THE ROLE OF RECOVERY MECHANISMS AND OXYGEN EFFECTS UPON CHANGES IN RADIATION SENSITIVITY IN SPERM TREATED IN MATURE MALES AND FERTILIZED FEMALES OF DROSOPHILA[1]

MARY L. ALEXANDER[2]

Section of Radiation Biology, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, and Institute of Animal Genetics, Edinburgh, Scotland

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THE influence of the cytoplasmic contents of the cell upon enhancement of radiation damage in chromosomal elements has been tested by irradiation of Drosophila eggs which contain large cytoplasmic volumes (TIMOFEEF-RESSOVSKY 1937; BONNIER, LÜNING and ARNBERG 1952). Although there were no indications of enhancement of genetic damage by radiation treatment of the cytoplasm, the increase in radiation sensitivity of sperm treated in fertilized females was recognized and tested by BONNIER (1954) and LÜNING (1954). These experiments with X rays showed that chromosome breakage measured as hyperploid males, was involved in the increase in sensitivity. BONNIER (1954) suggested the possibility of differences in oxygen concentration, differences in chemical protection or systems of metastable energy. Recovery mechanisms postulated by BAKER and VON HALLE (1953) led to the possible explanation that recovery in genetic damage occurred in sperm populations in the male testes until the time they were used in female insemination. The higher radiosensitivity of sperm treated in females could therefore result from the absence of postirradiation repair in sperm treated in females.

In the present experiments, recovery mechanisms have been tested by using 2 Mev neutrons. X-ray treatments were done in air and nitrogen to test the effectiveness of oxygen combined with tests for recovery mechanisms. The preliminary results for neutrons were reported earlier (ALEXANDER 1961) and for X rays by ALEXANDER and BERGENDAHL (1962a).

MATERIALS AND METHODS

The Oregon-R, Oak Ridge strain was used as a test stock of *Drosophila melanogaster;* the sc^{81} *B InS* w^a sc^8 Muller-5 (M-5) and sc^{81} *InS* w^a sc^8; *Cy/Bl* L^2

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[2] National Cancer Institute-National Institute of Health Special Fellow, 1960-61 at the Institute of Animal Genetics, Edinburgh, Scotland, under Grant No. CF-10,924. Present address: Genetics Foundation, Department of Zoology, University of Texas, Austin, Texas.

Marker-9 (M-9) were used to test for sex-linked recessive lethals; the *bw st* stock was used for detecting induced translocations involving the Y, second and third chromosomes. In tests where mature sperm were treated in males, four-day-old Oregon-R males received radiation treatment and were mated to females of either the M-5 or Marker-9 stock for recessive lethal tests or to *bw st* females for translocation studies. In tests where mature sperm were treated in fertilized females, the M-5, Marker-9 or *bw st* females were placed with mature Oregon-R males for three days in small mass matings of ten males and ten females. The fertilized females were separated from the males before radiation treatment. In the neutron tests, males and inseminated females were treated in air with 900 rads of neutrons with an average energy of 2 Mev produced from the Van de Graaff accelerator at the Hammersmith Hospital, London. All the males used in the tests were treated at the same time, but due to a limited target area for neutron treatment the inseminated M-5 females and *bw st* females were treated separately in two additional treatments. Some of the treated males were mated to M-5 females and others to *bw st* females immediately after treatment and separated after one day. The other portion of the males was held without mating for one day and mated to M-5 females and *bw st* females the second day. After treatment, the inseminated females were allowed to lay eggs for one day and then transferred to fresh food for another day. Offspring from the first and second day were tested separately in both male and inseminated female tests. Translocation tests with 2 Mev neutrons were repeated with the same procedures as in the above tests. In the second tests, inseminated females produced too few offspring the first or second day after treatment for genetic testing. Repeat tests were obtained from treated males but not inseminated females.

The same type of tests were repeated using 14 Mev neutrons from the Cockcroft-Walton accelerator at the Biology Division of the Oak Ridge National Laboratory, Oak Ridge. Mature males and inseminated M-5 and *bw st* females were treated with 2000 rads of 14 Mev neutrons while in nitrogen gas. Induction of sex-linked lethals and translocations were tested the first and second day after treatment of mature sperm in males and in inseminated females.

In the X-radiation tests, the Marker-9 stock was used for testing sex-linked recessive lethals and the *bw st* stock was used for testing for translocations involving the Y, second and third chromosomes. For one series of tests, a dose of 4000r of X rays was given at 200 kv voltage and 15 ma in an atmosphere of air and a similar dose was given in an atmosphere of nitrogen gas. To eliminate variation in X-ray dosage, all the males and inseminated females (Marker-9 and *bw st* female) for the air test were treated at one time. All the material for the nitrogen test were also treated at one time. In these tests the first and second days after irradiation were also tested separately.

The usual Muller-5 type tests were used to detect sex-linked recessive lethals using the Muller-5 or Marker-9 stock. With either stock, the F_1 females, heterozygous for the Muller-5 chromosome (or Marker-9) and the treated X chromosome, were mated to Muller-5 or Marker-9 males. The absence of normal males in the next generation indicated the presence of induced lethals in the X chromo-

some. Induced translocations involving the Y chromosome, second and third autosomes were tested by mating F_1 males heterozygous for *bw st* and the treated autosomes, individually, to *bw st* females. The appearance of only certain classes indicated the presence of a translocation resulting from breakage and reciprocal exchange of genetic material involving any two or three of the Y, second or third chromosomes.

Sex-linked recessive lethals and translocation induced in female chromosomes, when inseminated females were treated, were separated from those induced in the mature sperm. Sex-linked lethals induced in the Muller-5 or Marker-9 chromosome of the females were detected by the absence of Muller-5 or Marker-9 males in the F_2 generation. Translocations induced in maternal chromosomes would involve the *bw st* genes. The F_2 normal males from the translocation test will carry translocations induced in mature sperm but not those induced in female chromosomes. The female chromosomes carry the *bw* and *st* genes on the second and third chromosome and induced translocations will be carried heterozygous in the F_2 *bw st* class. Retesting F_2 normal males from crosses which give translocation segregation eliminates translocations induced in female chromosomes.

RESULTS

The results for sex-linked lethals and translocations induced in inseminated females and in males with 2 Mev neutron treatment in air are presented in Table 1 and illustrated in Figure 1. To test for recovery mechanisms modifying genetic damage, the percentages of biological damage appearing in mature sperm the first and second day after treatment were compared. The results do not show a significant reduction in either sex-linked lethals or translocations the second day after treatment as compared to the first day. In the inseminated female test, a chi-square value of 0.832 resulted for sex-linked lethals and a value of 0.036 for the first and second day comparisons of translocations. In treated males, the chi-square values were 2.532 for sex-linked recessive lethals and 3.158 for the translocation test. In the repeat test for translocations, a chi-square value of 1.634 was obtained for first and second day comparisons. The percentages of biological damage in the first and second day show no significant changes and the values for the two days appear to show random variation. The high and low values for the first and second days in the two independent translocation tests for males are reversed (Table 1). There were no consistent decreases in biological damage the second day after treatment to indicate that recovery mechanisms may be occurring in sperm treated in males and not in sperm treated in fertilized females. The first and second day tests were not significant; however, when comparisons were made for the total values for both days with sex-linked lethals and translocations, the values were significantly higher in sperm treated in inseminated females than those treated in males. A significant chi-square value of 8.927 resulted for the comparison of sex-linked lethals and 7.876 resulted for translocations induced in sperm treated in mature males and in inseminated females.

Inseminated females and mature males were treated with 14 Mev neutrons in

TABLE 1

Sex-linked recessive lethal and translocation damage in D. melanogaster *with 900 rads of 2 Mev accelerator neutrons in air*

Stage sperm treated	Period after treatment	Sex-linked recessive lethals: Lethals / Total no.	Sex-linked recessive lethals: Percent lethals	Translocations: Translocations / Total no.	Translocations: Percent translocations	Translocations (repeat): Translocations / Total no.	Translocations (repeat): Percent translocations
Inseminated females	First day	5/90	5.5	47/479	9.8	Sterile	
	Second day	51/611	8.3	59/623	9.5	Sterile	
	Total	56/701	8.0	106/1102	9.6	.....	
Mature males	First day	40/735	5.4	33/629	5.2	49/683	7.2
	Second day	17/488	3.5	43/552	7.8	21/400	5.3
	Total	57/1223	4.7	76/1181	6.4	70/1083	6.5

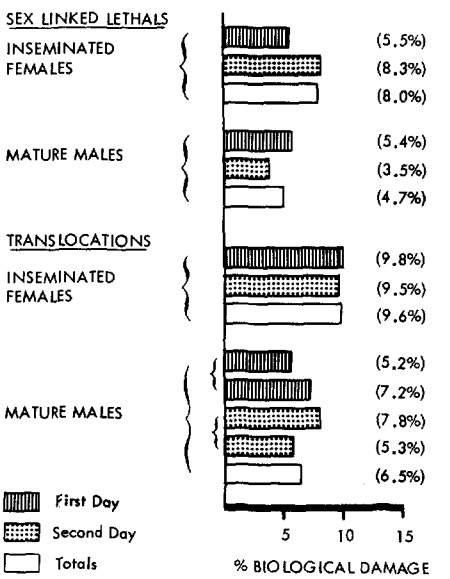

FIGURE 1.—Percent biological damage induced in *D. melanogaster* with 900 rads of 2 Mev neutrons.

an atmosphere of nitrogen gas since an oxygen effect has been observed with neutrons of this energy in previous tests (ALEXANDER 1958). The inseminated Muller-5 females did not survive the 4–5 hour treatment time in nitrogen gas. The *bw st* females survived the nitrogen gas treatment but produced too few offspring for testing. In treated males, 5.4 percent (31/571) sex-linked lethals were

observed the first day after treatment and 4.6 percent (72/1567) the second day. For translocations, 4.3 percent (95/2204) were observed the first day and 3.5 percent (91/2614) the second day. Chi-square values of 0.464 result from comparison of sex-linked lethals from the first and second day and 2.253 for translocations. Neither value is significant and no recovery mechanisms in induced genetic damage the second day after treatment were detected after 14 Mev neutron treatment.

In the X-radiation tests, the percentages of biological damage were compared for sperm treated in males and in inseminated females in atmospheres of air and nitrogen gas. The tests in air, as those in nitrogen, are comparable since both males and inseminated females were treated at the same time. The results for sex-linked lethals and translocations induced in sperm in mature males and inseminated females treated with 4000r of X-radiation in air are presented in Table 2 and illustrated in Figure 2. The sex-linked lethal damage induced in

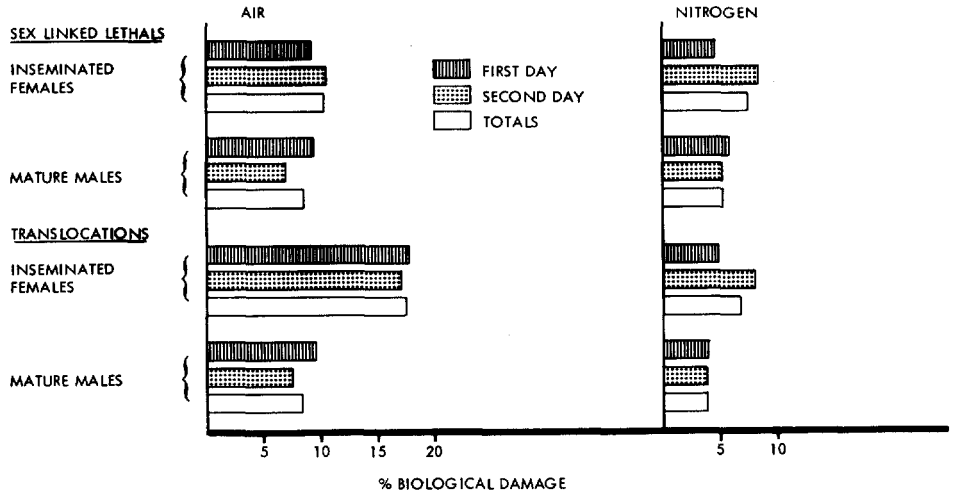

FIGURE 2.—Biological damage in *D. melanogaster* with 4000r of X ray in air and nitrogen.

sperm in inseminated females the first and second day are quite similar as are the translocation values. The chi-square values of 0.0868 for sex-linked lethals and 0.0465 for translocations were not significant for the first and second day test. For mature males, the chi-square value for the first and second day tests for sex-linked lethals was 3.904 and 1.678 for translocations. The chi-square value for sex-linked lethals gives a probability level at .05. Although the values were lower the second day for sex-linked lethals and translocation with treated males, there were no highly significant changes. The percentages in biological damage in the first and second day in treated males varied more than those for inseminated females.

When the values for translocations induced in sperm in inseminated females are compared to those for mature males for the air test, the percentages of translocations induced in inseminated females are much higher with a highly sig-

TABLE 2

Sex-linked recessive lethal and translocation damage in D. melanogaster *with 4000r of X-radiation in air*

Stage sperm treated	Period after treatment	Sex-linked recessive lethals: Lethals / Total no.	Sex-linked recessive lethals: Percent lethals	Translocations: Translocations / Total no.	Translocations: Percent translocations
		3		39	
Inseminated	First day	32	9.37	216	18.05
		43		82	
females	Second day	401	10.72	474	17.29
		46		121	
	Total	433	10.62	690	17.54
		109		52	
Mature	First day	1128	9.66	528	9.85
		57		42	
males	Second day	814	7.00	559	7.51
		166		94	
	Total	1942	8.54	1087	8.65

nificant chi-square value of 32.226. The percentage of sex-linked lethals was higher in inseminated females but the chi-square value of 1.6916 was not significantly different from the value for males. Translocations result from chromosome breakage and reattachment and the higher percentages of damage induced in sperm in inseminated females suggest a dependence of the higher sensitivity upon these processes.

The results for the nitrogen tests are presented in Table 3 and illustrated in

TABLE 3

Sex-linked recessive lethal and translocation damage in D. melanogaster *with 4000r of X-radiation in nitrogen gas*

Stage sperm treated	Period after treatment	Sex-linked recessive lethals: Lethals / Total no.	Sex-linked recessive lethals: Percent lethals	Translocations: Translocations / Total no.	Translocations: Percent translocations
		7		20	
Inseminated	First day	147	4.76	411	4.87
		62		55	
females	Second day	757	8.19	685	8.03
		69		75	
	Total	904	8.26	1096	6.84
		78		22	
Mature	First day	1338	5.83	557	3.95
		66		21	
males	Second day	1255	5.26	546	3.85
		144		43	
	Total	2593	5.55	1103	3.90

Figure 2. The reduction in biological damage by the anoxic conditions of nitrogen are obvious for sperm tested in males or inseminated females. In the male test, the first and second day comparisons for sex-linked lethals and translocations show no significant chi-square values. The sex-linked tests give a chi-square value of 0.519 and the translocation tests a value of 0.961. When the first and second day tests are compared in the inseminated female test, increases in sex-linked lethals and translocations are observed in the second day tests. This increase in the second day appears to be characteristic of nitrogen treatment with inseminated females. It does not appear in the mature male tests nor in the air tests as seen in Tables 2 and 3 and Figure 2. An increase in biological damage appeared the second day although the chi-square value for sex-linked lethals, 1.87, is not significant in these tests. The first and second day translocation comparisons give a chi-square value of 3.915 which would be significant at the .05 probability level. An increase in translocations the second day was observed in a previous test with X-ray treatment in nitrogen. In this test, translocation damage, in the inseminated female test increased from 2.4 percent (4/170) the first day to 6.3 percent (54/860) the second day with values of 4.7 percent (52/1112) and 5.7 percent (65/1142) translocations observed in the first and second day of the male test.

If the increase in the second day of the female tests is considered random variation then the comparison of biological damage in sperm in inseminated females and in mature males can be compared from the totals of both days. If comparisons are made with the totals, the translocation tests are highly significant in females with a chi-square value of 9.170. Sex-linked lethals are significant at the .02 probability level with a chi-square value of 5.115. In the nitrogen test as in the air test, the increase in translocations indicates that chromosome breakage is an important factor in the higher sensitivity in sperm in inseminated females.

The induction of genetic damage in the chromosomes of females combined with that induced in mature sperm does not account for the higher percentage of genetic damage observed when inseminated females are treated. In the sex-linked lethal test, lethals induced in the Marker-9 chromosomes of the female were easily separated from recessive lethals induced in paternal chromosomes. The absence of F_2 normal males indicates recessive lethals in the treated chromosomes of sperm (paternal) whereas the absence of F_2 Marker-9 males indicates recessive lethals in the female chromosome (maternal). In the male test, where maternal chromosomes were not exposed to irradiation, a "correction" rate for chromosomes treated in inseminated females can be obtained. The rates varied in the male test. In the male test, in air, there were 1.23 percent lethals observed in the untreated Marker-9 chromosomes. In the inseminated female test, an average of 3.99 percent lethals in the Marker-9 chromosomes was observed. In the nitrogen test, a lethal correction value of 0.19 percent was observed for untreated M-9 chromosomes. With inseminated females an average value of 3.73 percent recessive lethals was observed for the two days for treated maternal chromosomes. With the translocation test, there were several cases in the insemi-

nated female tests in which the translocation segregation was not recovered when retested. This indicates that the translocation had not been induced in mature sperm but in maternal chromosomes. Several cases were retested from treated males and did not show translocation segregation. These may result from spontaneous translocations from females or an error in the original classification of the translocation. The tests were not designed to test for exact rates for induced sex-linked lethals and translocations in female chromosomes. They were designed to eliminate the genetic damage originating in female chromosomes from the test. The higher values of biological damage in sperm treated in inseminated females cannot be explained by additional damage from the female chromosomes.

Nondisjunction of the maternal X chromosomes was observed in the neutron and X-ray tests. Nondisjunction XX and no X gametes from the female are observed as XXY females (M-9 phenotype) and X0 males (normal phenotype). The XXX and 0Y classes are lethal and are not recovered. The M-9 females and normal males appearing in the F_1 indicate nondisjunction since normal disjunction of the X chromosome produces M-9/+ females of normal phenotypes and M-9/Y males which are white-apricot, scute-8 phenotype.

With neutrons, ten nondisjunction individuals (four females and six males) were recovered the first day after treatment. This gives a value of five percent nondisjunction individuals whereas the second day only 14 nondisjunction individuals were observed in 1302 to give a value of approximately one percent. For the X-ray air test, three nondisjunction individuals were recovered out of 64 to give a value of 4.68 percent the first day. The second day, the percentage of nondisjunction was reduced to 3.48 percent with 28 nondisjunction individuals appearing in 804. In the nitrogen test, three normal males in 248 gave a value of 1.2 percent for nondisjunction. In the second day, the percentage dropped to .5 percent with only six nondisjunction individuals appearing in 1140. Higher percentages of nondisjunction appeared in the first day after treatment than in the second in each case and neutrons appeared to be somewhat more effective than X rays in producing nondisjunction. The X-ray dose was approximately four times higher than the neutron dose although the percentage of nondisjunction was approximately equal in the neutron or X-ray tests in air. When recessive lethal and nondisjunction percentages were compared, then equal percentages of recessive lethals were induced in the neutron and the X ray, nitrogen tests but the nondisjunction values were higher with neutron treatment than with X-ray treatment.

DISCUSSION

The higher sensitivity observed when sperm are treated in fertilized females than when treated in males has received several possible explanations. The theory of postradiation recovery in induced genetic damage in sperm treated in males and the absence of such recovery mechanisms in sperm treated in fertilized females will not entirely explain the sensitivity differences. The percentages of dominant lethals were reported to be lower the second day than the first day after X-ray treatment of mature males by BAKER and VON HALLE (1953). This

phenomenon was explained as resulting from recovery mechanisms acting to heal a portion of the lethal damage during the first day after treatment to produce fewer dominant lethals the second day. After neutron exposure from nuclear detonations, this phenomenon was not observed for males by BAKER and VON HALLE (1954). LÜNING and JONSSON (1956) found similar results for sex-linked lethals and translocations after neutron treatment of mature males. In the present experiments with neutrons, neither sex-linked lethals nor translocations showed a significant change in the first and second day tests for sperm treated in males or in inseminated females. However, when values for sperm treated in males and in those treated in inseminated females were compared, significantly higher values were obtained for the female tests. The absence of "recovery" in sperm the second day after treatment when treated in males indicates that recovery mechanisms are not necessary to explain the increase in sensitivity in sperm after fertilization. OSTER (1961) reported that translocation damage was similar in mature sperm treated with neutrons in mature males and in inseminated females. The data we obtained with 2 Mev neutrons do not agree with those results. In our tests, all the males were treated at the same time; whereas, the inseminated M-5 and *bw st* females were treated in two separate treatments. This gives a repeat experiment to test for dose variations. Also, the biological damage obtained for sperm treated in mature males was retested and almost identical values were observed as in the first test (Table 1).

Possible differences in the effectiveness of oxygen to enhance biological damage in sperm treated in males and inseminated females are not eliminated by 2 Mev neutron treatment in air. A slight oxygen effect was reported for Ehrlich ascites tumors with 2 Mev neutrons from the same source at the Hammersmith Hospital by HORNSEY, HOWARD-FLANDERS and MOORE (1960). The tests using 14 Mev neutrons were done in nitrogen gas to eliminate oxygen effects and these tests showed no evidence for recovery mechanisms after neutron treatment of mature males as did 2 Mev neutron treatment in air. Comparisons to sperm treated in fertilized females were not possible since inseminated females were either killed or sterilized by the long nitrogen treatment.

To test for the role of oxygen in the increase in sensitivity, treatments were made with X rays in atmospheres of air and nitrogen. In the air test, there was a consistent, if not always significant, reduction in sex-linked lethals and translocations the second day after treatment when mature males were treated. The reduction in sex-linked lethals the second day gave a P value of .05 whereas, the reduction in translocations was less and gave a P value of .2. The reduction in dominant lethals, sex-linked recessive lethals and translocations the second day after X-ray treatments in air appears to be characteristic of biological damage after treatment of sperm in males. It has been reported previously by BAKER and VON HALLE (1953), LÜNING (1954), NORDBACK and AUERBACH (1956) and ABRAHAMSON and TELFER (1956). In the present tests, reductions in the percentages of sex-linked lethals and translocations the second day were about equal in the male test (2.66 percent *vs.* 2.34 percent). As pointed out by NORDBACK and AUERBACH (1956), if recovery is occurring in sperm treated in males, both

intergenic and intragenic types of damage are involved. In nitrogen, there was less than a one percent drop the second day after treatment for sex-linked lethals and translocations in sperm treated in males. The slight differences in biological damage the first and second day after treatment of males indicates an anoxic phenomenon which could be due to a difference in postradiation recovery mechanisms. Although it is possible that differences in genetic recovery occur in air and nitrogen treatments, there also exists the possibility that a greater proportion of sex-linked lethals may be associated with breaks in air than in nitrogen when the same dose of radiation is used. If a portion of the sperm carry breakage types which are in some way selected out of the sperm population, then there would be a greater reduction in biological damage the second day in the air test than in the nitrogen test.

The possibility of unequal concentrations of oxygen in the sperm populations at the time of treatment was offered as an explanation for the reduction in biological damage the second day after treatment in males by OSTER (1961) and LÜNING (1961). If the results for treatment of males in nitrogen are considered, there is little change in the biological damage the first and second day. The presence of a higher oxygen concentration in the sperm population closer to an external opening at the time of irradiation could possibly be eliminated by anoxic treatment when males are treated. However, when the results for sperm treated in females under anoxic conditions are considered, there is an increase in damage the second day. A higher oxygen concentration could not explain these results with treatments in nitrogen.

Chromosome breakage is an important factor in the high sensitivity of sperm treated in inseminated females with X-ray treatments in air. LÜNING (1954) reported a higher percentage of hyperploid males resulting from sperm treated in females than those treated in males with X rays in air. ABRAHAMSON and TELFER (1956) found similar results for chromosome breakage types (translocations) as compared to yellow mutations and sex-linked recessive lethals. In the present tests, the relative increase in translocations in sperm treated in inseminated females is greater than that for sex-linked lethals when X-ray treatments were done in air. There was an 8.87 percent increase in translocations and only 2.08 percent in sex-linked lethals. With X-ray treatment in nitrogen, there is a similar increase in translocations and sex-linked lethals of 2.94 percent and 2.71 percent in sperm treated in inseminated females above those treated in mature males (see Figure 2 for comparisons). The increase in chromosome breakage appears to be eliminated in the anoxic atmospheres therefore indicating an oxygen effect which is in some way related to chromosome breakage but perhaps not with other types of chromosome damage as point mutations and small deletions.

With neutrons, there is also an equal increase in sex-linked lethals and translocations in sperm treated in females as compared to the value observed for treatment in males (3.3 percent and 3.2 percent). Although the neutron tests are similar in this respect to the nitrogen tests, the results with neutrons are probably

not due to an anoxic phenomenon. The results may reflect the effectiveness of neutrons for producing chromosome breakage.

Another characteristic of X-ray treatment in nitrogen is the increase in the percentages of translocations and sex-linked lethals the second day after treatment when sperm are treated in inseminated females. This increase in biological damage the second day suggests a postradiation modification. There are no differences in sensitivity in biological damage in sperm treated in males and in inseminated females if the tests for the first day are compared. The high increase in biological damage the second day after testing accounts for the significant increase in sperm after fertilization (see Table 3 and Figure 2). Since the increase in damage is in the second day, it appears as if enhancement in biological damage may depend upon the return of air to the environment for radiation damage to appear. If this phenomenon proves to be consistent, then the higher sensitivity of sperm treated in inseminated females may depend upon postradiation enhancement of genetic damage resulting from the presence of oxygen. This increase in biological damage the second day after treatment was observed for both sex-linked lethals and translocations as was the reduction in damage the second day when males were treated and therefore involves both intergenic and intragenic types. The low values the first day are not explained by the smaller numbers of F_1 individuals for the first day test since greater sterility the first day was characteristic for all the tests when inseminated females are treated. The results for nondisjunction of the maternal X chromosome in the nitrogen test showed no unusual results as compared to the air test. There is no basis for assuming any unusual effect of nitrogen on paternal chromosomes which might result in an increase in biological damage the second day of testing.

Postradiation modifications of mature sperm treated in the males of Drosophila by immediate posttreatment in gases have not been substantiated as yet. Immediate posttreatment in carbon monoxide gas after X-ray treatment in oxygen in the germ cell cycle of *D. virilis* males did not modify biological damage in either mature or immature germ cells (ALEXANDER and STONE 1955; SCHMID 1961). However, LÜNING and HANNERZ (1957) and LÜNING and HENZE (1957) reported a system of inactivation of recovery mechanisms in mature sperm with X-radiation by combining one radiation treatment in air with one in nitrogen. The test with *virilis* by ALEXANDER and BERGENDAHL (1962b) did not show a postradiation recovery period of 30 minutes as reported by LÜNING (1958) for *melanogaster*. There were no dose-rate or dose-fractionation effects observed in the *virilis* test with gamma-ray treatments in oxygen or nitrogen.

Many of the characteristics of the genetic results with Drosophila sperm in inseminated females are similar to those of the system in plants reported by CALDECOTT, JOHNSON, NORTH and KONZAK (1957). In barley, different postradiation modifications in seedling injury were observed by posthydration in oxygen and nitrogen gases. With X rays, the amount of radiation injury depended upon the water content of the embryo at the time of irradiation and the duration of the period of hydration. Posthydration in oxygen increased damage above that for nitrogen in embryos with a water content of four percent but not

those of sixteen percent water content. After posthydration in oxygen, the biological damage could not be modified by nitrogen but if the first posthydration was in nitrogen, then later oxygen hydrations would modify the biological damage. There were no postradiation effects with neutron radiations.

In Drosophila, the absence of a difference in the first and second day tests in sperm with neutrons indicate that there are no postradiation effects. With X-ray treatment the lower values obtained the second day for air treatment of sperm in males and the higher values the second day after X-ray treatment of sperm in nitrogen in inseminated females suggest possible postradiation modifications. The second day increase in biological damage in sperm treated in inseminated females when nitrogen is present at the time of irradiation would be expected if posthydration in nitrogen can later be modified by the presence of oxygen as the system in plants.

Later reports by NORTH, CALDECOTT and BERGBUSCH (1962) show that preirradiation temperature treatment of 75–85 degrees Centigrade will eliminate postradiation storage injury for 48 hours but the sensitivity to oxygen hydration is retained. This indicates a pretreatment dependence upon postradiation modification for some processes but not others.

Modifications of the genetic system of Drosophila by postradiation treatment appears to be a possibility in immature germ cells. Postradiation modifications by gas posttreatments have been reported for immature male germ cells by SOBELS and TATES (1961) and in female oocytes by ABRAHAMSON (1959). The literature on the relationship of germ cell stage and radiation effects is extensive and a discussion of this aspect will appear with data more related to this problem. More recent reviews of this aspect include those on mutation rates in meiotic stages by WHITING (1961) and on genetical protection by CONGER (1960). Several aspects of chromosome breakage and recovery have been included in an extensive and interesting review by EVANS (1962).

SUMMARY

An increase in biological damage in sperm treated in inseminated females was observed after treatment with either neutrons or X rays. Recovery mechanisms cannot account for the high sensitivity with neutron treatment. The absence of recovery mechanisms was indicated by equal percentages of biological damage observed the first and second day after treatment with 2 Mev neutrons in air or with 14 Mev neutrons in nitrogen.

With X-ray treatment, higher percentages of translocations and sex-linked lethals were observed in the treatment of sperm in inseminated females than in males. The enhancement in chromosome breakage, measured as translocations, was greater in sperm treated in fertilized females than the increase in sex-linked lethals with X-ray treatment in air. The presence of oxygen (air) appears to be correlated with the enhancement in chromosome breakage in sperm treated in fertilized females. In nitrogen, the relative increases in translocations and sex-linked lethals in sperm treated in females were similar for comparisons of two-day totals.

The possibility of a postradiation enhancement of radiation damage was observed when mature sperm were treated in inseminated females with X rays in the presence of nitrogen gas. The percentages of translocations and sex-linked lethals were higher in the second day after treatment than the damage for the first day.

The data indicate that an explanation for the increase in radiation sensitivity of mature sperm treated in fertilized females involves more than one factor, the relative importance depending upon the type of radiation and conditions at the time of irradiation. The neutron data show that the chromosomes change in some way to become more sensitive to radiation injury after fertilization. The X-ray data indicate that there may be differences in recovery occurring in genetic damage in sperm treated in males and inseminated females and that there is an increase in chromosome breakage which is associated with the increased sensitivity. There is also a correlation of an increase in chromosome breakage and the presence of oxygen and a possible postradiation enhancement of radiation damage which may be associated with the presence or absence of oxygen.

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