetics. *Journal of Investigative Dermatology* **60**, 509–515.

Carter, H. B. & Clarke, W. H. (1957). The hair follicle group and skin follicle population of Australian Merino sheep. *Australian Journal of Agricultural Research* **8**, 91–108.

Chase, H. B., Montagna, W. & Malone, J. D. (1953). Changes in the skin in relation to the hair growth cycle. *Anatomical Record* **116**, 75–81.

Fisher, C., Dale, B. A. & Kollar, E. J. (1984). Abnormal keratinization in the pupoid fetus (*pf/pf*) mutant mouse epidermis. *Developmental Biology* **102**, 290–299.

Gates, A. H. & Karasek, M. (1965). Hereditary absence of sebaceous glands in the mouse. *Science* **148**, 1471–1473.

Gemmell, R. T. & Chapman, R. E. (1971). Formation and breakdown of the inner root sheath and features of the pilary canal epithelium in the wool follicle. *Journal of Ultrastructure Research* **36**, 355–366.

Green, M. C., Alpert, B. N. & Mayer, T. C. (1974). The site of action of the ichthyosis locus (*ic*) in the mouse, as determined by dermal–epidermal recombinations. *Journal of Embryology and Experimental Morphology* **32**, 715–721.

Grüneberg, H. (1971). The Tabby syndrome in the mouse. *Proceedings of the Royal Society of London* B**179**, 139–156.

Halmi, N. S. & Davies, J. (1953). Comparison of aldehyde fuchsin staining, metachromasia and periodic acid–Schiff reactivity of various tissues. *Journal of Histochemistry and Cytochemistry* **1**, 447–459.

Hardy, M. H. (1949). The development of mouse hair *in vitro* with some observations on pigmentation. *Journal of Anatomy* **83**, 364–384.

Hardy, M. H. (1951). The development of pelage hairs and vibrissae from skin in tissue culture. *Annals of the New York Academy of Science* **53**, 546–561.

Hardy, M.H. (1969). The differentiation of hair follicles and hairs in organ culture. In *Advances in Biology of Skin* volume IX; *Hair Growth* (ed. W. Montagna and R. L. Dobson), pp. 35–60. Oxford and New York: Pergamon Press.

Josefowicz, W. J. (1975). The Development and Expression of the Asebia Mutation in Mice. MSc Thesis, University of Guelph.

Josefowicz, W. J. & Hardy, M. H. (1974). The development and expression of the mutation asebia (*ab/ab*) in mice. *Proceedings of the Canadian Federation of Biological Sciences* **17**, 173.

Josefowicz, W. J. & Hardy, M. H. (1978*a*). The expression of the gene asebia in the laboratory mouse. 1. Epidermis and dermis. *Genetical Research* **31**, 53–65.

Josefowicz, W. J. & Hardy, M. H. (1978*b*). The expression of the gene asebia in the laboratory mouse. 2. Hair follicles. *Genetical Research* **31**, 145–155.

Josefowicz, W. J. & Hardy, M. H. (1978*c*). The expression of the gene asebia in the laboratory mouse. 3. Sebaceous glands. *Genetical Research* **31**, 157–166.

Kindred, B. (1967). Some observations on the skin and hair of Tabby mice. *Journal of Heredity* **58**, 197–199.

McManus, J. F. A. & Mowry, R. W. (1965). *Staining Methods, Histological and Histochemical.* New York: Evanston, London: Harper and Row.

Mayer, T. C., Miller, C. K. & Green, M. C. (1977). Site of action of the crinkled (*cr*) locus in the mouse. *Developmental Biology* **55**, 397–401.

Mohn, M. P. (1958). The effects of different hormonal states on the growth of hair in rats. In *The Biology of Hair Growth* (ed. W. Montagna and R. A. Ellis), pp. 335–398. New York: Academic Press.

Montagna, W. & Parakkal, P. F. (1974). *The Structure and Function of Skin*, 3rd edition. New York: Academic Press.

Nay, T. (1960). Growth of the mouse coat. VII. Hair cycles and sebaceous glands in homozygous and heterozygous naked mice. *Australian Journal of Biological Sciences* **13**, 351–355.

Nay, T. (1972). Hair of asebic mice. *Mouse News Letter* **46**, 40.

Parakkal, P. F. (1970). Morphogenesis of the hair follicle during catagen. Zeitschrift für Zellforschung und mikroskopische Anatomie **107**, 174–186.

Pennycuik, P. R. & Raphael, K. A. (1984*a*). The tabby locus (*Ta*) in the mouse: its site of action in tail and body skin. *Genetical Research* **43**, 51–63.

Pennycuik, P. R. & Raphael, K. A. (1984*b*). The angora locus (*go*) in the mouse: hair morphology, duration of growth cycle and site of action. *Genetical Research* **44**, 283–291.

Raphael, K. A. & Pennycuik, P. R. (1980). The site of action of the naked locus (*N*) in the mouse as determined by dermal–epidermal recombinations. *Journal of Embryology and Experimental Morphology* **57**, 143–153.

Slee, J. (1957). The morphology and development of 'Ragged' – a mutant affecting the skin and hair of the house mouse. a, Adult morphology. *Journal of Genetics* **55**, 100–121.

Straile, W. E. (1965). Root sheath–dermal papilla relationships and the control of hair growth. In *Biology of Skin and Hair Growth* (ed. A. G. Lyne and B. F. Short), pp. 35–37. Sydney: Angus & Robertson.

Straile, W. E., Chase, H. B. & Arsenault, C. (1961). Growth and differentiation of hair follicles between periods of activity and quiescence. *Journal of Experimental Zoology* **148**, 205–222.

Theriault, L. L., Dugan, D. D., Simons, S., Keen, C. L. & Hurley, L. S. (1977). Lipid and myelin abnormalities of brain in the crinkled mouse. *Proceedings of the Society of Experimental Biology and Medicine* **155**, 549–553.

Wilkinson, D. I. & Karasek, M. A. (1966). Skin lipids of a normal and a mutant (Asebic) mouse strain. *Journal of Investigative Dermatology* **47**, 449–455.