COMPARATIVE STUDIES ON X-AUTOSOME TRANSLOCATIONS IN THE MOUSE. II. INACTIVATION OF AUTOSOMAL LOCI, SEGREGATION, AND MAPPING OF AUTOSOMAL BREAKPOINTS IN FIVE T(X;1)'S[1]

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THE nine X-autosome translocations observed in the mouse possess distinctive features that give each certain advantages and certain disadvantages in studies of gene action connected with X-chromosome inactivation. Thus, for T(X;?)16H, the translocation reported by SEARLE (1962), both genetic (LYON, SEARLE, FORD and OHNO 1964) and cytological (OHNO and LYON 1965) evidence indicates that X inactivation, at least in certain adult cells and tissues, is nonrandom, affecting almost invariably the nonrearranged X. While this situation provides a tool for determining gene inactivation in the normal X chromosome (LYON 1966) it lacks the opportunities, offered by the other translocations, of studying inactivation in autosomal genes. The fact that the autosome involved in this translocation is not known becomes less important when one realizes that its genes would presumably behave no differently in the rearranged chromosome (which is virtually always active) than they normally do.

In the two X;8 translocations (RUSSELL and BANGHAM 1959; RUSSELL 1961; RUSSELL, unpublished), the five X;1 translocations to be described in this paper (RUSSELL and BANGHAM 1961; RUSSELL, BANGHAM and SAYLORS 1962; RUSSELL 1963a) and the *flecked* translocation (CATTANACH 1961), nonrandomness appears not to be a factor. The *flecked* translocation, which is probably an insertion of about one-third of LG1 into the X (OHNO and CATTANACH 1962), has certain distinctive features, one of which is that one class of unbalanced segregants (autosomal duplication) is viable, providing the opportunity for studying autosomal dosage phenomena. Furthermore, the unbalanced males are fertile, in contrast to males carrying any of the other X-autosome translocations known in the mouse—an obvious practical advantage. On the other hand, there is no recombination between the inserted segment and normal autosomes, which means (a) that no allelic substitutions can be made and (b) that mapping of autosomal breakpoints is hampered. Mapping has been possible by comparing balanced and unbalanced types with respect to phenotype of certain LG1 markers (EICHER 1967; WOLFE 1967).

The five X;1 translocations described in the present paper and the preceding

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one (RUSSELL and MONTGOMERY 1969) recombine in their autosomal and X-chromosome portions. Various suitable markers are available in LG1. The fact that the same loci can be studied in relation to different rearrangement points has already led to the hypothesis that there is a center or region in the X chromosome from which inactivating influences spread along gradients (RUSSELL 1963a, 1964a,b). The present paper presents more detailed data on the effect of the various rearrangements on certain LG1 loci, location of autosomal rearrangement points, and segregation in translocation heterozygotes. This makes possible a critical examination of suggestions made earlier, a discussion of some new points raised by more recent work with mouse translocations, and a reexamination of the single-active-X-chromosome hypothesis, in view of GRÜNEBERG's (1967) criticism of this hypothesis and his alternate suggestion that both X chromosomes are partly active. The results confirm our earlier conclusions that the inactivation center or region is called into play in only one of two X-chromosome-equivalents present, and that a given locus may or may not come under the influence of this inactivation depending on various factors.

The translocations to be described are T(X;1)2Rl, T(X;1)3Rl . . . T(X;1)6Rl, to be referred to, for short, as R2, R3, R4, R5, R6a, R6b*. As in the preceding paper, the symbol R will be used not only to name the rearrangements but to indicate the position of the breakpoint in genetic formulae. Gene symbols shown in parentheses indicate that the genes are located in the rearranged chromosome.

INACTIVATION OF AUTOSOMAL LOCI

Whether or not a given autosomal locus is inactivated at all by a given rearrangement and, if so, how much, can be revealed by introducing an intact autosome carrying a lower allele at that locus. If the locus is subject to inactivation, and if the gene acts autonomously, this procedure will result in variegated females mosaic for (i) cells in which the given autosomal gene in the rearranged chromosome is suppressed ("uncovering" the lower allele in the intact chromosome) and (ii) cells in which the rearranged chromosome is active. Various parameters of variegation provide information on the degree of inactivation.

(a) The variegating phenotype, which could theoretically be either that of the hemizygous lower allele in the intact autosome, or that of some compound, indicates whether inactivation is to a nullo state or to some intermediate level. For example, the variegating color in R(+)/*c* females should be white in at least some hairs if inactivation is complete, but some shade or shades of gray if inactivation is to some intermediate level or is variable. The "background" color is determined by the two alleles present, regardless of location, being the color that would exist if there were no rearrangement. However, the total effect does depend on relative location. Thus, (c^{ch})/*c* females are *c*-variegated on a c^{ch}/*c* background, but (c)/c^{ch} females are uniformly c^{ch}/*c*, since *c* can presumably be no further inactivated.

* It should be noted that in an earlier abstract (RUSSELL and MONTGOMERY 1965) the symbols for R5 and R6 were exchanged as a result of a clerical error.

(b) Frequency of inactivation is indicated by the average proportion of the body having the variegating phenotype. If a given autosomal locus in the rearrangement comes under the full influence of X chromosome inactivation, 50% of the body, on the average, should have the variegating phenotype, providing X differentiation is random and there is no cell selection. Less than 50% of variegation could mean either that the locus is under less than full influence of the inactivation center or region (i.e., not necessarily inactivated every time the inactivation center is called into play), or that X differentiation is not random (i.e., the inactivation center on the translocated X called into play less than half the time), or that there is selection against those cells that are functionally hemizygous. The last two possibilities can be ruled out if, in the same rearrangement, variegation associated with another locus having its basic action in the same cell type does involve about 50% of the pertinent tissue. When the mean fraction, q, of cells with the recessive phenotype is small, one would expect the proportion of translocation females that fail to show variegation to exceed the theoretically expected $(1 - q)^n$ (where n is the number of anlage cells at the time of establishment of cellular phenotype), due to the fact that very small areas would be nondetectable. This possibility is considered in the subsequent examination of results.

Four LG1 loci were used in this work: *c* (*albino* locus), *p* (*pink-eye*), *tp* (*taupe*), and *pu* (*pudgy*). The first three affect coat color (and eye color, in the case of *p*); the last, the axial skeleton, causing defective segmentation and extreme shortening of the tail. The site of gene action of both *c* and *p* locus alleles is within the melanoblast, the *albino* locus genes probably controlling the structure of tyrosinase, while *pink eye* prevents orderly arrangement and crosslinking of pigment-granule fibers, causing the granules to be smaller and irregular (WOLFE and COLEMAN 1966, review). The manner of action of *tp* has not been studied in detail, although this gene, because of absence of melanocytes in one tissue (the nictitans), has been postulated to affect melanoblast differentiation by controlling the tissue environment (MARKERT and SILVERS 1956). The axial abnormalities of *pudgy* are the result of defective segmentation of somites (GRÜNEBERG 1961).

1. c *locus inactivation:* Table 1 shows results of crosses expected to produce $(+)/c^{ch}$, $(+)/c$, $(c^{ch})/c^{ch}$, or $(c^{ch})/c$ genotypes as well as non-translocation segregants. A glance at the female progenies reveals immediately that variegation is produced by all of the rearrangements except R2. Of the 36 wild-type self (i.e., uniformly colored) females classified in the R2 cross (which was made with c/c, thus giving maximum chance for detecting variegation), 17 were tested with p/p (see below), and every one of them turned out to carry the rearrangement. It is thus evident that R2 does not inactivate the *c* locus.

Among the other rearrangements, R3 was the only one for which, in segregating crosses (top of Table 1), every wild-type female was variegated, while every recessive (c^{ch}/c^{ch} or c^{ch}/c) female was self. R4, R5, R6a and R6b all gave a minority of wild-type self and recessive variegated females which can be shown to be, with few, if any, exceptions, the result of recombination between breakpoint and *c* locus, giving $+/c^{ch}$ or $+/c$, and $(c^{ch})/c^{ch}$ or $(c^{ch})/c$, respectively. Theoret-

TABLE 1

Introduction of c locus marker into T(X;1) heterozygotes

Cross ♀ × ♂	Phenotype of progeny*	R2 ♂	R2 ♀	R3 ♂	R3 ♀	R4 ♂	R4 ♀	R5 ♂	R5 ♀	R6a ♂	R6a ♀	R6b ♂	R6b ♀
♀ $\frac{(++)\dagger}{c^{ch}}$ × $\frac{c^{ch}}{c^{ch}}$ ♂	+; Self	50	36	73(+13)‡	0	17	2	37	11	35	12	21	8
	+; Variegated	0	0	0	63(+18)	0	24	0	13	0	22	0	10
or	c^{ch} or c^{ch}/c; Self	49	55	88(+27)	89(+32)	21	35	31	34	33	32	20	19
♀ $\frac{(++)\dagger}{c^{ch}}$ × $\frac{c}{c}$ ♂	c^{ch} or c^{ch}/c; Variegated	0	0	0	0	0	1	0	11	0	12	0	1
	Unclassified	1	4	19(+2) 1¶	21(+4)	1	3	6	3	0	3	0	2
	Total												
	from c^{ch}/c^{ch} ♂	..		279		97				129		81	
	from c/c ♂	195		75(+96)		7		146		20			
♀ $\frac{(+)}{+}$ **or** $\frac{(c^{ch})}{c^{ch}}$ × ♂ $\frac{c^{ch}}{c^{ch}}$ **or** $\frac{c}{c}$	+, c^{ch}, or c^{ch}/c; Self	..		42	19			50	24	69	45		
	+, c^{ch}, or c^{ch}/c; Variegated	..		0	21			0	16	0	23		
	Unclassified	..		6	1			0	3	0	4		
	Total												
	from c^{ch}/c^{ch} ♂	..		46						114			
	from c/c ♂	..		43				93		27			

* Here and in Tables 3, 4, 5, and 6, the symbols indicate background color: + = wild type (black), $c^{ch} = c^{ch}/c^{ch}$, p = p/p, $c^{ch}p = c^{ch}p/c^{ch}p$.

‡ Figures in parentheses indicate offspring from a cross of $(+)/c \times c/c$. While otherwise similar to the other crosses in this portion of the table, this cross would fail to reveal half of the possible R–c recombinants, namely the $(c)/c$ class, which would be indistinguishable from c/c nonrecombinant segregants.

† $(++)/c^{ch}+$ or $(++)/c^{ch}p$.

¶ Here and in Tables 3, 4, 5, and 6, figures for unclassified animals placed between the ♂ and ♀ columns represent mice whose sex could not be determined because of damage to the genital region.

ically possible alternative explanations to be considered are that the wild-type self females are R females lacking an X (see section on unbalanced segregants), or R females with "normal overlap" (not enough cells with $+^c$ inactivated to give detectable variegation). A number of wild-type self females derived from the crosses in the top portion of Table 1 were tested—namely, 2, 2, 7, and 6 for R4, R5, R6a and R6b, respectively. All turned out to be non-R. Moreover, the number of wild-type self females is approximately equal to that of recessive-variegated females—i.e., the expected reciprocal crossover class that must be R; and, in turn, the proportion of females belonging to both of these classes is roughly similar to recombination frequencies calculated from other data (see below).

The mean proportion of fur variegated with a *c* locus marker is close to 50% in R3, about 40% in R6a and R6b, about half that in R4, and even less in R5 (see Table 2; a more detailed discussion of this parameter will be given in a future paper). From these various facts, it is concluded that normal overlaps for *c* locus variegation are probably absent or very rare in the case of R3, R6a and R6b. They may possibly occur in R4, and, perhaps more likely, in R5. In R5, in fact, the 11 wild-type self females in Table 1 included 3 that were small, 2 of them being near-sterile and untestable. No further information was obtained on these and they could have been R.

2. p *locus inactivation:* Table 3 shows results of crosses in which the intact autosome being introduced carried only *p* as a marker. Variegation was observed in all cases except R4. Although the total data for that stock are scanty for these crosses, the following facts add to the information: of the 7 wild-type self females obtained in the $(+)/+ \times p/p$ cross (bottom portion of Table 3) four were tested and two of these turned out to be R; in the $(+)/p \times p/p$ cross (top half) two females that could have been R were noted as being possibly very slightly lighter in color. It may thus be tentatively concluded that R4 inactivates the *p* locus very rarely. In R3, the variegated animals had very few and/or small areas of *p* color; and one of the wild-type self animals, on test, turned out to be R—i.e., a normal

TABLE 2

*Inactivation of LG1 loci in various T(X;1)'s**

Locus	R2	R3	R4	R5	R6
c	0	++++	+++	++	++++
p	++	(+)	0 or (+)	+++	++++
tp	some†	some	some	some	some
pu	0	....	....	0	0

* Symbols within the table indicate mean proportion of the fur having the variegating color, as follows: ++++ 36–70%; +++ 18–35%; ++ 9–17%; + 5–8%; (+) < 5%; 0 no variegation. Percentages were determined by visual grading of 594 R females—from 41 to 246 per independent R stock. While the visual estimates may not accurately represent the actual proportion of fur involved (CATTANACH and ISAACSON 1967), their comparative value has been verified by re-grading samples of animals and arriving at mean percentages differing by not more than 2% from originally determined ones. Ranges were set by the geometric mean of 50 and 25%, 25 and 12.5%, etc.

† No quantitative data on amount of variegation (see text).

TABLE 3

Introduction of p *locus marker into T(X;1) heterozygotes*

Cross ♀	Cross ♂	Phenotype of progeny	R2 ♂	R2 ♀	R3 ♂	R3 ♀	R4 ♂	R4 ♀	R5 ♂	R5 ♀	R6a ♂	R6a ♀	R6b ♂	R6b ♀
$\frac{(+)}{p}$	$\frac{p}{p}$	+; Self	40	6†	26	9‡	4	2¶+2§	47	4	15	1	22	2
		+; Variegated	0	18	0	7*	0	0	0	26	0	9	0	11
		p; Self	31	35	26	13	2	7	37	35	8	6	13	17
		p; Variegated	0	1	0	1	0	0	0	1	0	0	0	0
		Unclassified	3	3 7	0	1	0	1	3	2 4	0	0	2	4
		Total	144		83		18		159		39		71	
$\frac{(+)}{+}$	$\frac{p}{p}$	+; Self	29	17	8	3	10	7‖			25	12		
		+; Variegated	0	9	0	2*	0	0			0	11		
		Unclassified	2	1	0	3	0	0			0	0		
		Total	58		16		17				48			

* Variegation barely perceptible.
† Two tested non-R.
‡ One tested R.
¶ One tested non-R.
‖ Four tested: two R, two non-R.
§ Very slightly lighter, overall; ? variegated. Died young, no offspring.

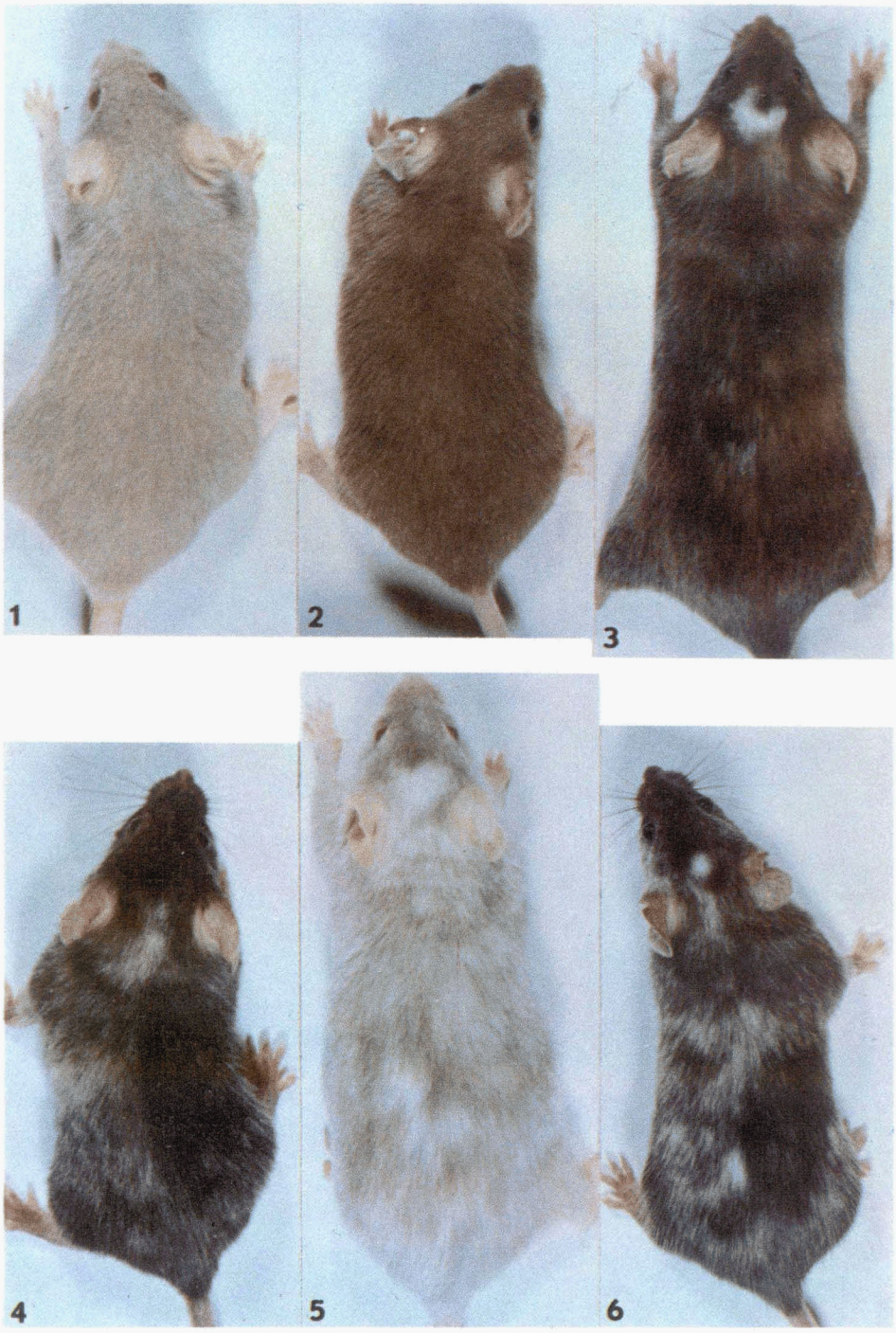
overlap. Full variegation (i.e., about 50%) was observed for R6, about half that for R5 and even less for R2 (Table 2).

A most interesting finding is that variegation on a *p* background is observable in $(p)/p$ females. In addition to the 3 animals shown in the top half of Table 3, there were 3 in miscellaneous crosses: $R3(p)/p \times p/p$ gave 1 variegated and 4 *p* self females; and $R2(p)/+ \times p/p$ gave 2 variegated on *p* and 1 *p* self (but R by test). This situation for *p* is in contrast to that found for *b*, where $R(b)/b$ is uniformly brown all over (RUSSELL and BANGHAM 1961), and would indicate that *p* is not an amorph, i.e. *p*/0 is lighter than *p*/*p*. The color difference is not extreme, and it is possible that, on the light background, it may occasionally be missed. This perhaps accounts for some of the discrepancy between wild-type self and pink eye variegated classes of females (top half of Table 3) which, apart from normal overlaps, should be reciprocal recombinants.

3. tp *locus:* Because *a*/*a*; *tp*/*tp* is a dark color, small areas of *taupe* variegation are difficult or impossible to detect in weanlings or older animals. Variegated animals are more easily recognizable at an earlier age. Since observations were not consistently carried out on young litters, the data in Table 4 cannot be used for quantitative comparisons. They do show that the *tp* locus is subject to inactivation in all the rearrangements, and is probably most frequently affected in R2. The *tp* locus has been shown to lie between *c* and *p* and gives about 3% recombination with *c* (RUSSELL 1963b, and unpublished).

4. pu *locus:* The LG1 recessive, *pudgy*, causes, in the homozygote, complete absence of the tail (except for an occasional small fleshy vertige). The possibility therefore exists that inactivation of the wild-type allele in up to half the cells of a $(+)/pu$ female would give some tail abnormality. Crosses of the type $(++)/c^{ch}+ \times c^{ch}pu/c^{ch}pu$ were made for all T(X;1) stocks but gave an extremely low yield due to the combined poor fertility of the partners. Thus, 48 R females of all stocks (3 to 14 per stock) mated to *pu*/*pu* males never produced any offspring, even though in many cases one or both partners had been fertile by other mates. Only 10 of 58 such matings (17.2%) yielded any progeny, giving an average of only 4.7 classifiable young each, which included a total of 9 R daughters (0.16 per R female mated to *pu*/*pu*). Of these, 4 each were R5 and R6a, and one was R2. All had normal tails. It may be concluded that in R5, R6, and R2, the *pu* locus is either not inactivated, or not inactivated with a high enough frequency to affect the total phenotype. On the inactivation-center hypothesis, no inactivation of this locus is expected in R5 or R6, the autosomal breakpoints of both of which lie between *p* and *pu* (see below). In R2, already *p* is only weakly inactivated (Table 2) and one might expect inactivation not to extend to *pu*, 22 units farther away. Obviously, more data are needed, and crosses with $+/pu$ males (with subsequent testing) are now underway.

5. *Variegating color:* Whenever a recessive allele was introduced at only one locus on the intact autosome, the variegating color was of the type that would be expected for a single dose of that allele. Thus, in all crosses in which the intact autosome carried c^{ch}, the variegating color was typical c^{ch}/c (see Figure 3: on an *a*/*a* background, this is a very characteristic "tan" shade; see Figure 2). Where


1
2
3
4
5
6

it carried *c*, the variegating color was clearly white. This indicates that inactivation is to a nullo-form, even in such rearrangements as R5 and R4, in which the *c* locus is affected less than half as often, presumably, as are other parts of the chromosome in which it is located. *Flecked* females with *c* in the intact autosome(s) also have "pure white" hair in flecked areas (CATTANACH 1961).

When the intact autosome carries *p*, the variegating color appears similar to *p/p* (compare Figure 4 with Figure 1) but is actually very slightly lighter, as demonstrated by the finding of faintly detectable variegation in (*p*)/*p* animals. Again the *p* locus is apparently being inactivated to the nullo state, for, if it were not, the variegating color would presumably be slightly darker than *p/p*. In the case of (+)/*tp* the variegating color is probably equivalent to *tp/tp*; although, *tp/tp* being so dark, this cannot be stated with certainty. It has not yet been possible to produce the critical type, (*tp*)/*tp*, with certainty: all *taupe* daughters from the R2(+)/*tp* × *tp/tp* cross have been self; but expected recombinants are rare, and the numbers are small since (+)/*tp* females produce very poorly (resembling, in this respect, *tp/tp* females).

When recessives are introduced at two loci in the intact autosome (Tables 5, 6), there may be two variegating colors, and a spreading effect is observed (Figures 3, 6). One color is that characteristic of the locus nearer the breakpoint in the inactivated chromosome, and the other that of both loci. Thus, in the case of R(+ +)/$c^{ch}p$, the colors are 0/c^{ch} ("tan", like c/c^{ch}) and 0/$c^{ch}p$ (near-white); while in the case of (+ +)R/$c^{ch}p$ the colors are 0/*p* ("lilac"-gray) and 0/$c^{ch}p$ (near-white). The frequency of animals with near-white spots (both loci inactivated) is very similar to that expected on the basis of frequency of inactivation of the more distant locus. Thus in R3, most R(+ +)/$c^{ch}p$ animals have only tan spots and the rare near-white spots are quite small; but in R6, most (+ +)R/$c^{ch}p$ animals show *both* "lilac" and near-white spots. The existence of occasional 0/c^{ch} spots in (+ +)R6/$c^{ch}p$ females cannot be completely ruled out. The complication of intermingling of hair of at least three levels of pigmentation might make small "tan" areas difficult to detect. On the other hand, it can be stated with near cer-

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FIGURE 1.—*a/a; p/p*, for comparison with variegation patterns.

FIGURE 2.—*a/a;* c^{ch}/c, for comparison with variegation patterns.

FIGURE 3.—*a/a*; R3(+ +)/$c^{ch}p$. Note that, on the black background, most of the variegation is with a color resembling c^{ch}/c (presumed gene action type: 0 +/$c^{ch}p$). Small areas, over the pelvic region and between ears, are near-white (presumed gene action type 0 0/$c^{ch}p$). Such near-white areas (spreading effect) are rare in R3 females, where *p* is very rarely inactivated.

FIGURE 4.—*a/a*; (+ +)R6/+ *p*. Note that on the black background, the variegation is with a color resembling *p/p* (presumed gene action type: 0/*p*).

FIGURE 5.—*a/a* R3(+ p)/($c^{ch}p$). Variegation with near-white (presumed gene action type 0 0/$c^{ch}p$) on a *p/p* background; *p* has been introduced into the translocated chromosome by recombination.

FIGURE 6.—*a/a*; (+ +)R6/$c^{ch}p$. A strong spreading effect is observed here: in addition to 0/*p* variegation, there is a considerable amount of near-white (0 0/$c^{ch}p$). Compare with Figure 3: in both cases, *c*+ and *p*+ are on the translocated, and $c^{ch}p$ on the intact chromosome; and the residual genotype is the same in the two animals. The strikingly different phenotypes are interpretated as due to different directions of spread of inactivation (see text).

TABLE 4

Introduction of tp *locus marker into T(X;1) heterozygotes*

Cross ♀	♂	Phenotype of progeny	R2 ♂	R2 ♀	R3 ♂	R3 ♀	R4 ♂	R4 ♀	R5 ♂	R5 ♀	R6a ♂	R6a ♀	R6b ♂	R6b ♀
$\frac{(+)}{+}$	$\times \frac{tp}{tp}$	+; Self	67	29	31	35*	14	22	21	19†	20	28‡	26	25‡
		+; Variegated	0	34	0	5¶	0	1?	0	8	0	2‖	0	2‖
		Unclassified	1	5	0	2	4	1 1	3	5	1	2 1		
		Total	136		73		43		56		54		53	

* Two obviously small.
† One tested R.
‡ Six obviously small.
¶ Four questionably variegated, but three of these verified R by test.
‖ Questionably variegated, but verified R by test.

TABLE 5

Introduction of c *and* p *locus markers into T(X;1)'s*

Cross ♀	Cross ♂	Phenotype of progeny	R2 ♂	R2 ♀	R3 ♂	R3 ♀	R4 ♂	R4 ♀	R5 ♂	R5 ♀	R6a ♂	R6a ♀	R6b ♂	R6b ♀
$\frac{(++)}{c^{ch}+}$	$\frac{c^{ch}p}{c^{ch}p}$	+; Self	39	1*	26	0	15	1‡	17	4	26	10†	20	2
		+; Variegated	0	42	0	30	0	19	0	11	0	13	0	15
or		c^{ch}, or c^{ch}/c; Self	43	42	31	28	23	22	18	8	22	15	25	14
		c^{ch}, or c^{ch}/c; Variegated	0	0	0	0	0	1	0	1	0	4	0	5
$\frac{(++)}{c+}$	$\frac{c^{ch}p}{c^{ch}p}$	Unclassified	4	3	3	4 7	3	1 0	1	0	2	2 2	4	5
		Total												
		from $(+)/c^{ch}$		0		105		85		0		96		90
		from $(+)/c$		174		24		0		60		0		0
$\frac{(++)}{++}$	$\frac{c^{ch}p}{c^{ch}p}$	+ or c^{ch}; Self							55	31	19	19	8	5
		+ or c^{ch}; Variegated							0	26	0	14	0	3
or		Unclassified								2	1	2		
$\frac{(c^{ch}+)}{c^{ch}+}$	$\frac{c^{ch}p}{c^{ch}p}$	Total												
		from $(+)/+$								114		0		16
		from $(c^{ch})/c^{ch}$								0		55		0
$\frac{(++)}{+p}$	$\frac{c^{ch}p}{c^{ch}p}$	+ or c^{ch}; Self	12	0			10	4	11	1	17	0	2	0
		+ or c^{ch}; Variegated	0	4			0	4	0	10	0	10	0	4
		p or c^{ch}p; Self	6	5			9	5	8	9	10	12	9	8
or		p or c^{ch}p; Variegated	0	0			0	2	0	0	0	1	0	1
$\frac{(c^{ch}+)}{c^{ch}p}$	$\frac{c^{ch}p}{c^{ch}p}$	Unclassified	0	1 0			2	4 3	1	3	2	3	3	4
		Total												
		from $(++)/+p$		28				15		0		6		0
		from $(c^{ch}+)/c^{ch}p$		0				28		43		49		31

* Tested normal overlap.
† Includes four non-R recombinants; and one translocation female lacking intact X.
‡ Translocation female lacking intact X.

TABLE 6

Progeny of crosses of $(++)/c^{ch}p$ ♀ × $c^{ch}p/c^{ch}p$ ♂ *

Phenotype of progeny	R2 ♂	R2 ♀	R3 ♂	R3 ♀	R4 ♂	R4 ♀	R5 ♂	R5 ♀	R6a ♂	R6a ♀	R6b ♂	R6b ♀
++; Self	213	11	175	0	61	10	116	15	97	5	99	1
++; Variegated	0	138	1	158	0	56	0	128	1	55	0	79
$c^{ch}p$; Self	191	177	177	157	73	97	141	121	59	64	77	67
$c^{ch}p$; Variegated	0	0	0	0	0	2	0	7	0	0	0	2
$c^{ch}+$; Self	23	27	31	30	19	30	34	0	24	0	21	0
$c^{ch}+$; Variegated	0	0	0	0	0	0	0	26	0	9	0	20
+p; Self	24	0	31	0	11	0	37	38	23	16	31	22
+p; Variegated	0	15	0	37	0	14	0	0	0	0	0	0
Unclassified	45	4 30	43	10 43	23	9 13	19	4 31	15	4 17	18	9 18
Total born	898		893		418		717		389		464	

* The table includes a relatively small number of animals (about 4% of the total) from matings of the same general type except that different *c* or *p* alleles were used, namely $(++)/c^{ch}p^{z} \times c^{ch}p/c^{ch}p$ and $(++)/c^{ch}p \times c\,p/c\,p$. Also included are some crosses (contributing about 13% of the total) in which the male introduced a sex-linked marker.

tainty that "lilac" areas were never found among the "tan" and black (and occasional near-white) areas of R3$(++)/c^{ch}p$. Quantitative details will be presented in a future paper.

The white color of the variegation found in the daughters of $(+)/c^{ch} \times c/c$ or $(c^{ch})/c^{ch} \times c/c$ crosses, leads to the conclusion that unbalanced segregants with autosomal duplications, such as are found in the case of the *fd* translocation (CATTANACH 1961), do not survive in the case of R3, 4, 5, and 6. If they did, their genotype, with respect to the *c* locus, would be $(+)/c^{ch}/c$ or $(c^{ch})/c^{ch}/c$ and the variegating color should be c^{ch}/c. (See section on unbalanced segregants.)

LOCATION OF AUTOSOMAL BREAKPOINTS

1. Breakpoint positions relative to c *and* p *loci:* The LG1 positions of the rearrangement points, relative to the *c* and *p* loci, have been determined in three-point crosses of the type $(+/+)/c^{ch}p$ ♀ $\times$ $c^{ch}p/c^{ch}p$ ♂, the third point being R (Table 6). The translocated autosomal portions of the females in this cross may thus be written R c^+ p^+, or c^+ R/R p^+, or c^+ p^+ R. Relative position of R will be considered first; calculation of recombination frequencies (for which this cross provides part of the data) subsequently. Table 7 shows the expectations for the three possible orders of points. These must consider the fact, discussed in earlier sections, that some of the translocations do not inactivate both *c* and *p* loci (see columns A, B, C of Table 7).

The results for individual rearrangements may now be considered in the light of these expectations. For R4, R5, and R6, the position of R relative to *c* and *p* could be determined from direct evidence. In the case of R2, where recombination with *c* has not occurred to date, and R3, where it is extremely rare, the 3-point cross could, in each case, rule out only one of the three possible orders. However, additional evidence made it possible to establish the position of R2 with considerable confidence.

For R5 and R6, as shown in Table 6, all chinchilla (non-pink eyed) females were variegated and all pink eyed (non-chinchilla) females were self. This would indicate (column A of Table 7) that the order is not R *c p*, since, in that case, such phenotypes could be obtained only as double crossovers. A distinction between the other two possible orders can be made by the finding of chinchilla-pink eyed variegated and wild-type self females, two groups that would have to be double crossovers if the order were *c* R *p*. It is thus concluded that for R5 and R6 the order is *c p* R.

For R3 and R4, column B of Table 7 should be used since *p* is affected only very slightly in R3 and perhaps not at all in R4. For R4, the results in Table 6 show, conversely from R5 and R6, all chinchilla females self and all pink eyed females variegated. Again, there are, in addition, wild-type selfs and chinchilla-pink-eyed variegateds, indicating an order for R4 of R *c p*.

In the case of R3, the results shown in Table 6 are sufficient only to rule out one order, namely, *c p* R, since no R–c^{ch} recombinants were obtained in that cross. Altogether, there has been only one case of probable R–*c* recombination and that was in a cross that could not establish relative positions. A mating of $(++)/c^{ch}$ + $\times$ + $p/+$ *p*, yielded a self daughter who was found to transmit no chinchilla in 66 offspring. The fact that she was nonvariegated did not in itself prove R–c^{ch} crossover, for *p* is only barely affected in the case of R3. However, the following

TABLE 7

Determination of location of autosomal breakpoint from cross of type (+ + +)/c^{ch}p ♀ × c^{ch}p/c^{ch}p ♂

Segregating chromosome*	Recombination events producing chromosome, if order is: R + + (1) (2) c^{ch} p	+ R + (1) (2) c^{ch} p	+ + R (1) (2) c^{ch} p	Phenotype‡ ♂♂	♀♀ if *c* and *p* loci affected (A)	♀♀ if *c* locus only (B)	♀♀ if *p* locus only (C)
(+ + +) $c^{ch}p$	nonrecombinant			w.t. st c^{ch}p f	w.t.$^{(nw)}$ ss c^{ch}p f	w.t.$^{(tan)}$ ss c^{ch}p f	w.t.$^{(p-)}$ ss c^{ch}p f
($c^{ch}p$) + +	(1)	(1)&(2)	(2)	c^{ch}p st w.t. f	c^{ch}p$^{(nw)}$† ss w.t. f	c^{ch}p$^{(nw)}$† ss w.t. f	c^{ch}p$^{(nw)}$† ss w.t. f
(+ p) c^{ch}+	(2)	(2)	(1)&(2)	p st c^{ch} f	p$^{(nw)}$ ss c^{ch} f	p$^{(nw)}$ ss c^{ch} f	p$^{(p^-)}$ ss c^{ch} f
(c^{ch}+) + p	(1)&(2)	(1)	(1)	c^{ch} st p f	$c^{ch(nw)}$ ss p f	$c^{ch(tan)}$ ss p f	$c^{ch(nw)}$ ss p f

* Parentheses indicate translocated genes.
† Variegation difficult to detect.
‡ Variegation, where present, is indicated by superscripts in parenthesis.
Explanation of symbols used for background color (all animals are *a/a*, non-agouti): w.t. = wild type (black); c^{ch} = c^{ch}/c^{ch} (only very slightly lighter than black); p = p/p (medium, "lilac" gray; pink eyes); c^{ch}p = $c^{ch}p/c^{ch}p$ (very light gray; pink eyes).
For variegating color, expectation is hemizygous expression of intact LG1 markers for affected loci–i.e., 0 0/$c^{ch}p$ (where loci are inactivated in the R chromosome); 0 $p/c^{ch}p$ or 0 +/$c^{ch}p$ (only *c* locus inactivated); and c^{ch} 0/$c^{ch}p$ or + 0/$c^{ch}p$ (only *p* locus inactivated). Symbols used for variegating color: nw = near-white (as in 0 0/$c^{ch}p$, 0 $p/c^{ch}p$, and c^{ch} 0/$c^{ch}p$); tan = resembling c^{ch}/c (as in 0 +/$c^{ch}p$); p^- = slightly lighter than p/p, see text, (as in + 0/$c^{ch}p$).
Fertility status is shown below the symbols indicating color: st = sterile; f = fully fertile; ss = semisterile.

indicate she was non-R: (a) she produced an average litter size of 9.3 by one year of age, well outside the range of 1.3–4.0 for R3 females (RUSSELL and MONTGOMERY 1969); (b) none of her 38 daughters, from a cross to c^{ch}/c^{ch}, was variegated; and (c) one daughter, though she gave a deficiency of sons and slightly reduced litter size, was cytologically normal (11 metaphases 40 chromosomes each, none long) and produced no X0 daughters in genetic tests.

Before considering R2, it should be noted that the 11 wild-type self females (Table 6) probably do not represent crossovers. Of the 11, 6 were recorded before we took over the stock and while it was still on an agouti background on which *p* variegation is relatively hard to detect; the remaining 5 all turned out to be

translocation heterozygotes, 3 representing normal overlaps and 2 lacking the intact X. (An additional 3 from other crosses all tested to be normal overlaps). With this in mind, the R2 results in Table 6 are of the same type as those for R3–i.e., they serve only to rule out one of the three possible orders, namely *c p* R. No cases of R–*c* recombination have been observed in any cross.

Although the order for R2 could thus, theoretically, be either R *c p* or *c* R *p*, the former is considered extremely unlikely since the *c* locus is not inactivated in any way by the rearrangement while the *tp* and *p* loci are (see above); i.e., it seems improbable that a locus should be completely skipped by an influence emanating from adjacent X chromosome material when this is obviously not the case in R4, R5, and R6. However, other theoretically possible causes of lack of *c* locus inactivation were examined. One of these is the existence of a multiple rearrangement, such as T(1;?, ?;X, X;1), in lieu of a simple reciprocal translocation. Arguing against this are, first, the fact that litter size in R2 is no more reduced than it is in the other T(X;1)'s (RUSSELL and MONTGOMERY 1969); and, second, the cytological analysis of meiotic configurations which reveals a high percentage of figures-of-four, but no figures-of-six (unpublished data of N. L. CACHEIRO at this laboratory).

The possibility has also been explored that R2 may be nonreciprocal as a result of one of two possible rearrangements. One of these is an X-1 translocation in which, however, the distal portion of X failed to attach to LG1 and was lost. Such a rearrangement can be rejected on several grounds. Even if the lost piece were small, it is doubtful that resulting translocation males would not be adversely affected by the complete absence of some X chromosome material. As shown in the preceding paper (RUSSELL and MONTGOMERY 1969), R2 males are quite normal. Furthermore, a compound chromosome consisting of almost the entire X plus all of LG1 distal to *c* would be quite a long one; actually, in R2, the "long" chromosome, though usually distinguishable, is considerably shorter than the "long" chromosome in some of the other T(X;1)'s (CACHEIRO and RUSSELL 1969). Finally, and most cogent, X chromosome loci on both sides of the breakpoint can be shown to be present (see below).

The other nonreciprocal rearrangement to be considered is insertion of a piece of LG1 into the X chromosome, one autosomal break being between *c* and *tp*, (see below), the other somewhere distal to *p*. (To consider the possibility of insertion, one must postulate a centromere–*c*–*p* order; otherwise *c*–*p* recombination giving the observed phenotypes would be nondisjunctional or beset by other difficulties). Such an insertion would differ from the *fd* insertion in that (a) the autosomal duplication type ("Type II", CATTANACH 1961) evidently does not survive and (b) there is recombination involving the translocated autosomal piece. If R2 were, in fact, an insertion, then crossovers between breakpoint and *p* in the cross $+(+)/c^{ch}p \times c^{ch}p/c^{ch}p$ would yield $c^{+}p$ recombinants deficient for one end portion of X and duplicate for the corresponding end portion of LG1; and $c^{ch}p^{+}$ recombinants duplicated for one end portion of X and deficient for the corresponding end portion of LG1. Apart from viability problems inherent in such conditions, the following results of tests for X imbalance make the insertion hy-

pothesis unlikely. It was shown that the hypothetical end piece of X does not contain either the *Ta* or *spf* loci, for when *Ta* or *spf* are introduced by the male in the above cross, (a) *p* recombinant daughters were not of 0/*Ta* or 0/*spf* phenotype; and (b) c^{ch} recombinant sons expected on the hypothesis to carry *both* $+^{Ta}$ and *Ta*, or $+^{spf}$ and *spf*, were of typical *Ta*/Y or *spf*/Y phenotypes. Furthermore, a mapping of the X chromosome breakpoint for R2 (RUSSELL and MONTGOMERY 1965, and to be published) shows this to be very probably located *between Ta* and *spf*. Unless more rigorous experiments should disprove this last result, the insertion hypothesis here considered must thus be rejected.

With the rejection of both the multiple-rearrangement and insertion hypotheses for R2, it appears that the most likely position of R2 with respect to *c* and *p* is an intermediate one. Three-point crosses involving R, *tp*, and *p* were made in an attempt to establish the position of R2 relative to *tp* and *p*. Matings of (+ +)/*tp p* and (+ *p*)/*tp* + females to *tp p*/*tp p* males yielded 25 and 75 classifiable offspring, respectively. The finding of a + *p*/*tp p* (non-*R*) female in the latter group probably rules out the order *tp*-R-*p*. All other recombinants found resulted from what must be *tp*–*p* crossovers. Since R2 is neither insertional nor a multiple rearrangement, the order *tp*–*p*–R can be rejected on the basis of the data that already disproved *c*–*p*–R. The position of R2 with respect to the three markers is thus assumed to be *c*–R2–*tp*–*p*. Recombination between R and *tp* from the three-point crosses, and from additional ones involving R and *tp* only, was 1.4%. It should be recalled (see above) that *c*–*tp* recombination in normal females is about 3%.

2. Recombination frequencies: Recombination frequencies are shown in Table 8 for R–*c*, R–*p*, and *c*–*p* in the different rearrangements. Figure 7 shows breakpoint positions in LG1. The data are derived from many crosses although the bulk come from matings of the type (+ +)/$c^{ch}p$ × $c^{ch}p$/$c^{ch}p$ (see Table 6). In most crosses, all recombinant females can be recognized from their phenotypes, and in such cases the calculation is based on all classified females. In a few crosses, e.g., R(+)/*c* × *c*/*c*, where only one class of recombinant females is identifiable, com-

TABLE 8

Recombination frequencies of breakpoints (R), c *and* p *loci in various T(X;1)'s*

Loci		R2	R3	R4	R5	R6
R–*c*	Number	629	657	346	131*	239*
	% recombination	0	0.2	6.9	30.5	27.6
R–*p*	Number	1369	30*	22*	939	855
	% recombination	9.7	(23.3)‡	(31.8)‡	8.1	2.2
c–*p*	Number	. . .	844	352	1077	1078
	% recombination	†	16.6	19.6	19.4	21.6

* Two-point crosses involving only the outside markers. Results from three-point crosses are included in the figures for the two shorter segments.

† Meaningless, if order is *c*–R–*p* (see text).

‡ Result doubtful because very low inactivation of the *p* locus in this rearrangement makes classification by phenotype alone unreliable.

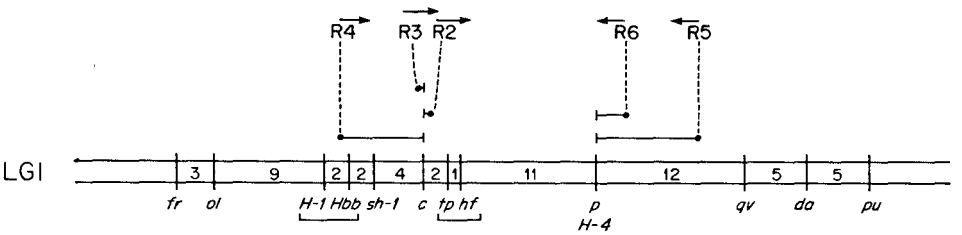

FIGURE 7.—Map of linkage group 1 of the house mouse (from GREEN 1966) above which have been marked mapped positions (·) of T(X;1) breakpoints R2, R3, R4, R5, and R6 in relation to the nearest autosomal marker used (as shown by connecting line). The arrow above each R symbol indicates direction of spread of inactivation within LG1. (Note that in GREEN's map distances shown are based on average recombination in the two sexes. The order within bracketed loci has not been established. The map differs slightly from more recent ones in Mouse News Letters and from unpublished results—e.g., *c–tp* recombination is 3% in females.)

putation is based on half the classified females. Included in the frequencies are also some results from fertility-tested males.

An example of the crosses that have entered into the results of Table 8 is the following list for R3. For R–*c* recombination: $(+\ +)/c^{ch}p$, $(+\ +)/c^{ch}+$, $(+\ +)/c^{ch}p^{x}$, (p^{x} is a radiation-induced allele of *p*), $(+\ p)/c^{ch}p$, $(+\ +)/c\ +$, each × $c^{ch}p/c^{ch}p$; $(+)/c$, $(+\ +)/c^{ch}p$, and $(+\ p^{x})/c^{ch}p$, each × $c\ +/c\ +$; $(+)/c^{ch}$ × c^{ch}/c^{ch}; $(+\ +)/c^{ch}p \times c\ p/c\ p$; $(+\ +)/c^{ch}+ \times +p/+p$ (tested ♀ ♀). For *c–p* recombination: $(+\ +)/c^{ch}p$ and $(+\ +)/c^{ch}p^{x}$, each × $c^{ch}p/c^{ch}p$; $(+\ +)/c^{ch}p$, $(+\ +)/c\ p$, and $(+\ p)/c+$, each × $c\ p/c\ p$. For other stocks, the variety of crosses is similarly great.

Although the presence of R reduces viability in females (RUSSELL and MONTGOMERY 1969), recombination frequencies derived from these data are probably not unduly distorted, since all calculations involve R and non-R females in reciprocal crossover classes, as well as R and non-R females in the noncrossover segregants. The inclusion of a very limited amount of data involving sex-linked genes (see Table 6, footnote) is also justified, since in these crosses all daughters (R and non-R within both crossover and noncrossover classes) carried the sex-linked marker, while none of the sons did. Slight distortions of calculated recombination frequencies could occur as a result of specific interactions of R with some of the autosomal recessives.

It may be noted that in the rearrangements where the autosomal breakpoint presumably lies outside the *c–p* region (R3, R4, R5, R6), recombination between these two loci is not reduced, even where R is close to one of them (R3, R6). The value for *c–p* recombination in normal females is generally taken to be about 16% (GREEN 1966), and the result for *non*-translocation $+\ +/c^{ch}p$ females in the T(X;1) stocks (Table 9) is in keeping with this value. If anything, the presence of R seems to give a slight increase in recovered cases of *c–p* recombination. In R5 and R6, the difference between the summed crosses (Table 8) and control data (Table 9) is significant (for each stock separately, $P = 0.02$ for R5 and $P = 0.006$ for R6; using pooled data of Table 9, $P = 0.02$ for R5 and $P = 0.0004$ for

TABLE 9

Test of presumed + +/c^{ch}p (non-translocation) animals resulting from crossovers between breakpoint and closest autosomal marker: segregation of autosomal alleles derived from the rearranged chromosome*

		R4		R5		R6 a & b	Total	
		♂♂	♀♀	♂♂	♀♀	♀♀	♂♂	♀♀
Recombinant animals tested		3	7†	2	12	5	5	24
Test progeny from above (sexes combined)	+ +	110	104	62	155	70	172	329
	$c^{ch}p$	123	93	72	136	73	195	302
	c^{ch}+	16	20	6	23	8	22	51
	+ p	19	25	12	24	12	31	61
Percent recombination, c–p		13.1	18.6	11.8	13.9	12.3	12.6	15.1

* Derived from R(+ +)/$c^{ch}p$ ♀ × $c^{ch}p/c^{ch}p$ ♂ in the case of R4 (i.e., R–c crossover) and from (+ +)R/$c^{ch}p$ ♀ × $c^{ch}p/c^{ch}p$ ♂ (i.e. p–R crossover) in the case of R5 and R6.

† In addition, two wild-type females derived from a R(+)/c^{ch} × c^{ch}/c^{ch} cross (i.e., p marker not present) produced 73 + and 74 c^{ch} young.

R6). However, when comparison is restricted to the cross most directly corresponding to that of the controls–i.e., (+ +)/$c^{ch}p$ × $c^{ch}p/c^{ch}p$ (Table 6) *versus* + +/$c^{ch}p$ × $c^{ch}p/c^{ch}p$ (Table 9)–significance of the difference disappears for R5 (P = 0.07), and almost does so for R6 (P = 0.04), which has the least extensive control data.

In the case of R2, where the breakpoint presumably lies between the c and p loci, c–R plus R–p recombinations (the former being zero, to date) do not quite add to control c–p frequency, even if one uses the R–p value of 11.4% from the female data of Table 6. This would indicate some inhibition of crossing over in the immediate vicinity of the breakpoint; however, the inhibition is apparently only moderate. As already shown, R2 probably lies between c and *tp*.

If recombination frequencies are taken at face value, breakpoints for the other rearrangements would be located as follows: R3 between c and *sh*-1; R4 probably fairly close to *H*-1 and *Hbb*; R6 close to p in the p–*qv* interval; and R5 probably between p and *qv*, but possibly between *qv* and *da*. However, recombination may not accurately indicate distance, (a) if there is crossover inhibition near the breakpoint (recombination frequency would underestimate distance), or (b) if there is some correlation between nonrecombination and unbalanced segregation (frequency calculated from surviving, euploid offspring would be an overestimate). A few crosses are in progress to determine locations of breakpoints more accurately.

3. *Tests of recombinants:* The position of R relative to c and p, deduced in the preceding section mainly from (+ +)/$c^{ch}p$ ♀ × $c^{ch}p/c^{ch}p$ ♂ crosses, is verified wherever other pertinent crosses have been made in the various stocks using R females, some of them presumed recombinants (Tables 1, 3, 4, 5). In addition, it seemed important to test presumed non-R recombinants of both sexes, for if the rearrangements were not simple reciprocal translocations, recombination could entail imbalances of various kinds.

Two types of recombinants from the $(+\ +)/c^{ch}p$ ♀ × $c^{ch}p/c^{ch}p$ ♂ cross were tested, namely (a) those between R and the closer marker (Table 9), and (b) those between *c* and *p* loci (Table 10). In the former case, presumed $+\ +/c^{ch}p$ offspring were crossed to $c^{ch}p/c^{ch}p$. (Since no *c*–R recombinant has, so far, been found in R2, and only one in R3, see above, Table 9 includes results only for R4, R5, and R6.) In the latter case presumed $c^{ch}+/c^{ch}p$ recombinants, in the case of R2, R3, and R4, and presumed $+\ p/c^{ch}p$ recombinants in the case of R5 and R6 were bred to $c^{ch}p/c^{ch}p$ to determine segregation of the respective + allele derived from the rearranged chromosome.

All of these presumed non-translocation recombinants (both Tables 9 and 10) proved to be fully fertile (for fertility data, see RUSSELL and MONTGOMERY 1969, Tables 4, 5, 6). Segregation among their offspring, with one possible exception, appeared perfectly normal in extensive tests (see also preceding section for another discussion of Table 9). Frequency in F_2 of the + allele derived by recombination from the rearranged chromosome varies from 47.1 to 51.5% in the various stocks (Table 10); and, in R3, 4, 5, 6a, 6b the differences from 50% are not significant. In R2, $P = 0.02$, with the biggest contribution to the difference from 50% coming from the shortage of $c^{ch}+/c^{ch}p$ males in F_2 (Table 10), especially among offspring of recombinant females. While it would be tempting to speculate as to possible causes, it should be pointed out that when as many comparisons are made as here, one expects one of them to be "significantly" different by chance. In any case, the reduction from 50% is only slight.

UNBALANCED SEGREGANTS

In a T(X;1) heterozygous female, the four chromosomes in question–two rearranged ones, the normal X and the normal autosome–can theoretically dissociate in various ways in the first division, all but one of these leading to unbalanced products. Table 11 summarizes the theoretical expectations for unbalanced segregants resulting from 2:2 dissociation, both adjacent-1 and adjacent-2 (code #1-4 in Table 11) and from 3:1 dissociation (code #5-12). The two translocated chromosomes are designated by the symbols $\frac{A^+}{X}$ and $\frac{X}{A}$, the former containing the autosomal locus being studied. The recessive marker is shown by the superscript r, the wild-type allele by +.

A priori, it may be assumed that segregants containing less than the equivalent of one X chromosome (code #1M, 2M, 5M, 7M, 8M), or those lacking an entire LG1 (6F, 6M), will not survive. At the other end of the spectrum, it is already known that certain unbalanced types (code #5F, 9M) are viable (RUSSELL and BANGHAM 1960, 1961; CATTANACH 1961). This leaves more than half the possible unbalanced genotypes for which evidence concerning viability must be examined.

Among this group of segregants are some whose phenotypes would reveal them as unbalanced–either regardless of recessive markers employed (#4M, 12M), or by use of specific markers (1F, 3F, 3M, 10F, 12F, and possibly 4F*, 4M*, 2F). Thus, in the cross $(+)/c^{ch} \times c/c$, balanced translocation females, $(+)/c$, should be variegated with white; while females with the type of imbalance being considered

TABLE 10

Test of presumed non-translocation animals resulting from crossovers between c *and* p *loci in translocation,* (+ +)/c^{ch}p, *mothers: segregation of the autosomal allele derived from the rearranged chromosome*

		R2	R3	R4	R5	R6a	R6b
Recombinant animals tested		13♂, 15♀	9♂, 16♀	4♂, 15♀	7♂, 13♀	8♂, 21♀†	5♂, 10♀†
♂ segregants	Number classified	755	453	387	464	575	355
	Percent +*	44.2	48.6	52.5	50.0	53.9	49.6
	P (for diff. from 50%)	0.002	0.54	0.33	1.00	0.06	0.87
♀ segregants	Number classified	764	399	356	424	591	328
	Percent +*	49.9	53.4	48.0	50.9	49.1	45.4
	P (for diff. from 50%)	0.94	0.18	0.46	0.70	0.65	0.10
Total segregants	Number classified	1519	852	743	888	1166	683
	Percent +*	47.1	50.8	50.3	50.5	51.5	47.6
	P (for diff. from 50%)	0.023	0.63	0.86	0.79	0.32	0.21

* $p+$ for R2, R3, R4; $c+$ for R5, R6a, R6b. The $p+$ and $c+$ alleles, respectively, presumably together with adjacent regions, are derived from the rearranged chromosome by crossing over.

† Of these, 1♂ and 11♀ for R6a, 1♂ and 6♀ for R6b resulted from *c*–R recombination in translocation females lacking *p* marker. Because of the low frequency of *p*–R recombination in R6, it is assumed that most, if not all, of these offspring resulted from crossing over in the *c*–*p* region, and are therefore comparable with the other individuals in the table.

in 1F, 10F and 12F, would be $(+)/c^{ch}/c$, and should appear variegated with c^{ch}/c. Only the former class was obtained (Table 1: R2, R3, R5, R6). Similarly, in crosses where the father had contributed $c^{ch}+^{p}$ (Table 1: R3, R4, R6), unbalanced types 3F, 3M, 4F*, and 4M* would be genotypically $c^{ch}/0$ and presumably resemble c^{ch}/c. Again, this was not found.

Progeny tests, in several cases, either corroborate indications given by the proband's phenotype or provide evidence not obtained in other ways. Thus, one may rule out the regular survival of classes 4F, 9F, and 12F: for, altogether, only three variegated sons have been produced by variegated females (see below), and each of these was the single one of this type in his sibship. The circumstances that would produce recessive-appearing segregants with occasional wild-type progeny (1F*, 4F*, 4M*, 12F*, 12M*) can also definitely be ruled out on the basis of very extensive evidence. Thus, the following number of recessive selfs were tested in R2, R3, R4, R5, R6a and R6b: 47, 59, 53, 22, 40, and 22 females, respectively, producing a total of 7835 young, all recessive (an average of 32.2 per tested female); and 28, 43, 16, 12, 16, and 9 males, respectively, producing a total of 4105 young, all recessive (an average of 33.1 per tested male).

It is thus obvious that the regular survival of most of the unbalanced segregants shown in Table 11 appears unlikely on the basis of one or more lines of evidence. Unbalanced segregants, found as occasional exceptions, were of only three types: variegated males (possibly code #9M in Table 11, but see below), recessive females with evidence of X loss (code #5F), and wild-type self females with only one X (paternal sex chromosome loss). These types will now be further considered.

Altogether three variegated males were found. Two of them, one in R3 and one in R6a, came from $(++)/c^{ch}p \times c^{ch}p/c^{ch}p$ crosses. In each, the variegating pattern was similar to that of females of the respective stock. Each was sterile and had 41 chromosomes, one of these being longer than the rest of the complement. The third variegated male, found in R2, came from a cross of $(++)/c^{ch}p \times tp\ p/tp\ p$, and the variegation appeared to be of *tp p* color, but covering only a small, rather discrete, area. Of 93 mitotic metaphases counted (from culture of external ear), 11, 60, and 19 had 39, 40, and 41 chromosomes, respectively (1 each had 38, 42, 43). This male was fully fertile, producing an average litter size of 5.5 in test matings to 9 miscellaneous females (total of 216 offspring); was not deficient for X-chromosome portions marked by *Blo*, *Ta*, *spf* and *sf*; transmitted c^{ch} and *tp p* in repulsion; and did not transmit the translocation. It was concluded that he was probably a mosaic of the type $\frac{c^{ch}++}{+\,tp\ p}///\frac{0}{+\,tp\ p}///\frac{\frac{c^{ch}++}{c^{ch}++}}{+\,tp\ p}$, i.e. a non-translocation male. (Note that this diagnosis could not be made with certainty from the cytological data, since the long chromosome in R2 is sometimes difficult to identify.)

The variegated males described above were found in progenies containing 1522 translocation females and 1828 presumed translocation males. If the variegated males in R3 and R6a are assumed to have had an extra X chromosome, the fre-

TABLE 11

Theoretical expectation of offspring resulting from unbalanced segregation in heterozygous translocation female $\frac{A^+X}{X\ A}A^rX$; *mating to* A^rA^rXY *male*‡

Code No.	Genotype: Maternal deriv.	Genotype: Paternal deriv.	Expected viability	Phenotype (if viable)	Condition for diagnosis by phenotype	Diagnostic progeny (from mating to r/r)
1F	$A^{rA^+}_{X}$	A^rX	?	variegated	*c* on A^P; c^{ch} on A^M	Deficiency of sons
1F*				(r/r, possibly)*		(some w.t. thru c.o.)*
1M	$A^{rA^+}_{X}$	A^rY	lethal	.....	.....	
2F	A^{rX}_{A}	A^rX	?	r/r	Possibly certain markers on X^P	Deficiency of sons
2M	A^{rX}_{A}	A^rY	lethal	.....	.....	.....
3F	X^{X}_{A}	A^rX	?	r/0	c^{ch}+^{p} on A^P	
3M	X^{X}_{A}	A^rY	?	r/0	c^{ch}+^{p} on A^P	
4F	$X^{A^+}_{X}$	A^rX	?	varieg. (heavily)		Variegated sons
4F*				(r/0, possibly)*	(c^{ch}+^{p} on A^P)*	(some w.t. thru c.o.)*
4M	$X^{A^+}_{X}$	A^rY	?	variegated ♂		
4M*				(r/0, possibly)*	(c^{ch}+^{p} on A^P)*	(some w.t. thru c.o., if proband fertile)*
5F	A^r	A^rX	viable	r/r	Marker on X^P; 39 chromosomes	0X daughters
5M	A^r	A^rY	lethal	.....	.....	.....
6F	X	A^rX	lethal	.....	.....	.....
6M	X	A^rY	lethal	.....	.....	.....
7F	A^+ X	A^rX	lethal?	.....	.....	.....
7M	A^+ X	A^rY	lethal	.....	.....	.....
8F	X A	A^rX	lethal?	.....	.....	.....
8M	X A	A^rY	lethal	.....	.....	.....
9F	$\frac{X}{A}\frac{A^+}{X}X$	A^rX	viable	varieg. (heavily)		variegated sons
9M	$\frac{X}{A}\frac{A^+}{X}X$	A^rY	viable	variegated ♂		.. [proband sterile]
10F	$\frac{X}{A}\frac{A^+}{X}A^r$	A^rX	?	variegated	*c* on A^P; c^{ch} on A^M	
10M	$\frac{X}{A}\frac{A^+}{X}A^r$	A^rY	?	wild type		.. [proband sterile]
11F	$\frac{X}{A}A^rX$	A^rX	?	r/r		
11M	$\frac{X}{A}A^rX$	A^rY	?	r/r		
12F	$\frac{A^+}{X}A^rX$	A^rX	?	varieg. (heavily)	*c* on A^P; c^{ch} on A^M	variegated sons
12F*				(r/r, possibly)*		(some w.t. thru c.o.)
12M	$\frac{A^+}{X}A^rX$	A^rY	?	variegated ♂		
12M*				(r/r, possibly)*		(some w.t. thru c.o., if proband fertile)*

* If X imbalance causes either nonrandom X differentiation or death of X-deficient cells.

‡ $\frac{A}{X}$ and $\frac{X}{A}$ denote the translocated chromosomes. Superscript + and r denote wild-type or recessive condition, respectively, of autosomal marker; P and M denote paternal and maternal.

quency of this condition among translocation heterozygotes is thus 0.06%. Since sex-linked markers were not present in matings that produced the two exceptional males, it is not known whether paternal or maternal nondisjunction was involved. The frequency is not significantly different from the 0.02% incidence of X^MX^PY, resulting from paternal nondisjunction in normal mice (RUSSELL 1961). It therefore appears that translocation females only very rarely, if ever, yield a segregation product containing the two translocated chromosomes plus the normal X. This conclusion is also supported by the failure to find translocation females with an extra X (code #9F in Table 11).

The reciprocal product, on the other hand, i.e., the intact autosome by itself, is produced with easily measurable frequency. This is particularly striking, since normal karyotypes only very rarely give spontaneous loss of a maternal X (RUSSELL 1961; RUSSELL 1968). In the cross $(++)/c^{ch}p$ ♀ × $c^{ch}p/c^{ch}p$; *Ta*(or *spf*)/Y, in which X^M losses were directly detectable since the male introduced an X-linked marker, there were 188 daughters (between 14 and 52 per R stock), 95 of them inheriting the intact autosome from their mother. Of these, 3 had apparently failed to receive the mother's intact X, an overall X^M loss frequency of 3.2%. The frequency in R4 alone was 14.3%, in the other stocks, combined, 2.3%. A frequency can also be estimated from the more extensive crosses in which X-linked markers were not present, but in which large numbers of daughters, assumed (on the basis of phenotype) to have received the mother's intact autosome, were tested. Of 222 and 20 that gave average litter sizes >4.9 and <5.0, respectively, 36 and 10 were further tested genetically and/or cytologically for absence of X. One X/0 was found in the former group, and 6 in the latter. Weighting by total numbers in the two littersize groups, the estimated overall frequency was 7.5%. Again, the frequency in R4 was highest, namely 17.0%, as compared to 4.5% for the other stocks, combined. X/0 frequency from spontaneous paternal sex-chromosome loss should be no higher than 1% (RUSSELL 1961). The balance of these frequencies may be assumed to be due to failure of the T(X;1) mother to contribute the intact X.

Whether this type of segregation is the result of three:one dissociation is as yet unknown. Observations indicate that what would be the two reciprocal classes of gametes of such dissociation–X^AA^XX (producing variegated X^AA^XXYA males after fertilization) and A (producing recessive AXA females)–are actually recovered in the ratio of about 1:100. X^AA^XXYA males are not known to be inviable after the time at which they can be classified by phenotype (about 6 days postnatally); but no evidence exists concerning their prenatal viability. The differential recovery of the two reciprocal classes could be affected at even earlier stages–e.g., by a greater probability of loss of the polysomic product at, or shortly after, ovulation (perhaps by going preferentially into the polar body). However, some mechanism for maternal X loss *independent* of 3:1 dissociation–such as, perhaps second-division nondisjunction, both in balanced and unbalanced products of the first division, might also be suggested as an alternative to the highly unequal recovery of the reciprocal products of 3:1 dissociation.

Paternal sex-chromosome loss in a female receiving the translocation from

her mother would manifest itself through lack of variegation (RUSSELL and BANGHAM 1960, 1961; CATTANACH 1961) in classes otherwise expected to be variegated. Since such nonvariegated types can, alternatively, result from normal overlaps or from recombination between breakpoint and marker, it is necessary to test genetically and/or cytologically. In R3, no c^+ self females were produced. In R2, there were 11 selfs among 149 c^+p^+ females (Table 6). Two of these were tested to be translocation, lacking the intact X; three were tested to be balanced translocation females (normal overlaps); and the remaining six, not completely tested, were probably also of the latter type. In R4, among 92 c^+ females, there were 12 selfs (Tables 6, 1), of which seven were tested. All seven of these turned out to be R–c crossovers (i.e. non-translocation), one of them also being X0 due to *maternal* loss. In R5, R6a, R6b, among 283 c^+p^+ females, there were 21 selfs (Table 6), 16 of which were tested and turned out to be non-R recombinants. An R6a translocation female lacking the paternal X was found in one of the miscellaneous crosses (Table 5). The overall frequency of paternal sex chromosome loss from these combined data is well within the limits of what has been found in non-translocation stocks (RUSSELL 1961).

DISCUSSION

All of the genetic evidence obtained for the five T(X;1)'s indicates that they are reciprocal translocations. This idea is consistent with mapping of breakpoints in relation to both autosomal loci (this paper) and X chromosome loci (to be published). The fact that no autosomal duplication segregants, equivalent to Type II *flecked* were found, is additional evidence against insertional rearrangements. Reciprocal translocations are further indicated by the results of tests of presumed non-R recombinants, which theoretically could entail imbalances if the rearrangements were not simple. Special care was taken to check the possibility that R2 might be an insertion or other type of nonreciprocal rearrangement that might explain non-inactivation of c in some way other than separation from an inactivation center. These tests were negative.

All of our T(X;1)'s, in contrast to *flecked*, which involves insertion of LG1 material into the X, have given recombination between breakpoint and autosomal marker, as well as among markers. The only recombination not yet observed is that between c and R2. However, this could be explained by inhibition of crossing-over in the immediate vicinity of R. No evidence for inhibition has been found at somewhat greater distances from R; and, in fact, a slight increase in recovered recombinants was observed in some of the rearrangements. If this should eventually prove significant, it could have several possible explanations. Thus, a correlation between nonrecombination and unbalanced segregation would lead to selection for recombinants. Alternatively (or additionally), an actual enhancement of recombination may occur in certain regions, as indicated by FORD and EVANS' (1964) finding of an increased chiasma frequency in autosomal portions of T(X;?)16H (genetic verification has not been possible since the autosome involved has not yet been identified). Whatever the cause, the magnitude of the effect observed in the T(X;1)'s is small.

The comparative study of several independent and different rearrangements, involving, in each case, the same two chromosomes (X and LG1) and maintained (for part of each stock) on a similar genetic background, allows a number of conclusions that could not be drawn from a single rearrangement. Basic to these conclusions are comparisons, between the rearrangements, of the *relative* inactivating effects within the same sets of loci.

In the absence of such comparisons, differential behavior of two loci in a particular rearrangement may be misleading. Thus, the finding that variegation in *flecked* was more extensive when the intact autosome carried *c* locus markers than when it carried *p* was explained by LYON (1963) in terms either of a diffusible p^+ product, or of a slower multiplication rate of cells where *p* was "uncovered." OHNO (1967) suggested that different functions of *c* and *p* could account for different amounts of inactivation. However, it is possible for *p* to variegate clearly more heavily than does *c*, as in R5. Similarly, comparison of "spreading effects" when both *c* and *p* markers are present in the intact autosome, e.g., the observation of $0/c^{ch}$ plus occasional $0/c^{ch}p$ areas in $R3(++)/c^{ch}p$, would argue against differential cell multiplication. As noted in RESULTS, the presence of small $0/c^{ch}$ areas in $(++)R6/c^{ch}p$ cannot be completely ruled out by visual observation. If the existence of such areas were to be proved, this would lend support to the suggestion of a diffusible p^+ product. The effect of such a product is, however, obviously insufficient to obscure the excess of *p* over *c* variegation in R5.

All of these results instead confirm the earlier suggestions (CATTANACH 1961; RUSSELL 1963a) of a gradient of inactivation similar to that found with Drosophila position effects (LEWIS 1950, review). In each case, the locus closer to the breakpoint in the inactivating chromosome is affected more strongly. This indicates (RUSSELL and MONTGOMERY 1965) that inhibitory influences can spread in both directions; and this, in turn, implies that inactivation cannot be associated with the X centromere, as suggested by some authors (e g., OHNO and LYON 1965). It is obvious that the translocations fall into two group, R2, R3, R4, *vs.* R5, R6; and if the marked autosomal regions are associated with the centromeric end of the X in one group, they must be associated with the distal end in the other. That is, in at least some of the translocations, autosomal loci are being inactivated by *non*-centromeric portions of the X.

The supposition that X-chromosome material, in general, has the power to inactivate attached autosomal material seems highly unlikely in view of the findings that the *c* locus remains active in R2, and that only one of the two translocated chromosomes shows asynchronous replication (CHU and RUSSELL 1965). The present evidence for lack of inactivation of the *pu* locus in R5 and R6 points in the same direction but needs to be strengthened by additional data. Similarly, it should be verified that such LG1 markers as *sh-1* and *fr* do not become inactivated in R3 and R2, and these tests are now in progress.

If X chromosome material in general does not have the power of inactivation, this power must reside in a specific region of the X, or in one or several points within this region (RUSSELL 1963a, 1964a). One limit to such a region must be, as pointed out, the R2 breakpoint in the X. Whether a limit on the other side can

be set by R6 will have to await determination of activity of loci in the reciprocally translocated chromosome. At any rate, the X chromosome region between R6 and R2 (RUSSELL and MONTGOMERY 1965) must contain within it one or more inactivation centers; or, possibly, inactivation may proceed from the region as a whole. It should be noted that CATTANACH and ISAACSON (1967) have produced evidence for a factor in the X chromosome which controls the extent of position-effect variegation when the X is in its "inactive" state. It is not yet known whether such a factor is equivalent to a single locus.

The finding of a gradient of inactivation within translocated autosomal material does not distinguish between inactivation center, centers, or region. It should be noted, however, that distance from breakpoint within the autosome alone does not determine degree of inactivation. Thus, R2–*p* and R3–*p* probably represent fairly similar distances; yet there is a considerable difference between the two rearrangements in degree of *p* variegation (Table 2). The result would indicate that the X-chromosome location of R2 was closer than that of R3 to an effective inactivation center or region. Again, the autosomal distance R3–*p* is slightly less than *c*–R6, yet *p* in R3 is barely affected while *c* in R6 is heavily inactivated (Table 2). This latter comparison is, however, somewhat complicated by the possibility that spread of inactivation could proceed with different efficiency in the two directions.

The position of the LG1 centromere will be deducible from the genetic data of this paper in conjunction with labelling studies of T(X;1)'s. CHU and RUSSELL (1965) found that the single longest chromosome of R2 (i.e., one of the two translocated chromosomes) showed frequent asynchronous labelling. On the assumption that late replication is correlated with genetic inactivity, this chromosome is thus presumably R p^+. No asynchrony was detected in the reciprocally translocated chromosome, which presumably carries the *c* locus. Although the cytological work showed differential labelling of proximal and distal regions of the longest chromosome, it was not extensive enough to designate one of these regions with certainty as being the late-replicating one. EVANS *et al.* (1965) showed that the late-labelling behavior of the normal X or of the X^{fd} of *flecked* was more constant proximally and need not extend to the distal end. Thus, a finding of proximal late labelling in the long R2 chromosome would lead to the conclusion that this chromosome had an X centromere and that the order in the intact LG1 was therefore centromere–*c*–*p*; conversely, distal late labelling would indicate the order in LG1 to be centromere–*p*–*c*.

Since it appears that in R2 only the translocated chromosome carrying the *p* locus is subject to inactivation, and since R2 +/*Ta* females show the typical +/*Ta* striped phenotype (RUSSELL and MONTGOMERY 1965), i.e., the *Ta* locus is presumably in the chromosome within which inactivation can occur, it may be concluded that the long R2 chromosome is *Ta–R–p*. The finding (RUSSELL and MONTGOMERY, to be published) that the X chromosome breakpoint of R2 lies between *Ta* and *spf*, indicates that, directionally, *c–p* corresponds to *Ta–spf*, i.e., if *c* is proximal to *p*, then *Ta* is proximal to *spf*, and *vice versa*. Location of the X

centromere will thus be determined automatically from location of the LG1 centromere.

It is of interest that the relative amounts of variegation for *c* and *p* found in the case of the *flecked* insertion (CATTANACH 1961) fit well with a gradient from the discussed inactivation region or center within such a region. The X chromosome breakpoint in *flecked* is located as follows: *Gy*–break–*Mo–Ta–Bn–spf*, recombination frequency with Mo^{Br} being about 2% (CATTANACH 1966). In view of the finding that *c–p* corresponds directionally to *Ta-spf* (see above), plus the finding that the *flecked* insertion is inverted (CATTANACH and ISAACSON 1965, citing OHNO; SLIZYNSKI 1967), the order in X^t should be *Gy-p-c-Mo–Ta–Bn–spf*. As has been discussed, the X chromosome region between the breakpoints of R6 and R2 has inactivating functions. Since R6 and R2 are about 6 and 23 units on the non-*spf* and *spf* side of *Ta*, respectively (RUSSELL and MONTGOMERY, to be published), this region lies on the *Ta* side of the *flecked* insertion. This is in keeping with the finding that inactivation for *c* is more frequent than for *p* in this rearrangement.

CATTANACH and ISAACSON (1965) prefer to think of at least one other inactivation center on the opposite side of the *flecked* insertion, since otherwise a considerable portion of the X in the X^t would not be involved in the inactivation process, leading to possible difficulties with dosage compensation. It should, however, be noted that in R2, the X chromosome portion in the *c*–R–*spf* chromosome is presumably not inactivated (see above), and the same may apply to even larger portions of the X in other T(X;1)'s (pending further work on the reciprocally translocated chromosomes). That the animal can support this type of imbalance in mosaic condition is perhaps no more surprising than the fact that it survives mosaicism for hemizygous autosomal lethals (see below) or for the long autosomal duplication existing in Type II of *flecked*.

That certain genes in the autosome can be suppressed completely as a result of X chromosome influences is shown by the variegating phenotypes, which clearly indicate that the allele present in the intact autosome behaves as if present in the hemizygous state. The decrease in inactivation with increasing distance from an inactivation center or region could theoretically take two forms: (a) gene action could be depressed to an intermediate level; or, (b) it could still be completely inhibited but not in all the cells in which the inactivation center or region in the translocated X has been called into play. Results indicate alternative (b) to be the case, for the variegating color in (+ +)R5/*c* + is clearly white in small areas, rather than one of the many intermediate shades associated with the numerous *c* alleles that have been discovered—in spite of the fact that *c* variegation in the case of R5 affects only about 13% of the body. The *flecked* translocation in combination with *c* also shows "clear white areas," even in a line selected for low percentage of variegation (CATTANACH and ISAACSON 1965). The finding of some hairs with intermediate amounts of pigmentation in *fd*/+; *c*/*c* females (GRÜNEBERG 1967) presumably indicates mixed population of melanocytes (black and white) within the same hairbulb. As an argument against this,

however, GRÜNEBERG presents a qualitative description of pigment concentration "generally" found distally in the intermediate hairs. While this finding cannot be fully evaluated until more quantitative data become available and until it is known what type of pigmentation patterns within hairs might be produced by hairbulbs with mixed melanocyte populations, it would seem that the presence of a preponderance of white hairs in *fd*/+; *c*/*c* and R(+)/*c* females indicates that when *c* locus inactivation occurs, it is to a nullo form.

The mosaicism for functional hemizygosity is a valuable tool for studies of gene action and developmental genetics. Already it has been shown that *p* is a hypomorph, rather than an amorph. Exploration of how various other LG1 loci act in the hemizygous state will be of considerable interest. A further possible use is the determination whether lethal alleles in the section of autosome subject to X inactivation act as cell lethals or can be supported in mosaic condition (RUSSELL *et al.* 1964; RUSSELL 1964b; and work in progress). The results, when analyzed in conjunction with known time of lethal action of the allele being employed, can be used to put limits on the stage of X differentiation (RUSSELL 1964b).

The finding that X-originated inactivation in T(X;A)'s appears to result in complete inhibition of the action of autosomal genes, rather than in depression to some intermediate level, argues very strongly against GRÜNEBERG's recent (1967) "alternative hypothesis" of gene action in the mammalian X, namely the idea that "partial inhibition of gene action happens in both X chromosomes of mouse females." If this hypothesis were correct, each cell of, e.g., an R(+)/*c* female should have a gene product equivalent to part-c^+ plus *c*, or, essentially part-c^+. If this were so, one should not expect (a) the mosaic phenotype (for threshold mechanisms do not exist at the *c* locus, where numerous intermediate alleles are known, all producing self coats), and (b) the existence of completely white mutant areas. One further criticism must here be made of GRÜNEBERG's attempt to replace the inactive X and the single-active-X hypotheses. He explains the behavior of X-linked genes in the T(X;?)16H translocation on the basis that they "escape from the effect of the inhibiting centre and thus become dominant over their alleles on the intact X which remain exposed to partial inhibition." While this might have applied to genes on the opposite side of the breakpoint from the "inhibiting centre," it will not hold for those on the same side, for both should then be partially inactivated according to his hypothesis. The T16H break was shown to be located between *Ta* and *Bn* (LYON 1966a) and is, therefore, between *Ta* and *spf*. Yet, both *Ta* (LYON *et al.* 1964) and *spf* (LYON 1966b) act fully "dominant" when on the translocated chromosome and fully "recessive" when on the intact X in a T(X;?)16H female. For several reasons, therefore, GRÜNEBERG's suggestion of both X's being partially active must be rejected.

The inactive X hypothesis stated not only that only one X was fully active, but that this X differentiated at random in different cells. Several apparent exceptions to the latter generalization are now known from a number of species. In no case, however, has there been an unequivocal distinction between primary nonrandomness, i.e., affecting the X-differentiation process itself, and secondary nonrandomness, i.e., subsequent cell-selection mechanisms superimposed on basically

random differentiation. The term nonrandomness will here be applied to the end result, without implications as to mechanisms. Of interest for the present discussion are several presumed T(X;A)'s with nonrandom X inactivation. In two human cases (MUKERJEE and BURDETTE 1966; GERMAN 1967, Figure 4) the evidence is cytological only and indicates one translocated chromosome to be always inactive. The opposite situation (normal X virtually always inactive) is found in the mouse for SEARLE's translocation, which has been studied both genetically and cytologically (LYON *et al.*, 1964; OHNO and LYON 1965); and in one cow, according to cytological evidence recently described (GUSTAVSSON *et al.* 1968).

These various cases make it necessary to examine the possibility of partial nonrandomness in the T(X;1)'s discussed in this paper. The first type of nonrandomness (translocated chromosome preferentially inactive) would result in phenotypes approaching the recessive, and this was not observed. Where at least one autosomal substitution leads to 50% variegation, partial nonrandomness of the second type may be ruled out. This would apply to R3 on the basis of *c* (even though *p* barely variegates), and to R6 on the basis of *p*. The possibility must, however, be examined for R2, R4, and R5, which give average amounts of variegation of about 15% (*p*), 20% (*c*), and 27% (*p*), respectively. One approach would be to find an autosomal locus closer to the inactivating portion of the X and to determine whether it approached 50% inactivation. There is, indeed, some evidence from the few R2(+)/*tp* animals scored at a young age (when the phenotypes are more easily distinguished) that *tp* variegation is much more extensive than is the variegation in R2(+)/*p*; although the observations are not exact enough to state whether it actually approaches 50%. Cytological studies involving labelling will eventually contribute to the evidence. The low *p* variegation in R2, and the low *c* variegation in R4 are quite plausible when one remembers that inactivation to the "right" can, as shown by R3, fade from maximum to practically nothing in an autosomal distance of 16 units. In R2 and R4, *p* and *c* are already 10 and 7, respectively, units to the "right" of the breakpoint. It is thus doubtful that evidence for nonrandomness exists in any of the T(X;1)'s.

OHNO (1967) has suggested that the nonrandomness in SEARLE's translocation is due to the fact that the X is divided near its middle and that "simultaneous inactivation of the two separate halves is beyond the means of the dosage compensation mechanism". He states, further: "It is our interpretation that RUSSELL's X-autosome translocations result in the variegated-type position effect, because in each a chromosome break occurred at the point very near to either the proximal or the distal end of the X." These interpretations are not borne out by the results. Thus, the position of the X chromosome break in R6, and even in R5, is fairly close to *Ta* (RUSSELL and MONTGOMERY 1965) which, in turn, is very close to the breakpoint in SEARLE's translocation (LYON 1966a). In other words, in R6 there is random inactivation in spite of a centrally located break in the X.

Since it is thus highly unlikely that "double inactivation . . . is possible only if the X chromosome material as a unit is preserved by the translocation-bearing X" (OHNO 1967) and since it has already been shown (above) that inactivation

is not associated with the X centromere, the hypothesis of an X inactivation region, or of a center or centers within it, is further confirmed. The T(X;1) results are completely consistent with the original idea that the center or region in only one of the two chromosomes comes into play and are at variance with GRÜNEBERG's recent hypothesis of inhibition emanating simultaneously from both X's. CATTANACH and ISAACSON (1967) believe to have found two or three alternate forms of a factor in the X, controlling degree of inactivation, and have postulated that these may arise from "changes in state," although they could not rule out meiotic crossing over. Our results to date have not shown alternate forms, but experiments in progress should reveal "changes in state" if these occur.

SUMMARY

Five X-1 rearrangements that arose at this laboratory provide an opportunity for studying the same loci in relation to different rearrangement points. Autosomal breakpoints were mapped relative to *c*, *p* and *tp* in LG1. The effect of the T(X;1)'s on activity at the *c*, *p*, *tp* and *pu* loci was studied.—Genetic evidence indicating that the rearrangements studied are reciprocal translocations comes from breakpoint mapping, from the fact that no animals resembling Type II *flecked* are found, from tests of presumed non-R recombinants, and from special tests in one of the rearrangements. Recovered recombination frequencies at medium distances from the breakpoints are not reduced and may be slightly enhanced; in the immediate vicinity of at least one of the breakpoints, recombination may be somewhat inhibited. Segregants with chromosomal imbalance, other than that involving an intact X, die prenatally. Translocation females only rarely, if ever, yield a segregation product containing the two translocated chromosomes plus the normal X. On the other hand, transmission of the intact LG1 alone (i.e., $0X^p$) is quite significantly higher than from normal females.—When single markers are used, the *c* locus is found to be inactivated more than *p* in some of the T(X;1)'s (R3, R4), but *p* more than *c* in others (R5, R6, R2); when both loci are marked together, the more strongly affected one may be inactivated alone, but the more weakly affected one is probably inactivated only in conjunction with the other (spreading effect). These findings are at variance with some suggestions (e.g., differential multiplication rates, different gene functions) that have been made by other authors to explain unequal amounts of *c* and *p* variegation. Instead, they confirm our earlier suggestion of a gradient of inactivation. The spread in both directions indicates that inactivation does not emanate from the X centromere, and that, in some of the translocations, autosomal loci are being inactivated by non-centromeric portions of the X.—Decrease in inactivation with increasing distance from inactivation center or region is the result of fewer cells being affected rather than of depression to an intermediate level. This completeness of inactivation, which produces mosaicism for functional hemizygosity, has already been used as a tool for studies of gene action and developmental genetics. It now presents a strong argument against GRÜNEBERG's recent hypothesis that

partial inhibition of gene action occurs in *both* X chromosomes. This hypothesis has also been rejected on other grounds.—Reports of other T(X;A)'s with nonrandom inactivation made it necessary to examine the possibility of partial nonrandomness in the five T(X;1)'s studied. This can be ruled out in two of them, R3 and R6, where at least one autosomal substitution leads to 50% variegation. Other evidence makes it somewhat unlikely for two others, R2 and R4. OHNO's recent interpretation that random inactivation is possible only if "X chromosome material as a unit is preserved" by the translocation can be rejected on the basis of breakpoint mapping in R6, a randomly inactivating T(X;1).—Evidence that it is neither the X centromere nor X chromosomal material "as a unit" that determines inactivation further confirms our earlier hypothesis of an inactivation center or an X chromosome region within which one or more inactivation centers are located. The T(X;1) results are at variance with GRÜNEBERG's recent hypothesis of inhibition emanating simultaneously from both X's. They are completely consistent with the original idea that an inactivation center or region comes into play in only one X chromosome, when two are present. Inactivation in this chromosome proceeds in both directions, the probability of a given locus being affected decreasing with distance, and the effect, when it does occur, being the equivalent of total inactivation.

LITERATURE CITED

CACHEIRO, N. L. A. and L. B. RUSSELL, 1969 Cytological studies of X-autosome translocations in the mouse. I. Mitotic chromosomes. Genetics **61**: s8.

CATTANACH, B. M., 1961 A chemically-induced variegated-type position effect in the mouse. Z. Vererbl. **92**: 165–182. ——, 1966 The location of Cattanach's translocation in the X chromosome linkage map of the mouse. Genet. Res. **8**: 253–256.

CATTANACH, B. M. and J. H. ISAACSON, 1965 Genetic control over the inactivation of autosomal genes attached to the X chromosome. Z. Vererbl. **96**: 313–323. ——, 1967 Controlling elements in the mouse X chromosome. Genetics **57**: 331–346.

CHU, E. H. Y. and L. B. RUSSELL, 1965 Pattern of DNA synthesis in X-autosome translocations in the mouse. Genetics **52**: 435.

EICHER, E. M., 1967 The genetic extent of the insertion involved in the *flecked* translocation in the mouse. Genetics **55**: 203–212.

EVANS, H. J., C. E. FORD, M. F. LYON and J. GRAY, 1965 DNA replication and genetic expression in female mice with morphologically distinguishable X chromosomes. Nature **206**: 900–903.

FORD, C. E. and E. P. Evans, 1964 A reciprocal translocation in the mouse between the X chromosome and a short autosome. Cytogenetics **3**: 295–305.

GERMAN, J., 1967 Autoradiographic studies of human chromosomes. I. A review. Proc. 3rd Intern. Congr. Human Genet.: 123–136. See Figure 4.

GREEN, M., 1966 Mutant genes and linkages. pp. 87–151. In: *Biology of the Laboratory Mouse.* Edited by E. L. GREEN. McGraw-Hill, New York. ——, 1967 Gene action in the mammalian X chromosome. Genet. Res. **9**: 343–357.

GRÜNEBERG, H., 1961 Genetical studies on the skeleton of the mouse. XXIX. Pudgy. Genet. Res. **2**: 384–393.

GUSTAVSSON, I., M. FRACCARO, L. TIEPOLO and J. LINDSTEN, 1968 Presumptive X-autosome translocation in a cow: Preferential inactivation of the normal X chromosome. Nature **218**: 183–184.

LEWIS, E. B., 1950 The phenomenon of position-effect. Advan. Genet. **3**: 73–115.

LYON, M. F., 1963 Attempts to test the inactive-X theory of dosage compensation in mammals. Genet. Res. **4**: 93–103. ——, 1966a Order of loci on the X chromosome of the mouse. Genet. Res. **7**: 130–133. ——, 1966b Lack of evidence that inactivation of the mouse X chromosome is incomplete. Genet. Res. **8**: 197–203. ——, 1966c X chromosome inactivation in mammals. pp. 25–54. In: *Advances in Teratology*. Edited by D. H. M. WOOLLAM. Logos Press, London.

LYON, M. F., A. G. SEARLE, C. E. FORD and S. OHNO, 1964 A mouse translocation suppressing sex-linked variegation. Cytogenetics **3**: 306–323.

MARKERT, C. L. and W. K. SILVERS, 1956 The effects of genotype and hair environment on melanoblast differentiation in the house mouse. Genetics **41**: 429–450.

MUKERJEE, D. and W. J. BURDETTE, 1966 Multiple congenital anomalies associated with a ring 3 chromosome and translocated 3/X chromosome. Nature **212**: 153–155.

OHNO, S., 1967 Three different consequences of X-autosome translocation. pp. 123–135. In: *Sex Chromosomes and Sex-linked Genes*. Springer Verlag, New York.

OHNO, S. and B. M. CATTANACH. 1962 Cytological study of an X-autosome translocation in *Mus musculus*. Cytogenetics **1**: 129–140.

OHNO, S. and M. F. LYON, 1965 Cytological study of Searle's X-autosome translocation in *Mus musculus*. Chromosoma **16**: 90–100.

RUSSELL, L. B., 1961 The genetics of mammalian sex chromosomes. Science **133**: 1795–1803. ——, 1963a Mammalian X chromosome action: Inactivation limited in spread and in region of origin. Science **140**: 976–978. ——, 1963b Linkage, *tp–c*. Mouse News Letter **29**: 73. ——, 1964a Another look at the single-active-X hypothesis. Trans. N.Y. Acad. Sci. (Ser. II) **26**: 726–736. ——, 1964b Genetic and functional mosaicism in the mouse. Symp. Soc. Study Develop. Growth **23**: 153–181. ——, 1968 The use of sex chromosome anomalies for measuring radiation effects in different germ cell stages of the mouse. pp. 27–41. In: *Effects of Radiation on Meiotic Systems*. Intern. Atomic Energy Assoc. Vienna, Austria.

RUSSELL, L. B. and J. W. BANGHAM, 1959 Variegated- type position effects in the mouse. Genetics **44**: 532. ——, 1960 Further analysis of variegated-type position effects from X-autosome translocations in the mouse. Genetics **45**: 1008–1009. ——, 1961 Variegated-type position effects in the mouse. Genetics **46**: 509–525.

RUSSELL, L. B., J. W. BANGHAM and C. S. MONTGOMERY, 1964 The use of X-autosome translocations in the mouse for the study of properties of autosomal genes. Genetics **50**: 281–282.

RUSSELL, L. B., J. W. BANGHAM and C. L. SAYLERS, 1962 Delimitation of chromosomal regions involved in V-type position effects from X-autosome translocations in the mouse. Genetics **47**: 981–982.

RUSSELL, L. B. and C. S. MONTGOMERY, 1965 The use of X-autosome translocations in locating the X chromosome inactivation center. Genetics **52**: 470–471. ——, 1969 Comparative studies on X-autosome translocations in the mouse. I. Origin, viability, fertility and weight of five T(X;1)'s. Genetics **63**: 103–120.

SEARLE, A. G., 1962 Is sex-linked *Tabby* really recessive in the mouse? Heredity **17**: 297.

SLIZYNSKI, B. M., 1967 Oocyte pachytene analysis of CATTANACH's *fd* translocation. Genet. Res. **9**: 17–22.

WOLFE, H. G., 1967 Mapping the hemoglobin locus in mice transmitting the *flecked* translocation. Genetics **55**: 213–218.

WOLFE, H. G. and D. L. COLEMAN, 1966 Pigmentation. pp. 405–425. In: *Biology of the Laboratory Mouse*. Edited by E. L. GREEN. McGraw-Hill, New York.