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THE MUTAGENIC ACTIVITY OF INORGANIC FLUORINE COMPOUNDS

by

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During the last three decades much experimental evidence has accumulated showing that many substances possess mutagenic activity, in some cases even greater than that of ionizing radiation. Increases

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in the frequency of gene mutations in human gametes, therefore, pose a grave threat to the genetic fund of mankind. Direct investigation of the mechanisms of such changes in man is virtually impossible, however, and indirect experimental and statistical methods have to be used. One of these methods is to study the effect of a given compound on the occurrence of chromosomal aberrations in human cell cultures and in somatic cells of experimental animals in vivo.

The present investigation concerns the influence of inorganic fluorine compounds on chromosomes in bone marrow and on the mytotic activity of epithelial cells of the cornea in female white rats. The animals were exposed 6 hours a day, six days a week, for 5 months, to the inhalation of cryolite (Na_3AlF_6) in concentrations of 3, 1, and 0.5 mg/m^3 (calculated as fluoride ion), which is frequently encountered in the air of electrolysis areas of aluminum plants, and also of a mixture of 0.5 mg/m^3 of cryolite and 0.35 mg/m^3 of hydrogen fluoride.

Four rats were used in each variant of the experiment for cytogenic analysis. Bone marrow preparations were stained with azure-eosin. All types of chromosomal and chromatid aberrations were considered in the metaphases, but for the analysis only undamaged metaphasal plates with the typical 42 chromosomes were selected. Gaps were not included in the count of aberrations; tears were distinguished from gaps by displacement of the fragment with respect to the chromatid axis. On the average 80 metaphasal plates were analyzed for each rat. Total corneal preparations were stained with hematoxylin; mytotic activity was determined by the number of mytoses per 15,000 cells in each cornea. The experimental results were processed by dispersion analysis.

As seen in Table I, there was no significant effect on the mytotic activity of the corneal epithelia immediately following inhalation and up to a month afterward. However, at the end of the recuperation period there was a definite increase in mytotic activity in the corneal epithelia of all the experimental animals ($P < 0.05$), probably attributable to seasonal variations in the rate of cell division.

TABLE 1

Mytotic Activity in the Corneal Epithelium Cells of
Experimental Animals (in %)

| Period of Experiment | Mytotic Activity ($\bar{x} + S_{\bar{x}}$) | | | | |
|---------------------------------|--|---------------------------------------|---------------|---------------|--|
| | Control | Cryolite (in mg/m^3) | | | Cryolite (0.5 mg/m^3) + HF (0.35 mg/m^3) |
| | | 0.5 | 1.0 | 3.0 | |
| At end of 5 months | 5.4 \pm 0.3 | 5.5 \pm 0.3 | 5.8 \pm 0.4 | 6.0 \pm 0.9 | 5.9 \pm 0.4 |
| After one month of recuperation | 6.0 \pm 0.3 | 6.0 \pm 0.3 | 6.3 \pm 0.3 | 6.3 \pm 0.2 | 6.9 \pm 0.7 |

The distribution of dividing cells among the phases of mytosis was also examined. The phase coefficient (equivalent to the ratio between the fraction of cells in the prophase and metaphase and the fraction in the anaphase and telophase) showed little variation and had a mean value of 1.4. Thus, long-term inhalation of fluorine compounds did not produce any significant cytostatic effect on the cells of the corneal epithelium.

Chromosomal aberrations were taken as the basic criterion of genetic damage, and, as seen in Table 2, the animals exposed to fluorides showed an increase in the percentage of aberrant bone marrow cells. Statistically significant differences were found, however, only with the highest concentration of cryolite and the mixture of cryolite and hydrogen fluoride. The absence of reliable difference after inhalation of the lower concentrations of cryolite probably reflects the small number of animals used. Whether threshold doses of mutagenic agents exist remains open to question.

That fluorine is primarily responsible for the chromosomal aberrations is seen in the fact that almost 3 1/2 times more aberrations occurred in the cells of animals exposed to the mixture of 0.5 mg/m³ of cryolite and 0.35 mg/m³ of HF as in those exposed to cryolite in a concentration of 0.5 mg/m³. In both of these experiments the sodium and aluminum concentrations were unchanged while the fluorine concentration in the cryolite-HF mixture was 0.7 times higher than in the cryolite alone. Moreover, fluorine is fully absorbed from HF which enters the lungs, whereas it is assimilated to a lesser extent and with greater difficulty from cryolite.

TABLE 2

Number of Cells (in %) with Chromosomal Aberrations in the Bone Marrow of Rats Subjected to Exposure to Fluorine Compounds

| Index | Control | Cryolite (in mg/m ³) | | | Cryolite (0.5 mg/m ³) + HF (0.35 mg/m ³) |
|--|------------|----------------------------------|-------------|-------------|--|
| | | 0.5 | 1.0 | 3.0 | |
| No. of cells with chromosomal aberrations, $\bar{x} \pm S_{\bar{x}}$ | 1.4 ± 0.88 | 1.50 ± 0.56 | 2.40 ± 0.59 | 6.50 ± 0.66 | 5.10 ± 0.55 |
| Reliability of the difference from the control, P | - | > 0.05 | > 0.05 | < 0.001 | < 0.01 |

Most of the aberrations observed were of the chromatid type. More than 70% of the damage was to individual fragments, which is typical of spontaneous mutations occurring under the influence of metabolic automutagens. Hence, in view of its marked biological activity, fluorine may be assumed to stimulate the formation of mutagenic metabolites in rats.

Although the percentage of mutations occurring in the experiments is not very large (5-6%), it is typical of inorganic mutagens. This, however, does not detract from their potential danger for humans. With long-term exposure, even weak mutagens can produce considerable damage to the practically irreversible mechanism of genetic changes. Because of the extensive industrial employment of inorganic fluorides, substantial quantities of fluorine enter the human environment and affect large numbers of people. Thus the present findings show the need for more detailed studies of the mutagenicity of fluorine in order to evaluate and predict its potential for genetic damage to man.

Other findings in this work showed that inhalation of cryolite in concentrations as low as 1 mg/m^3 and also cryolite combined with HF leads to retardation effects in the central nervous system, suppression of the activity of a number of enzymes, to morphological changes in internal organs and tissues, etc. At a concentration of 0.5 mg/m^3 , cryolite was not observed to have these effects.

Conclusions

1. Chronic inhalation by white rats of cryolite in concentrations of 3, 1, and 0.5 mg/m^3 , and also of a combination of 0.5 mg/m^3 of cryolite and 0.35 mg/m^3 of hydrogen fluoride, had no cytostatic effect on the epithelial cells of the cornea.

2. Cryolite concentrations of 3 mg/m^3 as well as a mixture of 0.5 mg/m^3 of cryolite and 0.35 mg/m^3 of hydrogen fluoride increases by $3 \frac{1}{2}$ to $4 \frac{1}{2}$ times (over controls) the percentage of cells with chromosomal aberrations in the bone marrow of rats.

3. The data indicate the need for further study of the mutagenic features of fluorine compounds in relation to their potential for harmful impact on the mechanism of inheritance in humans.

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A, W, B.