A mixture of BP + PY in the ratio of 1:1 reduced the toxic effect of BP. Under these circumstances the mutagenic effect of BP was inhibited by 50%, but if the ratio of BP to PY was 1: 2.5 the level of induced mutations came close to the control value.

Inhibition of the mutagenic activity of BP by polyhydric phenols, observed in these experiments, is thus evidently connected with the presence of reactable hydrogen atoms in these compounds, which inhibit free-radical self-oxidation reactions of the chemical mutagen. The number of reactable hydrogen atoms in a series of polyhydric phenols increased with an increase in the number of hydroxyl groups, and was responsible for the antimutagenic properties of the simple polyhydric phenols (for example, the trihydric PY compared with the monohydric PH). Our results are in agreement with data in the literature on reduction of the mutagenic effect of BP by pyrogallol in a bacterial system [4]. Its antimutagenic properties may also be explained by its ability to form semiquinone derivatives, which bind free radicals most actively.

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IDENTIFICATION OF MUTAGENS BY FREQUENCY ANALYSIS OF MICRONUCLEAR NORMOCHROMIC ERYTHROCYTES

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Analysis of the frequency of polychromatophilic erythrocytes (PCE) with micronuclei in mammalian bone marrow is a rapid and simple test used at the stage of detection of potential mutagens and carcinogens [1, 2]. Reports have recently been published that normochromic erythrocytes (NCE) with micronuclei may accumulate in the peripheral blood of mice exposed repeatedly to chemical mutagens [3, 4]. The authors cited recommend that this modified micronuclear test be used for the intravital detection of mutagens in experiments on mammals.

To assess the potential of this method, the frequency of NCE (mature) and PCE (young) with micronuclei in the peripheral blood and the frequency of chromosomal aberrations in the bone marrow cells of mice, receiving cyclophosphamide with their drinking water, were compared.

EXPERIMENTAL METHOD

Experiments were carried out on random-bred male SHK mice. Each experimental and control group consisted of 5-6 animals. Cyclophosphamide (CP, from Jenapharm, East Germany), given to the animals with their drinking water in a concentration of 0.01% for 2 weeks, was used as the model mutagen.

In experiment I peripheral blood films were prepared by mixing a drop of blood from the subclavian vein on a slide with a drop of embryonic calf serum. The films were fixed in methanol and stained by the Giemsa method (pH 7.0). In experiment II, colchicine was injected intraperitoneally into the animals in a dose of 2.5 mg/kg 2 h before fixation, after which a parallel series of peripheral blood films and preparations of metaphase chromosomes of bone marrow cells was made.

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TABLE 1. Frequency of NCE and PCE with Micronuclei in Peripheral Blood and of Metaphases with Aberrant Chromosomes in Bone Marrow of Mice Receiving Cyclophosphamide in a Concentration of 0.01% with the Drinking Water for 2 Weeks

Experiment	Group	No. of mouse	Peripheral blood		Bone marrow	
			NCE with micronuclei, %	PCE with micronuclei, %	metaphases with aberrations, %	number of aberrations per 100 metaphases
I	Cyclophosphamide, 0.01%	1	0,45	0,8	—	—
		2	0,75	2,8	—	—
		3	0,60	0,8	—	—
		4	0,50	1,0	—	—
		5	0,50	1,0	—	—
		6	0,60	1,6	—	—
	$M \pm m$		0,57±0,04*	1,33±0,32*		
	Control	1	0,15	0,4	—	—
		2	0,05	0,0	—	—
		3	0,25	0,4	—	—
		4	0,20	0,6	—	—
		5	0,30	0,0	—	—
		6	0,20	0,4	—	—
	$M \pm m$		0,19±0,04	0,30±0,10		
II	Cyclophosphamide, 0.01%	1	0,65	—	10,0	11,0
		2	0,65	—	6,0	7,0
		3	1,10	—	3,0	5,0
		4	0,70	—	4,0	5,0
		5	1,55	—	11,9 †	13.1
	$M \pm m$		0,93±0,18*		7,0±1,72*	8,2±1,64*
	Control	1	0,55	—	0,0	0,0
		2	0,10	—	1,0	1,0
		3	0,40	—	3,0	3,0
		4	0,25	—	0,0	0,0
		5	0,25	—	2,0	3,0
	$M \pm m$		0,31±0,08		1,2±0,58	1,4±0,68

Legend. *P < 0.05 compared with control. †Data obtained by analysis of 84 metaphases.

During analysis of the peripheral blood films the following parameters were studied: the number of micronuclear NCE in 2000 NCE counted, the number of micronuclear PCE among 500 PCE counted, and the number of PCE for every 2000 NCE. In the bone marrow preparations 100 metaphases per animal were analyzed and the number of single and paired fragments and of chromatic and chromosome changes counted. The results for the groups were compared by White's T test for comparison of unpaired samples and by Wilcoxon's W test for comparison of paired samples.

EXPERIMENTAL RESULTS

The number of PCE as a fraction of the total number of erythrocytes in the peripheral blood films of the control animals varied from 0.7 to 4.7%, on average 2.25 ± 0.38%. Under the influence of CP no change in this proportion was observed: it varied in the experimental animals from 1 to 4.7% (mean 2.47 ± 0.35%).

The mean frequency of micronuclear NCE in the peripheral blood of the mice was 0.19 ± 0.04%, and the number of micronuclear PCE was 0.30 ± 0.10% (Table 1). In the group of animals receiving the mutagen a significant increase was observed compared with the control. Whereas in the control animals the values of these parameters did not differ statistically significantly, in the experimental groups the frequency of micronuclear PCE was significantly higher than the frequency of micronuclear NCE (P < 0.05).

In experiment II, just as in experiment I, the frequency of NCE with micronuclei was three times greater in the experimental animals than in the controls (Table 1). This indi-

cates preservation of mature micronuclear erythrocytes in the peripheral blood of the mice and their accumulation during prolonged administration of the mutagen. Incidentally, despite the higher frequency of micronuclear NCE in the animals receiving colchicine (in both experiment and control), compared with the corresponding groups of experiment I, these differences were not statistically significant. In experiment II, besides counting NEC with micronuclei, the frequency of chromosomal aberrations was estimated in the bone marrow cells of these same animals. With respect both to the percentage of metaphases with aberrant chromosomes (7%) and to the number of chromosomal aberrations per 100 metaphases (8.2), the values of these parameters were significantly higher than in the control (1.2% and 1.4, respectively). Aberrations were in the form of single and paired fragments.

Thus, during subacute (for 2 weeks) exposure to CP with the drinking water, cytogenetic disturbances were found both in the bone marrow (a 5.5-fold increase in the number of cells with chromosomal aberrations) and in the peripheral blood of mice (a 4.5-fold increase in the frequency of micronuclear PCE, a threefold increase in the number of micronuclear NCE compared with the control).

Since not only deleted chromosomes, but also acentric fragments of chromosomes, are recorded as micronuclei the results of the micronuclear test, determined in bone marrow or peripheral blood cells, provide an indirect indicator of mutagenic exposure. The micronuclear test on mouse peripheral blood erythrocytes is of great interest because of the accessibility of the material (blood from the caudal vein) and the possibility of obtaining information repeatedly over a period of time. As an indicator of mutagenic exposure it is advantageous to use the frequency of micronuclear NCE rather than the frequency of micronuclear PCE, because of the small number of young erythrocytes in the blood (1-4% according to our experimental results).

Our data on preservation of mature micronuclear erythrocytes in the peripheral blood of mice and on their accumulation during long-term exposure to the mutagen are in agreement with data in the literature [4]. In experiments on Swiss mice with triethylenemelamine as the mutagen it was shown [4] that the accumulation of micronuclear NCE approaches its maximum when the duration of exposure to the substance is close to the lifespan of the erythrocytes in the mouse peripheral blood. According to Shalm et al. [5], this is 20-45 days.

Absence or weakness of selection against micronuclear NCE in mouse peripheral blood is indicated also by the identical level of young, polychromatophilic (0.30 ± 0.10%, data for six animals) and mature, normochromic (0.25 ± 0.04%, data for 11 animals) micronuclear erythrocytes in the control mice.

Thus analysis of the frequency of micronuclear NCE in the peripheral blood of mice during prolonged exposure (up to 20 days) to the factor chosen for investigation may be an effective test for detecting its mutagenic properties. The question of whether other species of mammals can be used in this method has not yet been examined and further study is required.

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