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The effect of some radioprotective substances on the redox equilibrium in the spleen

I. MUSTEA and Z. URAY
Oncological Institute, Cluj, Romania

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The redox potential of spleen tissue *in vivo* was measured, and changes were observed after the administration of radioprotective drugs with reducing properties (aminothiols, dimercaptans, hydrazine derivatives, glucose, sulphites). The redox potential can be regarded as an indicator of the level of the energy metabolism of a tissue. The depression of the energy metabolism could be correlated with the potency of radioprotective drugs. The time-course of this depression on administration of AET follows that of its radioprotective property.

1. Introduction

Some compounds which protect against the action of ionizing radiations have reducing properties (aminothiols, dimercaptans, hydrazine derivatives, glucose, sulphites). But these two properties do not correlate well (Bacq and Alexander 1965). When reducing substances are given to cells or tissues the equilibrium of their oxidation reduction systems is altered, by corresponding changes in the ratio of reduced to oxidized forms of components of the system. Nernst's equation defines the redox potential as

$$E = E_0 - \frac{RT}{nF} \ln \frac{\text{Red}}{\text{Ox}}, \tag{1}$$

where E_0 = standard redox-potential at pH 7, when the concentration of the reduced and oxidized forms are equals, thus Red/Ox = 1 ('mid potential'); R = universal gas constant (1·987 cal/mol/degree); T = absolute temperature (°K); n = number of electrons transferred in the oxidation-reduction process; F = Faraday constant (23·068 cal/V. equiv.). It follows that when the concentration of the reduced forms increases, the redox potential of the system tends towards negative values, and vice versa.

An understanding of the changes in the redox potential (ΔEh) is particularly important because it reflects the energy metabolism within a given tissue. The variation of the free energy (ΔG) is proportional to the difference in the redox potential.

$$\Delta G = -nF\Delta Eh. \tag{2}$$

The energy production is one of the major determining factors of a tissue's radiosensitivity (Bacq 1965, Bacq and Goutier 1968, Pora, Abraham, Uray and Holan 1969, Zins, Raymond and Dubois 1959).

Modifications in the redox equilibrium within a tissue may be estimated using Nernst's equation to determine the concentrations of the two forms of a redox system for which E_0 is known, usually the lactate-pyruvate system.

The lactate-pyruvate system has the advantage of high extracellular diffusion of the compounds involved. Their concentration in the blood of vessels coming from an organ reflects the situation within that organ. It has been used to

calculate the redox potential of the lactate-pyruvate system in different tissues and under various experimental conditions (Cotaescu, Cotaescu, Bejan, Silveanu and Dreichlinger 1962, Duyckaerts and Liebecq 1970, Gudbjarnason and Bing 1962, Hohorst, Arese, Bartels, Stratmann and Talke 1965, Marinescu, Ionescu, Trandafirescu and Pausescu 1963, Mustea, Popescu, Carabas and Rogozan 1965, Mustea, Muresian, Todorutiu and Popescu 1966).

In the present paper we report changes with time of the redox potential in the spleen under the influence of some radioprotective substances with reducing properties.

2. Materials and methods

The recordings of the splenic redox potentials were made on white male rats, weighing 220 ± 20 g, kept on a standard diet. The animals were anaesthetized with Nesdonal (Specia) 50 mg/kg, injected into the tail vein. The abdominal wall was opened by an incision penetrating into the left hypochrondrium, at the level of the spleen. A shiny platinum electrode, 2 mm thick, and insulated with nitrocellulose laquer up to 5 mm from its tip, was introduced into the spleen.

The calomel electrode was placed in the vicinity of this incision. The two electrodes were connected to an electronic voltmeter MV 11 (Clamann and Grahnert-Dresden) and coupled to an automatic recorder (Bonnet Maury-Jouan) with a recording speed of 60 mm/h.

The reducing substances, dissolved in 0·5 ml of physiological saline solution and neutralized to pH 7·0, were given by intravenous injection 20 min after anaesthesia and the recording continued for 75 min.

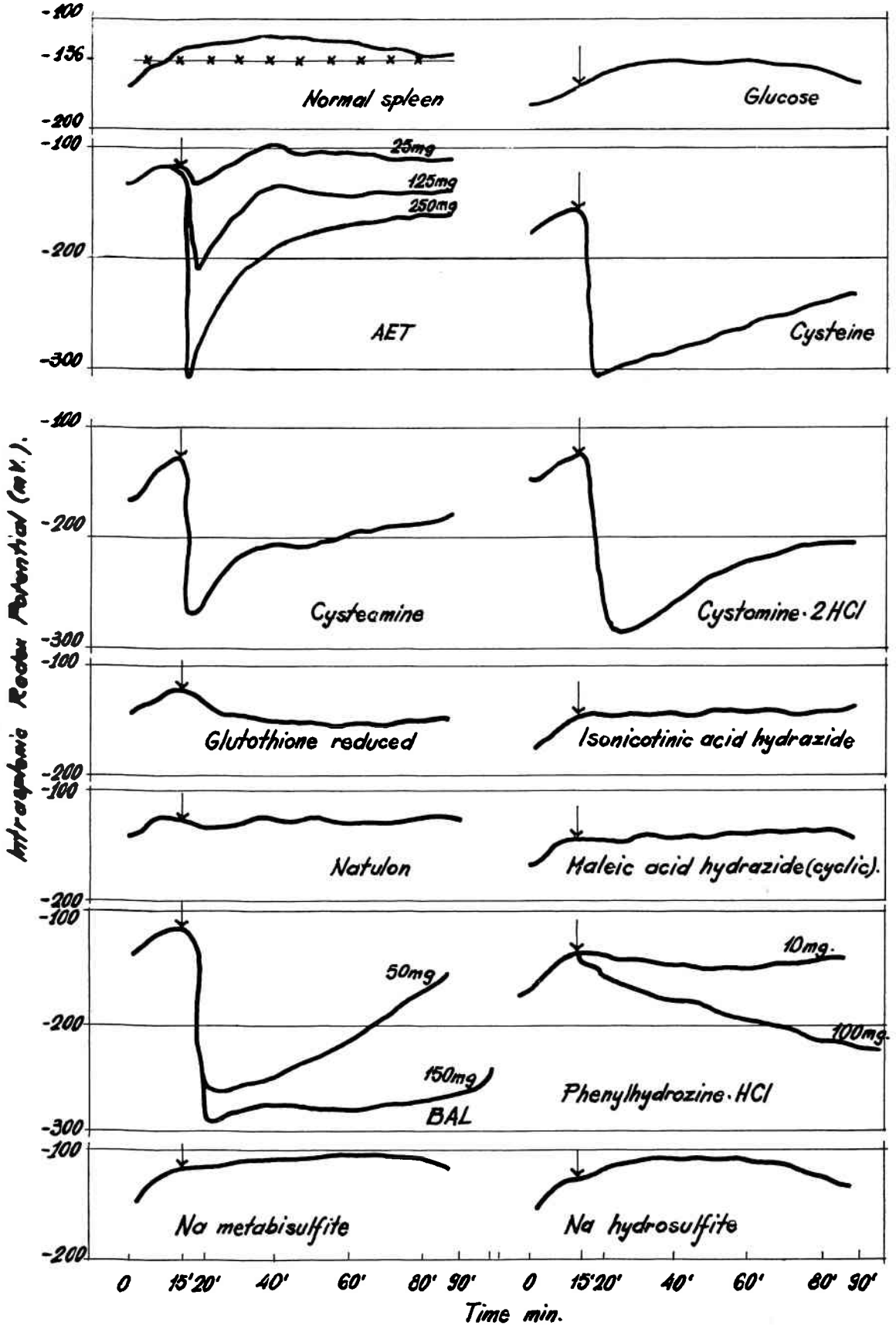
For each substance 3–6 recordings were made. The control redox potential of the spleen (figure) represents the mean calculated from the values obtained after 15 min of recording in the anaesthetized conditions (24 experiments).

The administered doses (in mg/kg) were as follows:

β-aminoethylisothiouronium BrHBr	25, 125, 250
Cystamine 2 HCl	150
Cysteamine	150
Cysteine	1000
Reduced glutathione	800
2,3-dimercaptopropanol 1 (BAL)	50, 150
Phenylhydrazine HCl	10, 100
p-(N′-methylhydrazinomethyl)-N isopropilbenzamide HCl (Natulan)	50
Maleic acid hydrazide (cyclic)	200
Isonicotinic acid hydrazide	200
Glucose	1500
Sodium metabisulphite	150
Sodium hydrosulphite	150

3. Results

The recordings of the splenic redox potential following administration of some reducing agents and plotted against the calomel saturated electrode, represent individual cases considered as characteristic for each substance (figure).



Intrasplenic redox potential development after administration of some radioprotective compounds with reducing properties.

The mean value of the splenic redox potential recorded by the platinum electrode at 15 min after its implantation is about −136 mV. Administration of some organic substances containing sulphur in radioprotective doses induced a sudden and marked increase in the negative redox potential, for AET cysteamine, cystamine, cysteine and BAL—up to values lying between −250 and −300 mV, less pronounced for reduced glutathione. Among the derivatives of hydrazine it is only phenylhydrazine hydrochloride which induced a slight modification in the negative redox potential. Maleic acid hydrazide (cyclic), isonicotinic acid hydrazide, natulan, glucose, and sulphites proved to be without effect. The time course of the splenic redox potential was different from one substance to another, the most rapid restoration of normal values being recorded for AET.

For identical working conditions (dose of anaesthetic, age, sex, and weight of the animals) our findings show for a given dose of a substance close or even superimposable patterns. The effect of varying doses on the modification of the intrasplenic redox potential has been tested with three doses of AET (25 mg, 125 mg and 250 mg, figure) and good agreement found.

4. Discussion

The diffusion of a reducing substance into the cells of a tissue depends on its concentration and results in a shift to negative values of the redox potential.

The activity of certain enzymes in the metabolism of carbohydrates and nucleic acids is perturbed and leads to a marked increase in lactic acid. This has been demonstrated for thiol compounds (Bacq and Liebecq 1965, Bacq and Goutier 1968, Zins *et al.* 1959) and for hydrazine (Fortney 1967). Simultaneously, the reducing agent may be fixed on various natural substrates and its elimination from the organism may also take place. The interval between administration of the compound and the restoration of the initial redox equilibrium is therefore dependent on the processes mentioned.

During the recordings the tissue pH remained constant. Therefore variations in the splenic redox potential reflect changes in the redox conditions or energy metabolism within this organ. When ΔE becomes negative, an increase of the reduced forms of the co-enzymes in the respiratory chain takes place, the energy producing activity tends to develop in the direction of glycolisis and anaerobic phosphorylation. When ΔEh shifts to positive values, respiration becomes efficient and the concentration of the oxidized forms of the coenzymes. Thus by ascribing to the redox conditions in an organism an important role in the energy producing activity, our experiments have pointed out, the existence of a correlation between the alteration of this activity in the spleen and the radiobiological effect reported in the literature for the corresponding substances (Bacq 1965, Faleeva 1969, Maggiora, Lozeron and Iadassohn 1966, Mustea and Uray 1971, Smith and McKinley 1968, Thayer, Carey, Carpenter and Mazuy 1969). The strongest inhibition is induced by the most effective protective compounds (AET, cysteamine, cystamine, cysteine, BAL) a moderate inhibition was recorded in the case of weak protectors (reduced glutathione, phenylhydrazine hydrochloride), while with those having little or no protecting effect at all (glucose, cyclic maleic acid hydrazide, isonicotinic acid hydrazide, natulan, sulphites) no or minimal alterations of this nature were found at the level of the spleen.

AET induces immediately after administration a marked decrease in the splenic redox potential for about 1–2 min. This is followed by a faster restoration of the initial value than with other radioprotectors. This particular pattern confers to AET the capacity of providing high radioprotection shortly after administration when a maximal depression of the energy metabolism occurs, and allows a quick restoration to normal, ensuring the efficiency of the recovery processes.

The decrease of the radioprotective effect or even a radiosensitizing effect of high doses of some compounds (BAL, phenylhydrazine) could be understood as being the consequence of a prolonged and severe disorder of the energy metabolism with possible effects on the recovery processes (Mustea and Uray 1971).

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On a mesuré le potentiel redox du tissu splénique *in vivo* et on a observé les changements après l'administration des substances radioprotectrices ayant des propriétés réductrices (aminothiols, dimercaptans, dérivés de l'hydrazine, glucose, sulfites). Le potentiel redox peut être considéré comme un indicateur du niveau du métabolisme énergétique des tissus. La dépression du métabolisme énergétique peut être corrélée avec la capacité radioprotectrice des substances utilisées. La durée de cette dépression après l'administration de l'AET suit celle des capacités radioprotectrices de la substance.

Es wurde das Redoxpotential von Milzgewebe *in vivo* gemessen und seine Veränderungen als Folge der Gabe von Strahlenschutzstoffen mit reduzierenden Eigenschaften (Aminothiole, Dimerkaptane, Derivate von Hydrazin, Dextrose und Sulfaten) studiert. Das Redoxpotential kann als ein Indikator für die enrgetische Stoffwechsellage angesehen werden. Die Depression des Energiestoffwechsels kann mit dem Potential des Strahlenschutzstoffes in Zusammenhang gebracht werden. Die Dauer der Depression nach Gabe von AET ist mit den Strahlenschutzeigenschaften des Stoffes streng korreliert.

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