aux analogies et filiations possibles, dans la cellule hépatique, entre la membrane externe des mitochondries et la membrane endoplasmique.

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## Role of cellular $P_i$ in $P_i$ transport and metabolism in human red cells

The significance of the $P_i$ compartment in human red cells is poorly defined with respect to its role in $P_i$ transport as well as in internal metabolism. As a result of $^{32}P_i$ uptake studies[1,2], it was suggested that inward transport may be due to a membrane esterification involving ATP rather than to simple diffusion. The view was proposed, moreover, that $P_i$ turnover in human red cells may be due to interaction between ester P at the cell surface and medium $P_i$, and that cellular $P_i$ may not be in the mainstream of either transport or metabolism[3]. These early concepts have since required some modification[4] but there has been little evidence reported in support of alternate views[5].

In the course of studies on $P_i$ release from human red cells, we observed[6] as have others[2,7–9] that medium $P_i$ is elevated when iodoacetic acid is added to the suspension. This result is due presumably to inhibition of $P_i$ esterification in glycolysis at the phosphoglyceraldehyde dehydrogenase step, in conjunction with continuing dephosphorylation of some ester P. A question which arose in this connection concerned the pathway of $P_i$ movement following dephosphorylation. Since there are ATPases associated with the red cell membrane[10–12], it seemed possible that $P_i$ could be released from the membrane directly into the medium. On the other hand, $P_i$ may be liberated into the cellular $P_i$ compartment from where it could pass into the medium. In order to resolve this question, $P_i$ concentrations were determined in extracts of iodoacetic acid-poisoned and control cells and compared with medium $P_i$ values in the present study. $^{32}P_i$ concentrations were also measured as $^{32}P_i$ was being released from previously labeled erythrocytes.

Erythrocytes were separated from blood which had been collected in acid–citrate–dextrose (N.I.H. Formula B) and washed with an equal volume of a salt medium containing, in mM: NaCl, 120; KCl, 5.6; $MgCl_2$, 2; $Na_2HPO_4$, 1; Tris, 24; and glucose, 10. The pH of this solution was 7.40 at 37°. After 3 additional washes, the red cells were suspended in the same medium containing 0.3 $\mu$C of $^{32}P_i$ (Abbott) per ml of medium and incubated for 1–2 h at 37° in a Dubnoff shaking incubator, cycled at 100/min. The labeled cells were centrifuged and washed twice with $P_i$-free media.

The packed red cells were resuspended with fresh medium so as to give a hematocrit of 25% in a total volume of 3.5 ml and were equilibrated for 10 min before time-zero samples were removed. A second sample was taken at 60 min. No significant differences were detected in pH and hematocrit during the incubation. Samples of the medium were placed on concentrically-ringed planchets, air-dried, and counted with an end-window Geiger–Müller counter.

Erythrocytes were collected by centrifugation and washed once at 5° with 20 vol. of cold, $P_i$-free medium in order to minimize the influence of trapped fluid. Protein-free extracts were prepared with 5% trichloroacetic acid and neutralized with NaOH at 0°. A modification of the Berenblum and Chain procedure[13] was employed for the $P_i$ determination. The step in this analytical procedure which results in the

TABLE I

$P_i$ CONCENTRATIONS IN HUMAN ERYTHROCYTES AND MEDIUM FOLLOWING A 60-min INCUBATION IN THE PRESENCE OF $5 \cdot 10^{-4}$ M IODOACETIC ACID

These values are means ± S.E. Values determined in the presence of iodoacetic acid were different from the controls ($P < 0.05$) when evaluated with the paired $t$ test. IAA = iodoacetic acid.

| *Number of experiments* | $(P_i)_m$ (mM) | | $(P_i)_c$* (mM) | | $(P_i)_c : (P_i)_m$ | |
|---|---|---|---|---|---|---|
| | *−IAA* | *+IAA* | *−IAA* | *+IAA* | *−IAA* | *+IAA* |
| 7 | 1.13 ± 0.04 | 1.45 ± 0.08 | 0.85 ± 0.11 | 2.23 ± 0.35 | 0.75 ± 0.11 | 1.62 ± 0.35 |

* Water content was assumed to be 0.65 of the red blood cell volume.

TABLE II

$^{32}P_i$ CONCENTRATIONS IN HUMAN ERYTHROCYTES AND MEDIUM FOLLOWING INCUBATION IN THE PRESENCE OF $5 \cdot 10^{-4}$ M IODOACETIC ACID OR IN 15 mM $P_i$

Data from 2 experiments have been averaged. IAA = iodoacetic acid.

| *Time (min)* | *Counts/min per ml* × $10^{-4}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $(^{32}P_i)_m$ | | | $(^{32}P_i)_c$* | | | $(^{32}P_i)_c : (^{32}P_i)_m$ | | |
| | *−IAA, 1 mM $P_i$* | *+IAA, 1 mM $P_i$* | *−IAA, 15 mM $P_i$* | *−IAA, 1 mM $P_i$* | *+IAA, 1 mM $P_i$* | *−IAA, 15 mM $P_i$* | *−IAA, 1 mM $P_i$* | *+IAA, 1 mM $P_i$* | *−IAA, 15 mM $P_i$* |
| 0 | 1.08 | 1.47 | 1.47 | 14.5 | 20.8 | 21.6 | 13.4 | 14.1 | 14.7 |
| 60 | 3.52 | 6.43 | 6.69 | 7.3 | 19.2 | 16.2 | 2.1 | 3.0 | 2.4 |

* Water content was assumed to be 0.65 of the red blood cell volume.

separation of $P_i$ is the transfer of phosphomolybdate from an aqueous to a lipid phase. Cellular $^{32}P_i$ concentrations were estimated from samples of this lipid extract.

Freshly drawn human erythrocytes contain 0.25–0.48 $\mu$mole of $P_i$ per ml of erythrocyte[14,15]. In 7 experiments, we found that $P_i$ concentrations in cells incubated with 1 mM $P_i$ for 1 h were 0.30–0.83 $\mu$mole/ml of erythrocyte. Judged by this indicator, our erythrocytes appear to have been in fairly good condition considering the extensive storage, washing, and incubation procedures. In Table I, these values have been converted to mM, *i.e.*, mmoles/l of cell water, by assuming that 0.65 of the red cell volume is water[14]. Cellular concentrations can thereby be compared more readily with the medium concentrations.

Elevation in medium $P_i$ following iodoacetic acid addition to a suspension of human red cells was confirmed in the present study (Table I). There was also a rise in the cellular $P_i$ concentration which was relatively greater than the elevation in medium $P_i$, *i.e.*, there was a 250 % increase in cellular $P_i$ in contrast to a 28 % increase in medium $P_i$. A more meaningful comparison is the direction of the concentration gradients. In the nonpoisoned cell, there appeared to be a small net movement from medium to cell whereas, in the iodoacetic acid-inhibited cell, there was a definite gradient from cell to medium. It appears probable, therefore, that cellular $P_i$ is the precursor compartment to medium $P_i$ and that the alternate possibility, *viz.*, $P_i$ liberation from an ester P compartment directly into the medium, is not the predominant pathway.

$^{32}P_i$ concentrations in cells and media were also determined during the course of $^{32}P_i$ release studies (Table II). At time zero, there was a 14-fold difference in $^{32}P_i$ concentration between previously labeled cells and medium, which decreased to a 2-fold difference after 1 h. In the presence of iodoacetic acid, there was more $^{32}P_i$ in both cell and medium at zero time but the ratio of these concentrations was about the same as in the control. After 1 h, the medium $^{32}P_i$ level increased further to twice the control level observed at this time. The cellular $^{32}P_i$ level remained high so that the cell:medium ratio appeared to be greater with iodoacetic acid than in the control. In general, these results on unidirectional movement support those obtained with net movement. In this connection, GERLACH, DEUTICKE AND DUHM[9] have observed a transient $^{32}P_i$ transfer from cell to medium in the absence of iodoacetic acid.

Experiments with high medium $P_i$ were also conducted in order to see if the $^{32}P_i$ changes brought about by iodoacetic acid could be duplicated by this procedure. It was expected that the cellular $P_i$ level would be raised by high medium $P_i$ and that $^{32}P_i$ incorporation into organic P would be reduced because of the increased probability that $P_i$ rather than $^{32}P_i$ would be esterified. This could result in a higher $^{32}P_i$ concentration within the cell as de-esterification of labeled organic P continues to liberate $^{32}P_i$ into the cellular $P_i$ pool. As shown in Table II, these predictions appeared to be correct since virtually the same results were obtained with high medium $P_i$ as with iodoacetic acid. The common change seems to have been the reduced $^{32}P_i$ esterification into organic P.

The present results support the view that cellular $P_i$ is directly involved in the outward transport of $P_i$ across the red cell membrane. The fact that the cellular $P_i$ and $^{32}P_i$ levels increased indicates that the permeability of the cell membrane to $P_i$ was not so great as to permit rapid equilibrium between the two compartments.

The present findings also suggest that cellular $P_i$ is dynamically involved with cellular metabolism. When esterification was inhibited, both cellular $P_i$ and $^{32}P_i$ levels were raised indicating that de-esterification and $P_i$ liberation into this compartment were taking place at significant rates. It would appear, moreover, that in the absence of iodoacetic acid the rate of esterification must be equally rapid in order to account for the low steady-state level found in the nonpoisoned cell. In other words, the low $P_i$ value in the normal red cell appears to be a consequence of active metabolism rather than the result of minimal metabolic participation.

The nature of the organic P which, when degraded, liberates $P_i$ into the cellular $P_i$ compartment is unknown. The $(Na^+, K^+)$-dependent ATPase releases $P_i$ internally[16,17] but the extent to which this could account for the increase in cellular $P_i$ when esterification is inhibited cannot be assessed from our data. It seems likely that whatever the reaction, ATP utilization may be involved because this is the compound which shows the major decrease in concentration when glycolysis is inhibited with iodoacetic acid[8].

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