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Superoxide dismutase activity in rabbit reticulocytes[1]

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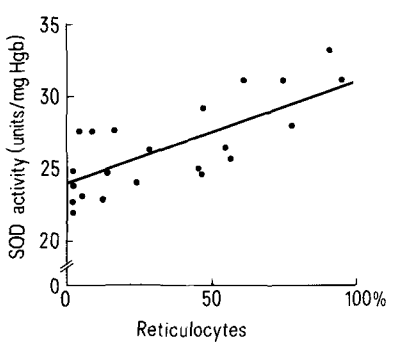
Summary. Reticulocytosis was induced in rabbits by bleeding anemia and erythrocyte superoxide dismutase activity was determined. Reticulocytes were found to contain about 1.3 times as much activity as mature erythrocytes.

Superoxide dismutase (SOD, EC 1.15.1.1) is ubiquitously present in oxygen-metabolizing cells, and serves as the first line of defense mechanism against oxygen toxicity[2,3]. The role of this enzyme is the subject of current intensive research, and clinical implications of SOD levels have been evaluated in physiological as well as pathological conditions. One example is the process of aging[4,5], and SOD activity was measured in whole organisms[6,7], several organs[4,8,9], and in certain types of cells[10,11]. Because of their relative ease of sampling and limited life span, erythrocytes are a suitable model for such a study. In the report of Bartosz et al.[10] bovine erythrocyte SOD was shown to decrease during aging. The purpose of this article is to extend further the above observation and to demonstrate that reticulocytes, the youngest erythrocytes in the circulatory system, have the highest SOD activity.

Materials and methods. Reticulocytosis was induced in 3 male rabbits weighing 2.1–2.5 kg by successive daily bleeding through ear vessels of about 10 ml/kg. When the peripheral reticulocyte count exceeded 30%, usually attainable on the 6th–8th days, heparinized blood was centrifuged and the plasma and buffy coat removed. After washing and diluting with saline, the erythrocyte suspension was then applied to a differential centrifugation in a gum acacia solution according to the method of Kimura et al.[12], and specimens with various reticulocyte concentrations were prepared. Daily bleeding was usually terminated on the 8th day and was followed by an i.v. injection of saccharated ferric oxide, 20 mg/kg. SOD activity was measured by the ferricytochrome c reduction inhibition method of McCord and Fridovich[2] at pH 10[13]. As proposed by McCord and Fridovich[2], 1 unit of activity was defined as the amount of enzyme activity which inhibits the rate of reduction of ferricytochrome c by 50%. SOD activity was then calculated on logit paper[14] and expressed as units/g hemoglobin, as the relationship between cell count and hemoglobin content was virtually parallel.

Results. The figure shows SOD activities of erythrocytes as a function of the reticulocyte population. The results for the 3 rabbits were combined as they were comparable. As shown in the figure, SOD activity increases as the reticulocyte population increases. The relationship is calculated in the following equation; $y = 0.07\ x + 24.08$, where y is an SOD activity, and x is a percentage of reticulocytes ($r = 0.71$, $p < 0.01$). Enzyme activity of reticulocytes was completely inhibited by 1 mM cyanide.

Discussion. The present study indicates that the erythrocyte SOD activity increases correspondingly with an increase in the reticulocyte population, and that reticulocytes have about 1.29 times as much activity as mature erythrocytes. This finding may be interpreted as a counterpart of observations of Bartosz et al.[10] that the activity of this enzyme decreases during erythrocyte aging. Furthermore, this increase is found to be accompanied by elevations of erythrocyte copper and zinc[15], both of which are constituent metals of the enzyme. Although the differences between the animal species studied and of methods of determination of



Rabbit erythrocyte SOD activity in relation to reticulocyte population.

enzyme activity should be taken into consideration, these results collectively suggest that erythrocytes in the peripheral circulation lose at least 50% of their SOD activity during the process of aging, starting from reticulocytes to the presumably oldest cells. Such a change of erythrocyte SOD activity appears to be a function only of the age of the cells themselves, and probably is not a reflection of the donor's age. Although this was not specifically determined in the present study, a lack of correlation found in other work between the erythrocyte SOD activity and the age of human subjects may serve as supporting evidence[16-18].

The biological significance of increased reticulocyte SOD activity still remains to be elucidated, but some possibilities may be suggested. Compared with mature erythrocytes, reticulocytes, which contain mitochondria, are known to engage in an active aerobic metabolism, for example involving the tricarboxylic acid cycle, with a markedly higher oxygen consumption[19]. Consequently, a higher SOD activity may be required in reticulocytes than in mature cells in order to scavenge possibly increased generation of superoxide anions, and thus effectively to maintain an oxidative metabolism. A number of erythrocyte enzymes are found to decrease with increased age of cells, i.e., higher activities are associated with younger cells[20-24]. It appears that a gradual lowering of various enzyme levels is a finding commonly associated with aging of erythrocytes. Alternatively, therefore, reticulocyte SOD may simply conform to this physiological pattern of senescence.

Correlation of elevated SOD activity of reticulocytes with their cellular functions has not been specifically evaluated. According to Walls et al.[25], young erythrocytes of humans are shown to be able to protect themselves effectively against thyroxine-peroxide induced hemolysis, while older cells exhibited less protection. Although their sensitivity was discussed primarily in relation to decreased glucose-6-phosphate dehydrogenase activity in older cells, there is a possibility that reduced SOD activity may also be a contributing factor to the above reaction.

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Metabolism of resorcinol and salicylate in *Aspergillus niger*

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Summary. In *A. niger*, resorcinol and salicylate are catabolized to β-ketoadipate, following ortho ring fission. The enzymes involved in the degradation are repressed in the presence of a primary substrate like glucose.

The degradation of resorcinol and salicylate has been studied extensively in bacteria[2-4], but comparatively little is known about the metabolism of these aromatic compounds in yeasts and fungi. However, Neujahr and coworkers have studied the resorcinol and phenol metabolism in *Trichosporon cutaneum* and *Candida tropicalis*[5,6]. *A. niger* is known to degrade various aromatic compounds[7,8]. Degradation of mandelic acid and benzoic acid in this organism occurs by the protocatechuate pathway while salicylate is catabolized by the β-ketoadipate pathway[9,10]. The objective of this study was to investigate the mode of degradation of resorcinol in this mould and compare it with that operative in bacteria.

A. niger was grown in shake cultures at 28±1 °C using resorcinol (0.5% W/V), salicylate (0.75%) and glucose (2% W/V) as carbon sources in a synthetic medium[11]. Oxygen uptake was measured using a washed cell suspension, as described earlier[10]. After oxygen consumption has