the frog, caused slowing of relaxation and the appearance of frequency-dependent contracture [2]. This is evidence that inhibition of Na^+/Ca^{++} exchange, found in isolated vesicles of the sarcolemma under the influence of the cardiotoxic antibiotic dexorubicin, which closely resembles rubomycin in its structure [9], is a universal mechanism of the cardiotoxicity of the anthracyclines, common to the myocardium of both poikilothermal and homoiothermal animals.

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MODIFICATION OF THE CYTOGENETIC EFFECT OF FOTRIN ON INDUCTION OF A METABOLIC SYSTEM BY PHENOBARBITAL

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Evidence has now been obtained to show that the level of mutagenic effects depends on the state of systems of the body responsible for the biotransformation of chemical compounds. The writers' previous investigations showed that even against the background of weak (1.5-fold) induction of the microsomal mono-oxygenase system (MMS), activating many indirect mutagens, the effect of cyclophosphamide is potentiated; the modification, moreover, is observed also in the case of long-term induction [2, 5]. Such levels of induction of MMS, incidentally, are observed under real external environmental conditions in the case of pollution by discharges from factories, automobiles, pesticide application, and so on.

The aim of this investigation was to study whether the effect of the direct-action mutagen fotrin can be modified against a background of different levels of long-term MMS induction.

EXPERIMENTAL METHOD

Experiments were carried out on 157 noninbred male rats weighing 200-250 g. The direct-action mutagen fotrin, containing 5 ethylenimine groups in its structural formula, was used.

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TABLE 1. Frequency of Cells with Chromosomal Aberrations (in %) in Bone Marrow of Rats Treated with Different Doses of Fotrin and PS (M ± m)

Dose of fotrin, mg/kg	Dose of PS, mg/kg			
	0	0,4	2	10
0	0,67±0,49	1,50±0,81	1,33±0,21	1,33±0,42
2	9,67±1,50	8,83±1,49	7,0±1,26	6,83±1,09
4	14,0±1,57	16,17±2,81	13,40±1,91	12,0±2,13
7	24,33±2,56	21,83±2,29	15,50±2,03	16,17±2,18

TABLE 2. Coefficients of Regression Equations Showing Frequency of Cells with Chromosomal Aberrations in Rat Bone Marrow on Dose of Fotrin, against the Background of Different Doses of PS.

Dose of phenobarbital, mg/kg	$\alpha \pm S_\alpha$	$\beta \pm S_\beta$
0	0,113±0,036	0,085±0,009
0,4	0,151±0,041	0,075±0,010
2	0,168±0,030	0,053±0,007*
10	0,147±0,033	0,057±0,008*

*$p < 0.05$ in versions with combined action of fotrin and PS compared with isolated action of mutagen.

The compound was synthesized and provided by the S. Ordzhonikidze Pharmaceutical Chemical Research Institute. As modifier we used phenobarbital sodium (PS), a classical MMS inducer which does not possess mutagenic activity in mammalian somatic cells [8]. The rats were given drinking water containing PS in concentrations of 8, 40, and 200 mg/liter, corresponding to average doses of 0.4, 2, and 10 mg/kg body weight, determined by monitoring water consumption. Induction of MMS was estimated in groups of animals (six to eight rats in each group) receiving PS for 5, 30, and 120 days. The groups took part consecutively in the experiments, and all the animals were killed at about the same time. The state of MMS was assessed from the concentrations of cytochromes P-450 and b_5, as described previously [2]. Each group contained six animals. Loading with the mutagen took place after exposure to the inducer for 120 days. A complete two-factor experiment was planned, in which one factor was the dose of fotrin (0, 2, 4, and 7 mg/kg), the other — the dose of the inducer. Fotrin was injected intraperitoneally 5 times at intervals of 24 h, 5 days before the end of exposure to PS. The animals were killed 6 h after the last injection of the mutagen. Metaphase preparations of rat bone marrow were obtained by the standard method. From each animal 100 metaphases were analyzed in coded preparations. The frequency of metaphases with chromosomal aberrations, single and paired fragments, and exchanges of chromatid and chromosome type were counted. To compare fractions of aberrant metaphases, Student's t test was used after transformation of the data for each animal (to stabilize the dispersion) by the equation $z = \arcsin \sqrt{\rho}$, where ρ denotes the fraction of metaphases with chromosomal aberrations [3]. Regression analysis was carried out by the method of least squares.

EXPERIMENTAL RESULTS

According to the results of cytogenetic analysis (Table 1) PS in all the doses used did not induce aberrant metaphases. Fotrin, when given alone in increasing doses, increased the frequency of cells with chromosomal aberrations. Dependence of the frequency of aberrant metaphases on the dose of the mutagen, whether given alone or in combination with PS (in all versions), was described by the regression equation $y = [1-e^{-(\alpha+\beta x)}]^2$, where y denotes the fraction of metaphases with chromosomal aberrations, x the dose of the mutagen, and α and β are coefficients. The coefficient β characterizes the increase in effect per unit dose of the mutagen, and it can be regarded as the basic parameter of modification.

PS in a dose of 0.4 mg/kg had no effect on MMS activity, and against this background neither the frequency of cells with chromosomal aberrations nor the coefficient β (Table 2) differed from those obtained in response to the isolated action of the mutagen. A dose of 2 mg/kg caused a lasting increase in enzyme activity of MMS up to 130-150% (the minimal statistically significant level of induction) throughout the period of exposure. During long-term induction of MMS by PS in a dose of 10 mg/kg, an increase in the cytochrome P-450 concentration to 223 and 226% respectively (the peak level of induction) was observed on the 5th and 30th days. PS in doses of 2 and 10 mg/kg reduced the cytogenetic effect of fotrin. The coefficient β was significantly lower in the case of the combined action of fotrin and PS in doses of 2 and 10 mg/kg compared with the action of the mutagen alone. There was no difference between the two versions according to this feature. Consequently, against the background of PS administration changes were observed in the quantitative parameters of the mutagenic effect. The qualitative (specific) parameters of action of mutagens are generally assessed by the appearance of the regression equations relating the fraction of metaphases to the concentration of mutagens, the ratio between induced single and paired breaks, and aberrations of chromosomal and chromatid types. These characteristics were unchanged by administration of the modifier, i.e., PS did not affect the specific features of action of the mutagen.

The influence of PS on the effect of fotrin is most probably realized at the stage of metabolism of this mutagen. Biotransformation of fotrin has received very little study, but it is claimed that one stage of detoxication involves the participation of enzyme systems; fotrin itself, moreover, is more reactive than any of its metabolites [4]. Since after induction of MMS by PS the effect of fotrin is reduced, as was also observed in experiments on *Drosophila* [1], the involvement of this system in metabolism of the mutagen may also be postulated. Reduction of the mutagenic effect of fotrin may be due to the more rapid detoxication of the compound in the case of activation of MMS.

Consequently, induction of MMS (minimal as well as maximal) leads to modification of the effect of mutagens; the effect of cyclophosphamide, a mutagen with indirect action, moreover, as the writers showed previously [5], is enchanced whereas that of fotrin, a mutagen of direct action, is depressed. Similar results also were obtained in experiments to study the effect of induction or inhibition of MMS on the manifestation of embryotoxic [7] and carbinogenic effects [6]. When the environmental hygiene is being evaluated, attention must therefore be paid not only to the presence of mutagens, carcinogens, teratogens, etc., but also to the possibility that the effects of these compounds may be potentiated by other pollutants and, in particular, by what are called "low-intensity chemical factors."

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