ADENOSINE TRIPHOSPHATE AND RADIATION-INDUCED CHROMOSOMAL ABERRATIONS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT.—Adenosine triphosphate (5 mg/ml) injected into male *Drosophila melanogaster* which were mated daily for twelve days decreased the percentage of x-ray induced dominant lethals in broods assumed to represent immature spermatozoa and spermatids. In four out of the twelve broods, there was a significantly lower percentage of dominant lethals (determined by the failure of the eggs to hatch after 24 hours). However, if the ATP-injected males were irradiated in air and posttreated in nitrogen, dominant lethal frequency was reduced only in the brood presumed to represent spermatids at the time of irradiation. The injection of ATP prior to radiation into males that were irradiated in nitrogen and post-treated in nitrogen, reduced the percentage of dominant lethals in four broods (which represented spermatids and spermatogonia at the time of irradiation). When the males were injected with ATP, irradiated in nitrogen and then permitted to recover in air, the dominant lethals were reduced in two broods (late spermatids and spermatocytes) but increased in two other broods (early spermatids and cells in first meiotic division). The exogenous ATP injections did not reduce the frequency of radiation induced sex-linked recessive lethals or translocations between 2nd and 3rd chromosomes. ATP injections did not reduce radiation induced deletion of the X chromosome or induced crossing-over in males.

The data gathered by many researchers in the last decade and a half have indicated that some radiation-induced genetic injury is reparable. Among the pioneer workers in this field, Lea (1946) calculated that 95% of the chromosomes broken by radiation rejoin. Numerous researchers have since employed various pre- and postradiation treatments with gasses and chemicals and have also used fractionation and dose rate experiments, from these studies it was interpreted that radiation-induced genetic damage was not irreversibly fixed and that a repair system existed. Treatments which decreased metabolism in the postradiation period such as carbon monoxide, anaerobiosis, cyanide, inhibited rejoining of radiation-induced chromosomal breaks in plants (Wolff and Luippold, 1955). Sobels (1964) reported that postradiation treatment with cyanide or N_2 inhibited a repair process thereby increasing the yield of mutations in spermatids of Drosophila. Wolff and Luippold (1955) believed that the increase in genetic damage was a result of an inhibition of a repair process which required energy from oxidative respiration to function properly. Indeed, adenosine triphosphate, (ATP) was a product of oxidative metabolism and when used exogenously did reduce radiation-induced chromosome aberrations in *Vicia faba* (Wolff and Luippold, 1955), in microspore division of *Tradescantia* (Beatty and Beatty, 1960, 1966) and in *Trillium* (Iwabuchii, Saho, Tanifuji, 1966) and did prevent the loss of X chromosomes in spermatogenesis of *D. melanogaster* (Mittler and U, 1966).

The work presented was the result of an investigation to determine whether exogenous ATP would reduce the frequency of radiation-induced recessive sex-linked lethals, dominant lethals, deletions of the X chromosome, translocations between chromosomes 2 and 3 and crossing-over in the spermatogenesis of *D. melcnogaster*.

MATERIALS AND METHODS

The male flies were irradiated in No. 000 perforated gelatin capsules with x-rays from a G. E. Maximar 250-III unit at 150 KV, 15 ma, 35 cm distance, at a rate of 140 R/min as determined by a Victoreen Model 570 condenser R-meter with 100R and 250R chambers. Approximately 0.1 μl (as determined by increase in weight) of either 0.85% NaCl, (controls) or 5 mg of ATP/ml in 0.85% NaCl was injected into the dorsal region between the 3rd and 4th tergites with a needle drawn to a tip of approximately 0.06 mm. The ATP was obtained as crystalline disodium from Nutritional Biochemicals Corp. The amount of ATP injected was equivalent to 666 mg of ATP/kg of Drosophila. The males were mated at a ratio of one to three females and transferred to new groups of females daily (every two days in the deletion test). By this "brood method" the effect of radiation on the cells in various stages of spermatogenesis could be ascertained, for the 0-1 day brood represented mature spermatozoa at the time of the radiation exposure, while those offspring from the 5-7 day broods represented cells in meiosis and broods from 8-12 days represented spermatogonia (Auerbach, 1954, Lüning, 1952).

The recessive sex-linked lethals and translocations were detected by the mating of y sc^{Sl} In dl-49 sc^{8}; bw; st p^{p} females to injected $X^{c2}yB/Y$ sc^{8} y+ males. The adult males were 2-6 hours old when injected and irradiated with 1000R or 1600R of x-rays in air. The F_1 males were backcrossed to virgin y sc^{Sl} In dl-49 sc^{8}; bw; st p^{p} females. The absence of brown and scarlet-pink to occur in the F_2 offspring indicated a 2-3 translocation. The F_1 females mated individually, and the absence of any Bar-eyed males in a culture of at least ten F_2 males indicated a recessive lethal on the ring X chromosome. The genetic markers of stocks used are fully described by Lindsley and Grell (1967).

The deletion of the X chromosomes was obtained by mating 2-6 hr. old Oregon-R males which had been injected and irradiated at 2000R to y cv v f/Y; K-pn ca/+ females. These females were obtained by an automatic virgin female technique from a cross between y cv v f; K-pn ca with *pn* males, in which only females were produced. A large deletion would result in inviable males, but could be detected in attached -X females by the presence of exceptional females. These flies would have a wild body color and the absence of one or more of the three mutants due to the presence of the dominant wild alleles in the deleted X. A whole X chromosome in combination with the attached X was usually lethal, although if these meta females emerged, they could be distinguished easily by their crumpled wings and rough eyes.

Crossing-over was induced in ru h th st cu s e^{s} ca/+ males by irradiation after they were injected with ATP. The males were then mated at the ratio of 1 male to 3 females for days 1-3 and for days 4-6. On the seventh day the males were mated individually every day until day 15. There were two series of experiments: in one, males were injected with ATP or saline, irradiated and permitted to recover in air; in the other, males were injected, pretreated in N_2 for 30 minutes, then irradiated in N_2 and permitted to recover in air.

In the dominant lethal tests, 2-6 hr. old $X^{c2}yB/Y$ sc^{9} y+ males were injected either with saline or ATP, x-rayed with 1600R and then mated daily to *y w f* females. These were the same stocks in which exogenous ATP had been shown to aid in reducing the number of radiation induced XO males by preventing the loss of a ring chromosome (Mittler and U, 1966). In the series in which the males were irradiated in N_2 and

TABLE 1.—Effect of ATP pretreatment upon radiation-induced recessive sex-linked lethals and translocation in Drosophila males. Totals are of all broods of the sperm sampled from days 0 to 12.

Pretreatment	Percent of Recessive Lethal	Total Gametes	Percent of Translocation	Total Gametes
		1000R		
ATP	2.91	4841	2.74	877
0.85% NaCl (control)	2.70	4849	2.57	817
		1600R		
ATP	3.53	1924	4.79	1316
0.85% NaCl (control)	3.30	1876	3.50	1287

posttreated in N_2 three day old isogenic Oregon-R males were injected and irradiated with 1600R of x-rays. The mated females were isolated in plastic tubes having one end covered by a nylon mesh through which eggs could be deposited. This method was a modification of that used by Abrahamson and Herskowitz (1957) to prevent counting eggs from an unmated female. A cluster of 18 tubes was placed on agar-cornmeal media darkened with black-strap molasses in a 150 mm petri dish with no live brewer's yeast. Females were permitted to lay eggs for 48 hours after mating. In the first series, the males were injected with ATP or saline (control), irradiated in air and permitted to recover in air. In the second series the flies were irradiated in air after ATP or saline injection but were posttreated for 40 minutes in N_2. In the third series, the males were injected with ATP or saline pretreated with N_2 for 10 minutes, then irradiated in N_2 and posttreated in N_2 for 40 minutes. (A high purity grade of commercial N_2 flowing at a rate of 1500 ml/min was used in N_2 exposures.)

RESULTS

The frequency of sex-linked recessive lethals and translocations between chromosomes 2 and 3 induced by x-rays was not influenced by pretreatment with ATP (Table 1). There was no significant difference between the control and any of the daily broods at 1000R or 1600R. The sum totals of these daily broods are presented in Table 1. The lower dose was employed to obtain more offspring at the radiation induced semi-sterile period of 5-8 days after irradiation, for it was in this period of spermatogenesis that exogenous ATP did prevent chromosome loss in *Drosophila melanogaster* (Mittler and U, 1966).

The injection of exogenous ATP did not reduce the percentage of radiation induced deletion of X chromosome in daily broods (Table 2). A chi square larger than 3.85 as determined by a 2 x 2 contigency table was considered to be a significant difference.

TABLE 2.—Effect of ATP upon radiation-induced deletion of the X-chromosome after 2000R. Total is from all daily broods sampled 1 to 12 days after irradiation.

Pretreatment	Total Gametes	Percent of Deleted X chromosomes
ATP	12,536	2.47% $X^2 = 1.58$
0.85% NaCl (control)	9,415	1.59%

Inconclusive results were obtained with exogenous ATP and radiation induced crossing-over frequencies in

TABLE 3.—Crossing-over frequencies induced in $\frac{rh\ h\ th\ st\ cu\ s\ e^s\ ca}{+\ +\ +\ +\ +\ +\ +\ +}$ males irradiated at 3000 R.

Treatment	Brood day						
	9	10	11	12	13	14	15
ATP-air-R-air[1]	(0.0) 0/109	(1.87) 58/3108	(1.75) 29/1657	(0.63) 38/6005	(0.83) 90/10916	(1.09) 114/13229	(0.61) 62/10184
Saline-air-R-air	(0.0) 0/307	(2.06) 51/2471	(2.69) 21/782	(0.98) 48/4880	(1.93)[a] 131/6799	(0.65)[b] 58/8904	(0.67) 48/7217
ATP-N_2-R-air[2]	(0.47) 8/1695	(0.56) 18/3199	(0.42) 15/4325	(0.34) 18/5267	(0.67) 39/5807	(0.51) 18/3514	(0.0) 0/3281
Saline-N_2-R-air	(0.10) 1/1003	(0.71) 34/4765	(0.47) 18/3818	(0.28) 16/5636	(0.37) 26/7123	(0.27) 10/3665	(0.52)[c] 23/4386

[a]$X^2 = 40.429$ [b]$X^2 = 10.766$ [c]$X^2 = 15.548$

[1]Injection with ATP 5 mg/ml irradiated air and permitted to recover in air.

[2]Injection with ATP pretreated with N_2 for 30 and then irradiated in N_2 and permitted to recover in air.

TABLE 4.—Effect of 5 mg/ml of ATP injected in male Drosophila on radiation-induced dominant lethals. Radiation was 1600R of x-rays in air or nitrogen. The flies were permitted to recover in air or posttreated in N_2 for 40 minutes. The chi square was calculated by means of a 2 x 2 contingency table with Yates' correction.

Brood day	Injection	Radiation	Posttreatment	Total number of eggs	Percent undeveloped	X^2
0-1	ATP	air	air	2216	43.32	1.004
	Control	air	air	1273	41.73	
	ATP	air	N_2	354	62.43	1.022
	Control	air	N_2	428	58.88	
	ATP	N_2	N_2	1500	30.066	3.272
	Control	N_2	N_2	1377	35.602	
1-2	ATP	air	air	5051	40.74	4.454
	Control	air	air	3966	38.55	
	ATP	air	N_2	1191	31.07	.003
	Control	air	N_2	2262	31.17	
	ATP	N_2	N_2	4376	32.586	.0094
	Control	N_2	N_2	3204	32.771	
2-3	ATP	air	air	5177	37.14	99.776
	Control	air	air	5567	46.69	
	ATP	air	N_2	2177	47.08	23.39
	Control	air	N_2	5301	40.99	
	ATP	N_2	N_2	2327	26.69	14.49
	Control	N_2	N_2	3056	33.31	

TABLE 4.—Continued

Brood day	Injection	Radiation	Posttreatment	Total number of eggs	Percent undeveloped	X^2
3-4	ATP	air	air	3628	49.89	.473
	Control	air	air	4481	50.66	
	ATP	air	N_2	4695	48.41	.063
	Control	air	N_2	5571	48.66	
	ATP	N_2	N_2	4120	28.73	19.788
	Control	N_2	N_2	3964	35.19	
4-5	ATP	air	air	4654	52.13	58.189
	Control	air	air	3529	60.58	
	ATP	air	N_2	2300	57.87	15.274
	Control	air	N_2	3533	62.98	
	ATP	N_2	N_2	3878	39.53	15.501
	Control	N_2	N_2	3239	35.94	
5-6	ATP	air	air	3047	60.55	8.552
	Control	air	air	3416	64.08	
	ATP	air	N_2	1436	75.21	1.969
	Control	air	N_2	1787	73.03	
	ATP	N_2	N_2	3118	43.87	0.8286
	Control	N_2	N_2	3635	42.09	
6-7	ATP	air	air	1920	66.61	8.336
	Control	air	air	2198	70.79	
	ATP	air	N_2	1792	71.49	0.386
	Control	air	N_2	2481	72.27	
	ATP	N_2	N_2	2223	53.26	0.548
	Control	N_2	N_2	2197	51.20	
7-8	ATP	air	air	1704	58.69	0.509
	Control	air	air	1428	59.94	
	ATP	air	N_2	1784	74.55	0.386
	Control	air	N_2	2158	73.68	
	ATP	N_2	N_2	2380	61.26	15.21
	Control	N_2	N_2	2733	55.95	

TABLE 4.—Continued

Brood day	Injection	Radiation	Posttreatment	Total number of eggs	Percent undeveloped	X^2
	ATP	air	air	990	64.24	0.601
	Control	air	air	748	62.43	
8-9	ATP	air	N_2	986	69.27	3.273
	Control	air	N_2	1109	65.66	
	ATP	N_2	N_2	981	46.59	0.202
	Control	N_2	N_2	1441	48.27	
	ATP	air	air	890	49.21	1.47
	Control	air	air	611	52.54	
9-10	ATP	air	N_2	1488	56.99	63.755
	Control	air	N_2	2133	43.51	
	ATP	N_2	N_2	1637	40.20	0.386
	Control	N_2	N_2	1537	41.96	
	ATP	air	air	884	44.68	0.904
	Control	air	air	785	47.01	
10-11	ATP	air	N_2	997	44.73	0.00
	Control	air	N_2	1411	44.72	
	ATP	N_2	N_2	1257	28.24	5.67
	Control	N_2	N_2	1939	33.88	
	ATP	air	air	186	46.77	4.091
	Control	air	air	281	37.37	
11-12	ATP	air	N_2	1562	44.37	9.939
	Control	air	N_2	1984	39.11	
	ATP	N_2	N_2	1444	20.152	15.250
	Control	N_2	N_2	1512	28.108	

the male Drosophila (Table 3). The ATP did not have any effect on broods of day 10, 11 or 12, however, in the series irradiated in air, the males injected with ATP had significantly less crossing-over in brood of day 13, however, in brood of day 14, the males injected with saline had significantly less crossing-over. In the nitrogen-irradiated series, the brood of day 15 which was produced by the saline injected males had significantly more crossing-over than the brood of the ATP treated males.

In the dominant lethal series (Table 4), the injection of ATP before irradiation in air and recovery in air did significantly enhance the number of eggs that did develop in broods of day 2-3, 4-5, 5-6, 6-7, however, the number of eggs that failed to hatch was increased in broods of day 1-2

and 11-12. In the second group in which the pretreatment consisted of saline or ATP before irradiation in air and then followed immediately by posttreatment with N_2, the protection due to ATP was limited to brood day 4-5 which represented spermatids at the time of x-ray injury. The ATP injection did significantly increase the percentage of dominant lethals as compared to the controls in brood day 2-3 and 9-10. In the third group of dominant lethal experiments in which the males were irradiated and posttreated in N_2, the injections of ATP did significantly decrease the dominant lethals in brood day 2-3, 3-4, 10-11, and 11-12. However, there was an increase in dominant lethals frequency in brood day 4-5. Irradiation in N_2 and posttreatment in N_2 induced the males to produce a lower percentage of dominant lethals (as compared to the above two other groups) in ten out of the twelve daily broods.

The irradiation in air resulted in a greater percentage of dominant lethals than irradiation in N_2. This occurred in all broods and was expected. The Drosophila males that were irradiated in air and posttreated in nitrogen had an increase in percentage dominant lethals compared to the eggs that were fertilized by males irradiated in air and permitted to recover in air on brood day 0-1, 5-6, and 7-8 and decrease in dominant lethals in brood day 1-2.

Discussion

Exogenous ATP did not have any effect upon radiation induced sex-linked lethals frequencies in the ring X chromosome. These lethals were apt to be "point mutations" rather than large deletions which would have resulted in the loss of the ring chromosome. Sex-linked lethals in Drosophila which have been induced by x-ray treatment in nitrogen were reported by Sobels (1964) to be decreased in mature spermatozoa by N_2 posttreatments while O_2 posttreatment reduced the lethals in spermatids. ATP in the work reported here did not have any influence on any of the stages of spermatogenesis with respect to radiation induced recessive lethals. Several of the broods indicated that ATP did lower the percentage of induced dominant lethals. Either the radiation in air damaged the mutation repair system to such an extent that the additional exogenous ATP could not be utilized or that there may exist major differences between repair and recovery of dominant lethals and that of recessive lethal mutations with respect to ATP utilization. Biswas and Matsuo (1966) also reported that pretreatment with ATP did not alter the rate of x-ray induced chlorophyll mutants in rice.

The injection of ATP failed to prevent radiation induced translocations between chromosomes 2 and 3, and deletion of the X chromosome or crossing-over in male Drosophila. These aberrations involved breakage of chromosomes. Extra amounts of ATP (if it is indeed helpful) should have aided in the recovery and decreased the percentage of the aberrations. In the translocation and deletion tests, the radiation damage occurred in cells during the various stages of spermatogenesis but the presence of certain adult phenotypes many cell divisions later were used to assess the damage. In the experiments with recessive lethals, the mutations were detected two complete life cycles later. The long interval between the time of exposure to radiation and the assessment of the damage would eliminate any chromosome that was not "properly" repaired.

The data obtained from radiation-induced dominant lethal experiments does indicate that in some broods there was a reduction in a percent-

age of those radiation induced lethals induced in air and permitted to recover in air as the result of the ATP injections. These broods represented immature spermatozoa, spermatids and spermatocytes at the time of irradiation. The reduction in the induced dominant lethals can be attributed to the repair of broken chromosomes aided by ATP. This is in accordance with a hypothesis proposed by Wolff and Luippold (1955) that the chemical bonds formed in rejoining chromosomes broken by radiation required ATP as a source of energy. Exogenous ATP did decrease the time of reunion of broken chromosomes in the root tip of *Vicia faba*, while inhibition of ATP formation by dinitrophenol increased the time the broken chromosomes were apart (Wolff and Luippold, 1956). Beatty and Beatty (1960) also reported that exogenous ATP reduced radiation induced chromosomal aberrations in the meiosis of *Trandescantia*. Swabuchi, Saho, Tanifugi (1966) found that ATP treatment reduced radiation induced chromosome aberration in ovular tissue of *Trillium*.

When the males were injected with ATP irradiated in air and posttreated in N_2 only in one brood (spermatids) was the dominant lethals reduced. This was unexpected for if the repair of broken chromosomes was inhibited by anerobiosis and dependent upon oxidative metabolism with resulting ATP formation, then exogenous ATP could have aided in the repair process. Either the exogenous ATP was utilized in the repair process only if oxygen was present or the nitrogen posttreatment with the resulting increase in dominant lethals overwhelmed whatever recovery effect the ATP imported to dominant lethals so that the end result was no decrease in dominant lethals. The work of Sankaranarayanan (1967) indicated that nitrogen posttreatment of 30 min. as used in the above dominant lethal experiments would have no effect at least on mature spermatozoa with respect to an increase in dominant lethals.

The chromosome imbalance theory of dominant lethals leads one to think that all dominant lethals are due to chromosome breakage, loss or rearrangement. The prevention of the loss of chromosomes or deletions as the only means of reduction of dominant lethals may not present the entire picture of dominant lethals. Von Borstel and Rekemeyer (1959) reported that the majority of radiation-induced dominant lethals which resulted in an early death of the embryos of Drosophila and Habrobracon was not similar to those that were produced genetically by the loss of chromosomes. A mitotic block was proposed to produce the dominant lethals. However, one cannot distinguish whether this mitotic block was due to chromosome loss or rearrangement or some other induced damage and change. Whatever these changes may be, the response by brood testing indicated in the work presented here that a percentage of radiation induced dominant lethals was greatest in early spermatids and the least in spermatogonia. Exogenous ATP did significantly reduce the percentage of induced dominant lethals in spermatozoa, spermatids and spermatocytes.

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