Félix, R. and M.E. de la Rosa. Genetics and Radiobiology Program of the National Commission of Nuclear Energy, Mexico. Cytogenetic studies with sodium cyclamate in D. melanogaster females.

It is now almost 30 years since the first highly effective mutagens were detected. Since then, much interest has been concentrated on the agencies which produce mutations and chromosome breaks. By the application of chemical compounds, some of whose effects on the constituents of the cell and nucleus are already known, it may be possible to obtain more information on the nature of the mechanisms involved in the production of genic and chromosomal alterations. Many agencies produce mutations and chromosome breaks. Their variety is such that it was wondered whether they do not achieve the same end effect by different means. Several mechanisms of mutagenesis were imagined, such as energy transfer to the chromosomes, chemical reactions with the genetic material, and interference with chromosome synthesis. Few of these would act equally well at all stages of the cell cycle. Thus, interference with chromosome synthesis should be restricted to interphase. Within the last two decades much interest has been concentrated on the variations in differential sensitivities exhibited by cells during gametogenesis in both plants and animals. These variations may be inherent in that they depend on processes or physiological states in the test object which, while sometimes recognized, cannot always be controlled, or they may be induced by different chemical and physical agents.

The reports of Sax and Sax (1968), D. Stone et al. (1969) and Legator et al. (1969) pointed out the cytogenetic damage induced by cyclamate and by its degradation product, cyclohexylamine, preceding the announcement of cyclamate's possible carcinogenic capabilities and the subsequent restrictions on dietary consumption.

Stone et al. (1969) demonstrated that cyclamate, in a minimum concentration of 200 microgram/ml can stiumlate chromosome breakage in human cells in vitro. Whereas a high dosage (equivalent to 15 g/175 Kg) was required to obtain a demonstrable increase in chromosome breaks, Stone pointed out that there is some evidence of synergistic actions on chromosome damage between X-irradiation and radiomimetic chemicals (Merz et al., 1961); between the chemical agents and virus (Nichols et al., 1965), and between the chemical agents themselves (Moutschen-Dahem, 1962).

A considerable number of workers are now engaged in testing compounds for their mutagenic activity. Since in many cases the publication of negative data is not warranted, there is often a repetition of effort with similar negative results.

This study is concerned with the production of X chromosome loss and non-disjunction in Drosophila females by sodium cyclamate. Obtaining identifiable meiotic stages is possible in the case of the female, since the meiotic divisions do not occur until after the oocyte is laid. To obtain fairly uniform samples of a single stage it is necessary to limit the period of egg collection, so that not more than one oocyte is recovered from each ovariole. On the other hand, in the male, meiosis occurs long before the completion of sperm development and the insemination of the female, and at best one can distinguish meiotic stages from pre and post-meiotic ones, with little hope of subdividing the various stages of development of the spermatocyte.

Radiation studies to date have largely been restricted to older stages of oocytes in the vitellarium. King et al. (1956) have described the structure of the ovariole in the adult female, and have designated 14 developmental stages of the oocyte. They recognize three sensitive groups on the basis of recessive lethal mutations, X chromosome losses, and dominant lethal effects. However, much of the work of others has been concerned with but two of the stages they describe, stages 7 and 14 (Parker, 1963), which are, respectively, the oldest stages in the newly emerged females and the fully mature, chorionated oocyte found in females ready to begin egg laying (usually during the second day of adult life). Apparently strains differ in the rate of egg production, as well as in the number of stage 14 oocytes in each ovariole in 4-day old females (Williamson and Stubblefield, 1970). In the control group of our experiment an average of 30 eggs were obtained in 24 hours which correspond mainly to stage 14 oocytes.

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 and chromosome X loss that gives particularly reliable evidence concerning the origin of each exceptional female and male makes use of a tester male stock with attached $Y^{S}X.Y^{L}$ chromosomes. This stock was derived from translocations between the X and Y chromo-

somes and has the markers (y) yellow and (B) Bar.

The females were taken from the cross of stocks having the sc^8Y chromosome: $y^2\ w^a/y^2\ w^a$; e/e x $y^2\ w^a/sc^8Y$; e/e. The existence of any secondary exceptions (from XXY mothers) among the $y^2\ w^a/y^2\ w^a$ females was made unlikely by the sc^8Y chromosome which covers the effect of yellow in XXY females. The males have an attached $Y^SX{\cdot}Y^L$ chromosome of the genotype: $In(1)EN,Y^S$ B $y{\cdot}Y^L$. When virgin females are isolated, the marker ebony insures the virginity of such females, as the genotype of the females from the cross: $y^2\ w^a/y^2\ w^a$: e/e x $In(1)EN,Y^S$ B $y{\cdot}Y^L$; +/+ is heterozygous for the ebony marker. The fertilization of an XX egg with an XY spermatozoon would produce meta-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 chromosome bearing spermatozoon would produce matroclinous yellow, white apricot 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 SY bearing sperm would become and XY patroclinous male, which can be identified by the Bar, yellow eyes in its phenotype.

Virgin females of the genotype $y^2\ w^a/y^2\ w^a$ were aged from 4 to 6 days and fertilized in cultures containing agar-cornmeal medium with sodium cyclamate. In each culture an aged female was mated with 3 attached $Y^SX{\cdot}Y^L$ males, and eliminated after 24 hours. All the cultures were kept at 25±1°C throughout the experiment.

The F_1 flies were scored for X-loss and non-disjunction from 13 to 15 days after the treatment with cyclamate of the P females. The female fruit fly has remarkable synthetic abilities, since during the period of maximum egg production it ingests a daily amount of yeast which approximately equals its body weight and manufactures from the raw material a quantity of eggs which approximately equals 1/3 its weight (King and Wilson, 1955).

Table 1. Progenies obtained from 28 cultures after feeding Drosophila adult females with media containing sodium cyclamate.

NaCy mg/ml	Control	0.05	0.10	0.20	0.40	0.80	1.60
♂ (m.p.c.)	17.14	16.57	17.75	13.21	12.50	13.25	11.32
♀ (m.p.c.)	12.46	9.79	13.46	10.82	8.29	9.11	7.82
♂/♀ (s.r.)	1.37	1.69	1.32	1.22	1.51	1.45	1.45
♂ (total)	480	464	497	370	350	371	317
♀ (total)	349	274	377	303	232	255	219

NaCy mg/ml, sodium cyclamate, milligram/milliliter; (m.p.c.) mean per culture; (s.r.), sex ratio.

As no exceptional progeny were obtained among 4,858 flies, a second experiment was started, maintaining during 6 days, 1 female previously aged during 4 days and mated to 3 males, from the stocks described above, in each culture. The agar-cornmeal medium contained no sucrose. After six days the P flies were eliminated. The feeding of the progenitors as well as the egg laying, embryonic and larval development of the progeny occurred in the medium with cyclamate. The results of this second experiment are contained in Table 2.

Table 2. Progenies obtained from adult and larval feeding during six days in media without sucrose and several concentrations of sodium cyclamate.

NaCy mg/ml	Control	10.00	40.00	100.00
♂(m.p.c.)	45.74	25.00	31.33	34.89
♀(m.p.c.)	25.16	11.39	12.00	17.33
♂/♀(s.r.)	1.82	2.19	2.61	2.01
♂ total	869	575	658	314
♀ total	478	262	252	156
(n.c.)	19	23	21	9
(n.d.p.)	-	-	-	4
(c.l.p.)	-	-	2	9

NaCy mg/ml, sodium cyclamate, milligram/milliliter; (m.p.c.), mean per culture; (s.r.), sex ratio; (n.c.), number of cultures; (n.d.p.), non-junctional progeny (c.l.p.), chromosome loss progeny.

From Day and Grell (1966) the non-disjunction frequencies are calculated by the expression:

$$\% \text{ non-disjunction} = \frac{4\ (\text{exc.}\ \varphi\varphi \times 100)}{\text{total} + \text{exc.}}$$

In multiplying the exceptional females by 4 and adding the exceptions to the denominator it is assumed that the number of exceptional males arising from non-disjunction is equivalent to the number of exceptional females and that one half of all XX and O oocytes are lost due to lethality.

The following expression was applied to calculate the frequencies of X chromosome loss among the

progenies: $\%\ \text{loss} = \frac{2\ (\text{exc. } ♂♂ - \text{exc. } ♀♀) \times 100}{\text{Total + exc.}}$

The excess of exceptional males over exceptional females is considered to arise from loss of an X-chromosome during meiosis and not from non-disjunction, as the excess of exceptional male progeny is best explained as arising from spontaneous loss (Mavor, 1924; Paterson et al., 1932; Sturtevant and Beadle, 1936; Uchida, 1962). Oocytes lacking an X will lead to viable progeny only when fertilized by an X sperm; hence a correction factor of 2 is used to account for the nullo-X oocytes fertilized by a Y sperm (Day and Grell; 1966).

From table 2, exceptional progenies was restricted only to the concentrations of 40 mg/ml and 100 mg/ml. The computation of such data gives the following percentages of non-disjunction and chromosome loss:

NaCy mg/ml	40	100
XO	0.44	2.07
XX·Y	-	3.31

A comparison among the data from Stone et al. (1969) on induced chromosome breakage in human cells in vitro with a minimum concentration of 0.20 mg/ml of cyclamate and the minimum effective dose from this experiment (40 mg/ml) demonstrates a difference of sensitivity, equivalent to two orders of magnitude. Although the two experimental designs are hardly comparable, it seems evident that Drosophila oocytes are much less sensitive to cyclamates than human cells in vitro.

In a third experiment female flies aged for 3 days were fed during 24 hours with sodium cyclamate dissolved in distilled water at concentrations of 50 mg/ml, 100 mg/ml, and 160 mg/ml. (See Technical Note by R. Félix). Each treated female was mated afterwards with 3 males in individual cultures containing normal agar-cornmeal medium. After 6 days of egg-laying the flies were eliminated and the progenies were scored after 13 to 15 days (Tab. 3)

Table 3. Progenies obtained from the feeding of adults with concentrated solutions of sodium cyclamate.

NaCy mg/ml	50.00	100.00	160.00
♂ (m.p.c.)	30.75	36.25	35.31
♀ (m.p.c.)	21.78	21.15	22.95
♂/♀ (s.r.)	1.41	1.71	1.54
♂ (total)	861	725	671
♀ (total)	610	423	436
(n.c.)	28	20	20

NaCy mg/ml, sodium cyclamate, milligram/milliliter; (m.p.c.), mean per culture; (s.r.), sex ratio; (n.c.) number of cultures.

No exceptional progeny among 3,726 flies were obtained after the feeding of Drosophila with sodium cyclamate solution, in spite of the 160 mg/ml concentration, which is close to the saturation point.

Comparing the 100 mg/ml concentration in the food medium with the same concentration in solution, the size of the progenies are remarkably alike.

An aspect of the experiments, which called our attention, is the deviation of the sex ratio, indicating a lowered proportion of the X·XY females (y^2w^a/In(1)EN, Y^S, By·Y^L) as compared with the XO males (y^2w^a). The deviation that is also found in the control groups, is considerably magnified by the prolonged treatment of adults with cyclamate, as shown in Table 2.

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