INFLUENCE OF OXYGEN, HELIUM, AND METABOLIC INHIBITION ON X-RAY INDUCED DOMINANT LETHALITY IN STAGE 7 AND STAGE 14 OOCYTES OF *DROSOPHILA MELANOGASTER*

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DOSE fractionation, centrifugation, or metabolic inhibition studies of X-ray induced dominant lethality (PARKER 1959) or detachment of attached-X chromosomes (PARKER 1959; PARKER and HAMMOND 1958) in Stage 7 and Stage 14 primary oocytes (KING, RUBINSON, and SMITH 1956) of *Drosophila melanogaster* have demonstrated that a significant amount of the X-radiation damage can be repaired within 15 minutes in Stage 7 oocytes. No repair was noted in similar studies of Stage 14 oocytes, during the approximately 24 hours between irradiation and fertilization. For this stage, repair may be associated with fertilization (PARKER 1959). There is also a large difference in the relative radiosensitivity of the two types of cells. The LD₅₀ for Stage 7 oocytes is approximately 3000r, for Stage 14 oocytes, 300r. The high sensitivity and "one-hit" survival curve of Stage 14 as contrasted with the low sensitivity and "multi-hit" curve for Stage 7 oocytes has been attributed to the difference in repair (PARKER 1959, 1963; PARKER and HAMMOND 1958; ABRAHAMSON 1961). Primary breaks in the two types of cells may reconstitute in the original configurations, rejoin illegitimately with an independently produced break, or perhaps may not be repaired. The first event leads to no observable effect, the second to a multi-hit dose response, while the third is a one-hit event. PARKER (1963) argues that rapid repair of damage in Stage 7 oocytes brings about primarily the first two events, while anaphase I, which may intervene between breakage and repair in Stage 14 cells, results in breaks spatially separated at the time of repair in Stage 14 oocytes so that the third event is predominant.

ABRAHAMSON (1959) studied the effect of various oxygen tensions on X-ray damage in oocytes of *D. melanogaster*. He reported that anoxia between fractionated X-ray doses caused an increase in rearrangements in Stage 7 cells over that observed when air was present between the irradiations. Further, giving nitrogen for one hour to Stage 7 oocytes immediately after irradiation caused an increased rearrangement frequency. SOBELS (1961) found that cyanide, a metabolic inhibitor, given after irradiation of *D. melanogaster* males caused an increase in translocation frequencies in spermatids over that observed when no posttreatment was given.

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No posttreatment effect is found in *Vicia faba* (WOLFF and ATWOOD 1954) or *Tradescantia* (WOLFF and LUIPPOLD 1958); in this material postirradiation anoxia only delays the reunion of X-ray induced breaks, and the frequency of chromosome interchanges is dependent upon the number of breaks in the cell at the time of rejoining but is independent of the length of time that breaks stay open. Furthermore, cellular metabolism is necessary for the repair of broken chromosomes, and inhibition of cellular metabolism delays reunion of breaks, leaving them available for interaction with subsequently formed ones (WOLFF 1956; WOLFF and LUIPPOLD 1955).

FALK (1961) also found no increase in translocations as a result of anoxia following a single X-ray exposure to spermatids irradiated in *D. melanogaster* pupae. He did, however, observe an increase when the radiation was administered following prolonged anoxia; he attributed this increase to a delay in spermiogenic development so that radiosensitive spermatids were the most mature germ cells present at the time of irradiation, rather than the more resistant later stages ordinarily present at the particular pupal age studied. OSTER (1957a,b) demonstrated a differential response of irradiated sperm and spermatids to the oxygen tension at the time of irradiation. He interpreted this as an indication that sperm have a lower intrinsic intracellular oxygen tension than spermatids. These observations indicate that both modifications in the cell sample and differential cellular oxygen concentrations are variables that must be considered when anoxia is used as a tool in analyzing radiation-induced X-ray damage.

The series of experiments reported in this paper were designed to test the effects of various oxygen tensions given before, during, and after the X-irradiation of adult females on the survival of Stage 7 and 14 oocytes. It is concluded that no major difference exists between these stages in response to the oxygen tension at the time of radiation. Studies with postirradiation and prolonged preirradiation anoxia, however, provide evidence for postirradiation repair of potential dominant lethal damage in Stage 7 but not in Stage 14 oocytes.

MATERIALS AND METHODS

The techniques used for irradiating and collecting homogeneous oocyte samples are based upon cytological observations of oogenesis reporting by KING *et al.* (1956) and on methods described by PARKER (1959). *Drosophila* females contain approximately 24 ovarioles. In newly emerged females, the most mature oocyte present in each ovariole is Stage 7. Flies matured for more than 2 days usually contain one Stage 14 oocyte per ovariole. Either newly emerged (zero to 5 hr) or matured (4½ to 5 day) Oregon-R females were irradiated so that either Stage 7 or 14 primary oocytes were the most mature cells treated. After irradiation the flies were mated individually with two Oregon-R males that had been matured 3 to 4 days. The males and females were transferred to fresh food every 12 hours, and the eggs laid during these periods were counted. Only egg samples of 24 or fewer eggs per female were included in the data so that the number of eggs sampled did not exceed the number of oocytes in the desired stage during irradiation. In all experiments the flies were kept on standard *Drosophila* cornmeal food to which bakers' black paste dye had been added. This dark background greatly facilitates egg-counting and has no effect upon the development of the eggs. It is estimated from the work of KING *et al.* (1956) that these irradiation and egg-collecting techniques give samples of eggs of which 85 percent or more had been treated in the desired stage.

Females were treated in a gas-tight Lucite chamber (checked at greater than 100 psi), through which the flow of gas could be regulated. Since no methods are available for estimating the internal gas concentrations of the oocytes, all concentrations reported in these experiments refer to ambient concentrations. Helium treatments are assumed to have no effect other than the production of anoxia. Inlet and outlet valves on the chamber were constructed so that almost immediate replacement of the atmosphere could be achieved. Therefore, the gas posttreatments were generally started within 15 seconds after the X-ray exposure was completed. The gases used in all of these tests were bubbled through fritted glass tubes submerged in water so that the humidity within the X-ray chamber remained high during every experiment. Wet and dry bulb thermometer readings indicated that the relative humidity under these conditions was no less than 85 percent.

By packing ice around the treatment chamber, except for the window through which the X rays were delivered, a temperature of $4 \pm 1^\circ\text{C}$ could be maintained throughout a treatment. A temperature of $24 \pm 1^\circ\text{C}$ was used in the other tests.

A General Electric Maxitron 250 machine was used for irradiation, operating under the following conditions: 250 KVP; 30 ma; 3 mm aluminum filtration, HVL 0.45 mm copper. Exposure rates were checked before each test with a Victoreen r-meter and were varied by altering the distance of the flies from the target. An exposure of 300r was used in all Stage 14 oocyte experiments and was delivered at approximately 60r per min. An exposure rate of 600r per min was used to deliver 3000r to the Stage 7 oocytes. The quality of the X rays was constant in all experiments.

The females were mated and the eggs were collected. The ratio of the number of larvae pupating to the total number of eggs laid gave the survival rate for each experiment. Concurrently with the experimental groups, control flies were given identical gas and temperature treatments but no X-ray exposure. Since some eggs failed to develop because of factors other than the interaction of the gas and X-ray conditions, a correction was made by dividing the survival of the irradiated groups by that of the control. The survival of the control, whether air, oxygen, or helium was the irradiation environment, was 90 ± 3 percent, except where an extended helium treatment was given to Stage 14 cells. This exception is noted in the results. Standard errors, which include the variability of both the X-ray and the control experiments, are given for these corrected survival rates.

RESULTS AND DISCUSSION

Table 1 shows the survival of Stage 7 and 14 oocytes irradiated in various oxygen concentrations. Substitution of oxygen for air during irradiation decreases

TABLE 1

The relative radiosensitivity of Stage 7 and 14 oocytes irradiated in various oxygen tensions

Experimental conditions*	Stage 7		Stage 14	
	Number of eggs	Percent survival $\pm$ SE	Number of eggs	Percent survival $\pm$ SE
Air, 24°C	2995	49.9 ± 1.43	2548	51.4 ± 1.44
Air, 4°C	1421	44.4 ± 1.94	1490	42.6 ± 1.83
Oxygen 10 min, 24°C	1718	39.8 ± 1.78	1636	41.9 ± 1.84
Oxygen 60 min, 24°C	1627	45.9 ± 1.92	1630	40.8 ± 1.67
Oxygen 60 min, 4°C	1786	42.1 ± 1.87	3311	38.4 ± 1.15
Helium 10 min, 24°C	1661	85.3 ± 2.00	1540	79.4 ± 1.59
Helium 60 min, 24°C	2441	82.8 ± 1.52	1037	80.3 ± 2.25
Helium 60 min, 4°C	1490	86.9 ± 1.89	1294	84.4 ± 1.94

* X rays were delivered during last 5 minutes of gas and temperature treatment. Stage 7 oocyte X-ray exposure, 3000r; Stage 14, 300r.

survival to approximately the same extent in the two stages. Reducing enzymatic removal of oxygen by lowering the temperature to 4° during irradiation decreases survival in air or oxygen for both stages. The intracellular oxygen should have been nearly maximal under these conditions, and the survival rates should represent almost the maximum effect that can be produced at this dose by increasing the primary damage. If under other conditions there is a significant decrease in survival below these levels, then factors other than increased primary damage should be considered as possible explanations. When helium was substituted for air, the increase in survival was about the same for the two oocyte stages. Stage 7 or 14 oocytes irradiated in helium following 1 hr of helium treatment at 4°C showed a survival the same as or perhaps slightly lower than those irradiated in helium following 1 hr of helium treatment at 24°C (see also GILES, BEATTY and RILEY 1951). Thus it seems that lowering the temperature during irradiation increases only the oxygen-dependent damage, and this is reasonable since lowering the temperature reduces the metabolic rate and concomitantly increases the accumulated unmetabolized oxygen.

Table 2 shows the effects of various durations of helium pretreatment on the survival of both Stage 7 and 14 oocytes irradiated in helium. Stage 7 cells become anoxic almost immediately, the full protective effect of helium being manifest after 5 minutes treatment. There is no further change in the survival of these Stage 7 oocytes with increasing intervals up to 1 hr of helium pretreatment. A slightly lowered survival rate is associated with flies irradiated after 1 hr of anoxia, and survival is decreased more as the pretreatment time is increased to 6 hours, and then to 14 hours. This is not a lowering of survival caused by the gas alone, since the survival of oocytes from flies receiving similar gas treatments, but no X rays, was approximately the same (91 percent) as was noted in tests in which either air or shorter helium treatments were used. Further, all X-ray tests have been corrected for any differences in the survival of the control cells. Approximately one percent of the 0 to 5-hour-old flies given helium pretreatments

TABLE 2

Effect of anoxia prior to X-irradiation on survival of Stage 7 and 14 oocytes

Time of treatment in helium*	Stage 7			Stage 14		
	Number of eggs	Percent survival $\pm$ se		Number of eggs	Percent survival $\pm$ se	
0	2995	49.9	1.43	2548	51.4	1.44
5 min	3199	85.4	1.26	2744	74.3	1.35
10 min				1540	79.4	1.60
15 min	1661	85.3	2.01	1313	77.0	1.81
30 min	1280	84.8	1.98	1386	80.8	1.85
60 min	2441	82.8	1.52	1037	80.0	2.19
2 hours				1199	78.7	2.86
6 hours	3810	72.0	1.47			
14 hours	1250	58.4	2.33			

* X-rays were delivered during last 5 minutes of treatment. Stage 7 oocyte X-ray exposure, 3000r; Stage 14, 300r.

of one hour or less died. However, approximately 10 percent of the newly emerged females treated 6 hours with helium, and 30 percent of those given 14 hours of anoxia, died in both the X-ray and control tests.

It can be seen in Table 2 that Stage 14 oocytes require approximately 10 minutes pretreatment with helium to reach the plateau level of survival and that they maintain this level following pretreatments of up to 2 hours duration. Approximately 50 percent of the flies matured for 4½ to 5 days died when they were treated with helium for 2 hours, and almost 100 percent died after longer anoxic treatments. The survival of nonirradiated Stage 14 cells that were helium-pretreated for 1 hr or less was normal (approximately 92 percent). In the tests in which 2 hours of helium was used as a pretreatment, the control survival rate was 80.2 percent. The radiosensitivity of the survivors of the 2-hour helium treatment is comparable to the sensitivity of Stage 14 cells given shorter intervals of helium prior to their irradiation. This is demonstrated by the similar survival rates of the X-irradiated germ cells when their survival is corrected for non-induced lethality. These data demonstrate that within the measurable limits, i.e., a 2 hour helium treatment, there is no decrease in survival of the irradiated Stage 14 oocytes that can be attributed to an interaction of the preirradiation anoxia and the X radiation.

Some of the characteristics of the response of the X-ray sensitivity of Stage 7 oocytes to prolonged helium pretreatment are shown in Figure 1. The sensitiza-

FIGURE 1.—Effect of helium given immediately before or after 3000r of X-irradiation on survival of Stage 7 oocytes. Broken lines, X rays delivered in helium; solid lines, X rays delivered in air; O, helium pretreatment at 23 to 25°C; Δ, helium posttreatment at 23 to 25°C; □, helium pretreatment at 3 to 5°C. Confidence intervals ($\pm 2 \times \text{SE}$) are included at each point.

tion associated with increasing time of helium pretreatment suggests that anaerobically there is a gradual shift in some cellular component that either causes more X-ray lesions to be produced or causes more of the lesions to result in dominant lethality. A similar sensitization of Stage 7 oocytes is observed to result from helium posttreatment; since posttreatment cannot affect the number of primary lesions produced by the radiation, it may be concluded that posttreatment (and inferentially, pretreatment) increases the probability that the primary lesions will result in measurable genetic damage. The magnitude of the sensitization is the same whether the lesions are produced in air or in helium. This is demonstrated by the 1.86-fold increase in dominant lethality of anoxically irradiated Stage 7 oocytes pretreated with 6 hours of helium compared with the 1.80-fold increase in those irradiated in air following 6 hours of anoxia. Reducing the temperature to 4°C during the helium pretreatment drastically reduces the rate of sensitization. Therefore, from Figure 1 it can be stated that storage in helium causes a temperature-dependent change in the constitution of Stage 7 oocytes. The result of this change is to increase the probability that X-ray-induced lesions will result in egg mortality.

Figure 2 shows that the sensitization effected by helium treatment in Stage 7 oocytes is reversible in air. These tests, in which the cells were allowed to recover from the effects of 6 hours of helium pretreatment prior to their irradiation, demonstrate that no irreparable damage had been induced in the oocytes by anoxia. It is assumed that with recovery in air, the initial condition of the oocytes, as regards X-ray sensitivity, is reestablished. The facts that both egg production and morphology, as well as survival, are the same before helium treatment and after recovery from helium treatment, indicate that the gas does not modify oocytes so that the stages that are more sensitive to X rays are sampled with the mating techniques. FALK (1961) has postulated that spermatid samples are modified in this manner to explain their increased radiosensitivity following anoxic pretreatment.

FIGURE 2.—Survival of Stage 7 oocytes pretreated with 6 hours helium and irradiated following various intervals of recovery in air. Confidence intervals ($\pm 2 \times \text{SE}$) are included at each point.

BEATTY and BEATTY (1957) found that helium given prior to the X-irradiation of *Tradescantia* microspores increased the frequency of the aberrations recovered. Their results were interpreted as indicating that helium pretreatment depleted energy necessary for the restitution of broken chromosomes. They further demonstrated (1960) an increased aberration yield in *Tradescantia* given post-irradiation anoxia, which also is presumed to lower the amount of energy available. The enhancement of X-ray damage associated with cyanide posttreatments of spermatids in *Drosophila* males was also explained in terms of blockage of metabolically dependent processes (SOBELS 1961). ABRAHAMSON (1961) suggested that in X-irradiated *Drosophila* females anoxia may be acting in several ways: it may influence either the rejoining frequency by preventing restitution, the breakage frequency by allowing potential breaks to become real breaks, or the chromosome structure itself by permitting the healing of broken chromosome ends.

Observations presented in the present paper suggest that under anaerobic conditions there is a progressive change in concentration of some cellular component in Stage 7 oocytes and that this change is reversible upon reestablishment of aerobic conditions. Further, the change associated with oxygen deprivation is not apparent when helium is administered at 4°C. The observations are compatible with the hypothesis that a component of a repair system, perhaps a metabolite or an enzyme, is depleted anaerobically and that oxygen is required for its resynthesis. Implicit in this hypothesis is that delay of repair results in increased damage; that is, that primary X-ray lesions become increasingly committed to the formation of damage with time. If it is assumed that dominant lethality results from chromosome aberrations, we can postulate that chromosome movement intervening between breakage and repair leads to increased chromosome rearrangement and decreased restitution.

The observation that chromosome rejoining in Stage 14 oocytes may be delayed until fertilization (PARKER 1959) indicates that there is no active system for the repair of chromosome breaks in these cells. Further, the observation that a 2-hr helium treatment produces no appreciable sensitization of Stage 14 oocytes to irradiation is in agreement with what we expect if the effect of prolonged anoxia is depletion of an existent repair system.

Conclusions: There is a system for repair of X-ray damage in the Stage 7 oocyte which is maintained at a functional level by an oxygen-requiring process. In the absence of oxygen the repair system is degraded at a rate that is measurable at 24°C but not at 4°C. Administration of oxygen to cells whose repair system has been depleted by oxygen deprivation results in regeneration of the system.

Under anaerobic conditions where the repair system is inoperative, the primary X-ray lesions become committed to the formation of measurable genetic damage. Thus a portion of lesions that would ordinarily reconstitute become committed to the formation of genetic damage if repair is inhibited; then when repair resumes, an increased amount of damage will be observed. It is postulated that the primary lesion of dominant lethality is chromosome breakage, and that when rejoining is

prevented, chromosome movement increases the probability of illegitimate rejoining at the expense of restitution in the original configuration.

This repair system is virtually inoperative in Stage 14 oocytes until after fertilization, and it is postulated that the relation between time of induction of primary lesions, chromosome movement, and repair may provide a partial explanation of the greatly increased sensitivity of Stage 14 oocytes compared with Stage 7 oocytes, where repair ordinarily occurs within 30 minutes of the time of irradiation, and presumably before much chromosome movement.

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SUMMARY

Experiments designed to test the effects of various oxygen tensions given before, during, and after the X irradiation of adult females on the survival of Stage 7 and Stage 14 oocytes support the following conclusions: No significant differences in the relative radiosensitivity between Stage 7 and Stage 14 oocytes is attributable to differential amounts of intracellular oxygen. Extended helium pre- or posttreatment causes a significant increase in X-ray induced dominant lethality in Stage 7 oocytes. This increase in lethality is proportional to the duration of anoxia. No such sensitizing effect of helium treatment is observed with the Stage 14 cells. The effect of anoxia in increasing dominant lethality is prevented when the flies are given anoxic treatments at 4°C. The effects of anoxic pretreatment are reversed when the cells are allowed to recover in air prior to irradiation. The results are interpretable in terms of a system that repairs chromosome breaks and that is depleted during prolonged anoxia.

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