tabolism and in cellular function is not clear. Theoretically this compound might act as an energy sump. If during anaerobic glycolysis no 2,3 DPGA is formed a net gain of 2 moles of ATP will occur for each mole of glucose converted to lactic acid. The formation of 2,3 DPGA from 1,3 DPGA will cause a proportional decrease in net yield of ATP. In addition recent work has suggested that 2,3 DPGA may play an important role in the regulation of oxygen transport in red cells. Since seal red cells have a relatively low energy requirement for cation transport but have an overall glycolytic rate equal to that found in high energy requiring cells (this Bulletin, abstract #33) it seemed of interest to measure ATP and 2,3 DPGA concentrations in these cells.

Measurements of ATP and 2,3 DPGA were performed on fresh blood obtained from the extradural vein of four seals, Phoca vitulina. The sum of ATP and 2,3 DPGA was determined using the technique of Bartlett. ATP concentrations were determined by a firefly tail method. The difference between sum and ATP gave calculated 2,3 DPGA. For comparison, similar measurements were performed on fresh human erythrocytes.

In the seal red cells the mean concentration of ATP was 0.43 mM/5.25 mM Hgb and of 2,3 DPGA was 6.80 mM/5.25 mM Hgb giving a 2,3 DPGA to ATP ratio of 15:8. In human red cells the mean concentration of ATP was 1.47 mM/5.0 mM Hgb and of 2,3 DPGA was 6.30 mM/5.0 mM Hgb with a ratio of 4.3.

Thus the ratio of 2,3 DPGA to ATP is substantially greater in the low energy requiring seal red cell than in the high energy requiring human red cell. This increased ratio, however, is produced by a lower ATP concentration rather than a higher 2,3 DPGA concentration. Studies of the stiochiometry of glucose to lactate conversion in the 2 cells reveal no essential difference (this Bulletin, abstract #33). A survey of existing values reported in the literature shows no consistent trend with respect to this ratio in high $Na^+$ versus low $Na^+$ cells. It is concluded that:

a. ATP is present in both types of cells and is presumably involved in energy delivery for cation transport.

b. 2,3 DPGA is present in seal red cells and may represent an energy sump.

c. The quantitative relationship between the 2 forms does not explain the difference in energetics between the 2 types of cells.

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## OUABAIN AND IODOACETATE INSENSITIVE, ETHACRYNIC ACID STIMULATED, ETHANOL INHIBITED UNIVALENT CATION TRANSPORT IN SEAL ERYTHROCYTES

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Mammalian erythrocytes show 2 different cation patterns. One pattern, like that in man, consists of relatively low $Na^+$ and relatively high $K^+$ concentrations. The other pattern, like cat and seal, consists of relatively high $Na^+$ and relatively low $K^+$ concentrations. Studies of the transport mechanisms involved in the high $Na^+$ group might be anticipated to deepen understanding of cation transport generally.

In this study, the effects of various inhibitors on $Na^+$ efflux and $K^+$ influx in the erythrocyte of the harbor seal, Phoca vitulina, were determined using isotopic $^{22}Na$ and $^{22}Na$ efflux was determined as the $T_{0.25}$ (the time for 25% equilibrium of radioactivity between cells and extracellular phase).

Mean steady state cell/plasma ion ratios ($\hat{r}$) in 6 seals were as follows: $\hat{r}(Na^+) = 0.91 \pm 0.08$: $\hat{r}(K^+) = 2.07 \pm 0.26$: $\hat{r}(Cl^-) = 0.59 \pm 0.04$. Thus both $Na^+$ and $K^+$ more closely approximate electro chemical equilibrium than in any previously studied erythrocyte. Results from the inhibitor studies were as follows:

| Study | $T_{0.35}$ (hours) | # | "p" |
|---|---|---|---|
| Control | 1.9 ± 0.4 | | |
| MIA ($10^{-4}$M) | 1.7 ± 0.5 | 12 | N.S. |
| Control | 2.0 ± 0.5 | | |
| Ouabain ($10^{-3}$-$10^{-4}$M) | 2.1 ± 0.6 | 12 | N.S. |
| Control | 1.9 ± 0.4 | | |
| Ethacrynic acid ($10^{-3}$M) | 1.4 ± 0.3 | 12 | $<.05>.02$ |
| Control | 1.9 ± 0.4 | | |
| Ethanol (5 x $10^{-1}$M) | 3.6 ± 0.5 | 10 | $<.001$ |

Unlike human erythrocytes, inhibition of anaerobic glycolysis by monoiodacetate (MIA) does not decrease net $Na^+$ efflux. This suggests a low ATP requirement for cation transport in seal red cells so that the basal ATP pool is sufficient to maintain this process during the 5 hours of study.

Inhibition of ATP-ase activity by ouabain does not change $Na^+$ transport in the seal erythrocyte. Since these cells contain a substantial concentration of ATP (this Bulletin, abstract #25) this suggests either incomplete inhibition of ATP-ase activity by ouabain or a non-ATP dependent energy mechanism.

Ethacrynic acid increases rather than decreases $Na^+$ efflux. A similar effect of ethacrynic acid on cation transport has been described in the isolated frog skin (Clin. Wochen. 44:30, 1966) where the presumed action is an increase in $Na^+$ permeability.

Ethanol, which does not affect $Na^+$ or $K^+$ flux rates in human erythrocytes, is a striking inhibitor of both $Na^+$ and $K^+$ transport in seal erythrocytes. Although the precise mechanism of ethanol is not known, it is of interest that a similar inhibition of $Na^+$ transport by ethanol has been reported in the isolated frog skin (Nature 200:476, 1963).

These studies suggest low energy requirements not involving major ATP expenditure for cation transport in high $Na^+$ erythrocytes. Presumably major differences in the molecular mechanisms for transport exist in high $Na^+$ versus low $Na^+$ erythrocytes to account for the different responses to ethacrynic acid and ethanol.