THE RADIOSENSITIVITY OF DROSOPHILA SPERMATOGONIA.

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IT has generally been accepted that *Drosophila* spermatogonia are less radio-sensitive as regards genetic effects than are sperm. This has been demonstrated with point mutations (LÜNING 1952; ALEXANDER 1954), with sex-linked lethals (KHISHIN 1955; MOSSIGE 1956; ABRAHAMSON and FRIEDMAN 1962) and with second chromosome recessive lethals (MEYER, in MULLER 1959; YTTERBORN 1962). These results have been obtained after doses from several hundred r to several thousand r. A similarly lower radiogenetic sensitivity has been found in mouse spermatogonia (RUSSELL, BANGHAM and GOWER 1958), while in the silkworm the situation appears to be more complicated (TAZIMA 1961; TAZIMA, KONDO and SADO 1961).

The high doses used by the above investigators may have caused selective killing of spermatogonia, and for this reason a sensitivity estimate obtained by less drastic treatment seemed to be of interest. The experiment proposed by WELSHONS and RUSSELL (1957) would not fulfill this requirement.

The motivation for the present investigation can hardly be better formulated than as stated in the first *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation* (1958, p. 175) where it says that "the curve of recovered mutations as a function of dose might turn out to be linear in the range of moderate doses, but to have considerably higher slope in the very low dose range where an appreciable proportion of the cells surviving irradiation belonged to the sensitive group."

Accordingly, the frequency of sex-linked recessive lethals has been investigated after treatments with doses of about 0 r, 55 r, 110 r, 160 r and 300 r. The experiments were started in January 1960, and the work to be reported here was completed in June 1962. Some of the results have been reported in abstract (OFTEDAL 1962a, b) and in summary (1962c).

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MATERIALS AND TECHNIQUE

Biological: A Canton S wild-type stock, maintained in mass culture, was used in all the present experiments. The frequency of sex-linked recessive lethals was determined by the Basc technique (SPENCER and STERN 1948; MULLER, HERSKOWITZ, ABRAHAMSON and OSTER 1954). Cultures were kept at 25°C throughout, except during irradiation, which was performed at room temperature. Eggs were collected by letting about 1500 Canton S females from ϕ , mated to a similar number of males, lay eggs for two consecutive 3-hour periods on two sets of eight yeasted wooden spoons mounted in a special cage. The eggs or hatching larvae were irradiated on the spoons on the following day, at 20 ± 3 hours after laying. The number of spermatogonia in the hatching larva is about 40 per testis (SONNENBLICK 1950). After treatment, each spoon was placed in a half-pint milk bottle with the usual cornmeal-molasses-agar yeasted medium. P_1 males eclosing in these bottles some nine to eleven days later were mated individually in vials to a single virgin Basc female. Eleven to fifteen days later, a single F_1 nonvirgin heterozygous $B/+$ female was transferred to a new vial. The flies were anaesthetized in CO_2 (OFTEDAL 1962d). The F_2 vials were scored for the presence of wild-type males 12 and 18 days later. Vials containing no wild-type males and at least ten Basc males and ten $B/+$ females by Day 18 were counted as lethals. With fewer flies of these types, an F_3 generation was raised. The lethals were then discarded. The limited capacity of the unit precluded keeping stocks of the 385 lethals found, or analyzing them further.

Physical: Irradiation was performed with a Keleket apparatus at 180 kev, 15 ma, 0.5 mm

FIGURE 1.—Irradiation phantom and dosimeters. The close fitting lid has been removed from the irradiation chamber which contains four yeasted egg-collection spoons. The Duplex "T"-chamber is seen in the position used for dose rate measurement and for monitoring. The Moxnes 500 r chamber in the diametrically opposite position was used for dose rate measurements. The peripheral positions were used for monitoring with Moxnes 500 r and 100 r chambers. The tubes in front are used for aerating the chamber during irradiation and sham treatments. The wooden frame is 2.8 cm thick, and rests on a 5 cm thick block of Presswood.

copper + 1 mm aluminium filtration, target distance = 53 cm, in an aerated chamber with full back scatter. Dose rate was about 24.5 r/min. Dose rate was checked before each day's irradiation, and each run was monitored by Victoreen type Moxnes ionization chambers (during 1960) or with a Duplex integrating dosimeter (1961-1962) (Figure 1).

Treatments: Mean doses given were 0 r, 55.8 ± 0.33 r, 108.9 ± 0.77 r, 162.9 ± 1.14 r (1960-1961), and 0 r and 307.4 ± 1.03 r (1962). These values are the arithmetic means and standard deviations of all runs at that level, calculated on the basis of the monitor readings. Controls were given sham irradiation lasting as long as the longest treatment. Each treated group (including controls) was made up of equal numbers of spoons from each of the two collection periods. An exception occurred in March 1961 (irradiation 39-40) when—owing to a breakdown of the X-ray apparatus—all spoons from two days' collections were used for controls. These controls are included in the total. Eggs were usually collected on four consecutive days. Two to several weeks passed between two such series. In some cases, all of one day's treated eggs were discarded, because the earlier collections in the series had already given a sufficient number of flies.

Records: For each irradiation day, two forms were filled out. The one contained all physical data pertaining to irradiation (kev, ma, filter, etc.), environment (temperature, barometric pressure, aeration) as well as experimental data (monitor readings, treatment times for different groups, etc.). The other form gave data on egg collection (number of layer females used, periods) and number of spoons, bottles and treatment times. Bottles were marked with a number for each irradiation day (1-73), a letter for each dose (A-E), a number for each spoon (1-4 or 1-8), and the date of treatment. Numbers of flies emerging in bottles were recorded on cards. The F_1 vials were set up and kept in metal trays holding about 135 vials. One or several cards (if more than 135 males were tested from a bottle) were made up to follow the vials. This card referred to the bottle signature, and gave number of vials set up, number of unsuccessful matings, number of transfers, number of unsuccessful F_2 's, number of vials scored for lethals, and number of lethals. On this card were also recorded the counts of flies in suspected lethal-containing vials. In addition, temporary lists of numbers of flies and vials in the different groups were kept for practical purposes. In a final check, two cases of discrepancies were found in the records, and one day's irradiation representing about 2300 vials was eliminated from the totals (irradiation 37).

RESULTS

A grand total of 105,937 X chromosomes were tested. In each series some 700 to 2,000 chromosomes were tested for each dose. The findings in the different series are given in Table 1 a and b, where numbers of chromosomes tested and lethals found, as well as frequencies in percent, are presented for each series of irradiations. Owing to the small number of lethals obtained in each series, the frequencies show rather wide variations. However, if results from sequential irradiations are pooled to make at least ten lethals in each group, a chi-square test does not indicate a statistically significant heterogeneity (Table 1a). A slight trend towards lower values for all treatments over the two years appears to be present, but since there are about the same numbers of chromosomes in each treatment in all series, this trend has not been taken into account.

In Table 1a, the results of comparisons between controls and each of the irradiated groups in all series are also given. A one-tailed t-test indicates that the differences between 0 r and 55 r points, or 0 r and 110 r points are of dubious significance, whereas the difference between 0 r and 160 r points is significant at the 5 percent level. This is also the case for the difference between the controls and the pooled treated series. (The use of the same control group in all these comparisons is, of course, not strictly in accordance with statistical theory.)

TABLE 1

Number of lethals found/chromosomes tested, and lethal frequencies in percent, after treatment of 20 ± 3 hour old eggs or larvae with different doses of 180 kev X rays

a. EXPERIMENTS 1960-1961

Series	A 0 r	B 55.8 r	C 108.9 r	D 162.9 r
1-4	5/1191	7/1118	8/1219	6/1030
	0.4198	0.6261	0.6563	0.5825
5-8	7/1021	4/767	8/1126	8/1005
	0.6856	0.5251	0.7105	0.7960
9-12	6/1322	7/1075	3/909	4/1218
	0.4539	0.6512	0.3300	0.3284
13-16	2/1568	7/1369	5/1096	8/1210
	0.1276	0.5113	0.4562	0.6612
17-20	2/1213	5/1459	4/1250	8/1632
	0.1649	0.3427	0.3200	0.4902
21-24	3/1337	7/1323	11/1324	7/1178
	0.2244	0.5291	0.8308	0.5942
25-28	8/1176	6/1536	3/1361	6/1096
	0.6803	0.3906	0.2204	0.5474
29-32	4/1731	10/1485	8/1770	7/1986
	0.2311	0.6734	0.4520	0.3525
33-36	5/1964	6/2465	8/2275	7/1829
	0.2546	0.2434	0.3516	0.3827
37-38	0/437	0/407	1/339	2/310
	0.0000	0.0000	0.2950	0.6452
39-40	6/4308			
	0.1393	0.0000	0.0000	0.0000
41-44	7/1758	6/1720	7/1562	9/1766
	0.3982	0.3488	0.4481	0.5096
45-48	2/1203	3/855	5/1055	4/897
	0.1663	0.3509	0.4739	0.4459

Series	A 0 r	B 55.8 r	C 108.9 r	D 162.9 r
49-52	3/923	4/706	2/1071	2/692
	0.3250	0.5666	0.1867	0.2890
53-56	6/1137	6/1702	7/1566	3/1808
	0.5277	0.3525	0.4470	0.1659
57-60	9/2283	3/2372	8/2013	8/2256
	0.3942	0.1265	0.3974	0.3546
61-64	1/1086	5/1179	1/1218	1/947
	0.0921	0.4241	0.0821	0.1056

Homogeneity*

χ^2	11.047	4.990	5.799	8.973
(d.f. = 5)	0.1 > P > 0.05	0.5 > P > 0.3	0.5 > P > 0.3	0.2 > P > 0.1

Differences	$\bar{X}_{B-A}$	$\bar{X}_{C-A}$	$\bar{X}_{D-A}$	$\bar{X}_{(B+C+D)-A}$
t-test	$\frac{\bar{X}_{B-A}}{s_{\bar{x}_{B-A}}} = 1.605$	$\frac{\bar{X}_{C-A}}{s_{\bar{x}_{C-A}}} = 1.551$	$\frac{\bar{X}_{D-A}}{s_{\bar{x}_{D-A}}} = 2.033$	$\frac{\bar{X}_{(B+C+D)-A}}{s_{\bar{x}_{(B+C+D)-A}}} = 1.968$
(d.f. = 15)				
(one tail)	0.1 > P > 0.05	0.1 > P > 0.05	0.05 > P > 0.02	0.05 > P > 0.02

Remarks: 37-38: 37 discarded (~2300 flies). See text; 45-48: 47 and 48 not used; 49-52: 52 not used; 53-56: 55 not used; 61-64: 64 not used.

* Series 1-8, 9-20, 21-25, 29-40, 41-52, and 53-64 were in each case pooled for the chi-square test.

b. EXPERIMENTS 1962

Series	A 0 r	E 307.4 r
65-67	8/3906	11/4060
	0.2048	0.2709
68-70	9/2730	5/2937
	0.3297	0.1702
71-73	5/1769	6/1333
	0.2826	0.4501

The totals are given in Table 2, and it will be seen that the highest measured effect—after 160 r—is only slightly more than 0.1 percent above the controls. The frequencies are plotted in Figure 2, which shows that the points pertaining to doses 0 r to 160 r do not obviously define a linear dose-effect curve. But it is also

TABLE 2

Total numbers of lethals found/chromosomes tested, and frequency of sex-linked recessive lethals in percent (with upper and lower limits of 95 percent expectation interval) after treatment of 20 ± 3 hour old eggs or larvae with different doses of X rays

A 0 r	B $55.8 \text{ r} \pm 0.33$	C $108.9 \text{ r} \pm 0.77$	D $162.9 \text{ r} \pm 1.14$	E $307.4 \text{ r} \pm 1.03$
76/25,650	86/21,538	89/21,154	90/20,860	
0.365%	0.485%	0.515%	0.525%	
0.296%	0.399%	0.421%	0.431%	
0.240%	0.325%	0.340%	0.355%	
22/8,405				22/8,330
0.385%				0.387%
0.262%				0.264%
0.175%				0.177%

Linear regression calculated for A, B, C and D
 $Y = 0.322 + 7.96 \times 10^{-4} X$
 $s_b = 2.75 \times 10^{-4}$ $t = 2.89$ $d.f. = 3$ $0.05 > P > 0.02$ for one-tailed test.
 Linear regression calculated for A, B, C, D and E
 $Y = 0.382 - 1.71 \times 10^{-4} X$

FIGURE 2.—Total frequencies in percent of sex-linked recessive lethals after treatment of spermatogonia. I: Regression line fitted to 0 r, 55 r, 110 r and 160 r points according to the least-squares method, $Y = 0.322 + 0.796 \times 10^{-3} X$. II: Regression line based on the hypothesis of variable sensitivity, $Y = 0.295 + 0.26 \times 10^{-2} X$. Points indicated with a dash designate controls and irradiations in 1960-61. Open circles designate controls and irradiations in 1962. Vertical lines indicate 95 percent expectation interval according to FISHER and YATES (1953).

impossible to distinguish between a linear regression and a nonlinear one. A linear regression fitted by the least-squares method indicates a sensitivity of $b = 0.796 \times 10^{-3}$ percent sex-linked lethals per r. This slope is significantly different from zero. (A slightly different slope is given in OFTEDAL 1962c, since at that time the exact doses had not been calculated.)

In an attempt to determine if the flattening off of the dose-effect curve were real, the experiment with 300 r was performed. This dose gave a nonsignificant—and indeed very minute—increase in mutation frequency in comparison with the simultaneously obtained control value. The value for 300 r is significantly outside the regression line calculated on the basis of the first four points.

On the basis of the above results, it was hypothesized that differential killing of genetically altered spermatogonia was the reason for the flattening off of the curve, and the absence of effect after 300 r. An attempt was made to estimate this killing effect directly by measuring the total number of offspring produced by irradiated males in comparison with simultaneous controls. The results are given in Table 3. First, an impression of the effects to be expected was gained by comparing the productivity of males irradiated at the age of two days, and subsequently mated for a single 24-hour period to ten Basc females each, with the productivity of a control group similarly treated. The females were given fresh bottles every three or four days for 15 days to insure maximal offspring production. Doses of 55 r and 160 r were used, with 12 males for each dose. Each irradiated group had its own control group of the same number of males, being handled in exactly the same way (mating vials kept in pairs, laying bottles stored in the same trays, transfers made in alternating sequence, etc.). The results are presented in Table 3, and demonstrate a slight change in productivity, in the expected direction.

TABLE 3

Productivity of males after irradiation

Stage treated	Mating procedure	Dose	Number of males	Total offspring per male ($\pm$ s.d.)	Productivity reduction		Sex ratio male/females
					flies (percent)	percent per r	
Sperm in 2 day old ♂♂	10 ♀♀ per ♂ for 24 hr	0	12	1105 $\pm$ 103			0.945
		55	12	1017 $\pm$ 68	88 (7.96)	0.145	0.960
		0	11	1172 $\pm$ 125			0.975
		160	12	1042 $\pm$ 64	130 (11.1)	0.069	0.962
Eggs or larvae 20 $\pm$ 3 hr after laying	5 ♀♀ per day for 10 days, starting <3 hr after eclosion	0	4	4587 (4 ♂♂)			0.930
		300	4*	3353 (3 ♂♂)	1234 (26.9)	0.090	0.968 (4 ♂♂)
		0	4†	2398 (4 ♂♂)			
		300	4§	4631 (3 ♂♂)			

* One male died on Day 4. One male sterile from Day 7.

† Three males sterile from Days 4 to 6.

§ One male lost, Day 4.

The effect of 300 r to spermatogonia in 20 ± 3 hour eggs or larvae has been measured by counting the total offspring produced by males in ten daily broods, mated to five fresh Basc females per day. This experiment measures a reduction in productivity which may be dependent on the amount of cell killing amongst the gonidia. The productivity curve of normal males under these conditions shows a minimum on Days 9–10 (OFTEDAL 1959), and this was thought to be the best point at which to break off the mating sequence. The females from each mating period were given optimal laying conditions (as above) and a similar control group was studied simultaneously (as above). A treated male plus a control male thus represents $2 \times 10 \times 5 = 100$ bottles or vials to be counted, which severely limits the number of males which can be handled at the same time.—The experiment has been done twice, with four treated males and four control males each time. The first experiment was reasonably successful, and resulted in a reduction of productivity of 27 percent, indicating an effect per r, after treatment of spermatogonia, of about the same order of magnitude as that found with sperm.—In the second experiment, three control males became sterile from Days 4, 5 and 6, and the result is not very informative. The irradiated males in the last experiment produced slightly more offspring than did the controls in the first experiment.

DISCUSSION

Earlier estimates of the genetic effects of radiation on *Drosophila* spermatogonia have been obtained mainly on the basis of single dose points, with the exception of ABRAHAMSON and FRIEDMAN's 1962 data, and the doses have been high. Table 4 summarizes some of the literature. It will be seen that the sensitivity is in general found to be some three or four times lower than that estimated for sperm, but reduction by a factor of up to 18 has been reported. Some of the data have been obtained after irradiation of larvae, others after irradiation of pupae or adult flies. In some of the studies, the occurrence of clusters of identical mutations has increased the statistical uncertainty of the results. In the present investigation, by testing a single offspring from each irradiated male, the cluster problem has been avoided altogether. A similar procedure has been used by ABRAHAMSON *et al.* (1962).

The doses used for the present investigation were chosen as low as was thought feasible, considering on the one hand the expected yield of mutations, and on the other the desire to avoid selective killing of cells. In retrospect, it seems probable that a dose sequence as used by SPENCER and STERN (1948) (0 r, 25 r, 50 r and 150 r) would have been better.

The dose-effect curve obtained in the first part of the present experiments could be suspected to be nonlinear, but the data did not prove this. The 300 r point demonstrates that the calculated regression line with $b = 0.796 \times 10^{-3}$ percent lethals per r does not describe the findings satisfactorily. It would be statistically unsound to calculate a new linear regression on the basis of the additional findings after 300 r. (This line—if calculated on the basis of the total data—would in any case be suspect, since the experimental points would not be randomly distributed around it, the intercept on the ordinate would have a low probability

TABLE 4

Radiosensitivity of spermatogonia in comparison to sperm. If simultaneous sperm data are not available, SPENCER and STERN's (1948) results have been used for comparison

Effect	Acute doses (r)	Ratio mature sperm to spermatogonia	References
Recessive lethals	Summary of early work	5	TIMOFEEFF-RESSOVSKY AND ZIMMER 1947
Recessive lethals	General statement	3	MULLER 1959
γ mutations		2	
w mutations	960 to mature males	18	LÜNING 1952
8 specific loci		4	
Minutes (if 2-break)	900	4	ALEXANDER 1954
Minutes (if single event)	to larvae	18	
Recessive lethals	1300 to larvae	3	KHISHIN 1955
Sex-linked recessive lethals	1000, 25000 to mature males	3	MOSSIGE 1956
Second chromosome recessive lethals	3000 to mature males	13.5	MEYER, referred to in MULLER 1959
Sex-linked recessive lethals	3000, 9000, 12000 to mature males	6	ABRAHAMSON and FRIEDMAN 1962
Second chromosome recessive lethals	900 to larvae	3	YTTERBORN 1962
Second chromosome recessive lethals	3000	3	PURDOM and McSHEEHY 1961
Specific loci in mice	300	2	RUSSELL, BANGHAM and GOWER 1958
Late embryonic (dominant) lethals	1000	2	TAZIMA, KONDO and SADO 1961
Specific loci (2) average in silkworm	1000 to eggs and larvae	0.6	TAZIMA 1961, (Table 3)

in relation to the control value, and the slope would be slightly negative [$b = -1.71 \times 10^{-4}$].) One is therefore forced to accept a nonlinear relationship to describe the observations.

Although nonlinear dose-effect curves of a shape similar to that found here have been obtained in a number of cases (MULLER *et al.* 1954; RUSSELL 1956; TRAUT 1962), these have been found mainly after treatments with doses one or two orders of magnitude higher than those used here. Two alternative interpretations of the findings are at hand. Nonlinearity in the direction observed here after acute doses may be caused either by saturation of the genetic test system—

which seems highly improbable with the present doses and the effect levels encountered—or it may be the result of differential killing of radiogenetically more susceptible cells.

The latter hypothesis will be adopted here. It is then presumed that the individual cell varies in sensitivity with time. It seems probable that this variation is correlated with mitotic cycle stage (WÜRLER 1962). Sensitivity variations of the order 1:2 to 1:50 during the cell cycle have been reported in mitotic or meiotic cells with regard to chromosome breakage, mainly in plant material. (See review by EVANS 1962.) Variation in mutagenic sensitivity in yeast has been reported by MAGNI (1962), and with regard to reproductive integrity in tissue culture cells by a factor of 7 by DEWEY and HUMPHREY (1962, footnote p. 521).

It is accordingly hypothesized that the genetic effect observed in the present experiments is predominantly the result of induction of mutations in a fraction of the spermatogonia, and that this same fraction is also more susceptible to the killing effect of radiation than is the rest of the cell population. Heterogeneity amongst *Drosophila* spermatogonia with regard to cytologically observable radiation damage has been found by DR. H. SLIZYNSKA (personal communication). Similar observations made on mammals by Russian workers are referred to in the second *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation* (1962).

On the basis of the present results it is not possible to say what proportion of the spermatogonia are in the sensitive phase at any one time, although there is some indication from the productivity experiment that this fraction may comprise about a quarter of the cell population. More data are needed for a reliable estimate of this aspect. Nor is it possible to assess the relative sensitivities in the two phases, or at what rate the changes take place.

The initial slope of the curvilinear dose-effect curve should indicate the true average genetic sensitivity of the spermatogonia in the present test system, since then killing will be minimal. In Figure 3, the initial slope has been drawn to 0.26×10^{-2} percent lethals per r. The choice of slope is at present arbitrary within rather wide limits. The value found by SPENCER and STERN (1948) for sperm seems to be close to the lowest extreme acceptable on the basis of the present results, being 0.215×10^{-2} percent lethals per r. On the other hand, the slope chosen here is some seven times as steep as the one found by ABRAHAMSON *et al.* (1962) for spermatogonia after doses of 3000 r–12000 r ($b = 0.377 \times 10^{-3}$ percent lethals per r).

In an attempt to explain the results presented above in the light of the hypothesized heterogeneity amongst spermatogonia, a model for the analysis of the radiogenetic effects found has been formulated. Its main point is that the observed genetic effect will be determined by the effect induced in the surviving sensitive cells relative to the total cell population sampled.

In general, the observed genetic effects of a given dose will be

$$E_{obs} = \frac{m(1 - k_m)}{N(1 - k_N)}$$

where m is the number of mutant cells induced in the total population to be

sampled N , and k_m and k_N are the respective fractions killed. If it is assumed that the cell population is heterogeneous as regards mutagenic sensitivity as well as regards sensitivity to killing, and that these sensitivities are correlated, nonlinear dose-effect curves will be obtained over certain dose ranges.

With high doses, effects induced in the surviving, resistant part of the cell population would be observed, and a linear dose-effect curve found, since the surviving cell population should be homogeneously resistant to killing. Presumably it is also less sensitive to mutation induction. At very low doses, effects induced in the sensitive cells would dominate, and these cells should also survive, leading to a linear dose-effect curve of steeper slope in this low range. In the intermediate range, where some killing of sensitive cells takes place, selective killing would then lead to a nonlinear dose-effect curve in the transition between the two slopes, from the steeper to the slacker.

Unexpectedly high genetic effects in the lowest dose range have been found—apart from in the present study—by ERIKSSON (1963) on specific locus mutations (waxy) in maize pollen after external gamma irradiation (for doses of 3 r, 6 r, 12 r and 25 r).

In order to make quantitative estimates, it is necessary to know—in addition to the genetic dose-effect curve in the relevant dose range—something about the way the sensitivities change during the cell cycle (in time and in magnitude), and about the shape of the dose-effect curves for killing (LD_{50} and extrapolation number, either or both of which will vary with sensitivity changes). In no material are all these parameters quantitatively known.

The data in the present study are likewise incomplete as regards other parameters than the observed genetic dose-effect curve. The mathematics of the model outlined above will therefore not be gone into. However, it may be permissible to mention that if in the present material it is assumed that a quarter of the spermatogonial population constitutes a homogeneous sensitive fraction, which has a genetic sensitivity of 2.6×10^{-5} sex-linked lethals per r, and survival according to an LD_{50} of 100 r and extrapolation number = 1.0, the theoretical Curve I in Figure 3 may be calculated. If, on the other hand, the sensitive fraction is taken to be one tenth of the cell population, and has a genetic sensitivity of 2.0×10^{-5} lethals per r, and survival according to an LD_{50} of 135 r and extrapolation number = 3.0, Curve II in Figure 3 is found. In both cases, effects induced in the remaining part of the cell population have been ignored.

A model of this sort is of considerable interest in materials where some of the parameters are available while others are not, and where—by drawing on a wider range of radiobiological data—predictions could be better made than by simple parallelism (often called extrapolation). However, the above interpretation of the present data is of purely academic interest unless it can be tested experimentally. One of the more encouraging aspects of the present model is that it can be tested rather simply. On the assumption that genetic damage is not completely repaired, whereas “sub-killing damage” is repaired completely during each cell cycle (ELKIND and SUTTON 1960), treatments protracted over several cell generations should lead to a higher yield of observable mutations, for doses in the nonlinear part of the dose-effect curve. Since *Drosophila* spermatogonia in late em-

FIGURE 3.—Observed mutation frequencies above control, and theoretical mutation curves based on data and model discussed in the text.

bryos or newly hatched larvae appear to have very short mitotic periods, protraction even over only some hours should alter the effects significantly. Data now in hand, from experiments with doses protracted over 8 hours, appear to support the validity of the above assumptions (OFTEDAL 1964).

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SUMMARY

The frequency of sex-linked recessive lethals in *Drosophila melanogaster* has been measured after irradiation of spermatogonia with 0 r, 55 r, 110 r, 160 r and 300 r doses of 180 kev X rays. A nonlinear dose-effect relationship was found, with maximum effect of 0.14 percent lethals at 160 r, and practically no effect at 300 r. Differential killing in conjunction with sensitivity variations is invoked to explain the results.

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