Hemolysis and peroxidation in heavy metal-treated erythrocytes; GSH content and activities of some protecting enzymes

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Summary. The activities of superoxide dismutase, catalase and glutathione peroxidase, and the level of reduced glutathione, were measured in heavy metal-treated erythrocytes. The hemolytic metals were found to significantly deactivate both catalase and glutathione peroxidase and to decrease the level of reduced glutathione, thus providing suitable conditions for the development of peroxidation.

Recently we have demonstrated that metal-induced hemolysis is preceded by peroxidation of membrane lipids[1]. Certain metals, such as iron and copper, have prooxidant catalytic activity and are able to initiate peroxidation in the erythrocyte membrane directly[2,3]. But some hemolytic metals can initiate peroxidation indirectly, by increasing hemoglobin autoxidation with the release of superoxide[4-6]. In this case, O_2^- and H_2O_2 (a product of O_2^- dismutation) can cooperate in the generation of species causing lipid peroxidation[7-9]. In the erythrocyte, O_2^- is broken down by superoxide dismutase (SOD), and H_2O_2 by catalase and glutathione peroxidase (GSH-Px). If these protective enzymes are inactivated stepwise in the course of increased O_2^- production, a peroxidation of the membrane lipids may occur. In this context it was of considerable interest to study the changes in the level of reduced glutathione (GSH) and activities of SOD, catalase and GSH-Px in erythrocytes treated with heavy metals in vitro.

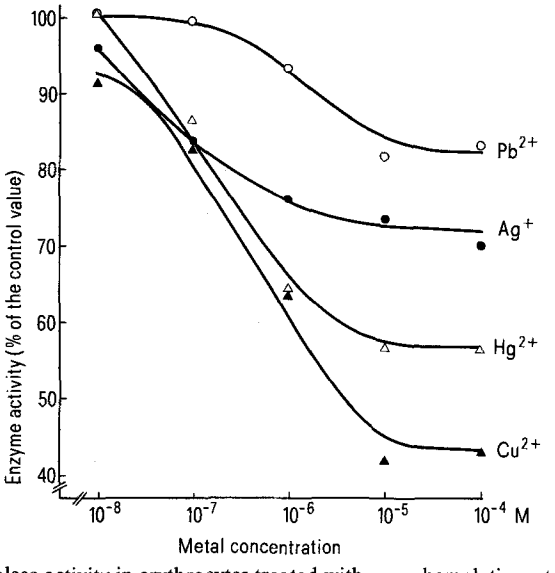
Methods. The experiments were carried out with guinea-pig erythrocytes incubated as previously described for 30 min in the presence of $HgCl_2$, $PbCl_2$, $CoCl_2$, $FeCl_3$, $CuCl_2$, $FeCl_2$ and $AgNO_3$. The final concentrations of the metals used are indicated in the table and the figure. The GSH content was measured by the method of Kolthoff et al.[10]. SOD activity was assayed as described by Maral et al.[11]. The catalase was estimated simultaneously by the method of Maral et al.[11] and by the method of Snyder et al.[12]. GSH-Px was estimated according to Utno et al.[13]. The enzyme activities were measured before the appearance of any noticeable hemolysis. During the time of incubation significant peroxidation was observed, as shown in our previous experiments[1].

Results and discussion. In erythrocytes incubated in the presence of heavy metals SOD activity was not changed (data not given). As shown in the table, the metals with significant hemolytic action (Ag^+, Hg^{2+}, Cu^{2+}, Pb^{2+}) at a final concentration of 10^{-4} M decreased the GSH content and the activities of the catalase and GSH-Px.

Cobalt, a metal without hemolytic action, does not induce a peroxidation of membrane lipids[1,3] and we have not found any noticeable decrease in the activity of enzymes tested. Also, no changes in the level of GSH were observed under the influence of cobalt. We were not able to establish the effect of copper on erythrocyte GSH-Px since Cu^{2+} rapidly oxidizes the GSH, thus preventing the estimation of the true enzyme activity. However, there is evidence[14] that Cu^{2+} inhibits the purified erythrocyte GSH-Px.

The inhibition of catalase in erythrocytes incubated in the presence of Ag^+, Hg^{2+}, Pb^{2+}, Cu^{2+} is shown in the figure 1. The effect on GSH-Px is similar.

The results obtained suggest that enzyme inactivation is rather unspecific. Some of the hemolytic metals are bound mainly to hemoglobin and, to a minor extent, to other cytosolic and membrane components[15,16]. For such a distribution the denaturation or precipitation of the enzymes



Catalase activity in erythrocytes treated with some hemolytic metals at different concentrations. The enzyme activity is expressed as a percentage of the control value.

The level of hemolysis, the activities of catalase and glutathione peroxidase and GSH content of erythrocytes incubated with heavy metals

Added metal (10^{-4})	Hemolysis (A_{540})	Enzyme activity - units/g hemoglobin Catalase $\times 10^3$ units	Glutathione peroxidase	GSH µM per 100 ml suspension
Control	0.050±0.009	(87.0±1.0)	16.62±0.15	296±14
Ag^+	0.666±0.020	(57.0±0.7)	7.65±0.15	246±15
Hg^{2+}	0.634±0.019	(52.0±2.0)	9.60±1.05	252± 7
Cu^{2+}	0.403±0.019	(27.0±4.0)	-	215±17
Pb^{2+}	0.216±0.011	(76.0±0.8)	11.70±0.60	243± 7
Fe^{3+}	0.206±0.016	(92.0±4.0)	13.80±1.50	293±18
Fe^{2+}	0.156±0.012	(89.0±4.0)	14.85±1.05	294± 4
Co^{2+}	0.044±0.010	(86.0±0.8)	16.20±1.05	287±17

The extent of hemolysis was estimated as previously described[1] after 60 min of incubation. The enzyme activity and the level of GSH were measured in the 30 min of incubation in twice-washed erythrocyte suspensions. Values are mean±SE (n=9).

tested by concentrations of the metals up to 10^{-4} M seems unlikely. No quantitative correlation between the extent of hemolysis and the degree of enzyme inactivation was found. This is probably due to the significant differences in the properties of the hemolytic heavy metals. Some of them may change the permeability of the erythrocyte membrane[17], thus leading to increased osmotic pressure which accelerates the hemolysis. Nevertheless, it is clear that the peroxidation and eventually lysis are associated with a significant inactivation of the enzymes protecting erythrocytes against peroxidative damage, as well with a decrease of the GSH content.

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Increase in superoxide dismutase activity induced by thyroid hormones in the brains of neonate and adult rats

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Summary. The activity of cytoplasmic superoxide dismutase (SOD) in the liver was about twice as high in adult rats as it was in neonates. In the brain and in the interscapular brown adipose tissue (IBAT), SOD activity was not changed during postnatal development, although it was slightly higher in the brain than in the IBAT ($p > 0.1$). Thyroid hormones produced an increase in SOD activity in the brain of newborn rats, as well as in those animals 30 and 60 days old. The same quantity of hormones did not produce any significant changes in the liver or in the IBAT.

Superoxide dismutase (SOD), prepared mainly from the liver, erythrocytes and brain, has been intensively studied[1]. Results obtained in the last few years showed that this enzyme may have a marked radioprotective influence and may be an important factor in the antiinflammatory action[2,3]. We have recently shown that noradrenaline (1.6 mg/kg/b.wt, i.p.) produced an increase in SOD activity in the interscapular brown adipose tissue (IBAT), but not in the liver of the rat[4]. However, higher doses of this neurohormone did produce a marked increase of SOD activity in both tissues[5]. The aim of present experiment was to test the possible influence of thyroid hormones on SOD activity in some tissues, with a special attention to the brain during postnatal development.

Material and methods. 8 groups of male Mill Hill hooded rats, aged 10, 30, 60, 90, 120 and 180 days, were used for the experiment. Animals were kept at room temperature (22 °C). In the preliminary experiments, 6 groups (each consisting of 8–10 animals of the 6 different ages) were used for testing SOD activity during postnatal development. 3 other groups of 10-, 30- and 60-day-old rats were treated intragastrally once daily, 3 days prior to sacrifice, with novothyral (Lek) which contains 10 µg of L-3,5,3′-triiodothyronine and 50 µg of L-3,5,3′,5′-tetraiodothyronine. The liver, IBAT and brain were removed within 3 min, the liver being perfused prior to the removal.

Tissues were minced and then dispersed with a loosely-fitting pestle in a Potter-Elvehjem homogenizer in 9 vols of the buffer, containing 0.05 M KH_2PO_4 and 10^{-4} M EDTA, pH 7.8. All the operations were performed at 4 °C. The homogenate was centrifuged for 15 min at 6000 × g in a Sorvall centrifuge. The supernatant was centrifuged for 90 min at 85,000 × g and used for the determination of SOD activity, as described by Misra and Fridovich[6]. This method is based on the capacity of SOD to inhibit autooxidation of adrenaline to adrenochrome. 1 unit of SOD activity was

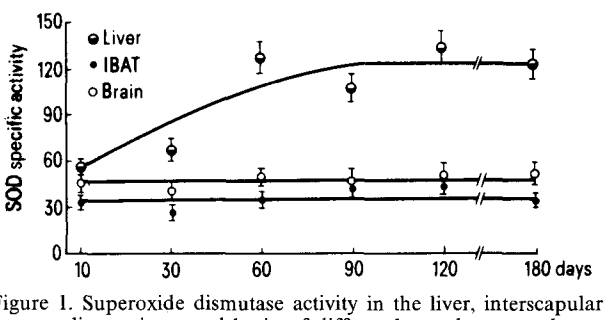

Figure 1. Superoxide dismutase activity in the liver, interscapular brown adipose tissue and brain of differently aged untreated rats. Mean ± SEM of 8 or 10 animals.