The Role of 2,3-Diphosphoglyceric Acid in the Potassium Transport of Human Erythrocytes

The concentration of 2,3-diphosphoglyceric acid (2,3-DPGA) in human erythrocytes is significantly high: more than 50% of all the acid-soluble organic phosphate esters is made up of this compound. The exact physiological function of 2,3-DPGA is not known as yet; however, a few decades ago, on the basis of comparative physiological data, the hypothesis was set up that some connection might exist between the 2,3-DPGA content of erythrocytes and the unequal distribution of cations[1-3]. Starting from this hypothesis, we undertook to investigate the connection between the 2,3-DPGA metabolism and K^+ transport of human erythrocytes.

In our previous work[4,5] we studied the effect of purine nucleosides in the presence of various glycolytic inhibitors, and our investigations led to the conclusion that there is some correlation between 2,3-DPGA metabolism of erythrocytes and K^+ *outflow*. The aim of our present work is to support from another angle these earlier experimental results. In these experiments we apply the results of MÁNYAI and VÁRADY[6,7], who have pointed out that $NaHSO_3$ is able selectively to split the 2,3-DPGA without considerably influencing the glycolysis and ATP content of the cells. This finding enabled us to investigate the cation transport influencing effect of 2,3-DPGA, independently from the glycolysis.

It is known[8] that in the presence of $10^{-3}M$ iodoacetic acid (IAA) the K^+ outflow of erythrocytes starts after the ATP content of the cells is exhausted. However, the rate of outflow is low: 1.6 meq K^+/l erythrocyte/h. By protracted experiment, after an incubation of 8 h at 37 °C the K^+ outflow suddenly starts with the high speed of 5.4 meq K^+/l erythrocyte/h. If, with the K^+ outflow, we investigate in parallel the increase of inorganic phosphate (P_i) and the 2,3-DPGA content of the cells, it can be seen that K^+ outflow begins only when the 2,3-DPGA content drops to 5–10% of its original level (Figure 1).

The breakdown of 2,3-DPGA occurring in the presence of IAA can be accelerated by $NaHSO_3$ to the required extent, depending on the concentration of $NaHSO_3$. When investigating the correlation between 2,3-DPGA breakdown (increase of P_i) and K^+ transport, it appears that the high rate of outflow of K^+ starts in each case when the P_i level of the blood reaches the value of 5 μM per ml. This value corresponds to a 90% splitting of the 2,3-DPGA content (Figure 2).

It is known that, in the presence of IAA + adenosine, a high rate of K^+ outflow sets in[4,5]. By adding a small amount of $NaHSO_3$ ($3 \cdot 10^{-3}M$) to the system, the high rate of K^+ outflow can be inhibited. However, at a higher concentration of $NaHSO_3$ this inhibition lasts only for a short period; the higher the $NaHSO_3$ concentration, the shorter the duration of inhibition. The high rate of K^+ outflow starts in each case when the 2,3-DPGA content almost totally disappears. However, while the strong breakdown process brought about by $NaHSO_3$ is still going on, no high rate of K^+ outflow can be observed (Figure 3).

On the basis of our experiments, it seems that the metabolic processes of 2,3-DPGA have a decisive function in the regulation of K^+ outflow. There is reason to assume

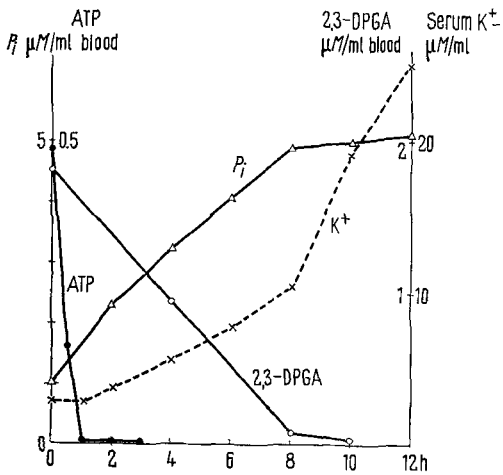

Fig. 1. Effect of $10^{-3}M$ IAA on the breakdown of ATP and 2,3-DPGA on the P_i content and K^+ transport of human blood at 37 °C. ATP and 2,3-DPGA were measured by the BARTLETT[9] procedure.

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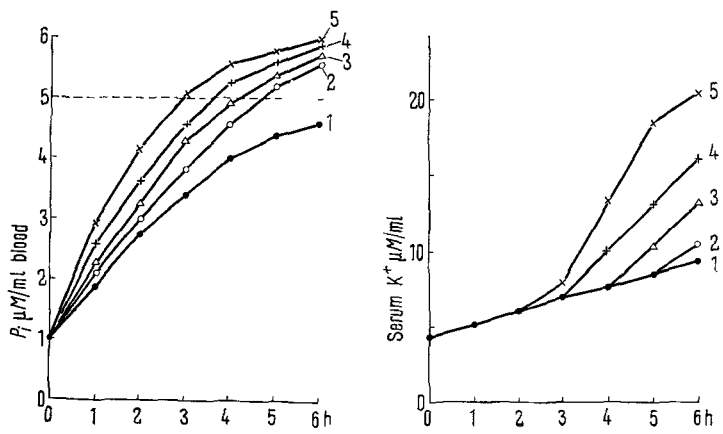

Fig. 2. Effect of $NaHSO_3$ on the P_i content and K^+ transport of human blood in the presence of $10^{-3}M$ IAA at 37 °C. 1 = Control; 2 = $3 \cdot 10^{-3}M$ $NaHSO_3$; 3 = $6 \cdot 10^{-3}M$ $NaHSO_3$; 4 = $10^{-2}M$ $NaHSO_3$; 5 = $1.5 \cdot 10^{-2}M$ $NaHSO_3$.

that our experimental results are in correlation with the shifting of equilibrium between synthesis and breakdown of adenylyl 2,3-DPGA, discovered by HASHIMOTO et al[10].

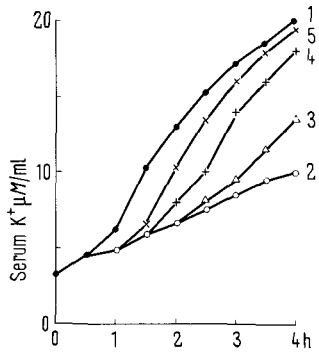

Fig. 3. Effect of $NaHSO_3$ on the K^+ transport of human blood in the presence of $10^{-3}M$ IAA + $10^{-2}M$ adenosine at 37 °C. For $NaHSO_3$ concentrations, see Figure 2.

Zusammenfassung. Der 2,3-Diphosphoglyzerat-Stoffwechsel menschlicher roter Blutkörperchen bedingt die Geschwindigkeit des infolge von ATP-Mangels auftretenden K^+-Austritts. Der rasche K^+-Austritt der Erythrocyten kann dann auftreten, wenn die Zellen kein 2,3-Diphosphoglyzerat mehr enthalten.

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Demonstration of a Negative Binding Effect of Bovine Growth Hormone Toward Potassium

Studies with enzymatically modified bovine growth hormone (BGH) preparations revealed that some of these fragments retained large amounts of inorganic residue despite dialysis or Sephadex gel exclusion chromatography. The same phenomenon, but to a lesser extent, was found to apply to undegraded BGH[1]. The bulk of this inorganic material consisted of Na^+, Mg^{++}, Ca^{++}, Al^{+++}, and SiO_3^{--}. These results suggested that some of the ions were quite intimately bound by the hormone. Several mono- and divalent cations were, therefore, added singly at various concentrations to BGH solutions on the premise that different external concentrations of these ions might induce specific conformational changes of the protein chain, which in turn might allow for optimal ion binding.

Reaction mixtures were prepared as 3 ml aliquots containing 1% BGH. Cations added singly to the hormone solutions were Mg^{++}, Ca^{++}, Na^+, or K^+, all as their chloride salts. The ions were tested over concentrations ranging from 0.005–$0.03M$. The BGH-Mg^{++} and the BGH-Ca^{++} solutions were prepared in $0.05M$ acetic acid to avoid precipitation of the hormone since addition of these divalent ions to basic solutions caused an immediate precipitation of the protein. Na^+ and K^+ were added to the hormone in $0.05M$ NH_3 solutions. These test solutions were passed over Sephadex G-25 gel columns (3.14 cm^2 by 40 cm) to effect separation of the hormone plus ion complexes from the unbound ions. Elution was done with deionized H_2O. Studies by GELOTTE[2] have shown that small inorganic cations, in particular Na^+ and K^+, were retained to extents up to twice the total volume of Sephadex G-25 columns due to the presence of negative charges in the gel. Surprisingly, the hormone emerged from the columns in the form of 2–3 usually poorly resolved peaks as exemplified in Figure 1 for the BGH-Mg^{++} test solution. Only in the case of the BGH-K^+ solution was a fair resolution into two peaks obtained. All the protein-containing tubes were pooled to give one fraction. These solutions were reduced to dryness by lyophilization.

Control solutions containing 1% hormone in 0.1 and $0.05M$ NH_3 emerged as one asymmetrical peak in the first case and as 2 symmetrical peaks in the latter case. Hormone solutions in $0.05M$ acetic acid yielded one symmetrical peak which emerged with the void volume of the

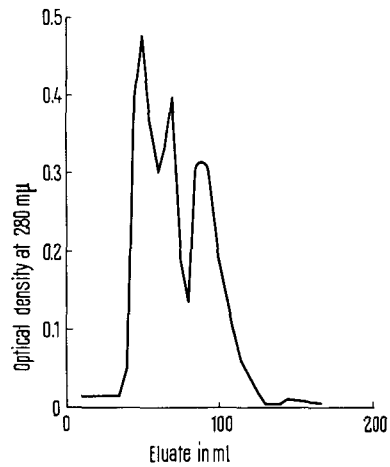

Fig. 1. BGH + $0.02M$ $MgCl_2$ solution passed over Sephadex G-25. The solution contained per ml: 10 mg BGH, 0.05 mM NH_3, 0.02 mM $MgCl_2$. Eluted with deionized H_2O at room temperature. Flow rate was 1 ml/min.

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