EFFECT OF SULFUR-CONTAINING RADIOPROTECTIVE SUBSTANCES ON TISSUE RESPIRATION AND PHOSPHORYLATION

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An earlier investigation [2] showed that mercamine (cysteamine) and cystamine, in concentrations of 10^{-3}-10^{-5}M, increase the oxygen consumption of the pigeon's and dog's erythrocytes. Similar data concerning the action of cystamine were obtained in experiments with human erythrocytes and with slices of liver and spleen [4, 8]. In experiments on the mitochondria of rats' liver and kidneys, using cystamine in a concentration of 100 mg/liter, some increase was also observed in the oxygen consumption, and respiration was depressed, but only with high concentrations much above the therapeutic levels (500-800 mg/liter) [9]. Cystamine in doses used clinically increased the oxygen consumption, the cardiac output, and the velocity of the blood flow in man [1].

An increase in oxygen consumption may be linked with the formation of high-energy phosphorus compounds or with other biochemical processes [6, 7]. Investigation of the effect of high cystamine concentrations on phosphorylation in the mitochondria of the liver, kidneys and brain of rats revealed a decrease in the utilization of inorganic phosphate [10]. Only in the kidney tissues, when cystamine was used in a concentration of 100 mg/liter, was a slight increase in its utilization observed.

The object of the present investigation was to study the effect of the three most active radioprotective agents—cystamine, cystamine, and AET (S, 2-aminoethylisothiouronium) on respiration and respiratory phosphorylation.

EXPERIMENTAL METHOD

The investigation was carried out on pigeon's erythrocytes and rat's liver homogenates. For the experiments with erythrocytes the methods described by I. F. Seits [5] in his monograph were used, and for the experiments with homogenates the usual method [6, 7] was used. The tissues were incubated in a medium containing potassium—phosphate buffer (0.005-0.015 M), glucose (0.05 M), succinate (0.01 M), magnesium chloride (0.005 M), versene (0.002 M), sodium fluoride (0.05 M), ATP (0.006 M), and hexokinase in dose of 0. 1 mg/ml medium; the pH of the medium was 7.4-7.6. Incubation was carried out in a Warburg's apparatus for 5 and 25 min at 37°. The intensity of oxidation and phosphorylation was judged by the absorption of oxygen and the decrease in inorganic phosphate. Phosphorus was determined by the method of Fiske and Subbarow. The preparations were used in concentrations giving a radioprotective effect to unicellular organisms and tissue cultures (10^{-3} M), and also in concentrations which can be attained in animals and man receiving radioprotective doses of the tested compounds (10^{-4}-10^{-5} M).

EXPERIMENTAL RESULTS

The results of these investigations showed that AET, in contrast to cysteamine and cystamine, did not affect the respiration of the pigeon's erythrocytes The oxygen consumption of the rat's liver homogenates was increased by AET just as by cysteamine and cystamine. One reson for the difference between the action of AET on the respiration of the erythrocytes and of the homogenates may have been poor penetration of the substances through the cell membrane of the erythrocytes.

The results showing the effect of cystamine on the fixation of inorganic phosphate by the erythrocytes showed that when a high concentration of the preparation (10^{-3} M) was used, phosphate fixation was signi-

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ficantly increased, but this effect was temporary and lasted only 5 min. In the course of the next 20 min the phosphate fixation slowed down and by the end of the observations it was not significantly different from the control. In lower concentrations (10^{-4} and 10^{-5} M) cystamine had no visible action on phosphorylation.

The results of the experiments with liver homogenates in the presence of mercamine were similar to those just described. In concentrations of 2×10^{-3}-2×10^{-4} M, during the first 5 min of the experiment an increase was observed, and during the next 20 min—a decrease, in the fixation of inorganic phosphate. When the preparation was used in a concentration of 4×10^{-5} M, considerable fluctuations were noted in the decrease in inorganic phosphate, and the results accordingly were not significant.

In a concentration of 10^{-3}, cystamine inhibited the fixation of inorganic phosphate throughout the period of observation (25 min). In concentrations of 10^{-4}-10^{-5}, cystamine had no effect on the phosphorylation process. The difference between the action of high concentrations of cystamine on phophorylation in the erythrocytes and the homogenates may be associated with differences in the rate of its reduction in these tissues [3].

In all concentrations tested, AET caused no change in the fixation of inorganic phosphate by the liver tissue.

Changes in the P:O ratio corresponded to the effect of the tested compounds on respiration and the coupled phosphorylation process. Mercamine and cystamine in concentrations stimulating the oxygen consumption and simultaneously depressing the fixation of inorganic phosphate lowered the P:O ratio. AET had practically no effect on the value of the P:O ratio.

It is concluded from the observations described above that, despite their effect in stimulating tissue respiration, the investigated preparations caused no prolonged increase in the fixation of inorganic phosphate. High concentrations of cystamine, on the other hand, caused depression of phosphorylation.

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