restriction endonucleases used at their ends. The precise location of these fragments one relative to the other can therefore be determined once the recognition sites of another (the 4th) restriction endonuclease have been mapped.

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EXPERIMENTAL STUDY OF THE ANTIMUTAGENIC PROPERTIES OF 5-METHYLRESORCINOL

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Metabolic activation of premutagens and precarcinogens has been shown to be connected with free radical formation. Among the inhibitors of free-radical processes the phenolic antioxidants are definitely interesting [3]. On the assumption that the antioxidative ability of phenolic compounds may determined their efficacy as antimutagens it was decided to study the effect of simple phenols on the induction of gene mutations in mammals *in vitro* [5]. The most effective compound proved to be 5-methylresorcinol (5-MR).

The antimutagenic activity of 5-MR during the action of benz(a)pyrene (BP), a typical environmental pollutant, and also of γ-rays, as a result of whose action products of free-radical oxidation of hydroperoxides accumulate, was studied *in vivo*.

EXPERIMENTAL METHOD

The action of 5-MR on the mutagenic activity of BP was studied *in vitro* by counting the number of induced direct gene mutations affecting the hypoxanthineguanine phosphoribosyltransferase (HGPRT) locus in cultures of somatic cells from V-79 Chinese hamsters by the method in [7] under conditions of metabolic activation by mouse liver microsomes [4].

BP (from Fluka, Switzerland) was used in a constant concentration of 0.04 mM, inducing a mutagenic effect, and 5-MR (from Merck, West Germany) in concentrations of 0.35 to 3.47 mM. Ethanol in a volume of 0.1 ml was used as the solvent. The toxic effects and mutagenic activity of BP and 5-MR, also used in the form of mixtures, also were investigated.

In an *in vivo* system mutagenic activity of BP and γ-rays was assessed, using as the criterion induction of micronuclei in polychromatophilic erythrocytes (reticulocytes) in adult (CBA × C57BL/6j)F_1 moust bone marrow. The presence of micronuclei in reticulocytes, which are the last precursor cells of mature erythrocytes to divide, reflects damage to chromosomes at the erythroblast stage. To determine the optimal time for recording the frequency of induction of micronuclei samples of bone marrow were taken from the animals 1, 2, 3, and 4 days after a single intraperitoneal injection of the chemicals or after irradiation. BP was injected in sunflower oil in a dose of one-third of the minimal lethal dose for mice [10]. A solution of 5-MR in water was injected in a dose of 0.1 LD_{50}, in the ratio of 1:0.5 (w/w) with BP. Films were stained by the method in [6], using the modification in [9], with fixation in methanol, washing in bidistilled water, and staining in 7% Giemsa solution at pH 6.8.

The animals were irradiated on the GUPOS apparatus with ^{137}Cs source of γ-rays (dose rate 470 R/min) at 0°C, with a total dose of 75 or 150 rads.

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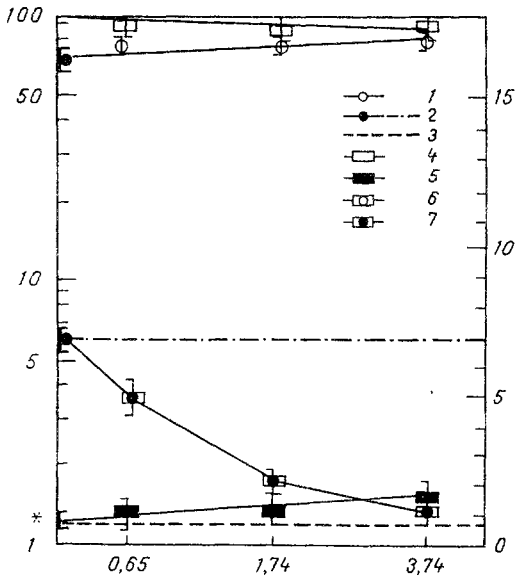

Fig. 1. Effect of 5-MR on level of gene mutations induced by BP under metabolic activation conditions. Abscissa, concentration of 5-MR (in mM); ordinate: on left, survival rate of cells (in % of control); on right, number of clones resistant to 8-azaguanine (per 10^5 cells). 1, 2) Survival rate of cells and number of induced mutations, respectively, for BP; 3) level of spontaneous mutations in initial cell population; 4, 5) survival rate of cells and number of induced mutations, respectively, for 5-MR; 6, 7) survival rate of cells (coincides with control) and number of induced mutations for mixture of BP + 5-MR.

EXPERIMENTAL RESULTS

Under the influence of BP in a concentration of 0.04 mM on the cell culture a fivefold increase in the spontaneous mutation rate for the HGPRT locus was observed, with a survival rate of the cells of 62-67%. In most of the concentrations used 5-MR did not increase the mutation rate significantly, nor did it exhibit its toxic effect.

Wtth combined treatment of the cells with a constant concentration of BP and increasing doses of 5-MR the survival rate of the cells was kept at the control level. If the molar ratio BP/5-MR was 1:9, the yield of gene mutations for the HGPRT locus was reduced by 2.6 times, whereas if the ratio was 1:44 it approximated to the mutation rate in the control (Fig. 1).

In the *in vivo* system 5-MR exhibited no mutagenic effects in the concentrations used. Under the influence of BP the peak of induction of micronuclei in mouse bone marrow reticulocytes was observed 2 days after injection, but after 4 days the number of micronuclei still remained higher than in the control. The mixture of BP + 5-MR reduced the number of micronuclei at all time intervals studied, with the greatest decrease at the peak of induction (by half), whereas after 4 days the level of induced micronuclei was close to that in the control (Table 1).

After irradiation of the mice induction of micronuclei reached a peak in 2 days, with a fourfold increase over the control level after exposure to γ-rays in a dose of 75 rads and a sevenfold increase after a double dose. At the end of three days the number of micronuclei was back to the control level.

If 5-MR was injected into the animals immediately after irradiation a marked decrease in the number of micronuclei was observed. If 5-MR was combined with a dose of 75 rads, their number at the peak of induction was reduced by half, and if combined with a dose of 150 rads it was reduced by 80%. The fall in the number of reticulocytes with micronuclei below the control level observed 3-4 days after irradiation with the high dose of γ-rays combined with 5-MR indicates nonspecific inhibition of erythropoiesis, evidently on account of gross injuries to the erythroblasts after the action of the two factors.

TABLE 1. Effect of 5-MR on Number of Micronuclei Induced in Polychromatophilic Erythrocytes of Mouse Bone Marrow by BP and γ-Rays (M ± m)

Experimental conditions	Number of cells ×10³	Time of investigation, days			
		1	2	3	4
Control	36	1,7±0,3	1,8±0,4	1,7±0,4	1,7±0,3
BP (150 mg/kg)	48	6,8±0,7	9,6±0,1	5,0±0.5	5,3±0,8
5-MR (75 mg/kg)	48	1,6±0,6	2,6±0,8	2,6±0,9	2,6±1,1
γ-rays:	48	3,0±0,9	3,7±1,0	0,5±0,3	1,0±0,4
75 rads	36	—	6,7±1,2	2,7±1,2	1,7±0,8
150 rads	36	—	12,6±0,8	2,0±0,1	1,0±0,4
γ-rays (75 rads + 5-MR (75 mg/kg)	36	—	3,7±0,3	1,0±0,5	1,0±0,2
γ-rays (150 rads + 5-MR (75 mg/kg)	36	—	2,6±0,3	0	0

5-MR was thus found to be an antioxidant with antimutagenic properties. These properties are attributable to the extremely easily removable hydrogen atoms of the hydroxyl groups of its molecule, and also of the methyl group, which prevents the formation of chemically active metabolites from carcinogens [8]. 5-MR can evidently actively destroy hydroperoxides formed after the action of radiation [2]. The use of BP and 5-MR in the ratio of between 1:5 and 1:25, in the present experiments, corresponds to that actually found in industrial waste products. For example, industrial waste from heat processing of oil shale contain BP and up to 40% of shale tars, a considerable proportion of which consists of fractions containing 5-MR [1].

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