Cytogenetic Effects of Cyclamates on Human Cells in vitro

Abstract. *Chromosome studies on human cells in vitro have shown that the artificial sweeteners, sodium and calcium cyclamate, can induce chromosome breaks in vitro in human cells, in both leukocyte and monolayer cultures.*

The effects of a variety of agents (irradiation, viruses, and antimetabolites) that can stimulate chromosomal aberrations, including breakage (*1*), as well as substances, such as steroid hormones (*2*) and components of fetal calf serum (*3*), that appear capable of regulating chromosomal changes which take place in vitro have been described. The report by Cohen *et al.* (*4*) on the induction of chromosome breaks in human leukocytes by small quantities of lysergic acid diethylamide (LSD) prompted investigations not only on the biological and genetic effects of LSD but also on chromosome breakage. The artificial sweetener sodium cyclamate, in relatively high concentrations, increases chromosome breaks in onion-root tips (*5*) and in human leukocytes in vitro (*6*); 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 (*7*). We now report (*6*) on the ability of sodium and calcium cyclamate to induce chromosome breakage in human leukocytes in vitro, as well as in monolayer cultures derived from human skin and carcinoma of the larynx.

Chromosome preparations were made from human leukocyte cultures by standard procedures (*8*). Usually, sufficient blood was collected for triplicate or quadruplicate cultures. One culture was used as a control, and the others were supplemented with different amounts of sodium or calcium cyclamate made up in sterile growth medium. All cultures were incubated for 72 to 84 hours at 37.5°C, and colchicine (0.5 μg/ml) was added for the last 3 hours. Four slides were prepared for each culture condition and coded, and, unless otherwise noted, 20 to 30 well-spread metaphase cells were chosen at random under low power (× 100) magnification on each slide and studied at high magnification (× 1000) for chromosome breaks. Approximately 100 metaphase spreads were studied for each control or cyclamate-supplemented culture. Very few quadriradial figures or double fragments were seen, and the data refer to clearly distinguishable chromosome breaks. A break was scored regardless of whether it was a single chromatid or an isochromatid break.

For the monolayer cultures, cells were cultured in Eagle's minimal medium supplemented with calf serum (10 percent). Cultures derived from human skin were produced from small biopsies (*9*), and cultures of human carcinoma of the larynx were purchased (Grand Island Biological Co.). Generally, the cells for chromosome analysis were grown either in the presence of or the absence of cyclamate for 5 to 6 days at 37.5°C, and colchicine (0.1 μg/ml) was added for the last 3 hours of culture. The cells were grown either in plastic bottles (Falcon Plastics) and harvested by trypsin treatment for chromosome preparation by a modification of the standard method (*8*), or cultured on glass slides in Leighton tubes for squashing (*10*). Subsequent procedure was identical to that with leukocytes.

Figure 1 shows the results obtained with more than 20 different subjects, both male and female, when the leukocyte cultures contained no cyclamates or were supplemented with sodium or calcium cyclamates at concentrations varying from 50 to 500 μg/ml. Chromosome breaks in the control cultures ranged from zero (in two cases) to a maximum of 12.3 percent, with an average value of 5.2 percent. In the cultures supplemented with cyclamates, the number of cells exhibiting breaks ranged from zero (in one case) to 35 percent, with an average value of 11.0 percent for cultures containing 250 to 500 μg of cyclamate per milliliter. The

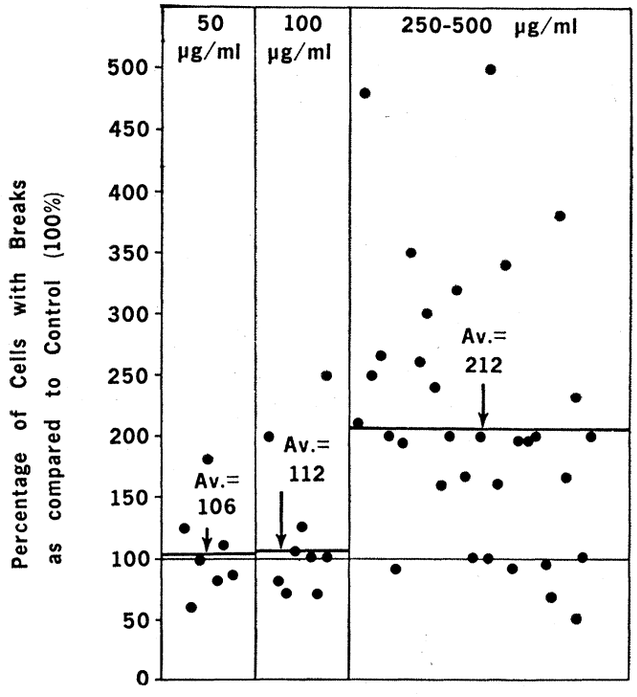

Fig. 1 (left). Effects of cyclamates on chromosome breaks in human leukocytes in vitro.

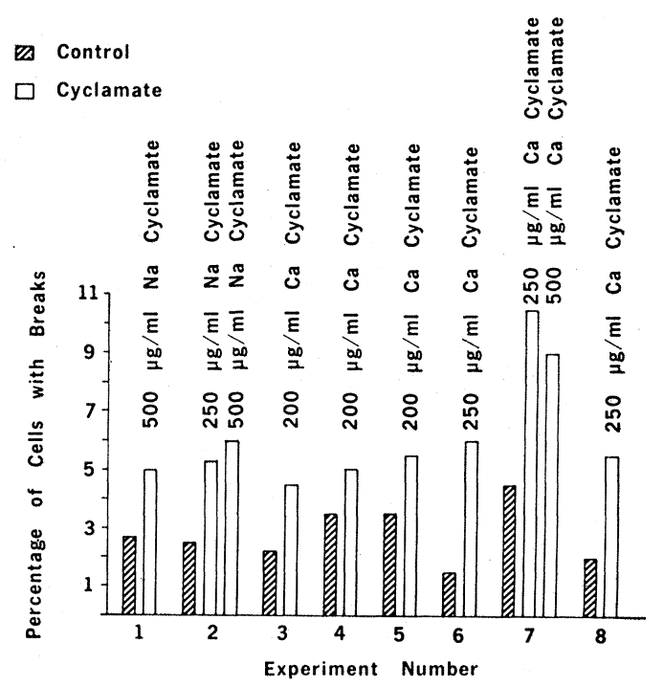

Fig. 2 (right). Cytogenetic effects of cyclamates on cultures of human leukocytes.

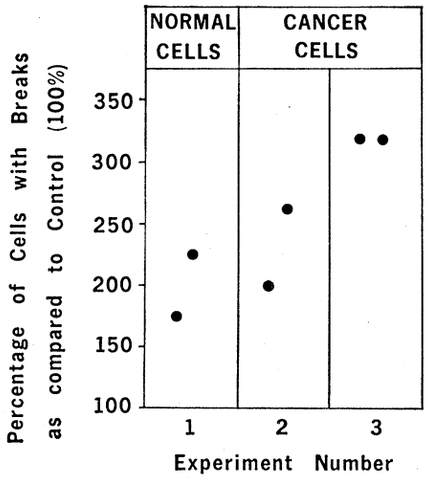

Fig. 3. Cytogenetic effect of cyclamates (200 μg/ml) on human monolayer cultures.

data have been plotted with each control value set at 100 percent (for plotting, the three samples exhibiting no breaks were given values of 1 break per 100 cells), and the results show that, while no clear effect of cyclamates on chromosome breaks at concentrations of 50 to 100 μg/ml can be seen, there is a significant effect at 250 to 500 μg/ml. In contrast to the action of cyclamate, leukocyte cultures supplemented with saccharin (500 μg/ml) showed no evidence of increased breaks.

In a second series of experiments on eight subjects, the procedure was slightly changed. The slides were not flamed during chromosome preparation; coded slides from two experiments were pooled prior to chromosome examination, and approximately 50 metaphases per slide were read (that is, a total of 200 metaphase spreads per culture. Figure 2 shows the results obtained in cultures supplemented with 200 to 500 μg of calcium or sodium cyclamate per milliliter. Chromosome breaks among control cells ranged from 1.5 to 4.5 percent, with an average value of 2.8 percent; in the treated cells, the range was 4.5 to 10.5 percent, with an average value of 6.2 percent. These results correspond well with the doubling found in the first series of experiments. Reported values from different laboratories are 3.7 (*4*), 1.4 (*11*), and 11.7 percent (*12*). Whether the variation in average control values found in the second series of our data, as compared to the first, is due to the smaller numbers of subjects studied, to the procedure of mixing the coded slides from two subjects, to the elimination of flaming the slides, to the greater number of mitotic figures studied, or to a more critical evaluation of breaks is not known.

Figure 3 shows the data obtained in one experiment with monolayer cultures of cells derived from normal human skin cultured in plastic bottles. Two bottles contained sodium cyclamate (200 μg/ml), and two bottles did not receive the supplement. Cells from each bottle were harvested separately, and four chromosome slides were prepared. In the presence of cyclamate, the cells show an approximate doubling of chromosome breaks. Figure 3 also shows the results obtained with cultures of cells derived from human carcinoma of the larynx. Experiment 2 was carried out in a manner similar to that described above; in experiment 3, cultures were made on glass slides in Leighton tubes, and chromosome squashes were prepared. Either way, the data indicate a two- to threefold increase in breakage by the presence of the supplements.

Our results indicate that cyclamate, in a minimum concentration of 200 μg/ml, can stimulate chromosome breakage in human cells in vitro. Whereas a high dosage (equivalent to 15 g/75 kg) was required to obtain a demonstrable increase in chromosome breaks, it should be pointed out that there is some evidence of synergistic actions on chromosome damage between x-irradiation and radiomimetic chemicals (*13*), between the chemical agents and virus (*14*), and between the chemical agents themselves (*15*).

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Hallucinogen-Tranquilizer Interaction: Its Nature

Abstract. *Study of the competition between hallucinogens and tranquilizers at cerebral synapses and on behavior in various species of animals indicates a continuum of effects from protection to dominance of tranquilizer toxicity as the dose of tranquilizer increases. Data on cat and monkey behavior, supplementing that on the rat, show that it is possible to arrive at a tranquilizer dose that can aggravate instead of protect, in accord with the competitive inhibitory nature of the interaction of hallucinogen and tranquilizer.*

Renewed interest in the problem of terminating the action of hallucinogenic drugs, particularly in the so-called "bad trip," has highlighted the necessity for a clearer understanding of the available facts. For example, can one reconcile the clinical accounts of the aggravation by chlorpromazine (CPZ) of the hallucinogenic effects of DOM (4-methyl-2,5-dimethoxy alphamethylphenethylamine) [also known as STP (*1*)], with the failure to obtain other than ameliorative effects in volunteers (*2, 3*)?

It is recognized that overlarge doses of CPZ, instead of allaying symptoms in mentally disturbed patients can, on occasion, themselves induce hallucination (toxic psychosis). Can, then, the therapeutic action, the antidotal action against exogenous hallucinogens, and the hallucinogenic action of large doses of CPZ be harmonized into an expected continuum?

A counterpart and a reasonable answer can be found in experiments with animals. In a previous comparative neuropharmacological survey (*4*) of cerebral synaptic transmission conducted by monitoring the output potentials evoked by a constant, submaximum input (*5*), we showed that the