CHEMICALLY INDUCED SOMATIC AND GONADAL MOSAICISM IN DROSOPHILA. I. SEX-LINKED LETHALS

E. A. CARLSON AND J. L. SOUTHIN

Department of Zoology, University of California, Los Angeles, California

Received January 9, 1963

THE sex-linked lethal test provides an objective, quantitative approach to mutagenesis in *Drosophila*. For radiation mutagenesis the analysis is usually limited to a single generation, the F_2 progeny of irradiated P_1 males or females. This procedure was established by MULLER (1928), who demonstrated that the bulk of induced mutations were present in these F_2 cultures. The sampling of single females from each F_2 culture revealed no important "sorting out" of altered components of the originally treated paternal X chromosomes in the F_3 generation. Similarly, the frequency of sex-linked lethals in the F_4 generation was shown to be insignificant in comparison with that obtained in the F_2 generation. In MULLER's experiments the sampling of one female from each culture for each generation enabled him to calculate the total mutation frequency with maximum accuracy. This method of sampling, however, does not provide a measure of the distribution of mutant and nonmutant tissue in the gonads of each generation tested. For chemical mutagenesis, furthermore, the distribution of sex-linked lethal mutations to the gonads is probably different from that found for radiation studies, where the mechanism of mutagenesis produces chiefly complete mutations. AUERBACH (1945) demonstrated a high degree of mosaicism for visible mutations with chemical mutagens. Following this, investigations comparing the ratio of lethal to visible mutations revealed a striking difference for various chemical mutagens suggesting a differential mutagenic effect on the classes of mutations themselves (FAHMY and FAHMY 1955). This was reinterpreted by CARLSON and OSTER (1961) on the basis of their studies with the monofunctional alkylating agent, ICR-100 (the compound is identified by the institutional numbering system of H. J. CREECH at the Institute for Cancer Research, Philadelphia, Pennsylvania). CARLSON and OSTER predicted that the same mechanism of mosaicism found by AUERBACH would lead to an *under-estimation* of F_2 lethals (where the absence of the treated paternal X chromosome is the basis of the lethal classification). Since mosaic sex-linked lethals would be represented by F_1 female gonads containing both mutant and nonmutant paternal X chromosomes, these would be misclassified as nonlethals or semilethals in the F_2 generation. This was directly demonstrated by CARLSON and OSTER (1962) using ICR-100. Also, BROWNING and ALTENBURG (1961), using several chemical mutagens, demonstrated that the ratio of sex-linked lethal mutations to specific visible mutations was dependent on their capacity for inducing mosaicism. It is the purpose of this paper

to demonstrate a sampling procedure which measures the distribution of mosaicism in the progeny derived from treated paternal X chromosomes and the effect of this mosaicism on the ratio of $F_2:F_3$ sex-linked lethal frequencies by brood.

METHODS

Oregon-R wild-type males were injected with an 0.1 percent ICR-100 solution in 0.7 percent saline. Details of the injection technique and the chemical structure of ICR-100 may be obtained in CARLSON and OSTER (1962). These injected males were individually brooded with virgin "*Basc*" (*sc⁸¹ B In49 w^a sc⁸*) females for two- or three-day intervals. A ratio of one injected male to two virgin *Basc* females was used in the P_1 generation. In each experimental series, approximately 50 P_1 males were injected. The F_2 progeny were isolated after 14 days incubation at 28°C. Approximately 25 females were isolated from each P_1 vial and these females were placed, individually, with two of their *Basc* brothers, in vials. From this F_1 mating, the F_2 sex-linked lethal test was scored, employing the *absence* of any (+) males and a minimum of seven *Basc* males as the definition of an F_2 lethal culture.

To set up an F_3 sex-linked lethal test, two approaches were used. In one experiment the sampling involved the mating of *one* F_2 heterozygous Bar female and two of her *Basc* brothers from each of the nonlethal cultures (those containing one or more (+) males among the F_2 progeny). In the second experiment, ten heterozygous Bar females were used from each F_2 vial. These were individually mated to one *Basc* brother and the bundled set of ten vials constituted a sampling of one F_2 line or one F_1 gonad.

Six broods were analyzed in the experiment using a sampling of ten females per F_2 "nonlethal" culture. However, the P_1 males for this experiment were first used in a separate experiment to inseminate *ed dp cl* virgin females for the first

TABLE 1
F₂ and F₃ mutation frequency by brood using sample size of one

Brood (in days after injection)	F_2 lethals	Total F_2 flies	F_2 lethal* frequency (percent)	F_3 lethals	Total† F_3 lines	F_3 lethal frequency (percent)	Total lethal frequency (percent)	Index of $F_2:F_3$ lethal frequency
	A	B	$C = \frac{A - (0.25\% B)}{B}$	D	E	$F = \frac{D - (0.25\% E)}{E}$	$G = C + F$	$H = C/F$
1	88	689	12.4	26	669	3.6	16.0	3.4
3	59	449	12.9	12	430	2.6	15.5	4.9
5	36	399	8.8	7	356	1.7	10.5	5.2
9	8	176	4.6	4	166	2.3	6.9	2.0
12	20	130	15.4	0	96	0	15.4	(>15.4)‡
Total	211	1,843	11.2	49	1,697	2.7	13.9	4.2

* Corrected for spontaneous lethals estimated at 0.25 percent.

† Calculated by adding the number of F_2 lethals corresponding to the surviving lines in the F_3 test. Each surviving nonlethal F_2 culture was sampled for one heterozygous Bar female. Each sample of one female constitutes one line.

‡ Actually infinity, but we are assuming at least one F_3 lethal to permit a comparison.

three days (see CARLSON and OSTER 1962, for work on visible mutagenesis at the dumpy (*dp*) and clot (*cl*) loci). The use of ten females per F_2 culture required about 20,000 sex-linked lethal tests in the F_3 . However, with an F_2 sampling of only one female, the total vials assayed in the F_3 generation was about 1,500. There were four broods set up in this smaller experiment. As in the larger experiment, these covered premeiotic and postmeiotic samples of sperm.

RESULTS

The major results of these two experiments are summarized in Tables 1-7. In Table 1 the mutation frequency for F_2 and F_3 sex-linked lethals is distributed over four broods. Note that the sampling of *one* F_2 "nonlethal" female for each F_3 test provides a measure of the *frequency* of mutation. It does not provide information on the degree of mosaicism in the F_2 "nonlethal" females sampled. In contrast, Table 2 presents an F_3 sex-linked lethal analysis which provides *both*

TABLE 2
F₂ and F₃ mutation frequency by brood using sample size of ten

Brood (in days after injection)	A Number of F_2 complete lethals	B Total F_2	C* Number of induced F_2 lethals	D F_2 induced lethal frequency (percent)	E Number of F_2 nonlethal cultures assayed	F Number of F_2 mosaic lethals in E	G† Number of F_2 lethals for E $G = \frac{(A)(E)}{B}$
				$D = C/B$			
4	97	821	95	11.6	563	91	66
6	69	688	67	9.7	346	57	35
9	38	580	37	6.4	257	18	17
11	68	371	67	18.1	208	11	38
14	69	462	68	14.7	311	7	46
18	45	493	44	8.9	443	12	40
Total	386	3,415	377	11.0	2,128	196	241

TABLE 2—Continued

Brood (in days after injection)	H Corrected total F_2 assayed	I Percent F_2 cultures with mosaic lethals	J Number of F_3 vials in E	K Corrected number of F_3 vials of F_3 lethals $K = \frac{(J)(H)}{E}$	L Number of F_3 lethal vials in J	M Number of F_3 induced lethals in J $M = L - (0.25\% J)$	N F_3 induced lethal frequency (percent) $N = M/K$
	$H = G + E$	$I = F/H$					
4	629	14.5	5,490	6,133	365	351	5.72
6	381	15.0	3,350	3,689	298	290	7.86
9	274	6.6	2,496	2,661	47	41	1.54
11	246	4.5	2,026	2,396	52	47	1.96
14	357	2.0	3,062	3,515	26	18	0.51
18	483	2.5	4,307	4,696	44	33	0.70
Total	2,369	8.3	20,731	23,079	832	780	3.38

* In Columns C and M, spontaneous lethals are subtracted using 0.25 percent as the spontaneous lethal frequency.

† In Column G an estimate is made of the number of F_2 complete lethals which would have occurred in the population listed in Column E.

the incidence of F_1 gonadal mosaicism among the F_2 nonlethals (8.3 percent for all broods) and the frequency of sex-linked lethals in the F_3 generation (3.4 percent for all broods). The method for calculating the frequency of mutation is presented in Table 2. A second method for calculating this frequency, using data from Tables 3, 4 and 5 is analyzed in the discussion. Table 6 provides a calculation of the fraction of gonad containing the lethal in each brood. Table 7 summarizes the major findings in these experiments and expresses the relation between F_2 and F_3 mutation frequencies and F_1 gonadal-complete-female: F_1 gonadal-mosaic-female frequencies.

TABLE 3

Distribution of nonlethal F_3 vials from F_2 nonlethal sets

Brood (in days after injection)	Size of nonlethal F_2 sets among sets sampled					Total sets of nonlethals	Total of nonlethal vials
	10	9	8	7	6		
4	405	47	11	6	3	472	4,603
6	246	29	10	2	2	289	2,815
9	213	17	6	1	2	239	2,338
11	170	18	7	1	1	197	1,925
14	275	21	5	2	1	304	2,993
18	373	40	8	2	8	431	4,168
Total	1,682	172	47	14	17	1,932	18,842

TABLE 4

Distribution of F_3 lethal vials among sampling sets of ten drawn from F_2 "nonlethal" cultures

Fertile vials per set	Number of F_3 lethals per F_2 set											Lethal sets
	0	1	2	3	4	5	6	7	8	9	10	
10	1,682	38	10	12	7	4	4	7	6	3	14	105
9	172	10	1	4	1	0	4	2	4	6	..	32
8	47	0	1	2	1	0	1	0	6	..	..	11
7	14	2	0	0	0	0	1	2	..	..	..	5
Total	1,915	50	12	18	9	4	10	11	16	9	14	153

TABLE 5

Lethal frequency of mosaic F_1 gonads sampled

Size of set	Vials with lethals											Total
	0	1	2	3	4	5	6	7	8	9	10	
10	0	3.8	2.0	3.6	2.8	2.0	2.4	4.9	4.8	2.7	14.0	43.0
9	0	1.11	0.22	1.33	0.44	0	2.66	1.55	3.55	6.0	...	16.89
8	0	0	0.25	0.75	0.5	0	0.75	0	6.0	..	...	8.25
7	0	0.29	0	0	0	0	0.85	2.0	..	..	...	3.14
Total	0	5.20	2.47	5.68	3.74	2.0	6.66	8.45	14.35	8.7	14.0	71.28

TABLE 6

Distribution by brood of mutant and nonmutant tissue in mosaically arising sex-linked lethals

Brood (in days after injection)	Number of sets detected	Lethal vials in A	Nonlethal vials in A	Semilethal vials in A	Doubtful* vials in A	Total vials in A	Fraction off gonad with induced mutant $G = \frac{B - (0.25\% F)}{F}$ %
	A	B	C	D	E	F	
4	91	365	476	38	8	889	39.5
6	57	298	226	11	29	564	51.4
9	18	47	98	13	0	158	25.9
11	11	52	43	6	2	103	45.6
14	7	26	41	2	1	70	26.1
18	12	44	82	1	2	129	25.6
Total	196	832	966	71	42	1,911	40.8

* Not retested and not containing enough progeny for classification.

† Correction for spontaneous lethals at 0.25 percent.

TABLE 7

The relation between mutant F_1 gonads and F_2 and F_3 mutation frequencies

Brood (in days after injection)	F_1 induced gonadal complete mutations (percent) A	F_2 induced mutation frequency (percent) B	F_1 induced gonadal mosaic mutations (percent) C	F_3 induced mutation frequency (percent) D	Total F_1 gonads carrying induced mutations (percent) E = A + C	Total $F_2 + F_3$ mutation frequency (percent) F = B + D	Ratio of F_1 gonadal completes to F_1 gonadal mosaics G = A/C	Ratio of F_2 to F_3 sex-linked lethal frequencies H = B/D
4	11.6	11.6	14.5	5.72	26.1	17.3	0.80	2.03
6	9.7	9.7	15.0	7.86	24.7	17.6	0.65	1.23
9	6.4	6.4	6.6	1.54	13.0	7.9	0.97	4.16
11	18.1	18.1	4.5	1.96	22.6	20.1	4.02	9.23
14	14.7	14.7	2.0	0.51	16.7	15.2	7.35	28.82
18	8.9	8.9	2.5	0.70	11.4	9.6	3.56	12.71
Total	11.0	11.0	8.3	3.38	19.3	14.4	1.33	3.25

DISCUSSION

The most significant observation that can be made from a comparison of Tables 1 and 2, is the existence of F_3 mosaic lethals in both experiments. However, the sampling technique using *ten* females shows the number of F_1 females which carried the mutant mosaically in their gonads. Note, in Table 2, that the frequency of such gonadal mosaic F_1 females is higher than the frequency of mutation which can be detected by an analysis of the F_3 tests. The F_3 mutation frequency may be determined directly by summing all the lethal vials for each brood, subtracting the expected spontaneous lethal frequency, and dividing by the total number of F_3 vials for each brood. This gives a value of 3.4 percent for all broods (see Table 2). This can be independently calculated by converting each set of vials in Table 4 into the fraction of lethal frequency corresponding to its degree of mosaicism (e.g., 0.1 mutants for sets with one lethal and nine non-

lethals, etc.). The actual portion of F_1 gonad which is mutant for all such 153 sets is $71.3/153 = 46.6$ percent. Since 196 such F_1 mosaic gonads were detected in F_3 tests, the number of gonadal-complete lethal equivalents is $46.6 \text{ percent} \times 196 = 91.3$. Altogether, 2,369 sets of vials were assayed. Thus, $91.3/2,369 = 3.9$ percent is the maximum frequency of mutation derived from the data without corrections for spontaneous lethals. For determining the spontaneous lethal frequency in Table 4, we can assume that usually a spontaneous mutation will occur singly. This is reflected in the high number of mosaics with only one lethal per set (50/153 in Table 4). If we take the mean number of lethals per set in the total, excluding the $1/n$ class, the value is $103/9 = 11.4$. Thus, $50 - 11.4 = 38.6$ or approximately 75 percent of the $1/n$ mosaic lethals were spontaneous and only 25 percent were induced. The correction, in Table 5, becomes $67.4/153 = 44.0$ percent and the number of gonadal complete lethal equivalents is $44.0 \text{ percent} \times 196 = 86.2$. From this, the mutation frequency becomes $86.2/2,369$ or 3.6 percent. This value is close to the directly determined value of 3.4 percent calculated in Table 2. Also, the spontaneous mutation frequency calculated from Table 5 is 75 percent of 5.20 or 3.9. Hence, $3.9/153 = 2.5$ percent, which now represents the amount of spontaneously occurring lethals in Table 4. From this we can estimate $2.5 \text{ percent} \times 196 = 4.9$ complete lethal equivalents spontaneously occurring among 2,369 F_1 gonads sampled; the spontaneous frequency then becomes $4.9/2,369 = 0.21$ percent.

An independent calculation supports this last figure. The spontaneous sex-linked lethal frequency for the Oregon-R stock used in these experiments resides between 0.1 to 0.3 percent (W. A. ANDERSON and E. A. CARLSON, unpublished; I. I. OSTER, personal communication). There were approximately 20,000 F_3 tests made, hence from 20 to 60 spontaneous mutations should have occurred, with most of them being located in the $1/n$ class of Table 4.

The sampling of *all* instead of ten F_2 heterozygous Bar females from each vial, in addition to presenting formidable numbers of F_3 tests, would present problems in calculating the induced frequency of lethals from the total number of mutations scored. The larger the sample size, the greater the fraction of such sets which would contain the $1/n$ (primarily spontaneous) category. It is true, nevertheless, that our sampling size *misses* those gonads, if any, containing less than ten percent of mutant paternal X-chromosomes. This problem will be resolved when sufficient data on the distribution of mutant and nonmutant progeny from individuals with gonadal mosaicism for a visible mutant (e.g., dumpy) have been accumulated.

Although the class n/n where $n = 10$ does not show a particularly excessive frequency among the other categories, the sum of classes n/n does show an excess of almost twice that which would exist for the nonexceptional categories. This may be due to lethals which were misclassified in the F_2 and recognized in the F_3 . It is also possible that some of this excess represents *semilethals* which were scored as nonlethals in the F_2 and which were perpetuated in the F_3 .

The results in Table 2, based on large numbers of F_2 and F_3 tests indicate a higher frequency of F_2 lethals and a lower frequency of F_3 lethals than that

reported by CARLSON and OSTER (1961) and BROWNING and ALTENBURG (1962). These earlier reports were based on smaller F_2 or F_3 samples. CARLSON and OSTER (1962) reported 11 F_2 lethals in 295 F_1 females (3.7 percent) and 16 F_3 lethals in 117 lines (13.7 percent) using a sampling of ten females per line as in our present experiments. BROWNING and ALTENBURG (1962) reported 115 F_2 lethals in 3,037 F_1 females (3.8 percent) and five F_3 lethals among 30 F_1 females (16 percent) tested for gonadal mosaicism.

It should be pointed out that BROWNING and ALTENBURG (1962) used those F_2 cultures in which there was a decided excess of (*Basc*):(+) males for retesting for the F_3 generation. This method would largely avoid the $1/n$ class (i.e., mainly spontaneous) which we have found in excess in our sampling of ten heterozygous Bar females from each nonlethal culture. It would also cut down on the incidence of $2/n$, $3/n$ and some $4/n$ classes because of the diminished likelihood that the ratio of (*Basc*):(+) males in such F_2 cultures would be significantly altered. This method also creates a problem in calculating the F_3 sex-linked lethal frequency. If the lethals obtained are divided by the sample selected for analysis (i.e., those cultures where the number of (*Basc*) males exceeds the (+) males) the frequency will be too high. If they are divided by the total number of F_2 cultures the frequency will be too low.

Apparent differences between sampling methods in the two experiments. The mutation frequency in F_3 tests of samples of one F_2 female per "nonlethal" culture is 2.7 percent. This is somewhat smaller than the F_3 mutation frequency (3.4 percent) determined directly for the experiment using a sample size of ten. There are several possible sources of error which might explain this failure in agreement. First, technical errors in scoring a large scale experiment are more apt to occur, with any F_2 lethals escaping detection being found in the F_3 tests as groups of all lethals in the n/n sets. This is certainly suggestive in the data of Table 4, where 28 sets of n/n are all lethals, a value two to three times that found for all other sets except the $1/n$ class which would be expected to carry the bulk of the spontaneous lethals which occurred. A second source of error comes from the mating of the 2,128 sets of F_2 females out of some 3,415 F_2 cultures which were available for analysis. The choice, to some extent, may have been non-random, with more samplings of ten taken from those sets of 25 F_1 females in which one or more F_2 lethals had occurred than in those F_1 sets where no lethals had occurred among the 25 F_2 cultures stemming from the treated P_1 male. Such a bias in selection would have no effect on the mutation frequency if all P_1 males received the *same* quantity of mutagen and reacted in the same way to the mutagen (e.g., if all the P_1 males were physiologically comparable and of similar mass). If this were not the case, then selecting those lines in which lethals had already occurred would tend to increase the F_3 mutation frequency. This bias could conceivably affect a considerable portion of all F_2 culture samplings since the 386 lethal cultures out of 3,415 tests when distributed among the sets of about 25 F_1 females derived from the initial P_1 injected males indicates that there were 119 sets with one or more lethals and 40 sets without an F_2 lethal (see Table 8). No bias would have arisen if 34,000 F_3 tests had been drawn from the 3,415 F_2

TABLE 8

Evidence for clustering among F_2 lethals

Brood (in days after injection)	Nonlethal sets of 25 F_1 females A	Lethal bearing sets of 25 F_1 females B	Total sets of 25 F_1 females A+B	Total number of lethals found C	Mean number of lethals per column B C/B
4	8	32	40	99	3.1
6	8	25	33	67	2.7
9	5	21	26	38	1.8
11	4	14	18	68	4.8
14	4	17	21	69	4.0
18	11	10	21	45	4.5
Total	40	119	159	386	3.2

cultures, but we had chosen a figure of 20,000 F_3 lethal tests as the largest scale manageable with our laboratory facilities.

It should also be borne in mind that there were far fewer F_3 tests in the small scale experiment and, thus, the range for the value 2.7 percent actually resides between 1.8 percent and 3.4 percent, and the difference between the two experiments, consequently, is not appreciably large. Because of these possible sources of heterogeneity in handling the two experiments, we do not see any contradiction in the two sampling methods used for determination of the F_3 mutation frequency.

Significance of the F_2 and F_3 results. In the large scale experiment the fluctuations in the F_2 and F_3 mutation frequencies by brood are considerable. These reflect, we believe, the activities of the chromatids in the stages of spermatogenesis in the injected males. If the sperm are mature or postmeiotic, the agent may cause the alteration of one strand of the DNA in the sperm X-chromosomes. If the gonad receives *both* types of paternal X chromosomes the F_1 female will not be scored as a lethal in the F_2 generation, but samples of her daughters may contain the mutant paternal X and consequently the F_3 test will show that the P_1 sperm had been mosaically altered. Such an F_1 female would be defined as a gonadal mosaic. However, if her polar cap received only mutant paternal X chromosomes, she would have been scored as an F_2 "complete" lethal (even though the mutant origin may have been mosaic in the zygote). Such females are called gonadal completes. An extensive analysis of visible mutations at the dumpy locus with ICR-100 (J. L. SOUTHIN and E. A. CARLSON, unpublished) has shown just such somatically mosaic individuals with gonads containing only the mutant bearing paternal chromosome. Such somatic mosaic but gonadal complete individuals should also exist for these F_2 lethal tests, but we cannot determine from our data what percentage of the F_2 lethals are mosaic in origin but have their gonads completely mutant. Also, a considerable number of somatic mosaics for induced sex-linked lethals probably occur but are not transmitted to the F_2 or F_3 progeny because the polar cap receives only the nonmutant paternal X chromosomes. Judging from studies of mutant transmissibility at the

dumpy locus, about five times as many mature sperm carried a mutational event with ICR-100 treatment than could be detected in the experiment by analysis of the gonad (see CARLSON and OSTER 1962 for a full discussion of transmissibility at the dumpy locus). With about 25 percent of P_1 postmeiotic sperm showing transmissibility in the large experiment (Table 7), we can estimate that almost every paternal X chromosome received one or more mosaically induced lethals. This is consistent with the high frequency of ICR-100 mutagenesis at the dumpy locus (0.88 percent phenotypic and 0.22 percent transmitted) and the fairly randomly spread distribution of ICR-100 induced sex-linked lethals (CARLSON 1963).

In the meiotic sperm, which gives a lower total mutation frequency, we believe two mechanisms are operating. First, there may be less ICR-100 which penetrates in mutagenic form and, secondly, the mosaic mutations which are induced are probably sorted out by reductional and equational division into one of the four spermatozoa which arise from the replicating primary spermatocytes.

If the premeiotic sperm are affected, especially spermatogonia, the alteration of one DNA strand in the duplex will eventually affect a portion of the descendant spermatozoa resulting in a cluster of F_2 lethals. Such clusters were found in the late broods (see Table 8) where the number of F_2 lethals per set of 25 F_1 females sampled from each treated P_1 male is greater in the premeiotic sperm than the postmeiotic sperm.

The effect of mosaic induction and meiotic distribution would be, first, a greater frequency of F_1 mosaic gonads in postmeiotic sperm than in premeiotic sperm. Secondly, we should expect a greater frequency of gonadal complete mutations among the descendants of premeiotic sperm than among the postmeiotic sperm (especially if we could, as in the visible studies, distinguish how many of the gonadal complete mutations are also somatic complete mutations). The two distributions by brood may be expressed by a ratio of the F_1 gonadal complete females to the F_1 gonadal mosaic females or even more useful, the ratio of the F_2 lethal frequency to the F_3 lethal frequency. In both cases we find a consistent agreement with our interpretation (see Table 7).

There are several further features which need to be explored. Is the meiotic stage (9–10 day sperm) less sensitive to ICR-100 than either the premeiotic or postmeiotic sperm (see Table 2 for total mutation frequency by brood)? Can visible mutagenesis (e.g., of dumpy) be used to predict the F_2 and F_3 sex-linked lethal frequencies or the F_1 gonadal complete to F_1 gonadal mosaic frequency? A theory, by CARLSON, espousing this prediction has been submitted elsewhere for publication.

The amount of gonadal tissue affected. From our estimates of Tables 4 and 5, we conclude, after corrections for spontaneous mutation, that the F_1 mosaic gonad (averaged for all broods) consists of 44 percent mutant gametes and 56 percent nonmutant gametes. A more direct analysis, by brood, is given in Table 6, where the average gonad is shown to consist of 41 percent mutant gametes with variations from 26 percent to 51 percent.

It is our belief that this high proportion of mutant tissue in the gonad does not

reflect the corresponding ratio of mutant to nonmutant tissue in the soma of these individuals. From our preliminary visible studies and those reported by CARLSON and OSTER (1962) we know that the mutant tissue is nonrandomly distributed (e.g., usually along a plane of bilateral symmetry). Secondly, we know that most of our phenotypic (somatic) mosaics are gonadal nonmutants and hence do not transmit. This suggests that the gonad is produced by a sample of a very few cells either at the time of pole cell migration or at the time of the germ cell formation. Finally, we have observed in ICR-100 tests that about one third of those somatic mosaic dumpy mutations which do transmit, transmit as gonadal complete mutations. All of these observations are consistent with the view that the polar cap is usually formed by migration of a very small number of nuclei from the embryonic pool of nuclei. Since this somatic mutant tissue is nonrandomly organized, we consider it likely that the posterior nuclei migrating into the polar cap bring in a nonrandom sample of nuclei from the embryo. The polar cap, if it eventually forms mutant germinal tissue, will usually introduce between 25 percent and 100 percent mutant pole cells into the gonad. A consequence of this, however, is a low transmissibility in mosaic mutant embryos when the nuclei are nonrandomly arranged and nonrandomly migrating into the polar cap. In contrast, a random selection of nuclei randomly arranged in an embryo, would give a high transmissibility (see Table 9).

If, in Table 9, we assume the conditions of random distribution and migration of nuclei in the embryo and polar cap, then with only two nuclei entering the polar cap, we would expect, for a transmissibility of 20–25 percent, an F_2 to F_3 mutation frequency in a ratio of 1:14. This is not found for the sex-linked lethal experiments discussed here, and it is considerably lower than what is found for ICR-100 induced transmissible dumpy phenotypic mosaics. Similarly, if we utilize the information that the ratio of F_1 gonadal complete to F_1 gonadal mosaic dumpy mutants is 1:2, then we should expect the total transmissibility to be 75 percent. Instead, we find 22 percent. Hence, we can rule out a randomly selected basis for our results.

In Table 10, however, we can assume a model in which the mutant tissue forms an aggregate which remains together. We can also assume that adjacent nuclei enter into polar cap formation. Using our data from sex-linked lethals, the average mosaic gonad which transmits will be 25 percent to 50 percent mutant (presumably arising from two to four cells in the polar cap) regardless of the stage at which the induced mutation becomes fixed in the early cleavage nuclei and, correspondingly, regardless of how small a fraction of the embryonic pool of nuclei contains the aggregate of mutant tissue. In this model, the transmissibility diminishes rapidly with the number of replications required to fix a mutant. For a mutagen with a $\frac{1}{4}$ mosaic distribution in the embryonic pool (resulting from one replication to fix the mutated or sensitized strand of DNA) we would satisfy all conditions of our data—a gonad of almost 50 percent mutant tissue (41 percent obtained in our data), a ratio of one gonadal complete mutant to two gonadal mosaic mutants from breeding tests of transmissible dumpy

TABLE 9
The relation of randomly arranged somatic and gonadal mosaicism to germ cell formation and transmissibility

Distribution of embryonic nuclei		Distribution of two nuclei to polar cap			Expected mutation frequency $G=C+(D/2)$
Mutant A	Nonmutant B	Complete mutant C	Mosaic mutant D	Nonmutant E	
1/2 = 0.50	1/2 = 0.50	1/4 = 0.25	2/4 = 0.50	1/4 = 0.25	1/2 = 0.50
1/4 = 0.25	3/4 = 0.75	1/16 = 0.06	6/16 = 0.38	9/16 = 0.56	4/16 = 0.25
1/8 = 0.13	7/8 = 0.87	1/64 = 0.016	14/64 = 0.22	47/64 = 0.77	8/64 = 0.13
1/16 = 0.06	15/16 = 0.94	1/256 = 0.004	30/256 = 0.12	225/256 = 0.88	16/256 = 0.06

TABLE 10
The relation of nonrandomly arranged somatic and gonadal mosaicism to germ cell formation and transmissibility

Distribution of embryonic nuclei		Mutation frequency Assuming all gonadal complete		Transmissibility Assuming all gonadal complete		Ratio of gonadal complete to gonadal mosaic	
Mutant	Nonmutant	C	D	E	F	G*	H†
A	B	C=A	D=A/2	E=A	F=A	(empirical)	H†
1/2(0.50)	1/2(0.50)	0.50	0.25	0.50	0.50	1:1	0.38
1/4(0.25)	3/4(0.75)	0.25	0.13	0.25	0.25	1:2	0.17
1/8(0.13)	7/8(0.87)	0.13	0.06	0.13	0.13	..	...
1/16(0.06)	15/16(0.94)	0.06	0.03	0.06	0.06	..	...

* 1:1 estimate based on X-ray data cited in Carlson and Osier 1962. 1:2 estimate based on ICR-100 data cited in Carlson and Osier 1962. No estimate available for 1/8 and 1/16 mosaics.
† Calculated by assigning 1.0 for each complete mutation and 0.5 for each mosaic mutation.
For 1:1 = $\frac{0.25}{0.125} + \frac{0.125}{0.125} = 0.38$.
For 1:2 = $\frac{0.083}{0.083} + \frac{(1/2 \times A/2)}{(1/3 \times A) + (2/3 \times A/2)} = 0.17$.

somatic mosaics, and a transmissibility of about 20 percent (25 percent predicted in the model).

A full, quantitative, treatment of this problem can best be made from studies with specific visibles (e.g., dumpy). Our results with the sex-linked lethals merely reveal the nonrandom selection of nuclei for the formation of the polar cap and, eventually, the germinal tissue in the gonad. Such a study would also require an extensive analysis of the effect of the early embryology of the gonad on the ultimate content of mutant gametes in the germinal tissue. These complications include (1) an uncertain number of contiguous nuclei at the posterior end of the embryo, ranging from two to eleven nuclei, but more usually three to seven nuclei, which are partitioned off to form a polar cap; (2) the exact time of formation of the cap which is believed to occur about the ninth synchronous nuclear division (512 nuclei present in the embryo); (3) the likelihood that some of the pole cells may migrate back to the embryo playing no further germinal role; (4) the degree of randomization of the remaining replicating pole cells, about 40 in number, which at the time of gonad formation move into the embryonic interior; (5) the uncertainty on the random or nonrandom migration of about five to 12 pole cells which enter the primordial ovary (which is smaller than the primordial testis). These generalizations of gonadal embryogenesis are given in more detail by SONNENBLICK (1950).

SUMMARY

Studies in ICR-100 mutagenesis using sex-linked lethal tests have revealed a high proportion of mosaically induced lethals. The mosaic gonads contain, on the average, about 41 percent mutant gametes. Postmeiotic sperm treated with ICR-100 produce more mosaic mutations than do premeiotic sperm. Also, postmeiotic sperm produce fewer gonadal complete mutations than do premeiotic sperm. These findings are interpreted as the sorting out of mutant and nonmutant genetic strands during mitosis (for spermatogonia) and early meiosis (for primary spermatocytes replicating at the time of treatment), with the final descendant spermatozoa containing all mutant or all nonmutant strands. The mosaically arising mutational events in premeiotic cells are thereby recorded as complete gonadal mutations. Postmeiotic sperm must await fertilization for distribution by mitosis of the mutant and nonmutant strands.

The calculation of the mutation frequency differs from the calculation of the number of gonads containing mutant and nonmutant gametes. The comparison of sex-linked lethal mosaicism and specific visible mosaicism indicates that the mosaicism of the gonads does not correspond to the degree of mosaicism in the embryonic pool of nuclei prior to polar cap formation. A model is offered which partially predicts the transmissibility of induced mutations, the expected mutation frequency, and the ratio of gonadal complete mutations to gonadal mosaic mutations.

ACKNOWLEDGMENTS

We are grateful to MR. ROY RAMSEY for his assistance in phases of these experiments and to MRS. EVELYN HAWKINS for her sacrifice of time and energy to meet the media requirements for the F_3 tests. We are grateful to PROF. H. J. MULLER for his penetrating and constructive criticisms of our manuscript. This investigation was supported by grants G 15569 and G 22924 from the National Science Foundation.

LITERATURE CITED

- AUERBACH, C., 1945 Chemically induced mosaicism in *Drosophila melanogaster*. Proc. Roy. Soc. Edinburgh B **62**: 211-222.
- BROWNING, L. S., and E. ALTENBURG, 1961 Gonadal mosaicism as a factor in determining the ratio of visible to lethal mutations in *Drosophila*. Genetics **46**: 1,317-1,321.
- 1962 The relatively high rate of mosaicism among the mutations induced in the X chromosome of *Drosophila* by monofunctional quinacrine mustard. Genetics **47**: 945.
- CARLSON, E. A., 1963 Distribution of sex-linked lethals induced by ICR-100 in spermatozoa of *D. melanogaster*. *Drosophila Inform. Serv.* **37**: 66-68.
- CARLSON, E. A., and I. I. OSTER, 1961 Chemical-mutagen induced mosaicism at the dumpy locus. (Abstr.) Genetics **46**: 856-857.
- 1962 Comparative mutagenesis of the dumpy locus in *Drosophila melanogaster*. II. Mutational mosaicism induced without apparent breakage by a monofunctional alkylating agent. Genetics **47**: 561-576.
- FAHMY, O. G., and M. J. FAHMY, 1955 Cytogenetic analysis of the action of carcinogens and tumor inhibitors in *Drosophila melanogaster*. V. Differential genetic response to the alkylating mutagens and X-irradiation. J. Genet. **54**: 146-154.
- MULLER, H. J., 1928 The problem of genic modification. Verh. V Intern. Kongr. Vererbungsw. 234-260. Z. Ind. Abst. Vererb., Suppl. I.
- SONNENBLICK, B. P., 1950 The early embryology of *Drosophila Melanogaster*. Pp. 62-167. *Biology of Drosophila*. Edited by M. DEMEREC. Wiley and Sons. New York.