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Hyperthermia and Radiation Induced Chromosome Loss in Anoxia in Drosophila

Sidney Mittler
Department of Biological Sciences
Northern Illinois University
DeKalb, Illinois 60115 USA

ABSTRACT

Male Drosophila *Oregon R*, and the mutagen sensitive strains, *y mei-9*^{a} and *y mei-9*^{a}*mei-41*^{D5} all with *B*^{s}*Yy*$^{+}$ were subjected to 42°C in anoxia and then irradiated with 2000 R of x-rays in anoxia. The hyperthermia treatment significantly increased the radiation induced loss of chromosomes in *Oregon R* in 5 out of 12 broods (two in CO_2 and three in N_2 series), but none in the broods of *y mei-9*^{a} and only in one of *y mei-9*^{a}*mei-41*^{D5}. The failure of excision repair deficient strains to be affected by hyperthermia in anoxia in increasing radiation induced genetic damage may signify that hyperthermia may decrease the efficiency of repair enzymes.

INTRODUCTION

The influence of hyperthermia on the enhancement of radiation induced damage has rapidly led to numerous investigations utilizing heat treatment in cancer therapy. The mechanisms of the radiation sensitization by the heat treatment is not fully understood. The impairment of repair enzymes by the hyperthermia has been suggested for the increase in damage to DNA and chromosomes (Dewey, Sapareto and Betten, 1977; Dube, Seal and Loeb, 1977; Corry, Robinson and Getz, 1977; and Mittler, 1980, 1981). Hyperthermia increased radiation induced chromosome loss, recessive and dominant lethals in *Drosophila melanogaster* (Mittler, 1979a, 1979b). Mittler (1981) utilized mutagen sensitive stocks which were deficient in several DNA repair pathways and reported that the *mei-9*^{a} strain deficient in excision repair did not respond to the increase in radiation induced chromosome loss as a result of pretreatment by hyperthermia. This may indicate that hyperthermia may affect the excision repair system. The investigation presented here was to study whether the hyperthermia would influence radiation induced chromosome loss in spermatogenesis of wild type and mutagen sensitive strains (with defective repair pathways) if the hyperthermia treatment and radiation were given in anoxia.

MATERIALS AND METHODS

Drosophila melanogaster males containing a Y chromosome with translocation of y^+ and B^s of the mutagen-sensitive strain *y mei-9*^{a}, the double mutants *y mei-9*^{a}*mei-41*^{D5}, and the wild type *Oregon R* stock were used to determine whether hyperthermia would have an effect on radiation induced chromosome loss in anoxia. Boyd and Setlow (1976) have proposed four tentative classes of DNA repair pathways for mutagen-sensitive Drosophila mutants. *mei-41*^{A1} was assigned to Class 1 (postreplication repair deficient, meiotic defective, residual postreplication repair not strongly sensitive to caffeine). *mei-9*^{a} was assigned to Class 3 in which the mutants are postreplication proficient with DNA metabolism partially caffeine sensitive and *mei-9*^{a} is also deficient in excision repair (Boyd, Golino and Setlow, 1971). The males were kept in anoxia either in N_2 or CO_2 for 12 minutes in a circular leucite container machined so that the two halves were grooved to contain four No. 000 gelatin capsules and designed to permit gases to pass. One day old adult males were placed in the capsules that had holes at either end and put into a leucite container with 500 ml/min CO_2, N_2 or air flowing through and kept submerged in a water bath at 42°C for 7 minutes for the hyperthermia exposure. With the gas still flowing the leucite container was lifted out of the water bath and placed in a G.E. Maximar III unit and was exposed to 2000 R of x-rays within 30 seconds at 250 KV, 15 ma, 32 cm at 419 R/min. The time for the hyperthermia plus radiation treatments totaled about 12 minutes and about 90% of the flies survived this treatment. Although adult Drosophila can survive 1 hour in anoxia and a half hour at 42°C in air, the combination of hyperthermia and anoxia sharply reduced the time that adult males could survive. The controls were kept in anoxia for 12 minutes, along with those groups of flies that received only hyperthermia or radiation. The males were then mated to y^2w^{ap}(1 ♂ : 3♀♀) and transferred every two days to a virgin group of females for 6 or 7 two day broods. This brooding tended to separate the effects on the various stages of spermatogenesis at the time of irradiation (Auerbach, 1954) in that offspring produced in 0-2 day brood represented mature or almost mature spermotozoa, 2-4 day brood represented late spermatids, while early spermatids by 4-6 day brood, 6-8 day broods represented spermatocytes, 8-10, 10-12, 12-14 day broods represented spermatogonia at the time of irradiation (Mittler, 1966).

The loss of the X or Y chromosomes in spermatogenesis were detected in the F_1 offspring by appearance of exceptional phenotypes. The loss of the X or Y resulted in XO males, y^2w^{ap}, yellow white speckled eye; the loss of the B^s translocation in a round eye wild body male; and the loss of the y^+ in a yellow body Bar eye male. Paternal nondisjunction was detected by the appearance of a wild body, Bar eye female. Each series was rerun at least three times and data pooled, with more males used in the radiation and the hyperthermia plus radiation experiments because of induced sterility.

RESULTS

The data obtained by hyperthermia and radiation in N_2 for *Oregon R*, *y mei-9*^{a} and *y mei-9*^{a}*mei-41*^{D5} strains are presented in Figure 1 and the data for radiation and hyperthermia in air and CO_2 for these strains are plotted in Figure 2. The double mutagen-sensitive strain y *mei-9*^{a}*mei-41*^{D5} is the most sensitive of the three to radiation induced chromosome loss in N_2 (Figure 1c) and in air and CO_2 (Figure

2c). The 2000 R of x-rays induced sterility in the males of both of the mutagen sensitive strains in the spermatogonia represented by the 8-10 and 10-12 day broods.

In the wild type strain *Oregon R*, the hyperthermia significantly increased the radiation induced chromosome loss in air in the spermatogonia, 8-10 and 10-12 day broods (Figure 2a). With respect to irradiation in CO_2, chromosome loss was significantly increased by hyperthermia treatment in broods 6-8 and 10-12 days (Figure 2a). The hyperthermia treatment significantly increased the radiation chromosome loss in N_2 broods 4-6, 8-10, and 10-12 days in *Oregon R* (Figure 1a).

The hyperthermia treatment in air, N_2 or CO_2 did not significantly increase the radiation induced chromosome loss in any of the stages of spermatogenesis of the mutant *y mei-9*^{a}. In the double mutant *y mei-9*^{a}*mei-41*^{D5} the hyperthermia treatment in the air series only increased the radiation induced chromosome loss in one brood, 0-2 days, which represented the mature or almost mature spermatozoa. There was no significant increase by the hyperthermia treatment in radiation induced chromosome loss in any of the broods in the CO_2 and N_2 series in *y mei-9*^{a}*mei-41*^{D5} stock. The 42°C treatment for 7 minutes did not significantly induce chromosome loss in any of the broods of the three stocks studied when given in CO_2 or N_2.

DISCUSSION

The radiation treatment of repair deficient mutants with 2000 R of x-rays in air and in anoxia severely reduced the number of offspring in those broods, 8-10, 10-12, 12-14 days which represented spermatogonial cells at the time of radiation. This did not occur in the spermatogonial cells of the wild type *Oregon R*. The hyperthermia treatment in anoxia (but not in air) reduced the number of spermatogonial cells of the repair deficient mutants. The reduction in offspring was not as severe when the heat treatment was given in air. There was no difference in the radiation induced loss of chromosome whether CO_2 or N_2 was used for the method of anoxia in the three strains.

The pretreatment with 42°C and irradiation of the wild *Oregon R* significantly increased the loss of chromosomes in spermatogenesis induced by radiation in 5 out of 12 possible broods in anoxia (two in CO_2 and three in the N_2 series). Similar hyperthermia treatments in anoxia did not increase radiation induced chromosome loss in any of the 22 broods produced by the two repair deficient strains. There were also no significant increases produced by hyperthermia on radiation induced chromosome loss in *y mei-9*^{a}, the excision repair deficient strain, in air. This is in agreement with the report by Mittler (1981) that the mutant, *y mei-9*^{a} did not respond to the hyperthermia radiosensitization in air. Since hyperthermia increased radiation induced chromosome loss in mutants of other repair pathways and in wild type Drosophila, this led to a hypothesis that the hyperthermia treatment could not impair an already inherited mutant block. The failure of the repair deficient strains to respond to hyperthermia radiosensitization in anoxia also tends to strengthen the hypothesis that hyperthermia may inactivate the repair enzymes. There was also a possibility that excision repair mutants were so sensitive to radiation that this sensitivity overrode whatever the effect of hyperthermia would add to the increase in radiation induced loss.

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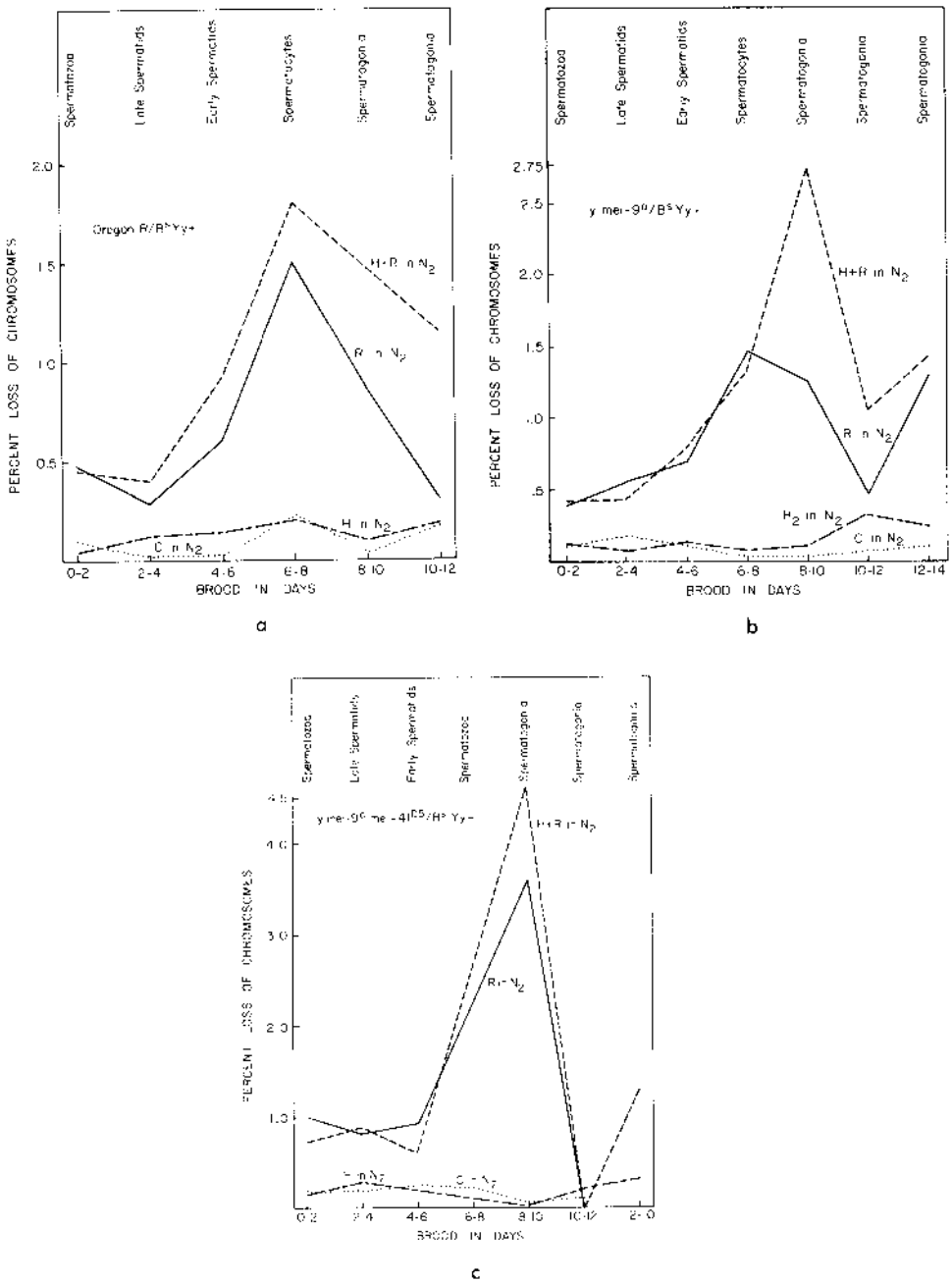

Fig. 1. The effect of hyperthermia upon radiation induced loss of chromosomes in spermatogenesis of (a) Oregon R/B^sYy^+, (b) y mei-9^a/B^sYy^-, and (c) y mei-9^amei-41^{D5}/B^sYy^+ when radiation of 2000R of x-rays and hyperthermia of 42°C were given in N_2.

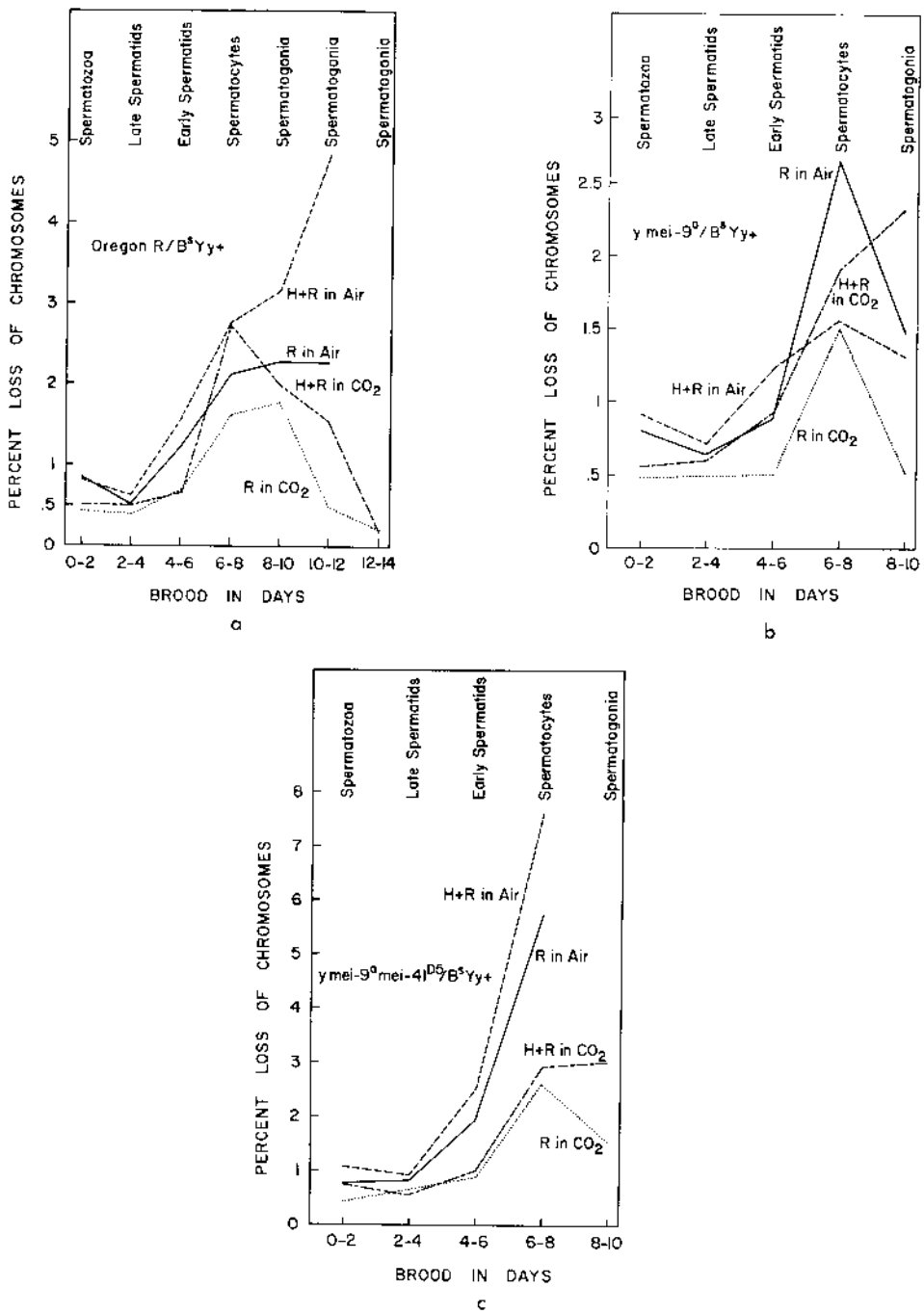

Fig. 2. The effect of hyperthermia upon radiation induced loss of chromosomes in spermatogenesis of (a) Oregon R/B^{S}Yy^{+}, (b) y mei-9^{a}/B^{S}Yy^{-}, and (c) y mei-9^{a}mei-41^{D5}/B^{S}Yy^{+} when radiation of 2000R of x-rays and hyperthermia of 42°C were given in air or CO_2.