De la Rosa, M.E. and R. Félix. National Institute of Nuclear Energy, Mexico City, Mexico. Cytogenetic effects of cycloheximide during spermatogenesis of D. melanogaster.

Cycloheximide inhibits protein synthesis in vivo in yeast (Siegel and Sisler, 1964a) and fungi (Sussman, 1965), as well as in mammalian cells (Ennis and Lubin, 1964). This antibiotic prevents the transfer of amino acid from aminoacyl-tRNA to protein (Siegel and Sisler, 1964b; Bennett et al., 1965). Whereas cycloheximide inhibits protein synthesis without interfering with RNA synthesis, DNA synthesis is also inhibited (Kerridge, 1958; Young et al., 1964). In certain animal cells DNA synthesis appears to be as sensitive or more sensitive to cycloheximide than protein synthesis (Cooney and Bradly, 1961; Bennett et al., 1965).

Ring chromosomes are potentially useful for studying the process of rejoining after chromosome breakage, since a certain portion of the restitutions should produce dicentric ring chromosomes or interlocked rings rather than structurally normal chromosomes. The relevance of ring chromosome studies to problems of induced chromosome breakage in Drosophila melanogaster sperm has been recognized for some time. In this experiment the effect of a physiological saline solution, and of cycloheximide on chromosome X or Y loss was measured in the progeny of pre-treated and irradiated "Oster males" by means of the genetic scheme designed by Oster (1958). Male flies from a stock containing a marked sc^8Y chromosome and the closed X, X^{c2} marked with the mutants yellow (y) and Bar (B) in the males, and yellow, forked (f), attached X chromosomes in the female (y f:=) were aged for 72 hours before the injection of the solutions, irradiation, or both, and mated to virgin "Oster females" with markers in the I, II and III chromosomes (y sc^{S1} In49 sc^8;bw;st p^p). The markers B and y, to identify the treated sex chromosomes of the males, makes the detection of X or Y chromosome loss fairly easy. The frequency of exceptional (X/O) males among the F_1 flies is determined by counting the yellow males, which represent cases of loss of the whole or part of the X or Y chromosomes. Normal males have non-yellow bodies, since they carry the normal dominant allelomorph of yellow in the sc^8 insertion of their Y chromosome.

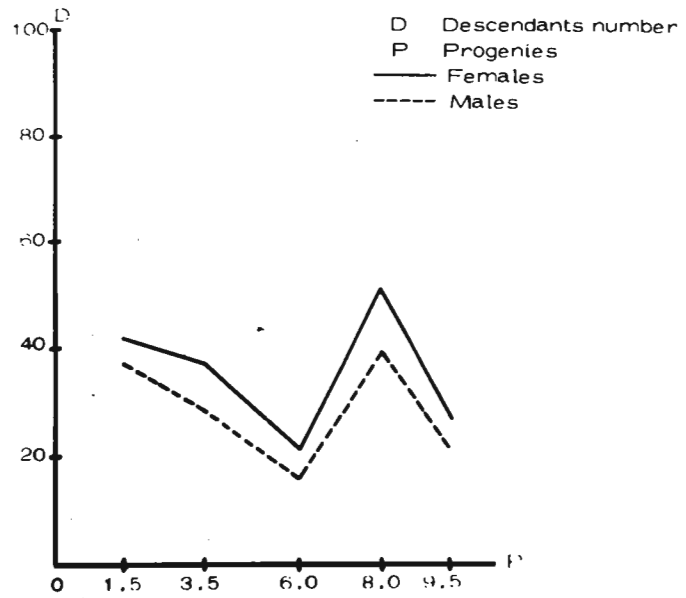

Figure 1. Progenies (average per culture/total) from the group treated with cycloheximide.

Several concentrations of cycloheximide (Schuchardt, München) dissolved in 0.7N NaCl solution were administered by injection in the gonadal area of aged "Oster males" in order to determine the concentration to be used without interfering with their viability or fertility. 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. A concentration of 50 µg/ml was used; at this level no mortality was recorded among the injected adults within fifteen days. A physiological 0.7N NaCl solution was injected to male controls, instead of using distilled water which obviates the problem of induced sterility and possible cell selection by osmotic shock. The source of radiation was a "Gamma-Cell 200" apparatus.

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

Three day old non-bred males were treated, placing them in separate vials (individual cultures) containing an aged male and five virgin females, which were renewed on the 2nd, 4th, 6th, 8th and 10th days; thereby five broods were obtained from gametes treated during specific stages of spermatogenesis. The fertilized females of each brood oviposited during 3 days in

the same vial, after which they were eliminated. The counting of the progenies to determine percentage of exceptional XO individuals was made 15 days after starting the cultures, before the following generation emerged.

According to Auerbach (1954) consecutive broods from males mated sequentially represent successively younger germ cells at the time of irradiation. Thus the spatial pattern of spermatogenesis in the treated testes becomes translated into a temporal pattern of genetical effects in successive broods which in turn "can be re-translated into the underlying sensitivity pattern of spermatogenesis".

In the mating procedure mentioned above the first cross among the treated or non-treated males and the five females in the same vial was designated the First Brood (0 - 1.5 days). These males were then mated in fresh cultures on the fourth day (1.5 - 3.5 days), or Second Brood. The following cultures correspond to the Third Brood, Fourth Brood and Fifth Brood. An approximate correspondence between the stages of spermatogenesis and the five cultures in which each male was successively bred is presented below.

1st. Brood - Days 0 to 1.5 post-treatment. Stage at time of treatment: mature sperm.

2nd. Brood - Days 1.5 to 3.5 post-treatment. Stage at time of treatment: spermiogenesis.

3rd. Brood - Days 3.5 to 6 post-treatment. Stage at time of treatment: second meiotic division and spermiogenesis.

4th. Brood - Days 6 to 8 post-treatment. Stage at time of treatment: first meiotic division and probably a few early second meiotic stages and late gonial cells.

5th. Brood - Days 8 to 9.5 post-treatment. Stage at time of treatment: spermatogonial cells and early first meiotic cells.

The following groups were conducted in parallel:

Group I. Injection of 0.7N NaCl solution.
Group II. Injection of 50 µg/ml cycloheximide solution.
Group III. Irradiation with 2,500 rads.
Group IV. Injection of 0.7N NaCl solution and irradiation with 2,500 rads.
Group V. Injection with 50 µg/ml cycloheximide solution and irradiation with 2,500 rads.

Effects of cycloheximide upon fertility. The administration of physiological serum (Table 1) to the males constitutes the test group which shows the fertility characteristic of each stage present during spermatogenesis, after injection of saline solution.

Table 1. Progenies (average per culture/total) from the group treated with 0.7N NaCl solution.

Progenies	0-1.5 days	1.5-3.5 days	3.5-6 days	6-8 days	8-9.5 days	Total
XX females	28/700	77/856	16/96	44/176	54/162	219/1990
XY males	26/639	64/708	17/104	39/155	53/160	199/1766
XO males	2	1	0	0	0	3

Table 2 sumarizes the data concerning the number of individuals obtained apart from specific stages of spermatogenesis, when physiological serum was administered before the irradiation with 2,500 r.

The differences between Tables 1a and 2 result from the modifications in the specific survival of successive stages of spermatogenesis, owing to its intrinsic radiosensibility.

Table 2. Progenies (average per culture/total) from the group treated with 0.7N NaCl solution and 2,500 r.

Progenies	0-1.5 days	1.5-3.5 days	3.5-6 days	6-8 days	8-9.5 days	Total
XX females	28/702	23/522	8/198	4/50	6/64	69/1536
XY males	24/592	16/505	7/167	5/60	4/44	56/1340
XO males	9	7	1	3	0	20

It is well established that the most radiosensitive stages of spermatogenesis are the

spermatid, and the spermatocites, which correspond in the tables to the progenies recovered between 3.5 - 6 days and between 6 - 8 days after treatment, respectively.

Table 3. Progenies (average per culture/total) from the group treated with 50 µg/ml cycloheximide.

Progenies	0-1.5 days	1.5-3.5 days	3.5-6 days	6-8 days	8-9.5 days	Total
XX females	42/1043	37/1343	21/451	51/672	27/288	178/3797
XY males	37/925	28/1039	16/345	39/507	21/230	141/3046
XO males	2	1	2	1	3	9

The data contained in Table 3 and Fig. 1 indicate that fertility diminishes when the administration of cycloheximide takes place during the stages of spermatid, and spermatogonia.

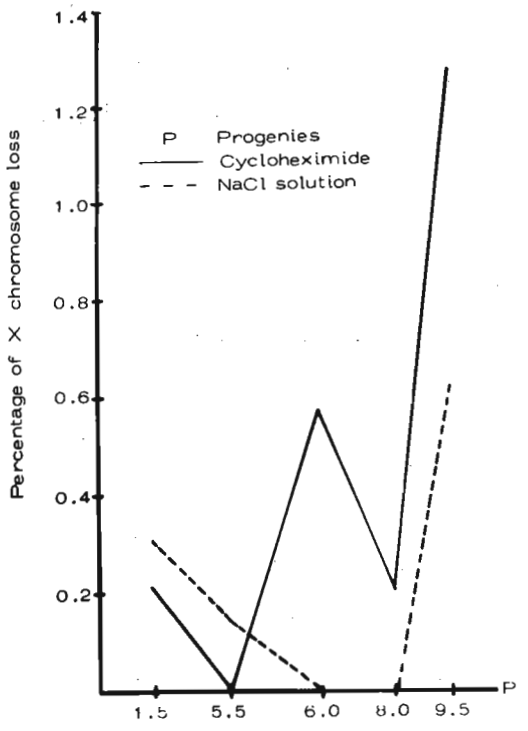

Figure 2. Percentage of X chromosome loss during spermatogenesis.

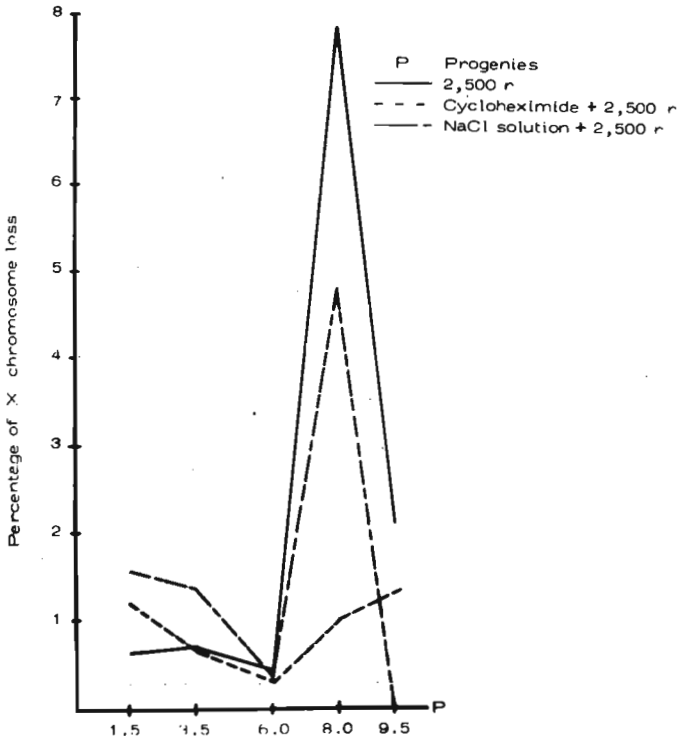

Figure 3. Percentage of X chromosome loss during spermatogenesis.

Radiation produced a diminution in fertility during all stages of gametogenesis preceding spermatozoa (Table 4).

Table 4. Progenies (average per culture/total) from the group treated with 2,500 r.

Progenies	0-1.5 days	1.5-3.5 days	3.5-6 days	6-8 days	8-9.5 days	Total
XX females	29/1354	23/1044	8/312	5/126	9/225	74/3061
XY males	26/1200	19/859	6/235	2/91	6/139	59/2524
XO males	8	6	1	6	3	24

Table 5 presents the progenies obtained from spermatozoa treated during such stage, and previous stages of spermiogenesis and spermatogenesis, with cycloheximide and gamma radiation.

A radioprotective effect of cycloheximide is noticeable from 3.5 to 9.5 days progenies, which includes stages from spermatogonia to spermiogenesis. Spermatids and late spermatocytes are the most affected cells in which a three-fold increase in the size of the progenies is obtained.

Table 5. Progenies (average per culture/total) from the group treated with 50 µg/ml cycloheximide and 2,500 r.

Progenies	0-1.5 days	1.5-3.5 days	3.5-6 days	6-8 days	8-9.5 days	Total
XX females	28/1031	29/1266	29/1020	11/375	12/343	109/4035
XY males	22/797	20/876	21/732	9/299	11/301	83/3005
XO males	10	6	3	3	4	26

The effects of cycloheximide upon X or Y chromosome loss. The data on exceptional individuals resulting from chromosome X or Y loss at successive stages of spermatogenesis following different treatments are contained in Table 6 and Fig. 2 and 3. It is observed that cycloheximide diminishes the frequency of the chromosome X or Y loss during non-irradiated states of spermatozoa and spermatid, increasing such losses during the stages of spermatocyte and spermatogonia.

Table 6. Percentage of X or Y chromosome loss during spermatogenesis (Definition 1, Traut, H., 1964).

Treatment	Spermatozoa	Spermatid	Spermatocyte		Spermatogonia
NaCl sol.	0.31 ± 0.55	0.14 ± 0.37	0.0 ± 0.0	0.0 ± 0.0	0.62 ± 0.78
NaCl sol.+2,500 r	1.56 ± 1.24	1.36 ± 1.16	0.59 ± 0.76	4.76 ± 2.18	0.0 ± 0.0
2,500 r	0.66 ± 0.81	0.69 ± 0.83	0.42 ± 0.64	7.79 ± 2.79	2.11 ± 1.45
cyloheximide sol.	0.21 ± 0.45	0.09 ± 0.30	0.57 ± 0.75	0.21 ± 0.45	1.28 ± 1.13
cycloheximide sol+2,500 r	1.23 ± 1.10	0.68 ± 0.82	0.37 ± 0.60	1.0 ± 1.0	1.34 ± 1.15

In the irradiated groups, there is a radioprotective effect of cycloheximide during spermatogonial stages and during meiosis, while an enhancement of chromosome X or Y loss is shown in spermatozoa. Such results are not compatible with the data obtained from experiments in which other protein-synthesis inhibitors, such as actinomycin D have been assayed (Félix and Rodriguez, 1968; Proust et al., 1972).

References: Auerbach, C. 1954 Z. Induk. Abst.- Vererb. 86:113-125; Bennett, L.L., V.L. Ward and R.W. Brockman 1965 Biochim. et biophys. acta 103:478; Carlson, E.A. and I.I. Oster 1962 Genetics 47:561-576; Cooney, W.J.B. 1961 In: Antimicrobial Agents and Chemotherapy, p. 237, Ed. by Finland, M. and G. Savage, Detroit 1962 American Society for Microbiology; Félix, R. and R. Rodríguez 1968 An. Inst. Biol. Univ. Autón. México 39, Ser. Biol. Exp. (1):13-21; Kerridge, D. 1958 J. Gen. Microbiol. 19:497; Oster, I.I. 1958 Rad. Biol. Proc. Sec. Austr. Conf. Rad. Biol. :253-267; Proust, J.P., K. Sankaranarayanan and F.H. Sobels 1972 Mutation Res. 16:65-76; Siegel, M.R. and H.D. Sisler 1964a Biochim. et biophys. acta 87:70; Siegel, M.R. and H.D. Sisler 1964b Biochim. et biophys. acta 87:83; Sussman, M. 1965 Biochem. and Biophys. Res. Commun. 18:763; Young, C.W., S. Hodas and J.J. Fennelly 1964 Proc. Am. A. Cancer Res. 5:279.