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ACTINOMYCIN D EFFECTS ON THE FREQUENCY OF X-CHROMOSOME LOSS AND NON-DISJUNCTION IN *DROSOPHILA MELANOGASTER* FEMALES.

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RESUMEN

En este experimento se midió la frecuencia de la pérdida del cromosoma X y de la no disyunción en la descendencia de hembras inyectadas con actinomicina D a las concentraciones de 0. 010 mg/ml y 0.100 mg/ml, o bien, con tratamiento previo con 0.100 mg/ml de actinomicina D e irradiación posterior. En cada frasco de cultivo se cruzó una hembra con dos machos y se les dejó en oviposición durante cuatro períodos consecutivos de dos días cada uno. La primera progenie corresponde en su mayor parte a ovocitos en el estado 7. El efecto fisiológico estimado según el grado de esterilidad inducido fue más evidente en la primera progenie.

El efecto más notable de la actinomicina D es el aumento de las frecuencias con que ocurren la pérdida del cromosoma X y la no disyunción, tanto en los grupos no irradiados como en los grupos irradiados. La frecuencia de la pérdida del cromosoma X comparada con el grupo testigo aumentó en dos órdenes de magnitud.

Los datos presentados están de acuerdo con un modelo que presupone un proceso dependiente de la síntesis proteínica que tiene influencia en los eventos de la pérdida del cromosoma X y de la no disyunción.

SUMMARY

In this experiment non-disjunction and X chromosome loss were measured in the progeny of females injected wi h actinomycin D at concentrations of 0.010 mg/ml and 0.100 mg/ml, or pre-treated with 0.100 mg/ml actinomycin D and irradiated. The females were mated with two males per vial and allowed to lay eggs for four consecutive two-day broods, the first brood corresponding mainly to stage 7 oocytes. The physiological effect of actinomycin D measured as the degree of induced sterility was more evident in the first brood.

The most noticeable effect of actinomycin D in the present experiment is the increase in the frequencies of X-chromosome loss and non-disjunction both in the irradiated and in the non irradiated groups. The frequencies of X-chromosome loss was increased by two orders of magnitude as compared with the control group.

The data presented are consistent with a model of a protein synthesis dependent process related to the events of X-chromosome loss and non-disjunction.

INTRODUCTION

The first recorded genetic effects induced by high energy radiations in *Drosophila* were reported by Mavor (1922). He showed that X-rays produce a marked increase in the frequency of non-disjunction. Ionizing radiations are known to increase also the chromosome loss (H. J. Müller, 1940; G. Pontecorvo, 1941). In neither process is the mechanism of induction well understood. Two mechanisms

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have been cited as possible causes for the failure of chromosomes to separate regularly from one another during meiosis, namely centromere malfunction (H. J. Müller, 1954), and chromosome stickiness (H. Traut, 1964).

Sturtevant and Beadle (1936) proposed the failure of metaphase pairing as an alternate hypothesis to explain primary non-disjunction. Such defect of chromosome coupling during metaphase would be the result of a failure of conjugation during zygotene or would result from complete separation of formerly paired homologous at diakinesis. The distribution at random of the two unpaired univalents with respect to each other would produce their irregular distribution at anaphase.

R. F. Grell (1962) formulated the "distributive pairing" theory of meiosis as the mechanism implicated in primary non-disjuction. The main feature of Grell's theory provides for two types of pairing, which are not competitive events: an early type, "exchange pairing", precedes and is a requisite for exchange, and a latter type concerns with segregation which is called "distributive pairing". Exchange pairing is determined by homology; distributive pairing is a size-dependent phenomenon.

The distributive pairing occurs after crossing-over, and may involve homologous or nonhomologous chromosomes; two chromosomes thus paired will pass to opposite poles of the division spindle at anaphase. R. F. Grell (1962) has shown that all co-oriented pairs of chromosomes are probably involved in distributive pairing, but this process becomes evident as different from exchange pairing only in certain situations of nonrandom assortment of nonhomologous chromosomes or in secondary non-disjunction.

The non-disjunction and chromosome loss lead to aneuploid gametes and generally to dominant lethality among the offspring. As this outcome is one of the main difficulties encountered in the study of both processes, it becomes necessary to recover viable progeny from aneuploid gametes, to recognize the origin of such progeny and to calculate what proportion of the aneuploid gametes they represent.

Traut (1964) investigated the dose-dependence of X-chromosome loss and non-disjunction induced by X-rays in oocytes of *Drosophila melanogaster*. He found that the dose-effect curve for X-loss suggests heterogeneous radiosensitivity of the oocyte sample and that the most sensitive oocytes are also specially suceptible to the induction of dominant lethals. Traut found difficult to interprete the dose-effect curve results of non-disjunction, propossing that perhaps radiation induced non-disjunction is not based on "hit events" as it is problematic to correlate the linear term obtained in his experiment with a one hit mechanism and it is also difficult to imagine such a mechanism which would produce a chromosomal change preventing the two X chromosomes from disjoining.

By the use of several techniques for microinjection of *Drosophila melanogaster* it is possible to administer high concentrations of toxic compounds to the gonadal tissue without killing the adult injected fly (O. G. Fahmy, 1959; E. A. Carlson, 1962; O. Strömnaes, 1962).

The antibiotic actinomycin D, at low concentrations permits deoxyribonucleic acid (DNA) replication, preventing DNA-mediated RNA (m-RNA) synthesis (E. F. Reich, 1961) while at higher concentrations both RNA synthesis and DNA replication are inhibited.

Susuki (1965) found an increase in: a) the mortality of injected females, b) the frequency of complete sterility of survivors, and c) the crossover values in the region spanning the centromere in the chromosome 3, after the injection of actinomycin D at concentrations of 0.010 mg/ml and 0.100 mg/ml. At a concentration of 0.001 mg/ml actinomycin D did not appear to influence crossing-over.

To account for the observed increase of crossover values, Susuki cites the postulate on the active functioning of a gene resulting in structural properties of such locus that prevent crossing-over from occurring in that region. Actinomycin D may inactivate loci modifying extrinsic and intrinsic

factors and the consequent structural changes would permit crossing-over to occur more frequently.

The role of proteins in the process of fixation of certain premutational lessions have been demonstrated hy the modification of yields of radiation induced mutations by postradiation treatment with various metabolic inhibitors. The experiments of Sobels and Tates (1961), Sobels (1963) and Clark (1963) showed the effect of chloramphenicol and metabolic inhibitors on the process of X-ray induced mutations in *Drosophila melanogaster*.

Another type of interference with the recovery process of chromosomes injured by radiation is the inhibition of the oxidation systems necessary for production of the adenosine triphosphate (ATP) needed to repair injured chromosomes (S. Wolff, 1955). Mittler (1966) found protection in *Drosophila* of cells in spermatogenesis which were in or near meiosis during the administration of X-rays, when adenosine triphosphate were injected either inmediately before or after the irradiation treatment. The ATP did not afford any protection against radiation induced non-disjunction. whereas the chromosome loss was significantly less in the groups treated with ATP hoth before and after irradiation.

The effect of actinomycin D, decreasing the frequency of recesive lethals recovered after X-irradiation of *Drosophila* when the antihiotic is added to the culture medium prior to the irradiation treatment was demonstrated hy Burdette (1961).

Mukherjee (1964) obtained further information on the pattern of stage sensitivity of reproductive cells to modification of radiation induced mutation yields by actinomycin D in *D. melanogaster* males. He found a significant reduction in the mutation frequency induced by gamma rays, in the actinomycin D series as compared to the saline series.

The experiments reported in this paper were undertaken in order to obtain information on the effect of actinomycin D, probahly through a protein synthesis dependent process which mediates on nondisjunction and chromosome loss.

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GENERAL EXPERIMENTAL PLAN

Bridges (1913) identified non-disjunction by the recovery of exceptional females and males among the progeny, by their being matroclinous and patroclinous, respectively, in phenotype for sex linked characters. In the present work an improved method for detecting non-disjunction that gives particularly reliable evidence concerning the origin of each exceptional female and male makes use of a tester male stock with attached XY chromosomes. This stock was derived from translocations between the X and Y chromosomes and has the markers (y) yellow and B (Bar).

Drosophila melanogaster virgin females of the genotype y cv v f car/y were aged from 12 to 24 hours and injected with a solution of actinomycin D. or a saline solution from 2 to 6 hours before the X-ray treatment. Descriptions of the markers used can be found in Bridges and Brehme (1944). The females were taken from the cross of stocks having the sc^8Y chromosome: the females from a y cv v f car ♀ ♀ y cv v f car/sc^8Y ♂ ♂, and the males from a y ♀ ♀ x y/sc^8Y ♂ ♂ stock. The existence of any secondary exceptions (from XXY mothers) among the y/y cv v f car females was made unlikely by the sc^8Y chromosome which covers the effect of yellow in XXY females.

Immediately after the irradiation treatment the females were placed in mass cultures with an excess of males and twenty four hours latter they were transferred to vials.

For our purpose the experiment was divided into nine groups: Group I. females

not treated but otherwise handled in the same way as the treated flies; Group II, irradiated with 1,000 R; Group III, irradiated with 2,000 R; Group IV, irradiated with 3,000 R; Group V, injected with a solution of actinomycin D at a concentration of 0.010 mg/ml; Group VI, injected with a solution of actinomycin D at a concentration of 0.100 mg/ml; Group VII, injected with a solution of actinomycin D and irradiated with 2,000 R; and Group VIII, injected with a 0.7N NaCl saline solution, before irradiation with 2,000 R.

In each vial a female was mated individually with two males having an attached XY chromosome of the genotype: $Y^S.X.Y^L$ In (I) EN, $Y^S.B$ y Y^L. The fertilization of a XX egg with an XY spermatozoon would produce super-females with low viability which were excluded from the following analysis, whereas the fertilization of eggs of the same non-disjunctional chromosomal constitution with a non X or Y chromosome bearing spermatozoon would produce matroclinous yellow females of the same genotype as their mothers. These females are easily identified from their normal Bar eyed sisters. The no-X egg when fertilized with an XY bearing sperm would become an XY patroclinous male, which can be identified by the Bar eyes in its phenotype.

The female and males of each vial were transferred every two days to complete 4 broods of 48 hours each, in order to sample different stages of gametogenesis.

The F1 flies arising from eggs laid in each two-day period were scored for X-loss and non-disjunction from 13 to 16 days after the P females were irradiated.

When several exceptional males or females were in the same vial they were recorded as a non-disjunctional or chromosome loss event.

The computation of the X-loss and non-disjunction frequencies is based on the definitions given by Traut (1964) which consider either the number of regular males (definition 1) or the number of regular females (definition 2) counted in the F1 (in this experiment XY males and XX females are scored as exceptional progenie).

	X-loss frequency	Non-disjunction-frequency
Definition 1	$\frac{\text{XY males}}{\text{XO males + XY males}}$	$\frac{\text{XX females}}{\text{XO males + XX females}}$
Definition 2	$\frac{\text{XY males}}{\text{XXY females + XY males}}$	$\frac{\text{XX females}}{\text{XXY females + XX females}}$

MATERIALS AND PROCEDURES

All the flies used throughout the experiment were reared in the agar-cornmeal medium regularly employed at the laboratory.. All the cultures were kept at 25± 1°C before and after the treatments.

The source of radiation was a X-ray Stabilipan Siemens instrument operating at 250 kV and 15 mA with an exposure rate of 186 R/min. The distance from the window was 40 cm and a 0.5 mm Copper filter was used.

The injection was performed with a micro-syringe attached with a Santotube Q needle and actioned by an air-compressor machine as outlined by Félix (1964, 1968). Small amounts of solutions (0.60 microliter) were injected dorsally between the fifth and sixth abdominal tergites.

A physiological 0.7 N NaCl solution was injected into female controls and the actinomycin D was dissolved in the same solutions, instead of using distilled water which obviates the problem of induced sterility and possible cell selection by osmotic shock. The actinomycin D solutions were prepared less than 1 hour before each series of injections.

RESULTS AND DISCUSSION

Female Drosophila studies have largely been restricted to older stages of oocytes in the vitellarium. From the 14 developmental stages as defined by King, Rubinson and Smith (1956) the two stages that have heen most studied are stages 7 and 14, both correspond to meiotic prophase, 7 is the oldest stage found in newly emerged females, and stage 14 corresponds to the fully mature, chorionated oocyte in females after the second day of adult life. Controlling the conditions of egg laying and limiting the number of eggs collected from each newly emerged female it is possihle to obtain homogenous samples of eggs from stage 7. If more than 30 eggs are collected some of them may have been in carlier stages at the time of treatment. The first two day brood of this experiment corresponds mainly to stage 7.

There is a considerable difference in sensitivity to X-ray treatment between the two stages. With stage 7 a reasonable number of eggs will survive after the irradiation with 2000 R, which was the dose applied in the groups 3, 7 and 8, while a dose of 600 R, kills the 75 per cent of eggs in stage 14 (D. R. Parker, 1959).

When compared with uninjected females or females injected witth saline solution, the actinomycin D series appeear to have noticeable physiological effect, which is more apparent in the induction of sterility between the first and the second brood, indicating that actinomycin D is being absorbed by the cells and interfering with cellular physiology. These physiological effects would seem to indicate that actinomycin D is biologically effective in *Drosophila* (R. Mukherjee, 1964). The total effect of actinomycin D on fertility through the four broods is similar to that induced by the treatment with 3000 R. With regard to such effect there is not a big discrepancy between the data obtained from the two groups to which different concentrations of actinomycin D were given (Tab. 5 and 6).

The most noticeable effect of actinomycin D in the present experiment is the increase in the frequencies of X-chromosome loss and non-disjunction, both in the non irradiated and in the irradiated groups (Tab. 5, 6 and 7).

The females injected with a 0.010 mg/ml actinomycin D concentration as well as the females pre-treated with a 0.100 mg/ml actinomycin D concentration and irradiated show that the X-chromosome loss was increased by two orders of magnitude as compared with the control group (Tab. 9).

Assuming that the primary effect of actinomycin D when injected into *Drosophila* is the inhibition of DNA-meditated RNA synthesis the data presented here are consistent with a model of a protein dependent process related to the events of X-chromosome loss and non-disjunction.

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Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	1 191	1 184	821	743	3 939
regular males	1 283	1 286	943	703	4 215
n.d. females	3	1	1	—	5
c.l. males	1	1	—	1	3
% n.d. females	0.25	0.08	0.12	—	0.12
% c.l. males	0.08	0.08	—	0.13	0.07

Table 1. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods from untreated females.

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	384	201	141	307	1 033
regular males	442	273	155	347	1 217
n.d. females	—	1	—	9	10
c.l. males	29	—	—	6	35
% n.d. females	—	0.49	—	2.84	0.95
% c.l. males	7.02	—	—	1.93	3.27

Table 2. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods from females irradiated with 1 000 R.

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	735	170	79	160	1 144
regular males	726	138	94	242	1 200
n.d. females	2	14	—	—	16
c.l. males	23	2	—	—	25
% n.d. females	0.27	7.60	—	—	1.37
% c.l. males	3.03	1.16	—	—	2.13

Table 3. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods from females irradiated with 2 000 R.

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	156	264	147	91	658
regular males	146	256	145	111	658
n.d. females	1	8	—	—	9
c.l. males	5	1	—	3	9
% n.d. females	0.63	2.94	—	—	1.34
% c.l. males	3.10	0.37	—	3.19	1.34

Table 4. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods from females irradiated with 3 000 R.

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	340	122	78	51	591
regular males	370	159	85	49	663
n.d. females	2	—	2	—	4
c.l. males	11	3	2	3	19
% n.d. females	0.58	—	2.50	—	0.67
% c.l. males	3.13	2.40	2.50	5.55	3.11

Table 5. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods from injected females (0.010 mg/ml actinomycin D).

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	366	215	109	100	790
regular males	378	220	110	115	823
n.d. females	4	1	—	1	6
c.l. males	1	—	2	1	4
% n.d. females	1.08	0.46	—	0.99	0.75
% c.l. males	0.27	—	1.80	0.99	0.50

Table 6. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods injected females (0.100 mg/ml actinomycin D).

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	125	117	190	210	642
regular males	138	147	215	300	800
n.d. females	7	11	2	3	23
c.l. males	17	2	1	2	22
% n.d. females	5.30	8.59	1.04	1.40	3.45
% c.l. males	11.97	1.68	0.52	0.94	3.31

Table 7. Frequency and percentage (definition 2) of exceptional females and males among the frequency in two-day broods from pre-treated females (0.100mg/ml actinomycin D) 1-2 h. before irradiation with 2 000 R.

Days:	0-2	2-4	4-6	6-8	TOTAL
regular females	107	217	283	134	741
regular males	173	243	319	213	948
n.d. females	7	1	1	1	10
c.l. males	3	—	4	—	7
% n.d. females	6.14	0.45	0.35	0.74	1.33
% c.l. males	2.72	—	1.39	—	0.93

Table 8. Frequency and percentage (definition 2) of exceptional females and males among the progeny in two-day broods from pre-treated females (0.7N NaCl) 1-2 h. before irradiation with 2 000 R.

	(1) % n.d. females	(2) % n.d. females	(1) % c.l. males	(2) % c.l. males
Control	0.11± 0.05	0.12± 0.06	0.07± 0.04	0.07± 0.04
Actinomycin-D (a)	0.59± 0.30	0.67± 0.33	2.78± 0.61	3.11± 0.70
Actinomycin-D (b)	0.72± 0.29	0.75± 0.30	0.48± 0.24	0.50± 0.25
2 000 R	1.31± 0.33	1.37± 0.34	2.04± 0.40	2.13± 0.42
2 000 R + act.D (b)	2.79± 0.57	3.45± 0.70	2.67± 0.56	3.31± 0.69
2 000 R + sol (c)	1.04± 0.33	1.33± 0.42	0.73± 0.28	0.93± 0.35

(1) definition 1; (2) definition 2; (a) 0.010 mg/ml actinomycin D solution; (b) 0.100 mg/ml actinomycin D solution; (c) 0.7N NaCl solution.

Table 9. Effect of actinomycin D on non-disjunction and X-chromosome loss calculated from the exceptional progenie recovered from 0 to 8 days after treatment.