# Preliminary Notes

PN 1256

## Adenosine triphosphate-dependent transport of rubidum into erythrocyte ghost

The rate of active transport of cations into erythrocytes has been said to parallel the level of ATP in the cells. It has been shown clearly that the transport is dependent on the level of ATP but not on glycolysis[1]. Recently, Na-, K-dependent ATPase has been considered to be an intimate component of active transport in erythrocytes[2,3]. JÄRNEFELT[4] has proposed a mechanism whereby the microsomal ATPase if brain tissue involves an intermediate which binds $Na^+$ in the presence of ATP. A similar mechanism has been presented for the calcium pump of the endoplasmic reticulum of skeletal muscle[5].

An attempt is made here to show that the ghosts of human erythrocytes are capable of taking up Rb in the presence of ATP. The properties of this uptake are compatible with those of a Na-, K-dependent ATPase. Human-erythrocytes ghosts were prepared as described by POST *et al.*[2]. The ghost-cells used in these experiments may be heterogeneous with respect to their cationic permeability as suggested by HOFFMAN[6]. Some cells have the pump mechanism by which $Rb^+$ is actively incorporated into them.

TABLE I

THE RELATIONSHIP BETWEEN THE ATPASE ACTIVITY AND THE BINDING OF $^{86}Rb$ BY GHOST CELLS

The incubation mixture contained 40 $\mu$moles of triethanolamine–HCl (pH 7.6), 1 $\mu$mole of ATP, 1 $\mu$mole of $MgCl_2$, 0.3 $\mu$mole of $^{86}Rb$ (25000 counts/min), ghost cells (prepared from 0.3 ml of erythrocytes) and other components as indicated below in a final volume of 0.5 ml. Incubation was carried out at 37° for 1 h. Liberation of $P_i$ from ATP was determined by a slight modification of the method of FISKE AND SUBBAROW[7]. The binding of $^{86}Rb$ by the ghosts was assayed as described in Fig. 1.

| *Additions (+) or omissions (—)* | *ATPase µmole Pi/h* | *$^{86}Rb$ binding (counts/min)* |
|---|---|---|
| Nil | 0.95 | 2960 |
| + $Na^+$ (0.1 M) | 0.96 | 3500 |
| + $K^+$ (0.1 M) | 0.99 | 1760 |
| — ATP | — | 650 |
| + Ouabain ($10^{-5}$ M) | 0.95 | 100 |
| ($5 \cdot 10^{-6}$ M) | 1.10 | 340 |
| — $Mg^{2+}$ | 0.06 | 950 |
| + $Na^+$ (0.1 M), $K^+$ (0.1 M) | 1.23 | 1250 |

A study of the increase of $^{86}Rb$ in the ghost cells in relation to time is illustrated in Fig. 1. The rate of incorporation was increased in the presence of ATP. A saturation level was reached in about 2 or 3 h, when the ATP added was hydrolyzed almost completely. Contrary to this finding the sodium binding by the microsomes of brain tissue[4] or the calcium uptake by the skeletal muscle endoplasmic reticulum[5] reached

saturation in a very short time, even in the presence of sufficient amount of ATP. ATP-dependent rubidium uptake by ghost cells was affected by various reagents (Table I). It was depressed in the presence of $K^+$ but not by $Na^+$. This is in accordance with a usual conception that $Rb^+$ and $K^+$ are similar electrochemically and have similar affinities for the carrier of $K^+$ transport. It is well known that an active cationic transport through the membrane of the cell is inhibited by ouabain with little effect on passive transport. In erythrocytes, ouabain slows the decline of ATP level, with little effect on the ATP-generative processes[8]. This is due possibly to the inhibitory action of ouabain on the membrane ATPase. Both rubidium uptake and $Na^+$-, $K^+$-dependent ATPase of ghost cells were inhibited markedly by the addition of ouabain.

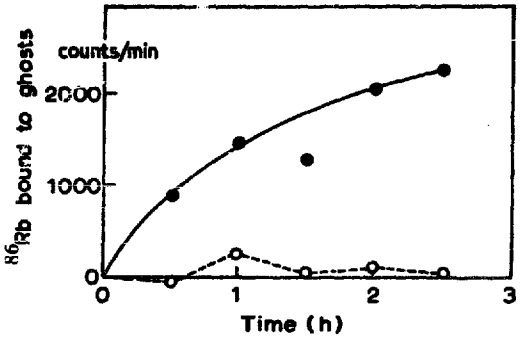


Fig. 1. The binding of $^{86}$Rb by ghost cells of erythrocytes in relation to time. Ghosts were suspended in 0.08 M triethanolamine–HCl buffer (pH 7.6) containing $^{86}$Rb (25000 counts/min) and 1 $\mu$mole of $MgCl_2$ in the presence (●—●), or absence (○—○), of 1 $\mu$mole of ATP in a total volume of 0.5 ml. Incubation was carried out at 37°. Binding of $^{86}$Rb was assayed, at appropriate intervals, by measuring the decrease in radioactivity of the supernatant after removal of the ghosts by centrifugation.

The characteristics of the ATP-dependent binding of $Rb^+$ by ghost cells are similar to the known properties of the active transport of $K^+$ and $Mg^{2+}$-activated $Na^+$-, $K^+$-dependent ATPase, which is considered to be an intimate component of the active cation transportation pump.

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