VARIEGATED-TYPE POSITION EFFECTS IN THE MOUSE

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UNTIL recently, V-type position effects were known only in Drosophila and Oenothera (see review by LEWIS 1950). Last year, we reported the first two cases of V-type position effect in a mammal, the mouse (RUSSELL and BANGHAM 1959). It was tentatively concluded that these were due to X-autosome translocations. Further work (RUSSELL and BANGHAM 1960) has supported this conclusion. The present paper will present the results obtained to date in experiments involving these first two cases, as well as a third one which has been investigated by us since then.

All three of the original variegated mutants arose in the course of specific locus mutation rate studies (RUSSELL 1951; RUSSELL, BANGHAM and GOWER 1958) and all were the offspring of irradiated wild-type males mated to females homozygous for seven recessive visibles (a = nonagouti, b = brown, c^{ch} = chinchilla, p = pink eye, d = dilution, se = short ear, s = piebald). As will be shown, the three cases involve variegation of the wild-type alleles at the b, c, and p loci, respectively. In two of the cases (b and p locus variegation), the mutant was sired within the first week following irradiation of the father, so that it can be assumed that the genetic change was induced in spermatozoa; in the third case (c locus variegation), the cell stage of induction may have been spermatocytes, spermatids, or spermatozoa. All three of the original variegated mutants were females which were clearly partially sterile and which transmitted variegation to their daughters.

Variegation involving the brown locus has been studied in considerably more detail than the other two cases and will be discussed first.

I. VARIEGATION INVOLVING THE b LOCUS

A. Progeny of variegated females; interpretation

The original mutant, on mating to a brown male, produced offspring of three types: variegated, wild type, and brown. The distribution is shown in Table 1. Almost all of the F_1 of the mutant were tested with brown mates and gave the following results: brown animals of both sexes in turn produced only brown progeny; wild-type animals produced wild-type and brown progeny in approximately equal numbers; the variegated daughters, which, it should be noted,

[1] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

were all clearly partially sterile, yielded progeny classes similar to those produced by their mother. Subsequently the stock has been maintained by continuously outcrossing variegated females to homozygous brown males. The results of all such matings are shown in Table 2.

TABLE 1

Progeny of the original brown-variegated mutant female and a homozygous brown male

	Variegated		Wild type		Brown	
	♂	♀	♂	♀	♂	♀
Fertile	—	0	1	0	4	2
Partially sterile	—	9	0	1?	0	1
Sterile	—	0	4	0	1	0
Not fertility tested	—	0	3	0	0	0
Total	0	9	8	1	5	3

TABLE 2

Offspring of brown-variegated females outcrossed to b/b *males*

	Variegated		Wild type		Brown	
	♂	♀	♂	♀	♂	♀
Fertile	—	0	3	27	9	48
Partially sterile	—	64	0	9	0	16
Sterile	—	2	12	0	2	1
Not fertility tested	—	209	263	9	325	287
Total	0	275	278	45	336	352

It should be noted that all variegated animals are females. Such animals are mottled with small brown areas irregularly interspersed with wild type. It can roughly be estimated that from 40–90 percent of the total fur is brown. Of 66 variegated females which were tested for fertility none was fully fertile and almost all were partially sterile. (The percentage of completely sterile variegated females, 2.7 percent, is not out of line with the proportion of female sterility found in many stocks of mice.) Average size of litters from the 64 partially sterile variegated females is 3.8, and the range for individual females is 1.8–5.0, with about three quarters of the females having average litter sizes in the range from 3.0 to 4.5, inclusive. By contrast, the average litter size for 32 fertile females in the stock is 7.9, with a range of 5.7–11.0. The average size of litters from the original mutant was 3.4.

Brown progeny are about evenly divided between the sexes and the bulk of those tested for fertility are fully fertile, with a few of the males sterile and some of the females partially sterile. On the other hand, the sex ratio among the wild-type progeny is very uneven. The bulk of the males tested are sterile and the bulk of the females fertile. Test matings to brown mates gave results similar to those found for the F_1 of the original mutant: brown animals of both sexes produced only browns (a total of 475); the wild-type males produced 83 brown and 87

wild-type young; and the fertile wild-type females produced 520 brown and 500 wild-type young, of which, in turn, 39 were fertile and one partially sterile. The partially sterile wild-type females will be discussed in a separate section (see section I.E).

The results discussed in this section and in sections I.B and I.C fit the interpretation which is outlined in Figure 1. According to this interpretation, variegation involves a reciprocal translocation between chromosome 8 and the X chromosome, the break in chromosome 8 being in the proximity of the *b* locus (which carries the wild-type allele). Variegated females are assumed to be heterozygous for this translocation. Their progeny may be explained as follows. (a) Unbalanced gametes do not ordinarily contribute to the surviving progeny but cause embryonic death and, thus, are the cause of the partial sterility of the variegated females. (b) Balanced gametes carrying the intact chromosomes produce fertile brown sons and daughters. (c) Balanced gametes carrying the reciprocally translocated chromosomes produce daughters that are variegated and partially sterile; and sons that, instead, are nonvariegated (i.e., wild type), and completely sterile. (In preliminary studies, OAKBERG (private communication) finds that the sterility is due to degeneration of primary spermatocytes in pachytene.) (d) Crossing over between the brown locus and the break point gives (i) wild-type young which carry the intact chromosomes and are, therefore, fertile; and (ii)

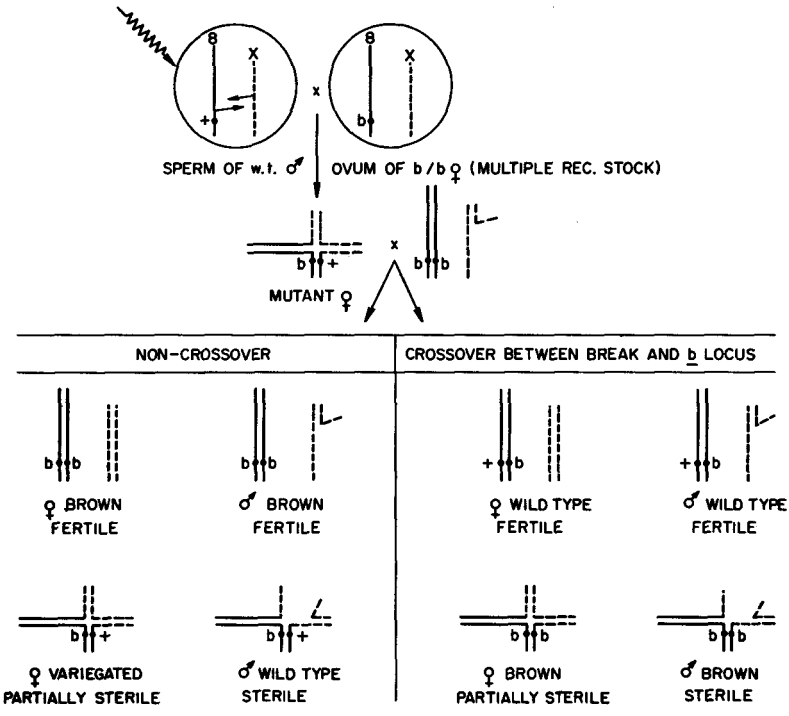

FIGURE 1.—Interpretation for the origin of brown-variegation and for the progeny of brown-variegated females crossed to *b/b* males (see text sections I. A, B, C).

brown translocation bearers, which are partially sterile, if female, and sterile, if male. Crossover frequencies will be discussed in section I.C.

Nondisjunction of the X's might be expected in appreciable frequency if the break in the X were near the centromere. This event would produce—in addition to duplication and deficiency types—the following progeny: (a) brown X0 females (intact chromosomes); (b) animals carrying the reciprocal translocation plus two intact X's, i.e., of an XXX constitution (not known whether viable, but, if so, probably variegated females); and (c) animals carrying the reciprocal translocation and of the XXY constitution. The latter would be viable males of normal size and with normal development of the external genitalia (RUSSELL and CHU 1961). By the reasoning outlined below (sec. I.E.3), they should be of variegated phenotype. Such a class has not been found; and there is additional, independent evidence (sec. I.C.2) that it is not produced with appreciable frequency, if at all. Although class (a) could be among the brown semisterile females and class (b) among the variegated females, the absence of class (c) would argue that the frequency of nondisjunction of the X is not appreciably increased by the X;8 translocation.[2]

Some evidence that animals carrying the reciprocal translocation may be slightly less viable than their nontranslocation sibs can be derived from the data of Table 2. If viability were equal, the number of wild-type males should equal the number of brown males. Actually, there are only 83 percent as many wild-type males as there are brown males and the difference from equality is significant. The bulk of the former class, of course, consists of translocation bearers, while only a relatively small proportion of the latter class is of that type (as the result of crossing over between the break point and the *b* locus). In the case of the females, the data go in the same direction but the difference is much smaller and not significant. The corresponding comparison here is that of the variegated plus wild-type classes *versus* the brown class, a ratio of 320 to 352. It is the relatively greater inviability of the male translocation bearers which accounts almost entirely for the fact that the over-all sex ratio is slightly in favor of females (614 males to 672 females).

B. Proof of variegated-type position effect

In view of the fact that we were dealing with a case unprecedented in mammals, it seemed necessary to bring formal proof that it is the *rearranged position* of the wild-type gene at the *b* locus—rather than the structural heterozygosity or a permanent change in b^+—that causes variegation. The procedure followed was modelled on that used by DUBININ and SIDOROV (1935) for hairy variegation in Drosophila. The ultimate aim of this procedure is substitution of a wild-type gene derived from a normal chromosome for the presumably rearranged wild-type gene that is variegating (see Figure 2).

In the first step, *b* was introduced into the rearrangement through crossing

[2] Note added in proof: Cattanach (Genet. Res. **2**: 156–158, 1961) has recently described XXY males in a stock carrying a presumed sex-linked rearrangement.

CROSS			FEMALE PROGENY			
			non-c.o.		c.o.	
(1)	$R(b^+)/b$ ♀ ×	b/b ♂	$R(b^+)/b$	b/b	$R(b)/b$*	b^+/b
			v - ps	b-f	b - ps	wt-f
(2)	$R(b)/b$ ♀ ×	$b^{+'}/b^{+'}$ ♂	$R(b)/b^{+'}$*	$b^{+'}/b$	$R(b)/b^{+'}$*	$b^{+'}/b$
			wt - ps	wt-f	wt - ps	wt-f
(3)	$R(b)/b^{+'}$ ♀ ×	b/b ♂	$R(b)/b$	$b^{+'}/b$	$R(b^{+'})/b$	b/b
			b - ps	wt-f	v - ps	b-f

FIGURE 2.—Procedure used for proof of V-type position effect in brown-variegation. Only the female progeny of each cross is shown. Those segregants of each cross which are used in the subsequent one are marked with an asterisk. The phenotype is indicated under each genotype as follows: v = variegated, b = brown, wt = wild type; f = fertile, ps = partially sterile.

over (the required crossover class being detected through fertility tests among the brown daugters of variegated females mated to brown males). The second step was to place this rearranged *b* opposite the wild-type allele in an intact chromosome (which, in Figure 2, is designated with a prime to distinguish it from the b^+ that was originally in the rearrangement). The fact that the heterozygotes turned out to be phenotypically wild type, rather than variegated, proved that it is not structural heterozygosity that causes variegation, since the $R(b)/b^+$ and $R(b^+)/b$ types are structurally equivalent and both heterozygous for *b*. In the final step, the b^+ from the intact chromosome was introduced into the rearranged one through crossing over. Variegated, partially sterile females were again obtained, proving that it was the rearranged position of the b^+ rather than any change in the b^+ that caused the original variegation.

C. *Linkage*

1. *Linkage with the X chromosome:* The types of progeny produced by variegated females (see Tables 1, 2) give strong indications of sex linkage. More recently, it has been possible to confirm the indications with direct genetic evidence. Linkage experiments were carried out with the sex-linked, dominant marker Tabby, *Ta*, one of the few in the mouse that can be introduced through the male. Brown-variegated, heterozygous *Ta* females were produced and were mated to brown hemizygous *Ta* males. Sixteen classes were expected from such a cross, namely, the eight classes shown in Figure 1, each segregating for *Ta*. Figure 3 shows diagrammatically the alternative expectations for X independence and X linkage, respectively.

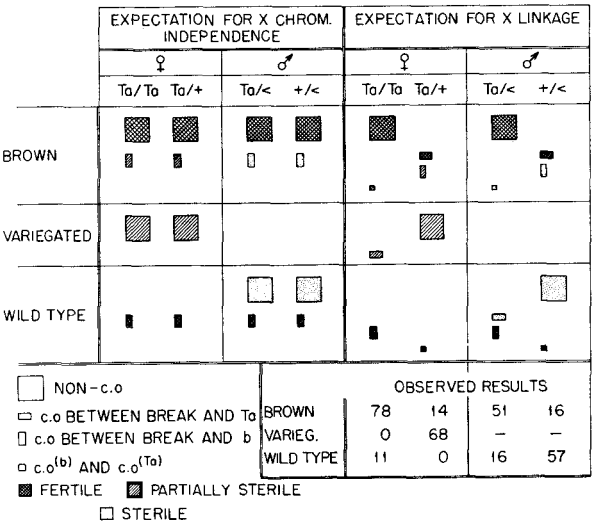

FIGURE 3.—Types of progeny from a cross of $T(b^+; Ta^+)/b$; $Ta/+$ ♀ × b/b; Ta/Y ♂: expectation for X chromosome independence and X linkage, respectively, and observed result.

If the X chromosome is not involved in the translocation, one expects equality of *Ta* segregation within each of the coat-color classes, as shown on the left side of the diagram. If, on the other hand, the translocation involves the X, one would expect the distribution shown in the right half of the diagram. Since *Ta/Ta* can be distinguished from *Ta/+* without difficulty, and *Ta*/Y from +/Y, eight classes of segregants can be classified without the further subdivision arrived at through fertility tests. The actual numbers of animals obtained in these eight classes are shown in the bottom right-hand corner of the figure. It is obvious that the bulk of the females are brown *Ta/Ta*, and variegated *Ta/+*; and the bulk of the males are brown *Ta*/Y, and wild type. This distribution is the one expected on the hypothesis of sex linkage (see right-hand portion of diagram).

It has thus been proved that brown variegation is due to a rearrangement between chromosome 8 and the X. As shown in section I.A, the assumption of a reciprocal translocation between these chromosomes fits all the data.

2. *Location of break point:* A further breakdown of the classes shown in the bottom right-hand portion of Figure 3 into fertility subclasses permits calculation of the crossover frequency between the break point and Tabby on the one hand, and the *b* locus on the other hand. The results are shown in Table 3. Crossover percentages calculated on the basis of the male progeny only are probably more accurate than those calculated on the basis of all tested animals, for the following reasons: (a) only 13 percent of the 140 males classified by coat color died before fertility test and these were distributed between crossover and noncrossover classes approximately in proportion to the totals; (b) the distinction between fertile and sterile can be made with considerably greater accuracy than the distinction between fertile and partially sterile which has to be made in the case of the females. The female data, on the other hand, are complicated in various ways

TABLE 3

Results of the cross T(b⁺;Ta⁺)/b;Ta ♀ × b/b; Ta/*Y* ♂ ; *and crossover frequencies between break point and* Ta *locus, and between break point and* b *locus calculated from the data (see Figure 3)*

Color	Fertility type	Females *Ta/Ta*	Females *Ta/+*	Fertility type	Males *Ta/Y*	Males +/Y
Brown	Fertile	45 + 24?*	5	Fertile	45	3
	Part.ster.	3	6	Sterile	0	9
Variegated	Part.ster.	0	57			
Wild type	Fertile	5 + 5?*	0	Fertile	8	0
				Sterile	6	51

Crossover frequency:	Based on: ♂♂ only	all animals
Break point and *Ta* locus	7.4%	6.3%
Break point and *b* locus	13.9%	13.2%

* Fertility type questionable due to poor general health of *Ta/Ta* (see text, sect. I.C2).

through the presence of the *Ta/Ta* classes. Thus, the relative inviability of these animals led to the death of large numbers of them (indicated by "?" in Table 3) before they had enough litters to allow an accurate decision as to their fertility type. The inclusion of 24 of these questionable animals in the brown *Ta/Ta* (noncrossover) class may—if any of them were, in effect, not wholly fertile—have led to an underestimate of crossing over. Furthermore, it should be noted that no variegated *Ta/Ta* females were found. This type may be lethal, which would also lead to an underestimate of crossing over. On the other hand, an overestimate of crossing over may be derived from the number of animals assigned to the double crossover category on the basis of their presumed partial fertility: this partial fertility may be due to the poor general health of some *Ta/Ta* females, rather than an indication of their being translocation bearers.

In spite of these various complicating factors, the crossover values calculated on the basis of all tested animals agree closely with those calculated on the basis of males only. Roughly, the frequency of crossing over between the break point and *Ta* locus is 6–7 percent, and between the break point and *b* locus 13–14 percent.

The crossing over frequency between the break point and the *b* locus can also be estimated from the larger body of data in Table 2. The genotypes of brown males and females and of wild-type males are not revealed by their coat color alone, and only relatively small proportions were fertility tested. However, by the interpretation shown in Figure 1, all wild-type females are due to crossing over and represent half of the crossover females. An estimate of crossover frequency can, therefore, be obtained from the ratio of 2× wild-type females/total

females, which gives a value of 13.4 percent, in good agreement with the 13–14 percent calculated from the independent data shown in Table 3. Because of the inclusion, in this calculation, of the problematic class of partially sterile wild-type females, which will be discussed below (sec. I.E), this figure may be a slight overestimate.

If the frequency of about 13 percent of crossing over between the break point and the *b* locus corresponds to a relatively great physical distance, then the action of the position effect must extend over a considerable length of chromosome 8. BAKER (1954) describes a V-type position effect in *D. virilis* acting over a distance (in heterochromatin) of one third the metaphase length of chromosome 5. Crossing over frequency between the break point and the affected locus was, however, low. Distances in euchromatin over which position effects in Drosophila have been found to act are quite variable (see reviews by LEWIS 1950; HANNAH 1951). In general, however, the crossover frequencies involved are smaller than in the present case of b^+ variegation in the mouse. On the other hand, a crossover frequency of almost comparable magnitude has been reported in Oenothera (CATCHESIDE 1947).

Tentative conclusions may be drawn concerning the location of the break in the X chromosome. *Ta* is relatively centrally located within the total length of 32 units that have been mapped for the X chromosome (GREEN and DICKIE 1959). If crossing over were not affected by the translocation (which, however, is unlikely), the crossing over frequency of 6–7 percent between the break point and the *Ta* locus would indicate that the break point lies either between *Mo* and *jp*, or between *Ta* and *Bn*. It is possible that future work will yield a more accurate location of the break point in the X. Similarly, it will be of interest to map the break point in chromosome 8 and to determine whether there is a spreading effect to other loci on that chromosome (e.g., misty, *m*).

The results of Table 3, unfortunately, cannot provide the answer as to whether translocation has placed the *Ta* and *b* loci on the same chromosome, or whether each translocated chromosome contains one of these loci. It should be noted that there is no obvious position effect on Ta^+: no patches of fur of the $Ta/0 = Ta/Ta$ type were noted on female translocation heterozygotes carrying *Ta* on their intact X chromosome.

The results shown in Figure 3 and Table 3 support those discussed above (sec. I.A) in giving no evidence of XXY males, a class which would be expected if the translocation had led to a high frequency of nondisjunction of the X's. Such males would be of the $X^{Ta}/X^+/Y$ constitution, which has a $Ta/+$ phenotype (RUSSELL and CHU 1961). No such animals were found.

D. Cytology

Preliminary cytological observations have been made in tissue cultures prepared from a brown-variegated, partially sterile female and from a wild-type, sterile son of a brown-variegated female.

In these two animals, 17 and 25 cells, respectively, were examined. The modal number of chromosomes was 40 (36 cells out of 42). No metacentrics were ob-

served. In almost all of the cells, one chromosome of excessive length was clearly discernible (see Figure 4). This is assumed to be one of the translocated chromosomes. Whether, corresponding to this, there is a shorter-than-normal chromosome in the complement cannot be stated since no detailed karyotype has as yet been prepared.

E. Suppression of variegation

1. *Wild-type males:* As has already been noted, male translocation heterozygotes are wild type, while females are variegated. It has been shown in Drosophila that the presence of an extra Y chromosome can suppress variegation and the removal of a Y can enhance it (see reviews by LEWIS 1950; HANNAH 1951; GRELL 1959). It was therefore considered not impossible that, in the mouse, the single Y chromosome normally present in a male might be sufficient to suppress

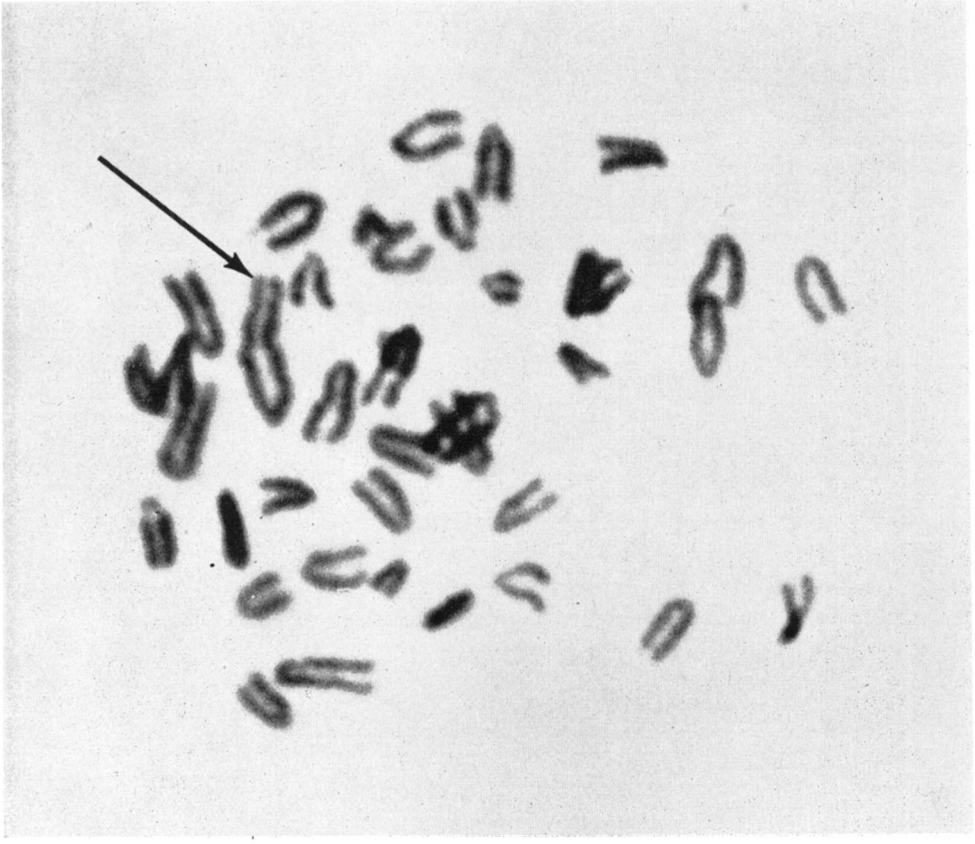

FIGURE 4.—Chromosomes from a spleen tissue culture preparation of a male mouse presumably heterozygous for a reciprocal translocation between chromosome 8 and the X (wild-type, sterile son of a brown-variegated female, see text, sections I. A–D). The chromosome which is of excessive length (indicated by arrow) is assumed to be one of the translocated chromosomes. × 3750.

variegation. However, more recent findings, which will be discussed in the following section (E.2), have led to a different interpretation, which will be outlined in section E.3.

2. *Partially sterile, wild-type females:* By the interpretation outlined in Figure 1, all wild-type females are expected to be fertile (with b^+ on the intact chromosome as the result of crossing over). It may, however, be seen from Table 2 that of 36 wild-type females tested for fertility, nine turned out to be only partially fertile. Three possible interpretations were considered: (a) the partially sterile wild-type females are of the expected genotype (intact chromosomes, XX) and the reduced fertility is due to some cause not connected with the translocation (e.g., random variations in fertility); (b) the partially sterile wild-type females are of some exceptional genotype but do not carry R(+); or (c) the partially sterile wild-type females carry R(+) but have some modification in their genotype which leads to suppression of variegation. Mating to brown males should produce variegated daughters only if the third explanation is correct. Actually, of the nine females in this category, only one, female 983, produced variegated daughters. Four other females had fewer than six daughters each and the results are, therefore, not conclusive for any one female by herself, although, as a group, the four females produced eight wild-type and six brown daughters. The remaining four females had ten or more daughters each, yielding a total of 25 wild type to 28 browns.

On mating the partially sterile wild-type females to Tabby males, two of them produced offspring. One of these was female 983 (who had previously thrown variegated daughters by a brown mate); the other was female 577 (who had given only nonconclusive results from a brown mate, namely, one wild-type and two brown daughters). Both of these females yielded *Ta/0* as well as *Ta/+* daughters. This result indicates that female 983 and female 577 lack either the whole intact X chromosome or an X chromosome portion containing the *Ta* locus. In the former case, the chromosome complement could, theoretically, be either 8; T(8;X); T(X;8), or 8; 8; X (with the maternal chromosome 8 carrying b^+ as a result of crossing over). However, the 8; 8; X genotype may be ruled out for both females (female 983 had variegated daughters; and female 577's wild-type sons were sterile). For the interpretation of lack of an X chromosome portion containing the *Ta* locus one may consider two possible chromosome complements: (a) X; 8; T(8;X). which is the result of the loss of one maternal chromosome and represents a deficiency for a portion of chromosome 8 (as well as portion of the X); or (b) X; 8; 8; T(X;8), which is the result of unbalanced segregation of the translocation figure in the mother and represents a duplication for a portion of chromosome 8 (as well as deficiency for portion of the X).

These various hypotheses (and subhypotheses, depending on the location of the *Ta* and *b* loci relative to each other and to the centromeres) were examined in relation to the results. First and second generation progeny of female 983 by *Ta*/Y and *Ta*/Y; *b/b* males fit perfectly the hypothesis that female 983 lacks the intact X. The data for female 577 are not yet as good but, so far, fit the same

hypothesis. (Under certain assumptions, more complex interpretations can also be put on these two cases.)

On the other hand, various lines of evidence indicate that three of the other seven partially sterile wild-type females probably do not lack either an entire X chromosome (chromosome count of 40) or an X chromosome portion (all of many tested daughters fully fertile). Results for the remaining four are inconclusive.

It is thus tentatively concluded that of the five partially sterile wild-type females for which some diagnostic data are available, two were probably of the chromosome constitution 8; T(8;X); T(X;8); and three may have been of the chromosome constitution 8; 8; X; X and were partially sterile for some reason not connected with the translocation.

3. *Influence of the sex chromosomes on variegation:* In view of the fact that translocation heterozygotes with sex chromosome constitutions X0 or XY are wild type, whereas those with sex chromosome constitution XX are variegated, it may be suggested that, in the mouse, two X chromosomes are required to produce variegation, and that removal of one X suppresses variegation. This is in contrast to the hypothesis mentioned in section E.1, namely, that the presence of the Y in male translocation heterozygotes might act as a suppressor of variegation. In Drosophila, it is generally true that when V-type position effects are due to rearrangement of genes from euchromatin to the vicinity of heterochromatin, variegation is markedly enhanced by the X0 condition (see reviews by LEWIS 1950 and HANNAH 1951 for specific references). The present case described for the mouse is obviously in the opposite direction.

It should be noted that one case is known in Drosophila where variegation due to rearrangement of the gene lt^+ from the proximity of heterochromatin to that of euchromatin was suppressed in the X0 male (SCHULTZ 1936). No information exists concerning heterochromatic and euchromatic regions in the chromosome complement of the mouse, and it can therefore not be stated whether the case described in this section parallels the case of the light gene in Drosophila. One respect in which parallelism fails, in any case, is in the phenotype of XY males which are wild type in R(b^+)/b mice but variegated in R(lt^+)/lt Drosophila. Furthermore, even in Drosophila, no generalizations can be based on the R(lt^+) case, since it has been shown that modification by extra Y chromosomes of the position effect at the "heterochromatic" *ci* locus is in the opposite direction from that at the *lt* locus (GRELL 1959).

If it should be true that two X chromosomes are required to produce variegation in R(b^+)/b mice, this case would parallel, in some respects, the condition found for the gene zeste, *z*, in *D. melanogaster*, where two doses of an X chromosome region including w^+ are required to produce the mottled phenotype (GANS 1953).

F. The lethal effects of the b$^+$ *rearrangement*

Preliminary data are available concerning the effect of R(b^+) in combination with lethal mutations at the *b* locus. Tests have been initiated with seven inde-

pendently induced mutations at the *b* locus, all of which act like *b* in the combinations b/b^l, and $+/b^l$, but are homozygous lethal. Brown-variegated females, $R(b^+)/b$, were mated to heterozygous brown lethal, b^+/b^l, males. If the combination $R(b^+)/b^l$ is viable, the expectation among daughters is 1 brown:2 wild type:1 variegated. The actual number found were 12:36:3. Even though these numbers are as yet small, the results already differ significantly from expectation.

Two interpretations are possible. (a) It may be assumed that the various cases of b^l represent point mutations. Under that assumption, one may conclude that the rearrangement $R(b^+)$ inactivates the b^+ allele to a recessive lethal form in a sufficiently large number of somatic cells to kill the entire animal when a nonviable allele, b^l, is present on the homologous chromosome. (b) On the other hand, if some of the b^l mutations represent deletions, rather than point mutations (and, since two of the seven were induced in postspermatogonial stages, this is a definite possibility), the lethal effect of the combination may indicate that the spreading effect extends to additional loci. That is, the rearrangement may inactivate genes adjacent to the *b* locus to a recessive lethal state in a sufficiently large number of somatic cells to cause death of the embryo when the homologous chromosome carries a deletion for the loci involved. It will be of interest to determine, when larger numbers become available, whether the different b^l mutations give different degrees of lethality in combination with $R(b^+)$.

II. VARIEGATION INVOLVING THE *c* LOCUS

Variegation of the c^+ gene has not yet been as thoroughly studied as variegation of the b^+ gene. However, the results, to date, are analogous, except for the fact that the amount of crossing over between the break point and the *c* locus appears to be considerably smaller than that between the break point and the *b* locus in the case of b^+ variegation (sec. I.C).

Offspring obtained from the outcross of variegated females to c^{ch}/c^{ch} (chinchilla) males are shown in Table 4. The following analogy to the b^+ variegation case (see Table 2) may be noted: (a) all variegated animals are females; (b) the bulk of the variegated females are partially sterile, and none is fully fertile; (c) males are of two types, namely, wild type, with all those tested, to date, sterile; and chinchilla, with all those tested, to date, fertile. The testes of the sterile males histologically resemble closely those of the $R(b^+)/b$ males (see sec. I.A) (OAKBERG, private communication).

TABLE 4

Offspring of c *variegated females outcrossed to* c^{ch}/c^{ch} *males*

	Variegated		Wild type		Chinchilla	
	♂	♀	♂	♀	♂	♀
Fertile	—	0	0	—	13	3
Partially sterile	—	27	0	—	0	0
Sterile	—	6	11	—	0	0
Not fertility-tested	—	28	50	—	78	69
Total	0	61	61	0	91	72

Animals with *c* locus variegation are mottled with diffuse patches—occasionally rather large—of the mutant tissue. When c^{ch} is carried on the intact chromosome, the mutant patches are of a color closely resembling c^{ch}/c. It thus appears that the action of the rearranged c^+ is completely suppressed to the state of the amorph, albino, in some somatic cells. It can roughly be estimated that 40–80 percent of the fur is of the mutant type, most of the animals being in the 50–60 percent range.

The main point in which the case of c^+ variegation differs from that of the case of b^+ variegation is the absence, to date, of crossing over between the break point and the *c* locus. Thus, no wild-type females have appeared; none of the fertility-tested chinchillas has been sterile; and none of the fertility-tested wild-type males has been fertile. From the data in Table 4 it may be estimated that the frequency of crossing over between the break point and the *c* locus is less than 1.5 percent.

In the case of the c^+ variegation, animals presumably heterozygous for the translocation appear to be relatively less viable than in the case of the b^+ variegation. Thus, there are only about 67 percent as many wild-type males as there are chinchilla males, and only about 85 percent as many variegated females as there are chinchilla females (roughly corresponding figures in the case of b^+ variegation are 83 percent and 91 percent, respectively). Furthermore, the c^+-variegated females are poorer producers than the b^+-variegated females: the intervals between litters are longer; a larger percentage produce no young at all; and among those that do produce young, the average litter size is smaller, namely, 2.7 (range 1.7–4.0) as compared to 3.9.

III. VARIEGATION INVOLVING THE *p* LOCUS

In the third case of presumed V-type position effect, the variegated portion of the fur is very much smaller than in the other two cases and cannot easily be characterized as to color. Variegated daughters are produced when variegated females are mated to $p\ c^{ch}/p\ c^{ch}$ (pink-eyed chinchilla) males, and the stock is maintained by continuous outcrosses of this type. Mating of variegated females to c/c (albino) males did not produce variegated daughters (in a rather limited test, but mating to p/p males did. It is, therefore, concluded that the variegating gene is p^+. (The gene *p* has a diluting effect on both the eumelanin and phaeomelanin of the coat).

Offspring produced from outcrosses of variegated females to $p\ c^{ch}/p\ c^{ch}$ males are shown in Table 5. Again, as in the other two cases (see Tables 2, 4), all variegated animals are females. The bulk of these are partially sterile (none is fertile) yielding an over-all average litter size of 2.4, with the range for individual females being 1.8–3.1. By analogy with the other two cases, the wild-type males may be assumed to represent the *male* translocation bearers. Indeed, all the members of this class that have been fertility tested to date have proved to be sterile. The testes of the sterile males histologically resemble closely those of the $R(b^+)/b$ males (see sec. I.A) (OAKBERG, private communication). The homozygous reces-

TABLE 5

Offspring of p *variegated females outcrossed to* p c^{ch}/p c^{ch} *males*

	Variegated ♂	Variegated ♀	Wild type ♂	Wild type ♀	$p\ c^{ch}/p\ c^{ch}$ ♂	$p\ c^{ch}/p\ c^{ch}$ ♀	$p\ +/p\ c^{ch}$ ♂	$p\ +/p\ c^{ch}$ ♀	$+\ c^{ch}/p\ c^{ch}$ ♂	$+\ c^{ch}/p\ c^{ch}$ ♀
Fertile	—	0	0	0	10	7	0	0	1	3
Partially sterile	—	16	0	0	0	0	0	0	0	0
Sterile	—	4	16	0	0	0	3	0	0	0
Not fertility-tested	—	8	30	6	36	32	4	5	2	6
Total	0	28	46	6	46	39	7	5	3	9

sive animals of both sexes, which may be assumed to represent segregants bearing the intact chromosomes, have, true to expectation, proved fully fertile (however, only 17 have been tested to date). The six wild-type females—again, by analogy with the b^+ variegation case—could represent crossovers between the break point and the *p* locus. On the other hand, in view of the fact that the variegated portions of the fur are so small in this stock, these six females might merely be normal overlaps. Breeding tests should distinguish between these alternatives.

On the hypothesis of a reciprocal translocation between chromosome 1 and the X chromosome (compare the case of b^+ variegation), there are three possible arrangements depending on the position of the break point relative to the *c* and *p* loci: (a) the break point may be *between* the loci, which would result in their being separated to two different translocated chromosomes; (b) the order may be break point, *c* locus, *p* locus; or (c) the order may be break point, *p* locus, *c* locus. It may be noted that regardless of which of these configurations exists, one expects *two* classes of variegated females: namely, in addition to the noncrossover class, which is variegated on a wild-type background, a class which is variegated either on a pink-eyed or a chinchilla background. These latter animals would represent either crossovers between the loci, or crossovers between the break point and one of the loci. The fact that such females have not yet been observed within the small $p+/p\ c^{ch}$ and $+\ c^{ch}/p\ c^{ch}$ classes may merely indicate that variegation, which is very slight in any case, cannot easily be seen on a lighter colored background. Fertility tests of the $p\ +/p\ c^{ch}$ and the $+\ c^{ch}/p\ c^{ch}$ classes, and particularly the males within these classes, should make it possible to decide between the three possible configurations of the rearrangement. Thus, in the case of configuration (b), all $p\ +/p\ c^{ch}$ males should be sterile and all $+\ c^{ch}/p\ c^{ch}$ males should be fertile; in the case of configuration (c), the situation would be the reverse; and in the case of configuration (a), both classes of males might contain a mixture of fertiles and steriles, unless crossing over between the break point and one of the loci were very infrequent or nonexistent.

DISCUSSION

For many years, V-type position effects have been known in only one animal, Drosophila, and only one plant, Oenothera. The finding of such position effects

in the mouse increases the probability that this phenomenon will eventually be demonstrated in a wide variety of species.

At the same time it should be noted that all three cases described for the mouse may represent a rather specialized mechanism, namely, one which involves sex-linked rearrangements. The number of autosome-autosome translocations is presumably a large multiple of the number of X-autosome translocations, but no V-type position effect has yet been found that does *not* involve the X. It thus appears possible that only the X has the power to produce such effects in the mouse. It is even conceivable that this power is restricted to a specific region of the X; and, in this connection, it will be important to determine whether the breakage point in the X is in the same region in all three translocations.

It is of interest that a large number of mottling mutations have been reported for the X chromosome of the mouse; and that, in fact, the bulk of sex-linked mutations of the mouse are of this type, whereas there seem to be no exactly comparable autosomal mutations. The question may, therefore, be raised whether the sex-linked mottlings are not due to some kind of position effect.

In addition to the above points, there are other features, already discussed at greater length in the paper, which distinguish the V-type position effects in the mouse from those in Drosophila. One of these distinguishing characteristics is the consistent phenotypic difference between the sexes in animals heterozygous for R(+) and the recessive: while females are variegated, males are wild type. On the basis of our finding that X0 females heterozygous for R(+) and the recessive are likewise wild type, we have suggested that two X chromosomes are required for variegation. If true, this would represent a lack of parallelism between mouse and Drosophila V-type position effects.

It should also be noted that in one of the three cases described here for the mouse, the crossover frequency between the breakage point and the variegating locus is 13–14 percent. This value is higher than crossover frequencies encountered in Drosophila position effects.

SUMMARY

1. Three cases of presumed variegated-type position effect in the mouse have been described. These involve the *b*, *c*, and *p* loci, respectively. All three were induced by irradiation in postspermatogonial stages.

2. In the case of one of these (*b* locus), the classical proof has been brought that variegation is due to a position effect produced through rearrangement of the wild-type allele.

3. The data fit the interpretation that the rearrangements are X-autosome translocations. The most direct evidence for this is the close linkage that has been found between the break point and the sex-linked marker *Ta* in the case of the b^+ variegation (c.o = 7 percent).

4. The frequency of crossing over between the variegating gene and the break point is high in the case of the b^+ variegation (13–14 percent), and low in the

case of the c^+ variegation (< 1.5 percent). No determination has yet been possible for the p^+ variegation.

5. In all three cases, the females heterozygous for the translocated wild-type gene and the recessive marker on the intact chromosome are variegated and partially sterile. Corresponding males are wild type and completely sterile (probably as a result of degeneration of primary spermatocytes in pachytene).

6. At least one female translocation heterozygote has been found which lacked the intact X. This X0 female was wild type. Since translocation heterozygotes with the sex chromosome constitution X0 or XY are wild type, while XX females are variegated, it is suggested that, in the mouse, two X chromosomes are required to produce variegation.

7. Since no case of variegated-type position effect has as yet been found which does *not* involve the X chromosome, it appears possible that only the X has the power to produce V-type position effects in the mouse. The question may also be raised whether some or all of the many sex-linked mutants that produce mottling in the mouse are not due to some kind of V-type position effect.

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