all tumor cells injected into the blood stream are exposed to a powerful cytostatic influence, which evidently causes rapid death of the overwhelming majority of them [6]. The few surviving tumor cells, after multiplication and subsequent study at the population level, differ from the population of parental STHE cells quite consistently (i.e., for at least 5-10 passages *in vitro*) in their MA and RDA values. It must therefore be assumed that the surviving tumor cells, which possess these properties, evidently pre-exist in the heterogeneous population of parental cells as rare genetic variants, possessing selective advantages *in vivo*. As a result of *in vivo* selection the tumor cell population becomes significantly richer in these variants.

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HYDRAZINE SULFATE AS A CELL MEMBRANE STABILIZER

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The discovery that hydrazine sulfate (HS) possesses experimental antitumor activity, its distinctive beneficial effect in clinical oncology [1, 4, 9], and the absence of any marked side effects such as are characteristic of most antitumor agents [1, 2], have attracted attention to the study of the mechanism of its action.

The aim of this investigation was to study the action of HS on the stability of erythrocyte, mitochondrial, and lysosomal membranes and also of artificial phospholipid membranes.

EXPERIMENTAL METHODS

Experiments were carried out on mature rats obtained from the Rappolovo Nursery, Academy of Medical Sciences of the USSR.

The action of HS on the cell membranes was judged by determining hemoglobin release from erythrocytes, and in the study of the organelles, by activation of marker enzymes under the influence of factors modifying membrane function. Subcellular structures were isolated by differential centrifugation of liver homogenate [5, 6]. Mitochondrial ATPase activity or lysosomal acid phosphatase (AP) activity was determined and preparations of these enzymes were isolated as described in [6, 7].

To obtain erythrocytes the rats were decapitated and the blood collected and mixed in the proportion of 1 ml with 3 ml of 0.15 MNaCl solution, containing 1 U heparin, cooled to 4°C. The suspension of erythrocytes from each animal was divided into four equal portions. Two samples were incubated at 25°C for 60 min, after which HS was added to one sample (experimental) in a concentration of 10^{-4} M (with a uniform distribution of HS in the body fluids, this concentration corresponds to a sessional therapeutic dose of 60 mg/kg). After incubation at 25°C the samples were cooled for 60 min at 4°C. Two other samples were frozen at −15°C for 60 min; HS was added to one of the samples in the same concentration.

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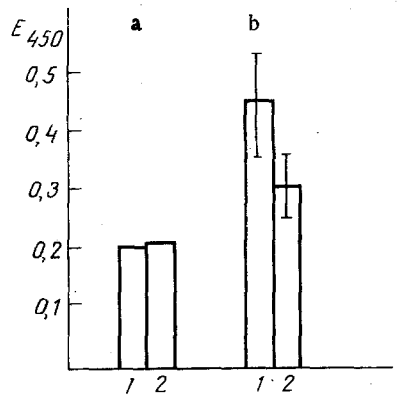

Fig. 1. Effect of hydrazine sulfate (10^{-4} M) on hemoglobin release from rat erythrocytes after incubation at 25°C (a) and after freezing and thawing (b): 1) control (nothing added), 2) HS (10^{-4} M) added.

The samples were then thawed to room temperature and all samples (from different conditions of incubation) were centrifuged at 105,000g for 60 min. The hemoglobin concentration was determined in the supernatant by measuring absorbance at 450 nm.

HS (10^{-4} M) was added to suspensions of freshly isolated organelles in 0.025 M sucrose, made up to 0.05 M Tris-HCl buffer, pH 7.4, and the samples were incubated for 15 min at 25°C. Control samples (without HS) were subjected to the same treatment. Next, ATP or p-nitrophenol phosphate was added to the samples, which were incubated for 30 min at 25°C. ATPase activity was expressed in micromoles P_i/mg protein, and AP activity in micromoles p-nitrophenol/mg protein. The incubation mixture for determination of enzyme activity contained 0.1% Triton X-100.

Bimolecular phospholipid membranes were formed from the total fraction of bovine brain phospholipids [11]. The conductance of these membranes was determined in 0.1 M KCl or NaCl solutions. Because of the experimental conditions, hydrazine chloride was used.

The significance of the results was determined by Student's t test and the Wilcoxon–Mann–Whitney U test.

RESULTS

Addition of HS to the mitochondrial or lysosomal suspension caused no marked increase in ATPase or AP activity. To rule out any possible effect of HS on ATPase or AP activity, special experiments were carried out in which the action of HS on preparations of these enzymes was studied. HS in a concentration of 10^{-4} M was found not to inhibit the enzymes. On the other hand, HS considerably prevented any increase in mitochondrial ATPase or lysosomal AP activity in organelles treated with 0.1% Triton X-100.

In just the same way HS increased hemoglobin release during incubation of erythrocytes at 25°C. After freezing and thawing of the erythrocyte suspension in the presence of HS the hemoglobin release was reduced.

These observations demonstrate the specific action of HS on membrane permeability. The possibility cannot be ruled out that this may also explain the slowing of glucuronide formation or inhibition of metabolism of alkylating compounds in a system of isolated microsomes [8].

In the experiments with artificial membranes HS did not affect their permeability for Na^+ and K^+ ions. The fact is evidence, first, that HS does not affect the lipid component of membranes, and it can accordingly be postulated, second, that HS, in its action on natural membranes, binds selectively with hydrophilic groups of the proteins of these membranes. In a discussion of the possible point of application of HS, it is worth noting that the radius of the hydrazine ion (0.6–0.7 nm) is about equal in size to the water pore of the membrane. It is quite possible that the hydrazine ion binds, for example, with carbonyl groups of membrane proteins and, during the action of injurious factors, it protects the cell membranes by forming "cross-linkages" in the pores. The "stabilization" of the biomembranes thus taking place may account for the antitumor action of HS.

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