De la Rosa, M.E. and R. Félix. National Institute of Nuclear Energy, Mexico City, Mexico. Chromosome X loss and non-disjunction in oocytes of D. melanogaster, induced by treatment with actinomycin D.

Actinomycin D in minute concentration is toxic to mammalian cells (Reich et al., 1961) and microorganisms (Kirk, 1960). The antibiotic selectivity inhibits RNA synthesis also in microorganisms and mammalian cells (Harbers and Muller, 1962). It seems likely that the antibiotic is primarily toxic to only those cellular activities which require the direct participation of DNA itself. The results with normal or virus infected cells indicate that actinomycin inhibits selectively the DNA-directed synthesis of RNA. The binding of actinomycin to DNA, which requires the presence of guanine in a helical configuration, is responsible for the inhibition of DNA-dependent RNA snythesis by RNA polymerase and is considered to account for the biological properties of actinomycin.

The present experiment was designed to evaluate the effects of actinomycin-D on chromosome X loss and non-disjunction, when injected to adult females of Drosophila melanogaster.

Actinomycin D dissolved in 0.7N NaCl at concentrations of 1 microg./ml., 10 microg./ml. or 100 microg./ml. were injected into localized regions of gonadal tissue, with a micropipette made at the laboratory. The physiolcgical 0.7N NaCl solution was used instead of distilled water to obviate 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.

Since Carlson and Oster (1962) have shown that the amount of liquid expelled after injection varies from fly to fly, estimates of the amounts injected were not made. Solutions were simply injected at the ventrolateral region of the fourth or fifth abdominal segment, until the abdomen was noticeably distended. 90 to 95% of the adults treated in this way survived.

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 X and Y chromosomes and has the markers y (yellow) and B (Bar). The treated female stock had the markers y^2 (yellow-2) and w^a (white apricot) in the X chromosomes, and ebony (e) in the third chromosome; this marker insures that the isolated females are virgin, when the phenotype is examined before the mating.

P	$y^2\ w^a/y^?\ w^a;e/e$	x	$In(1)EN,\ Y^S{\cdot}B\ y{\cdot}Y^L;+/+$
F_1 regular:	$y^?\ w^a/In(1)EN,\ Y^S{\cdot}B\ y{\cdot}Y^L;e/+$		$(B\ y/y^2)$
	$y^2\ w^a;e/+$		$(y^?\ w^a)$
exceptional:	$y^2\ w^a/y^?\ w^a;e/+$		$(y^?\ w^a)$
	$In(1)EN,\ Y^S{\cdot}B\ y{\cdot}Y^L;e/+$		$(B\ y)$

The computation of 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 F_1 (in this experiment X·Y males and XX females are scored as exceptional progenie).

	X-loss frequency	non-disjunction frequency
Definition 1	$\frac{\text{X·Y males}}{\text{X/O males+ X·Y males}}$	$\frac{\text{X/X females}}{\text{X/O males+ X/X females}}$
Definition 2	$\frac{\text{X·Y males}}{\text{X/X·Y females+ X·Y males}}$	$\frac{\text{X/X females}}{\text{X/X·Y females+ X/X females}}$

The process of oogenesis in adult Drosophila has been revised in detail (King, 1970) and in so far as studies on germ cells stage sensitivity are concerned, the essential features are as follows: an ovary of an adult female Drosophila melanogaster consists of a parallel cluster of ovarioles, each of which is differentiated into an anterior germarium and a

posterior vitellarium (Koch, Smith and King, 1967). The development of the egg chamber has been subdivided into a series of consecutive stages, ending with stage 14, the mature primary oocyte (King, Rubinson and Smith, 1956). At eclosion all oocytes are in previtellogenic stages, and the most posterior egg chamber in each ovariole is generally in stage 7. During the first day of adult life vitellogenesis begins, and stage 14 oocytes appear during the second day.

The differences in response of stages 7 and 14 to irradiation are both qualitative and quantitative. Stage 14 shows a higher incidence of all types of genetic damage which have been looked for, with break-rejoining delayed until fertilization, while breaks induced in stage 7 rejoin in about 10-15 minutes (Parker, 1955; Parker and Hammond, 1958; King, Darrow and Kaye, 1956).

In the case of chemical treatments administered by injection, a longer period of treatment is required than with irradiation, and the responsible mutagenic reaction(s) is expectedly prolonged after the period of treatment. Thus, if newly emerged adult females are treated by injection, the most advanced germ cell stages present during the 24 hours following the treatment (unless the treatment itself delays development), is the stage(s) immediately preceeding stage 7, stage 7 itself, and stages 7-13 which have developed during the 24 hr period after the treatment. The sensitivity of the stage 14 oocyte to adult injection can be studied with more confidence, since no further development of this cell takes place during or after treatment. Consequently, although an accurate study of the mutational response of the premeiotic stage 7 primary oocyte can not be made after chemical treatment, a comparison can be made of the mutational responses covering approximately stages 7-13 with the mutational response of stage 14.

The treatment to the oocyte stages with actinomycin D at the concentrations named above, was parallel to a control group, without treatment, and to a group injected with 0.7N physiological saline solution. The actinomycin D solution was prepared half an hour before the injection dissolving this substance in a drop of 25 per cent ethanol, and diluting in the saline physiological solution to the concentration desired.

In order to treat oocytes of the 7-13 stages, virgin females were isolated from 0 to 24 hours after eclosion; they were injected and then maintained in cultures without males during 24 hours before transferring them for breeding, to vials, each with 2 males.

So that the oocytes would be in the 14 stage in the moment of being treated, the virgin females were isolated and allowed to age during 4 days, after which they were injected and immediately bred. In each case, only the eggs deposited during the first 24 hours in every individual culture, corresponding to the treated oocytes, were collected.

The scoring of first generation descendants was made 13 days after the date of treatment of the P females, so that it might include all the progeny, classifying them in four phenotypes that correspond to "exceptional" individuals and "normal" individuals, following the scheme previously described.

The exceptional females originate from non-disjunction and the exceptional males from the loss of the X-chromosome which takes place during oogenesis. The individuals originating from non-disjunction, of the constitution $y^2\ w^a/y^2\ w^a/In(1)EN,\ Y^S \cdot B\ y \cdot Y^L$, are metafemales with a very low viability. In the present experiment not one individual whose phenotype corresponded to such genetic constitution was found.

Effect of actinomycin D upon fertility. The administration of actinomycin D in different concentrations, during the 7-13 and 14 stages of oogenesis of D. melanogaster, produced a diminution in the fertility of the females injected. The data contained in Table 1 illustrates the dose-effect relation which is most noticeable in the oocytes of stage 14, corresponding to the stage of oogenesis with the greatest sensitivity to the antibiotic. The number of descendants originating from the 7-13 stages of treatment, isolated during the first 24 hours of oviposition, is greater than the number of descendants originating from the 14 stage, isolated during the same period. The effect that actinomycin D had upon the fertility of the oocytes can be related to the inhibitory activity of genetic transcription which shows this compound. The intensity of the effect seems to depend upon the doses administered, within the limits of concentration applied in this experiment. It is necessary to take also into consideration the notable differences in metabolic activity that exist between the two stages, in order to explain this reduction resulting from sterility or from induced meiotic delay.

Table 1. Total progeny and average per vial from each treatment of 7-13 and 14 oocyte stages.

Group	Total progeny/number of vials		Average progeny per vial	
	Stages 7-1	Stage 14	Stages 7 13	Stage 14
Control	819/29	1276/23	28	42
Saline solution	934/27	519/17	31	17
Actinomycin 1 µg/ml	1125/26	589/14	36	19
Actinomycin 10 µg/ml	1441/75	843/48	19	17
Actinomycin 100 µg/ml	332/17	604/40	19	15

The frequency of non-disjunction and loss of X-chromosome. Tables 2 and 3 contain data on the numbers of normal and exceptional offspring that were obtained from oocytes treated during the 7-13 and 14 stages of oogenesis.

Table 2. Normal and exceptional progenies from each of the groups treated during 7-13 oocyte stages.

Group	Normal progeny		Exceptional progeny	
	females	males	females	males
Control	346	483	0	0
Saline solution	400	532	1	0
Actinomycin 1 µg/ml	493	686	0	0
Actinomycin 10 µg/ml	606	835	0	0
Actinomycin 100 µg/ml	111	219	0	2

Table 3. Normal and exceptional progenies from each of the groups treated during 14 oocyte stage.

Group	Normal progeny		Exceptional progeny	
	females	males	females	males
Control	530	742	0	1
Saline solution	262	256	1	0
Actinomycin 1 µg/ml	307	281	1	0
Actinomycin 10 µg/ml	319	513	9	2
Actinomycin 100 µg/ml	264	340	4	4

The percentages of exceptional offspring obtained among the descendency of the oocytes of D. melanogaster treated with different concentrations of actinomycin D during the stages of oogenesis studied, are contained in Tables 4 and 5, following the definitions given by Traut (1964).

Table 4. Chromosome X loss and non-disjunction from each of the groups treated during 7-13 oocyte stages.

Group	Chromosome X loss (%)		Non-disjunction (%)	
	def.1	def.2	def.1	def.2
Control*	0	0	0	0
Saline solution	0	0	0.18 ± 0.42	0.24 ± 0.48
Actinomycin 1 µg/ml	0	0	0	0
Actinomycin 10 µg/ml	0	0	0	0
Actinomycin 100 µg/ml	0.90 ± 0.94	1.76 ± 1.32	0	0

* The spontaneous frequencies for non-disjunction and X chromosome loss for this stock amount to about 0.11% and 0.07% respectively.

The administration of actinomycin D in a 100 µg/ml concentration during the 7-13 stages increases the frequency of the loss of the X chromosome, which is statistically significant. The group which was injected with physiological serum, has, likewise, a significant number of individuals originating from non-disjunction.

Table 5. Chromosome X loss and non-disjunction from each of the groups treated during 14 oocyte stage.

Group	Chromosome X loss (%)		Non-disjunction (%)	
	def.1	def.2	def.1	def.2
Control*	0	0	0	0
Saline solution	0	0	0.38 ± 0.61	0.38 ± 0.61
Actinomycin 1 µg/ml	0	0	0	0
Actinomycin 10 µg/ml	0.38 ± 0.61	0.62 ± 0.78	1.72 ± 1.31	2.74 ± 1.65
Actinomycin 100 µg/ml	1.17 ± 1.08	1.49 ± 1.22	1.17 ± 1.08	1.49 ± 1.22

* The spontaneous frequencies for non-disjunction and X-chromosome loss for this stock, amount to about 0.11% and 0.07%, respectively.

The frequency of the loss of the X-chromosome during the 14 stage (Table 5) because of the treatment with actinomycin D, increases significantly in the individuals of the groups injected with physiological serum and actinomycin D in the concentrations of 10 µg/ml, and 100 µg/ml.

The frequency of non-disjunction, as the frequency of the X-chromosome loss, resulting from the inhibition of protein synthesis, is greater when the treated oocytes are found in stage 14. The oocytes at this stage are in a very intense stage of metabolism, in its proximity to fecundation.

References: Bridges, C.B. 1913, J. Expt. Zool. 15:587-606; Carlson, E.A. and I.I. Oster 1962, Genetics 47:561-576; Harbers, E. and W. Muller 1962, Biochem. and Biophys. Res. Commun. 7:107; King, R.C. 1970, In: Ovarian Development in Drosophila melanogaster. Acad. Press, New York and London; King, R.C., A.C. Rubinson and R.F. Smith 1956, Growth 20:121-157; King, R.C., J.B. Darrow and N.W. Kaye 1956, Genetics 41:890-900; Kirk, J. 1960, Biochem. et biophys. acta 42:167; Koch, E.A., P.A. Smith and R.C. King 1967, J. Morphol. 121: 55-70; Parker, D.R. 1955, Genetics 40:589 (Abstr.); Parker, D.R. and A.E. Hammond 1959, Genetics 43:92-100; Reich, E., R.M. Franklin, A.J. Shatkin and E.L. Tatum 1961, Science 134:556; Traut, H. 1964, Mutation Res. 1:157-162.