# INDUCTION OF MICRONUCLEI IN MOUSE ERYTHROCYTES BY $Cr^{6+}$

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The phenomenon of the appearance of micronuclei (MN) in a nuclear erythrocyte in anemia, described by Howell [5] and later by Jolly [6], has recently been applied in a completely different field — as a screening test for evaluation of the mutagenic activity of chemicals. MN (Howell–Jolly bodies) are formed in the cytoplasm of erythroblasts at the anaphase stage from acentric chromatids, chromosomal fragments, or whole chromosomes after treatment with a mutagen. A few hours after completion of the last mitosis the erythroblasts lose their nucleus but the MN remain and are easily identified in the cytoplasm of the enucleated cells [4, 8].

The aim of the present investigation was to study genetic activity of $Cr^{6+}$, using as the criterion induction of MN in mouse bone marrow erythrocytes. The choice of $Cr^{6+}$ as test object was determined by the widespread use of chromium in industry, its presence in mineral springs, its ability to accumulate in living organisms, and its mutagenic and carcinogenic properties [1]. Practically no investigations devoted to the use of the MN test for evaluating the mutagenic activity of small doses of salts of the heavy metals, including chromium, except a study [2] which led to the conclusion that the MN test cannot be used to predict the carcinogenic properties of heavy metals, although this is contradicted by existing evidence on correlation between carcinogenic and mutagenic activity of carcinogenic metals with variable valency.

## EXPERIMENTAL METHOD

Experiments were carried out on hybrid (CBA × C57Bl/6J)$F_1$ mice aged 60-80 days and weighing 27-30 g. To determine the optimal time of recording the frequency of MN induction samples of bone marrow were taken 24, 48, and 72 h after a single intraperitoneal injection of $K_2Cr_2O_7$, containing 99.9% of the active water-soluble substance, in doses of 1, 5, and 10 mg/kg body weight, by the method in [8]. Films were stained by the modification in [3, 7]: air-dried (for 24 h) films were fixed in methanol (5 min), washed in bidistilled water, stained in 7% Giemsa solution [instead of the standard May–Grünwald solution (Fluka) [8]] for 10 min, and washed in phosphate buffer, pH 6.8. The stained films were cleared in xylol and mounted in Canada balsam.

## EXPERIMENTAL RESULTS

MN induced by $K_2Cr_2O_7$ in young mouse erythrocytes are shown in Fig. 1 (a-e). They are circular in shape and their diameter is about 1/25-1/10 of the diameter of the erythrocyte. Some cells contained two MN (Fig. 1d). Quantitative data are given in Table 1. All doses of $K_2Cr_2O_7$ proved to be effective, and the maximal frequency of MN induction for all doses was observed 48 h after injection of the compound. In this investigation the least effective dose of $K_2Cr_2O_7$ for MN induction in mouse marrow cells was found to differ from that given in the literature [2], in which the concentrations used were 10-50 times higher.

Figure 1 (e, f) shows MN in nucleated bone marrow cells, evidence of a common mechanism of chromosomal injury, ending with MN formation, in different types of cells.

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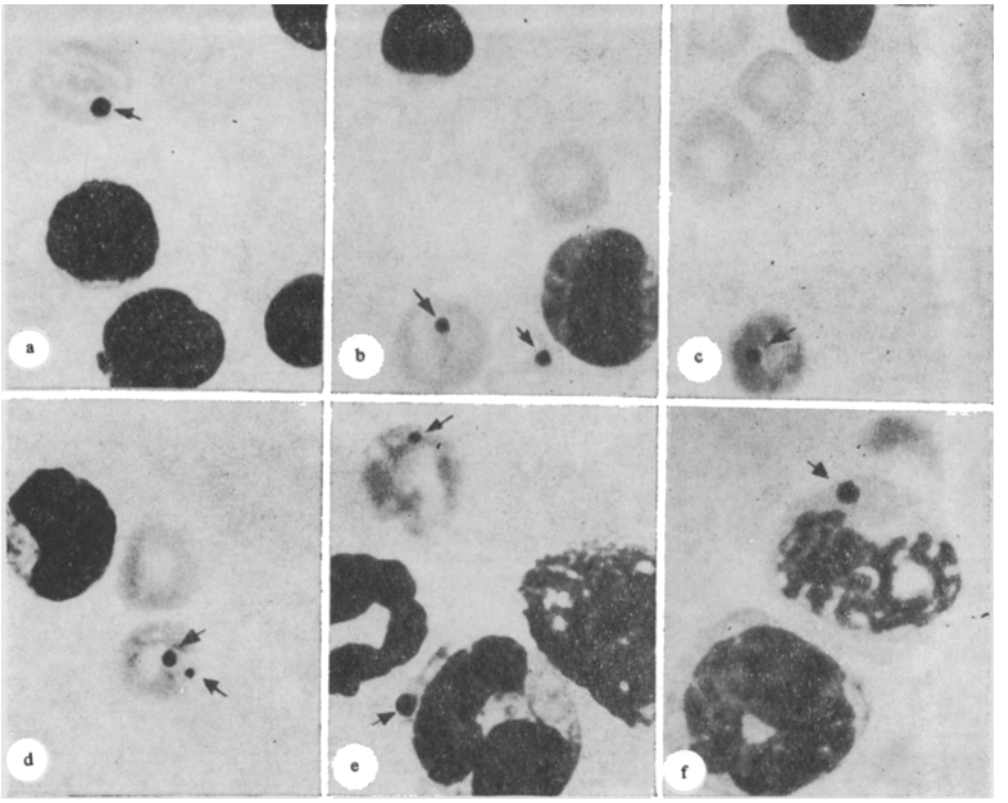

Fig. 1. Mouse bone marrow. Arrows indicate MN induced by $K_2Cr_2O_7$ in young erythrocytes (a-e). MN found in cells containing a nucleus (e, f) may be masked by the varying shape of the nuclear lobes and, consequently, they were excluded from quantitative analysis. Magnification 900.

TABLE 1. Changes in Number of MN (in %) in Mouse Bone Marrow Erythrocytes after Injection of $K_2Cr_2O_7$ ($\bar{x} \pm S_{\bar{x}}$)

| Dose, mg/mg | Number of cells counted, thousands | Times of investigation, days | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Control (12) | 12 | 2,75±0,49 | 2,81±0,38 | 2,90±0,45 |
| 1 (12) | 12 | 4,25±0,45 | 6,25±1,70* | 5,75±0,51* |
| 5 (12) | 12 | 4,75±0,71** | 7,50±1,29* | 7,25±0,97* |
| 10 (12) | 12 | 6,50±0,54** | 8,75±1,11* | 8,25±0,74* |

Legend. Number of animals given in parentheses. *P < 0.001; **P < 0.01 compared with control.

In spite of its apparent simplicity, the method of counting MN is extremely demanding with respect to the ionic composition of the water, the pH of the solutions, and the quality of the dyes and embryonic calf serum used to disperse the erythrocytes. Yet it has been recommended by a number of workers as a screening method which is even capable of replacing the much more difficult procedure of metaphase analysis of chromosomes, for which special knowledge is required [7, 8]. These extreme views cannot be fully accepted. Without belittling the virtues of the MN induction test, it must be emphasized that metaphase analysis is more informative and must still be awarded priority when the mutagenic potential of chemical compounds is to be estimated.

The contradictory data on the mutagenic activity of chromium compounds [2] can be explained by a change in the valency of chromium, namely reduction by the microsomal enzymes of the cell [1] to the less active trivalent form.

## LITERATURE CITED


1. Yu. V. Pashin, V. I. Kozachenko, T. A. Zatsepilova, et al., Tsitol. Genet., No. 5, 66 (1981).
2. L. Fabry, C. R. Soc. Biol., 174, 889 (1980).
3. B. Gollapudi and O. P. Kamra, Mutat. Res., 64, 45 (1979).
4. J. A. Heddle, Mutat. Res., 18, 187 (1973).
5. W. H. Howell, J. Morphol., 4, 57 (1890).
6. J. Jolly, Arch. Anat. Micr., 9, 133 (1907).
7. M. Salamone, J. Heddle, E. Stuart, et al., Mutat. Res., 74, 347 (1980).
8. W. Schmid, Agents Actions, 3, 77 (1973).