THE INDUCTION BY X-RAYS OF HEREDITARY CHANGES IN MICE

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INTRODUCTION

Although numerous experiments have been carried out, both before and since the classical work of MULLER with Drosophila, to determine if X-rays induce hereditary changes in mammals, the results heretofore obtained have all been either negative or, when considered individually, decidedly inconclusive. The production of sterility in the X-rayed individuals has been proved (ALBERS-SCHÖNBERG 1903, BAGG and LITTLE 1924, DOBROVOLSKAÏA-ZAVADSKAÏA 1928, SNELL 1933a, and many others); so also has the occurrence of defective embryos (indicated in some cases only by the reduction in number of viable young) in litters conceived between raying and the onset of sterility (REGAUD and DUBREUIL 1908, MARTIUS and FRANKEN 1926, YAMAMOTO 1929, MURPHY 1930, STRANDSKOV 1932, SNELL 1933a); moreover, at least two investigations (MARTIUS and FRANKEN 1926, and MARTIUS 1927 describing the work of SCHUGT and KIKKAWA) have indicated the production of complete sterility and of subnormal growth in some or all F_1 individuals when both parents, or when the female parents alone, were rayed, this last perhaps due to faulty nutrition of young whose mothers had been "burned" by the X-rays (NÜRNBERGER 1926). The occurrence, however, of any kind of heritable variation as a result of the treatment has remained uncertain. BAGG and LITTLE (1924, see also BAGG 1925, and later papers by the same authors), in pioneer studies on X-ray induction of mutations, reported the appearance in the third and subsequent generations of the descendants of two different pairs of X-rayed mice of a recessive mutation irregularly affecting eyes, feet, and viscera. In the experiments of DOBROVOLSKAÏA-ZAVADSKAÏA (1928), the well known dominant mutation "tailless" (or short-tailed) appeared several times among the first and second generation progeny of treated male mice. This experiment also yielded an F_1 male the top of whose cranium was unhardened, an F_2 male with one digit of a front foot missing (both these variants failing to survive), and an F_2 male showing a nervous motion of the head. This last variation and the tailless mutation were perpetuated. STRANDSKOV (1932) found one male with a duplicated

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[1] The investigations described in this paper were nearly all carried out while the writer was NATIONAL RESEARCH COUNCIL Fellow at the UNIVERSITY OF TEXAS.

penis in the second generation following treatment of male guinea-pigs. No similar variation appeared in the control. Considered together, these three experiments seem to indicate that an effect on offspring results from the X-ray treatment; analysed separately, with due allowance for the nature of the control, statistically significant proof of induced genetic changes is found to be lacking.

Since the results of former investigations proved inconclusive, it appeared of interest following the discovery by MULLER of X-ray mutations in Drosophila to re-investigate the possibility of inducing hereditary changes in mammals through the application of X-rays. Such a re-investigation was under-taken by the writer in 1931. An analysis of the effect of X-rays on the fertility of treated males has already been published (SNELL 1933a). A description of the technique used in raying is given in this earlier paper, and will not be repeated here. A preliminary report of the high rate of induced change appearing in later generations of mice has also been given (SNELL 1933b), together with detailed accounts of some of these changes (SNELL, BODEMANN, and HOLLANDER 1934; SNELL and PICKEN, in press). This paper presents in detail the evidence that approximately one third of the offspring of males rayed with doses in the neighborhood of 600 r-units carry induced translocations.

For a more complete bibliography of the subject than is here given, reference may be had to papers by DOBROVOLSKAÏA-ZAVADSKAÏA (1928), NÜRNBERGER (1927 and 1930), and HERTWIG (1932a).

STOCKS AND GENETIC TECHNIQUE

Since early investigations of the genetic effects of X-rays on Drosophila indicated a high rate of production of recessive mutations, and particularly of recessive lethals, the experiments with mice were planned with a view to revealing these types of genetic changes. A careful survey was made of the inbred stocks of mice available for work of this sort in different genetics laboratories, and of the different systems of matings which might be used to reveal induced mutations. The aim was to set up the experiment in such fashion as to give the greatest chance of discovering any mutations that might be induced with the least use of pens and of time; or more concisely, to give the maximum probability of mutation detection per pen per week.

The P_1

Five stocks of mice were finally selected for the experiment, as follows:

1. The *R*-stock was an inbred stock of mice homozygous for five recessive mutant genes, *a*, *b*, *d*, s_e, and *p*. As *d* and s_e are very closely linked these five genes served to mark four chromosomes. The stock was fur-

nished by Prof. WILLIAM H. GATES. All the X-rayed males were from this stock.

2. The *L*-stock was an inbred stock homozygous for one recessive mutant gene, *a*. It was obtained from Dr. L. C. STRONG of the ROSCOE B. JACKSON MEMORIAL LABORATORY. It was characterized by high fertility and a high degree of uniformity. Approximately one half of the original untreated parent females came from the stock.

3. The *Ag*-stock was an inbred stock also supplied through the kindness of the ROSCOE B. JACKSON MEMORIAL LABORATORY. It was homozygous for the wild-type genes, except that some individuals carried *a*.

4. The *A*-stock was an inbred strain homozygous for A^w and c^{ch}. It was supplied by Dr. GREGORY PINCUS.

5. The *F*-stock consisted of the first generation progeny from a cross between the *Ag*-stock and the *A*-stock. It was characterized by a considerable degree of hybrid vigor, the females breeding well and producing large litters. Tumor incidence was rather high in old females, however.

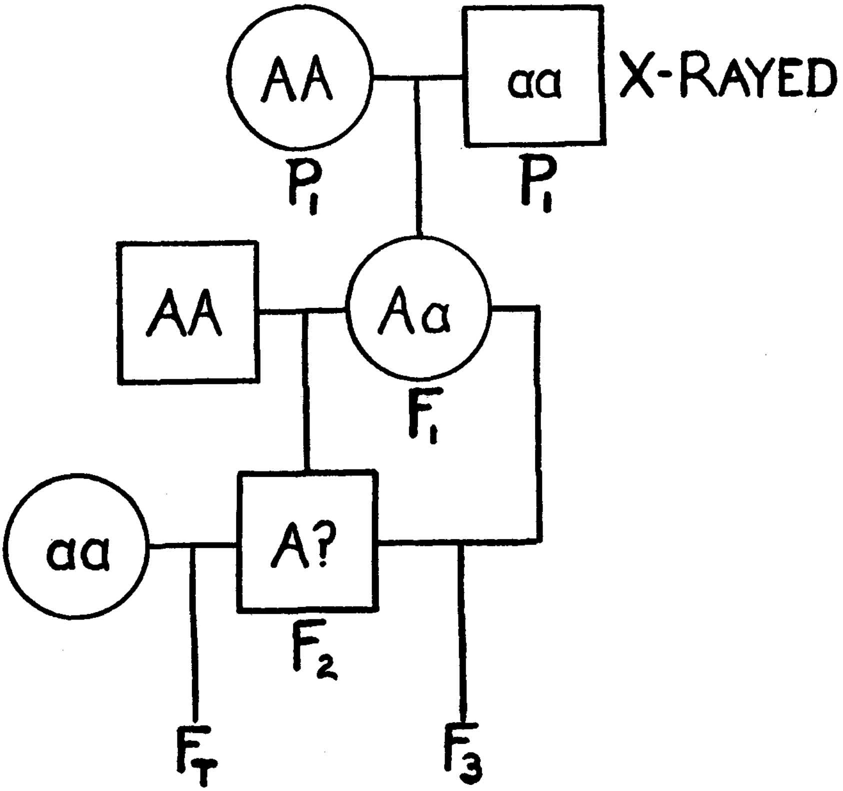

FIGURE 1.—Diagram showing system of mating.

All of the original untreated female parents not from the *L*-stock were from this stock.

The system of matings used is outlined in figure 1. For simplicity, only one of the marker genes involved in the cross is indicated in the figure.

The F_1

Males of the *R*-stock were mated to females of the *L*-and *F*-stocks, the offspring of these matings being designated as the F_1. Each *R*-stock male was mated once before raying to furnish the control, then several times during the one or two weeks fertile period following raying to produce F_1 test litters. The details as to the method of timing matings, the duration of the fertile period following raying, and the effect on F_1 litter size have been described in a previous paper (SNELL 1933a) and will not be repeated here. In subsequent generations the treatment of the test and control groups was identical. The pens occupied by individuals of the two groups were selected at random so that there could be no significant difference in location or in feeding. Moreover, the records were so kept that it was not known when a given litter was examined whether it belonged to the test or to the control.

The F_2

The F_2 was produced, not by mating F_1 individuals *inter se*, but by mating them to mice from the *L*-, *F*-, or *Ag*-stocks. With a few exceptions, F_1 mice from *F*-stock females were mated back to the *F*- or *Ag*-stocks, F_1 mice from *L*-stock females to the *L*-stock. The phenotype of the F_2 was thus like that of the P_1 females, but most of them carried one or more recessive marker genes.

The F_T

To test F_2 individuals for recessives, they were mated to *R*-stock individuals, and, with a few exceptions, at least five offspring reared until they were old enough so that their phenotypes could be determined (usually about ten days). This composed the F_T generation.

The F_3

The F_3 was produced by mating F_2 individuals back to their F_1 parents. Where the F_1 was a male, as many as three daughters were often backcrossed; where the F_1 was a female, usually only one son was backcrossed. The average number backcrossed in each case is shown in table 5 in the column headed "Mean number of F_2 mated to each F_1." The phenotype of F_3 individuals was determined by one or more examinations made between the first and the fourth weeks of their age. At about four weeks of age, they were killed, and an autopsy performed to detect possible abnormalities of the internal organs.

With a few exceptions, all pregnant females were isolated in separate, freshly cleaned pens, and examined daily until the birth of the litter.

The above system of matings was used because calculations showed it to be better adapted than any other for the detection of lethal and visible mutations.

The detection of recessive lethal mutations depends on the alteration of ratios in the F_3 generation. If a lethal occurs on a marked chromosome in the germ tract of a P_1 male, an F_1 individual inheriting it will be of the genotype AL/al, where l is the lethal mutation and a the marker gene. Assuming complete linkage, one half of the F_2 individuals will be of the genotype AL/al like their parent. Such heterozygous individuals can be distinguished from their homozygous sibs by the test mating. When backcrossed to their F_1 parent, the lethal which they carry will prevent the appearance of F_3 individuals homozygous for the recessive marker gene, as all such individuals will be homozygous for the lethal also and will die. Hence the failure of a recessive to appear following the backcross of an F_2 individual, proved by the test mating to carry the recessive, is evidence for the presence of a linked lethal. Twenty offspring from such a mating, if all of them show the dominant phenotype, are sufficient to establish a strong presumption that a lethal is present, while if no lethal is linked with the marker gene, one litter is usually sufficient to show it. By no other system of matings can the presence or absence of lethals in mice be so easily determined. Even with this method, however, only a fraction of all treated chromosomes carried by F_1 individuals are tested, the maximum being four (the number of marked chromosomes) out of twenty (the haploid number), and the proportion actually realized considerably less than this.

In the case of recessive visible mutations, over fifty percent of all treated chromosomes carried by F_1 individuals are tested by the system of matings used. In previous attempts to detect induced mutations in mammals, F_2 individuals have been mated together to produce the F_3. Our method of backcrossing F_2 individuals to their F_1 parents gives just double the chance of detecting visible mutations per F_1 individual. The practice of backcrossing from one to three F_2 individuals to each F_1 was adopted because calculations showed the use of larger numbers to be subject to diminishing returns. Three daughters of a single F_1 male will, on the average, carry seven-eighths of all his treated chromosomes. These three daughters can be backcrossed in a single pen. The inclusion of a fourth daughter would necessitate the use of a second pen, and would increase the fraction of the treated chromosomes available for testing by only one-sixteenth. The number of F_3 individuals raised from each backcross was determined by similar considerations, the actual figures being given in table 5 in the column headed "Mean number of autopsied F_3 mice per F_2." As has been pointed out by PAULA HERTWIG (1932b), previous investigators have, in some cases, raised a very inadequate number of F_3 litters.

The autopsy that was performed consisted of a standardized examination of salivary glands, thyroid, trachea, heart, lungs, thymus, digestive organs, kidneys, testes and ovaries and their ducts, the accessory glands of the reproductive system, and parts of the skeletal and circulatory systems. It was undertaken in the belief that, because of the relatively simple external anatomy but complex internal anatomy of mammals, many mutations may not be externally visible. The belief was substantiated by the results; the only mutation found affects primarily the shape of the spleen, and would not have been detected in the absence of the autopsy.

While the experiment was not originally planned with the detection of induced translocations in mind, results obtained by Dr. H. B. GLASS at the UNIVERSITY OF TEXAS with translocations in Drosophila soon suggested the possibility of detecting them in mice by watching for F_1 individuals that consistently produced small litters. The practice followed throughout the experiment of outcrossing F_1's to mice from untreated stocks made it easier to discover such individuals than would have been the case if the F_1's had been mated *inter se*. By good fortune the system of matings used in the experiment was thus as well adapted to the detection of translocations as to the detection of visible and lethal mutations.

EVIDENCE FOR THE PRODUCTION OF TRANSLOCATIONS

The presence among the F_1 test mice of a considerable number of individuals that consistently produced small litters is indicated by table 1, which shows the frequency distribution of F_1 mice with respect to the mean size of the F_2 litters which each produced. From many of the F_1 mice only a single F_2 litter was obtained, from others six or seven or more, the average being 1.8 F_2 litters per F_1 mouse. The position of many of the

TABLE 1

Frequency distribution of F_1 mice with respect to the mean size of the F_2 litters produced by each F_1 mouse.

MEAN SIZE OF F_2 LITTERS BY EACH F_1 MOUSE	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL
Number of F_1 test ♂♂	1	2	5	8	2	7	10	10	13	1			59
Number of F_1 test ♀♀	3	5	4	6	2	3	3	11	15	2	1		55
Number of F_1 control ♂♂					2	7	12	8	9	5	2	1	46
Number of F_1 control ♀♀					4	12	10	15	9	4	3		57

F_1 mice in the frequency distribution is thus determined by averaging the number of young in several F_2 litters. It will be seen from the table that whereas none of the 103 F_1 control mice produced litters averaging less than five, 34 of the 114 F_1 test mice produced litters averaging from one to four. The distribution of the controls is unimodal, with the mode at eight; the distribution of the test animals is strikingly bimodal, with modes at four and nine. Thus there is clearly a tendency for some of the test animals to produce small litters. These animals will be referred to as "semi-sterile."

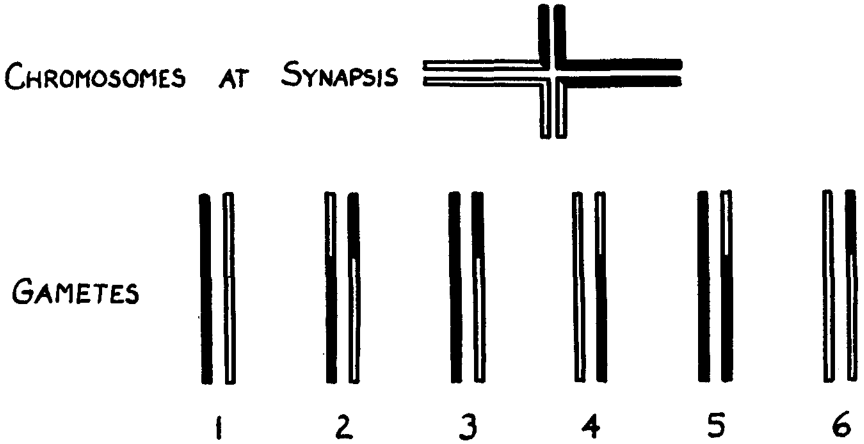

FIGURE 2.—Diagram showing the types of gametes formed by an individual heterozygous for a reciprocal translocation (based on translocations involving the second and third chromosomes of *Drosophila melanogaster* as studied by GLASS, and by DOBZHANSKY and STURTEVANT). Type 1 is entirely normal. Type 2, when combined with a normal gamete, gives an individual heterozygous for the translocation like the heterozygous parent. The chromosomal balance of such individuals is normal. Types 1 and 2 are formed with equal frequency, and together make up at least 50 percent of the total. Types 5 and 6, in the case of certain translocations in Drosophila, are not formed at all. Types 3, 4, 5, and 6, when combined with normal gametes, give zygotes with chromosomal unbalance, usually non-viable. Simple translocations in Drosophila likewise produce a certain proportion of gametes with chromosomal unbalance. Whether the translocations in mice herewith described are simple or reciprocal, and whether they produce two or four types of gametes with chromosomal unbalance, is undetermined.

Ten of the semi-sterile F_1 animals (♀♀$F_1$109, $F_1$99, $F_1$145; ♂♂$F_1$93, $F_1$107, $F_1$146, $F_1$262, $F_1$271, $F_1$285, $F_1$292) were saved for further study. In an analysis of the descendants of one of them, ♂$F_1$146, SNELL, BODEMANN, and HOLLANDER (1934) have shown that the semi-sterility is due to the presence of a translocation. Matings between $F_1$146 and normal females produced normal mice, semi-sterile mice, and abnormal embryos approximately in the ratio 29: 29: 42. Similar ratios were produced by semi-sterile sons of ♂$F_1$146. The abnormal embryos usually do not come to term, and hence account for the small size of the F_2 litters. Their occurrence when semi-sterile mice are outcrossed to normal mice from untreated stocks rules out the possibility that they are due to the segregation of a recessive lethal gene. All the facts are in accord with the idea that semi-sterile mice in the F$_1$146-stock are heterozygous for a translocation, and that the small litter size is due to the formation of zygotes which are non-viable because they have inherited unbalanced chromosome combinations. The types of gametes probably formed by individuals heterozygous for the translocation are indicated in figure 2.

While none of the other semi-sterile stocks has been tested as thoroughly as the F$_1$146 stock, considerable data are available in regard to the remaining nine of those selected for intensive study. Of these, the F$_1$271-stock is perhaps the best tested, and is of particular interest because the translocation appears to be linked with the recessive marker gene, "brown" (*b*). The evidence is summarized in table 2. All the mice listed in the first column of table 2 are derived from matings between semi-sterile individuals of the F$_1$271-stock and normal individuals of untreated stocks. It will be seen that they fall into two classes, the first producing litters

TABLE 2

Descendants of ♂$F_1$271 showing probable linkage between semi-sterility and the gene for brown (b).

COLONY NUMBER	GENOTYPE	MOTHER'S COLONY NUMBER	FATHER'S COLONY NUMBER	NUMBER OF LITTERS	MEAN SIZE OF LITTERS
(a) Probably semi-sterile					
♀ F$_2$295	$AaBbDS_e/ds_e$	*Ag*94 *BB*	F$_1$271 *Bb*	1	3
♀ F$_2$719	*AaBb*	*Ag*75 *BB*	"	3	3
♀ F_T3698	$aabbds_e/ds_e$	*R*343 *bb*	"	2	3
♂F_T3770	$aabbds_e/ds_epp$	*R*342 *bb*	"	5	3.2
♂F_T3771	A^WabbDS_e/ds_ePp	"	"	4	3.5
♂$N_8$7	*aaBbNnWw**	$N_4$378 *BB*	F_T3770 *bb*	2	2.5
♀ $N_8$166	*aabb*	*R*477 *bb*	$N_8$7 *Bb*	1	4
♀ $N_8$167	$aabbds_e/ds_e$	"	"	1	3
(b) Probably normal					
♀ F$_2$720	$AaBBDS_e/ds_e$	*Ag*75 *BB*	$F_1$271 *Bb*	4	6.7
♀ F_T3697	$AaBbDS_e/ds_ePp$	*R*343 *bb*	"	1	6
♀ $N_8$0	*A?BBNnWw*	F$_2$719 *Bb*	$N_4$413 *BB*	2	9.5
♀ $N_8$1	*aaB?NnWw*†	"	"	2	11
♀ $N_8$2	*A?B?nnww*†	"	"	1	12
♀ $N_8$4	*aaBbNnww**	N$_4$378 *BB*	F_T3770 *bb*	2	10
♂$N_8$17	*aaBbNnWw**	$N_4$376 *BB*	"	1	11
♀ $N_8$23	*aaBbnnww**	$N_5$405 *BB*	"	1	9
♀ $N_8$24	*aaBbnnWw**	"	"	1	7

* Gene for brown (*b*) may have been derived from untreated stock.

† Not tested for presence of gene for brown (*b*).

averaging four or less than four young, the second producing litters averaging six or more than six young. The first class is listed as "probably semi-sterile," the second as "probably normal." There are 8 individuals in the first class, 9 in the second, a good approximation to the 1:1 ratio expected on the hypothesis that semi-sterility is due to the segregation of a translocation. The genotypes of the individuals in question are given in the second column, and their parents' colony numbers and genotypes with respect to brown in the next two columns. In five cases the individuals were not tested for the presence of the gene for brown; in two cases the mating which produced them was such that the gene for brown might have been derived from an untreated stock. The significant data come from the remaining ten cases. In seven of these ten the treated chromosome bearing the gene for brown has been inherited, and in all seven the individual is probably semi-sterile; in the remaining three the untreated chromosome bearing the gene for non-brown (*B*) has been inherited, and in all three the individual is probably normal. A linkage is thus indicated, the odds against the treated chromosome segregating with semi-sterility due to chance along in all of the ten cases being 1023 to 1. The fact that the semi-sterility of some of the ten individuals was only tested by a single litter, and the possibility that ♂$F_1$271 actually carried more than one X-ray-induced translocation, somewhat reduce the presumption of linkage, but the evidence for linkage may be regarded as quite strong even if not entirely conclusive.

Data derived from other semi-sterile stocks all point to the same interpretation of semi-sterility as that given in the case of the $F_1$146- and the $F_1$271-stocks.

In the first place, all of the semi-sterile F_1's so far tested appear to transmit the tendency to produce small litters to a part of their descendants. An incomplete presentation of the data showing this is given in tables 3 and 4, in which are listed all F_1 mice suspected of semi-sterility. In the last column of each table are listed the average sizes of the F_T litters produced by each F_2 mouse from each of the semi-sterile stocks. The number in parenthesis is the number of litters on which each average is based. It will be seen that in many of the stocks some of the F_2 mice exhibit the same tendency to produce small litters that was characteristic of their semi-sterile parents. Thus four F_2 females from ♂$F_1$107 produced litters averaging 2, 3.5, 4, and 7 young. The first three were probably semi-sterile. Some further data pointing in the same direction will be presented in a paper by Miss ELSIE BODEMANN. When all the data are considered, there can be no reasonable doubt that the tendency to produce small litters possessed by all genuinely semi-sterile mice is hereditary.

In the second place, all ten of the specially tested semi-sterile stocks produce abnormal embryos, many of which are similar in type to the abnormal embryos found in the $F_1$146-stock. These abnormal embryos appear in all cases not only when two semi-sterile individuals are mated together, but also when semi-sterile individuals are mated to individuals from normal, untreated stocks. The data will be presented in a paper by Miss ELSIE BODEMANN. These facts are in accord with the assumption that

TABLE 3

Tabulation of evidence concerning all F_1 males suspected of semi-sterility.

F_1♂	X-RAY DOSE	NUMBER F_2 MICE	NUMBER F_2 LITTERS	MEAN SIZE F_2 LITTERS	NUMBER F_3 MICE	NUMBER F_3 LITTERS	MEAN SIZE F_3 LITTERS	MEAN SIZE ALL LITTERS	CORRECTED MEAN SIZE	P	ABNORMAL EMBRYOS	AVERAGE SIZE OF F_T LITTERS PRODUCED BY EACH F_2 FEMALE†
(a) Control												
352		10	1	10	27	5*	5.4	6.2	6.7	.02		6(1) 8(1)
354		11	2	5.5	9	1	9	6.7		.19		8(1)
151		8	1	8	26	4	6.5	6.8		.13		7(2)
152		10	2	5	38	5*	7.6	6.9	7.2	.08		
(b) Test												
261	800	1	1	1	—	—	—	1.0		<.01		7(1)
116	400	7	4	1.7	1	1	1	1.6		<.01		8(1)
139	600	4	2	2	—	—	—	2.0		<.01		3(1)
285	800	6	2	3	17	7*	2.4	2.6	2.6	<.01	yes	3.5(2) 8(2)
262	800	9	3	3	5	2	2.5	2.8		<.01	yes	5(3) 2.4(5) 4(2)
271	800	12	3	4	2	2	1	2.8		<.01	yes	3(1) 3(2) 6.7(4)
119	600	15	5	3	4	1	4	3.2		<.01		2(1) 3(1)
93	600	18	6	3	9	2	4.5	3.4		<.01	yes	6(1) 6.3(3) 9(2)
107	400	11	4	2.7	52	15*	3.5	3.3	4.0	<.01	yes	2(2) 3.5(2) 4(1) 7(1)
256	800	8	2	4	20	6	3.3	3.5		<.01		3.3(3) 7(1) 8(1)
292	800	8	2	4	6	2	3	3.5		<.01	yes	3(2) 7(1) 8(1)
112	400	16	4	4	9	3	3	3.6		<.01		4(1) 8(1)
146	600	26	7	3.7	18	5	3.6	3.7		<.01	yes	Nine semi-sterile out of eighteen tested F_2
98	400	13	3	4.3	26	6*	4.3	4.3	4.4	<.01		6(1) 9(1)
288	800	8	1	8	1	1	1	4.5		.02		
240	800	13	3	4.3	40	6	6.7	4.8		<.01		6(1) 7(1) 9(1)
180	600	7	1	7	27	6	4.5	4.9		<.01		4(1)
230	800	15	3	5	8	1	8	5.7		.02		2(1) 10(2)
294	1200	13	2	6.5	10	2	5	5.7		.02		6(1) 8(1) 8(1)
53	800	6	1	6	—	—	—	6.0		.29		
281	800	8	1	8	17	3	5.7	6.2		.07		2(1)5(1)
280	800	11	2	5.5	8	1	8	6.3		.13		5.5(2) 6(1) 10(1)
195	600	7	1	7	25	4*	6.2	6.4	7.2	.05		4(1) 7(1)
158	600	8	1	8	69	8	8.6	8.6		>.9	yes	4.5(2) 5(1)

* One or more litters not recorded at birth.

† Average size of all F_T litters produced by F_2 control ♀♀ =6.93.

a semi-sterile mouse gives small litters because it is heterozygous for a translocation.

The incidence of translocations among the F_1 mice from treated sires is surprisingly high. Approximately one-third of all F_1's in the test group are thus affected. The evidence indicating this high incidence is presented in tables 3 and 4.

TABLE 4

Tabulation of evidence concerning all F_1 females suspected of semi-sterility.

F_1♀	X-RAY DOSE	NUMBER F_2 MICE	NUMBER F_2 LITTERS	MEAN SIZE F_2 LITTERS	NUMBER F_3 MICE	NUMBER F_3 LITTERS	MEAN SIZE F_3 LITTERS	MEAN SIZE ALL LITTERS	CORRECTED MEAN SIZE	P	ABNORMAL EMBRYOS	AVERAGE SIZE OF F_T LITTERS PRODUCED BY EACH F_2 MALE†
(a) Control												
55		5	1	5	3	2	1.5	2.7		.01		6(1) 7(1)
89		8	1	8	7	4	1.7	3.0		$<.01$		8.5(2)
124		9	2	4.5	21	7	3	3.3		$<.01$	no	4.1(9)
130		6	1	6	10	3*	3.3	4.0	5.0	.02		7(2)
47		13	2	6.5	9	3	3	4.4		.04		11(1)
87		9	1	9	9	3*	3	4.5	9.0	.08		9(1)
80		5	1	5	9	2	4.5	4.7		.16		8(1)
(b) Test												
120	600	1	1	1	—	—	—	1.0		.03		
238	800	3	3	1	—	—	—	1.0		$<.01$		
257	800	1	1	1	—	—	—	1.0		.03		
184	800	2	1	2	4	3	1.3	1.5		$<.01$		1.5(4)
241	800	4	2*	2	—	—	—	2.0	3.0	.01		
270	800	4	2	2	—	—	—	2.0		.01		
99	400	7	2	3.5	4	3	1.3	2.2		$<.01$	yes	2.7(6) 5.5(2) 7.5(2)
145	600	3	1	3	7	4*	1.7	2.0	2.2	$<.01$	yes	4.5(2) 5(2)
231	800	3	1	3	6	3	2	2.2		$<.01$		5.2(4) 7.3(3)
251	800	7	3	2.3	—	—	—	2.3		$<.01$		
101	400	2	1	2	6	2	3	2.7		.01		5.2(4)
237	800	4	1	4	7	3*	2.3	2.7	3.0	$<.01$		8.7(3)
216	800	3	1	3	6	2*	3	3.0	3.5	.02		6.5(4)
97	400	8	2	4	2	1*	2	3.3	4.0	.03		3(1) 5(3)
242	800	4	1	4	3	1	3	3.5		.08		7(1)
109	600	6	1	6	16	5	3.2	3.7		$<.01$	yes	4.3(7) 6.4(5)
94	600	6	1	6	16	5*	3.2	3.7	3.8	$<.01$		
289	800	8	2	4	—	—	—	4.0		.13		
100	400	9	1	9	25	7	3.6	4.2		$<.01$		5(1)
154	600	15	3	5	7	2	3.5	4.4		.04		5.5(2)
219	800	4	1	4	10	2*	5	4.7	4.0	.16		1.7(3) 5.5(2)

* One or more of litters not recorded at birth.

† Average size of all F_T litters produced by F_2 control ♂♂ = 6.74.

These tables give all F_1 animals, both test and control, which, by virtue of the small size of the litters they produced, may be suspected of semi-sterility. The first column in each table gives the colony number of the F_1 animal. The second column gives the X-ray dosage in r-units applied to the fathers of the treated group. The next eight columns give data on litter size. The last of these, headed "Corrected mean size," gives the mean size of all the F_2 and F_3 litters of each F_1 mouse which were observed and recorded within 24 hours of birth; all litters recorded more than 24 hours after birth are omitted. Such litters are often depleted when finally examined, and, when included, tend to give a mean litter size that is too low. The next column, headed "P", gives the probability that the difference between the mean size of the F_2 and F_3 litters produced by each F_1 (from the column headed "Mean size of all litters"), and the mean size of the F_2 and F_3 litters produced by all control F_1 of the same sex, could occur by chance alone. The means used are the uncorrected means, including all litters whether recorded at birth or some days thereafter. P is calculated by the method for the difference of two means given by R. A. FISHER (1930). The next column, "Abnormal embryos," shows which animals were used for embryological studies, and which of these gave embryos with open brains, found to be typical of translocation stocks. (♂$F_1$292 gave embryos of a slightly different but related type.) I am indebted to Miss ELSIE BODEMANN for the data in this column. The last column, as explained above, indicates the incidence of semi-sterility among the F_2 mice derived from each F_1.

Table 3 contains all F_1 males whose combined F_2 and F_3 litters averaged less than 7 young. For the control group as a whole, the average size of the combined F_2 and F_3 litters was 8.48 ± .18, so that an average of 7 may be taken, more or less arbitrarily, as suggesting semi-sterility. Four males from the control group produced litters averaging less than this. However, it is only in the case of male $F_1$352, whose 6 litters averaged 6.2 young, that the difference is significant ($P = .02$). Moreover, the significant difference in this one case, if not due to chance alone, apparently can be explained by the fact that 3 of the F_3 litters were not recorded at birth, and probably had been somewhat depleted by the time they were first examined. With these 3 litters omitted, the average size of the remaining F_2 and F_3 litters becomes 6.7, a figure that does not differ significantly from 8.48. It may be concluded that none of the F_1 control males were semi-sterile.

Turning to the consideration of the F_1 test males, we find that in the case of 23 of them, the combined F_2 and F_3 litters averaged less than 7 young. Moreover, in the case of 19 of these 23, the difference between this average and the average for all F_2 and F_3 litters from F_1 males of the control group (8.48), is very probably significant ($P \leqq .02$), so that the individuals in question may be accepted as semi-sterile. The remaining test males, listed in column 1 of table 3, require further consideration. Male $F_1$53 produced only one litter, an F_2 litter containing 6 individuals. With these meagre data, no safe conclusion about this male can be drawn. Male $F_1$281 produced 4 litters averaging 6.2 young. $P = .07$. Two daughters produced F_T litters of 2 and 5 respectively, suggesting that one of them, at least, was semi-sterile. On the strength of this, we may assume that ♂$F_1$281 was himself probably semi-sterile. $F_1$280 was a border-line case, with little suggestion of semi-sterility coming from one daughter who produced two F_T litters averaging 5.5 young. Male $F_1$195 was probably normal, his apparent semi-sterility being attributable to the inclusion of one litter not recorded at birth. Male $F_1$158 was not detectably semi-sterile, but he produced occasional abnormal embryos and abnormal young at term, and evidence now in press indicates that he carried a translocation causing the formation of a few defective zygotes, but not enough to effect litter size appreciably. He is therefore included with the semi-sterile animals, though not semi-sterile himself. Assuming ♂♂$F_1$53, $F_1$280, and $F_1$195 to be normal, we arrive at 21 (36.5 percent) as a probable figure for the number of F_1 test animals carrying translocations. Some of these males undoubtedly carried more than one translocation. This is indicated by the high degree of semi-sterility exhibited by some of them, and, in the case of ♂$F_1$93 (unpublished data obtained by Mr. WILLARD HOLLANDER), by the large proportion of semi-sterile mice among his offspring.

The incidence of semi-sterility is not quite so easily determined in the case of the F_1 females. Apparently some of them produced small litters due to causes other than the presence of a translocation. The pertinent data are presented in table 4, which includes all F_1 females whose combined F_2 and F_3 litters averaged less than 5 young. Of the control females, 7 fall within this category, and in the case of 5 of them the difference between the mean size of the litters which they produced and the mean size ($6.96 \pm .21$) of all F_2 and F_3 litters from F_1 control females is probably significant ($P \leqq .04$). Female $F_1$47 may be dismissed as a border-line case, the small size of whose litters is probably due to chance alone. The apparent semi-sterility of ♀$F_1$130 is probably attributable to the inclusion of two litters not examined at birth. With these litters excluded, the mean size of the remaining litters becomes 5.0. Females $F_1$55, $F_1$89, and $F_1$124, however, definitely show a subnormal fertility. Moreover, in the case of ♀$F_1$124, there is some evidence that semi-sterility was transmitted to a son (♂$F_2$466). The 9 F_T litters produced by this son averaged 4.1 young (or omitting two litters not recorded at birth, 4.9 young). On the other hand, five litters of embryos obtained from ♂$F_2$466 contained 38 normals, 4 solid moles, and 1 dead embryo showing a distended and twisted central nervous system (data kindly furnished by Miss ELSIE BODEMANN). The absence of embryos with the brain deformities characteristically produced by chromosome unbalance, and the large proportion of normals, strongly argue against the conclusion that ♂$F_2$466 carried a translocation. The cases of ♀♀ $F_1$55, $F_1$89, and $F_1$124 must be left somewhat uncertain, but much the most probable interpretation appears to be that they produced small litters because of poor health or some abnormal physiological condition, rather than because they had inherited translocations, or a recessive lethal may have been involved, particularly in the case of $F_1$89.

Turning to the F_1 test females, it will be seen (table 4) that there are 21 the mean size of whose litters averaged less than 5 young. Most of these are undoubtedly semi-sterile, but considerable uncertainty attaches to the last six listed in the table excluding ♀♀ $F_1$109 and $F_1$94. Female $F_1$154 was very likely normal. The mean size of the litters she produced is 4.4, compared with a mean size of 6.96 for all F_2 and F_3 control litters from F_1 females. The difference between these figures could occur one time in twenty-five due to chance alone ($P = .04$), and since there were fifty-five test females, equivalent differences would be expected to occur one to several times without specific cause. There is, moreover, no evidence that female $F_1$154 produced abnormal embryos, and her one tested son since he produced litters averaging 5.5 young, was quite probably normal. Similar arguments apply to ♀♀ $F_1$97, $F_1$242, $F_1$289, and $F_1$100, though the suspicion of semi-sterility in these cases is somewhat greater than in the case of $F_1$154. Female $F_1$219 would be taken for normal were it not for one apparently semi-sterile son. Perhaps as reasonable a conclusion as any is to assume semi-sterility for two of the six doubtful females. This leaves a total of 17 semi-sterile females. Hence approximately 17, or 30.9 percent, of the F_1 test females carry translocations.

Combining the figures for males and females, we find that approximately 38 mice, or 33.3 percent of the total carry one or more X-ray induced translocations. This may be compared with the figure for *Drosophila melanogaster* reported by MULLER and ALTENBURG (1930) of 117 translocations in 883 flies from X-rayed males (13.3 percent), and the figure for corn indicated by the investigations of STADLER (1931) of about 25 percent following treatment of mature pollen, the dose in each case being roughly determined by the maximum tolerance of the species.

In addition to the semi-sterile mice, there were a number which proved to be completely sterile, giving no litters at all. Six were F_1 test males. These males were mated with (put in the same pen with) one or more normal females, usually for periods of three or more weeks. Most of the females had been, or were later, proved fertile by matings to other males.

No pregnancies resulted. One F_1 test female ($F_1$241) was likewise proved sterile. One control male and two control females also failed to give young, but they were not so thoroughly tested as the above-mentioned test animals, the male particularly having been mated to only one female, so there is some reason to doubt if they were actually sterile.

The seven sterile animals in the X-rayed group may be interpreted as extreme cases of semi-sterility. This interpretation is particularly plausible in the case of ♀ $F_1$241. Two proven semi-sterile females, $F_1$120 and $F_1$257, verged on complete sterility, giving one litter of one in five matings, and one litter of one in three matings, respectively. At least two different males were used in each case. Female $F_1$241 may well have been merely slightly more "semi-sterile" than these two. The interpretation does not fit so well in the case of the males. Only one semi-sterile male, $F_1$261, verged on complete sterility. This male gave one litter of one. He died at three and one half months of age before very thorough tests of his fertility had been made. As F_1 males could be mated to two or three females at once, and shifted frequently from one pen of females to another, it was usually possible to obtain several litters even from those with the lowest fertility, provided they were fertile at all. There is thus some reason to believe that the six above mentioned test males were truly sterile. However, the above interpretation may be the correct one in the case of some or all of the seven sterile F_1 test animals. If so, the figure of 33.3 percent for the incidence of semi-sterility in the test group is conservative.

Another explanation, at once plausible and interesting, is that the six sterile F_1 test males were sterile because they had inherited a Y chromosome which had been fragmented or deleted by the X-ray. Male Drosophila lacking a Y chromosome are viable but sterile. *A priori*, we might expect the same to be true of male mice.

ABNORMALITIES OF DEVELOPMENT ATTRIBUTABLE TO THE TRANSLOCATIONS

Studies by SNELL, BODEMANN, and HOLLANDER (1934,) SNELL and PICKEN (in press), and Miss ELSIE BODEMANN (unpublished) show that some of the gametes produced by mice heterozygous for a translocation produce non-viable embryos; non-viable, presumably, because of chromosome unbalance. Many of these embryos, particularly in the case of certain translocation stocks, die at or shortly after implantation. The nature of the abnormality causing death in these embryos has not been definitely ascertained. Others develop beyond implantation; their abnormalities, so far as we have been able to determine, are confined to the central nervous system, or to structures immediately affected in their development by the central nervous system, and consist primarily in the failure of the neural groove to close at its anterior end. Embryos thus affected occasionally come to term, but never live more than a short time after birth.

A detailed description of the defective embryos will not be given here. It is of interest, however, to mention several abnormal individuals, not elsewhere described, whose abnormality is perhaps attributable to the translocations.

Female $F_2$390, by normal ♀L119 and semi-sterile ♂$F_1$107, exhibited a peculiar, hesitating, staggering walk, which suggested the appellation "drunken." This persisted as long as she lived. ♂$F_3$1670 and ♂$F_3$2700, half-sibs of $F_2$390 by ♀$F_2$389 and ♂$F_1$107 were still-born and showed a pronounced swelling of the top of the head, though the skin was unbroken. Fifteen progeny of $F_2$390 were normal (though two were still-born), as were the twelve sibs of ♂$F_3$1670 and ♂$F_3$2700, and fifty less closely related descendants of ♂$F_1$107.

Female F_T3914 was still-born and showed a swelling of the head similar to that described above. She was derived from test female $F_1$267, a female who was not definitely semi-sterile (average size of F_2 and F_3 litters was 7).

Unfortunately the relation of brain abnormalities to translocations had not been discovered at the time the above individuals appeared, and as a result their brains were not saved for future study. It seems not unlikely, however, that they had a mild form of brain abnormality resulting from chromosome unbalance.

It has already been noted that DOBROVOLSKAÏA-ZAVADSKAÏA (1928) found an F_1 male from treated parents the top of whose cranium was unhardened. In view of our results, this case is plausibly explained as the result of an X-ray induced deficiency.

EVIDENCE FOR THE NON-PRODUCTION OF RECESSIVE LETHAL MUTATION

The method used to test for the production or non-production of recessive lethal mutations has been described in a previous section. A lethal is indicated if mice homozygous for one of the marker genes fail to appear in the F_3 litters produced by the backcross of F_2 mice heterozygous for the marker to their F_1 parent. In no case where the tests were sufficiently extensive to be significant did the homozygous F_3 mice fail to appear. The figures on completed tests are given in the last two columns of table 5. It will be seen that 41 F_1 experimental females and 51 F_1 experimental males were tested for the absence of a lethal on one or more of the marked chromosomes. The total number of marked chromosomes tested for the absence of a lethal was 209 in the test group, 166 in the control.

TABLE 5

Summary of F_1, F_2, and F_3 mice.

	NUMBER OF F_1 PRODUCING ONE OR MORE F_2 MICE	TOTAL NUMBER OF F_2 LITTERS	NUMBER OF F_2 MICE					NUMBER OF F_1 PRODUCING ONE OR MORE F_3 MICE	NUMBER OF F_2 PRODUCING ONE OR MORE F_3 MICE
			LIVE-BORN		STILL-BORN				
			♀	♂	♀	♂	?		
Test	59♂♂	113	273	315	12	10	9	53♂♂	128♀♀
Test	55♀♀	77	212	241	10	3	2	46♀♀	50♂♂
Control	46♂♂	60	233	209	9	3	3	34♂♂	77♀♀
Control	57♀♀	69	237	258	9	9	4	49♀♀	49♂♂

	TOTAL NUMBER OF F_3 LITTERS	NUMBER OF F_3 MICE					MEAN SIZE OF F_2 PLUS F_3 LITTERS	ESTIMATED NUMBER OF SEMI-STERILE F_1 MICE	ESTIMATED PERCENT OF SEMI-STERILE F_1 MICE
		LIVE-BORN		STILL-BORN					
		♀	♂	♀	♂	?			
Test	211	727	722	25	16	7	6.53±.18	21♂♂	35.6
Test	113	293	319	23	15	9	5.93±.23	17♀♀	30.9
Control	115	493	502	10	10	12	8.48±.18	0♂♂	0.0
Control	105	332	342	7	4	9	6.96±.21	0?♀♀	0.0?

	NUMBER OF F_1 PRODUCING ONE OR MORE OF THE AUTOPSIED F_3 MICE	NUMBER OF F_2 PRODUCING ONE OR MORE OF THE AUTOSIPED F_3 MICE	MEAN NUMBER OF F_2 MATED TO EACH F_1	NUMBER OF AUTOPSIED F_3 MICE	MEAN NUMBER OF AUTOPSIED F_3 MICE PER F_2
Test	50♂♂	121♀♀	2.41	615	5.08
Test	41♀♀	42♂♂	1.02	254	6.05
Control	32♂♂	73♀♀	2.28	384	5.26
Control	49♀♀	49♂♂	1.00	356	7.47

	NUMBER OF F_1 WITH ONE OR MORE CHROMOSOMES TESTED FOR ABSENCE OF LETHAL	NUMBER OF CHROMOSOMES TESTED FOR ABSENCE OF LETHAL
Test	51♂♂	136
Test	41♀♀	73
Control	34♂♂	81
Control	45♀♀	85

The greatest presumption of the presence of a lethal exists in the case of control ♀ $F_1$48. A mating between this female and a son who had inherited the marker gene *p* failed to produce any *pp* offspring in a total of 13 young. The odds against this occurring by chance alone are 41 to 1. However, in view of the large number of F_1 animals being tested, such an occurrence is better explained as due to chance than as due to the presence of a lethal. In two other cases in the control and in ten cases in the treated group the recessive marker genes failed to appear, but in all these cases too few F_3 mice were raised for the results to be significant. Nine of the ten incompletely tested mice in the treated group were semi-sterile. The small litters which they produced account for the non-completion of the tests in these nine cases.

The 209 treated chromosomes tested for the absence of a lethal are equivalent to the full chromosome complement of ten and one-half mouse spermatozoa (haploid number = 20). This number of treated sperm would yield, on the average, at least three translocations. It appears likely, therefore, that X-ray treatment of mature sperm in mice produces recessive lethals with a lower frequency, perhaps a very much lower frequency, than it produces translocations, though the possibility that some recessive lethals may have been induced on the marked chromosomes, but at loci so loosely linked with the marker loci as to escape detection, lends an element of uncertainty to this conclusion.

Whether or not translocations in mice sometimes behave as recessive lethals when homozygous, as is frequently the case in Drosophila, can only be determined by future investigations.

THE PRODUCTION OF VISIBLE MUTATIONS

Only one visible mutation, an irregular dominant causing a narrowing and constriction of the spleen and a considerable reduction in viability, was found among descendants of X-rayed animals. In affected individuals, the spleen was more or less narrowed, and showed changes in shape ranging from a slight constriction to a separation into two parts, usually unequal, giving it the shape of an exclamation point. However, some individuals, shown by progeny tests to be affected, had perfectly normal spleens. The changes in the spleen were usually visible at birth through the skin, but thereafter could be detected only by autopsy. The size of the individual as a whole was somewhat reduced, not only at birth but also in maturity. Vigor was markedly reduced, most affected individuals being very difficult to raise, and if raised, showing somewhat subnormal fertility.

Owing to the poor viability of the affected individuals, and the difficulty of detecting affected individuals except by autopsy or breeding tests, the stock was finally lost, but not until considerable data had been gathered on the inheritance of the trait. A partial pedigree of the descendants of ♂$F_1$182, the original affected male, is given in figure 3. Several lines of descent from this male have been omitted because the phenotype of some of the individuals concerned was not adequately determined. Determination at birth was often difficult, and frequently individuals suspected of being affected would die and be eaten before an autopsy was possible. The phenotype of individuals shown in the pedigree, however, is believed to have been accurately determined, unless otherwise indicated by a question mark. The data permit of only one interpretation, namely, that the character is inherited as an irregular dominant. Several matings between affected individuals were made, but if the homozygote survived it was not detectably different from other affected individuals. It is impossible to say whether the condition was due to a mutation in the strict sense, or to a deletion as is the case with "notch" in Drosophila.

The rate of production of dominant internally visible mutations was 1 per 91 (50+41; figures from table 5, column headed "Number of F_1 producing one or more of the autopsied F_3 mice") treated spermatozoa, or approximately 1 per 1820 treated chromosomes. This is obviously very much lower than the rate of production of translocations.

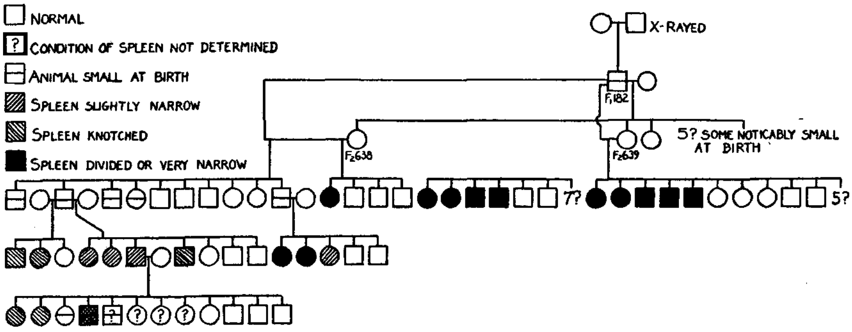

FIGURE 3.—Pedigree chart showing inheritance of X-ray-induced mutation, causing alterations in the shape of the spleen, reduction in vigor and reduction in the size of the animal as a whole. Inheritance as an irregular dominant is indicated.

No other visible mutations were found. Calculation indicates that, with the system of matings used, at least one half, on the average, of the treated genes borne by F_1 individuals should segregate in the homozygous condition in the F_3. Hence at least 990 treated chromosomes were tested for recessive visible mutations detectable at birth, and 910 treated chromosomes for recessive visibles detectable only by autopsy. Since no such mutations were found, we must conclude that X-ray treatment of mouse spermatozoa, if it produces them at all, at least produces them with a much lower frequency than it produces translocations.

ABNORMALITIES PROBABLY NOT ATTRIBUTABLE TO THE X-RAY TREATMENT

A number of abnormalities were found, some of them occurring more than once, which probably were not due to the X-ray treatment.

The commonest consisted of the reduction or absence of the thirteenth rib on one or both sides. This occurred in approximately 46 percent of the short-eared dilute (ds_e/ds_e) mice of the F_3 generation, both tests and controls, but in less than 1 percent of the non-short-eared non-dilute F_3 mice, and is therefore attributable in most cases to the short-ear or to the dilute factor, or to a factor closely linked with them.

In about 20 percent of the short-eared dilute F_3 mice, the muscular wall of the diaphragm was imperfectly formed, having a slit down the middle through which a small piece of the liver protruded into the pleural cavity. This hernia of the diaphragm occurred only in mice of the short-eared dilute phenotype, and is attributable, therefore, to the action of one or both of these genes, or of a gene closely linked with them. It is noteworthy that the gene for short-ear has already been shown (SNELL 1931) to cause alterations in the shape of the skull and a muscular waviness of the tail in addition to its primary effect on the size of the ears.

In eight F_3 mice, four of them from the control, the portal vein passed ventral to the duodenum instead of dorsal to it. In two of the cases it was ventral also to the transverse colon.

Test ♀ $F_2$724 (still-born) had a reduced upper jaw, and the lower jaw was reduced or lacking. Test ♀ $F_3$2543 (still-born) had a reduced lower jaw. Control ♀ $F_3$1689 (still-born) had almost no lower jaw. In the case of all of these females, two of the three grandparents were from the *L*-stock. Pure *L*-stock individuals occasionally are born with this same defect (agnathia). It is probably the same as the "lethal head and jaw abnormality" found by LITTLE and BAGG (1924) in both test and control lines of their X-ray experiment. The inheritance of what is probably the same trait in guinea pigs has been analysed by WRIGHT (1934).

Test ♀ $F_3$1977 had fourteen ribs on both sides. Six sibs were normal.

Test ♂ $F_3$2339 had large paired pockets, full of food, lying under the skin of the throat and opening by narrow passages into the mouth on each side of the lower jaw. Twenty-one sibs were normal.

In test ♀ $F_3$2539, the left uterus in the region of the kidney, instead of being attached to the dorsal body wall, was attached to the ventral face of the kidney itself. Seventeen sibs were normal.

Test ♀ $F_2$739 was still-born and had greatly reduced eyelids. Apparently death had occurred some little time before parturition. Seven sibs were normal. The mother, $F_1$289, was semi-sterile.

Test ♂ F_T1192 had an abnormal tail which kinked sharply up over his back due to malformation of the vertebrae. Several sibs and half sibs and numerous progeny were normal.

In test ♀ $F_3$756, the left digastricus muscle lay ventral instead of dorsal to the submaxilary gland. Sixteen sibs were normal.

Test ♂ $F_2$473 was decidedly undersize from 10 days of age until he was accidentally killed at 5 1/2 months. The shortness of the nose and shape of the head gave an appearance similar to that found in dwarf mice, though the size was considerably larger than that of the true dwarf. The mother, $F_1$109, was semi-sterile. The two sibs that were raised were normal. One son showed similar characteristics at 2 weeks of age, but failed to survive beyond 3 weeks. Four more offspring were normal at 2 weeks when they were killed; a number were normal at birth but failed to survive.

Control ♀F_T3512 lacked a right front leg. When examined at birth, there were a few bruises on the right side and bottom of the body, but the skin was unbroken, showing that the leg had not been eaten by the mother or lost in any similar accidental fashion. On autopsy at 28 days, it was found that the right clavicle and scapula and the upper end of the humerus were present. Thirty sibs were normal.

Control ♂F_T1658 was still-born. Its tail consisted merely of a slender thread about one-third normal length. Examination under a dissecting binocular showed that, although the head had been eaten, no damage had been done in the region of the tail. Eight sibs, ten half-sibs, and fifteen F_3 individuals derived from the same F_1 male were all normal.

In control ♀$F_3$367, the anterior third of the left kidney was reduced in size and showed a finer and more transparent structure than the normal part of the same kidney. The line of demarcation between the two parts was sharp. The abnormality, if it had a genetic basis at all, was perhaps the result of a somatic mutation. Four sibs were normal.

Seven of the above cases in which the abnormality appeared in one individual only were in the test group, three in the control. The difference may well have been due to chance alone; if not, slight changes in chromosome constitution of some of the individuals in the test group is the most likely explanation.

ACKNOWLEDGMENTS

The investigation reported in this paper was conceived under the stimulus of the discovery by Prof. H. J. MULLER that X-rays cause an enormous increase in the mutation rate of *Drosophila melanogaster*. Communications between the writer and Prof. MULLER revealed that almost identical plans—essentially those outlined in an above section of the paper—for an X-ray experiment with mice, had been prepared independently by each of us. Prof. MULLER, with the aid of several of his students, had developed an animal colony at the UNIVERSITY OF TEXAS for the purpose of executing this experiment. I am indebted to him for his kindness in putting this colony at my disposal. I am also indebted to him for valuable suggestions made during the course of the experiment.

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SUMMARY

1. X-rayed male mice from an inbred stock carrying five recessive genes were mated before the onset of X-ray sterility to untreated females carrying the dominant alleles of most or all of these genes. Three generations of progeny were raised, the matings being so planned as to give the greatest possible chance that any induced visible or lethal mutations would be detected. A control was provided by three generations of mice, similarly mated, and derived from the original parents of the treated group before the application of the X-rays. This paper describes results obtained in the second and third generations.
2. All individuals in the third generation, in which recessive visible mutations should appear, were autopsied with a view to the detection of mutations affecting only the internal organs.
3. Approximately 33 percent of the immediate progeny of the X-rayed males consistently produced litters of sub-normal size. This tendency to produce small litters is transmitted to later generations, and is the result of the death *in utero* of a certain proportion of the embryos. It has been designated "semi-sterility."
4. Evidence is presented showing that semi-sterility is the result of translocations carried in the heterozygous condition. The segregation of the translocations produces zygotes with chromosome unbalance which develop abnormally, and almost always die before term. The primary effect is on the central nervous system.
5. One female with a nervous disorder, and three still-born young with enlarged crania, all in the X-rayed group, were perhaps cases of relatively slight chromosome unbalance.
6. One translocation was linked with the marker gene "brown" (*b*).
7. No evidence indicating the occurrence of lethal mutations was obtained. In the test group, 209 chromosomes, in the control group 166 chromosomes, were tested and proved not to carry lethals closely linked with the marker genes.
8. One visible mutation, a variable dominant causing a reduction in width and a change in shape of the spleen, a considerable reduction in vigor, and frequently a reduction in size of the animal as a whole, was

found in the X-rayed group as a result of the autopsy. It was not determined whether the homozygote is viable. A deficiency, rather than a mutation in the strict sense, may be responsible.

9. A number of other abnormalities were found which are not attributable to the X-ray treatment.

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