EVIDENCE FAVORING A FRAME SHIFT MECHANISM FOR ICR-170 INDUCED MUTATIONS IN *DROSOPHILA MELANOGASTER*

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MONOFUNCTIONAL quinacrine mustard, 2-methoxy-6-chloro-9-(3-[ethyl-2-chloroethyl]aminopropylamino) acridine dihydrochloride, ICR-170*, consists of two portions: an acridine ring and an alkylating mustard. The complex chemical structure of ICR-170 suggests that mutations could be produced by several different mechanisms. If the nitrogen mustard component of the molecule is the effective part of the molecule, ICR-170 should act simply by radio-mimetic alkylation. Alkylating mustards have long been known to be mutagenic in *Drosophila* (AUERBACH 1945). It is also possible that the quinacrine ring is the mutagenic component and that ICR-170 acts as an acridine. The work of ORGEL and BRENNER (1961) has shown that many acridines are mutagenic in phage T4. A third possibility for ICR-170 would be a complex one, in which both components are directly involved in the mutational process.

Studies of phage T4 have supported the theory of BRENNER, BARNETT, CRICK and ORGEL (1961) which argued that acridine type mutants were due to the addition or deletion of one or more base pairs. Such mutations would be caused by shifts of the reading frame in the DNA (CRICK, BARNETT, BRENNER and WATTS-TOBIN 1961; STREISINGER *et al.* 1966). These acridine type "frame shift" mutations result in changes of a long sequence of adjacent amino acids which constitute a major portion of a protein. The acridine type mutations are characterized also by the complete loss of protein function, by noncomplementation, and by reversion specificity (ORGEL and BRENNER 1961).

Alkylating agents differ from the acridines (BAUTZ and FREESE 1960); they readily induce mutations of the base substitution or "missense" type. This mutational specificity has been critically determined by KRIEG (1963) for ethyl methanesulfonate and by LOEBBECKE (1963) for nitrogen mustard. Agents which produce base substitution types of mutants readily produce temperature sensitive mutations in many different structural genes (EDGAR, DENHART and EPSTEIN 1964). EDGAR (personal communication) has shown that temperature sensitive mutants are not induced by acridines in phage T4.

In microbial systems, particularly phage T4, it is possible to distinguish readily these alternative mechanisms proposed for ICR-170. Experiments in T4 phage support the conclusion that ICR-170 is mutagenic and acts as an acridine agent (SEDEROFF 1966). AMES and WHITFIELD (1966) have used ICR-170 on *Salmo-*

* This agent was identified as ICR-100 in earlier papers from CARLSON's laboratory. Its present code name at the Institute for Cancer Research, Philadelphia, is ICR-170.

nella typhimurium and have come to similar conclusions. Our analysis of ICR-170 induced mutations supports the existence of a similar or identical mechanism of mutagenesis in *Drosophila*.

MATERIALS AND METHODS

Oregon-R wild-type males were injected with 0.1% ICR-170 in 0.7% saline. They were mated to Basc: *sc⁸¹ B In49 w^a sc⁸* virgin females and brooded with virgin females as indicated in Table 4. Individual F_1 females were inseminated by their Basc brothers and placed in separate shell vials. The F_2 progeny were examined for lethals, using a criterion of at least seven Basc males, no wild-type males, and the presence of both classes of Bar-eyed females (homozygous and heterozygous for Basc). In each alleged case, the heterozygous females were retested to verify the F_2 lethal. Stocks of F_2 postmeiotic and F_2 premeiotic lethals were kept. These were assigned numbers and maintained by selection of heterozygous Basc females and hemizygous Basc males.

For localization tests, each lethal stock was crossed to $\gamma^2 v f car$ males (γ^2 , 0.0 = yellow body; v , 33.0 = vermilion eyes; f , 56.7 = forked bristles; car , 62.5 = carnation eyes). In each test, ten F_1 females, heterozygous for the lethal and the $\gamma^2 v f car$ chromosomes, were placed in half-pint jars with ten F_1 Basc brothers. About 15 to 20 bottles were used for each localization. This yielded a mean of some 1500 male progeny per localization. Temperature conditions were maintained in a range of 24° to 28°C.

For some localizations, a second test was performed with $F_1 l/\gamma^2 v f car$ virgin females crossed to $\gamma^2 v f car$ males. The female classes among the progeny provided a basis for deciding whether certain classes missing in the males were due to gross rearrangements (e.g. inversion) or the presence of two or more lethals on the X chromosome. Tests were also made (1964) on some lethals localized in 1962 and 1963, to check the constancy of crowding and other factors which might affect the reliability of the localizations.

To test for temperature sensitive lethals, each lethal stock was transferred to fresh medium and cultured at 16°C. All of these mutations were originally induced, cultured, scored, and maintained as stocks at 24° to 28°C. The parents were discarded before eclosion and the progeny were later scored. The criterion for scoring was simply the reverse of the Basc test. If the lethal effect was suppressed, then wild-type males would emerge.

RESULTS

In Table 1, the ICR-170 induced point mutation lethals are listed in their map order with their limits and the number of F_2 males used for their map determination. For loci close to γ , v , f , or car , the usual procedure employing the standard error at a 5% confidence level was not used. The determination of the fiducial limits for small numbers of recombinants was presented by STEVENS (1942), and such calculated fiducial limits at the 5% level are also indicated in Table 1.

The apparent structural alterations are listed in Table 2. In each case the male and female F_2 progeny from the cross of $F_1 \gamma^2 v f car/l\varnothing \times \gamma^2 v f car \delta$ are indicated. The absence, or near absence, of expected classes of males is not encountered for the corresponding female data in all of the tested cases. This rules out a gross structural change as the mechanism of the peculiar distributions occurring among the F_2 males. In the case of $l(1)Q29$, there are probably two lethals, one to the left of v (near 25) and one to the right of car (near 65.0). For $l(1)Q60$, two lethals—one to the left of v (near 30) and the other between f and car (about 60), would be consistent with the distribution shown. The situation

TABLE 1

Localization of ICR-170 induced sex-linked lethals

Symbol	Brood	Locus	Limits	Males used	Symbol	Brood	Locus	Limits	Males used
<i>l(1)Q217</i>	1	— 0.0	.04 to 0.6	1587	<i>l(1)Q75</i>	1	29.3	±0.7	856
<i>l(1)Q20</i>	1	— 0.0	.04 to 0.7	1812	<i>l(1)Q33</i>	1	29.9	±0.5	1845
<i>l(1)Q5</i>	1	— 0.0	.02 to 0.3	1642	<i>l(1)Q6</i>	1	30.2	±0.4	2108
<i>l(1)Q212</i>	4	0	0 to 1.4	537	<i>l(1)Q44</i>	1	31.7	±0.3	1572
<i>l(1)Q36</i>	1	+ 0.0	0. 0 to 0.2	1416	<i>l(1)Q53</i>	1	32.0	±0.3	2042
<i>l(1)Q78</i>	2	+ 0.0	0. 0 to 0.2	1944	<i>l(1)Q240</i>	5	—33.0	32.2 to 32.95	1549
<i>l(1)Q87</i>	2	± 0.0	±0.0	1801	<i>l(1)Q54</i>	1	—33.0	32.5 to 32.95	1473
<i>l(1)Q209</i>	5	+ 0.0	.02 to 0.4	1598	<i>l(1)Q26</i>	1	+33.0	33.01 to 33.4	1982
<i>l(1)Q77</i>	1	+ 0.0	.03 to 0.4	1203	<i>l(1)Q211</i>	4	+33.0	33.1 to 34.0	1302
<i>l(1)Q221</i>	3	+ 0.0	.02 to 0.6	812	<i>l(1)Q66</i>	2	+33.0	33.1 to 33.4	1007
<i>l(1)Q220</i>	1	+ 0.0	.04 to 0.6	1485	<i>l(1)Q225</i>	1	36.0	±0.4	1611
<i>l(1)Q85</i>	2	+ 0.0	0.15 to 0.7	1686	<i>l(1)Q52</i>	1	36.9	±0.2	1542
<i>l(1)Q219</i>	1	1.3	±0.1	1410	<i>l(1)Q43</i>	1	37.6	±0.5	1499
<i>l(1)Q9</i>	1	1.4	±1.0	568	<i>l(1)Q58</i>	1	38.2	±0.4	2283
<i>l(1)Q40</i>	1	1.5	±0.3	1994	<i>l(1)Q45</i>	1	38.3	±0.4	2403
<i>l(1)Q39</i>	1	1.7	±0.4	1291	<i>l(1)Q234</i>	4	38.9	±0.7	958
<i>l(1)Q41</i>	1	2.0	±0.3	2210	<i>l(1)Q22</i>	1	39.1	±0.5	1511
<i>l(1)Q218</i>	1	2.8	±0.5	1421	<i>l(1)Q202</i>	4	40.1	±0.6	1215
<i>l(1)Q61</i>	2	6.5	±0.7	1597	<i>l(1)Q68</i>	2	40.4	±0.5	1794
<i>l(1)Q81</i>	2	6.8	±0.9	975	<i>l(1)Q2</i>	1	40.9	±0.6	1293
<i>l(1)Q216</i>	1	8.6	±0.8	1385	<i>l(1)Q64</i>	2	41.4	±0.5	1809
<i>l(1)Q27</i>	1	9.3	±0.6	2081	<i>l(1)Q12</i>	1	42.0	±0.5	2163
<i>l(1)Q215</i>	1	10.4	±1.0	822	<i>l(1)Q228</i>	2	42.2	±0.5	1809
<i>l(1)Q72</i>	1	10.6	±0.7	1967	<i>l(1)Q59</i>	2	44.5	±0.8	887
<i>l(1)Q56</i>	1	11.2	±0.7	1877	<i>l(1)Q28</i>	1	44.6	±0.6	1629
<i>l(1)Q83</i>	2	12.1	±0.8	1778	<i>l(1)Q224</i>	1	47.8	±0.7	1014
<i>l(1)Q248</i>	1	12.2	±0.7	2051	<i>l(1)Q226</i>	2	47.9	±0.6	1625
<i>l(1)Q49</i>	1	12.7	±0.7	2055	<i>l(1)Q79</i>	2	48.0	±0.6	1569
<i>l(1)Q206</i>	5	13.0	±1.2	712	<i>l(1)Q76</i>	1	49.1	±0.6	1118
<i>l(1)Q1</i>	1	13.1	±0.7	2133	<i>l(1)Q13</i>	1	49.7	±0.5	1765
<i>l(1)Q201</i>	5	13.4	±0.9	1285	<i>l(1)Q89</i>	2	51.5	±0.5	1705
<i>l(1)Q231</i>	5	13.5	±0.8	1500	<i>l(1)Q70</i>	2	51.9	±0.6	793
<i>l(1)Q233</i>	5	15.2	±0.8	1807	<i>l(1)Q4</i>	1	52.0	±0.5	2130
<i>l(1)Q11</i>	1	16.0	±0.8	1712	<i>l(1)Q16</i>	1	52.6	±0.3	3509
<i>l(1)Q232</i>	5	16.5	±2.0	281	<i>l(1)Q63a</i>	2	53.0	±0.5	1249
<i>l(1)Q57</i>	1	16.8	±0.8	1537	<i>l(1)Q73</i>	1	53.9	±0.4	1894
<i>l(1)Q208</i>	5	17.0	±1.2	744	<i>l(1)Q69</i>	2	54.1	±0.4	1162
<i>l(1)Q74</i>	1	18.2	±0.8	1929	<i>l(1)Q80</i>	2	54.2	±0.5	946
<i>l(1)Q82</i>	2	18.7	±1.3	696	<i>l(1)Q238</i>	4	54.1	±0.3	2293
<i>l(1)Q15</i>	1	19.6	±0.7	2008	<i>l(1)Q71b</i>	1	54.4	±0.4	1654
<i>l(1)Q67</i>	2	20.3	±1.1	857	<i>l(1)Q18</i>	1	56.7	56.69 to 56.71	3495
<i>l(1)Q65</i>	2	20.5	±0.8	1577	<i>l(1)Q31</i>	1	56.7	±0.0	1317
<i>l(1)Q21</i>	1	20.5	±0.8	1846	<i>l(1)Q214</i>	4	56.7	none	480
<i>l(1)Q236</i>	5	21.0	±0.7	2164	<i>l(1)Q86</i>	2	56.7	±0.0	1558
<i>l(1)Q205</i>	5	21.1	±0.9	1284	<i>l(1)Q34</i>	1	+56.7	56.8 to 57.3	1755
<i>l(1)Q48</i>	1	21.3	±0.9	1153	<i>l(1)Q23</i>	1	57.8	±0.1	3020
<i>l(1)Q237</i>	5	21.7	±0.8	1617	<i>l(1)Q222</i>	3	57.9	±0.1	1766
<i>l(1)Q204</i>	5	22.6	±0.9	1042	<i>l(1)Q24</i>	1	58.3	±0.1	2302

TABLE 1 (CONTINUED)

Localization of ICR-170 induced sex-linked lethals

Symbol	Brood	Locus	Limits	Males used	Symbol	Brood	Locus	Limits	Males used
<i>l(1)Q30</i>	1	22.7	±0.7	1794	<i>l(1)Q55</i>	1	60.6	±1.0	1145
<i>l(1)Q25</i>	1	23.0	±0.5	3211	<i>l(1)Q62</i>	2	+62.5	62.6 to 63.0	1743
<i>l(1)Q17</i>	1	23.6	±0.7	1610	<i>l(1)Q19</i>	1	+62.5	62.6 to 62.8	4068
<i>l(1)Q244</i>	2	24.2	±1.0	977	<i>l(1)Q203</i>	5	+62.5	62.6 to 63.6	985
<i>l(1)Q3</i>	1	26.5	±0.6	1801	<i>l(1)Q50</i>	1	+62.5	62.6 to 63.1	1852
<i>l(1)Q227</i>	2	27.4	±0.6	1869	<i>l(1)Q14</i>	1	64.2	±1.3	300
<i>l(1)Q235</i>	5	28.1	±0.3	3567	<i>l(1)Q42</i>	1	64.4	±0.2	1885
<i>l(1)Q10</i>	1	28.6	±0.5	1701	<i>l(1)Q210</i>	4	64.5	±0.6	2163
<i>l(1)Q7</i>	1	28.8	±0.5	1882	<i>l(1)Q51</i>	1	65.2	±0.4	2007
<i>l(1)Q223</i>	1	29.2	±0.5	1501	<i>l(1)Q8</i>	1	65.5	±2.0	330

Lethals numbered from 1 to 90 are F_2 lethals (postmeiotic and meiotic); lethals numbered from 200 to 248 are either premeiotic or F_3 postmeiotic and meiotic lethals (mosaic lethals).

Brood 1 = 0-2 days; Brood 2 = 3-5 days; Brood 3 = 6-8 days; Brood 4 = 9-11 days; Brood 5 = 12-15 days.

in *l(1)Q63* can also be interpreted by assuming one lethal at or close to *v* (near 30) and a second lethal to the left of *f* (near 50). For *l(1)Q71a* there may also be two lethals, one to the right of forked (about 59) and the other at or just to the right of *car* (near 62.5+). The lethal *l(1)Q207* appears to contain one lethal a few map units to the right of γ (about 7) and a second lethal about two thirds of the distance between *v* and *f* (near 49). For *l(1)Q239* one lethal would be very

TABLE 2

Multiple lethals induced by ICR 170

Crossover class	<i>l(1)Q29</i> ♂ ♀		<i>l(1)Q60</i> ♂ ♀		<i>l(1)Q63</i> ♂ ♀		<i>l(1)Q71a</i> ♂ ♀		<i>l(1)Q207</i> ♂ ♀		<i>l(1)Q239</i> ♂ ♀		<i>l(1)Q47*</i> ♂
$\gamma v f car$	207	178	660	237	389	324	533	198	1105	179	1256	188	826
++++	0	306	0	335	0	312	0	280	0	351	2	326	0
$\gamma + + +$	8	189	224	162	0	180	0	100	0	179	6	172	0
+ <i>v f car</i>	106	143	0	130	0	148	335	135	41	123	356	128	404
$\gamma v + +$	0	87	3	111	10	110	1	55	27	101	9	98	0
++ <i>f car</i>	2	59	0	71	0	72	310	133	0	89	1	86	267
$\gamma v f +$	2	14	0	11	27	43	0	11	56	11	1	14	0
+++ <i>car</i>	0	13	0	11	0	11	2	20	0	8	2	8	0
$\gamma + + car$	0	7	0	6	0	10	0	2	0	6	8	2	0
+ <i>v f +</i>	6	12	26	2	0	18	0	16	1	11	0	6	0
$\gamma + f car$	6	35	0	10	43	80	35	21	1	20	18	20	40
+ <i>v + +</i>	0	36	0	32	0	28	0	23	0	26	2	23	0
$\gamma v + car$	0	1	0	1	1	1	0	1	0	0	5	1	0
++ <i>f +</i>	0	2	0	0	0	10	0	3	0	0	0	2	0
$\gamma + f +$	1	2	0	0	0	3	0	0	0	0	0	0	0
+ <i>v + car</i>	0	0	0	0	0	0	0	0	0	0	0	1	0
Totals	338	1084	913	1119	469	1350	1416	998	1231	1104	1666	1075	1546

The seven apparent rearrangements are shown by this analysis to be multiple mutants. If these were rearrangements, the female classes would parallel the male classes of crossing over.

* Female data were not obtained for *l(1)Q47*.

FIGURE 1.—The localizations of the ICR-170 induced sex-linked lethals are depicted with their calculated limits. The average degree of potential allelism is 3.5; this may be calculated from the numbers of alleles indicated parenthetically next to the regions of overlapping: 0.0—(3); 0.0(2); 0.0+(6); 1.5(4); 6.8(2); 9.5(3); 10.5(3); 13.0(7); 16.0(4); 18.5(2); 21.0(7); 23.0(4); 27.0(2); 29.0(5); 30.0(2); 31.5(2); 33.0(5); 38.0(4); 40.5(3); 42.0(3); 44.5(2); 48.0(3); 49.0(2); 52.0(4); 54.0(5); 56.5(4); 58.0(2); 63.0(4); 64.0(3).

close to v (near 33.0) and a second lethal would be to the right of car (near 64). Similar interpretations may be applied to $l(1)Q27$ and $l(1)Q47$. In the case of $l(1)Q27$ lethals near 25 and near 50 would satisfy the distribution; and for $l(1)Q47$ two lethals near f (56.7) and car (62.5) could account for the absence of recombinants between these two markers. In $l(1)Q47$ the stock was lost before a retest could be run for the female data.

There are, undoubtedly, other instances of double lethals which occurred in these chromosomes but which were not detected because the two lethals were within a few map units of each other or because an independent semi-lethal occurred in the same chromosome as a lethal. Such cases cause a depression in the number of male progeny for a given region but not to an extent that is as dramatic as the cases in Table 2. Among the lethals in Table 1, $l(1)Q44$, $l(1)Q40$, $l(1)Q26$, $l(1)Q45$, $l(1)Q4$, $l(1)Q18$, $l(1)Q23$, and $l(1)Q24$ show such moderate reductions of crossover frequencies. In one instance, $l(1)Q238$, a lethal was localized to 54.1 ± 0.3 , but there were ten (γv) male crossovers and one (v)

FIGURE 2.—The upper graph shows MULLER's distribution of X-ray induced lethals based on a sample of 93. In the lower graph, a similar distribution of 110 ICR-170 induced sex-linked lethals is shown. The lethals are distributed into intervals of five map units beginning at 0 on the left and ending with 70 map units on the right, except for the first five map units which are in intervals of 2.5 units. To calculate the number of lethals in a region, multiply the height of the bar by the number of map units in the interval. The only region which shows an apparent difference is the area between 10.0 and 20.0 which has three mutations in the X-ray map and 14 mutations in the ICR-170 map. The fiducial limits do overlap at a 5% level of significance for the equivalent frequencies: 3 (0.6 to 8.0) *vs.* 14 (7.8 to 21.3) among 93 lethals. There are, therefore, no significant differences in the two distributions.

male, all 11 showing rudimentary wing (r , 54.5). These were the only rudimentary flies among 2293 male progeny. The presence of the lethal less than 0.5 map units from the rudimentary mutation makes it likely that the partially depressed lethals cited above are instances of double lethal or lethal and semi-lethal combinations. It is also possible that some of the double lethals in Table 1 are actually triple lethals.

TABLE 3
Analysis of multiple lethals arising from a common male

Mutant	Brood	P ₁ male used	Locus
A. Clusters of premeiotic lethals			
Cluster A			
<i>l(1)Q204</i>	5	30	22.6 ± 0.9
<i>l(1)Q205</i>	5	30	21.1 ± 0.9
<i>l(1)Q236</i>	5	30	21.0 ± 0.7
<i>l(1)Q237</i>	5	30	21.7 ± 0.8
Cluster B			
<i>l(1)Q206</i>	5	30	13.0 ± 1.2
<i>l(1)Q232</i>	5	30	16.5 ± 1.9
<i>l(1)Q233</i>	5	30	15.2 ± 0.8
<i>l(1)Q231</i>	5	30	13.5 ± 0.8
B. Nonclusters of premeiotic and meiotic ($\bar{F}_2$) lethals			
<i>l(1)Q210</i>	4	28	64.5 ± 0.6
<i>l(1)Q211</i>	4	28	+33.0 (33.1 to 34.0)
<i>l(1)Q212</i>	4	28	0.0 ± 1.4
<i>l(1)Q234</i>	4	30	38.9 ± 0.7
<i>l(1)Q238</i>	4	30	54.5 ± 0.3
<i>l(1)Q201</i>	5	39	13.4 ± 0.9
<i>l(1)Q203</i>	5	39	63.1 ± 0.5
<i>l(1)Q208</i>	5	34	17.0 ± 1.2
<i>l(1)Q209</i>	5	34	+0.0 (0.0 to 0.4)
<i>l(1)Q240</i>	5	34	-33.0 (32.2 to 32.95)
<i>l(1)Q221</i>	3	19	+0.0 (0.02 to 0.6)
<i>l(1)Q222</i>	3	19	57.9 ± 0.1
C. Nonclusters of mosaic (F_3) postmeiotic lethals			
<i>l(1)Q215</i>	1	12	10.2 ± 1.1
<i>l(1)Q216</i>	1	12	8.6 ± 0.8
<i>l(1)Q217</i>	1	16	-0.0 (0.04 to 0.6)
<i>l(1)Q218</i>	1	16	2.8 ± 0.5
<i>l(1)Q219</i>	1	17	1.3 ± 0.1
<i>l(1)Q220</i>	1	17	+0.0 (0.04 to 0.6)

Figure 1 indicates the detailed localization of all the lethals tested. The localization limits which overlap indicate potential allelism. No direct test of allelism is possible, however, in conventional tests of sex-linked lethals. Figure 2 clearly demonstrates the similarity of MULLER's distribution of X-ray induced sex-linked lethals and those induced with ICR-170. The sample size in the two cases are comparable, MULLER using 93 lethals while 110 lethals were used in the ICR-170 analysis. Only one region (10-20) shows an apparent, but not significant, difference and this may be due to the high degree of overlapping allelism at 13.0 which has seven possible alleles.

Table 3 lists the premeiotic and meiotic "clusters." These "clusters" may be true clusters if the lethals give allelic localizations; and nonclusters if the lethals are unambiguously scattered along the map. Two presumed clusters of four alleles each (one at 14.5 ± 0.5 , the other at 21.8 ± 0.4) exist for brood 5, male 30. This was interesting because two clearly independent lethals must have been induced in separate spermatogonia.

There were five instances of nonclustering among the meiotic and premeiotic broods. One of these is of particular interest because it represents a premeiotic brood of male 30 which has two premeiotic true clusters. In this case, however, the two lethals are located at 38.9 ± 0.7 and 54.5 ± 0.3 and must be considered of independent origin. Note, too, that a premeiotic "cluster" may, on genetic analysis, actually represent independent mutations (males 34 and 39 of brood 5). Thus, the mere presence of two or more lethals from a premeiotic sampling of a treated male should not be considered *prima facie* evidence for clustering. This is particularly true where the frequency of induced mutations is high.

TABLE 4

Distribution of ICR-170 sex-linked lethals by brood

Map region	Broods 1 and 2 postmeiotic 0-5 days	Brood 3 meiotic 6-8 days	Broods 4 and 5 premeiotic 9-15 days	Total mutants
0-5	14	1	2	17
6-10	6	0	0	6
11-15	5	0	2*	7 (+3)
16-20	8	0	1	9
21-25	5	0	1†	6 (+3)
26-30	8	0	1	9
31-35	5	0	2	7
36-40	9	0	2	11
41-45	5	0	0	5
46-50	5	0	0	5
51-55	9	0	1	10
56-60	7	1	1	9
61-65	7	0	2	9
Totals	93	2	15 (+6)‡	110 (+6)‡

* Includes one cluster of four represented as a single localization.

† Includes one cluster of four represented as a single localization.

‡ The parentheses indicate the six lethals which were not included in the premeiotic localizations.

Three pairs of postmeiotic, mosaic, lethals were selected for localization. These were picked up in the F_3 generation and each lethal is derived from a separate F_1 female. Thus the spermatozoa of the P_1 male, in each instance, received independent exposure to the ICR-170 and any lethal mutations would necessarily arise from independent mutational events. This is indicated in Table 3, but in each of these three sets of postmeiotic lethals, the localizations are close to one another. The probability of this happening, in each case, is very low. For male 16 or 17 it is $(14/93)^2 = 2.3\%$; for male 12 it is $(6/93)^2 = 0.4\%$. That all three of these sets should be so close, though clearly nonallelic, is surprising, but the small size of this sample precludes any premature speculation. The fact that these F_3 mosaic lethals are separately localized, however, does support the inferences of SOUTHIN and CARLSON on the origin of the F_3 lethals induced by ICR-170.

Table 4 indicates the brood distribution of the lethals used for the map localizations. There appears to be no striking deviation of distribution by brood, but the numbers of these lethals are not sufficient to rule out more subtle regional differences. The retests of 16 lethals after one to two years (Table 5) clearly demonstrates the reliability of using overlapping localization limits for determining probable allelism with a sample size of about 1500 males per localization.

Table 6 presents the data for 214 lethals tested by growth at 16°C . Among these tested stocks, no temperature sensitive lethals were observed. The average number of Basc males per tested lethal was 25. The lower temperature extended the generation time from ten days at 25°C to 22 days at 16°C , but the total number of progeny per mated pair was not significantly reduced.

TABLE 5
Retests of localized lethals

Lethal No.	Localization in 1962-63	Localization in 1964
<i>l(1)Q2</i>	40.9 $\pm$ 0.6	39.8 $\pm$ 0.3
<i>l(1)Q7</i>	28.6 $\pm$ 0.7	30.1 $\pm$ 0.9
<i>l(1)Q17</i>	23.6 $\pm$ 0.7	24.6 $\pm$ 1.5
<i>l(1)Q20</i>	-0.0	0.0
<i>l(1)Q26</i>	+33.0	33.0
<i>l(1)Q29</i>	29.1 $\pm$ 0.5	26.7 $\pm$ 1.3
<i>l(1)Q48</i>	20.7 $\pm$ 1.2	22.8 $\pm$ 2.0
<i>l(1)Q49</i>	12.7 $\pm$ 0.9	12.1 $\pm$ 1.5
<i>l(1)Q52</i>	37.2 $\pm$ 0.5	36.4 $\pm$ 1.4
<i>l(1)Q55</i>	61.3 $\pm$ 0.2	58.4 — 61.4
<i>l(1)Q209</i>	0.0 $\pm$ 0.4	0.1 — 1.0
<i>l(1)Q210</i>	64.5 $\pm$ 0.6	62.5 — 63.3
<i>l(1)Q211</i>	33.8 — 34.2	33.2 — 34.1
<i>l(1)Q232</i>	16.5 $\pm$ 1.9	12.0 $\pm$ 1.4
<i>l(1)Q235</i>	28.3 $\pm$ 0.4	25.8 $\pm$ 1.7

TABLE 6

Results of tests for temperature sensitive sex-linked recessive lethals

Code No.	Wild-type males	Basc males	Basc females	Basc/l females	Code No.	Wild-type males	Basc males	Basc females	Basc/l females
1	0	49	54	99	108	0	14	35	52
2	0	18	16	33	109	0	23	24	43
3	0	16	6	39	110	0	12	17	28
4	0	18	13	27	111	0	18	16	53
5	0	17	17	55	112	0	22	14	5
6	0	12	8	41	113	0	42	25	65
7	0	13	19	9	114	0	26	28	58
8	0	23	28	56	115	0	17	24	45
9	0	17	16	21	116	0	14	7	16
10	0	19	17	55	117	0	17	15	34
11	0	34	21	54	118	0	17	17	59
12	0	35	30	58	119	0	21	12	53
13	0	13	11	33	120	0	13	22	22
14	0	15	15	41	121	0	19	14	59
15	0	32	29	51	122	0	31	28	38
16	0	13	10	14	123	0	26	11	13
17	0	23	48	47	124	0	9	9	16
18	0	18	7	19	125	0	11	7	24
19	0	21	17	42	126	0	17	10	24
20	0	15	14	31	127	0	21	21	72
21	0	13	20	28	128	0	17	30	35
22	0	15	21	22	129	0	25	29	76
23	0	10	14	22	130	0	34	24	60
24	0	16	21	39	131	0	15	12	46
25	0	14	22	52	132	0	18	16	46
26	0	9	10	13	133	0	18	11	33
27	0	16	18	18	134	0	39	30	59
28	0	10	14	13	135	0	25	16	44
29	0	16	7	13	136	0	39	31	61
30	0	9	1	10	137	0	12	18	31
31	0	13	9	29	138	0	13	26	51
32	0	13	23	27	139	0	23	28	44
33	0	9	6	14	140	0	12	14	89
34	0	9	14	18	141	0	15	16	54
35	0	17	20	37	142	0	24	27	75
36	0	11	7	16	143	0	14	13	66
37	0	22	23	54	144	0	24	31	50
38	0	17	23	17	145	0	19	8	41
39	0	18	13	42	146	0	13	11	58
40	0	99	7	13	147	0	19	26	48
41	0	10	10	20	148	0	21	26	50
42	0	16	13	8	149	0	24	9	55
43	0	11	7	15	150	0	13	15	63
44	0	26	13	28	151	0	13	15	18
45	0	15	5	37	152	0	21	19	47
46	0	15	17	45	153	0	16	25	23
47	0	27	19	32	154	0	20	18	33
48	0	20	10	60	155	0	31	23	39

TABLE 6—Continued

Code No.	Wild-type males	Basc males	Basc females	Basc/l females	Code No.	Wild-type males	Basc males	Basc females	Basc/l females
49	0	24	23	35	156	0	17	23	61
50	0	17	21	44	157	0	7	9	16
51	0	11	7	15	158	0	13	11	40
52	0	21	9	39	159	0	7	10	48
53	0	10	11	12	160	0	22	20	67
54	0	13	22	52	161	0	14	12	46
55	0	22	24	45	162	0	22	44	44
56	0	7	11	24	163	0	18	16	36
57	0	22	27	50	164	0	59	38	63
58	0	24	16	34	165	0	20	21	25
59	0	11	8	38	166	0	32	61	49
60	0	37	25	62	167	0	35	42	74
61	0	18	17	19	168	0	62	54	43
62	0	23	22	28	169	0	15	7	19
63	0	23	36	75	170	0	22	17	23
64	0	30	22	69	171	0	15	13	33
65	0	22	16	33	172	0	32	29	55
66	0	18	37	75	173	0	17	27	28
67	0	22	18	29	174	0	27	22	34
68	0	69	55	127	175	0	20	30	30
69	0	29	29	115	176	0	16	16	16
70	0	34	30	56	177	0	25	26	41
71	0	17	35	62	178	0	32	79	122
72	0	43	50	77	179	0	21	20	0
73	0	37	27	39	180	0	10	12	40
74	0	13	16	25	181	0	21	13	18
75	0	20	23	13	182	0	36	24	61
76	0	13	16	79	183	0	12	6	47
77	0	12	13	19	184	0	32	47	54
78	0	17	11	42	185	0	30	27	53
79	0	17	17	54	186	0	21	21	35
80	0	24	31	95	187	0	27	23	26
81	0	29	30	44	188	0	16	15	18
82	0	11	21	46	189	0	13	12	1
83	0	14	14	37	190	0	16	28	35
84	0	31	15	74	191	0	20	32	58
85	0	21	24	63	192	0	17	26	42
86	0	19	10	24	193	0	11	20	73
87	0	13	9	72	194	0	39	20	31
88	0	17	8	42	195	0	52	32	85
89	0	12	10	62	196	0	19	17	25
90	0	13	10	114	197	0	39	37	66
91	0	22	26	50	198	0	14	24	20
92	0	17	10	23	199	0	18	23	29
93	0	18	31	54	200	0	19	18	35
94	0	19	13	77	201	0	50	37	71
95	0	19	22	42	202	0	14	12	20
96	0	24	13	71	203	0	10	10	9
97	0	20	25	85	204	0	49	55	66
98	0	13	15	31	205	0	36	29	48

TABLE 6—Continued

Code No.	Wild-type males	Basic males	Basic females	Basic/l females	Code No.	Wild-type males	Basic males	Basic females	Basic/l females
99	0	13	27	47	206	0	47	57	76
100	0	35	41	96	207	0	17	15	37
101	0	9	33	37	208	0	55	69	86
102	0	12	7	31	209	0	13	8	35
103	0	21	11	44	210	0	25	46	52
104	0	17	7	17	211	0	65	58	66
105	0	10	19	41	212	0	58	62	86
106	0	12	32	83	213	0	45	36	67
107	0	11	10	26	214	0	12	15	16

The 214 lethals tested for temperature sensitivity were obtained from several experiments using males injected with ICR-170. The F_1 males and females were raised in shell vials kept at 16°C. There was an average of 24 Basic males for each lethal tested.

DISCUSSION

ICR-170, a monofunctional quinacrine mustard, induces a high mutation frequency at the dumpy locus (2, 13.0). Males injected with an 0.1% ICR-170 solution in 0.7% saline will often yield a 1% dumpy mutation frequency (CARLSON and OSTER 1962). The pattern of mutation from postmeiotic sperm is overwhelmingly (95%) mosaic. Of these mosaic or fractional dumpy mutations, about 25% are transmitted by the F_1 mutants to their progeny. Similar studies using ICR-170 to induce postmeiotic sex-linked lethals have shown about 12 to 13% F_2 sex-linked lethals; an additional 14 to 15% F_1 gonadally mosaic females result in an F_3 sex-linked lethal frequency of 5 to 7% (CARLSON and SOUTHWORTH 1963). The preponderance of the mosaicism and the virtual absence of gross chromosome rearrangements among the dumpy mutations induced by this agent differs from similar studies employing X rays (CARLSON 1959). These features suggest that the induced mutations affect one strand of a duplex DNA molecule or that the mutation is fixed by a copy error within the first few divisions of the zygote after fertilization.

The high frequency of mutation at the dumpy locus and the low frequency of mutation for most of the recessive visible loci in the "twelveple" stock (a chromosome bearing 12 specific autosomal visibles, including dumpy) made it seem questionable, at first, that the events at the dumpy locus were point mutations, despite the transmissibility of 20 to 25% of the exceptional dumpy flies found and the abundant presence of allelic types (e.g. dp^{oi} , dp^{lv}) which are less extreme than the type which resembles or accompanies deficiencies for this region (dp^{olv}).

A number of sex-linked lethals were isolated in the F_2 from postmeiotic and premeiotic broods. These were localized to determine, first, if their induction was uniformly distributed along the X chromosome or if they could be attributed to allelism at a few loci which would show exceptionally high mutation frequencies, comparable to that of the dumpy locus. Second, it was inferred from the earlier studies that all ICR-170 induced dumpy alleles were point mutations—that is, no variegated position effects were found nor were there any deletions

showing the phenotype of the Minute(2)Z locus at 12.9 associated with the dumpy mutants induced; these breakage events would be expected and are found for X-ray mutagenesis of this region (CARLSON 1959). Consequently, if the events at the dumpy locus were typical for ICR-170 treatment, the sex-linked lethals should also be free of gross rearrangements. The localization of the sex-linked lethals would readily uncover instances of such induced gross rearrangement.

These localizations would also provide a test for the theories proposed for the brood distribution of mosaicism in the experiments using dumpy. The shift from mosaic to complete dumpy mutations during meiosis (reflected by the premeiotic broods) is paralleled by a change in the relative frequencies of F_2 and F_3 sex-linked lethals for these broods (CARLSON and SOUTHIN 1962; SOUTHIN 1966). The F_2 lethals increase in frequency premeiotically, giving an illusion of extremely sensitive spermatogonia. This is concomitant with a decrease in F_3 sex-linked lethals for this same brood. This phenomenon has been interpreted by CARLSON and SOUTHIN as a clustering effect in the P_1 testes arising from the mitotic and meiotic segregants of a single strand originally altered in a P_1 spermatogonial stem cell. The sorting out of a mutant strand into the descendant chromosomes results in a higher probability of obtaining, in an ejaculation, two or more gametes which can form lethals. Such clusters can be demonstrated by the similarity of the localizations on the chromosome map. In contrast to these premeiotic clusters, the F_3 postmeiotic lethals which stem from a common injected male are derived from separate F_1 gonadally mosaic females. These F_3 lethal descendants of a common male can be localized to see if their distribution on the map is nonallelic as one would expect, *a priori*, for individual sperm or spermatids at the time of exposure.

The localization of the F_2 postmeiotic sex-linked lethals would also provide a basis for distinguishing the susceptibility of the genome to ICR-170. The high mutation rate at the dumpy locus and the low rate for the "twelveple" markers could be reflected in the sex-linked lethals by a high sensitivity to the mutagen for only a few loci. Alternatively, the high mutation frequency at dumpy may be atypical, with many or most other loci capable of being mutated by this agent at lower frequencies.

Our results show that the mutagenic effect of ICR-170 is not restricted to the dumpy locus. Several regions (loci) of the X chromosome respond to this agent with a frequency which may be comparably high. Only 20 to 25% of the dumpy mutants recovered from postmeiotic sperm transmit the mutant defect to their progeny; if a similar transmissibility exists for sex-linked lethals, the frequency of mutation at apparently allelic regions approaches 1%. This can be calculated from the total frequency of the detectable gonadal complete F_2 and gonadal mosaic F_2 sex linked lethals, which was about 25% in the series yielding these lethals (SOUTHIN and CARLSON 1963). The inferred induced frequency is four times 25% or 100% of the postmeiotic sample used here, assuming, of course, that the same proportion of nontransmitted sex-linked lethals exists as in the nontransmitted dumpy mutants (i.e., four times as many may be inferred with a transmissibility of about 20 to 25%). MULLER (1922) estimated that there

are at least 500 genes on the X chromosome. If every X chromosome carried one induced mutation, then the average probability for a mutation in any one gene would be 0.2%. A mean allelism for a presumptive allelic region can be estimated from the number of overlapping localizations in Figure 1. (See legend for Figure 1 for the details of this calculation.) This value is about three alleles per allelic region. This would then provide ($3 \times 0.2\%$) or 0.6% as the approximate average induced rate of mutation for such an allelic region. If the number of genes in *Drosophila* is 10,000 (rather than 2,500 on MULLER's 1922 estimate), this value would be reduced to 0.1% to 0.2% which is about one-fifth of the frequency encountered at the dumpy locus.

The precise number of genes in the haploid genome of *D. melanogaster* has not been demonstrated satisfactorily. RITOSSA and SPIEGELMAN (1965) use 2×10^{11} daltons (8.0×10^7 nucleotide pairs) for the DNA content of the haploid genome. This would yield 50,000 genes, each bearing 300 nucleotide pairs. Their molecular weight determinations were based on unpublished results of G. RUDKIN. The basis for the estimate of 10,000 genes, which we prefer, is based on the size of the scute-19 segment of *D. melanogaster* salivary chromosomes. See CARLSON (1966) for an evaluation of these estimates.

Many more regions (loci) of the X chromosome did not show an apparent allelism (i.e. the limits for the localizations did not overlap). This indicates an ubiquitous distribution of the lethal mutations induced by ICR-170 in contrast to the low mutability of most of the specific visible mutations associated with the "twelvple" stock. Other tests of ICR-170 with visibles employing the "Maxy" stock (13 specific sex-linked visible markers) by BROWNING and ALTENBURG (1962) have demonstrated mutability for this class, although none of these markers have provided similar frequencies to that of dumpy. In the isolation of the ICR-170 induced sex-linked lethals, several independently induced viable mutations of rudimentary (*r*, 1, 54.5), yellow (*y*, 1, 0.0) and singed (*sn*, 1, 21.0) were found, suggesting that these loci are mutable with this agent.

The distribution of the ICR-170 induced sex-linked lethals is comparable to the maps prepared for X-ray induced mutations (MULLER 1928). There are no regions free of mutagenic effect; the relatively high frequency near the yellow region (0 to 5 map units) has been interpreted as a consequence of the shortened map length of this region which is associated with the reduced frequency of crossing over near the tips of chromosomes in *Drosophila* (MULLER 1928). Although ICR-170 is a complex compound with a side group structurally classified as an alkylating mustard, it differs in its effect on the X chromosome from the alkylating mustards and other chemicals cited by FAHMY and FAHMY 1956. These agents (1:2, 3:4 diepoxybutane, 2:4:6-tri-(ethyleneimino)-1:3:5-triazine, and *p*-N-di (chloroethyl-phenylalanine) were claimed by the FAHMYs to have had a greater production of sex-linked lethals to the right of carnation (62.5) than that found for X rays. The absence of such an effect with ICR-170 makes a generalization on preferential chemical mutagenesis at this gross level less likely. It lends support to an alternative interpretation by AUERBACH and WOOLF (1960), that among the various mutagens used there are no major differences in the mutability of chromosomes by region.

The localizations of the sex-linked lethals also permitted a test of the inferred paucity of structural alterations induced by ICR-170. CARLSON and OSTER (1962), using data from analysis of the dumpy region argued against a breakage mechanism comparable to that obtained for ionizing radiation. This was based on the absence of Minute phenotypes associated with dumpy alleles, the absence of variegated position effects, and the lower frequency of extreme (dp^{olv}) phenotypes among the total dumpy alleles recovered in the ICR-170 series. In the localization tests there were seven lethals whose pattern of crossover classes among the male progeny suggested inversions or other gross alterations in the vicinity of the lethal. Retests using male and female data showed conclusively that all of the retested lethals were caused by double or triple lethals induced in the chromosome by ICR-170. Thus, none of the 98 postmeiotic lethals and none of 25 premeiotic and meiotic lethals in this experiment involved concomitant gross structural changes. In contrast, a dose of 5000r to postmeiotic sperm would have produced a similar percent of F_2 sex-linked lethals but one fourth of these would have been associated with gross structural rearrangements. MULLER (1928) reported 23 of 91 sex-linked lethals with gross rearrangements affecting recombination; he used a 5000r dose resulting in a 16.5% F_2 (gonadal complete) sex-linked lethal frequency.

The frequency of sex-linked lethals obtained for this localization analysis was reported by CARLSON and SOUTHWICK (1962). The average postmeiotic frequency for F_2 sex-linked lethals was 12.5%. The overall $F_2 + F_3$ sex-linked lethal frequency for these broods was 15%. The random expectation for two lethals occurring in one X chromosome for this frequency would be 1.1%. This estimate is actually lower than it should be, however, because the 15% frequency is based on recovered (i.e. germinal) lethals and not on the total of induced lethals. The somatically distributed lethals should far outnumber the germinally distributed lethals. Thus each original sperm likely had an average of one induced mutation. In such a case, the number of induced multiple mutants calculated from a Poisson distribution would be 26%. A transmissibility of 20 to 25% would reduce the number of instances of multiple mutants on the X chromosome to 6%. The fact that seven double lethals were obtained among the 123 mutants sampled, suggests very strongly that the total mutation frequency, 15%, is a consequence of the transmissibility of an induced rate somewhere between 75% and total saturation of the X chromosomes. The appearance of these seven confirmed double mutants, the lethal associated with an independent mutant to rudimentary, and the numerous cases of mildly depressed crossover ratios all support the view that the F_2 and F_3 mutation frequency of 15% cannot represent the induced frequency of mutations but rather that it must be several times higher.

We believe that the high frequency of dumpy mosaics induced by ICR-170 represents true mutations at this locus. Of the dumpy mosaics, 20 to 25% transmit the mutation to their progeny (CARLSON and OSTER 1962). There can be no doubt that the transmitted dumpies are true mutations. Some of the mosaic mutants should have wild-type gonads if the distribution of mutant tissue did not include the primordial germ cells in the polar cap. Even if this were not the case,

no more than 75% of the total dumpy mutants induced by ICR-170 could possibly be nongenetic. To test this, *ed dp cl* males were injected with ICR-170 and mated to wild-type virgin females. Perhaps the nontransmitting mosaics arise from physiological abnormalities in the zygotes. However, this reciprocal test should yield up to 75% of the regular dumpy mutation frequency, such mosaic mutations would be classified as phenocopies of dumpy. No significant mutation frequency over control values (about 0.03%) was observed in these experiments (SOUTHIN; SEDEROFF, unpublished). Thus, there is no basis for doubting the genetic nature of the ICR-170 mosaics in spite of the extremely high mutation frequency at the dumpy locus.

The mutagenic mechanism of ICR-170: The majority of mutations induced by ICR-170 are produced in postmeiotic sperm and give rise to mosaic or fractional mutations. This result would appear to argue for an alkylation reaction analogous to the production of mutation in free phage by alkylating agents which give rise to mottled plaques (LOVELESS 1958). Acridines also may be effective as mutagens on free phage, presumably by attachment to the phage DNA before infection. Mutants induced by proflavin on free phage arise as mottled plaques and have been shown to be acridine type (frame shift) mutations (RITCHIE 1964).

The available data argues against an alkylation reaction as the basis for ICR-170 mutagenesis. The mutagenic activity in phage T4 induced by ICR-170 is due to an acridine type mechanism (SEDEROFF 1966). AMES and WHITFIELD (1966) have demonstrated that the same mechanism of action exists in bacteria. The data of BROCKMAN and GOBEN (1965) suggest that ICR-170 is an acridine type agent in *Neurospora* as well. ICR-170 is an efficient mutagen in all of these systems. Thus, there is no basis for expecting the mechanism of action to be different in *Drosophila*.

The localization of sex-linked lethals is strong evidence for the point mutational nature of the ICR-170 induced mutations. The absence of rearrangements, in contrast to the effects of the radiomimetic alkylating mustards (AUERBACH 1945), implies that the alkylating portion of ICR-170 may be effective as a mutagen but not effective in the production of chromosome breaks. We have tested the alkylating mustard part of the molecule ICR-177 (3-[ethyl-2-chloroethyl] amino propylamine) on mature sperm and it is not an effective mutagen at the dumpy locus in *Drosophila*. Similarly, we have found that proflavin is not effective in inducing dumpy mutations in mature sperm.

Further support for the acridine mutagenic route of ICR-170 is the observation that all *transmitted* dumpy mutations are *ol*, *lv*, or *olv* alleles (a test for *l* alone has not been extensively tried out). (There are seven major alleles at the dumpy locus: *olv*, *lv*, *ol*, *ov*, *o*, *v*, and *l* where *o* = oblique wings; *v* = thoracic vortices with bristle disturbances; *l* = lethality. All three of these effects are recessive and complement one another, expressing only the effect in common [*o/v* = (+); *o/ov* = (*o*); *ol/lv* = (*l*).] Spontaneous mutations, and ultraviolet, X-ray, and ethyl methanesulfonate induced mutations at the dumpy locus do give homozygous viable (*ov*, *o*, *v*) alleles as well as the *ol*, *lv*, and *olv* alleles

among the transmitted mutants (CARLSON 1959; CARLSON and OSTER 1962; SOUTHIN 1966; JENKINS (personal communication)).

The work of EDGAR, DENHART and EPSTEIN (1964) demonstrated that temperature sensitivity is a good criterion for detecting missence mutation in bacteriophage. Such temperature sensitive conditional lethals, if found in *Drosophila*, would be evidence in support of a base-substitution type of mechanism of mutagenesis.

The absence of temperature sensitive lethals among the ICR-170 induced sex-linked lethals may be interpreted in several ways. It may be argued that this particular kind of temperature sensitive lethal condition does not occur in *Drosophila*. This argument is ruled out by D. SUZUKI (personal communication) who has found that 10% of the sex-linked lethals induced by ethyl methanesulfonate are suppressed at approximately 16°C. If ICR-170 acted as an alkylating agent, similar to ethyl methanesulfonate, we would have expected to find 10% of the sex-linked lethals induced by ICR-170 to be temperature sensitive. Since 214 mutants were tested, we would have expected to find about 20 of the temperature sensitive type. If the action of ICR-170 does not parallel the results of known alkylating agents (radiomimetic and non-radiomimetic) it suggests that the mutations produced by ICR-170 involve a new type of alkylation effect (with non-breakage of the chromosome and lethals which are not temperature sensitive). Alternatively, these mutations may be of the acridine type. We prefer the latter hypothesis because this is consistent with the predictions of acridine mutagenesis: the extreme phenotypic effects of the transmitted mutations at the dumpy locus and the absence of chromosome breakage associated with them. ICR-170 therefore appears to act as an acridine agent in *Drosophila*. Frame shift mutations should result in a drastic alteration of the gene product; they should be nonfunctional (amorphic) and not suppressible by lower temperature. The characteristics of both dumpy and sex-linked lethals are consistent with these expectations. We propose as a working model that ICR-170 produces a permanent or long-lasting intercalation of the acridine ring in the sperm DNA, the alkylating portion of the molecule attaching the ICR-170 to DNA. Frame shift mutations would arise when this acridine intercalated DNA replicates.

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SUMMARY

The monofunctional quinacrine mustard ICR-170 induces sex-linked recessive lethals throughout the length of the X chromosome. The pattern of distribution is similar to that obtained by X rays. In contrast to radiation, however, the ICR-170 sex-linked lethals are free of gross rearrangements. None were found in a sample of 123 lethals induced during spermatogenesis at a frequency of about 15% in injected males. Apparent allelism for some lethals suggests that there are several regions (loci) in the X chromosome with mutation frequencies comparable to that of the dumpy locus. No striking differential mutability by region exists for premeiotic and postmeiotic sex linked lethals. Tests for temperature sensitive lethals among these mutations were negative. Furthermore, the high frequency of mutation at the dumpy locus cannot be attributed to ICR-170 induced phenocopies. Several lines of evidence favor the hypothesis that ICR-170 acts as an acridine in *Drosophila*. The probable origin of these ICR-170 mutants is permanent intercalation of the acridine in the sperm DNA resulting in mosaically-arising "frame shift" (acridine) mutations subsequent to replication.

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