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DK/Lm: A Strain of Laboratory Mouse with an Unusual Expression of the Lethal Yellow (A^y) Phenotype

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Summary

DK/Lm is a new inbred mouse strain with over 20 generations of brother–sister mating. The genotype of the DK/Lm mouse at the black-brown locus is *b/b* and heterozygosity at the agouti locus (A^y/a) is maintained. DK/Lm-A^y/a mice become sable in phenotype at the first moult, whereas C57BL/6J-A^y/a mice do not. The sable phenotype is defined as that of a mouse whose basic colour is yellow (phaeomelanic) but whose dorsal aspect is more or less darkened by the presence of nonyellow (eumelanic) pigment. At about 6 months of age the DK/Lm mouse gradually reverts to yellow in phenotype.

Mice of the two strains are compared. Observations are discussed and related to hypotheses regarding gene action at the *a* and *e* loci. The new strain is a useful experimental model for study of the relationship between gene action at the agouti locus and the important pleiotropic effects influenced by this locus.

1. Introduction

We describe herein a new inbred mouse strain designated DK/Lm. The genotype of the DK/Lm mouse at the black-brown locus is *b/b* and heterozygosity at the agouti locus (A^y/a) is maintained by matings between yellow and brown mice. DK/Lm-A^y/a mice, when born, appear yellow and are indistinguishable from other lethal-yellow mice such as C57BL/6J-A^y/a. However, DK/Lm-A^y/a mice become sable in phenotype at the first moult, whereas C57BL/6J-A^y/a mice do not. The sable phenotype is defined as that of a mouse whose basic colour is yellow (phaeomelanic) but whose dorsal aspect is more or less darkened by the presence of nonyellow (eumelanic) pigment (Silvers, 1979). At about 6 months of age the DK/Lm mouse gradually reverts to yellow in phenotype. This strain originated from undocumented matings among mice received from MRC Radiobiology Unit, Harwell, descended from mice TOE/544.3d (*A/a Rw/+ go/go*), By/22.13d (A^y/a *b/b*), RW/186.3d (*A/A Rw/+*), JUN/80.1 g, h, i and JUN/79.4 a, b, c. Subsequently, the lethal yellow allele (A^y) also has been backcrossed onto JU/FaCtLm-$+^c$, and yellow (A^y/a *B/B*) mice of this stock exhibit the same striking phenotype.

The sabling and other phenotypic characteristics of the DK/Lm mouse, which appear to be influenced by the genotype of the mother, suggest an additional avenue of study of the pleiotropic effects that are associated with the yellow alleles at the agouti locus. In phenotypically yellow mice these pleiotropic effects include increased rate of growth, obesity, diabetes and tumour incidence, as well as an alteration of the eumelanin–phaeomelanin switch and in the size of the white area in mice that are white-spotted.

2. Materials and Methods

(i) *Mice*

Mice were fed Purina Mouse Breeder Chow (11% fat) and housed under standard conditions. DK/Lm mice do not breed well and do not express the full phenotypic potential unless fed as described above.

Table 1 lists the types of matings which produced mice for this study, and the numbers of mice of each phenotypic category that were weighed and graded for colour. In addition hairs were examined from several yellow animals that were the offspring of DK/Lm-A^y/a males and Ju/FaCtLm-$+^c/+^c$ *a/a* females.

(ii) *Evaluation of pigment phenotype*

Coat colour was scored weekly by the same person. Each A^y/a animal was assigned a number on a scale of 1 (entirely yellow) to 5 (very dark sable). Plate 1 illustrates the range of colour. Scores of + or − (for

Table 1. *Origin of mice used in this study: mice from the following matings were weighed and graded for coat colour*

Female	Male	Number of litters	Number of yellow males	Number of yellow females	Number of black males	Number of black females
C57BL/6J-A^y/a	× DK/Lm-A^y/a	2	5	6	4	1
DK/Lm-A^y/a	× C57BL/6J-a/a	2	3	4	5	3
DK/Lm-a/a	× C57BL/6J-A^y/a	2	2	6	4	2
C57BL/6J-a/a	× DK/Lm-A^y/a	2	3	5	3	1
DK/Lm-a/a	× DK/Lm-A^y/a	1	3	2	4	1
C57BL/6J-a/a	× C57BL/6J-A^y/a	5	8	5	4	7

example, 3+ or 3−) were assigned as deemed appropriate. Consistency of scoring was determined by comparing scores for the same mice over periods of time when no changes were taking place and was consistent within the range of + or −. Colour changes occurred only during the period of moult and were easily determined. The average of scores within each type of mating and before and after the first moult are shown in Fig. 1. Fifty-two yellow mice were scored.

(iii) *Microscopic examination of hairs*

Hairs were plucked from the same area of the rump of mice before and after the first moult. The hairs were affixed to microscope slides using egg white and were allowed to dry for about a week. They were then immersed sequentially, for approximately 1 week each, in 100% alcohol, 50% absolute alcohol/50% xylene, 100% xylene. Upon removal from xylene they were very quickly covered with mounting medium, before the xylene could evaporate, a cover slip was added, and the slide was allowed to dry for at least another week.

(iv) *Weight Gain*

Mice were weighed weekly. Ninety-one mice from 14 litters were weighed over a period of approximately 140 days (20 weeks).

(v) *Litter Size*

Fourteen litters were counted, with a total of 112 offspring.

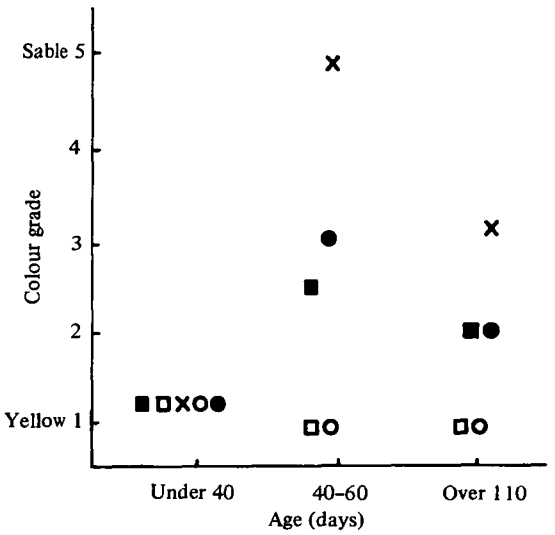

Fig. 1. Coat colour of A^y/a mice was scored weekly. Yellow = 1, very dark sable = 5 (see Plate 1). Scores of plus or minus (for example 3+ or 3−) were also given. Scores were consistent within the range of plus or minus. Variability among mice within each group was less than the variability which resulted from the necessarily subjective method of scoring. When no differences were observed, males and females were grouped together. The data are based upon the following numbers of mice: ○, C57BL/6J-A^y/a ($n = 13$); ×, DK/Lm-A^y/a ($n = 5$); ●. DK/Lm♀ × C57BL/6J♂, all offspring ($n = 15$); □, C57BL/6J♀ × DK/Lm♂, female offspring ($n = 6$); ■, C57BL/6J♀ × DK/Lm♂, male offspring ($n = 5$).

3. Results

(i) *Pigment Phenotype* (see Fig. 1 and Plate 1)

C57BL/6J × C57BL/6J mice are born yellow and remain yellow. DK/Lm × DK/Lm mice, however, are born yellow and darken dramatically at first moult so that coat colour becomes notably darker between days 40 and 60. The F_1 mice also darken, but not as much as do the DK/Lm mice. Subjectively, it appeared that F_1 offspring of DK/Lm mothers darkened approximately one grade more than did the F_1 offspring of C57BL/6J mothers. The latter is the only group in which males and females differed; the males darkened somewhat while females did not.

(ii) *Microscopic examination of hairs*

Study of the hairs (Plate 2) at high magnification clearly shows that even those animals macroscopically classified as clear yellow possess hairs with eumelanin granules. The yellow animals studied here differ therefore not by virtue of their ability or inability to synthesis eumelanin, but rather by the amount of eumelanin that they do synthesize. As a rule, darker

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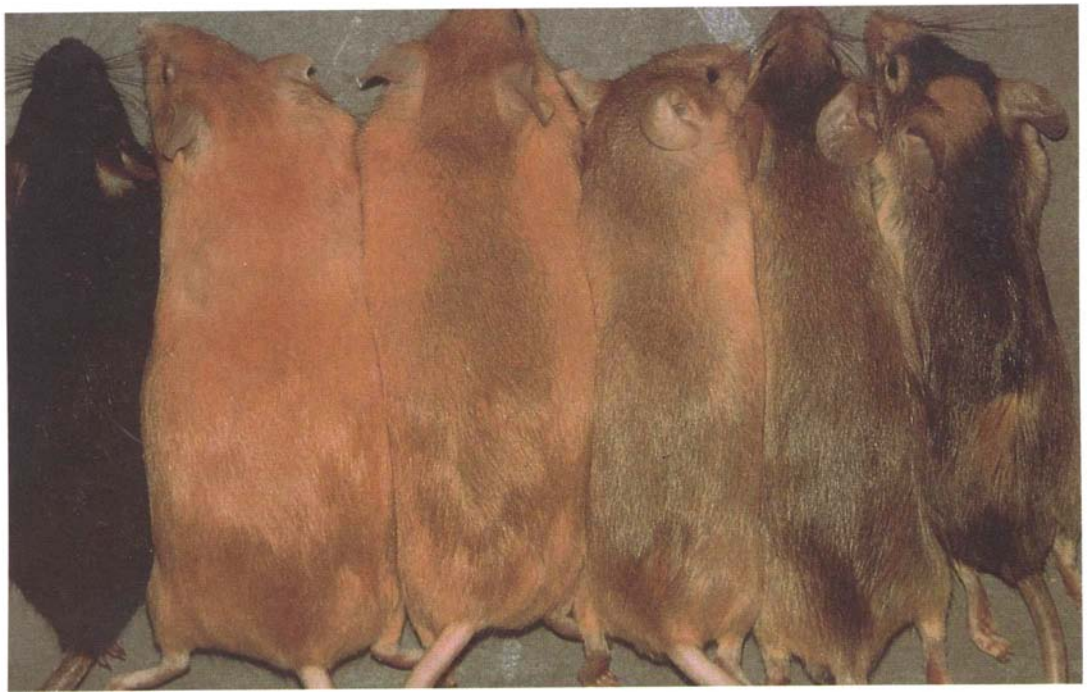

Five mice of the genotype $A^y/a\ B/-$, with a black ($a/a\ B/B$) mouse for comparison. From left to right the genetically yellow mice represent "grades" of pigmentation 1 through 5. The yellow spots on the grade 5 mouse are areas of the juvenile coat that have not yet been shed.

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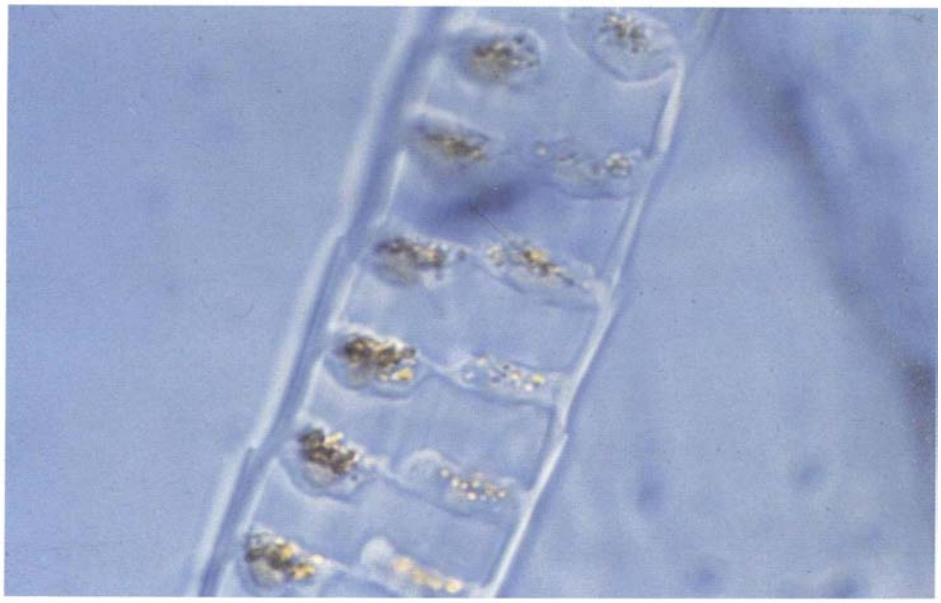
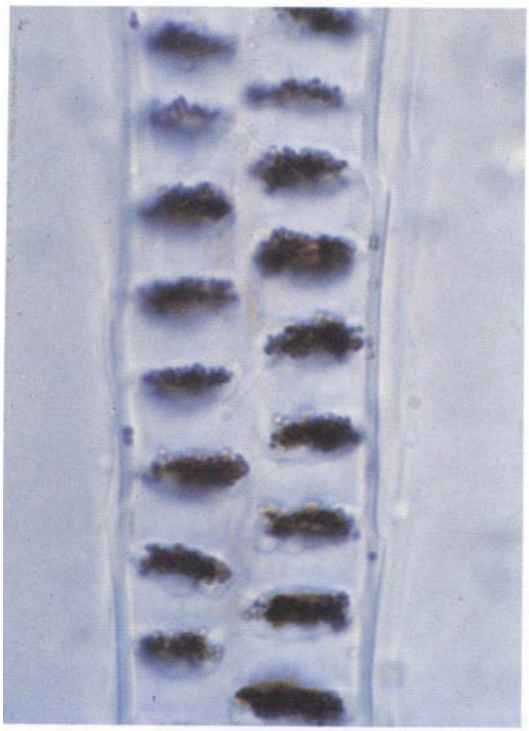


a



b

Photographs of hairs (1125x) taken from DK/Lm mice of the genotype $A^y/a\ b/b$.
a, Juvenile, b, Adult.

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animals had more hairs that contained eumelanin granules as well as more abundant eumelanin granules in each hair than did lighter animals. In addition, in lighter animals, eumelanin was limited for the most part to the over-hairs (awls, auchene, monotrichs) whereas the smaller zigzags were most often clear yellow. In darker animals, zigzags also contained eumelanin granules. Yet even in the darkest animals, totally eumelanic hairs were rare and were always over-hairs, never zigzags. This observation is in accord with previous reports which show that hairs produced by larger hair follicles tend to favour eumelanin production over phaeomelanin production (Galbraith, 1969; Galbraith & Wolff, 1974). The darkening effect, then, is achieved mainly by increased eumelaninization of the over-hair population.

Hairs containing eumelanin granules were almost always mosaic, i.e. consisted of a mixture of eumelanin and phaeomelanin granules. There was no patterned distribution of these two pigments although an exception occurs in B6DK × JU offspring which, surprisingly, have small numbers of agouti hairs (unpublished). Eumelanin and phaeomelanin granules were distributed throughout the length of the hair shaft where they comingled in individual medullary cells (Plate 2). Both types of granules also are found in the less heavily pigmented cortical cells. Although considerable variation in pigmentation existed among hairs, individual hairs exhibited a fairly constant ratio of eumelanin/phaeomelanin granules from tip to base. Each follicle therefore seems to behave as an autonomous pigmentary unit relative to its neighbours. In addition, this observation indicates that once hair follicle melanocytes have established a ratio of eumelanin/phaeomelanin synthesis, that ratio is maintained with only slight variations throughout the period of hair growth and melanocyte activity.

A progressively greater volume of individual medullary cells was filled with pigment granules in the following order; all yellow granules, yellow/brown granules, all brown granules, yellow/brown granules, yellow/black granules, all black granules. This is consistent with Russell's (cf. Searle, 1968) observations for yellow, brown, and black genotypes. Since several of the above granule phenotypes occur in the same animal, it is clear that the density of granule distribution is in accord with the type of granule(s) produced and transferred to keratinocytes rather than the genotype of the melanocyte–keratinocyte system.

DK animals increased their load of nonyellow pigment granules enormously in the adult hairs (Fig. 1, Plate 2). Indeed, the major pigment contained in adult DK dorsal hairs is brown eumelanin. All hair types, including zigzags, contained mainly brown granules which were distributed uniformly throughout the hair shaft with a lesser number of yellow granules. As described above, there was greater variation in eumelanin distribution among hairs than within individual hairs.

A comparison of F_1 progeny of different matings according to the degree of eumelaninization can be summarized as follows: None of the F_1 progeny darken to the extent of DK/Lm mice and there is little difference between juvenile and adult hairs of yellow progeny from C57BL/6J-a/a × DK/Lm-A^y/a and C57BL/6J-A^y/a × DK/Lm-A^y/a or a/a. On the other hand, adult hairs have more eumelanin than juvenile hairs in progeny of DK/Lm-a/a × C57BL/6J-$A^y a$ and DK/Lm-A^y/a × C57BL/6J-a/a. Therefore, any maternal effect noted here is a function of the strain of the dam rather than her agouti locus genotype.

(iii) *Weight gain*

Mice included in this study were from a group which were raised under similar environmental conditions. The weights of mice of each sex, offspring of each type of mating, were graphed separately. Results were similar in each case. Fig. 2 is one such graph, selected because the sample sizes were relatively large, illustrating the actual weights of yellow males from four types of matings. Yellow mice of the DK/Lm strain are more nearly normal in weight than are yellow mice of the C57BL/6J strain. This is consistent with previous observations of mice mutant at the agouti locus. In general, the mice which have large amounts of eumelanin in their hair pigmentation are not obese, while those that are mostly phaeomelanic are obese. The F_1 mice appear to be intermediate in rate of gain.

However, examination of Fig. 3 shows that the nonyellow littermates from the comparable matings also differ in rate of weight gain. This is not surprising, as the DK/L mice are smaller than the C57BL/6J mice at maturity, and they differ in eumelanic pigmentation. DK/Lm mice are b/b, in genotype, while C57BL/6J mice are B/B, the F_1 mice of course being heterozygous for the two alleles at the black/brown locus.

In an effort to provide a more meaningful comparison of rate of weight gain, we, therefore, subtracted the average weight of the nonyellow male mice from the average weight of their yellow littermates, divided by the average weight of the nonyellow mice, and again graphed the data, excluding the offspring of the B6 female × DK male mating, which included only one nonyellow mouse. Fig. 4 shows no apparent differences in this comparison between the DK × DK litters and the DK female × B6 male litters. There is, however, a clear difference between these two and the B6 × B6 litters. This result is consistent with previous observations of lethal-yellow mice which vary in amount of eumelanic pigmentation as a result of genetic or environmental manipulation. The genetically yellow mice with large amounts of eumelanic pigment do not exhibit the obesity which is one of the pleiotropic effects otherwise associated with the $A^y/-$ genotype (Silvers, 1979).

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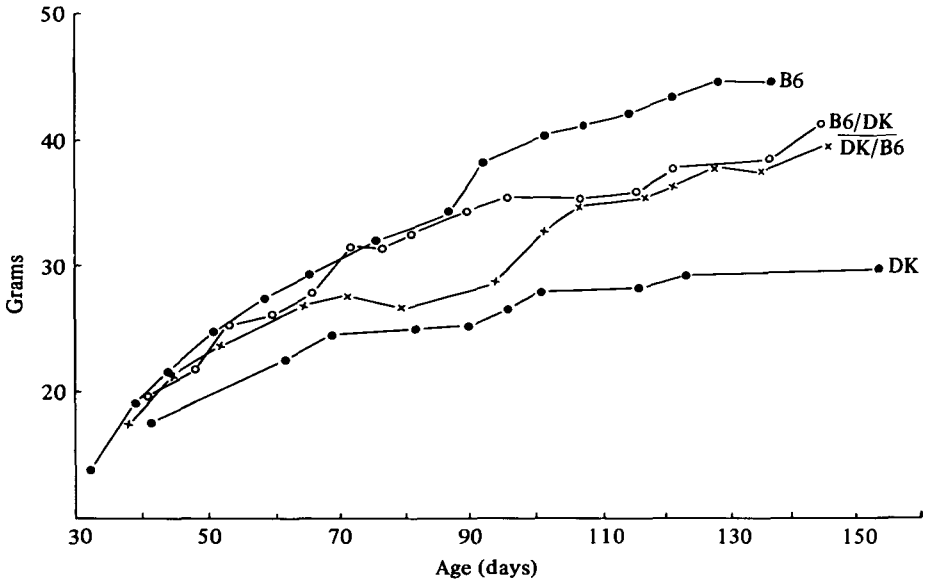

Fig. 2. Average weights of the yellow male mice from each of the separate types of matings.
B6 = C57BL/6J × C57BL/6J (n = 7).
DK = DK/Lm × DK/Lm (n = 3). B6/DK = C57BL/6J female × DK/Lm male (n = 7). DK/B6 = DK/Lm female × C57BL/6J male (n = 7). Results for females were similar.

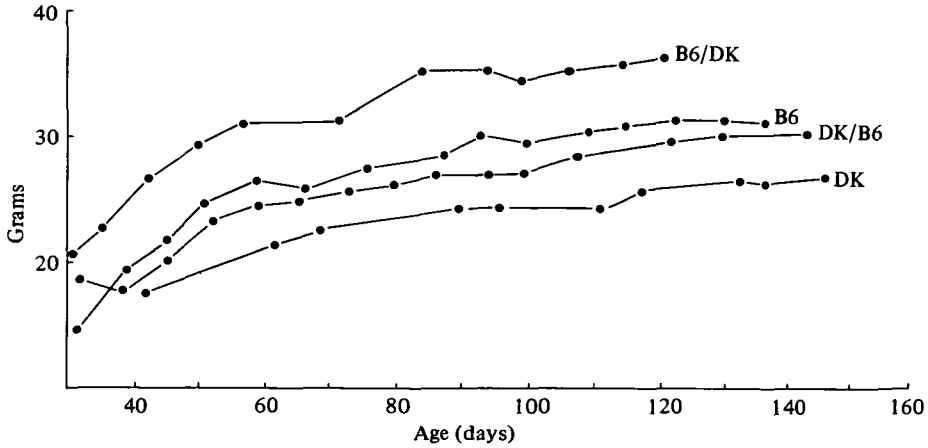

Fig. 3. Average weights of the non-yellow male mice from each of the separate types of matings.
B6 = C57BL/6J × C57BL/6J (n = 4).
DK = DK/Lm × DK/Lm (n = 3). B6/DK = C57BL/6J female × DK/Lm male (n = 1). DK/B6 = DK/Lm female × C57BL/6J male (n = 8). Results for females were similar.

(iv) *Litter size*

Average litter size in DK/Lm × DK/Lm (3 litters) is 11, C57BL/6J (3 litters) under these conditions average 5·6, and the F_1 (7 litters) average 9 young. Litters were excluded if both parents were yellow. Some of these litters were the same used in studies of weight gain and pigmentation changes; some were not.

4. Discussion

All experimental mice in this study carry the same allele (A^y) which affects rate of weight gain, obesity, blood glucose levels, tumour incidence and pigmentation. All controls are homozygous for the recessive allele, a. The data report effects of the background genome of two different inbred strains (DK/Lm and C57BL/6J) upon the phenotype of lethal yellow (A^y/a) mice.

Differences in pigmentation are obvious grossly and microscopically. C57BL/6J-A^y/a mice are grossly yellow throughout their lives, but with microscopic evidence that eumelanic melanosomes are also produced. DK/Lm-A^y/a mice are yellow until the first adult hair generation, when the new growth of hair contains a dramatically increased amount of eumelanin and the pelage becomes very dark, especially dorsally. From gross observation of hybrids, it appears that there is some maternal influence upon this darkening process.

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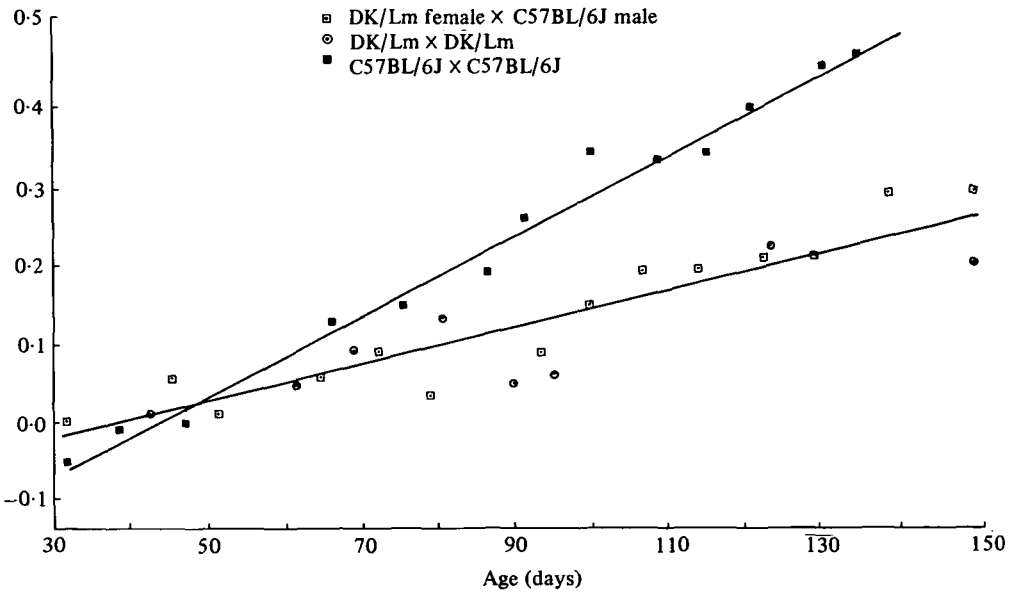

Fig. 4. Each data point represents the following calculation. The average weight of non-yellow male mice was subtracted from the average weight of their yellow male littermates, and the result was divided by the average weight of the non-yellow male mice. This was done to adjust for differences among the non-yellow control mice produced by the different matings. When this adjustment was made, no differences were observed between the data generated for the DK × DK litters and those from the DK female × B6 male litters. B6DK litters were excluded because of small sample size. Litters resulting from B6 × B6 matings showed a distinctly increased rate of gain in yellow compared with non-yellow mice when compared with DK × DK matings and DK × B6 matings.

Clearly, diet does affect the process. Only mice fed Purina Mouse Breeder Chow (11% fat) expressed full dark pigmentation. In fact, it has not been possible to maintain DK/Lm mice over more than three generations on other diets. Rate of weight gain is also markedly different in C57BL/6J-A^y/a mice than in DL/Lm-A^y/a mice, with the latter more nearly approximating control levels. The other important pleiotropic effects which result from presence of the A^y allele, viz. increased serum insulin level and increased incidence of tumours, were not evaluated in this study.

Tamate & Takeuchi (1984) proposed the hypothesis that the agouti locus controls changes in the follicular environment, which surrounds the melanocyte, by means of a 'messenger' which is recognized by a cell surface receptor found on melanocytes. The extension-locus alleles presumably influence the receptor itself or some other step, internal to the cell, by which the message is transduced. Tamate & Takeuchi (1981), reported that A^y/a skin which is making yellow pigment in tissue culture responds to MSH, or to dibutyryl cyclic AMP by production of black pigment, and suggested that the yellow/nonyellow 'switch' is mediated through the influence of MSH, working together with some other factor to influence levels of cAMP within melanocytes.

Some lines of melanoma cells are unable to form pigment (these cells are eumelanic) unless MSH or dibutyryl cyclic AMP are added to the culture medium (Wong *et al.* 1974). The addition of MSH is known to cause a rise in the level of cyclic AMP, and is thought to produce its effect upon pigmentation via one or more protein kinases. Other factors which affect the response of these cells to MSH are Ca^{2+}, Mg^{2+}, Mn^{2+} and prostaglandin E_2 (Wong *et al.* 1974). Because injection of α-MSH into yellow (A^y/a) mice induces formation of nonyellow pigment, (Geschwind, Huseby & Nishioka, 1972) we may assume some parallels between the observations *in vivo* and *in vitro*. On the basis of current evidence, it seems most likely that the agouti locus in some ways controls the 'message' which is sent to the cell (Silvers & Russell, 1955; Mayer & Fishbane, 1972; Poole, 1974). The extension locus may control receipt of this message (Lamoreux & Mayer, 1975; Tamate & Takeuchi, 1984). Alternatively, the extension locus may contain the structural gene for dopachrome oxidoreductase as suggested by Barber *et al.* (1985).

The manifold metabolic effects in $A^y/-$ and yellow A^{vy}/a mice demonstrate that the defect in message production (or utilization) by these animals is not confined to a single cell type. Thus, the study of mice of the genotype A^y/a or A^{vy}/a as models for factors controlling cancer, obesity and diabetes, and any physiological relationships among these conditions, must concentrate on identification of the messengers to which the melanocyte is a sensitive indicator, or of the ions and molecules which mediate the effectiveness of the messengers. The DK/Lm-A^y/a mouse and the less studied JU/Ct/Lm-A^y/a mouse which exhibits a similar phenotype may be useful in identifying the chemical 'messengers' which, in the abnormal condition, result in such deleterious effects.

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