ACTINOMYCIN D EFFECTS ON THE FREQUENCY OF RADIATION-INDUCED MUTATIONS IN *DROSOPHILA*^{1,2}

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RECENT work indicates that one class of mutations induced by radiation may involve a series of discrete steps leading from an induced premutational lesion in the genetic material and ending in the fixation of the lesion as a self-replicating mutational event. Evidence for this path of mutation induction comes from the modification of yields of radiation-induced mutations by postradiation treatment with various metabolic inhibitors (WITKIN 1956; DOUDNEY and HAAS 1959; KIMBALL, GAITHER and WILSON 1959).

WOLFF (WOLFF and LUIPPOLD 1955; WOLFF 1959; WOLFF 1960) has found that protein synthesis is necessary for the rejoining of X-ray-induced chromosomal breaks in *Vicia faba*. KIMBALL and co-workers (KIMBALL, GAITHER and WILSON 1959; KIMBALL 1961) have found that treatment of *Paramecium aurelia* with metabolic inhibitors of protein synthesis reduces the recovery of recessive deleterious mutations induced by radiation. SOBELS and TATES (1961), SOBELS (1963) and CLARK (1963) obtained similar results using chloramphenicol and metabolic inhibitors for the analysis of the process of X-ray-induced mutations in *Drosophila melanogaster*. These experiments have been inferred to implicate a role of proteins in the process of fixation of certain premutational lesions. Indeed, SETLOW and CARRIER (1963) have evidence for enzymes in *Escherichia coli* which repair thymine dimers induced in DNA by ultraviolet irradiation.

BURDETTE (1961) found that the frequency of sex-linked recessive lethals induced in *Drosophila* by X rays is reduced when larvae are reared on culture medium containing actinomycin D prior to radiation. Since actinomycin D inhibits DNA-mediated RNA synthesis (REICH 1964) which is necessary for the production of proteins, BURDETTE's data may also indicate the necessity for proteins in the fixation of some radiation-induced mutations. The experiments reported in the present paper were undertaken to obtain further information on the pattern of stage sensitivity of reproductive cells to modification of radiation-induced mutation yields by actinomycin D.

MATERIALS AND METHODS

Oregon-R males less than 24 hours old were used throughout the experiments. These males

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were injected in the gonadal area as outlined by SUZUKI (1963). Since CARLSON and OSTER (1962) have shown that the amount of liquid expelled after injection varies from fly to fly, estimates of the amount of solution injected into each fly were not attempted. Actinomycin D dissolved in 0.7N NaCl at a concentration of 1.0 $\mu\text{g}/\text{ml}$ or a 0.7N NaCl solution were injected into the males. Within six hours of injection, half of each injected group was treated with 600 rads of gamma rays delivered from a 6000 curie cobalt⁶⁰ therapeutic source at an intensity of 52 rads/min. Thus there were four sets of test conditions: actinomycin D + γ -rays, saline + γ -rays, actinomycin D and saline. Sperm from the treated males were tested for recessive sex-linked lethal mutations by the standard Muller-5 (M-5) technique and brood pattern analysis (IVES 1963). Each male was individually mated with a harem of six virgin M-5 females. Males were transferred, without etherization, to vials with six fresh virgin females at 24 hour intervals for eight successive broods. After each mating period, the group of six mated females was transferred twice to fresh vials at 48 hour intervals. This practice, suggested by IVES (1963), prevents overcrowding in any vial and assures maximal recovery of radiated sperm. All F_1 females which had emerged within 18 days of the laying period in each brood were pair mated with their brothers and the F_2 scored for incidence of lethal mutations.

RESULTS

The frequencies of sex-linked recessive lethal mutations were obtained in three successive experiments. A chi-square analysis indicated that the data from the three experiments were homogeneous and the pooled data are listed in Table 1. It can be seen that saline and actinomycin D injection alone does not result in changes from the level of spontaneously occurring mutations (Columns 3 and 4, Table 1).

The mutation frequency in the saline + γ -ray series shows a progressive rise to a peak of 6.02 percent in the fifth brood (Column 2, Table 1). This period of peak radiosensitivity is presumed to represent cells in meiosis at the time of treatment since sterility induced by the radiation occurs in that brood and is in accord with the results of IVES (1963). IVES (1963; personal communication) suggests that the use of large harems in daily transfers of the males permits an

TABLE 1

Frequencies of radiation-induced recessive sex-linked lethal mutations in successive one-day broods after each treatment

Days after treatment	Treatment											
	1 Actinomycin D + γ -rays			2 Saline + γ -rays			3 Actinomycin D			4 Saline		
	Number tested	Mutations No.	%	Number tested	Mutations No.	%	Number tested	Mutations No.	%	Number tested	Mutations No.	%
1	1,768	26	1.47	2,240	38	1.7	685	0	0	907	0	0
2	2,065	22	1.06	2,288	36	1.57	573	0	0	744	2	0.26
3	2,233	40	1.79	2,322	65	2.8	638	2	0.31	714	0	0
4	2,542	76	2.98	2,877	148	5.1	632	1	0.15	653	1	0.15
5	1,289	43	3.34	1,777	107	6.02	663	0	0	631	0	0
6	1,319	34	2.57	1,590	63	3.96	618	1	0.16	626	0	0
7	1,515	4	0.26	1,561	11	0.70	1,164	1	0.08	665	0	0
8	1,611	3	0.18	1,523	0	0	514	0	0	582	1	0.17
Totals	14,342	248	1.72	16,178	468	2.89	5,487	5	0.09	5,522	4	0.072

FIGURE 1.—Mutation percentages in successive one-day broods after radiation of saline and actinomycin D treated males.

exhaustive recovery of sperm batches and may be responsible for the detection of spermatocytes in Brood 5. In the seventh- and eighth-day broods, the rate of mutation in gonial cells shows a sharp drop.

Data with actinomycin D + γ -rays show a similar pattern in the frequency of induced mutations in successive broods with the peak being reached in the fifth brood (Column 1, Table 1). However, the mutation frequency is reduced with actinomycin D treatment. The mutation percentages in each brood of the actinomycin D + γ -ray and saline + γ -ray series are plotted in Figure 1, with their 95 percent confidence limits. It can be seen that the frequencies of mutation in the third to the sixth broods are significantly lower in the actinomycin D + γ -ray series than in the saline + γ -ray controls. Actinomycin D does not appear to be effective in Broods 7 and 8, which presumably represent cells which were gonial at the time of treatment (Columns 1 and 2, Table 1). This may indicate that the repair mechanism is absent or that a different class of mutations occurs in gonial cells.

In the absence of assays for the chemical effects of actinomycin D, sterility and induced mortality were used as biological indicators of the effectiveness of the antibiotic. All deaths occurred within four days of treatment and it can be seen (Table 2) that actinomycin D increases mortality of the treated flies relative to

TABLE 2

Percentage of males surviving after each treatment in successive one-day broods

Days after treatment	Treatment and number of flies treated			
	1 Actinomycin D + γ -rays (180 flies)	2 Saline + γ -rays (149 flies)	3 Actinomycin D (40 flies)	4 Saline (41 flies)
1	82.2	90.0	82.5	83.0
2	63.3	82.0	62.5	78.0
3	53.8	78.5	60.0	78.0
4	52.0	78.5	60.0	75.6

saline treatment. Fertility of the males as indicated by changes in the number of progeny per male in each brood is also decreased by the actinomycin D (MUKHERJEE unpublished). These physiological effects would seem to indicate that actinomycin D is effective in *Drosophila*. Studies (BEERMAN 1963; LAUFER, NAKASE and VANDERBERG 1964) indicate that actinomycin D does inhibit DNA-mediated RNA synthesis in Diptera and it is probable that this effect is responsible for the results reported here.

DISCUSSION

These results indicate that the frequency of radiation-induced mutations in *Drosophila* can be modified by preradiation treatment with actinomycin D. This effect of the antibiotic is specific to cells recovered in Broods 3 to 6 which are presumed to have been spermatids and spermatocytes at the time of treatment.

Mutagenesis by radiation may occur in a number of ways. One class of mutations is induced directly by radiation (MULLER, CARLSON and SCHALET 1961) whereas others may not be fixed immediately. Changes in the genetic material (which may correspond to the chromosome breaks observed by WOLFF 1959) implied by the latter class of events may encounter a number of possible fates: (1) the damage may not be fixed and result in a dominant lethal; (2) the lesion may be "repaired" to its original state; or (3) the lesion may be "repaired" with a genetic change incorporated at the site of repair which is detectable as a recessive lethal. If the second and third processes require specific proteins, suppression of protein synthesis would be expected to decrease the recovery of recessive lethal mutations. The experiments reported and the findings of KIMBALL (1964) in *Paramecium* are consistent with this model if one assumes that protein synthesis is inhibited in the absence of messenger RNA after actinomycin D treatment.

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SUMMARY

Oregon-R males of *Drosophila melanogaster* were subjected to the following treatments: (1) actinomycin D + 600 rads of gamma rays; (2) 0.7N NaCl + 600 rads of gamma rays; (3) actinomycin D; and (4) 0.7N NaCl. Each treated male was tested for radiation-induced sex-linked recessive lethal mutations by the Muller-5 technique, for eight successive daily broods.

The actinomycin D + gamma ray series showed a significant reduction in mutation frequency relative to the frequencies in the saline + gamma ray series in all the sperm samples from the third- to the sixth-day brood. The data are consistent with the model of a protein dependent process of mutation fixation of radiation-induced premutational genetic damage.

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