COMPARATIVE STUDIES ON X-AUTOSOME TRANSLOCATIONS IN THE MOUSE. I. ORIGIN, VIABILITY, FERTILITY, AND WEIGHT OF FIVE $T(X;t)$ 'S¹

LIANE BRAUCH RUSSELL AND CLYDE SAYLORS MONTGOMERY

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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NOT long after the earliest descriptions of X-autosome translocations in the mouse (RUSSELL and BANGHAM 1959, 1960) these rearrangements played an important role in the development of the inactive X chromosome hypothesis (LYON 1961; RUSSELL 1961). Salient features that entered in the formulation of this hypothesis were, first, that autosomal loci when placed in the vicinity of X chromosome material assumed "mosaic" expression in certain heterozygotes, similar to what had been noted for some X chromosome genes (LYON 1961; RUSSELL 1961); and, second, that the presence of *two* X chromosome equivalents was required to allow variegation in X-autosome translocation heterozygotes, regardless of sex (RUSSELL and BANGHAM 1960; RUSSELL 1961; CATTANACH 1961).

Since the formulation of the inactive X hypothesis, a number of X-autosome translocations have been described in mammals. Altogether, there are now nine in the mouse. Seven of these have occurred at this laboratory (RUSSELL and BANGHAM 1961; RUSSELL, BANGHAM, and SAYLORS 1962; RUSSELL 1963; and RUSSELL, unpublished); one was found by CATTANACH (1961) and one by SEARLE (1962). Some of these mouse translocations have been extensively studied genetically and cytologically and will be discussed. In addition, two presumptive X-autosome translocations in human infants (MUKERJEE and BURDETTE 1966; GERMAN 1967), and one in a cow (GUSTAVSSON, FRACCARO, TIEPOLO, and LINDSTEN 1968) have been reported on the basis of cytological evidence. A presumed Y-autosome translocation was briefly reported for the mouse by FALCONER, SLIZYNSKI and AUERBACH (1952), and another has been described in hamster tissue culture (STUBBLEFIELD 1966).

The fact that five of our seven X-autosome translocations involved the same autosome, LG1, (linkage group 1) allows comparative studies which have already led to a modification of the inactive X hypothesis, renamed the "single-active-X hypothesis" (RUSSELL 1963, 1964b). According to the modified hypothesis, the X bears an inactivation center² from which influences proceed along gradients. In

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² The existence of a specific inactivation center has not been formally proved. However, genetic limits have been established for a portion of X chromosome which must contain one or more such centers or regions (RUSSELL and MONTGOMERY 1965, and to be published). Although the phrase "inactivation center" will be used for ease of discussion in the present paper, these limitations in our knowledge should be implied.

a T(X;A), the autosomal segment that has been joined to that portion of the X that bears the inactivation center (but not that joined to the other portion) also comes under the influence of this center (see Figure 1). In those cells in which the intact X is active, a major part (but not all) of this compound chromosome is inactive and certain autosomal genes are suppressed, thus allowing recessives on the corresponding portion of the intact autosome to express themselves in essentially hemizygous condition. Autosomal genes in the translocation may fail to be inactivated either because they are attached to that portion of the X not bearing the center; or because, though in the proper chromosome, they are too far from the center. Further study of these translocations made it possible to locate the center within certain limits (RUSSELL and MONTGOMERY 1965).

Although some of the conclusions from the comparison of X;1 translocations have thus been reported, detailed comparative studies have not heretofore been published. The present paper describes the origin of these translocations, and the viability, fertility, and weight of the heterozygotes (homozygotes cannot be obtained since males are sterile). These "vital statistics" are of particular interest in connection with the presumed functional hemizygosity for autosomal genes, and with indications that X-autosome translocations may be rarer than would be expected from the yield of other translocations.

ORIGIN AND SYMBOLISM

All of the sex-linked rearrangements were detected in the course of various specific-locus mutation-rate experiments in which wild-type animals (irradiated or control) are mated to a test stock of constitution a/a ; b/b ; $c^{ch}p/c^{ch}p$; $d\ se/d\ se$; s/s . Five of the markers used, in four of the linkage groups, affect coat color. In

FIGURE 1.—Interpretation of inactivation in X-autosome translocations by the modified single-active-X-chromosome hypothesis. Contents of the two circles represent alternate conditions, each of which presumably exists in about half the cells of the body. Solid bars are autosomal portions, dotted bars, X chromosome portions; black regions represent inactivity; a, b, c are generalized symbols for autosomal recessives; position of a postulated inactivation center (see Footnote 2 p. 103) is marked by an asterisk. (Modified from RUSSELL 1963.)

such experiments, the majority of the exceptional progeny showing a coat-color change are self-colored, but some show the mutant color in only part of the coat. Testing of the latter reveals many of them to be either mosaics for one of the test-stock markers, or to carry new sex-linked or autosomal dominant mottling factors (RUSSELL 1964c). A very few, however, have turned out to be X-autosome translocations. Two of four possible autosomes have been involved, namely LG8 and LG1.

Following standardized mouse genetics nomenclature, the rearrangements are named $T(X;8)$'s and $T(X;1)$'s with suffixes 1R1, 2R1, 3R1 . . . etc., in the order of discovery. For the sake of brevity in the following discussions, the rearrangements will be designated R1, R2, R3 . . . etc. R1 and R7 are $T(X;8)$'s, and one of these has been described earlier (RUSSELL and BANGHAM 1961). The remainder, which are $T(X;1)$'s, will be discussed in this paper.

The origin of the $T(X;1)$ rearrangements is summarized in Table 1. Four were found in daughters of pre-sterile-period matings of males irradiated with doses of 300 or 600r X rays at either 80–84r/min or 10r/min. Three of these (R2, R4, R5) were presumably induced in spermatozoa, and one (R3) in spermatocytes (or possibly spermatids). R6a and R6b were found in two successive litters of a control mating. It is assumed that the mother, who was $(101 \times C3H)F_1$, already carried the rearrangement. In her mating to the multiple-recessive, test-stock male, she produced nine wild-type sons and the two variegated daughters, and yielded an average litter size of 2.8. Her 101 mother also had given a low average litter size, 3.6, and may have been the original mutant. R6a and R6b, although presumably the same rearrangement, have been maintained in separate stocks and most data for them will be presented separately.

Preliminary data on two of the rearrangements, R2 and R3, have been presented earlier (RUSSELL and BANGHAM 1961, Sections III and II, respectively). Some or all of the rearrangements have entered into more general discussions concerning mammalian X chromosome activity (RUSSELL 1962; 1963; 1964a, b, c; RUSSELL and BANGHAM 1964; RUSSELL and MONTGOMERY 1965).

TABLE 1
Origin of $T(X;1)$'s

Symbol	Original designation	Dose (r)	Dose rate (r/min)	Irradiation-conception interval (days)	Probable cell stage of induction	First report
R2	191G	300	83.7	4	Spermatozoa	(2)*
R3	212G	300	84.0	24	Spermatocytes	(1)*
R4	4AS	600	80.5	2	Spermatozoa	(3)*
R5	1SaSd	600	10.0	1	Spermatozoa	(4)*
R6a	26FUFo	...	From 2 successive litters of (101 × C3H)F ₁ ♀ × multiple recessive ♂. Spontaneous origin, probably in a 101 ♀			
R6b	30FUFo	...				
(3)*						

* References as follows: (1) RUSSELL and BANGHAM 1959; (2) RUSSELL and BANGHAM 1961; (3) RUSSELL, BANGHAM, and SAYLORS 1962; (4) RUSSELL 1963.

The symbol R will be used in subsequent discussions not only to name the rearrangements but also to indicate the position of the breakpoint in genetic formulae, e.g., $\frac{(+)}{c^{ch}} \frac{R(+ +)}{tp\ p}$. Gene symbols shown in parentheses, either with or without the accompanying symbol R, indicate that the genes are located in the rearranged chromosome, e.g., $(c^{ch}+)/c^{ch}p \times c^{ch}p/c^{ch}p$.

GENERAL FEATURES OF RESULTS AND PROCEDURE

It was shown early (RUSSELL and BANGHAM 1961) by a series of crosses that variegation is the result of the rearranged position of the variegating autosomal genes, rather than being caused either by structural heterozygosity or by any change in the wild-type allele. Evidence that the rearrangements are reciprocal translocations between the X and LG1 comes from various sources, both genetic and cytological. Linkage with both X and LG1 markers and mapping of break-points in both chromosomes are most cogent (RUSSELL and MONTGOMERY 1970; also RUSSELL, BANGHAM and SAYLORS 1962; RUSSELL 1963; RUSSELL and MONTGOMERY 1965). Cytological evidence from meiotic material (studies in progress) is consistent with reciprocal translocations. Mitotic metaphases of all T(X;1)'s show one of the two translocated chromosomes to be longer than the longest pair in the complement (RUSSELL 1964a; CACHEIRO and RUSSELL 1969).

In all the various T(X;A)'s discovered by us, the general breeding pattern is essentially similar. When females variegated on a wild-type background are crossed to chromosomally normal males which carry an appropriate marker in the intact autosome that corresponds to the one involved in the rearrangement, the most prevalent classes obtained are the following (Table 2): (a) females that resemble the mother in type of variegation, in the fact that they are semisterile, and in the progeny they produce; (b) wild-type, sterile males; and (c) males and females of the marker genotype that are fully fertile and do not transmit variegation or wild type. Rarer classes obtained are: (a) semisterile females that, depending on the marker used, are either variegated on the marker background or of uniform marker phenotype (see below); (b) marker males that are sterile; and (c) wild-type males and females that are fully fertile and do not transmit variegation. The chromosomal basis for these various classes is shown in Table 2. The rarer group of classes represents recombination between the breakpoint and the autosomal marker. The (a) and (b) classes in both groups are balanced translocation heterozygotes; the (c) classes have received the intact chromosomes from their mother.

A great many variations in this basic scheme are possible. Thus, the marker used may be an amorph or a hypomorph (a difference that affects the phenotypes of both classes of semisterile females); two or more markers on the intact autosome may be used in combination; allelic substitutions can be made in the translocated chromosome. These variations will be considered in the more detailed discussion of results, particularly in the next paper in this series.

The unbalanced types formed from two-two segregation, both adjacent-1 and

TABLE 2

General breeding pattern for females carrying T(X;1)'s or T(X;8)'s

		$\frac{A^+}{X} \frac{A^r}{A} \frac{X}{A} X$	$\times$	$A^r A^r X Y^*$	Autosomal symbolism $\frac{(+)}{r} \times \frac{r}{r}$
paternal chroms.	maternal	$A^r X$		$A^r Y$	
$\frac{A^+}{X} \frac{X}{A}$	$\frac{X}{A}$	varieg. on wild type, "semisterile"		wild type, sterile	$(+)/r$
A^r	X	marker, fertile		marker, fertile	r/r
$\frac{A^r}{X}$	$\frac{X}{A}$	marker or varieg. on marker, "semisterile"		marker, sterile	$(r)/r$
A^+	X	wild type, fertile		wild type, fertile	$+/r$
$\frac{A}{X}$	A	} Unbalanced complements from two-two segregation (adjacent-1 and adjacent-2) are apparently lethal			
$\frac{A}{X}$	X				
$\frac{X}{A}$	A				
$\frac{X}{A}$	X				

* A, X, Y denote intact autosome (LG1 or LG8), X and Y chromosomes, respectively. $\frac{A}{X}$ and $\frac{X}{A}$ are the two reciprocally translocated chromosomes. Superscript r denotes a recessive marker; +, the corresponding wild-type allele.

adjacent-2, appear to be lethal, causing the "semisterility" of translocation females. Three-one segregation can apparently occur, though rarely, and is detectable by certain XO and XXY individuals with balanced autosomal complement. These types played a major role in the development of the inactive X hypothesis (RUSSELL 1961; LYON 1962). Unbalanced segregation is further discussed in the next paper.

Because of complete sterility of translocation males, the rearrangements have to be carried through the female line. The translocation females, as will be shown below, not only yield less than half the normal litter size but have a productivity that is reduced in other ways as well, and are less viable than controls. In some cases it is difficult even to maintain a stock (R4 has now been lost); and to obtain the comparative results to be presented has taken several years.

For routine maintenance, all of our T(X;1) stocks are continuously backcrossed to a/a segregants of our $A/a; c^{ch}p/c^{ch}p$ stock 22A (small areas of variegation are more easily detectable on a/a than on agouti). They are, therefore, on a reasonably uniform background. In addition, many different types of crosses, ranging from 19 to 37 per T(X;1) stock—altogether close to 200—have been performed for a variety of purposes. For some of the results to be presented, information from various crosses can be pooled within each stock. This is the case for

viability and fertility. The bulk of the data for each stock comes from crosses to 22A.

VIABILITY AND FERTILITY

Survival to seven days of age, males and females: Classification based on coat color can be made at about seven days of age, and survival from conception to that age can thus be estimated from the segregation ratios of crosses in which translocation heterozygotes are phenotypically distinguishable. Viability data concerning female offspring can be used from all crosses in which segregating R and non-R daughters can be reliably classified. Excluded were crosses in which the marker introduced on the intact autosome was at a locus not subject to complete inactivation; those in which the background color would make variegation difficult to detect; and those in which viability problems resulting from the marker used would complicate the picture. Viability data concerning male offspring can be used from only a few crosses, since, in most cases, phenotype alone—without fertility test—is insufficient to distinguish R and non-R sons. Phenotypic classification was taken at face value in crosses where a segregating marker is extremely closely linked to the breakpoint (less than 0.5% recombination: namely, *c* in R2 and R3; see below). Data are also used from those crosses in which, although the closest segregating marker is somewhat more distant (though still less than 10 units away, e.g., *p* in R5 and R6, *c* in R4), a large enough percentage of the male progeny has been fertility tested to allow one to estimate with some reliability the relative proportion of R and non-R in the small number of untested male progeny.

Results are shown in Table 3. The percentages of R progeny can be compared with two sets of values: (I) with a theoretical expectation of 50%; and (II) with a "control" that attempts to make allowance for the possibility that relative via-

TABLE 3
Seven-day survival of $T(X;1)$ males and females

	R2	R3	R4	R5	R6 (a and b)
Female progeny					
Total classified	756	670	316	595	727
Percent with rearrangement	41.8	47.0	36.7	44.7	44.8
P (for difference from 50%)	< .00001	.12	< .00001	.0098	.0054
P (for difference from "control")*	.0014	.21	< .00001	.26	.25
Male progeny					
Total classified	580	650	153	266	385
Percent with rearrangement	50.7	47.1	45.6	46.3	56.2
P (for difference from 50%)	.74	.14	.27	.23	.015
P (for difference from "control")*	.64	.07	.15	.13	.17

* $+/c^{chp}$ (nontranslocation) animals in the various stocks, in matings to c^{chp}/c^{chp} yielded the following progeny (pooled results): 315 ♂ ♂, 252 ♀ ♀ $++$; 288 ♂ ♂, 261 ♀ ♀, c^{chp} ; 46 ♂ ♂, 34 ♀ ♀, c^{ch} ; 47 ♂ ♂, 49 ♀ ♀, p ; 1292 total.

For comparison with R2, 3, 4, used $(252 + 49)/596 = 50.50\%$ for female progeny; $(315 + 47)/696 = 52.01\%$ for male progeny.

For comparison with R5, 6, used $(252 + 34)/596 = 47.99\%$ for female progeny; $(315 + 46)/696 = 51.87\%$ for male progeny.

bility may be affected by the segregation of markers as well as by the segregation of the rearrangement. This "control" is provided by the pooled results from the various stocks of crosses of $++/c^{ch}p$ (non-R) recombinants with $c^{ch}p/c^{ch}p$. It is much more suitable for comparison with the male viability data, 73% (1483 out of 2034) of which come from $(++)/c^{ch}p \times c^{ch}p/c^{ch}p$ crosses, than with female viability data, over 55% (1689 of 3064) of which come from a large variety of other crosses.

It is obvious from Table 3 that survival of male translocation heterozygotes to seven days is not significantly reduced in the case of any of the rearrangements, regardless of whether comparison is made with the 50% expectation or with "control." On the other hand, frequency of female translocation heterozygotes is less than 50% in all stocks, the difference being highly significant in four of the five rearrangements (R2, 4, 5, 6). Comparison with "control" which, as discussed, is not so good for females, still yields a highly significant difference in two cases (R2 and R4). Giving all stocks equal weight, about 43% of all females at 7 days are R females. This represents a loss of 24% of R, relative to non-R females [i.e., $(57-43)/57$].

Survival and well-being of young adult females: Incidental information on survival and general well-being during the first few months of life comes from the females set up for breeding. Since males are not used for stock maintenance or experimental crosses, but only in fertility tests, the numbers observed are not extensive enough for reliable results.

The incidence of young-adult death and of sterility of females set up for breeding is shown in Table 4. Females were mated at weaning age, between one and

TABLE 4

Incidence of young-adult death and sterility of females set up for breeding in $T(X;1)$ stocks

	R2	R3	R4	R5	R6a	R6b
Translocation heterozygotes						
Number of ♀♀ mated	198	240	93	143	108	84
Percent ♀♀ died aged 1-7 months†	2.5	7.1	7.5	2.1	1.9	4.8
Number of mated survivors*	184	218	84	118	101	73
Percent sterile ♀♀	4.4	11.0	8.3	4.2	11.9	11.0
Percent death and sterility	6.9	18.1	15.9	6.3	13.7	15.7
Non-translocation segregants‡						
Number of ♀♀ mated	48	61	65	32	50	28
Percent ♀♀ died aged 1-7 months†	6.3	3.3	3.1	0	4.0	0
Number of mated survivors*	45	59	63	32	48	28
Percent sterile ♀♀	0	5.1	4.8	3.1	0	0
Percent death and sterility	6.3	8.3	7.8	3.1	4.0	0

* Includes only females mated to known fertile males.

† About 43% of the animals in these groups died by 6 weeks of age, another 40% between 6 weeks and 4 months. Of the remaining 17%, most were recorded "missing" and probably died considerably earlier.

‡ Diagnosed by coat-color phenotype; non-recombinant or recombinant for marked autosomal portion. Excludes known X/0 females.

four per cage, isolated when pregnant, and returned as soon as their litter was weaned. Comparison can be made within each rearrangement stock, between R and non-R segregants. The great bulk of the deaths occurred between one and four months of age. Some, if not most, of the sterility may be an indication of lack of general well-being, rather than of specific defects of the reproductive system, since many of the sterile females were also noted as being small, thin, or sickly. In each stock, the combined percentage of young-adult death and sterility is higher in the R than the non-R group, and the same is true for percent sterility alone. The "died young" class alone, however, is more frequent in the R group of only four of the six stocks. Pooling data for all stocks, the proportion of young-adult deaths is not significantly higher for R females (4.4%) than for non-R females (3.2%; $P = 0.37$). On the other hand, the proportion of sterile R ♀♀, 8.2%, is quite significantly higher than that of sterile non-R ♀♀, 2.5% ($P = 0.002$). Since there were, in general, more females per male in the case of non-R than in the case of R matings (and thus a better chance for the latter to be productive), the difference here found may actually be an underestimate of the true difference.

Percentage of young raised to weaning: T(X;1) females, on the average, raised fewer of their young to weaning age than did their non-R sibs. Tabulations were made on two types of crosses in each stock, namely, (a) $(++)/c^{ch}p \text{♀} \times c^{ch}p/c^{ch}p$ (22A) ♂ and (b) $c^{ch}p/c^{ch}p \text{♀}$ segregants mated with the same type of males, approximately concurrently. Only young born within the females' first year of life were included. Summing all stocks, R females failed to raise 321 of 2882 young born, i.e. 11.4%, while their non-R sibs failed to raise only 139 of 2659 young born, or 5.2%. The difference is significant ($P < 0.00001$). Average litter sizes involved were 2.56 and 6.65, respectively, so that the offspring of R females might have been expected to have a better chance because of less competition. On the other hand, it is a well known fact that mouse females in general often fail to raise litters of one or two. Figures for individual stocks were 8.7, 14.6, 16.1, 10.1, 8.8% not raised, for R2, R3, R4, R5, R6, respectively.

Since apparently one-quarter of all translocation females do not survive to 7 days of age (see above), the question arises whether this could account for the excess in young not raised by R females. If all females missing by 7 days did indeed die between birth and 7 days, this would, in fact, be equivalent to 6% of born young not raised, i.e., approximately the observed percentage. However, it appears much more likely, from limited observations, that most of the deficiency of R females by 7 days is accounted for by prenatal loss.

Productivity of females: Data on average litter sizes, at birth, produced by translocation heterozygotes and contemporary non-translocation segregants (including recombinants) in the same stocks are shown in Table 5. (The productive females in Table 5 are the fertile survivors of Table 4.) For all stocks combined, the number of offspring born to date in the two groups are 8104 and 9425, respectively. Average litter size calculations, however, are based only on young born before the mother was one year old (6989 and 8204, respectively), since litter size drops in old age and data might otherwise be biased by length of mating.

TABLE 5

Average litter sizes produced by $T(X;1)$ females and contemporary controls

	R2	R3	R4	R5	R6a	R6b
<i>Translocation Heterozygotes:</i>						
Number of productive ♀ ♀	176	194	77	113	89	65
Total number offspring born	1888	1845	758	1682	1122	809
Number offspring born in ♀ ♀'s first year	1591	1698	682	1428	917	673
Number of litters born in ♀ ♀'s first year	647	702	277	468	335	250
Average litter size*	2.46	2.42	2.46	3.05	2.74	2.69
Litters per productive ♀ *	3.68	3.62	3.60	4.14	3.76	3.85
Number of ♀ ♀ with > 2 litters*	107	126	50	81	62	44
Range in average litter size for ♀ ♀ with > 2 litters	1.3-4.3	1.3-4.0	1.3-4.0, and 1 @ 5.0	1.3-5.0	1.5-4.0	1.8-4.0
<i>Non-translocation Segregants:</i>						
Number of productive ♀ ♀	45	56	60	31	48	28
Total number offspring born	1620	1628	2197	1149	1649	1182
Number offspring born in ♀ ♀'s first year	1445	1442	1869	884	1511	1053
Number of litters born in ♀ ♀'s first year	206	225	275	127	218	141
Average litter size*	7.01	6.41	6.80	6.96	6.93	7.47
Litters per productive ♀ *	4.58	4.02	4.58	4.10	4.54	5.04
Number of ♀ ♀ with > 2 litters*	43	44	52	27	42	28
Range in average litter size for ♀ ♀ with > 2 litters	5.2-9.5	4.3-8.8,	4.0-10.7	4.7-9.2	4.5-9.4	5.0-9.8 and 1 @ 3.6

* Based only on litters born in ♀ ♀'s first year of age.

An interesting finding is that litter size of translocation females in every one of the stocks is considerably less than one-half that of non-translocation females. For R2, 3, 4, 5, 6a, 6b, the percentages are 35.1, 37.8, 36.2, 43.8, 39.5, 36.0, respectively; the combination of all stocks gives 37.9 or 38.1 for the unweighted or weighted averages, respectively. Table 5 also shows the average number of litters per female produced during the interval for which average litter size is computed. Heavy breeding activity might be expected to reduce litter size: however, the R females averaged a smaller number of litters than non-R controls. It is likely that, were the numbers of litters per female equal in the two groups, the litter size of R females would be an even smaller percentage of the litter size of controls. (The *number* of litters should not be used to compare productivity of the two groups, since mating conditions were not exactly standardized; e.g., there were, in general, more females per cage and, therefore, longer periods of separation from the male in the non-R group.)

Table 5 also shows ranges in average litter size, based only on females having three or more litters in their first year. It may be seen that in three of the stocks there was no overlap at all between the R and non-R groups, and that, in the remainder, overlap occurred only for a very small number of individuals.

Fertility of males: Fertility tests of males are summarized in Table 6. Alto-

TABLE 6

Fertility tests of males resulting from crosses of the type (+)/marker × marker/marker

	R2	R3	R4	R5	R6a		R6b	
Marker utilized†	c^{ch}	c^{ch}	c^{ch}	p	p	c^{ch}	p	c^{ch}
Cross‡	A	A, B	A, B	A	A	B	A	B
Wild-type males								
Number tested§	32	35	21	22	17	7	4	11
Number fertile	0	0	3	2	0	1	0	1
Average litter size range of fertile ♂ ♂ *	..	..	5.1–5.7	6.0–6.5	..	6.6	..	5.3
Recessive males								
Number tested	27	43	17	12	7	9	4	9
Number fertile	27	43	16	12	7	8	4	5
Average litter size range of fertile ♂ ♂ *	4.7–8.3	4.6–7.5	5.3–8.5	4.3–8.7	4.3–6.8	4.3–8.5	5.0–6.3	4.8–7.8 and 1 @ 1.7

* Based only on males having > 2 litters by the time mate was 1 year old.

† Where an additional linked marker is present, as in cross A, it is one giving more recombination with the breakpoint. "Wild-type" and "recessive" categories (below in same column) refer only to the marker shown on this line, irrespective of condition of the other marker.

‡ Cross A = $(++)/c^{ch}p \times c^{ch}p/c^{ch}p$ Cross B = $(++)/c^{ch}+ \times c^{ch}+/c^{ch}+$; or $(++)/c^{ch}p \times c^{ch}+/c^{ch}+$; or $(++)/c^{ch}+ \times c^{ch}p/c^{ch}p$.

§ Of 148 males that turned out to be sterile, 83.1% were mated starting at an age of less than 6 weeks. More than 1 female was used in many of the fertility tests.

gether 277 males were tested, from 34 to 78 per rearrangement. Nearly all males (see footnote to Table 6) were mated from less than six weeks of age onward and, in the case of those that appeared sterile, the mate was fertility tested elsewhere or additional mates were added. In addition to fertility tests, we have obtained extensive data on testis weights and have studied testis histology (with E. F. OAKBERG), but these results, which corroborate those of the fertility tests, will be presented elsewhere.

The tested males in Table 6 are classified according to phenotype of the marker utilized that is most closely linked to breakpoint, regardless of other markers that may be segregating in the cross; e.g., sons of $R(++)/c^{ch}p \times c^{ch}p/c^{ch}p$ matings are classified as "wild type" if their phenotype is $+^{c+p}$ or $+^{c}p$, and as "recessive" if it is $c^{ch}p$ or $c^{ch}+$. When the closest available markers were used (c for R2, R3, R4; p for R5, R6—see RUSSELL and MONTGOMERY 1970), wild-type males were found to be sterile and recessive males fertile in all or virtually all cases. The few exceptions in R4, R5 and, when the more distant marker c was used, in R6 are presumably cases of recombination between breakpoint and marker. In support of this conclusion are the following points: (I) litter sizes produced by exceptional wild-type fertiles fall within the range produced by recessive fertiles; (II) the exceptional wild-type fertiles yield no variegated daughters and they sire segregants expected on the basis of their being non-R recombinants (e.g., $++/c^{ch}p$, $c^{ch}+/c^{ch}p$, etc.); (III) the percentage of exceptional males found is what would be expected

from recombination frequencies calculated on the basis of female progeny (RUSSELL and MONTGOMERY 1970).

WEIGHT

Translocation females are often noticeably smaller than their normal sisters, while translocation males do not obviously differ from their brothers. Litters containing either *both* types of females or *both* types of males were weighed at weaning. Because of the considerable variability between litters, the means for two groups being compared are based on a paired entry from each litter. For example, if there were two non-R females and one R female in a given litter, only the *average* weight of the former two was included in the total for the non-R group. The number of comparisons is therefore smaller than half the number of animals. Statistical significance was calculated by the method of paired comparisons. It may be noted that in almost all cases, the non-R animals carry recessives, p , c^{ch} , c , etc., or combinations of recessives, while the R animals do not. Any weight-reducing effect of the translocation may thus be minimized.

In all four of the translocations still available for weighing, R females are quite significantly lighter than their normal sisters. For the different stocks, weight reductions range from 8.3 to 30.2%; and, in between 67 and 100% of the individual paired comparisons, the R entry is the smaller one. Weaning weight of translocation males did not differ significantly from that of their normal brothers in any of the stocks.

It thus appears that the presence of T(X;1)'s has a marked effect on body size in females but not in males.

TABLE 7

Paired comparisons of weaning weights of translocation bearers and normal sibs

	R	R2 non-R	R	R3 non-R	R	R5 non-R	R6 (a and b) R non-R
Number of ♀ ♀	21	20	8	8	24	26	64 68
Number of paired comparisons		18		7		21	55
Number of pairs where R < non-R		18		7		14	47
Mean R/mean non-R (percent)		69.8		84.5		91.7	86.6
P		<0.00001		0.002		0.007	<0.00001
Number of ♂ ♂	16	16	4	6	23	28	48 52
Number of paired comparisons		15		4		21	42
Number of pairs where R < non-R		6		3		10	27
Mean R/mean non-R (percent)		104.8		92.7		99.9	97.3
P		0.2		0.4		0.9	0.05

DISCUSSION

Translocations are relatively easily recovered following irradiation of appropriate stages in the male mouse, and large numbers have been studied for various purposes (RUSSELL 1962, review). In the earlier work, neither the means nor the impetus was available for determining whether any of the recovered rearrangements were sex-linked. The X-autosome translocations that have actually been discovered were found as a result of experimental conditions present for other purposes, namely autosomal markers for specific-locus experiments in the case of our translocations and of CATTANACH's insertion; and a sex-linked marker for a sex-chromosome-loss experiment in the case of SEARLE's. In a specific attempt to recover X-autosome translocations, on the assumption that they would probably induce female semisterility and male sterility (RUSSELL and BANGHAM 1959, 1961; LYON *et al.* 1964), LYON and MEREDITH (1966) studied 38 rearrangements recovered in daughters of irradiated spermatozoa. None of these turned out to be X-linked. The difference from expectation, on the assumption that each chromosome had an equal chance of being involved in a translocation, was significant at the 5% level. GRIFFEN and BUNKER (1967) report finding no X-linked translocation in a sample of 118 semi-sterile daughters of irradiated males. SEARLE *et al.* (1968) irradiated spermatogonia and cytologically scored close to 2000 translocations in spermatocytes of the same testes. Translocations involving the sex chromosomes were not seen, although it is not stated that this type of rearrangement was definitely ruled out for each of the translocations scored.

A rough estimate may be made concerning whether or not the X-autosome translocations discovered at this laboratory corroborate the apparent dearth of such rearrangements. In accordance with findings that translocations are relatively rarely recovered from irradiated oocytes (RUSSELL 1962) and spermatogonia (W. L. RUSSELL 1954, review; United Nations Report, 1966, review), none of our T(X;A)'s stemmed from such treatment. The known, relatively high, frequency of translocation induction from acute irradiation of post-spermatogonial stages, on the other hand, allows one to make reasonable estimates of the total number of translocations that probably occurred in those of our specific-locus experiments that met these experimental conditions. Five such experiments yielded 40,829 young from irradiated spermatozoa, spermatids, and spermatocytes, 10% and 90% of these involving acute X-ray doses of 600r and 300r, respectively (single exposures in each case). Based on the quantitative results of SNELL, HERTWIG (W. L. RUSSELL 1954, review), and AUERBACH and SLIZYNSKI (1956), an estimate of 24% and 6% translocation yield from 600r and 300r experiments, respectively, appears reasonable. The progeny of the pertinent experiments can thus be estimated to contain 3,158 translocations. About half of these, or 1,579, would have come from X-bearing germ cells; and, on the simplified assumption that each of the 20 chromosomes had an equal chance of being involved in a rearrangement, 10%, or 158, are expected to involve the X chromosome. Four linkage groups carry markers that might produce detectable variation—namely LG1 (c^{ch} , p), LG2 (d), LG5 (a), and LG8 (b)—and 4/19 of the

158, or 33, of the $T(X;A)$'s would, on our simplified assumption, involve one of these linkage groups. Since, on the modified single-active-X hypothesis (RUSSELL 1963), the marked autosomal portion could become attached to a part of the X not bearing an inactivation center, or be too far from the center to be affected, the $T(X;A)$'s yielding variegation would constitute only a fraction of the $T(X;A)$'s involving a marked autosome. The magnitude of this fraction would depend on relative location of the inactivation center and the marked locus: if both are in distal or both in proximal portions of their respective chromosomes, the types of exchange producing variegation would be relatively rare. If, however, the inactivation center is fairly centrally located in the X chromosome, as appears possible (RUSSELL and MONTGOMERY 1965, 1970), then in about one-half of all $T(X;A)$'s, this center and the autosomal marker would be on the same chromosome, regardless of location of the latter. For the purpose of the present argument, we thus assume that of the estimated 33 $T(X;A)$'s involving a marked autosome in this sample, about 16 could result in variegation.

The actual number of $T(X;A)$'s positively identified on the basis of variegation in experiments with acute, high-intensity X rays to postspermatogonial stages was four, namely R1, R2, R3, R4—i.e., about one-fourth of those expected. However, the positively identified cases may represent only a portion of the true yield, since almost one-half of the non-self offspring of irradiated males, were, for one reason or another, not adequately tested (RUSSELL 1964c). If the true number of $T(X;A)$'s in the sample was somewhere between four and eight, this would constitute between about one-fourth and one-half the number estimated to have been produced on the assumption that a $T(X;A)$ is as likely to be recovered as is a given $T(A;A)$. We thus conclude that there is, indeed, a slight but not an extreme dearth of $T(X;A)$'s. It is of interest that one of the $T(X;1)$'s was of spontaneous origin, for spontaneous translocations are apparently rare (W. L. RUSSELL 1954, review; United Nations Report, 1966).

With regard to distribution among the four marked autosomes, it is striking that five of our seven $T(X;A)$'s as well as the *flecked* translocation, involve LG1, and none involve LG2 or LG5. One possible factor in this unequal distribution is the availability of two coat-color markers in LG1 but only one in each of the others. In addition, one or more of the following factors should be considered: (a) relative lengths of marked chromosomes; (b) relative positions of inactivation center and autosomal marker (see above); (c) location, near some of the autosomal markers, of "critical" genes, i.e. genes that must be active in double dose in all cells to allow survival. That relative length is not the only factor is indicated by the findings that LG1, involved in six of the nine mouse $T(X;A)$'s, is probably a "medium" chromosome (unpublished results of N. L. CACHEIRO of this laboratory), as is LG2 (EVANS, LYON, and DAGLISH 1967), which is involved in none of the nine; while LG8, probably a long chromosome (SLIZYNSKI 1952), and possibly the second longest in the complement (CACHEIRO and RUSSELL 1969), has yielded only one-third as many recovered $T(X;A)$'s as has LG1. LG5, also considered to be a long chromosome (SLIZYNSKI 1952), has not yet been found involved in any known $T(X;A)$'s. The failure to discover $T(X;5)$'s and $T(X;2)$'s

by the methods employed could be due to the location of genes used to mark these linkage groups; or, to "critical" genes located near the marker loci. If the inactivation center is, indeed, fairly central in the X (see above), the former possibility is less likely.

Since the existence of "critical" genes, i.e. genes whose functional hemizyosity in half the cells cannot be tolerated, would normally result merely in absence of certain types, positive evidence concerning such genes will be difficult to obtain. However, enough information exists to show that there is a whole range in tolerance to hemizyosity, depending, perhaps, on the total number of genes that are hemizygous and/or on the specific genes involved. At one extreme are the perfectly viable deficiencies, a number of which have been recovered (RUSSELL 1962, review), including many at the *d* locus, which has not yet been involved in an X-autosome translocation. That is, hemizyosity can evidently exist in all cells without causing any apparent harm. Next in the scale of increasing effects is total-body hemizyosity that is deleterious but not lethal. Among 33 heterozygous LG2 deficiencies, detected because they involved *d* and *se*, 17 were less viable than normal, most of these also acting to reduce body size. In this more deleterious group were some that included *sv* in addition to *d* and *se* (*d*-0.16-*se*-2-*sv*), but none of the deficiencies was long enough to extend as far as *tk*, another 4 units beyond *sv* (RUSSELL *et al.* 1966). Next in order, are conditions that lead to lethality when whole-body but that are compatible with life when in the mosaic state. Thus, the functional hemizyosity produced by a T(X;A) can be viable even where it exists for a lethal gene (RUSSELL *et al.* 1964; RUSSELL 1964c). Animals that very probably were *Def/c^hp///++/c^hp* have been obtained (RUSSELL, unpublished), even though *c-p* deficiencies have never been recovered as total-body mutations in radiation experiments yielding hundreds of thousands of offspring and over 100 separate *c* or *p* events. Several sex-linked lethals, later established as stocks, first arose in mosaic males, $X^1/Y///X^+/Y$ (RUSSELL 1964c). Allophenes in which one component is lethal are said to have been produced (MINTZ 1967).

The results presented in the present paper, which indicate that females heterozygous for T(X;1)'s are considerably less than perfect with respect to various vital statistics, may be taken as evidence for the next step in the progression, i.e. part-body functional hemizyosity that is deleterious. The final step, the existence of "critical" genes, thus seems very plausible. It could contribute to the dearth of T(X;A)'s in general, as well as to the shortage of specific T(X;A)'s. Whether or not "critical" genes, by acting sometimes only as cell lethals, might furnish the explanation for certain cases of apparently non-random X inactivation needs to be further elucidated. Such cases would not be detected through variegation of the autosomal marker and could thus further contribute to the apparent shortage of T(X;A)'s found in specific-locus experiments. SEARLE *et al.*'s (1968) failure to find X-autosome translocations in spermatocytes following irradiation of spermatogonia may indicate cell selection in the germline (Y and X are late replicating in spermatogenesis); or, alternatively, some difficulty in the production of such rearrangements in spermatogonia.

The deleterious effects of mosaicism for functional hemizygoty for LG1 are definite but not extreme. About one-quarter of the heterozygous females (pooling all stocks) do not survive to 7 days of age; about 6% in excess of control frequency are completely sterile, probably for lack of general well-being. They raise their young to weaning with somewhat lower efficiency than do their non-translocation sibs; and their weight is between 8 and 30% less than that of their sisters (cf. size reduction caused by LG2 deficiencies.)

A comparison of the various translocations shows R4 females to be most severely affected with respect to 7-day survival and the raising of their young. This stock, in fact, became extinct before it could be fully studied (no weight data available). R2 also has a rather poor 7-day survival and the lowest weight. However, it has relatively few sterile females and raises its young quite well. Differences between the other stocks are probably not significant.

It is interesting that the average litter size produced by the T(X;1) females is about 38% of controls (stocks pooled)—i.e., considerably less than the one-half of normal which would be expected if a quadrivalent were invariably present and if alternate and non-alternate segregations were equally frequent. Depression below 50% could have chromosomal or physiological causes. Among the former could be: more nearly random segregation from a quadrivalent (complete randomness would yield 33% balanced progeny); or a considerable frequency of non-quadrivalent associations, i.e. trivalent plus univalent, or bivalent plus bi-valent (preliminary cytological data, however, indicate this not to be the case, at least for one of the rearrangements). Among physiological causes of litter size depression could be the fact that the T(X;1) females are generally less fit to be mothers. (The lowered survival of their R daughters, even if all of it were due to prenatal death, would cut only to 47% an expected 50% of normal.) Physiological factors could also act to “increase” litter size, e.g. as a result of improved intra-uterine survival when there was less competition. No firm decision between the various alternatives is possible from breeding data alone. It may be noted that in the case of the flecked (*fd*) insertion, females heterozygous for the rearrangement (both types I and II) do not show a lower litter size than would be expected from the lethality and relative inviability of certain segregating classes of offspring (CATTANACH 1961).

Weights had also been determined for animals carrying the *fd* insertion or T(X;?) 16H. In the latter case, a significant reduction was found for heterozygous females at birth, although these were said to look “grossly normal in body size at weaning” (LYON *et al.* 1964). The mean weight at 3 weeks for heterozygous *fd* females of both types was slightly lower than that of their chromosomally normal sisters, but no statistical comparison is provided (CATTANACH 1961).

It is to be noted that wherever T(X;1) males could be tested in the present investigation, namely with respect to 7-day survival and weight, they were apparently normal. With regard to coat color too, of course, they behave as if no rearrangement were present. Their only defect is their complete sterility, and this will be discussed when more detailed results are published. The normality, in other respects, of the males indicates that the rearrangements did not involve

loss of chromosomal material. It is, further, in complete agreement with the single-active-X hypothesis, according to which the single X of the male, and thus the autosomal portions attached to it, are fully active.

We are grateful to W. L. RUSSELL, of this laboratory, for making available to us the X-autosome translocations recovered in the course of his mutation rate experiments.

SUMMARY

A study of certain vital statistics was carried out in 8000 animals of five independent $T(X;1)$'s to determine some of the effects of the presumed mosaicism for functional hemizygosity of autosomal genes. All of the rearrangements originated at this laboratory. Four of them, R2-R5, were radiation-induced in post-spermatogonial stages; one, R6, was spontaneous.—Observed frequency of autosomally variegated $T(X;A)$'s was between about one-quarter and one-half that calculated on the assumption that the X enters into viable translocations as readily as does the average autosome. Explanation in terms of poorly tolerated functional hemizygosity of certain autosomal loci is supported by the finding that all of our $T(X;1)$'s have slightly deleterious effects in heterozygous females: 24% do not survive to 7 days of age; 6%, in excess of controls, are sterile for lack of general well-being; their weaning weight is between 8 and 30% lower than that of normal sisters. $T(X;1)$ males, in agreement with the single-active-X hypothesis, are normal in every respect tested, except for their complete sterility, already reported earlier.—The average litter size produced by $T(X;1)$ females (stocks pooled) is 38% of control, i.e. considerably less than the 50% which would be expected if a quadrivalent were invariably present, and alternate and non-alternate segregations were equally frequent. The chromosomal basis of the low litter size remains to be elucidated.—Various explanations for the unequal distribution of recovered $T(X;A)$'s among four marked autosomes are discussed, namely, relative lengths of the marked autosomes; relative positions of X-inactivation center and autosomal marker; and, perhaps most likely, location, near some of the markers, of "critical" genes, i.e. genes whose functional hemizygosity in half the cells cannot be tolerated. The large variation in tolerance to hemizygosity that is now known for different genetic conditions makes it probable that such genes exist at one end of the range. An intermediate condition is apparently illustrated by the definite, though not extreme, deleterious effects of part-body functional hemizygosity for LG1, demonstrated in this paper. "Critical" genes would account for the dearth of $T(X;A)$'s in general, as well as for the shortage of specific $T(X;A)$'s.

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