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SENSITIVITY DIFFERENCES IN THE SUCCESSIVE STAGES OF SPERMATOGENESIS IN *DROSOPHILA* AFTER IRRADIATION IN NITROGEN OR AIR

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SUMMARY

The influence of nitrogen treatment on the pattern of radiosensitivity in the successive stages of spermatogenesis in *Drosophila* was studied. X-irradiation was administered either in air or in an atmosphere of nitrogen, and the frequencies of induced sex-linked lethals and II–III translocations in five successive one-day broods (with 10 females per male per brood) were compared. Four levels of radiation exposure, namely 1000 R, 2000 R, 3000 R and 4000 R were used to irradiate the males. In the air controls, the observed frequencies of lethals and translocations in broods A to E, when plotted graphically, produce asymmetric "U"-shaped curves, with an initial high frequency in brood A, low frequencies in broods B and C, and then a sharp increase through brood D to a very high frequency in brood E. The differences between the frequencies in broods A and B are more pronounced at higher than at lower doses. After radiation exposure in nitrogen, (1) a reduction in the mutation and translocation frequencies is observed, the effect in the latter being more pronounced, and (2) the frequencies obtained in broods A, B and C being nearly equal. This indicates that the differential yield of mutations between mature and almost mature spermatozoa originates from differences in oxygenation. Nitrogen treatment affects the fertility and fecundity of the treated males with the mean number of progeny produced by each fertile male being reduced by about 22 and 78% with 30- and 60-min exposure to nitrogen, respectively. Some of the causal factors that might underlie the nitrogen effect are discussed.

INTRODUCTION

The problem of the differential response of the various stages of spermatogenesis to the mutagenic action of ionizing radiations has been investigated in *Drosophila*

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by a number of workers. All the available evidence indicates that the successive stag of sperm development manifest a wide spectrum of radiosensitivity. For example, was found[4,12–15] that spermatids are characterized by a higher sensitivity to t mutagenic action of X-irradiation than are mature sperm. BONNIER AND LÜNIN and ABRAHAMSON AND TELFER[1] showed that the mutation rate of spermatozoa irr diated in females is significantly higher than those irradiated in the males. LEFEV AND JONSSON[11], LEFEVRE[10], AND MOSSIGE[22] found sensitivity differences amo sperm batches derived from successive ejaculates of the irradiated male, those us for the first mating being the most sensitive. Recently SOBELS[28,29] confirmed the o servation of LEFEVRE AND JONSSON[11] that the first ejaculate of the 7-day-old mal is even more radiosensitive than the sperm irradiated in the female. Moreover, h findings[25,26] on post-radiation modification in mature sperm and spermatids indica that differences in radiation-induced mutation frequencies depend on conditio during and after irradiation exposure. For a recent review, see SOBELS[29].

The present series of experiments was designed to study *(a)* the effect of nitr gen treatment on the males before and during irradiation on the sensitivity patte of the stages of spermatogenesis as measured by the relative frequencies of sex-link lethals and translocations, and *(b)* the influence of anoxia on fertility and fecundi of the treated males.

MATERIALS AND METHODS

For all experiments, males 3–4 day old of the *yellow Bar* stock were used. Tes for sex-linked lethals were made by mating these males to females of the genet constitution *y sc^{sl}In 49 sc^{s}; bw; st p^{p}*. The presence of autosomal eye-color marke brown and scarlet, permits the scoring of II–III translocations among the sam progeny as those used for lethal tests. The brood-pattern technique developed b AUERBACH[4], KHISHIN[8], MOSSIGE[20] and others was used to delimit the successiv stages of sperm development.

The frequencies of lethals and translocations were studied in five one-da broods, with 10 females per male per brood. With this mating procedure, presumabl mature sperm is sampled in brood A, nearly mature sperm in brood B, late spermati in brood C, and early spermatids and spermatocytes in broods D and E, with som possible overlap. The flies of the parental and F_1 generations were reared at 25 ± 1 and those of the F_2 at 22–23°.

X-irradiation was administered at 150 kV, 15 mA at a dose rate of 500 R/mi Four levels of radiation exposure, namely 1000 R, 2000 R, 3000 R and 4000 R, we used to irradiate the males. In the control experiments, the flies were irradiated i air. In the N_2 series, the flies were kept in a continuous flow of N_2 (99.9% purity before and during irradiation, for a period of 30 min. At the 2000 R level, addition experiments were performed to test the effectiveness of 15 and 60 min exposure t N_2. The duration of N_2 treatment before and during irradiation varied with the dos but was so adjusted that the total treatment lasted exactly 15, 30 or 60 min. Th details of the procedure are set forth in Table I.

In the fertility and fecundity studies, the males were exposed to N_2 for 30 c 60 min, after which they were returned to a normal atmosphere. The treated male were utilized to investigate the influence of anoxia on fertility and fecundity b

raising four one-day broods with 10 females per male per brood. In making brood changes, the males were not treated with ether. The inseminated females of each brood were distributed in pairs among vials, and were allowed to lay eggs for two days, after which they were discarded. Vials that did not produce any progeny were classified as sterile, and the F_1 progeny were scored after about two weeks.

TABLE I

DETAILS OF NITROGEN TREATMENT BEFORE AND DURING IRRADIATION

Dose (R)	*Duration of N_2 treatment*		*Total (min)*
	Pretreatment (min)	*During irradiation (min)*	
1000	28	2	30
2000	11	4	15
2000	26	4	30
2000	56	4	60
3000	24	6	30
4000	22	8	30

RESULTS

The description and comparison of the results can be conveniently presented in three sections.

(a) Sex-linked lethals

The frequencies of sex-linked lethals obtained in the five broods at the four levels of radiation exposure studied are summarized in Table II and graphically presented in Fig. 1. For all broods in general, the higher the radiation dose, the higher the mutation frequency. At each exposure level, the mutation frequencies in the five successive broods show a distinct pattern. Furthermore, these frequencies in the N_2 series are usually lower than those in the comparable controls. For example, in the control experiments, with a dose of 3000 R, the lethal frequencies are 9.1% in brood A, 5.3% in brood B, 7.1% in brood C, 11.8% in brood D and 25.9% in brood E. The comparable values in the N_2 series are 5.3% in brood A, 4.7% in brood B, 4.9% in brood C, 7.2% in brood D and 8.1% in brood E. This pattern of difference between the air controls and the N_2 series is uniformly observed at other levels of radiation exposure studied except that at low doses (2000 R and 1000 R) the absolute magnitude of the difference tends to become progressively smaller. In fact, with 1000 R, the mutation frequencies observed with N_2 treatment are similar to those observed in the air controls in broods A, B and C and significantly lower only in broods D and E.

A careful inspection of Table II will reveal that the mutational response of broods A and B differs depending on whether the radiation is administered in air or in N_2. In the controls, the lethal frequencies in brood B are much lower than in brood A with 4000 R and 3000 R; whereas with 2000 R, the magnitude of the difference is much reduced and with 1000 R, the frequencies are almost identical. In other words, with decreasing doses, the differences between broods A and B gradually disappear. As a consequence, the left limb of the approximately "U"-shaped curves shown in Fig. 1 (dotted lines) is steeper at high doses (3000 R and 4000 R),

TABLE II

FREQUENCIES OF SEX-LINKED LETHALS IN FIVE SUCCESSIVE ONE-DAY BROODS AFTER EXPOSURE TO DIFFERENT DOSES OF X-IRRADIATION IN AIR OR IN NITROGEN

X-ray dose	*Treatment and duration*	*Brood A*			*Brood B*			*Brood C*			*Brood D*			*Brood E*		
		Number of chromosomes tested	*Number of lethals*	*% of lethals*	*Number of chromosomes tested*	*Number of lethals*	*% of lethals*	*Number of chromosomes tested*	*Number of lethals*	*% of lethals*	*Number of chromosomes tested*	*Number of lethals*	*% of lethals*	*Number of chromosomes tested*	*Number of lethals*	*% of lethals*
1000 R	air control	2036	39	1.91	1133	21	1.85	1464	39	2.66	848	42	4.95	389	32	8.23
	N_2, 30 min	3061	51	1.67	2113	46	2.18	2668	57	2.14	2578	64	2.48	2069	68	3.29
2000 R	air control	878	44	5.01	954	33	3.46	905	33	3.65	583	56	9.61	21	5	23.81
	N_2, 15 min	1534	57	3.72	777	23	2.96	1135	36	3.17	708	31	4.38			
	N_2, 30 min	3183	115	3.61	2929	108	3.69	2250	89	3.96	1697	87	5.13	313	20	6.39
	N_2, 60 min	2547	95	3.73	1808	66	3.65	1873	70	3.74	986	64	6.49			
3000 R	air control	2011	183	9.10	1227	65	5.30	1558	111	7.12	525	62	11.81	54	14	25.92
	N_2, 30 min	2673	141	5.27	1266	60	4.74	2069	102	4.93	1125	81	7.20	693	56	8.08
4000 R	air control	535	69	12.90	672	50	7.44	984	79	8.03	228	36	15.79	76	13	17.11
	N_2, 30 min	757	59	7.79	657	44	6.70	1642	110	6.70	1109	110	9.92	559	74	13.24

TABLE III

FREQUENCIES OF II–III TRANSLOCATIONS IN FIVE SUCCESSIVE ONE-DAY BROODS AFTER EXPOSURE TO DIFFERENT DOSES OF X-IRRADIATION IN AIR OR IN NITROGEN

X-ray dose	*Treatment and duration*	*Brood A*			*Brood B*			*Brood C*			*Brood D*			*Brood E*		
		Number of gametes tested	*Number of translocations*	*% of translocations*	*Number of gametes tested*	*Number of translocations*	*% of translocations*	*Number of gametes tested*	*Number of translocations*	*% of translocations*	*Number of gametes tested*	*Number of translocations*	*% of translocations*	*Number of gametes tested*	*Number of translocations*	*% of translocations*
1000 R	air control	1560	14	0.90	1014	3	0.30	967	16	1.65	521	13	2.50	176	7	3.98
	N_2, 30 min	2284	14	0.61	1605	10	0.62	2199	20	0.91	1578	11	0.70	1202	17	1.41
2000 R	air control	974	47	4.83	842	27	3.21	864	26	3.01	500	37	7.40	98	21	21.43
	N_2, 15 min	1122	18	1.60	652	12	1.84	1011	15	1.49	600	25	4.17			
	N_2, 30 min	2972	59	1.99	2490	31	1.24	1772	34	1.92	1370	58	4.23	234	16	6.84
	N_2, 60 min	2062	30	1.45	1379	17	1.23	1255	20	1.59	718	15	2.09			
3000 R	air control	616	55	8.93	421	21	4.99	861	45	5.23	95	9	9.47	26	5	19.23
	N_2, 30 min	1201	30	2.50	712	18	2.53	1424	34	2.39	774	45	5.81	505	42	8.32
4000 R	air control	533	57	10.69	402	23	5.72	635	42	6.61	134	12	8.96	52	7	13.46
	N_2, 30 min	665	33	4.96	507	26	5.13	1132	53	4.68	737	73	9.90	305	49	16.07

less so with 2000 R and almost parallel to the abscissa with 1000 R. In the N_2 series, however, the differences in mutation frequencies between broods A and B are not statistically significant. Thus with 4000 R, the frequencies of lethals are 7.8% in brood A and 6.7% in brood B; with 3000 R 5.3% in brood A and 4.7% in brood B; with 2000 R 3.6% in brood A and 3.7% in brood B; and finally with 1000 R, these frequencies are 1.7% in brood A and 2.2% in brood B. It appears as if both the first- and second-day sperm samples are equally sensitive to irradiation in N_2. In addition, in the N_2 series the mutation frequencies observed after exposure to 1000 R in broods D and E are much lower than in the corresponding controls. As a result, the mutation curves (Fig. 1) tend to be more or less flat, rather than "U"-shaped.

In contrast with other broods, the mutation frequencies in brood E present a somewhat inconsistent picture regarding the effect of dose. As shown in Table II, these frequencies are 17.1% with 4000 R, 25.9% with 3000 R, 23.8% with 2000 R and 8.3% with 1000 R. While the absolute frequencies observed in this brood are subject to large sampling errors in view of the small number of chromosomes that could be tested, it is likely that at higher doses the most sensitive cells are selectively eliminated and this results in an apparent lack of dose–frequency relationship.

Also presented in Table II are data relating to the effects on the males of N_2 treatment for 15 and 60 min, with a dose of 2000 R. The results indicate that the lethal frequencies remain more or less constant in each brood irrespective of the duration of exposure to N_2. An apparent exception is found in brood D, where, with a 15-min treatment, the frequency of lethals is 4.4%, with 30 min it is 5.1% and with 60 min it increases to 6.5%.

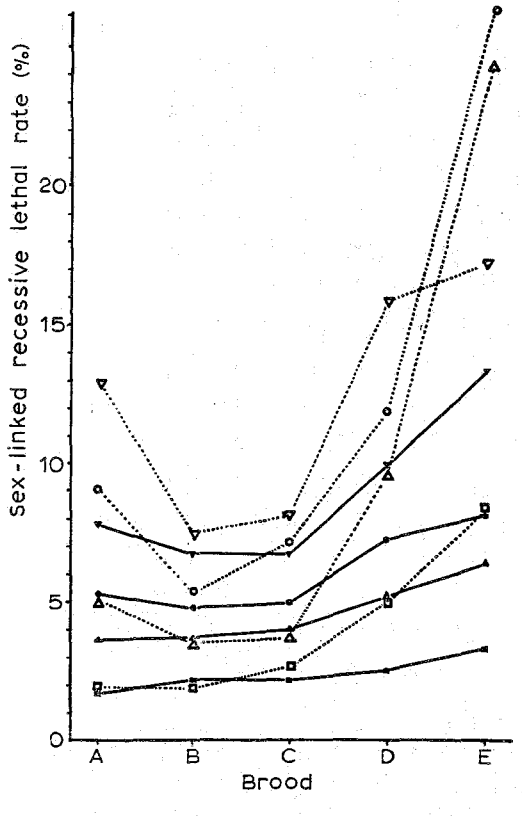
(b) Translocations

The data on the frequencies of induced II–III translocations are shown in Table III and Fig. 2. The general shape of the translocation curves is strikingly similar to those for lethals. It is clear that the dose–frequency relationship (or the apparent lack of it in brood E of the air controls) and the brood pattern at each exposure level are entirely in line with the data on the frequencies of lethals. As for lethals, it looks as if the most sensitive stages to the induction of translocations are the sperm cells sampled in the D and E broods, which were at the early spermatid or spermatocyte stages at the time of the irradiation.

A comparison of Tables III and II and of Figs. 2 and 1 will reveal that for a given dose, the frequency of translocations is relatively much lower than that of lethals. It is interesting to observe that irradiation under anoxic conditions considerably reduces the translocation frequencies as compared with irradiation in air. For example, with 1000 R, these frequencies are reduced from 1.65% to 0.91% in brood C, from 2.5% to 0.70% in brood D, and from 3.98% to 1.41% in brood E. With 2000 R and 3000 R, this effect is seen in all the broods; with 4000 R however, these frequencies are lower than in the controls only in broods A, B and C, while in broods D and E they are slightly higher than in the controls. This apparent increase in the translocation frequencies in these highly radiosensitive cells probably results from the fact that under anoxia, cell-killing is less severe: consequently more cells carrying translocations come to light. On the average, there is a considerably greater nitrogen effect for translocations in broods B and C than for lethals. This is not unexpected since nitrogen simply acts as a dose-reduction factor, and at these overall frequencies

the frequency of translocations will be related to the dose with an exponent of between 1.5 and 2, whereas lethals will only increase in linear proportion.

As with lethals, 15 or 60 min N_2 treatment given to the males before and during irradiation produces approximately the same relative amounts of reduction in translocation frequencies as the 30-min exposure. Only in brood D, does prolonged treatment with N_2 (60 min) seem to be more effective as compared with 15- or 30-min exposures (2.1% with 60 min *versus* 4.2% with 15 or 30 min). This situation is the reverse of that found for lethals in this brood.



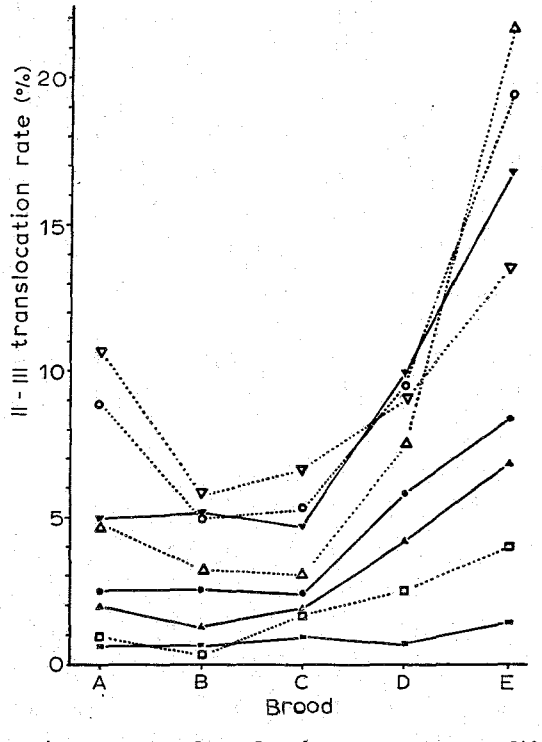


Fig. 1. Frequencies of sex-linked lethals in five successive one-day broods after exposure to different doses of X-irradiation in air or nitrogen. Open symbols, air controls; closed symbols, nitrogen series. ▽ ▼, 4000 R; ○ ●, 3000 R; △ ▲, 2000 R; □ ■, 1000 R.

Fig. 2. Frequencies of II–III translocations in five successive one-day broods after exposure to different doses of X-irradiation in air or nitrogen. Open symbols, air controls; closed symbols, nitrogen series. ▽ ▼, 4000 R; ○ ●, 3000 R; △ ▲, 2000 R; □ ■, 1000 R.

(c) Fertility and fecundity studies

Table IV presents the results of studies dealing with the effect of nitrogen treatment on the fertility and fecundity of the treated males. If males are exposed to N_2 for 30 or 60 min, their fertility is reduced relative to the control (Table IV, column 4). The numbers of tested males are so small that a meaningful quantitative evaluation of the effect is difficult. Also adversely affected by N_2 treatment is the fecundity of the males (defined here as the mean number of F_1 progeny produced by each fertile male per brood under the conditions of the experiment, namely, with 10 females per male per brood). There is a progressive decrease in the numbers of progeny sired by each treated fertile male in the successive broods, with 30-min as well as with 60-min N_2 treatment (Table IV). For example, with 30-min treatment, the absolute fecundity

TABLE IV

EFFECT OF N_2 TREATMENT ON THE FERTILITY AND THE FECUNDITY OF TREATED MALES

Duration of N_2 treatment (min)	*Number of males treated*	*Number fertile*	*% fertility*		*Number of F_1 progeny sired in broods*				
					A	*B*	*C*	*D*	*Grand tot (all brood*
				Total	1315	1866	1565	1276	6022
0 (control)	17	17	100	Absolute fecundity	77.4	109.8	92.1	75.1	354.2
				Relative fecundity (in %)	100.0	100.0	100.0	100.0	100.0
30	48	43	89.6	Total	3846	2580	2797	2739	11962
				Absolute fecundity	89.4	60.0	65.0	63.7	278.2
				Relative fecundity (in %)	115.5	54.6	70.6	84.8	78.5
60	58	50	86.2	Total	997	1396	1031	546	3970
				Absolute fecundity	19.9	27.9	20.6	10.9	79.4
				Relative fecundity (in %)	25.7	25.4	22.4	14.5	22.4

decreases from about 90 in brood A to 64 in brood D and with 60 min in N_2, from 20 to 11.

The very small numbers of offspring produced in the 60-min series indicates that with prolonged exposure, the adverse effect of N_2 is considerably more than would be predicted from short exposures. If the number of F_1 progeny produced by the control flies in each brood be taken as standard (100%) it is possible to make an estimate of the relative fecundity of the treated males, Such estimates are shown in Table IV, along with the absolute fecundities. It is clear that the average number of F_1 progeny produced by a fertile male in the treated series, while varying over a wide range, is reduced by about 22% with 30 min treatment and by 78% with 60-min treatment (Table IV, rightmost column). The fluctuations in fecundity between broods in the treated and untreated series are not entirely unexpected, since it is known that characters such as fecundity manifest enormous variability not only between individual flies but also between experiments.

DISCUSSION

The results presented above confirm the existence of pronounced differences in sensitivity between the stages of spermatogenesis in *Drosophila*, which can be further modified by anoxic conditions. With respect to stage sensitivity, these results agree with those of other workers who have used similar techniques of sampling the germ cells (see for example MOSSIGE[21,22] AND TRAUT[31]). In the air-control experiments, at higher doses, asymmetric "U"-shaped curves are obtained for mutations as well as translocations, with an initial high frequency in brood A, a decrease in broods B and C and then a sharp increase through brood D to a high frequency in brood E. At lower doses, the differences between the frequencies in broods A and B become smaller, thereby leading to a near flattening of the left limb of the curve. Mutation frequencies in the first- and second-day sperm after irradiation of the males are known to be different, those of the first day being higher than those of the second[2,3,5,17–19,23,30]. A decrease in the frequency of the rearrangements has also been observed in sperm utilized on the second day as compared with that of the first day, although this

finding has not always been consistent[18]. The role of several factors such as the age of the male at irradiation[16], age of sperm[20], differential sensitivity due to differences in oxygen tension near the opening of the male genital tract[16,22,24], recovery with time and mating sequence with individual females[11], differences in initial sensitivity and/or sperm mixing[11,18,32–34] has been studied and discussed in an attempt to explain the observed differences between first- and second-day sperm. Recent studies of SOBELS[28] indicate that the pronounced differences in radiosensitivity between fully-mature motile spermatozoa (brood A) and the immotile late spermatids (brood B) are in fact oxygen-dependent, which arises from the relatively greater degree of oxygenation in the former than in the latter. Furthermore, higher RBE values were recorded by DAUCH *et al.*[7] after exposure to fast neutrons in sperm sampled on the second than in those of the first day after irradiation for both lethals and translocations. Since neutrons, of the energy spectrum used in their studies, show considerably lower dependence on oxygen, these results are entirely in line with what would be expected for cells differing in their degree of oxygenation.

Further evidence along the lines postulated above comes from the data of the experiments of the N_2 series. In these experiments, at all levels of radiation exposure studied, there are no statistically significant differences between the mutation frequencies of broods A and B. This is to be expected, since N_2 treatment effectively excludes oxygen available to the population of sperms during irradiation and may thus reduce their susceptibility to X-ray-induced damage. This agrees well with SOBELS' observation, who sampled essentially the same stages from the first ejaculates of 7-days-old and 1-h-old males, respectively.

A comparison of the mutation and translocation frequencies in broods A and B on the one hand and in E on the other in the air-control experiments reveals that these frequencies, for any given dose, are always much higher in the latter than in the former. It is known[27] that the spermatids have a higher intrinsic sensitivity to radiation damage in the presence of oxygen than the cells sampled in broods A or B. This is manifested by their greater oxygen-enhancement ratios (about 3.3) which are about one and a half of that estimated for mature or nearly mature sperm. Since this factor outweighs the difference in radiosensitivity between the cells sampled in broods A and B, it is to be expected that the right limb of the "U"-shaped curves would be higher than the left, and this is actually observed.

Special mention must be made of the results of the experiments with 2000 R in the N_2 series, where in most broods there were no detectable differences in mutation and translocation frequencies with 15-, 30- or 60-min N_2 treatment. This shows that effective anoxia is already attained in 15 min. Brood D, however, is an exception. There is an increase in lethal frequencies with an increase in the duration of N_2 treatment (4.4% with 15 min, 5.1% with 30 min and 6.5% with 60 min), but for translocations the trend is reversed (4.2% with 15 and 30 min and 2.1% with 60 min). The reasons for this discrepancy are not clear.

Finally, the observations that the fecundity of the N_2-treated males is reduced and that this reduction is related to the duration of N_2 treatment raise questions about the influence of anoxia on the mating ability of the treated flies and on the physiology of the sperm. After the flies recovered from anoxia, they were found to mate normally. However, it is likely that in spite of normal matings, the treated males may not be able to ejaculate enough sperm or that the sperm itself is inactivated so

that this interferes with fertilization. LEFEVRE AND JONSSON[9] studied sperm utilization in *D. melanogaster* and found that the inability to transfer sperm in the later matings of a sequence results not from the lack of mature sperm in the seminal vesicles, but rather from the lack of accessory-gland secretion. It is likely that even the functioning of the accessory gland is unfavorably affected by anoxia. SANKARANARAYANAN (personal communication) has observed that even the sperm derived from single ejaculates of 7-day-old males which were exposed to N_2 for 110 and 140 min caused a drastic and progressive increase in the proportion of unhatched eggs during the 10 successive periods of egg-collection that he studied. An understanding of the mechanism by which N_2 brings about these changes must await further studies.

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REFERENCES

1 ABRAHAMSON, S., AND J. D. TELFER, Sex-chromosome loss and translocation frequencies in *Drosophila melanogaster* after X-raying sperm in males or in females, *Genetics*, 39 (1954) 955–956.
2 ABRAHAMSON, S., AND J. D. TELFER, The relative constancy of the X-ray-induced mutation frequency of *Drosophila melanogaster* sperm in inseminated females, *Genetics*, 41 (1956) 677–684.
3 ALEXANDER, M. L., The role of recovery mechanism and oxygen effects upon changes in radiation sensitivity in sperm treated in mature males and fertilized females of *Drosophila*, *Genetics*, 47 (1962) 1505–1518.
4 AUERBACH, C., Sensitivity of the *Drosophila* testis to the mutagenic action of X-rays, *Z. Induktive Abstammungs-Vererbungslehre*, 86 (1954) 113–125.
5 BAKER, W. K., AND E. S. VON HALLE, The basis of the oxygen effect on X-irradiated *Drosophila* sperm, *Proc. Natl. Acad. Sci. (U.S.)*, 39 (1953) 152–161.
6 BONNIER, G., AND K. G. LÜNING, Sex-linked lethals in *Drosophila melanogaster* from different modes of X-ray irradiation, *Hereditas*, 39 (1953) 193–200.
7 DAUCH, F., U. APITZSCH, A. CATSCH AND K. G. ZIMMER, RBE Schneller Neutronen bei der Auslösung von Mutationen bei *Drosophila melanogaster*, *Mutation Res.*, 3 (1966) 185–193.
8 KHISHIN, A. F. E., The response of the immature testis of *Drosophila* to the mutagenic action of X-rays, *Z. Induktive Abstammungs-Vererbungslehre*, 87 (1955) 97–112.
9 LEFEVRE, G., AND U. B. JONSSON, Sperm transfer, storage, displacement and utilization in *Drosophila melanogaster*, *Genetics*, 47 (1962) 1719–1736.
10 LEFEVRE, G., The influence of age and mating patterns before and after irradiation on the incidence of induced mutations in *Drosophila melanogaster*, *Genetics*, 48 (1963) 897–898.
11 LEFEVRE, G., AND U. B. JONSSON, X-ray-induced mutability in male germ cells of *Drosophila melanogaster*, *Mutation Res.*, 1 (1964) 231–246.
12 LÜNING, K. G., X-ray induced dominant lethals in different stages of spermatogenesis in *Drosophila*, *Hereditas*, 38 (1952) 91–107.
13 LÜNING, K. G., X-ray induced chromosome breaks in *Drosophila melanogaster*, *Hereditas*, 38 (1952) 321–338.

14 LÜNING, K. G., Studies on the origin of apparent gene mutations in *Drosophila melanogaste Acta Zool. (Stockholm)*, 33 (1952) 193–207.
15 LÜNING, K. G., *Studies on X-ray Induced Mutations in Various Stages of Spermiogenesis i Drosophila melanogaster*, Bonnier, Stockholm, 1952, p. 12.
16 LÜNING, K. G., Effect of oxygen on irradiated males and females of *Drosophila*, *Hereditas*, 4 (1954) 295–312.
17 LÜNING, K. G., AND A. HANNERZ, The recovery phenomenon after irradiation in *Drosophil melanogaster*, I. Recovery or differential sensitivity to X-rays, *Hereditas*, 43 (1957) 550–562.
18 LÜNING, K. G., AND J. SÖDERSTRÖM, The recovery phenomenon after irradation in *Drosophil melanogaster*, II. Recovery of recessive lethals, *Hereditas*, 43 (1957) 563–570.
19 LÜNING, K. G., AND A. HENZE, The recovery phenomenon after irradiation in *Drosophil melanogaster*, III. The inactivation dose of the recovery process, *Hereditas*, 43 (1957) 571–57
20 MOSSIGE, J. C., Sperm utilization and brood patterns in *Drosophila melanogaster*, *Am. Natura ist*, 89 (1955) 123–127.
21 MOSSIGE, J. C., The relative biological efficiency of 31 MeV betatron X-irradation and 17 KeV X-rays as measured by recessive sex-linked lethals in *Drosophila melanogaster*, *Progress i Radiobiology*, Oliver and Boyd, Edinburgh, 1956, pp. 137–143.
22 MOSSIGE, J. C., Differential yields of mutations from the first and second matings after irra diation of mature sperm in *Drosophila melanogaster*, in: F. H. SOBELS (Ed.), *Repair from Genet Radiation Damage and Differential Radiosensitivity in Germ Cells*, Pergamon, Oxford, 1963, pp 253–274.
23 NORDBACK, K., AND C. AUERBACH, Recovery of chromosomes from X-ray damage, in: G HEVESY (Ed.), *Advances in Radiobiology*, Oliver and Boyd, Edinburgh, 1957, pp. 481–485.
24 OSTER, I. I., On recovery in X-irradiated germ cells. *J. Cellular Comp. Physiol.*, 58, *Suppl.* (1961) 203–207.
25 SOBELS, F. H., Radiosensitivity and repair in different germ cell stages of *Drosophila*, in F. H SOBELS (Ed.), *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Gern Cells*, Pergamon, Oxford, 1963, pp. 179–204.
26 SOBELS, F. H., Radiosensitivity and repair in different germ cell stages of *Drosophila*, in: S. J GEERTS (Ed.), *Genetics Today*, Vol. II, Pergamon, Oxford, 1965, pp. 235–255.
27 SOBELS, F. H., The role of oxygen in determining initial radiosensitivity and post-radiatio recovery in the successive stages of *Drosophila* spermatogenesis, *Mutation Res.*, 2 (1965) 168 191.
28 SOBELS, F. H., Oxygen dependent differences in radiosensitivity between fully mature an almost mature spermatozoa, *Drosophila Information Service*, 41 (1966) 150.
29 SOBELS, F. H., Processes underlying repair and radiosensitivity in spermatozoa and spermatid of *Drosophila*, in: International Atomic Energy Agency Panel, *Genetical Aspects of Radio sensitivity, Mechanisms of Repair*, 1966, in the press.
30 TRAUT, H., Die Dosisabhängigkeit der Strahleninduzierten Mutationsrate bei Berücksichti gung des Reifegrads der Keimzellen, untersucht an *Drosophila melanogaster*, *Habilitations schrift*, Heidelberg, 1962.
31 TRAUT, H., Dose-dependence of the frequency of radiation-induced recessive sex-linked lethal in *Drosophila melanogaster*, with special consideration of the stage sensitivity of the irradiate germ cells, in: F. H. SOBELS (Ed.), *Repair from Genetic Radiation Damage and Differentia Radiosensitivity in Germ Cells*, Pergamon, Oxford, 1963, pp. 359–374.
32 TRAUT, H., Evidence for differential radiosensitivity rather than recovery in sperm sample from X-irradiated *Drosophila melanogaster* males, *Genetics*, 50 (1964) 167–171.
33 TROSKO, J. E., Effect on genetic damage of post-treatments given X-rayed *Drosophila* males *Genetics*, 49 (1964) 401–409.
34 TROUT, W. E., Differential radiosensitivity as an explanation for so-called recovery in *Droso phila* sperm, *Genetics*, 50 (1964) 173–179.