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The production of recessive lethals by calf-thymus DNA in *Drosophila*

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1. INTRODUCTION

The possibility of inducing mutations by DNA was first suggested just before the war by Gershenson (1939*a*, *b*), who obtained various types of heritable visible mutations by the addition of thymonucleic acid to larval food. Rapoport (1940), working with DNA from different sources, however, could not confirm these results. Muller (1941), too, failed to obtain sex-linked recessive lethals from larvae that had been fed on DNA-containing food. Renewed interest in the genetical effects of DNA arose with the claims of Gershenson & Kiselyeva (1958) that calf-thymus DNA in larval food produced visible mutations, but no sex-linked lethals. It is difficult to imagine that any treatment should produce visible changes without producing lethals, and as most of the visible mutations occurred on the 2nd chromosome, it seemed possible that DNA might act preferentially on autosomes. This assumption received support from experiments by Fahmy & Fahmy (1961, 1962), in which DNA injected into males produced Minutes, but no sex-linked lethals. Since attached-X females had been used in their tests and it is known that most Minutes are lethal in hemizygous condition, practically all these Minutes must have been autosomal. In order to test for a specific response of the 2nd chromosome to DNA, Alexander & Auerbach (unpublished) carried out experiments with three different samples. A pilot experiment with calf-thymus DNA prepared by Dr Gershenson yielded an increased frequency of autosomal lethals. However, further experiments on a dual-purpose stock with commercial salmon sperm DNA and calf-thymus DNA prepared by Dr Kølmark according to the recipe given by Dr Gershenson gave only negative results. This raised the possibility that DNA from different sources or prepared in different ways might behave differently in such tests. For the present work, a second sample of calf-thymus DNA was kindly provided by Dr Gershenson.

Experiments were undertaken with two objectives in mind: (1) to compare mutation rates in the X- and 2nd chromosomes, and (2) to establish whether in the previous tests the mutagenic action of DNA had been underestimated because DNA, like chloro-ethyl methane sulphonate—CEMS—(Mathew, 1964), produces a high frequency of mosaics. This was investigated by scoring both complete and mosaic lethals in the X- and 2nd chromosomes. When mosaic tests yielded positive results, the experiment was extended to later generations in order to test for the recurrence of mosaics in lines derived from treated males.

2. MATERIALS AND METHODS

The major part of the present work is concerned with the effects of adding calf-thymus DNA to larval food. The rest relates to the results obtained in a small experiment by injection of commercial salmon sperm DNA into adult males.

In the experiment with salmon sperm DNA, sex-linked recessive lethals and Minutes were scored. Wild-type Oregon-K males were injected with two concentrations of DNA, 0·5% and 1·0% respectively, in 0·4% saline and subsequently were individually mated to three Muller-5 females. The treated males were discarded after 3 days and the F_1 progeny were examined for Minutes. Sex-linked lethals were scored in the usual way in F_2.

The experiments with calf-thymus DNA differed from that with salmon sperm DNA inasmuch as the treatment was given at the larval stage and the mutations looked for were sex-linked and 2nd chromosome recessive lethals. Two series of experiments were carried out: the first with 10% sodium salt of DNA and the second with 13% (the concentration used by Gershenson) in larval food. In the first series, only male larvae were tested while in the second one, following a suggestion from Dr Muller (personal communication), both sexes were studied to eliminate a possible influence of germinal selection on the frequency of sex-linked lethals. Young wild-type Oregon-K males and *Cy/Bl* L^2 virgin females were mass mated in bottles with the DNA-containing medium. The parents were discarded after 2 days and the larval progeny were allowed to feed on this treated medium. The Curly males and females that emerged were individually mated to sc^{sl} *InS* w^a sc^8; *Cy/Bl* L^2 females and males respectively and the progeny were tested for both X- and 2nd chromosome lethals in the usual way. In series 1, progeny from two broods of 3 days each were tested, while in series 2 only the first brood progeny were tested. Since the treatment was given to heterozygous Curly (*Cy*/+) larvae, any pre-existing lethal on the wild-type 2nd chromosome of one of the larvae would show up in all F_3 cultures of all broods derived from that larva. All such pre-existing lethals were excluded.

The incidence of mosaicism was studied by extending the experiment to later generations. The sex-linked mosaics were detected by the same procedure as adopted earlier in the case of CEMS (Mathew, 1964), i.e. by testing on an average twenty females per non-lethal F_2 culture and examining the X-chromosome under test in F_3. In the experiment with CEMS, all F_2 cultures were selected from 'lethal lines' only, while in the present work, the F_2 cultures (and also F_3 cultures for autosomal mosaics) were taken from both 'lethal' and 'non-lethal lines'. In tests for autosomal mosaics, both males and females from F_3 non-lethal cultures can be used. In the present work only males (ten per culture) were tested. These males were individually mated to *Cy/Bl* L^2 females and their Curly progeny were inbred to score lethals in F_5. A lethal in F_5 will testify to mosaicism of the corresponding F_1 male, and the proportion of lethal-bearing males in the F_3 cultures will reflect the size of the mutated tissue in the F_1 gonad. In some cases, the 2nd chromosome test was extended up to F_9; i.e. mosaic lethals were scored in F_5, F_7 and F_9. Autosomal lethals that arise in P_1, F_1, F_2 and F_3 are detected in F_3, F_5,

F_7 and F_9 respectively. Lethals obtained in different generations from the same as well as different treated parents were finally cross-tested for allelism.

3. RESULTS

Injection

Injection of 1% salmon sperm DNA produced only one clear Minute in more than 2000 F_1 progeny and two recessive lethals in about 700 X-chromosomes. Neither Minutes not lethals were obtained in similar numbers of progeny from males injected with 0·5% DNA. In experiments by Fahmy & Fahmy (1961, 1962), injection of 1% *Drosophila* DNA had yielded about 1% Minutes.

Feeding

The results obtained by adding calf-thymus DNA to the larval food are summarized in Tables 1 to 8.

Sex-linked lethals

(*a*) *Completes*

Table 1 shows that the frequency of induced sex-linked lethals in male larvae was not increased over the control level. The observed value of 0·8% in treated females, although not significantly different from the controls, is well outside the range of the usually observed spontaneous mutation frequencies in females, and this suggests a real, though slight, effect of the treatment on female germ cells.

Table 1. *Frequencies of complete sex-linked lethals*

Treatment	Brood (3 days)	No. of chromosomes tested	Complete lethals	
			No.	%
Male larvae				
DNA 10%	A	1044	1	0·1
	B	895	2	0·2
	Total	1939	3	0·2
DNA 13%	A	635	2	0·3
Control	A	544	1	0·2
Female larvae				
DNA 13%	A	651	5	0·8
Control	A	614	0	0·0

The clear failure to obtain complete lethals in males might then be attributed to the influence of germinal selection. This assumption agrees with the finding of sex-linked mosaic lethals (see below) in both male and female larvae. Since, with the method used, mosaic lethals first occur in the F_1 females, whether or not they have been induced in P_1 males or females, no difference in germinal selection between the sexes would be expected.

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(*b*) *Mosaics*

The data on mosaics (see Table 2) are expressed in two different ways: (1) as the proportion of mosaic cultures among all F_2 cultures tested (column 3), and (2) as the proportion of lethals among all F_3 cultures independent of their origin from any particular F_2 culture (last column). The first approach will show the total number of mosaic cultures, but does not take account of the number of lethals by which the mosaic cultures were ascertained. Although in both sexes more mosaics were obtained in the treated than in the control series, the differences were not significant with the numbers used.

The second approach takes account of the consideration that apparent mosaicism for an induced lethal in a P_1 male may, instead, be due to spontaneous mutation in an F_1 female. This is especially true for lethals that are found singly in groups of F_2 females. In order to overcome this uncertainty, the values in the last column of Table 2 were calculated as the overall frequency of lethals among all F_3 cultures. Lethals in both sexes were several times as frequent in the treated as in the control series, and the combined χ^2 gave a significant value ($\chi^2 = 5{\cdot}1$ for 1 D.F.). This, together with the fact that eight out of eleven lethals in F_3 of the treated series had occurred as pairs in the same group, strongly suggests that DNA had produced mosaic sex-linked lethals in larvae of both sexes.

In summary, then, the results of the sex-linked lethal test indicate that feeding of DNA to larvae has a slight, but definite mutagenic action on the X-chromosome.

Table 2. *Frequencies of mosaic sex-linked lethals*

Treatment	No. of F_2 non-lethal cultures tested (n_1)	Mosaic F_2 cultures		No. of ♀♀ tested from F_2 cultures ($n_1 \cdot n_2$)	Lethal bearing ♀♀ = lethals in F_3	
		No. (X)	% (X/n_1)		No. (Y)	% ($Y/n_1 \cdot n_2$)
Male larvae						
DNA 13%	56	4	7·1	945	6	0·6
Control	72	1	1·4	1161	1	0·1
Female larvae						
DNA 13%	67	3	4·5	1052	5	0·5
Control	76	1	1·3	1206	1	0·1

Legend of symbols:

n_1 = number of F_2 non-lethal cultures tested, each by a group of n_2 ♀♀ (n_2 = approximately 20).

$n_1 \cdot n_2$ = total number of ♀♀ tested.

X = number of mosaic cultures (number of groups of ♀♀) that contained at least one lethal-bearing ♀.

Y = total number of lethal-bearing ♀♀ among the $n_1 \cdot n_2$ tested ♀♀.

2nd chromosome lethals

(*a*) *Completes*

It will be seen from Table 3 that both male and female larvae showed noticeable increases in the frequency of complete lethals over the controls (combined $\chi^2 = 3{\cdot}8$).

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The frequency of lethals in females was higher than in males, but the difference was not significant.

Table 3. *Frequencies of complete lethals in 2nd chromosome*

Treatment	Brood (3 days)	No. of chromosomes tested	Complete lethals No.	Complete lethals %
Male larvae				
DNA 10%	A	869	6	0·7
	B	715	13	1·8
	Total	1584	19	1·2
DNA 13%	A	216	3	1·4
Control	A	358	1	0·3
Female larvae				
DNA 13%	A	426	12	2·8
Control	A	351	3	0·9

(*b*) *Mosaics*

In Table 4, the data on mosaics have been presented in the same way as those for the X-chromosome (see Table 2). It will be seen that both alternative methods of testing for mosaicism (fourth and penultimate columns) showed increased

Table 4. *Frequencies of mosaic lethals in 2nd chromosome*

Treatment	Brood (3 days)	No. of F_3 non-lethal cultures tested (n_1)	Mosaic F_3 cultures No. (X)	Mosaic F_3 cultures % (X/n_1)	χ^2 (1 D.F.)	No. of ♂♂ tested from F_3 cultures ($n_1 \cdot n_2$)	Lethal-bearing ♂♂ = lethals in F_5 No. (Y)	Lethal-bearing ♂♂ = lethals in F_5 % ($Y/n_1 \cdot n_2$)	χ^2 (1 D.F.)
Male larvae									
DNA 10%	A	66	9	13·6		577	12	2·1	
	B	111	10	9·0		901	13	1·4	
	Total	177	19	10·7		1478	25	1·7	
DNA 13%	A	74	9	12·2	4·5	457	17	3·7	14·2
Control	A	85	2	2·4		649	3	0·5	
Female larvae									
DNA 13%	A	62	12	19·4	6·3	416	24	5·8	18·7
Control	A	72	3	4·2		485	3	0·6	

The symbols are the same as those used in Table 2.

frequencies in treated series, and all differences were significant for each sex separately (see χ^2 values in Table 4). As in the case of completes, females yielded more mosaics than males, but the difference was not statistically significant.

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Size of mutated sector in F_1 mosaic gonads

The proportion of lethal-bearing progeny in an F_2 (sex-linked) or F_3 (autosomal) culture is a fair index of the size of the mutated sector in the F_1 gonad. The relevant data are shown in Tables 5 and 6. It will be seen from Table 5 that more than half of all mosaic cultures (twenty-eight out of forty-seven) yielded only one lethal each. However, a comparison with the mutation rate in the controls (last column in Table 2 and penultimate column in Table 4) shows that most of these singly occurring lethals were of induced origin. Even where several lethals were found

Table 5. *Distribution of mosaic cultures according to the number of lethals per tested group*

	X-chromosome			2nd chromosome					
		No. of lethals per tested group (approximately 20)			No. of lethals per tested group (approximately 10)				
Sex	No. of mosaic cultures	1	2	No. of mosaic cultures	1	2	3	4	5
Males	4	2	2	28	18	6	4	–	–
Females	3	1	2	12	7	1	2	1	1
Both sexes	7	3	4	40	25	7	6	1	1

in a group, the mosaic sector rarely reached 50% and usually formed a much smaller proportion of the F_1 gonads. There was a peculiar difference in this respect between mosaics for sex-linked and autosomal lethals. While the sector size (see Table 6) for sex-linked lethals never exceeded 2/20, sector size for autosomal lethals often was higher than 2/10 and in one case reached 5/10. If the sector size reflects degree of germinal selection during the development of F_1 gonads, such a difference might, for instance, arise if autosomal lethals were due to smaller deficiencies than sex-linked ones; but this possibility has not been examined.

Table 6. *Average fraction of mutated tissue in F_1 gonad as calculated by the proportion of lethal-bearing progeny*

		Males	Females	Both sexes
X-Chromosome	(1) No. of F_2 mosaic cultures	4	3	7
	(2) No. of ♀♀ tested from (1)	70	48	118
	(3) No. of lethal-bearing ♀♀ in (2)	6	5	11
	(4) Percentage of lethal-bearing ♀♀ = mutated fraction of F_1 gonads = (3)/(2)	8·6	10·4	9·3
2nd Chromosome	(5) No. of F_3 mosaic cultures	28	12	40
	(6) No. of ♂♂ tested from (5)	213	102	315
	(7) No. of lethal-bearing ♂♂ in (6)	42	24	66
	(8) Percentage of lethal bearing ♂♂ = mutated fraction of F_1 gonads = (7)/(6)	19·7	23·5	21·0

Mosaics in later generations

The incidence of mosaicism in generations later than F_1 was studied by testing the seemingly non-lethal progeny of F_1 mosaics. The results showed that in many treated lines, the F_1 mosaics produced again F_2 mosaics. In a further extension of the experiment, some of these F_2 mosaics, in turn, produced mosaic progeny. In one case, the mosaic parent produced several mosaic progeny. For an interpretation of these results, it was necessary to establish whether or not lethals obtained in different generations from the same treated male were allelic to each other. This question cannot easily be resolved in the case of sex-linked lethals because of the inherent difficulty of cross-testing. For autosomal lethals, on the other hand, cross-testing can easily be carried out.

Table 7. *Allelism among 2nd chromosome lethals in different generations from the treated male No.* 60

Generation in which lethal arose	Generation in which lethal was detected	Serial No. of lethals	1	26	27	28	56	88	91	92
P_1	F_3 (completes)	1								
F_1	F_5* (mosaics)	26	−							
		27	−	+						
		28	−	+	+					
F_2	F_7 (mosaics)	56	−	−	−	−				
F_3	F_9** (mosaics)	88	−	+	+	+	+			
		91	−	−	−	−	+	+		
		92	−	−	+	−	−	+	+	

\+ = allelic. − = non-allelic.

* = The three lethals in F_5 came from the same F_3 ♂.

** = The three lethals in F_9 came singly from three different F_7 brothers tracing back to the same F_3 mosaic ♂.

Tests for allelism between lethals from the same male

Cross-tests were performed in lines derived from three different males (Nos. 17, 50 and 60) which had yielded lethals up to F_9, i.e. mosaics up to F_3. The detailed data for male No. 60, which had produced most mosaic lethals, are given in Table 7. This male had produced one complete (in F_3) and seven mosaic lethals (three in F_5, one in F_7 and three in F_9). The complete lethal was not allelic to any of the seven mosaic lethals; this is as expected because complete and mosaic lethals arise from independent spermatozoa. Among mosaic lethals, on the other hand, a very high incidence of allelism was observed. The pattern of allelism was peculiar. Thus, one lethal (No. 88) was allelic to all of the remaining six mosaic lethals, not all of

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which were allelic to each other. This is clearly demonstrated in Fig. 1. This type of relationship could be explained on the basis of overlapping deficiencies of which lethal No. 88 will have the greatest extension, covering the regions of all the others.

The results obtained in the other two males are shown in Table 8. In male No. 17, all the mosaic lethals were allelic to each other. In the other male (No. 50), the

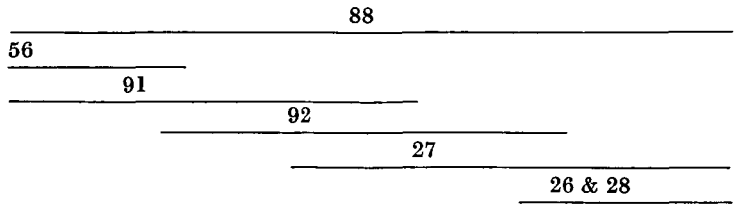

Fig. 1. Pattern of allelism (complementation map) among 2nd chromosome mosaic lethals from male No. 60.

pattern of allelism showed the same type of overlapping relationship that had been observed in male No. 60, with the difference that one of the lethals, detected in F_9 (83), was not allelic to any of the other mosaic ones. This lethal may possibly have arisen spontaneously, or alternatively its region may not have overlapped any of those covered by the small number of lethals in this line. The results of these crosses suggest that within each line one particular region had been affected by the treatment and that the instability induced in that region had been transmitted as such to later generations. Moreover, in male No. 60 the original instability must have replicated as such in order to yield several mosaic progeny.

Table 8. *Allelism among 2nd chromosome lethals in different generations from males* 17 *and* 50

Male No. 17

	Serial No. of lethals	6	33	61	62	73
F_3	6					
F_5	33	−				
F_7	61*	−	+			
	62	−	+	+		
F_9	73	−	+	+	+	

Male No. 50

	Serial No. of lethals	9	42	43	60	83
F_3	9					
F_5	42*	−				
	43	−	+			
F_7	60	−	+	−		
F_9	83	−	−	−	−	

* Lethals 61 and 62 were derived from the same F^5 ♂; lethals 42 and 43 were produced by the same F^3 ♂.

Tests for allelism between lethals from different males

While the allelism tests described above were restricted to lethals tracing back to the same treated male, Gershenson (1965) found a high degree of allelism between

lethals from different males, indicating preferential action of the treatment on one region in the 2nd chromosome. Similar allelism tests have been carried out on all nineteen complete lethals in the present series 1. These lethals were derived from ten different males in the following distribution: one male with six lethals, four males with two lethals each and five males with one lethal each. Among the six lethals from the same male, there were two clusters, each with two lethals. Since lethals in the same cluster probably trace back to the same spermatogonium, each cluster has been counted as one. Thus, altogether there were seventeen independent lethals, yielding 136 possible cross-tests. Of these, only two crosses showed allelism. These two crosses involved three single lethals, one of which was allelic to the other two. This may be attributed to overlapping deficiencies. Gershenson (1965), in a larger sample of lethals, found many more such cases and concluded that the specifically affected region was large enough to accommodate both overlapping and non-overlapping deficiencies. The overall incidence of allelism in the present sample works out to be 1·5%, which is much higher than that observed either by Yoshikawa & Mukai (1963) among spontaneous 2nd chromosome lethals (0·7%) or by Wallace (1950) among gamma-ray induced ones (0·3%). It is, however, still smaller than that found by Gershenson; conceivably, this is due to the fact that regions of overlap between deficiencies are more easily missed in a small than in a large sample of lethals.

4. DISCUSSION

The main object of the present investigation was to test whether, as previous results had suggested, it is only the autosomes that respond to DNA. The results obtained with the aid of a dual-purpose stock showed that the increase in mutation rate in the 2nd chromosome was indeed much more pronounced than in the X-chromosome. In treated males the ratio of induced 2nd chromosome to X-chromosome lethals was about 6:1, while the corresponding figure in the controls was only 1·5:1, i.e., close to the value expected from the relative lengths of the two chromosomes. In treated females, too, the frequency of lethals in the 2nd chromosome was considerably higher than that in the X-chromosome. Yet, the X-chromosome does not seem to be completely refractory to the treatment, as has been discussed before. This conclusion is in agreement with the finding by Sobels (personal communication) that injection of *Drosophila* DNA produced deficiencies in the X-chromosome. If it is accepted that DNA has a slight but real mutagenic effect on the X-chromosome, then the absence of complete lethals in treated males must be attributed to germinal selection against induced hemizygous lethals. Mosaic lethals from treated males are no longer exposed to germinal selection because they arise in F_1 females. In earlier investigations, no mosaic lethals were scored, but mosaicism for visible mutations has been reported by various workers after treatment by feeding (Gershenson, 1940; Gershenson *et al.*, 1948) or injection (Fahmy & Fahmy, 1961, 1962; Sobels, personal communication).

The extension of the 2nd chromosome test to later generations yielded interesting evidence on the nature of the delayed mutations leading to mosaicism. As in

previous experiments with CEMS (Mathew, 1964) it was found that (1) mosaicism for a particular mutation may repeatedly occur in the same line and (2) several mosaic progeny may arise from a mosaic parent, indicating the replication of the original induced instability. In the case of CEMS, it could not be decided whether it was the same locus that repeatedly gave rise to a mutation in the same line. Location tests showed that all mosaic lethals in the same line occupied closely neighbouring loci on the X-chromosome but, as sex-linked lethals could not be cross-tested for allelism, it was not possible to test for identity of the mutated regions. In the present work, this difficulty was overcome because 2nd chromosome lethals could be cross-tested for allelism (non-complementation). When this was done for lethals in the three mosaic lines, it was found that in one of them all lethals were allelic, while in the other two the pattern of allelism was of a kind expected from overlapping deficiencies. This suggests that the primary damage had induced an instability in one particular small region which then had been transmitted over several cell generations. In lines where one mosaic gave rise to several mosaic progeny, this instability must have replicated as such.

Cross-tests between lethals from different males were carried out to test for a regional specificity of the action of DNA, such as has been found in the recent experiment by Gershenson (1964). Although allelism between separately induced lethals was found, its frequency was much lower than in Gershenson's tests on a larger sample. A regional specificity of DNA action has also been reported by Fahmy & Fahmy (1963) who found that, after injection of DNA, more than 80% of the induced Minutes were located in the proximal region of the 4th chromosome. If the 2nd chromosome, but not the X-, should contain such vulnerable regions, this would explain the much lesser response of the latter to the treatment by feeding of DNA.

As regards the nature of DNA-induced mutations, evidence from a variety of sources suggests that they are fairly large deletions. Gershenson's (1964) lethals, as mentioned before, were due to overlapping deficiencies. In the present work the same conclusion was drawn from the pattern of allelism among mosaic lethals. Moreover, it seems that the deletions connected with the rather small number of sex-linked recessive lethals were large enough to lead to their complete elimination in males by germinal selection. The generally small sector of mutated tissue in the mosaic gonad also points to the presence of lesions which are large enough to slow down rate of replication even in cells heterozygous for the induced lesion. After injection of DNA, Fahmy & Fahmy (1961, 1962) obtained Minutes that could be confirmed as cytologically observable deficiencies, while Sobels (personal communication) found that almost all sex-linked visible mutations (at specific loci) were due to deficiencies.

Regarding the mechanism of DNA action, it is difficult to imagine any specific chemical interaction between alien DNA and the chromosomal material. It is more likely that the effect of the treatment is due to some unspecific action of macromolecules on the genetic material. Evidence that this may be so comes from studies by Kaufman *et al.* (1959, 1961) in which injection not only of DNAase, but

also of RNAse and bovine plasma albumin yielded mutations in *Drosophila*. Similarly, in experiments by Fahmy & Fahmy (1963), polymethacrylic acid produced even higher frequencies of Minutes than did DNA. If the effect of the treatment depends on the structure of the molecule rather than on its chemical specificity, it becomes understandable that the results obtained by the feeding method differed between preparations of calf-thymus DNA (see Introduction). Chemical purity or the degree of polymerization may influence the effectiveness of a preparation. The nature of the introduced macromolecule, the method of treatment and the type of germ-cell treated appear to determine which chromosomal regions show specific responses. Larval feeding in Gershenson's experiment (1965) acted preferentially on the Lobe region of the 2nd chromosome. In the injection experiments by Fahmy & Fahmy (1960), about 80% of the Minutes produced by DNA, but only 30% of those produced by polymethacrylic acid, were found in the 4th chromosome. While the macromolecules used by Kaufman *et al.* (1959, 1961) produced lethals on the X-chromosome of treated males, calf-thymus DNA failed to do so in the present experiments and in those by Gershenson (1964).

It may be imagined that a macromolecule, by attaching to a chromosomal region, may produce deficiencies by interfering with replication, but it is difficult to devise an explanation for the finding that DNA produces localized instabilities which may be transmitted over several generations and may even replicate as instabilities.

SUMMARY

Calf-thymus DNA was mixed with the larval food of *Drosophila melanogaster* and complete and mosaic lethals were scored in both the X- and 2nd chromosomes. There was a decided mutagenic effect on the 2nd chromosome in both sexes. No complete sex-linked lethals were produced in males, but the production of a moderate number of complete lethals in females and of mosaic lethals in both sexes suggests that, in the absence of germinal selection, the X-chromosome is not completely refractory to the treatment.

The extension of the 2nd chromosome test to later generations showed that mosaics often gave rise to further mosaic progeny. Tests for allelism showed that lethals in the same mosaic line formed series of overlapping deficiencies, indicating that the primary effect in these cases was a transmitted instability of a narrow chromosomal region. Cases in which several mosaics arose out of one mosaic parent show that this instability must have replicated as instability.

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