A cause-and-effect relationship under a certain environmental condition or after exposure to chemicals in the human population is a perplexing subject for study because of the intricacy of the population and long latent periods. *In vitro* experiments provide in general a method advantageous for the investigation of the direct action of drugs on cells and tissues, since there are absent complex metabolic interactions occurring in the whole body.

Sodium cyclamate was widely used as an artificial sweetner in foods and beverages (Rubin, 1970). Since the first description that sodium cyclamate increases chromosome breaks in onion root tip cells (Sax and Sax, 1968), cytogenetic as well as carcinogenic effects of this drug on human subjects have been studied by several workers (Stone *et al*., 1969, Stolz *et al*., 1970 and Rubin, 1970), suggesting the possibility of carcinogenicity of this drug, and subsequently the restriction and inhibition on dietary consumption.

This preliminary paper describes some aspects of cytogenetic effects of sodium cyclamate on human leucocytes in culture.

**Materials and methods.** As a preliminary experiment for the determination of appropriate doses an established *in vitro* cell line of monkey kidney (Vero) was used. The cells have been maintained in Eagle's MEM with 10 percent calf serum. Cells were counted and cultured in Leighton tubes with or without sodium cyclamate of varying concentrations. The cells were trypsinized everyday in five Leighton tubes, stained with 0.5 percent trypan blue, and counted with the aid of a hemocytometer.

Human leucocyte cultures were made for the purpose of chromosome examinations. To blood, about 10 ml, withdrawn from a healthy male donor into a heparinized syringe, 0.2 ml of diluted (1:10) phytohemagglutinin-P was added. After centrifugation at 350 rpm for 5 minutes, leucocyte rich plasma was mixed with three volumes of Eagle's MEM, with or without sodium cyclamate. Final concentration of cyclamate in the medium was 0.01 M.

Cultures were incubated at 37°C, and colchicinized two hours

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prior to the chromosome preparations. Slides were prepared by the routine air-drying method with Giemsa staining.

Results. The effect of sodium cyclamate on the growth of the Vero cells are shown in Fig. 1. It was shown that the cell growth was not affected in the concentrations of 0.01 M and the less, while the concentrations higher than 0.05 M caused a remarkable growth inhibition. On this basis the 0.01 M concentration was used exclusively for the following chromosomal studies.

In untreated control cells cultivated for 3 and 6 days, 3.4 percent of cells showed aneuploid karyotypes, while no chromosome breakage was noted. On the other hand, in 3-day-cultures with cyclamate, cells with two breaks were found out of 176 cells scored, and 6-day-cultures furnished 27 cells with breaks and acentrics out of 149 (Table I). Some representative figures of such chromatid breaks and acentrics are presented in Fig. 2.

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>No. of cells observed</th>
<th>No. of cells with simple breaks (%)</th>
<th>No. of cells with abnormal karyotypes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>Control: 118</td>
<td>0</td>
<td>4(3.4)</td>
</tr>
<tr>
<td></td>
<td>Treated: 176</td>
<td>2(1.1)</td>
<td>40(22.7)</td>
</tr>
<tr>
<td>6 days</td>
<td>Control: 89</td>
<td>0</td>
<td>4(4.4)</td>
</tr>
<tr>
<td></td>
<td>Treated: 149</td>
<td>27(18.1)</td>
<td>57(38.3)</td>
</tr>
</tbody>
</table>

Table II. Chromosome-number distribution in leucocytes exposed to sodium cyclamate

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>Chromosome count</th>
<th>Total No. of cells observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;43</td>
<td>44</td>
</tr>
<tr>
<td>3 days</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6 days</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent pseudodiploid cells. Acrocentric chromosome was not taken into the count.

Karyotype analyses made in the 3rd- and 6th-day samples revealed the occurrence of many aneuploid cells in the treated series some of which contained several markers or unusual elements (Fig. 3). The frequency of such aneuploid cells was strikingly higher in 6-day-cultures than in 3-day-cultures (Table II). Noticeable are the facts that some of cells having 46 chromosomes were pseudodiploid, and that a considerable proportion of hyperdiploid and hypodiploid cells was noted (Fig. 3).

Discussion. So far as I am aware, three reports have been available on cytogenetic damages induced by cyclamate or by its
metabolites in human and rat cells (Stone et al., 1969, Legator et al., 1969, Stolz et al., 1970). They reported as many as 15–20 percent of the cells showing chromosome breaks in general sense, though Brewen et al. (1970) failed to find a significantly higher rate of breaks after short time exposure to cyclohexylamine. The present study revealed that the frequency of cells with breaks was not significantly higher in 3-day-cultures, while an increase of such cells was noted in 6-day-cultures. From the data here obtained it is most likely that cyclamate has some mutagenic and carcinogenic action as have been suggested by Legator et al. (1969) and Stone et al. (1969). Previous investigations on the effect of cyclamate upon human leucocytes were undertaken for the most part on cultures within 3 days (Stolz et al., 1969, Stone et al., 1969, Brewen et al., 1970). Their major interest dealt with the chromosome breakage without special attention to aneuploid cells. In contrast, the present study noted a high incidence of karyotypically abnormal cells, being more frequent in 6-day-samples than in 3-day-ones. A fact of interesting is that most of those cells did not contain acentric fragments. This seems to suggest that they have undergone one or more cell divisions after rearrangement. Detailed chromosomal investigations between 3 and 6 days of cultures may illustrate the above point, studies having been now in progress.

Fig. 1. Growth curves of Vero cells with or without sodium cyclamate. Each point represents the average number from five culture tubes.
Summary. Chromosome studies are carried out in human leucocytes cultivated in the presence of 0.01 M sodium cyclamate, an artificial sweetner. Many cells with aneuploidy and breaks, and chromosome rearrangements were produced by cyclamate. Mutagenic
action of this agent may be suggested by the cytogenetic data to
certain extent.

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References
genetic effects of cyclohexylamine and N-OH-cyclohexylamine on human leuco-
studies in rats of cyclohexylamine, a metabolite of cyclamate. Science, 165,
1139–1140.
Rubin, H. (1970): Bladder tumors in rats fed cyclohexylamine or high doses of
Sax, K., and H. J. Sax (1968): Possible mutagenic hazards of some food addi-