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

The artificial sweetener sodium cyclamate, increases chromosome breaks when added in relative high concentrations in human leukocytes in vitro (Stone et al., 1968), as well as in monolayer cultures derived from human skin and carcinoma of the larynx (Stone et al., 1969). The same compound fails to induce chromosomal damage in Haworthia variegata Haw (Majumdar and Lane, 1970). This inability is probably due to the fact that chemical agents that break animal chromosomes may not induce chromosomal aberrations in plants, and different plant genera may react in different ways to the same agent as is found in mammals (Brodie, 1965).

Legator (1968), and Legator et al. (1969) demonstrated that cyclohexylamine, a breakdown product of cyclamate, also induces chromosome breaks in vitro in rodent cells in culture, as well as in vivo, in rat spermatogonia.

The Food Additives and Contaminants Committee provides evidence on the transformation of cyclamates to cyclohexylamine in persons ingesting cyclamates for 2 to 3 days (Food Additives and Contaminants Committee, 1967).

In another line of research a cyclamate-saccharin mixture was fed to male and female rats (FDRL strain, Wistar derived). The doses received in the food varied from 0 to 2,500 mg/Kg/day. Cyclohexylamine was assayed in the urine using the procedure of Derse and Daun (1966), omitting the oxidative steps. The research was carried on, in order to determine whether the conversion of cyclamate to cyclohexylamine takes place in the gastrointestinal tract or systematically as a biotransformation product. In the latter case, the reason for the differences on metabolic handling and the possibility for its genetical control would be of particular interest, considering the similar findings in man (Oser et al., 1968).

Kojima and Ichibagase (1966) and Leahy et al. (1967) also found that cyclamate is metabolized to cyclohexylamine in dogs and man. The most difficult areas in which to determine a cause-and-effect relationship in the human population are in the assessment of carcinogenicity, mutagenicity, or teratogenicity after exposure of the subject to a specific compound. Because of the long latent period between exposure and expression of effects as well as the high background rate of damage, it is difficult to detect effects of a given agent in the population even after years of exposure. Induction of chromosome damage is one of the methods used to evaluate potencial carcinogenic, mutagenic, or teratogenic effects of the cyclamate metabolite.

The development of bladder neoplasms was reported in the Wistar strain of rats fed with cyclamate of saccharin (Price et al., 1970). There is no evidence that the use of cyclamate or saccharin has caused cancer in man, malformations in children, or any other abnormality in humans other that a rare skin hypersensitivity. However, in view of the requirements of the Delaney clause of the Food Additives Amendment, the removal of cyclamates from the classification of substances generally recognized as safe resulted in the prohibition of their use in general purpose food products.

The present report contains preliminary results on the sensitivity of oocytes of D. melanogaster using the adult feeding method. The process of oogenesis in adult females has already been described in detail (King et al., 1956), and the main features of germ-cell stage sensitivity to some mutagens are well known (Pelecanos, 1964). The chemical treatments administered by adult feeding require a longer period than the irradiation treatments, as the responsible mutagenic reaction is expectedly prolonged after the period of treatment. If newly emerged adult females are treated by adult feeding during 24 hours the stages affected are the stages immediately preceeding stage 7, stage 7 itself, and stages 7-13, which have developed during such a period.

The sensitivity of stage 14 oocytes, reached at the 3rd day of adult life can be studied with more confidence, as there is no further development of oogenesis after the treatment.

The procedure adopted for the present study was to collect newly-emerged virgin females which were aged afterwards during 4 days and mating each of them with two males in vials containing regular agar-cornmeal food-medium, making further collection of less than 24 early eggs. The post-treated group includes the isolation of newly-emerged virgin females in regular medium, aging them during 4 days in food with cyclohexylamine, and further mating and oviposition in regular medium (Table 1). In the pre and post-treated group the embryonic, larval and first 4 days of adult life prior to mating took place in a medium with cyclohexylamine (Table 2). Afterwards, the flies were transferred to the regular agar-cornmeal medium employed during all the experiment, and mated in order to collect samples of stage-14

oocytes.

Cyclohexylamine (Merck) was added to the food medium and homogenized with a stirrer (Félix, 1970) at the temperature of 40 ± 2° C. The examination of the progenies was done 13 days after oviposition.

In order to test the toxicity of cyclohexylamine, several concentrations were tested, feeding all the stages of development. The concentration of 8.60 mg/ml killed adults before 2 hours, while feeding with concentrations from 4.30 mg/ml to 6.88 mg/ml gave adult survival during 24 hours without development of the eggs layed during such a period. Cyclohexylamine at concentrations of 0.86 mg/ml did not noticeably affect the life-cycle of adult and larvae.

Table 1. Progenies obtained from adult feeding of females with cyclohexylamine.

cyc mg/ml	Control	0.08	0.86
♂ (m.p.c.)	10.40	11.17	10.54
♀ (m.p.c.)	6.40	7.00	7.42
♂/♀ (s.r.)	1.63	1.60	1.42
♂ (total	260	201	274
♀ (total)	160	226	193
(n.c.)	25	18	26

cyc mg/ml, cyclohexylamine, milligram/milliter; (m.p.c.) mean per culture; (s.r.), sex ratio; (n.c.), number of cultures.

An improved method for detecting non-disjunction and chromosome X loss was applied, that gives particularly reliable evidence concerning the origin of the exceptional recovered progeny. Females were taken from one stock with sc^8Y chromosome, to avoid the existence of any secondary exceptions from XXY mothers (y^2 w^a/sc^8Y; e/e). The tester stock which provides males has an attached $Y^SX{\cdot}Y^L$ chromosome with the markers yellow and Bar (In(1)EN, Y^S B $y{\cdot}Y^L$/ $\frac{y^2}{su\text{-}w^a\ bb}$/0). When virgin females are isolated, the marker sc^8 in the Y chromosome, which contains the normal allele of y identifies XXY females which show gray phenotype instead of the yellow color showed by normal XX females. 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.

Table 2. Progenies obtained from larval and adult feeding of females with cyclohexylamine.

cyc mg/ml	Control	0.08	0.86
♂ (m.p.c.)	10.40	7.57	7.81
♀ (m.p.c.)	6.40	7.57	6.81
♂/♀ (s.r.)	1.63	1.33	1.15
♂ (total)	260	174	125
♀ (total)	160	131	109
(n.c.)	25	23	16

cyc mg/ml, cyclohexylamine, milligram/milliter; (m.p.c.), mean per culture; (s.r.), sex ratio; (n.c.), number of cultures.

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 or Y 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 XY bearing sperm would become an XY patroclinous male, which can be identified by the Bar eyes in its phenotype.

Virgin females of the genotype y^2 w^a/y^2 w^a; e/e were aged during 4 days. In each vial an aged female was mated with 2 attached Y^S X B $y{\cdot}Y^L$; +/+ males, and eliminated after the collection of stage 14 oocytes. All the cultures were kept at 25 ± 1° C throughout the experiment.

P y^2 w^a/y^2 w^a;e/e ♀♀ x In(1)EN,Y^S By·Y^L;+/+ ♂♂

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

y^2 w^a;e/+ ($\frac{y^2}{}$ $\frac{w^a}{}$) ♂♂

exceptional: $y^2\ w^a/y^2\ w^a;e/+$ $(\underline{y^2\ w^a})$ ♀♀

$In(1)EN,Y^S\ B\ y\cdot Y^L;e/+$ $(\underline{B\ y})$ ♂♂

Data on the progenies obtained in the treated and control groups are included in Tables 1 and 2. No exceptional progenies were found either among 894 treated flies, from the adult feeding group, nor among 539 treated flies from the larval and adult feeding group.

Among 1,433 stage-14 oocytes treated with the maximum concentrations permissible for the female adult, no exceptions from non-disjunction and X-chromosome loss were recovered. However, the equivalence between the effects of the dosages employed in this experiment, and those from previous findings named above, is evidently difficult. Such factors as absorption, protein binding and excretion are to be considered to make pertinent comparisons with mammalian or human intake cyclamates and its transformation to cyclohexylamine.

It seems from this experiment that Drosophila shows a low sensitivity to the ingestion of cyclohexylamine when the genetic events mentioned before are recorded.

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Sreerama Reddy, G. and N.B. Krishnamurthy. University of Mysore, Manasagangotri, Mysore-6, India. Preliminary survey of Drosophilids in Nilgiris and Kodaikanal ranges.

Maiden trips were made to Nilgiris and Kodaikanal ranges to explore the Drosophilids. Nilgiris is about 100 miles to the south of Mysore and Kodaikanal is about 300 miles also to the south of Mysore. Both these localities are hill stations characterised by the combination of temperate and tropical forests. The sholas are evergreen with moist and humid atmosphere in most part of the year. Shifting cultivation forms the common biotic factor in both the localities. The annual rain fall ranges from 1524 to 2540 mm. The highest peak of Nilgiris is 2580 meters and that of Kodaikanal is 1900 meters.

Collections were made in the beginning of December 1970. In the Nilgiris, flies were trapped at 11 different altitudes ranging from 840 to 2580 meters, whereas in Kodaikanal the flies were trapped at six altitudes ranging from 1000 to 1900 meters. A total of 1470 flies were collected from both the localities, of which 1009 flies come from Nilgiris and 461 from Kodaikanal. The details of the collection record are depicted in tables 1 and 2. The various species collected from both the localities are D. melanogaster, D. ananassae, D. melarkotliana, D. takahashi, and D. immigrans. The species like D. repleta, D. kikkawai, D. mysorensis, D. hoozani like species and D. busckii are found in Nilgiris and absent in Kodaikanal range. In addition, two new species of Drosophila which will be described elsewhere were also found in the traps. In Kodaikanal range one individual belonging to the Genus Leucophenga was found in an orchard near Valegiri at an altitude of 1000 meters. It is quite remarkable to note that D. immigrans is wide spread in its distribution in almost all places scanned. This shows that D. immigrans thrives well in a moist and humid climate. Further the most interesting feature of our collection study is the lack of Drosophila flies