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Letters to the Editor

Calcium-Calcium Exchange and Calcium-Strontium Exchange in Red Cell Ghosts

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In his recent article, Dr. Porzig (H. Porzig, 1973. *J. Membrane Biol.* **11:** 21) claimed that I did not take into account the possible differences in affinities of calcium and strontium for erythrocyte ghosts. Dr. Porzig, however, apparantly ignored the fact that I also infused erythrocytes with strontium (E. J. Olson & R. J. Cazort, 1969. *J. Gen. Physiol.* **53:**311). In this experiment a substantial amount of calcium entered or was adsorbed on the erythrocyte ghosts without displacing any strontium. If Dr. Porzig's contention about the relative affinities of calcium and strontium is correct, strontium should have been displaced from these ghosts by the entering calcium. Therefore, it appears to me that the different conclusions drawn by Dr. Porzig and myself result from a conflict of data rather than an oversight on my part.

If one assumes that the cellular concentration of calcium is the same as the concentration of calcium in the infusing solution, one can consider from Fig. 11 of Dr. Porzig's paper that calcium is transported against a concentration gradient. However, there is no reason that I can see for this assumption to be considered valid. Furthermore, Dr. Porzig did not report dry weight analysis so that it is impossible to determine whether or not the strontium induced a water shift.

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It can be inferred from tracer flux experiments that the Ca/Sr affinity ratio for the sites that mediate Ca-Ca and Ca-Sr exchange across the membrane is at least 10:1 (H. Porzig, 1970. *J. Membrane Biol.* **2:**324; H. Porzig,

1973. *J. Membrane Biol.* **11**:21). This ratio seems to be similar on both sides of the membrane. Therefore, in Dr. Olson's experiments where the cells contained 1.5 mM Sr, only a small fraction of total Ca uptake can be expected to occur via the Ca-Sr exchange pathway. Moreover, Ca-poor ghosts, in contrast to Ca-containing ghosts, are known to bind a considerable amount of extracellular Ca to unspecific membrane sites (H. Porzig, 1972. *J. Membrane Biol.* **8**:237). Most likely a large fraction of the Ca uptake observed by Olson and Cazort (1969, *J. Gen. Physiol.* **53**:311) was due to unspecific membrane binding and not to penetration into the cell interior. This conclusion is supported by two observations: (1) The Ca uptake in Dr. Olson's study was complete already when he started his measurements (zero time of his experiments). (2) The ghost cells in Dr. Olson's experiments were not washed in a large volume of Ca-free EDTA-containing solution prior to Ca analysis. In my experiments this procedure usually removed a considerable amount of externally absorbed Ca, which otherwise tends to obscure changes in the cellular Ca content.

As indicated in the legend to Fig. 11, the cellular Ca content at the beginning of this experiment was 0.75 mM/liter ghosts. This value was measured directly in the cells and not calculated from the Ca concentration of the hemolyzing solution. All cellular Ca and Sr concentration changes were calculated on the basis of the hematocrit value of the suspension which was measured *after* the addition of Sr. Therefore, a Sr-induced osmotic shrinkage of the cells and a resulting increase in the outwardly directed Ca concentration gradient can certainly not explain the Sr-activated Ca-outflow in Fig. 11. Volume shifts due to an asymmetry in Na and K fluxes were avoided by incubating the cells which contained KCl as the main osmotic constituent in isotonic KCl solutions. In fact, the hematocrit did not change significantly during the course of an experiment. Therefore, no dry weight analysis was necessary.

The Sr-induced Ca transport against the Ca concentration gradient in net flow experiments is confirmed by the results of radioactive flux measurements (Fig. 7) which show the same counterflow phenomenon. Moreover, in control experiments the addition of 10 mM $MgCl_2$ instead of 10 mM $SrCl_2$ did not induce any Ca counterflow (*see* Fig. 7). All these conditions are clearly defined in my papers.

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