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Letter to the Editors

Comments on: On the Red Blood Cell Ca^{2+}-Pump: An Estimate of Stoichiometry

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The paper by Larsen, Hinds and Vincenzi (1978) discusses the question of the stoichiometry of active calcium transport in resealed human red cell ghosts. The authors conclude that the extrusion of one calcium ion requires the splitting of one ATP molecule.

In intact red cells, when comparing lanthanum-sensitive calcium extrusion and ATP splitting, we previously found a Ca^{2+}/ATP stoichiometry of 2:1 (Sarkadi *et al.*, 1977). Larsen *et al.* (1978) suggest that the difference obtained is due to the problems of our method applied for assessing ATPase activity in intact red cells. They raise the possibility of an underestimation of the rate of ATP splitting in the iodoacetamide+tetrathionate-treated intact red cells, and thus an overestimation of the Ca^{2+}/ATP stoichiometry.

Our comments on the paper of Larsen *et al.* follow:

1. The complete inhibition of red cell glycolysis by 2 mM iodoacetamide+2 mM tetrathionate has been carefully checked in various experimental conditions (Szász, 1970). There is no measurable lactate production in these cells and, as shown in Fig. 7 in the paper of Sarkadi *et al.* (1977), there was no change in the 2,3-DPG content of the cells during the experimental period. We have recently found that a similarly effective blocking of red cell metabolism and similar results for the ATPase activity can be obtained by applying 5 mM iodoacetamide alone, which in this higher concentration blocks both glyceraldehyde 3-phosphate dehydrogenase and 2,3-DPG phosphatase (*unpublished results*). Therefore, any untoward reaction between iodoacetamide and tetrathionate, producing misleading results, is highly unlikely.

2. The underestimation of the total cellular ATPase activity, due to some reutilization of inorganic phosphate in the iodoacetamide+tetrathionate treated red cells, would not change the ratio of lanthanum-sensitive/lanthanum-insensitive ATP splitting. However, the lanthanum-insensitive fraction is about 50% in the intact red cells and is only about 10–15% in the resealed ghost preparation of Larsen *et al.* There is no significant difference between our results for the ratio of the total calcium-dependent ATP splitting to calcium transport and that of Larsen *et al.*

3. As we have recently reported, a considerable decrease in the lanthanum-insensitive ATPase activity is detected in red cells stored in ACD for longer periods than three weeks (Szász, Sarkadi & Gárdos, 1978*a*) or in cells loaded with calcium to higher concentrations than 3 mM (Szász *et al.*, 1978*b*). By measuring ^{140}La uptake, we have shown (Szász *et al.*, 1978*b*) that the above decrease in the lanthanum-insensitive ATP splitting is due to a small lanthanum influx and to the inhibition of calcium-stimulated ATPases by low concentrations of lanthanum in the cell interior. Larsen *et al.* in their experiments used

resealed ghosts of outdated, ACD-stored red cells, into which a considerable amount of lanthanum may permeate. This possibility is supported by their findings that the passive calcium influx into these ghosts is about 10% of the active calcium extrusion. A small increase in cellular lanthanum concentration may well inhibit calcium-stimulated ATP splitting, not connected to the calcium pump, and thus resulting in an overestimation of the ATP requirement of the pump.

In summary, our belief is that the method used by us is appropriate for measuring ATPase activity in intact red cells. Our explanation for the Ca^{2+}/ATP stoichiometry lower than 2:1, obtained by Larsen *et al.*, is that a lanthanum influx into their resealed ghosts may have caused the inhibition of calcium-stimulated ATPase not connected to the pump. A criticism of our results by Dr. Schatzmann (*personal communication*), namely, that blocking of the calcium pump by external lanthanum might cause an uncoupling of the ATPase activity from calcium transport, is still open to discussion.

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