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A comparative study of the coats of chimaeric mice and those of heterozygotes for *X*-linked genes

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SUMMARY

The coats of Ta–Ta^+ and c–c^+ chimaeric mice have been compared with those of Ta heterozygotes and animals heterozygous for an X-autosome translocation which causes c-variegation. With one exception all those features typical of the heterozygotes were found in their chimaeric counterparts, thus showing that the creation of two cell populations by X-inactivation can ultimately lead to the observed heterozygous phenotypes. The one exceptional feature not found in c-variegated chimaeric mice was the progessive increase in the proportion of pigmented hairs in c areas with age which occurs in X-autosome translocation heterozygotes.

The coat of a $Mo^{dp}/+$–Ta chimaeric animal was also investigated. Ta hairs showing only the colour effect of Mo^{dp} were numerous, suggesting that Mo^{dp} and Ta affect different cell populations. However, Ta hairs showing the structural effect of Mo^{dp} were very rare. The two observations suggest Mo^{dp} can operate independently upon two different cell types – melanocytes and hair follicle cells.

1. INTRODUCTION

According to the inactive-X theory of dosage compensation the mammalian female is a natural mosaic for clones of cells having either the maternally derived or paternally derived X chromosome genetically inactive (Lyon, 1961). Consistent with the hypothesis is the observation that women heterozygous for certain X-linked genes, whose products are distinguishable at the cellular level, possess two cell populations (Davidson, Nitowsky & Childs, 1963; Danes & Bearn, 1967; Migeon *et al.* 1968; Romeo & Migeon, 1970). In one, only the product of the maternally derived allele is detected and, in the other, only that of the paternally derived allele. Most tissues comprise a fine admixture of the two cell types. The variegated or banded coats exhibited by female mice heterozygous for X-linked coat colour, or coat structure genes, or for X-autosome translocations which cause variegation for autosomal genes translocated to the X, have also been taken to result from the creation of two cell populations by X-inactivation (Lyon, 1968). However, in this case the 'patch size' is large, regularities in the 'variegation' are apparent and, as Gruneberg (1969) has stressed, there are many detailed features of these heterozygous phenotypes which require additional postulates before they

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can be taken to be consistent with the inactive-*X* hypothesis. These include such factors as regularities of cell lineage, cell mingling, unequal cell multiplication, etc.

The development of techniques for the production of chimaeric mice by the fusion of embryos at the eight-cell stage provides a means of determining whether the 'variegations', observed in heterozygotes for *X*-linked genes can result from the presence of two differing cell populations. Regularities of coat colour and coat structure patterns have already been demonstrated in chimaeric mice and attributed to regularities in melanoblast and hair-follicle cell lineages, and in fusions utilizing the *X*-linked gene, *Tortoiseshell*, variegated phenotypes typical of the heterozygote have been obtained (Mintz, 1971). Should all features characteristic of heterozygotes be found in their chimaeric counterparts, the difficulties they have created for the inactive-*X* hypothesis must be considered invalid.

The present communication reports the results of comparative studies on the *albino* (*c*)-variegation found in the coats of c–c^+ chimaeric mice and in those of females heterozygous for the *flecked* (*fd*) *X*-autosome translocation, T(1; *X*)Ct (Cattanach, 1961) and on the effect of the sex-linked gene, *Tabby* (*Ta*), on the coats of Ta–Ta^+ chimaeric mice and on that of the normal *Ta* heterozygote. The coat of a *Dappled–Tabby* (Mo^{dp}–*Ta*) chimaeric animal will also be described. Where possible, chromosome markers were included in one component of the fusions so that the distribution and frequency of the two cell populations in tissues other than hair could be estimated.

2. METHODS

(i) *Production of chimaeric mice*

Donors of eggs were induced to ovulate and mate to males of appropriate genotypes by i.p. injection of 4 i.u. PMS (Gestyl) followed in 41–43 h by 3 i.u. HCG (Pregnyl). Eight-cell embryos from these hormonally primed females were collected on day 2 (day of plug is day 0), fused by pairs, cultured over-night, then inserted by glass pipette into the uterus of hormonally primed, sterile-mated host females which were at day 2 of pseudopregnancy.

The methods of fusion and culture were a composite of those summarized by Mintz (1967*a*), Biggers, Whitten & Whittingham (1971) and Wegmann (referred to by Wegmann & Gilman, 1970). The culture medium was the foetal calf-serum – Earle's BSS – lactate medium described by Mintz (1964). It was adjusted to pH 7·0 at the time of preparation by addition of a 7·5 % $NaHCO_3$ solution and phenol red as indicator. The pronase solution for removing the zona pellucida was that developed by Mintz (1962) and modified by Whitten (see Biggers *et al.* 1971), consisting of 0·5 % pronase in phosphate buffered saline (PBS) with polyvinyl-pyrollidone (PVP) added. Rinsing of embryos was done either in the culture medium or in PBS. All solutions were sterilized by filtration.

The handling of eggs, their pronasing, washing and culture were all carried out in sterile plastic culture dishes (Falcon). Paraffin oil (Boots) used for the microdrop cultures was first sterilized by dry heat at 150 °C for 1 h, gassed with 5 % CO_2 in

air, and pre-equilibrated with a sample of the culture medium for 24–48 h (see Biggers *et al.* 1971). The culture dishes containing denuded embryos adhering to one another by pairs were placed in a small plastic sandwich box and incubated at 37 °C for 22–26 h. An approximate 5–10 % CO_2 atmosphere was maintained in the box by dissolving 3–6 mineral-water tablets (Sparkloid soluble tablets, Boots) in a vial of water and then sealing the box with masking tape.

Surrogate hosts were 'PT' females, homozygous for *a*, *b*, c^{ch}, *p*, *d*, *se*, *s*, and maintained at Harwell as a closed outbred colony. These were nulliparous females hormonally primed by i.p. injection of 4 i.u. PMS (Gestyl) followed in 41–43 h with 3 i.u. HCG (Pregnyl). To induce a state of pseudopregnancy, surrogate hosts were mated to genetically sterile males heterozygous for the T145H translocation. Fused embryos at the late morula-blastocyst stage were injected by glass pipette into a single horn of the uterus of a female anaesthetized by i.p. injection of sodium amytal. Each surrogate host received 5–6 fused embryos.

(ii) *Genotypes fused*

Three different genotype fusions were carried out. The first comprised the fusion of embryos from the cross of *Ta*/*Ta* ♀♀ × *Ta Y* ♂♂ with those from T1Ald/T1Ald *cc* ♀♀ × T1Ald/T1Ald *cc*♂♂. This fusion was made to provide information on the *c*-variegation that results from a $c - c^+$ combination and also on the banding pattern expected to result from a Ta–Ta^+ combination. The Robertsonian translocation, T1Ald, is a good chromosome marker and was included in this experiment to provide a means of assessing the distribution of the two cell populations in various tissues.

The second type of fusion was that of embryos from the cross of *Ta*/*Ta* ♀♀ × *Ta Y* ♂♂ with those from the cross of T145H p^+/T145H$^+$ *p* ♀♀ × T145H$^+$ *p*/T145H$^+$ *p* ♂♂. This combination should again yield chimaeric *Ta* progeny. The reciprocal translocation, T145H, is not a reliable chromosome marker; it was incorporated into this experiment with the aim of testing whether the sterility, typical of the male heterozygote (Lyon & Meredith, 1966), is determined at the cell or organism level. Thus, it was considered possible that T145H/+ germ cells might be able to complete meiosis and produce functional sperm in a chimaeric male when the other component cell line was that of a chromosomally normal male. The use of T145H further offered the possibility of determining whether the aneuploid embryos from the translocation heterozygote could form viable cell lines when present with a normal cell population (*Ta*) in a chimaeric animal.

The third type of fusion comprised the combination of genotypes differing with respect to two *X*-linked genes. Embryos from the *Ta*/*Ta* ♀♀ × *Ta Y* ♂♂ cross were fused with those from the cross of Mo^{dp}/+ ♀♀ × Mo^+Y, T163H/T163H ♂♂. The aim of this experiment was to check for interaction between *Ta* and Mo^{dp}, both of which affect coat structure. The Robertsonian translocation, T163H, was utilized as a chromosome marker.

(iii) *Tests on fusion products*

All mice derived from the fusion experiments were mated when 6 weeks' old to normal animals carrying the recessive gene markers employed in each particular fusion. Thus, those from the *Ta* – T1Ald *c* combination were mated to *c* animals, those from the *Ta* – T145H/*p* combination to *p* animals, and those from the *Ta*–*Mo*^{dp} combination to + animals. The progeny were classified for *c* or *p* in appropriate crosses at birth, and for *Ta* and *Mo*^{dp} at 3 weeks of age.

Hair samples were taken from all animals derived from the fusion experiments when they were 5–6 months old. For overtly chimaeric mice, an attempt was made to sample hair selectively from the band and interband areas. However, especially in the case of the fine *Ta* banding, this proved to be very difficult. All samples were taken from the mid-dorsal region of the body and each comprised about 30–40 hairs. These were arranged side by side on a slide flooded with alcohol, then dried, covered with a coverslip and subjected to detailed analysis with a Zeiss photomicroscope at a magnification of × 200. For comparative purposes hair was similarly sampled and studied from *Ta* and *c*-variegated *fd* mice. All slides were coded.

When the chimaeric mice were about 1 year old and it was deemed that they were as sufficiently progeny-tested as practicable they were killed and chromosome preparations were made from the tissues of all those carrying chromosome markers. Fredga's (1964) method was used for corneal epithelium, a modified method of Ford & Hamerton (1956) was used for bone-marrow cells colchecinized *in vitro*, and the method of Evans, Breckon & Ford (1964) was applied to testis. Chromosome preparations were also obtained from fibroblast cultures of skin taken from the band and interband areas in the mid-dorsal areas of the body.

The heads of all animals carrying *Ta* were sent to Professor H. Grüneberg for analysis of the teeth. The results of this study will be the subject of a separate publication.

3. RESULTS

(i) *Evidence of chimaerism*

Eight animals were produced from the various fusions (Table 1). Before analysis of their hair, evidence of chimaerism was sought from three sources: gross phenotype, progeny tests and chromosome studies.

(*a*) *Gross phenotype*

Five animals, CH/1, 2, 5, 7 and 9, showed grossly obvious 'variegation' in their coats, of a kind to be expected from the particular genotypes fused. The coat of CH/11 was variegated with a pattern typical of *Mo*^{dp}/+ heterozygous females, but this did not constitute evidence of chimaerism since the genotype of one component of the fusion could have been *Mo*^{dp}/+. As well as this, CH/7 was a phenotypic intersex. It was male-type, but the penis was small, the ano-genital distance was less than that of a normal male, and the scrotum was ill-defined. It was placed with a series of different females but there was no evidence of mating.

Table 1. *Results of fusion experiments*

Genotype of fused embryos A	Genotype of fused embryos B	Chimaera	Phenotype	Evidence of chimaerism* 1	2	3
Ta/*Ta* or *Ta Y*	T1Ald/T1Ald *cc*	CH/1†	*c*-var. ♂	+	−	+
		CH/2†	*c*-var. ♂	+	+	+
Ta/*Ta* or *Ta Y*	T145H p^+/T145H$^+$ *p* or T145H$^+$ *p*/T145H$^+$ *p*	CH/5†	*Ta*/+ ♀	+	−	−
		CH/6†	+ ♀	−	+	−
		CH/7	*Ta*/+ ♀	+	−	−
		CH/8	*Ta* ♂	−	−	−
		CH/9	Extreme *Ta*/+ ♂	+	+	−
	Mo^{dp}/+, T163H/+ or Mo^{dp} *Y*, T163H/+	CH/11	Mo^{dp}/+ ♀	−	+	+

* 1, Gross phenotype; 2, progeny test; 3, chromosome studies.
† Born by Caesarian section. ♀ indicates intersex.

At dissection, the gonads of CH/7 were found to be located in the abdomen and each was attached to a normal epididymis and vas. All other reproductive organs were those of a normal male. The left gonad was extremely small. On sectioning it was found to be predominantly testis, tubules containing dividing spermatogonia and a few spermatocytes being present. The interstitial tissue appeared hypertrophied in relation to the tubules and, especially in one region, resembled that of an ovary. The right gonad was considerably larger but much of the surface was occupied by fluid-filled cysts. On sectioning, one lobe was found to be testicular and similar to that of the left gonad. The remainder comprised what could have been ovarian tissue but there was a great deal of other abnormal tissue which appeared to be partially differentiated as in a teratoma (Stevens & Hummel, 1957). A few structures resembling follicles could be found.

The intersex condition may be taken to indicate that CH/7 is an *XX*/*XY* chimaera (Mintz, 1968, 1969) and since *p*-variegation was not detected, it is most probable that this animal derived from the fusion of a T145Hp^+/+*p* embryo with one carrying *Ta*. It is also conceivable that the + component was an aneuploid product of T145H. It is not known which component was *XX* and which was *XY*.

(*b*) *Progeny tests*

Two of the four fertile animals which were known to be chimaeric from their gross phenotype, CH/2 and 9, also proved to be so on progeny test (Table 2). From the work of others (Mintz, 1968; Mystkowska & Tarkowski, 1968; Tarkowski, 1961) this means that the fused components of these animals must have been of like sex (*XY*/*XY* in both cases). CH/1 and CH/5 both left progeny attributable to only one component of their genotype but, in both, this same component predominated very heavily in the coat (*c* in CH/1 and *Ta*$^+$ in CH/5). Hence it is not

Table 2. *Results of progeny tests on chimaeras*

		Progeny					
Animal	Phenotype	*Ta*/+ ♀♀	*Ta* ♂♂	+ ♀♀	+ ♂♂	*c* ♀♀	*c* ♂♂
CH/1	*c*-var. ♂	0	0	0	0	147	
CH/2	*c*-var. ♂	23	0	0	13	64	59
						p ♀♀	*p* ♂♂
CH/5	*Ta*/+ ♀	0	0	9	8	3	4
CH/6	+ ♀	3	2	0	1	1	2
CH/8	*Ta* ♂	56	0	0	57	0	0
CH/9	Extreme *Ta*/+ ♂	53	0	0	58	5	3
						Mo^{dp}/+ ♀♀	Mo^{dp} ♂♂
CH/11	Mo^{dp}/+ ♀	4	5	19	19	13	0*

* Mo^{dp} is a pre-natal lethal in the male.

known whether the components were of unlike sex, or of like sex with one component by chance not represented in the germ cells.

In addition, two animals not obviously chimaeric in the coat proved to be so on progeny test, CH/6 and CH/11 (Table 2). CH/11's progeny showed that her two components were genetically $Mo^{dp}/+(XX)$ and *Ta*/*Ta* (*XX*).

For the chimaeras produced from the second type of fusion, involving T145H, the progeny tests provided information about the exact genotype of the T145H component. CH/5 and CH/6 produced both + and *p* progeny, so that one component of each must have been T145H +/+*p* (*XX*). CH/9 produced *p* daughters and sons as well as *Ta*/+ daughters and + sons. The absence of + daughters suggests that the chimaerism did not involve T145H and in agreement with this conclusion is the finding that of 400 spermatocytes examined none carried the translocation. This cytological evidence also virtually eliminates the possibility that the non-*Ta* component was an aneuploid T145H product. It must be concluded that CH/9 was a *Ta* (*XY*)–*pp* (*XY*) chimaera.

CH/8 was a phenotypically normal male showing the uniformly abnormal coat of the hemizygous *Ta* male. Its progeny were all attributable to a *Ta*/*Y* genotype (Table 2), and at sacrifice all the spermatocytes examined from each testis (200 cells) appeared to be chromosomally normal. There was thus no evidence of chimaerism in this animal. It is thus possible that the + component was not included in the embryoblast (Mintz, 1968; Mystkowska & Tarkowski, 1968; Tarkowski, 1961) but it is also possible that it was an aneuploid product of T145H which proved to be inviable even at the cell level in a chimaeric animal.

(c) *Chromosome studies*

Since T145H was not considered a sufficiently good marker for mitotic studies only three of the eight chimaeras were studied cytologically. All three showed evidence of their chimaerism chromosomally (Table 3). The proportions of the two types of cell varied both among animals and among tissues within an animal as has been found by Nesbitt (1971) in mouse *X* chromosome inactivation mosaics.

Table 3. *Results of chromosome studies on CH/1, CH/2 and CH/11*

	CH/1		CH/2		CH/11	
Tissue	No. cells	% T1Ald	No. cells	% T1Ald	No. cells	% T163H
Corneal epithelium						
Eye 1	68	100	72	100	43	77
Eye 2			47	79	51	76
Bone marrow	100	100	100	59	100	89
Skin fibroblasts						
Overall	792	99	1189	92	333	48
From + areas	*	*	424	82	116	54
From mixed areas	269	98·5	187	92·5	217	44
From *c* or Mo^{dp} areas	523	99·2	578	99	†	†
Testis						
(1) Sample 1	100	100	100	45	—	—
Sample 2	100	100	100	47	—	—
(2) Sample 1	100	100	100	77	—	—
Sample 2	100	100	100	79	—	—

* There were no clear + areas in CH/1.
† There were no clear Mo^{dp} areas.

In CH/1, in which the *c* (T1Ald) component predominated in the coat and gave rise to all of the progeny, this cell line also predominated in all other tissues examined. In CH/2 the *c* (T1Ald) component comprised only about half the coat and formed a lower proportion of the other tissues than in CH/1. A point of interest is that the testes of this animal differed in proportions of cells carrying T1Ald and the overall frequency in the testes (62 %) differed significantly ($\chi^2_3 = 12{\cdot}03$) from the proportion among the progeny (Table 2). This discrepancy might be attributable to gametic selection in favour of the cell line carrying T1Ald, or to disproportionate contributions by the two testes.

A further point concerning CH/2 was that there appeared to be a correlation between hair colour and skin fibroblast composition. Clearly further animals must be studied before this can be considered meaningful.

In CH/11 both cell lines were present in all tissues examined, despite the fact that the *Ta* component was difficult or impossible to see macroscopically in her coat.

Thus, considering all lines of evidence it is clear that seven of our eight animals were chimaeras, and therefore suitable for detailed studies of their coats.

(ii) *Hair analysis*

The coats of the seven chimaeras were compared with those of heterozygotes for *X*-linked genes and the *fd* translocation, both at the gross or macroscopic level, and by the study of individual hairs. There were three kinds of variegation to be considered: that due to *albino*, *c*, and expressed in pigment cells, that due to *Ta*, and that due to Mo^{dp} and *Ta* in animal CH/11.

Table 4. *Hair analysis of* c*-variegated animals*

Animal	Area	No. hairs			Proportion of mixed hairs (%)	Mixed hairs: Proportion polarized (%)	Mixed hairs: Proportion reverse polarity (%)
		c	*M*	+			
CH/1 ♂	*c*	282	0	0	0	0	0
	+	182	53	17	21	49	11
CH/2 ♂	*c*	229	2	0	1	50	0
	+	36	79	159	29	34	0
Young *fd* ♀	*c*	99	20	0	16	15	40
	+	20	21	78	17	47	0
Young *fd* ♀	*c*	101	34	4	25	24	18
	+	11	42	79	30	50	12
Old *fd* ♀	*c*	85	48	3	35	11	25
	+	36	18	101	12	17	11
Old *fd* ♀	*c*	82	36	2	30	19	17
	+	34	28	82	19	11	11

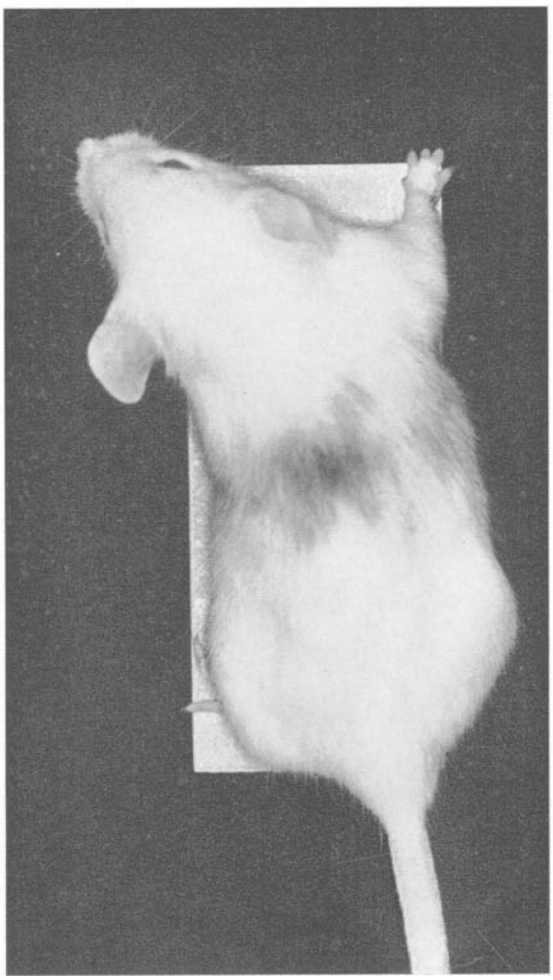
M = mixed or variegated single hairs.

(*a*) *Albino variegation*

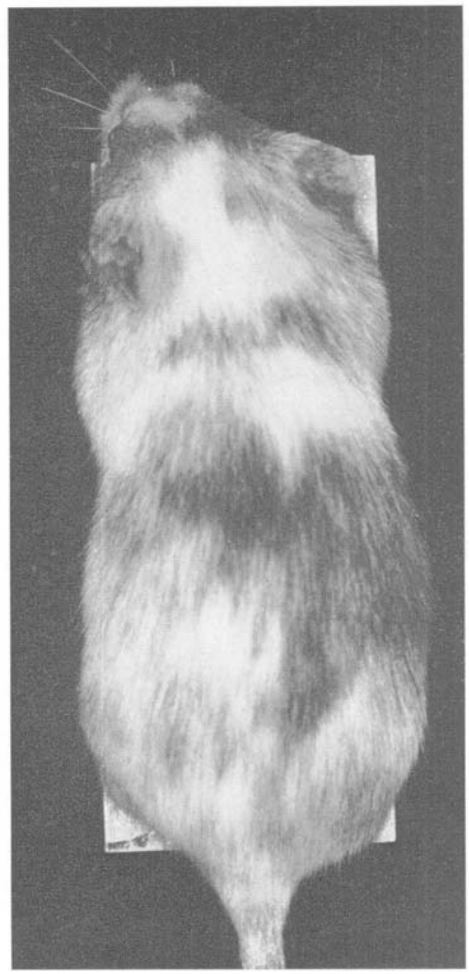
About 90 % of the coat of CH/1 was unpigmented (Plate 1) with pigmented hair limited to a single transverse band on each side of the body, each located in the region of the upper rib-cage. On the back the bands were substantially interspersed with *c* hair but on the belly the two areas were more discrete. The pigmented areas appeared *agouti*; they were too limited in size to permit the detection of any of the very much finer *Ta* banding that may have been present. The ears, eyes and tail appeared to be devoid of pigment.

In CH/2 the proportion of pigmented hair was quite substantial (50 %) (Plate 1). The distribution of + and *c* hair clearly showed banding in accordance with the archetypal melanoblast pattern described by Mintz (1967*b*), with a greater admixture of the two hair colours on the rump than on other regions. As in CH/1, the + and *c* areas were more discrete on the belly than on the back and there was a sharp mid-line effect on the belly. The pigmented hair appeared to be *agouti*, but there was a suggestion of *Ta* banding in some of the larger pigmented areas. The ears, eyes and tail were clearly *c*-variegated.

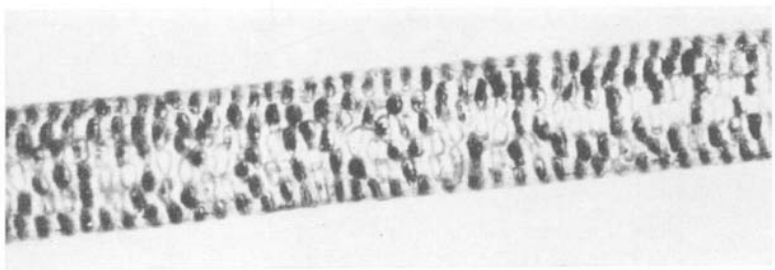
While being in full accord with Mintz's (1967*b*) archetypal melanoblast pattern the *c*-variegated phenotypes expressed by CH/1 and CH/2 were macroscopically indistinguishable from those of females heterozygous for the *fd* *X*-autosome translocation, T(1; *X*)Ct. In these animals the archetypal melanoblast pattern is also found, and a greater admixture of the two hair colours on the rump, back-belly differences and ventral and dorsal mid-line effects are also regularly observed. However, in one respect chimaeric and *fd* *c*-variegated mice clearly differ; as *fd* animals age the *c* areas progessively become partially pigmented, with the result that in the aged animal much of the 'white' hair shows some pigmentation (Cattanach &



The *c*-variegation of CH/1.



The *c*-variegation of CH/2.



A partially pigmented (mixed) hair from CH/2.

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Isaacson, 1965). It seems that each hair growth cycle gives hairs with more pigment than before (Cattanach, unpublished). This was not found in either CH/1 or CH/2 even though they were over a year old at the time of sacrifice.

For the detailed study of individual hairs the coats of two 6-week-old *fd* females and two 4-month-old *fd* females were investigated as well as those of CH/1 and CH/2. All four *fd* animals were selected as showing similar amounts of *c*-variegation to CH/2. None was available with such extensive *c* areas as CH/1 at the time of the experiment.

As Gruneberg (1966, 1969) has indicated, the *c*-variegated coats of *c*-variegated *fd* mice do not contain only pigmented and unpigmented hair. Mixed hair with pigmented and unpigmented sectors may also be found and, in such hair, pigmentation tends to be polarized towards the tip. Mixed hair is generally found in those areas where there is an admixture of pigmented and unpigmented hair. According to Gruneberg (1969), in the large *c* areas, the few hairs with pigment usually show a reverse polarity (unpigmented tip and pigmented base).

The results of our study on the coats of the *fd* females (Table 4) are in full agreement with Gruneberg's observations. Mixed hairs clearly were present in the coat, and, at least in the young females, a high proportion tended to be polarized; in the *c* areas, mixed hairs tended to show a reverse polarity. The only difference between our observations and those of Gruneberg (1969) was that while he found few hairs with pigment in large *c* areas, we found quite a high proportion, especially in the older animals. However, from independent observations we have concluded that the age effect is dependent upon genetic background (Cattanach, unpublished).

Turning to the chimaeric animals, it may be seen that mixed hairs were also found in these animals (Plate 1) and that a very high proportion were polarized (Table 4). On the basis of this criterion, no distinction can be made between CH/1 and CH/2 and the *fd* females studied. The principal difference lay in the extreme shortage of pigment-containing hairs in the *c* areas. In the case of CH/1, the + areas were so minimal that pigmented hairs resulting from a migration of pigmented melanocyts from neighbouring pigmented areas might not be expected. This cannot be true for CH/2 whose coat showed extensive areas of pigmentation and yet the *c* areas were effectively free of pigmented hairs. The difference from *fd* females is emphasized when it is remembered that the pigmentation of *c* hairs in *fd* animals is enhanced with age and yet CH/1 and CH/2 possessed clear *c* areas even when over 1 year old. It may be noted that the difference only appears to apply to the *c* areas; the proportion of mixed hairs in the + areas of the chimaeric and *fd* mice studied differed little.

(*b*) *Tabby variegation*

Although all seven of the chimaeras had a *Ta* component, macroscopic *Ta* banding was only obvious on CH/5, 7 and 9.

CH/5 exhibited the finely banded coat typical of *Ta* heterozygotes. Although the bands were widely distributed over the body and the degree of *Ta* effect was well

within the range of *Ta*/+ heterozygotes, the bands were very fine, giving a predominantly wild-type appearance to the coat. By contrast, CH/9 showed an extreme expression of *Ta* in his coat. At first sight he appeared to be hemizygous *Ta*, but his tail was hairy, not naked as in the hemizygote, and there were several fine dark bands in the left lumbar region. This extreme *Ta* effect lay at the limit of or beyond the range of the normal *Ta* heterozygote. The third banded animal, CH/7, possessed a coat grossly indistinguishable from that of a typical *Ta*/+ heterozygote.

Detailed descriptions of the coat of the normal mouse and the abnormalities caused by *Ta* have been given by Gruneberg (1966, 1969). The essential features are that in the *Ta* hemizygote the zigzags, auchnes, awls and guard hairs, which comprise the normal coat, are generally absent and replaced with a single aberrant hair type which has been classified as an abnormal awl. The coat of the heterozygous female contains both normal and *Ta* hairs but all inter-grades between normal and *Ta* are also found. The *Ta* bands and the inter-band areas predominantly comprise *Ta* and normal hair, respectively, but the difference is only one of proportion. In the present study, recognition of anomalous hair types was based entirely on the criteria described by Gruneberg (1966, 1969), but in order to simplify the presentation of the data only those abnormalities caused by *Ta* will be indicated.

In addition to the chimaeric mice, the coats of two wild-type males, two hemizygous *Ta* males and four heterozygous *Ta* females were studied. Two of the heterozygotes were selected as showing the type of *Ta* banding pattern exhibited by CH/5 and CH/7. The other two were selected because they showed large areas of *Ta* and normal-appearing + areas which were relatively clearly defined. It was hoped that in these animals there would be a lower admixture of hair from adjoining *Ta* and + regions, thus providing optimal conditions for detecting two distinct hair populations.

Our findings on the coats of normal, hemizygous *Ta* and heterozygous *Ta* animals (Table 5) agree well with those of Gruneberg (1966, 1969). Only abnormal awls were found in the hemizygous *Ta* males and a range from abnormal to normal hairs were found in both *Ta* and + areas of heterozygotes, only the proportion of this *Ta* effect differing in the two types of regions. Among the chimaeric group CH/6 and CH/8 showed uniformly normal and hemizygous *Ta* coats, respectively, as the gross phenotypes suggested, but the coats of CH/5 and CH/7 exhibited all of the same features as those described in *Ta* heterozygotes. They were thus indistinguishable from the heterozygote at all levels examined. CH/9 perhaps proved to be of the greatest interest; the majority of the coat was almost entirely that of the hemizygote, only two normal awls being found, but in the few dark bands a high proportion of normal hairs was detected. Thus, while the dark bands of predominantly wild-type *Ta* heterozygotes and *Ta* chimaeras (CH/5 and CH/7) principally contained *Ta* hair, the dark bands of CH/9, whose coat was predominantly *Ta*, principally contained normal hair. The dark colour of the bands does not therefore result from the composition of the hair within it.

Table 5. *Classification of* Ta *and normal hair types in chimaeric mice and* Ta/+ *and* Ta/*Y controls*

Animal	Area sampled	Z	Auch.		A		G.H.		% Z	% *Ta*	Total
			Ta	N	*Ta*	N	*Ta*	N			
Wild-type (2 ♂♂)	(Random)	219	—	10	—	87	—	4	68	0	320
Ta (2 ♂♂)	(Random)	—	—	—	364	—	—	—	0	100	364
Wide-banded *Ta*/+	+ areas	99	—	2	3	54	—	2	62	1.8	160
	Ta areas	—	—	—	148	—	—	—	0.6	99	149
Wide-banded *Ta*/+	+ areas	43	—	—	58	22	1	1	33	44	131
	Ta areas	—	—	—	137	—	1	3	0	97	141
Fine-banded *Ta*/+	+ areas	67	5	3	19	40	—	1	50	14	135
	Ta areas	38	2	2	76	18	—	1	28	55	137
Fine-banded *Ta*/+	+ areas	59	—	8	9	38	—	—	51	8	115
	Ta areas	62	6	5	55	33	1	2	53	47	117
CH/1	(Random)	36	2	43	2	148	—	2	57	0·3	534
CH/2	(Random)	30	5	39	13	123	—	10	62	2·5	500
CH/5	+ areas	94	—	25	9	48	—	—	53	0·5	172
	Ta areas	99	2	18	25	48	—	1	52	13.0	200
CH/6	(Random)	184	—	4	—	62	—	2	70	0	262
CH/7	+ areas	102	2	8	23	55	—	1	53	12	190
	Ta areas	97	—	5	58	34	—	—	50	30	194
CH/8	(Random)	—	—	—	316	—	—	—	0	100	316
CH/9	Bands	42	—	2	104	28	—	—	24	59	176
	Ta areas	—	—	—	228	2	—	—	0	99	230

Z = zigzags; Auch. = auchenes; A = awls; G.H. = guard hairs.

Table 6. *Correlation between* Mo^{dp} *hair colour and structure and* Ta *abnormalities in CH/11*

Hair structure	Hair colour	*Ta*	Non-*Ta*
Mo^{dp}	*Mo^{dp}**	1	190
Mo^{dp}	+	–	9
+	*Mo^{dp}**	14	93
+	+	14	181
		29	473

* Mixed hair taken as *Mo^{dp}* in colour.

The samples from CH/1 and CH/2 showed that there was a small contribution by the *Ta* component to the coats of both animals. All four combinations of *Ta* and normal and pigmented and unpigmented hair were found. Clearly *Ta* and *c* operate upon different cell populations, hair follicle cells and melanoblasts, respectively (Mintz, 1971).

(*c*) Mo^{dp}–Ta *abnormalities*

Chimaera CH/11, as previously mentioned, showed variegation typical of a *Mo^{dp}*/+ heterozygote, with a possible but uncertain suggestion of *Ta* banding in the pigmented areas.

The coat of *Mo^{dp}* animals has not been studied in detail but Gruneberg (1969) has described the abnormalities caused by *Brindled* (*Mo^{br}*), which is considered to be its allele. Although the *Mo^{dp}* males die pre-natally and hence cannot be studied, many of the coat abnormalities seen in the heterozygote resemble those found in its *Mo^{br}* equivalent. Medullary cells appear to be fused, perhaps due to the presence of liquid (absence of air) as suggested by Gruneberg (1969), and a flattening of the hair is also apparent. In addition to the structural abnormalities, pigment also tends to be missing. However, there is not a complete correlation between structural abnormalities and lack of pigment; four classes of hair may be found. Mintz (1971) has reported that a third allele, *Tortoiseshell* (*To*), exhibits both a hair follicle and a melanoblast pattern and it seems probable that this also applies to *Mo^{dp}*. The variegated coat of the *Mo^{dp}* heterozygote appears to result from the melanoblast pattern, closely resembling that of *fd* mice. Like *fd* but unlike *Mo^{br}*, which shows the hair follicle banding pattern in the heterozygote, the two hair colours of *Mo^{dp}* females are clumped by the spotting genes, *recessive spotting* (*s*) and belted (*bt*) (Cattanach, unpublished).

CH/11 has been shown to be derived from the fusion of *Ta*/*Ta* and *Mo^{dp}*/+ embryos. Since the *Mo^{dp}* component is represented in the heterozygous condition, CH/11 provides only a partial test for interaction between *Mo^{dp}* and *Ta*. Wild-type hair would be expected, as in a *Mo^{dp}* heterozygote, but the presence of hairs showing the effects of both genes would constitute evidence of an interaction.

Hair samples were taken from apparently *Mo^{dp}* and apparently + regions of

CH/11's coat, but each kind of sample contained both normal and Mo^{dp}-type hairs, the difference being merely in frequency of the two types. Hence, in Table 6 hairs from all regions have been pooled. There were very few hairs showing *Ta* abnormalities, and of these only one also showed Mo^{dp} effects on hair structure. The difference in frequency of *Ta* abnormalities between structurally Mo^{dp} and + hair is statistically highly significant ($\chi^2_3 = 19{\cdot}9$). This is consistent with the idea that the two genes were acting in the two component cell lines of the same cell type, although the possibility cannot be excluded that the shortage of combined Mo^{dp}–*Ta* structural abnormalities was caused by difficulty in recognizing the double abnormality. By contrast, the pigment effect of Mo^{dp} was present in about half the *Ta* hairs, suggesting that this pigment defect was expressed through a different cell population. Although based on a single animal, these observations are in accord with Mintz's (1971) conclusion, based on her studies on *To* chimaeric mice, that a gene can effect more than one cell type. The puzzling aspect of this interpretation of the present data is that they require an association between Mo^{dp} colour and structural effects. However, it is possible that this is an artifact caused by difficulties in the detection of Mo^{dp} structural effects in pigmented cells.

4. DISCUSSION

Since none of the male chimaeric mice produced in this study was found to carry the T145H translocation, it was not possible to distinguish whether T145H male sterility is determined at the cell or organism level. Similarly, the *Ta*–T145H chimaeric animals did not provide any satisfactory evidence to indicate whether or not the aneuploid products of T145H could survive at the cell level. However, the investigation of the coats of the *c*-variegated and *Ta* chimaeric animals provided an answer to the question of whether or not the heterozygous *Ta* and *fd* phenotypes can result from the presence of two cell populations as stipulated by the inactive-*X* hypothesis (Lyon, 1961). With the exception of the age effect on *c*-variegation, clearly they can. The characteristic 'mosaic' patterns of *fd* and *Ta* heterozygotes are indistinguishable from those of their chimaeric counterparts and, hence, there is no reason to suspect that differing mechanisms may be responsible. The two chimaeric patterns are postulated to derive from the clonal origin of the melanoblasts and hair follicle cells, respectively (Mintz, 1967*b*, 1971), and it is therefore to be expected that the creation of two cell populations by *X*-inactivation would similarly allow these patterns to be detected. It is not necessary to postulate threshold phenomena (Gruneberg, 1969) to account for the repeatable *fd* and *Ta* heterozygote patterns. Similarly, the 'mixed' areas and even 'mixed' hairs typical of *fd* and *Ta* heterozygotes are also found in their chimaeric counterparts and hence there is no need to seek postulates beyond the developmental consequences that can modify the archetypal patterns (Mintz, 1967*b*, 1971). The same developmental causes presumably are responsible for the presence of two cell populations in single hairs of women heterozygous for G-6-PD and HGPRT (Gartler *et al.* 1971*a*, *b*; Goldstein, Marks & Gartler, 1971).

The noteworthy feature of the data concerning the Mo^{dp}–*Ta* chimaeric animal, CH/11, is that they suggest that Mo^{dp} can independently affect coat colour and coat structure and this would indicate that the gene operates on two distinct cell populations, melanoblasts and hair follicle cells. It is therefore not surprising that Lyon (1963) observed that *Ta* hair was to be found in the white (Mo^{dp}) areas of repulsion heterozygotes. Had she scored Mo^{dp} by its coat structure effect, such an extensive interaction with *Ta* might not have been found.

The chimaeric mice described do not readily provide an explanation for a number of features of the mosaicism found in heterozygotes. Thus, although CH/1 and CH/2 showed polarization of the pigment in *c*-variegated hairs as clearly as do *fd* mice, the responsible mechanism remains obscure. It may be that some system exists within the hair follicle which tends to localize available pigment in the hair tip. Similarly, an explanation for the dark bands of the *Ta* heterozygote (as opposed to the yellowish colour of the hemizygote) is also not forthcoming from these studies, but the fact that the bands of CH/9 consisted primarily of normal hair suggests that the dark colour is caused by a mechanism other than the hair composition.

While the data presented provide satisfactory evidence that chimaeric mice display mosaic phenotypes similar to those of their heterozygous counterparts, they do not distinguish whether the same mechanisms are always responsible. Differences between the two types of animal may tend to be concealed. This is best illustrated in the comparison of the *c*-variegated phenotypes. Partially pigmented hair was found in the coats of chimaeric mice, thus demonstrating that more than one melanocyte contributes towards the pigmentation of single hairs. Presumably the same mechanism leads to partially pigmented hair in *fd* mice but in these animals a second mechanism must also be operating. As *fd* mice age the *c* areas tend to become uniformly partially pigmented and since this reversal is restricted to *c* areas it is unlikely that the active and inactive *X*-chromosome exchange their roles; it seems more probable that the inactivation process is breaking down as the animals age, permitting activity of the + allele in the rearranged *X* (Cattanach & Isaacson, 1965). Whatever the precise mechanism, it is evident that the same end-effect, i.e. partially pigmented hairs, can result from more than one process in the heterozygote. This difference between chimaeric and *fd* mice was observed in the present experiment by the permanence of the pigment-free hair in the *c* areas of the chimaeric mice and perhaps also by the higher proportion of partially pigmented hairs in the *c* areas of both old and young *fd* females. Such a distinction might be hard to recognize with the more complex *Ta* banding patterns but it is of interest to note that Gruneberg (1969) has reported that changes in the Mo^{br} heterozygous phenotype occur with age.

A further difference between chimaeric mice and their heterozygous counterparts should also be mentioned. Three out of the seven animals that were demonstrably chimaeric showed phenotypes that were at the extremes or possibly beyond the range of that found in the equivalent heterozygotes (CH/1, 6, 9). This appears to be a characteristic feature of chimaeric mice (Mintz & Palm, 1969; Wegmann &

Gilman, 1970) and is probably related to the fact that about 25 % of embryo fusions yield animals which are not demonstrably chimaeric. The cause may be the earlier establishment of the two clonal populations in chimaeric mice together with the regulation from the double-sized blastocyst to the normal-sized post-implantation embryo (Mintz, 1971). Cell selection may also have played a part in CH/1 and CH/2 where the T1Ald component may have been favoured.

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