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1. OBJECTIVE

The purpose of this project is to produce chromosomal inversions by irradiation or chemical treatment of sperm in mice and to detect inversions by observing the frequency of first meiotic anaphase bridges of their sons. For each new inversion of considerable length, we will (1) determine its linkage group, (2) mark it genetically, if possible, or place it with a genetically marked homologous chromosome, (3) study its cytological, physiological, and anatomical effects, and (4) use it, possibly in combination with other inversions or Robertsonian metacentrics, to produce test systems for precise estimates of mutational loads in irradiated populations.

2. MAIN RESEARCH ACCOMPLISHMENTS

1. Detection of inversions. Our usual procedure is to treat strain DBA/2J males with irradiation or a chemical mutagen, mate them immediately to strain C57BL/6J females, and save offspring conceived in the first two weeks after treatment. When the male offspring are 60 days of age, one testis is removed, sectioned, and stained. High frequencies of bridges in the first meiotic anaphase are indicators of inversions. Repeatedly we have found a low percentage of bridges (about 3.5 per cent) in the first anaphases of almost every male (Roderick, 1971). The reasons for these bridges are not known. But they do necessitate our distinguishing them quantitatively from a higher frequency of bridges that indicate an inversion. Other indicators of inversions that we do not usually see in normal animals are broken anaphase bridges and telophase bridges, neither of which are included in our count for anaphase bridges. Although anaphase bridges are the main indicator, we use these other characteristics as well in searching for new inversions. Numerous associated broken bridges suggest that the inversion is relatively near its centromere, whereas numerous telophase bridges suggest that the position of the inversion is much farther from its centromere. The frequency of anaphase bridges is an indication of the length of the inversion itself.

In the past year we have screened an additional 157 males for new inversions. We have also examined the sectioned testes of an additional 917 males in the handling and studying of the inversions we have produced. The smaller the inversion, the fewer the anaphase bridges, and the more anaphases we must score to be able to distinguish the bridge frequency due to the inversion from that of normal controls (Roderick, 1971). Now we classify as presumptive inversions any male whose frequency of first meiotic anaphase bridges remains above 15 per cent with continued sampling. We study animals with 15 per cent or above but usually keep only those with at least 20 per cent, since the smaller inversions are difficult to maintain. If the linkage of a particular small inversion is found quickly using our multiple linkage testing system (Roderick, 1971), we will keep the inversion. Also, we might keep a smaller inversion if it can be made homozygous quickly and with certainty.

As we breed a prospective new inversion heterozygote, we simultaneously backcross him to one of the parental strains, usually DBA/2J. This particular backcross enables us to check the linkage of the new inversion with nine isozyme loci and two coat color loci simultaneously (Roderick, 1971). We estimate, with the location of these various loci and the number of backcross animals observed, that we are testing about 1/4 to 1/3 of the genome of the mouse in this single cross. If unsuccessful in the initial cross, we use other appropriate linkage testing stocks. So far in the nine known inversions we have produced, the linkage of five has been determined, three of which were linked to isozyme loci.

In this past year, collaborating with Dr. Eva M. Eicher, we have imported and established stocks of Mus m. castaneus and a few newly derived stocks from feral populations of Mus m. musculus. These stocks will be useful in that they carry new isozyme variants. These variants will be put into our initial cross to detect inversions. With the additional isozyme loci, we shall be able to test the linkage of newly induced inversions more efficiently in the first backcross.

We also plan to examine the testes of males of these stocks and crosses between them for naturally occurring inversions.

The naturally occurring inversion we discovered in Mus m. molossinus (Roderick, 1971) has now been confirmed cytologically by Professor Alfred Gropp (personal communication). Unfortunately this inversion is too small to be of any use to us at this time.

2. Inversions produced. A male is characterized as carrying a presumptive inversion if, upon continued counting of his first meiotic anaphases, 15 per cent or more show clear bridges. Only after the animal produces offspring half of which show the same characteristically high bridge frequency do we classify him as carrying a known inversion, and the inversion is given a symbol.

Table 1 summarizes the results of screening for new inversions. Roderick (1971) calculated the rate of induction of inversions of a length to produce at least 11 per cent of anaphases with bridges. This estimate for sperm and spermatids, based on a total of 541 animals with an average radiation dose of 814 R was

$$15/541/814 = 3.4 \times 10^{-5} \text{ per R per gamete}$$

or 2.8 per cent of animals screened.

Now because our data concern only longer inversions, we estimate the induction rate of inversions producing at least 15 per cent anaphase bridges to be

$$15/859/1557 = 1.1 \times 10^{-5} \text{ per R per gamete}$$

or 1.0 per cent of animals screened.

This rate is smaller and indicates, as the array of inversions also does (Table 2), that longer inversions are less frequently induced than smaller ones.

In another experiment, ethylmethane-sulfonate (EMS) and triethylene melamine (TEM) were administered to animals through oral and intraperitoneal routes. Preliminary experiments of Soares and Petersen (in preparation) indicate there is not a significant difference between the effects of these mutagens induced by these different routes. If we assume no difference, the animals in these experiments received an average of 311 mg/kg of EMS or 0.19 mg/kg of TEM. An estimate of the rate of induction of inversions for EMS is therefore

$$1/311/156 = 2.06 \times 10^{-5} \text{ per mg/kg EMS I.P. or oral) per gamete}$$

or 0.6 per cent of animals screened.

The estimate for TEM is

$$3/.19/114 = 1/39 \times 10^{-1} \text{ per mg/kg TEM I.P. or oral) per gamete}$$

or 2.6 per cent of animals screened.

Flupromazine and triflupromazine, chemical mutagens used in other studies, provided us an opportunity to obtain preliminary data on their importance for inducing inversions. None were found.

It does not appear that TEM or EMS will be substantially more effective than irradiation in producing inversions. Of considerable importance, however, is the fact that two of the inversions induced by these chemicals are probably at least twice as long as the longest ones produced by irradiation (Table 2). At this time we are using only TEM to induce inversions.

Table 2 gives a summary of each of the 19 inversions produced. In(5)9Rk and the presumptive inversion in male No. 6567 are exceptionally long. Unfortunately, although male 6567 has sperm in the tubules of his testis, there is no sperm in the seminal fluid. With the collaboration of Dr. Wesley Beamer, we are providing this male with regular pituitary hormonal treatment in the hopes of bringing about his fertility. Male 6567 is otherwise normal in appearance. The inversions in males 6845 and 6857 will probably be available soon for study since both have produced offspring. The inversion in male 6857 is unusual in that it is characterized by a very high frequency of associated first meiotic telophase bridges. This with the relatively low bridge frequency suggests that this inversion is a relatively small one located toward the distal end of one of the longer chromosomes.

For one reason or another, not all inversions can be maintained. Some males carrying a new inversion were sterile. Some died before they could be mated, possibly because of infection after surgery to remove a testis, but more likely because the inversion made them less resistant to the stress of surgery. Some were discarded because the inversions were small. Small inversions are difficult to maintain and they are far less useful for our primary objective than longer inversions. Furthermore there are a sufficient number of large inversions discovered on a regular basis to justify discarding small ones.

We have studied nine inversions extensively. They were given symbols only after we showed that their characteristic high frequency was passed to half of the sons. Table 3 summarizes these data for these inversions and shows as well the high correlation between the original male's percentage of anaphase bridges and that of his inversion carrying descendants. The percentage of inversion carriers is about 50 per cent as expected, and the other 50 per cent show the percentage of anaphase bridges characteristic of normal males. Our original estimate of the control frequency was close to 3.5 per cent but the best estimate now, based on these data, is 4.2 per cent. It appears that the control frequency has risen recently, but we have no explanation to account for it unless quicker scanning attracts the eye more easily to an anaphase with a bridge rather than one without a bridge.

The correlation between the anaphase bridge frequency of each original male and the average frequency of his inversion-carrying descendants is very high. The rank-order correlation coefficient between the two arrays is +0.93 which is highly significant for this number of comparisons. This correlation indicates, as one would suspect, that inversions are stable in their lengths and do not change over generations.

We have plotted the individual bridge frequencies for sons of heterozygotes for In(1)1Rk and In(5)2Rk (Roderick and Hawes, 1970). The distribution for the longest inversion, In(5)9Rk, is shown in Figure 1. Distribution for stocks with other inversions are similar in that all are bimodal with approximately half of the males showing the background frequency and half showing the frequency of the original inversion-carrying male. Genetic evidence shows clearly that the variation around the means of the two groups in each set is due to sampling error.

3. Homozygosity of inversions. Inversions In(1)1Rk, In(5)2Rk, In(10)6Rk, and In(5)9Rk are now maintained in homozygous condition. Inversion In(2)5Rk appears to be homozygous lethal in males but partially homozygous viable in females. This type of viability difference between males and females is also known for homozygous condition of certain translocations. We believe In(5)9Rk can be easily maintained by repeated backcrossing to a stock homozygous for pallid (pa/pa), a locus with which this inversion does not recombine.

4. Linkage of inversions. The linkage is known for five of the nine

named inversions and is shown in Figure 2. The limits of the inverted segment are known rather precisely only for In(1)1Rk and In(2)5Rk. These limits are set by those loci that do not recombine with the inversion and that do not recombine with each other in the presence of the inversion. So far in these two inversions no recombination has occurred along their lengths which suggests that they are sufficiently short so that double-crossing over is physically impossible. Evidence for this is given in a later section concerned with the balanced-lethal system using the first inversion. It is worth noting that the limits of these two inversions is approximately the same, and this is expected because their characteristic bridge frequencies are very similar. One expects that a minimal estimate of the length in centi-Morgans of an inversion would be half the frequency of anaphase bridges. This distance for these two inversions would be approximately 17 or 18 centi-Morgans. The actual effective length of the inversions however is almost twice that distance as shown in Figure 2. The lower estimate may be too low and the higher estimate too high because crossing over immediately adjacent to the breakpoints is completely inhibited. The actual lengths may well be somewhere in between the two estimates.

The positions of In(5)2Rk and In(10)6Rk are known from their recombination frequencies with specific genes on their chromosomes. The extent of these inversions is only approximate and is estimated in centi-Morgans as about half the frequency of first meiotic anaphase bridges.

The location of In(5)9Rk presented us with a totally new problem. The anaphase bridge frequency is over 70 per cent which is over twice the frequency manifested by the largest inversions we have previously studied. If it is also over twice the length of those inversions, it must have an effective length of approximately 70 centi-Morgans. Since this would be an underestimate of the length of this inversion, we suspect that In(5)9Rk may encompass the whole chromosome 5, which is equivalent to placing the centromere on the opposite end. We are presently attempting to test this hypothesis through cytological means.

In(5)9Rk presents a novel situation furthermore in that it is sufficiently long to permit two strand double crossing-over with the non-inverted homolog. Genes closer to the center of the inversion have a greater likelihood of crossing over with the inversion. The limits of the inversion can then be estimated conservatively by its recombination with genes toward the ends of the inversion. The preliminary data we have of recombination of the inversions with genes on Chromosome 5 are:

In9 and <u>bf</u> (buff)	0.125
In9 and <u>rd</u> (retinal degeneration)	0.204
In9 and <u>Pgm-1</u> (Phosphoglucomutase - 1)	0.176
In9 and <u>Hm</u> (Hammertoe)	0.220

Initial evidence further shows that recombinations between genes within the inversion, in the presence of the inversion, is reduced. The reduc-

tion in recombination is in the neighborhood of 50 per cent. It will be difficult to pinpoint the ends of this inversion by genetical means, since the data now indicate there are no marked loci beyond the inversion.

Longer inversions such as this one may not be as useful for our objective as shorter ones, since much recombination between the inversion and its non-inverted homolog can occur. But the longer inversions may be useful when we attempt to combine one or more inversions on the same chromosome.

5. Cytology of inversions. During the past year we have developed reproducible techniques for both trypsin-Giemsa and fluorescent banding of chromosomes in the mouse. Both techniques are based on methods already described in the literature (Sun et al., 1973; Wurster, 1972; Miller et al., 1971; Francke and Nesbitt, 1971). To obtain fluorescent banding patterns in our mice, it was necessary only to increase the washing time following staining to ensure removal of all excess stain. Our procedure is to stain air-dried preparations for 8 to 10 min with quinacrine mustard dihydrochloride (50 µg/ml), wash the slides under running tap water for 15 to 20 min, and coverslip the stained preparations using the Tris-maleate buffer (pH 5.6) described by Miller et al. (1971).

Obtaining Giemsa banding patterns required more modification of preexisting techniques. Chromosomes from our mice were sensitive to much less drastic treatment than that previously reported in the literature for either human or mouse chromosomes. Staining times were also shortened appreciably. Air-dried preparations are treated in a horizontal position with 0.025 per cent trypsin, made up in Ca⁺⁺-free and Mg⁺⁺-free Earle's saline, for 7 to 10 sec. They are rapidly rinsed in two changes of the same saline and stained 4 to 6 min in 4 per cent phosphate-buffered Giemsa. Pre-treating the slides with heat (6 to 24 hr at 65 to 70 C) followed by a 10-min incubation in 56 C phosphate buffer (Sun et al., 1973) just prior to trypsinization improves the banding definition.

We can now report we have actually seen chromosomal inversions cytologically in Mus musculus. Using the techniques described above we have demonstrated chromosomal banding differences in In(1)1Rk and In(2)5Rk. In both cases the differences can be explained as inverted segments in the central region of the chromosome involved. Figure 3 shows the first five chromosomes for heterozygotes for In(1)1Rk and In(2)5Rk. The characteristically strong band in the center of normal chromosome 1 is replaced in the inverted chromosome by two bright bands toward the ends of the chromosome with a poorly defined area in between. In In(2)5Rk the difference is not as dramatic but a common variation has been detected in several photographs from two different mice. Distal to the characteristic prominent negative band at the centromere end are three banded regions separated by two poorly stained bands. In the normal chromosome 2 the distal band is the wider of the two while in the inversion chromosome the more proximal band is wider.

We will now use the banding methods described to analyze the remaining inversions. The techniques can also aid in the search for naturally occurring inversions and in the preliminary determination of chromosomes involved in newly discovered or induced inversions. With the trypsin-Giemsa method it is possible to obtain extremely fine resolution of banding patterns. It may be possible to detect deletions in chromosomes found to be carrying lethals in the balanced lethal system.

We have successfully produced banding patterns in first meiotic anaphase bridges using quinacrine fluorescence. The stain can be applied to the same sectioned preparations used for routine bridge counts. While these patterns are not definitive enough to determine the chromosomes involved in new inversions, they are characteristic in each known inversion. This technique could be used to follow individual inversions in matings combining two or more known inversions.

6. Balanced lethal test system. The major purpose of these studies is to produce inversions that can be used in balanced lethal systems like those that have had such practical advantages in the study of genetics in Drosophila. In(1)lRk is the most suitable inversion so far produced for this purpose, because (1) it is long, (2) it covers a chromosomal segment which has good genetic markers, and (3) it is homozygous viable. Id-1 (isocitrate dehydrogenase - 1), Sp (splotch), ln (leaden) and Dip-1 (dipeptidase - 1) are the most useful loci in the inverted segment. Splotch is a viable dominant spotting marker that is lethal when homozygous. There also appears to be a slight reduction in viability of Sp/+ heterozygotes. The mutant allele is fully penetrant but quite variable. Some animals exhibit a large clear white belly spot, whereas in a few only the tips of the feet are white. In only one or two cases of the several hundred we have observed have we had trouble distinguishing a Sp/+ animal from the wild-type +/+. Leaden is a fully penetrant recessive coat-color marker with no effect in the laboratory on fertility or viability. Loci Id-1 and Dip-1 are polymorphic in feral populations and are fully penetrant codominant loci. Both are determined by starch gel electrophoresis. Neither has any detectable effect on fertility or viability.

Figure 4 shows the mating scheme of this system which makes use of the two external phenotypic markers. Animals homozygous for the inversion are treated with the mutagen and mated to animals of the splotch-leaden stock. All offspring are potential bearers of recessive visibles or lethals. Propagation from the splotch (Sp/+) animals is shown in Figure 4. The wild-type (+/+) alternate animals can also be tested with a minor modification of the breeding scheme. In the case of the splotch animals, each is mated to another animal from the splotch-leaden stock of the same genotype as its parent from that stock. Each test animal at this point (Generation IIa) becomes the progenitor of two more generations of descendants ending in the test Generation IV. In Generation III the leaden marker distinguishes the normal splotch from a splotch-inversion double heterozygote. The latter should be identical to the test parent with

respect to the inverted and normal segments. All four genotypes of Generation III can be distinguished either through coat color or pattern, or because they die. The test animal can be mated to his offspring of identical genotype, or offspring of that genotype can be mated inter se. In the test generation one should expect a ratio of 2:1 of splotch animals to wild-type animals. Any appreciable and consistent reduction in wild-type animals suggests a lethal has been induced at a locus near the inversion. A complete absence of wild-type animals suggests a lethal has been induced within the inverted segment. Also the wild-type class may exhibit recessive visibles or subvitals. In using the alternate animals of Generation IIa, the mating system is altered only slightly and is different in genotypes only in Generation III. All four genotypic classes can be distinguished, and only the non-leaden splotch animals are used for mating to produce Generation IV. In this case matings can only be made between animals of this genotype in Generation III, since the test animal of Generation IIa cannot be used.

The induction rate for recessive lethals per genome in the mouse for spermatogonia has been estimated by Luning and Searle (1971) to be about 10^{-4} per R per genome. For sperm and spermatids, which we are irradiating, it may be over twice that rate. Since the genome of the mouse is approximately 1200 centi-Morgans in length (Searle et al., 1970; Henderson and Edwards, 1968), our inversion (about 34 centi-Morgans) is approximately 2.8 per cent of the genome. We can give about 1000 R to sperm and spermatids to induce lethals and detrimental. Our expectation would be:

$$10^{-4} \text{ (per R per genome)} \times 2 \text{ (increased factor for sperm and spermatids)}$$

$$\times 1000 \text{ R} \times 0.028 \text{ (fraction of the genome)} = 0.006.$$

If we also include about 20 centi-Morgans on either side of the inversion which the system would scan, and if we also include the probability of inducing some recessive visibles and detrimental in this region of the chromosome, our probability of finding a recessive may be in the neighborhood of 1/50. With the system we can also screen simultaneously for dominant mutations as well as mutations at the two isozyme loci included within the inversion.

Initially we have used high doses of radiation to sperm and spermatids to test the ability of the system to uncover recessives. Table 4 shows the number of animals we have tested from Generation IIa and those which are still in progress. Forty nine have been completed and 15 are in progress. Nineteen animals were mated for several months without offspring and were classified as sterile. They cannot be included in the number tested for recessives. For each test animal of Generation IIa, we raise at least 30 offspring in Generation IV and examine the ratio of Sp/+ to +/+ in them. Also at least one Sp/+ and +/+ animal are examined for their alleles at loci Id-1 and Dip-1. Table 5 gives the numbers of animals tested for all genotypes in Generation IV. Only the two

parental genotypes occurred in 127 animals. This combined with previous data (Roderick, 1971) brings to 155 the number of animals where at least 3 or more markers have been tested and found not to recombine in the presence of the inversion. Loci ln, Sp, and Id-1 are centrally located in the inversion and would be most likely involved in a double recombination when it occurs. We are searching for the products of double recombination, since that would provide us with allelic variation within the inverted segment.

An important feature about the isozyme loci in this inversion is their codominance. We therefore have the opportunity of finding new allelic mutations which could be seen as a band in a new location in both heterozygotes and homozygotes. Codominance also permits us to see the absence of a band in the heterozygotes if a deletion of either locus is induced. We would expect all mutations at either of these loci to occur in the B band because the inversion homozygote exposed to the mutagen is homozygous for b at both loci.

In the balanced lethal study, the Generation IV descendants of one test animal (female 8010-1) showed a gross departure from a 1:2 (Table 6) the nine wild-type animals are perfectly viable and healthy in appearance, and thus this does not appear to be a recessive detrimental within the inverted segment. Most likely it is a lethal outside the inverted segment but closely linked.

The lethal could be in the center of the inverted segment, but our data indicate that double crossovers within the segment could not be so frequent to produce 12 +/+ animals in this size of sample. We can assume that we should have produced about 1/2 as many +/+ animals as Sp+/+, or 59. Only one crossover between the inversion and the lethal is necessary to produce a viable +/+ animal, and therefore there were, among the gametes which produced this hypothetical 59 animals, 118 opportunities for recombination. The crossover value then is at maximum $12/118 = 10.2$ per cent.

We can assume that the genotypes of most of the parents in these crosses In1 (+) 1/ +(Sp) + where the splotch locus is within the inversion and the lethal locus is outside. If a crossover occurs between the inversion and the lethal, then a splotch animal could have the genotype In1 (+) +/+ (Sp) +. This animal mated to the lethal carrying splotch animal above would produce +/+ animals in the normal ratio. Thus we must characterize each of the matings between descendants of ♀ 8010-1 as to whether they have +/+ animals of the normal proportion, or whether they have them of the greatly reduced proportion. The mating between 8491-2 and 9491-5 (Table 6) appears to have a nearly normal ratio of +/+ to Sp+/+ animals, and we suspect therefore that one of the parents is a crossover. If we eliminate the offspring of that mating from the table, the crossover value between the inversion and the lethal is 5.9 per cent.

This lethal is not allelic with the known loci within the inversion or immediately outside the inversion.

The important fact of having this lethal is in the verification of the system to detect lethals within a reasonable number of animals tested (i.e. one of 49). It is also important to have a recessive lethal which can be propagated and studied in itself.

7. Robertsonian metacentrics. The Tobacco mouse, *Mus. m. poschiavinus*, has 7 metacentric and 6 acrocentric chromosomes instead of the usual 20 acrocentric haploid chromosomes of laboratory mice. These metacentric chromosomes probably arose by Robertsonian translocation (Gropp et al., 1970) since the chromosome arms are individually identifiable with specific acrocentric chromosomes of laboratory mice (Zech et al., 1971).

Fortunately the tobacco mice are interfertile with laboratory mice. We have separated the metacentric chromosomes in several different stocks and plan eventually to establish a stock homozygous for each one. So far we have identified by quinacrine banding techniques a homozygous stock for T1, T5, and T6. Another stock is homozygous for only T1 and T6. Other stocks are segregating for the other metacentrics.

We plan to put inversion In(1)lRk into the largest metacentric (T1) which includes Chromosome 1 as well as Chromosome 3. This stock then could be further treated with a mutagen to produce a more complex inversion which might effectively reduce recombination for a major part of Chromosomes 1 and 3. Inducing inversions in the metacentrics alone may also achieve more efficient balanced lethal systems.

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TABLE 1. SCREENING FOR INVERSIONS WITH VARIOUS MUTAGENIC AGENTS.

Treatment	male	
of sire	offspring observed	inversions found*
None	29	0
Saline, I.P.	15	0
700 R	158	0
750 R	29	0
800 R	293	4
850 R	28	1
900 R	1,024	10
1000 R	25	0
Ethylmethane-sulfonate (EMS)		
200 mg/kg, oral	4	0
" , I.P.	11	0
250 mg/kg, oral	3	0
" , I.P.	8	0
300 mg/kg, oral	43	1
" , I.P.	45	0
390 mg/kg, oral	13	0
" , I.P.	25	0
400 mg/kg, I.P.	4	0
Triethylene melamine (TEM)		
0.15 mg/kg, oral	17	0
0.20 mg/kg, oral	14	0
" , I.P.	83	3
Flupromazine (FP)		
40 mg/kg, I.P.	36	0
Triflupromazine (TFP)		
75 mg/kg, I.P.	44	0
Total	1,907	19

*Males are classified as inversion carriers only if they show frequencies of first meiotic anaphase bridges consistently above 15%. All males screened were conceived within two weeks of treatment of their sires.

TABLE 2. INVERSIONS INDUCED IN THE MOUSE

Male No.	Genetic Stock*	Inversion Symbol	Anaphase bridge frequency	standard error	Induced by	Comments
471	1		0.379 ±	0.033	800 R	functionally sterile
587	2		0.176	0.034	"	died before mating
618	"	In(1)1Rk	0.340	0.008	900 R	
639	"		0.151	0.026	"	fertile, discarded
816	"	In(5)2Rk	0.192	0.010	850 R	
2414	"		0.340	0.048	900 R	small, died
2425	"		0.158	0.033	"	discarded
2457	"	In3Rk	0.143	0.016	"	"
2576	"		0.164	0.050	"	"
2624	"		0.175	0.050	"	"
2913	"	In4Rk	0.188	0.013	"	
2916	"	In(2)5Rk	0.345	0.014	"	
3563	"	In(10)6Rk	0.262	0.018	"	
4587	"	In7Rk	0.150	0.024	800 R	discarded
4609	"	In8Rk	0.179	0.019	"	
5204	3	In(5)9Rk	0.737	0.020	.2 mg/kg TEM I.P.	
6567	4		0.741	0.033	300 mg/kg EMS oral	
6845	5		0.294	0.055	.2 mg/kg TEM I.P.	
6857	"		0.228	0.047	" " "	

- * 1. Heterogeneous stock; male was Ra +/+ Sd
 2. (C57BL/6J × DBA/2J)F₁
 3. (C3H/HeJ × DBA/2J)F₁

4. C57BL/6J × (C3H/HeJ × DBA/2J)F₁
 5. (DBA/2J × C57BL/6J)F₁

TABLE 3. COMPARISON OF PERCENT OF ANAPHASE BRIDGES BETWEEN ORIGINAL INVERSION-HETEROZYGOTES AND THEIR DESCENDANTS WITH HIGH FREQUENCIES.

	Number of original ♂	Percentage of original ♂	High descendants		Normal descendants		P*
			N	Percentage	N	Percentage	
In(1)1Rk	618	61/181 = 33.70	155	1264/3721 = 33.97	145	124/3480 = 3.56	0.94
In(5)2Rk	816	29/137 = 21.17	48	273/1440 = 18.96	50	55/1500 = 3.67	0.54
In3Rk	2457	20/135 = 14.81	10	45/320 = 14.06	8	11/320 = 3.44	0.97
In4Rk	2913	23/100 = 23.00	38	139/760 = 18.29	24	19/480 = 3.96	0.26
In(2)5Rk	2916	59/154 = 38.31	51	346/1020 = 33.92	69	76/1380 = 5.51	0.29
In(10)6Rk	3563	29/100 = 29.00	24	123/480 = 25.62	16	12/320 = 3.75	0.49
In7Rk	4587	7/40 = 17.50	9	26/180 = 14.44	9	11/180 = 6.11	0.64
In8Rk	4609	39/180 = 21.67	12	36/240 = 15.00	14	15/280 = 5.36	0.08
In(5)9Rk	5204	68/97 = 70.10	37	276/370 = 74.59	35	23/350 = 6.57	0.44

High and normal descendants are all offspring of inversion heterozygotes.

*P is the probability, based on a χ^2 test, that the difference between the percentages of the original male and his inversion-carrying descendants is due to chance.

TABLE 4. ANIMALS TESTED FOR RECESSIVE MUTATIONS IN THE BALANCED-LETHAL
TEST SYSTEM

Mutagen	Completed			In Progress
	♀♀:♂♂	Induced Recessives	Additional Steriles ♀♀:♂♂	♀♀:♂♂
800 R	12:16		8:2	0:0
850 R	1: 1		1:0	0:0
900 R	7:12	1	4:4	0:15

TABLE 5. GENOTYPES OF MALES OF THE TEST-GENERATION WHERE ALL LOCI AND THE INVERSION WERE SCORED*.

THE CROSS**:

	$\frac{\text{In}(b + + b)}{+ (a \text{ Sp } \ln a)}$	x	$\frac{\text{In}(b + + b)}{+ (a \text{ Sp } \ln a)}$
	$\frac{\text{In}(b + + b)}{\text{In}(b + + b)}$		$\frac{\text{In}(b + + b)}{+ (a \text{ Sp } \ln a)}$
♀♀	14		25
♂♂	33		55

* Females were not scored with respect to anaphase bridges. At the ln locus only +/? and ln/ln could be distinguished.

** Loci within the inversion are arranged in the order Id-1, Sp, ln, Dip-1.

TABLE 6. RESULT OF GENERATION IV FROM TEST FEMALE 8010-1.

Type of Mating	Dam	Sire	Offspring	
			+/+	Sp/+
			♀♀:♂♂	♀♀:♂♂
Daughter × son	8174-3	8174-4	0:0	14:14
Granddaughter × grandson	8491-1	8491-6	1:0	4:4
	8491-2	8491-5	5:1	9:7
	8491-3	8491-4	0:0	2:10
	8540-1	8540-3	1:0	12:8
	8605-1	8605-3	0:0	0:3
	8605-2	8617-1	1:1	6:2
	8617-3	8617-6	0:0	1:0
Matings between more distant descendants	8299-	8617-5	1:1	3:6
	9339-1	9339-2	0:0	6:7
		Total*	9:3	57:61

* Departure from a ratio of 1:2 of +/+ to Sp/+: $\chi^2 = 33.9$; 1 df: $P < 0.001$

Figure 1. Percent anaphase bridges of sons of animals heterozygous for inversion In(5)9Rk. Each male's frequency of bridges was based on a total of 10 anaphases of the first meiotic division. Theoretical curves, based on what would be expected from the mean frequencies of each mode, are drawn in for comparison.

Figure 1

Figure 2. Inversion map of the mouse

Figure 3. Chromosomes 1 through 5 from a male heterozygous
for In(1)1Rk and a male heterozygous for In(2)5Rk

In(1)1Rk

In(2)5Rk

Chromosome

* Inversion Chromosome

Figure 4. The balanced lethal test system using inversion In(1)lRk.

3. CONTINUATION PLANS

1. Inversion In(1)1Rk. We will continue to use the balanced-lethal test system to induce recessive lethals and detrimentals by irradiation of sperm and spermatids.

2. Induced lethal. We will conduct further linkage tests to locate the position of the induced recessive lethal. We will begin studies of the time of lethality and the fitness of animals heterozygous for the lethal.

3. Other inversions. We will continue to study the other inversions particularly the most recent three induced. Their linkages will be ascertained and they will be incorporated into balanced lethal test systems if feasible. Attempts will be made to combine two or more inversions into a single test system.

4. Effects of inversions. We shall continue to study the mechanism by which reduction in recombination is achieved without loss of fertility. Also we will study the effects of inversions on crossing-over in other chromosomes.

5. Cytogenetic analysis. By quinacrine and Giesma staining we will try to show visually the inverted segments of the other inversions. We will also continue our efforts to characterize cytologically the anaphase bridge chromatid for each of the inversions.

6. Further screening. We shall continue to induce and detect inversions using the chemical mutagen TEM.

7. Linkage test system. We shall continue our work toward incorporation of new isozyme loci into our linkage testing system.

4. STUDENTS AND VISITING INVESTIGATORS

The Jackson Laboratory is primarily a research institution, but its Staff members are directly concerned with training students for careers in biomedical research. This training is accomplished through summer and year-round programs of research participation wherein each student carries out a research project under a Staff member's sponsorship. Visiting Investigators join in collaborative research efforts of mutual interest. During this past year the following students and Visiting Investigators have participated in projects directly relating to research of this contract. Their work was done in collaboration with the Principal Investigator in his laboratory.

GROUP

Name and address

Title of project

SUMMER PROGRAM FOR PRECOLLEGE STUDENTS

Kimberly R. Long
Bald Eagle-Nittany High School
Mill Hall, Pennsylvania

Determining the rate of somatic crossing-over in mice by the paracentric inversions.

SUMMER PROGRAM FOR COLLEGE, GRADUATE, AND MEDICAL STUDENTS

Nancy L. Stover
Johns Hopkins University

The mapping of a linkage group to the mouse Chromosome 3.

POSTDOCTORAL TRAINING PROGRAM

Eugene R. Soares, Ph.D.
The Jackson Laboratory

Chemical mutagenesis and new for assessing dominant lethals.

Mutagenic and carcinogenic properties of alkylating agents.

VISITING INVESTIGATOR PROGRAM

Kyle W. Petersen, Ph.D.
University of Maryland
School of Medicine and
Food and Drug Administration
Washington, D.C.

Dominant lethal studies in five strains of mice using triethyl-enemelamine

James E. Womack, Ph.D.
Abilene Christian College
Abilene, Texas

Developmental genetic expression of mouse esterases.

The Principal Investigator was also co-director with Dr. Victor A. McKusick of Johns Hopkins University in presenting another Short Course in Medical Genetics at the Jackson Laboratory in the summer of 1972.

5. BIBLIOGRAPHY

Two papers were prepared for publication under this contract since the last progress report. Draft copies of each are attached:

DAVISSON, M. T., and T. H. RODERICK. 1973. Cytological characterization of two paracentric inversions in mice. To be submitted to Cytogenetics and Cell Genetics.

RODERICK, T. H., and N. L. HAWES. 1973. Nineteen paracentric inversions in mice. To be submitted to Genetics.

6. PRESENT STATUS OF RESEARCH IN THIS FIELD

Studies in mammalian mutagenesis are still hampered by the relatively inefficient systems available to assess and maintain recessive lethals. Furthermore, there is essentially no information available for mammals on the dominant effects of recessive lethals. The two major techniques for inducing recessive lethals have been the specific locus technique of W. L. Russell and the entire genome scan for recessive lethals of K. G. Luning. The former technique is relatively inefficient in estimating rates of induction of lethals since only seven loci can be tested simultaneously. But the system is efficient for maintaining lethals once they have been induced. The latter system is more efficient in screening for lethals, but is less efficient in maintaining them.

Our approach is the first study to induce, genetically define, and use inversions in mice. With a balanced lethal system now available we have been able to screen about 5 per cent of the genome for recessive visibles, detrimentals, and lethals. Recessives induced with the system are easily propagated for study. Of 49 test animals where the father has received at least 800 R to the sperm or spermatids, one recessive lethal closely linked to the inverted segment has been revealed.