MUTATION FREQUENCIES IN SUCCESSIVE SPERM EJACULATES OF X-RAYED *DROSOPHILA MELANOGASTER* MALES

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IN brood pattern studies of irradiated *Drosophila melanogaster* sperm, the mating periods used by AUERBACH (1953) were of three days, and by STRÖMNÆS (1959), and SÄVHAGEN (1960) of one day. Even shorter mating periods have been used by LÜNING (1961) who studied the change in mutation frequency on the first day after irradiation. The brood pattern technique has led to a better understanding of the rate of spermatogenesis and the radiosensitivity of the different stages of spermatogenesis.

The last stage in spermiogenesis, mature sperm, is considered to be those sperm available for fertilization on the first two to three days after irradiation. If they constitute a population of sperm which all have the same radiosensitivity, no difference in induced mutation frequency would be expected to appear in sperm available for fertilization on first and second day after irradiation. However BAKER and VON HALLE's (1953) finding of a significant difference led to the discussion of whether differential sensitivity existed, or if recovery from damage of the genetic material occurred from one day to the next. (See also LÜNING 1958, LÜNING and HANNERZ 1957, LÜNING and HENZE 1957, LÜNING and SÖDERSTRÖM 1957 and LÜNING, SCHUWERT and JONSSON 1958).

That the age of the male at time of irradiation also may influence the sensitivity of mature sperm can be deduced from the works of DEMPSTER (1941), STRÖMNÆS (1949), and BONNIER and LÜNING (1950). Sexual maturity of *D. melanogaster* males may vary between strains (STRÖMNÆS 1959a) and this can contribute to a change in the sensitivity pattern (STRÖMNÆS 1959b).

STRÖMNÆS and KVELLAND (1962) have in a study of the sexual activity of *D. melanogaster* males found that a few males irradiated when 0 to 2 hours old had fertile matings during the next 12 hours, while most of the males irradiated at the age of 72 hours had mated already within the first hour. In an investigation in which every mating was observed, KVELLAND and STRÖMNÆS (1964, unpublished) found that a change in the induced mutation frequency did occur within the first 12 hours for males that had been irradiated at 0 to 2 hours of age and males that had been irradiated at the age of 24 hours. During the same mating period, no corresponding change was found in sperm coming from males irradiated at the age of 72 hours. They also found a lower mutation frequency in the second mating period which started 24 hours after the first. Their data indicated further that the mutation frequency in the first mating period varied sig-

nificantly between male age groups, while no significant variation in the mutation frequency could be established between age groups in the second mating period. In order to study these and other variations in more detail, the present study was undertaken.

MATERIALS AND METHODS

The wild-type Oslo males employed in this investigation were from 0 to 2 hours old when collected. The males were divided into four groups: the first group of males was irradiated immediately after collecting, the second group of males was kept without females and irradiated when they were 12 ± 1 hours old, and the third and fourth group of males were also kept without females and irradiated when the males were 24 ± 1 hours or 72 ± 1 hours old respectively. All males were treated with 3000r of X rays. After irradiation the males were placed in mating tubes with ten virgin Muller-5; *cn bw*; *e¹¹* females. The males were permitted to mate ad libitum for 12 hours, then stored for 12 hours without females, and again permitted a 12-hour mating period. For the first group of males the first mating period is labelled Series 0A, and the second mating period is labelled Series 0B. For the second group of males the first mating period is labelled Series 12A, and the second mating period is labelled Series 12B. For the third group of males the corresponding labelling is Series 24A and Series 24B, and for the fourth group of males Series 72A and Series 72B. Each individual mating was recorded, and the females were isolated for egg-laying. The details of the procedure are the same as those described by STRÖMNÆS and KVELLAND (1962).

The number of Minutes was scored among the F_1 offspring; the F_1 females were saved for testing of induced recessive sex-linked lethals which were scored in F_2 , and the F_1 males were saved for testing of autosomal 2-3 translocations which were also scored in F_2 . Whenever in doubt about the scoring of a mutant, it was tested for another generation.

Frequencies were not calculated nor recorded in the tables whenever too few observations were made. Thus, the percentages were not calculated when the total number of offspring was less than 100. The tables do not contain the data for a sixth sperm batch because the numbers of offspring were too small to have any meaning by themselves. They are, however, included in the total for each mating period.

EXPERIMENTAL RESULTS

The differences in induced mutation frequencies between mating periods: The data for consecutive sperm batches (i.e. sperm ejaculates) and for the total of each mating period are given in Table 1 for Minutes, for recessive sex-linked lethals in Table 2, and for 2-3 translocations in Table 3. The first mating period has a higher mutation frequency than the second mating period for all groups of males and for each mutation type. The significance of the difference between the totals of each mating period has been tested by the chi-square method, and the result of this analysis is pictured in Table 7. It can be seen from the last table that Series 0A *vs.* Series 0B exhibits no significant difference in the frequency of induced Minutes and sex-linked lethals, and that Series 12A *vs.* Series 12B exhibits no significant differences in the frequencies of induced Minutes and translocations. All other differences between mating periods reached statistical significance.

The reduction in induced mutation frequency, from first to second mating period, has been calculated, and the percent reduction for the different mutations and male age groups is given in Table 8. The average reduction of Minutes for

TABLE 1

Minutes in successive sperm batches (ejaculates) of wild-type Oslo males X-rayed with 3000r when males were 0-2, 12, 24 or 72 hours of age

Series	Sperm batch										Total M/N [*] M × 10 ⁻³
	1	2	3	4	5	6	7	8	9	10	
0A	M/N* 10/2560	M/N 0/92	M/N 5/2878	M/N 4/1084	M/N 0/564	M/N 2/606	M/N 2/1330	M/N 1/572	M/N 5/511	M/N 0/75	10/2652
0B	M × 10 ⁻³ 2.12	M × 10 ⁻³ 2.20	M × 10 ⁻³ 1.73	M × 10 ⁻³ 3.69	M × 10 ⁻³ 3.30	M × 10 ⁻³ 1.50	M × 10 ⁻³ 1.74	M × 10 ⁻³ 9.75	M × 10 ⁻³ 2.81	M × 10 ⁻³ 2.81	44/20317
12A	M/N 7/2775	M/N 1/589	M/N 0/237	M/N 2/1639	M/N 3/1810	M/N 6/2982	M/N 1/1130	M/N 5/1302	M/N 0/75	M/N 0/75	8/3623
12B	M/N 7/5639	M/N 3/2314	M/N 2/1639	M/N 3/1810	M/N 6/2982	M/N 1/1130	M/N 5/1302	M/N 0/75	M/N 0/75	M/N 0/75	12/10270
24A	M/N 36/9457	M/N 18/4713	M/N 9/5344	M/N 13/3004	M/N 4/2675	M/N 0/75	M/N 0/75	M/N 0/75	M/N 0/75	M/N 0/75	59/16688
24B	M/N 22/10372	M/N 2.12	M/N 4.32	M/N 4.32	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	41/20477
72A	M/N 29/4705	M/N 6.16	M/N 4.32	M/N 4.32	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	45/9643
72B	M/N 13/4999	M/N 2.60	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	M/N 1.49	27/9581

The first 12-hour mating period is labelled Series 0A, 12A, 24A and 72A, and the second mating period of the same males Series 0B, 12B, 24B, and 72B. Between first and second mating period is a 12-hour celibacy period.

* M/N = number of Minutes over number of offspring.

TABLE 2

Recessive sex-linked lethals in successive sperm batches of wild-type Oslo males X-rayed with 3000r when males were 0-2, 12, 24, or 72 hours of age

Series	Sperm batch										Total 1/N [*] %
	1	2	3	4	5	6	7	8	9	10	
0A	1/N 78/1135	1/N 6/58	1/N 75/1269	1/N 21/449	1/N 5/43	1/N 0/25	1/N 1/53	1/N 5/163	1/N 3/17	1/N 1/10	84/1193
0B	%1 6.87	%1 5.10	%1 5.91	%1 4.68	%1 4.28	%1 4.47	%1 3.08	%1 5.93	%1 5.72	%1 0/49	519/8979
12A	%1 6.08	%1 5.86	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	119/1516
12B	%1 8.11	%1 5.86	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	%1 9.91	260/4501
24A	%1 5.80	%1 4.82	%1 5.91	%1 5.91	%1 5.91	%1 5.91	%1 5.91	%1 5.91	%1 5.91	%1 5.91	571/7322
24B	%1 8.42	%1 7.69	%1 6.37	%1 6.37	%1 6.37	%1 6.37	%1 6.37	%1 6.37	%1 6.37	%1 6.37	515/9262
72A	%1 5.73	%1 5.40	%1 5.40	%1 5.40	%1 5.40	%1 5.40	%1 5.40	%1 5.40	%1 5.40	%1 5.40	384/3975
72B	%1 9.26	%1 10.92	%1 8.03	%1 8.03	%1 8.03	%1 8.03	%1 8.03	%1 8.03	%1 8.03	%1 8.03	259/4291

The first 12-hour mating period is labelled Series 0A, 12A, 24A and 72A, and the second mating period of the same males Series 0B, 12B, 24B, and 72B. Between first and second mating period is a 12-hour celibacy period.

1/N = number of lethals over number of sperm tested.

TABLE 3
*Translocations in successive sperm batches of wild-type Oslo males X-rayed with 3000r when
 males were 0-2, 12, 24, or 72 hours of age*

Series	Sperm batch																Total T/N %T
	T/N	%T	1		2		3		4		5						
0A	43/667	6.45	2/25													45/692	6.50
0B	111/2870	3.87	49/1229	3.99				32/799	4.01		5/248	2.02		1/6		198/5152	3.84
12A	28/583	4.80	6/143	4.20				3/48								37/777	4.76
12B	51/1432	3.56	13/536	2.43				26/422	6.16		5/122	4.10		0/7		95/2521	3.77
24A	155/2488	6.23	69/1297	5.32				24/522	4.60		6/222	2.70		1/30		255/4559	5.59
24B	115/2594	4.43	47/1321	3.56				27/641	4.21		14/325	4.31		5/104	4.81	211/5020	4.20
72A	93/1104	8.42	53/590	8.98				20/227	8.81		9/90			5/48		177/2058	8.60
72B	44/1128	3.90	23/602	3.82				11/336	3.27		5/139	3.60		0/13		83/2218	3.74

The first 12-hour mating period is labelled Series 0A, 12A, 24A and 72A, and the second mating period of the same males Series 0B, 12B, 24B, and 72B. Between first and second mating period is a 12-hour refractory period.
 T/N = number of 2-3 translocations over number of sperm tested.

all age groups is about 43 percent. The reduction in sex-linked lethals increases with the age of the males, such that the reduction for the oldest males (Series 72A to Series 72B) is about twice the reduction for the youngest group of males (Series 0A to Series 0B). The percent reduction of translocations is highest from Series 72A to 72B and from Series 0A to Series 0B. The respective values are roughly twice the values found for the two remaining groups.

A comparison between mating periods within the same sequential sperm batches (Tables 1, 2, 3) shows that with a few exceptions all sperm batches in the first mating period have a higher mutation frequency than the corresponding sperm batches in the second mating period, although many of them do not reach statistical significance.

The differences in induced mutation frequencies between sperm batches within mating periods: The assumption may be made that mature sperm exhibit differences in radiosensitivity depending for example on whether or not they have achieved motility at the time of irradiation. A change in mutation frequency related to such a physiological change might show up by testing individual sperm batches. There is also the possibility that all mature sperm have the same radiosensitivity, and that a certain fraction of the sperm can recover from genetic damage. Thus, a drop in mutation frequency might be expected to occur at some time after irradiation. Separating the effects of the two possible changes is difficult since the sequential number of sperm batches is not independent of time, and also because in young males the individual variation in sexual maturation is quite large. Some males release their first sperm batch early in the mating period, while others do not do so until late in the period. The largest variations seemed to be between sperm batches for sex-linked lethals, and chi-square tests were therefore made of the homogeneity between sperm batches within each mating series. None of the variations tested was statistically significant.

The differences in induced mutation frequencies between hourly intervals within mating periods: The numbers of Minutes, recessive sex-linked lethals, and translocations obtained in hourly intervals are given in Tables 4, 5 and 6 respectively. The frequencies of the same mutational types in accumulated hourly intervals are given in Figures 1, 2 and 3 respectively. These figures are convenient aids in searching for drops in the mutation frequency with time.

It can be seen from Table 4 that no fertile matings occurred in the first 5 hours in Series 0A. This is ascribed to sexual immaturity of the males. Few offspring were obtained for this reason in Series 0A and Series 12A. The percentages of Minutes in accumulated hourly intervals (Figure 1) give no indication of a significant drop in the frequency of Minutes in any of the series tested.

However, the accumulated frequencies for Series 0A exhibits a drop between the 8th and 9th hour, and Series 12A between the 6th and 7th hour. The data are in both cases too scanty in the early part of the mating period to permit a chi-square analysis.

The results in Table 5 and Figure 2 show that the frequencies of sex-linked lethals are less in the last 2 hours than in the earlier part of Series 0A. This difference is statistically not significant, but the frequency of sex-linked lethals in

TABLE 4
Minutes during hourly intervals given as number of Minutes over number of offspring

Series	Hours											
	1	2	3	4	5	6	7	8	9	10	11	12
0A						0/44	1/146	0/10	0/363	3/676	3/750	3/663
0B	12/6008	3/1005	1/1045	3/1536	2/930	3/1096	5/1292	5/1726	2/1475	2/1771	2/1060	4/1373
12A	0/185	0/165	0/116	2/121	0/15	0/138	0/391	0/197	0/314	2/699	2/715	2/566
12B	4/3761	0/766	1/617	3/562	1/268	0/409	0/450	0/782	1/820	1/474	1/745	0/616
24A	9/2671	4/1809	4/1279	5/718	3/544	2/855	4/1315	6/1562	6/1871	7/1357	7/1182	2/1525
24B	13/7052	7/1604	0/1037	2/1395	4/1180	4/1197	1/672	2/1218	1/1359	0/1130	2/1409	5/1224
72A	11/2896	8/1409	3/925	2/489	2/533	4/429	4/486	1/402	1/369	5/553	5/480	0/672
72B	12/4301	0/842	1/432	1/387	1/342	3/575	0/529	0/431	3/617	4/520	2/246	0/334

TABLE 5

Recessive sex-linked lethals during hourly intervals given as number of lethals over number of sperm tested

Series	1	2	3	4	5	6	7	8	9	10	11	12
0A						0/26	6/71	0/5	14/172	19/291	20/334	25/294
0B	167/2700	24/425	29/462	35/722	23/393	10/453	33/598	54/748	38/629	50/818	24/468	32/563
12A	9/64	5/68	8/50	4/41		5/48	11/146	8/95	6/114	25/307	21/332	17/251
12B	83/1662	24/337	18/276	11/245	4/114	17/183	9/181	23/349	26/351	12/197	17/346	16/260
24A	108/1158	64/772	53/571	20/288	20/204	39/385	49/613	62/728	51/786	35/577	34/592	36/648
24B	194/3291	34/753	38/464	32/581	29/556	26/505	12/319	38/594	22/554	31/507	38/652	21/486
72A	100/1209	68/631	45/406	33/230	17/208	27/195	12/168	20/202	3/142	17/219	17/199	24/284
72B	108/1854	23/380	18/160	8/198	9/156	15/213	18/248	8/169	22/328	13/240	1/102	10/129

TABLE 6

Translocations during hourly intervals given as number of translocations over number of sperm tested

Series	1	2	3	4	5	6	7	8	9	10	11	12
0A						0/11	1/34	0/1	9/102	14/199	14/181	7/164
0B	56/1533	16/247	10/288	14/377	7/242	16/295	17/351	17/421	19/403	14/410	7/269	5/316
12A	2/51	3/31	1/20	1/23	0/3	0/16	2/65	1/29	2/74	4/161	15/145	6/159
12B	35/975	6/175	3/151	3/117	2/61	5/116	3/114	5/201	9/220	3/106	8/159	13/126
24A	42/694	32/567	20/311	14/174	16/116	18/195	16/330	27/482	21/490	14/362	13/386	22/452
24B	74/1808	15/351	8/258	20/387	15/285	10/270	10/215	10/253	10/328	12/282	9/307	18/276
72A	57/661	29/318	20/189	4/89	10/108	6/87	5/67	9/97	6/94	13/113	11/96	8/143
72B	38/1056	6/169	3/100	3/70	5/97	3/92	5/137	2/115	6/198	5/115	4/56	3/103

TABLE 7

Chi-square analyses of the difference between mating periods within male age groups, and of the differences between male age groups within mating periods

Differences tested between series	Minutes χ^2	P-value	Sex-linked lethals χ^2	P-value	Translocations χ^2	P-value
0A vs. 0B	2.47	0.20-0.10	3.01	0.10-0.05	10.80	=0.001
12A vs. 12B	2.04	0.20-0.10	8.25	<0.01	1.53	0.30-0.20
24A vs. 24B	8.06	<0.01	33.46	<0.001	9.97	<0.01
72A vs. 72B	4.42	<0.05	37.80	<0.001	44.19	<0.001
0A-12A-24A-72A	4.70	0.20-0.10	14.98	<0.001	25.24	<0.001
0B-12B-24B-72B	6.83	0.10-0.05	1.28	0.30-0.20	1.48	0.70-0.60

the first 6 hours of Series 12A is significantly higher than the frequency of lethals in the rest of this period.

The accumulated frequency on the 6th hour (i.e. mean frequency for the first 6 hours) is 11.44 (Figure 2), while all later accumulated frequencies are less, the mean being 7.07 percent for the last 6 hours (calculated from Table 5). Similarly it is found in Series 24A that the first 6 hours have a significantly higher frequency of lethals than the rest of that period. The first 6 hours have

FIGURE 1.—Minute mutations per thousand are given for data accumulated at the hours indicated on the abscissa. Series 0A = $\times$ — $\times$; Series 12A = $\circ$ — $\circ$; Series 24A = $\bullet$ — $\bullet$; Series 72A = $\blacktriangledown$ — $\blacktriangledown$. The corresponding B-series are indicated by broken lines.

TABLE 8

Percent reduction in induced mutation frequency from the first to the second 12-hour mating period

Series	Minutes (percent)	Sex-linked lethals (percent)	Translocations (percent)
0A to 0B	43	18	41
12A to 12B	47	26	21
24A to 24B	43	29	25
72A to 72B	40	37	57

a mean frequency of 9.00 percent lethals (Figure 2), while the last 6 hours have a mean of 6.77 percent lethals (calculated from Table 5). In Series 72A a significant drop at the 5 percent level in the frequency of lethals can be found between the first 6 hours and the rest of the mating period. It can be seen from Figure 2 that accumulated frequencies reach a peak on the 6th hour.

For the second mating period, in all age groups of males, no significant drop in the frequency of sex-linked lethals can be found.

The data on translocations are scanty during the first mating period for the young males (Series 0A and Series 12A, Table 6). The data for all series have been searched for a significant change in the induced mutation frequency of translocation (Figure 3), and it was found that the frequency in the first half of Series 24A is significantly higher than the frequency in the second half. No other series exhibits a significant drop in the mutation frequency.

FIGURE 2.—Percent sex-linked lethals are given for data accumulated at the hours indicated on the abscissa. Series 0A = ×—×; Series 12A = ○—○; Series 24A = ●—●; Series 72A = ▼—▼. The corresponding B-series are indicated by broken lines.

FIGURE 3.—Percent translocations are given for data accumulated at the hours indicated on the abscissa. Series 0A = ×—×; Series 12A = ○—○; Series 24A = ●—●; Series 72A = ▼—▼. The corresponding B-series are indicated by broken lines.

DISCUSSION

The present experiment was designed in such a way that the radiation-induced mutation frequency of Minutes, recessive sex-linked lethals, and translocations could be compared in males 0–2, 12, 24, and 72 hours old at time of irradiation. It was possible to study simultaneously the frequency of mutation induced at any time during two 12-hour mating periods separated by a 12-hour male celibacy period. The mutation frequency could also be studied in individual sequential sperm ejaculates (sperm batches).

DEMPSTER (1941), STRÖMNÆS (1949), and BONNIER and LÜNING (1950) have reported that the frequency of induced dominant lethals increased with increased age of the male at time of irradiation. The present data on translocations and sex-linked lethals obtained in the first 12-hour mating period gave support to their findings. The age effect on the induction of Minutes was not realized in either mating period, neither was it realized in the second mating period for sex-linked lethals and translocations. The difference between age groups reached statistical significance in the first mating period for both sex-linked lethals and translocations. Series 72A had the highest mutation frequency of all series tested and for all mutation types studied.

BAKER and VON HALLE (1953) found after irradiation of three to four-day old *D. melanogaster* males in air that the sperm available for fertilization during the first 24 hours after treatment had a higher frequency of dominant lethals than the sperm available in the next 24 hours. Similar results were obtained by NORDBACK and AUERBACH (1956) for sex-linked lethals and translocations. Data confirming their findings have been presented in this paper; however, it should be noted that these data were obtained from irradiated 24-hour and 72-hour old males. In these groups of males, a significant drop was observed from one mating

period to the next in the frequency of induced Minutes, sex-linked lethals, and translocations. For males younger than 24 hours at the time of irradiation a significant change in mutation frequency of sex-linked lethals was found for the 12-hour old males, and a significant change in the frequency of translocations was found for the 0 to 2-hour old males.

BAKER and VON HALLE (1953) had also one group of males which were not mated during the first 24 hours. The sperm released by these males in the next 24 hours had the same mutation frequency as those sperm coming from another group of males in their second day of mating. This, BAKER and VON HALLE interpreted as evidence for the existence of no differential sensitivity of sperm coming on the first and second day after irradiation. This would be true if no ejaculation or resorption of sperm occurs in unmated males. NORDBACK and AUERBACH's (1956) experiment led to the conclusion that recovery occurs from intergenic as well as intragenic damage to the genetic material.

GLASS (1955) studied the dosage curve for induced Minutes and came to the conclusion that almost all Minutes induced at low dosages were very small deficiencies (single event), while at larger dosages an increasing proportion of Minutes were produced by two hits. HERSKOWITZ (1946) thought that the majority of induced recessive sex-linked lethals are associated with breakage while the nonbreakage mutations constitute a relatively small part of the lethals. VALENCIA (1954) found for a dosage of 2800r, that 71 percent of the induced lethals were point mutations and 29 percent structural changes. Translocations arise from two breaks on different chromosomes. Thus, the three types of mutations studied in this paper are predominantly of different origin.

If the recovery hypothesis is correct, it would be anticipated that the reduction in mutation frequency from first to second mating period would be the same for all age groups of males. This is true for Minutes where the reduction is about 43 percent. For sex-linked lethals and translocations the explanation for the drop in mutation frequency does not seem to be that simple. That some of the differences between the two mating periods may be ascribed to recovery is acceptable. However, in addition to this factor it seems necessary to assume differential sensitivity between sperm released during the first and second mating periods in order to explain the variations in the difference between mating groups from one age group of males to another. That differential recovery may exist cannot be excluded, but it would imply that the recovery of presumptive translocations should be twice as high for the 72-hour old males as compared to the 24-hour old males.

The differential sensitivity may vary with age of the males, since it is likely that the volume of fully mature motile sperm and mature nonmotile sperm can vary with age, and motile and nonmotile sperm may have different sensitivities. By testing successive sperm ejaculates, it should be possible to determine the time when sperm of less sensitivity become available. They would be expected to be available earlier in very young males than in older males. The present data have been inspected with this in mind and support for the presented surmise has been found. Further support is found in data published by LÜNING (1961) who found

that a change in mutation frequency occurred after 4 hours for males irradiated when 0 to 24 hours old, while no change in mutation frequency was found the first 12 hours after irradiation of 3- to 4-day old males.

LEFEVRE (1963) found that in young males the mutation frequency drops from ejaculate to ejaculate during the first day after irradiation, while this is not true in older males. Our data indicate that in young males the time from irradiation to fertilization contributes more to the observed variation in mutation frequency than the sequential ejaculate number.

The time after irradiation, when sperm with less radiosensitivity appear, will also depend on accessory gland secretion. That such secretion is essential for sperm transfer has been demonstrated by LEFEVRE and JONSSON (1962).

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SUMMARY

Four groups of unmated wild-type Oslo males were irradiated with 3000r of X rays when the males were 0–2 hours, 12, 24, or 72 hours of age. After irradiation the males were permitted to mate ad libitum for 12 hours, which was followed by a 12-hour period without females, and again a 12-hour mating period. All matings were observed and recorded, and the offspring from each individual mating were examined for the induction of Minutes, recessive sex-linked lethals, and translocations.

An age effect is reflected in the mutation frequency of the sperm ejaculated during the first mating period, but not during the second mating period. The induced-mutation frequencies observed in the first mating period were always higher than the frequencies induced in the sperm available in the second mating period. The difference in induced mutation frequency between the two mating periods is ascribed partly to recovery and partly to differential sensitivity.

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