The metabolic potential of erythrocyte ghosts*

Although the structure of the human erythrocyte ghost has been extensively studied, no metabolic investigations with morphologically intact post-hemolytic cells have as yet been reported. A technique suitable for measuring metabolic turnover in cellular ghosts as contrasted with non-cellular post-hemolytic debris or stroma is reported here. Ghosts derived from hemolysates produced with water, made subsequently isotonic with either NaCl or KCl, centrifuged, washed with Krebs-Ringer bicarbonate buffer, suspended in plasma, and equilibrated kinetically under 5% CO_2–95% O_2 at 37° C have been found useful in studying glycolytic reactions as affected particularly by nucleosides. The preparation has been found to be cellular with assorted sizes of ghosts and heterogeneous in pigmentation, but relatively free of noncellular fragments and unhemolyzed erythrocytes. Cell counts have been made, and the metabolic behavior of such tissue compared simultaneously with intact erythrocytes. Ghosts so prepared are orange red in color containing approximately 20% of hemoglobin, pools of ester, inorganic and lipid phosphorus, and small amounts of potassium and sodium ions, the latter dependent upon the electrolytes used to render the hemolysate isotonic.

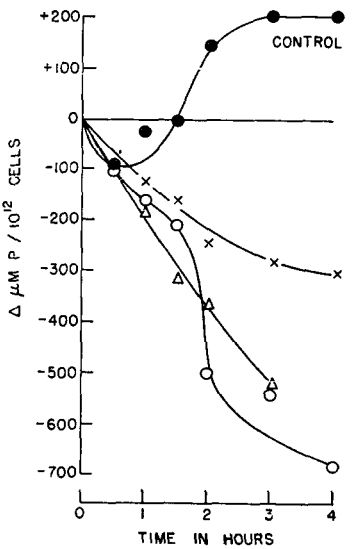

Fig. 1. Effect of adenosine on plasma inorganic phosphorus of ghost plasma suspensions at 37° C. Cells from one day old, heparinized, white cell free, whole blood in homologous heparinized plasma. 50 ml cell suspensions (1:1 packed cells to plasma) were equilibrated with 0.388 m*M* adenosine and 0.88 ml of 0.11 *M* phosphate buffer under 5% CO_2–95% O_2. Average pH = 7.42 ± 0.05. ○ Isotonicity of hemolysate restored with 0.62% NaCl; ● Same. No adenosine; ×, △ Isotonicity of hemolysate restored with 0.79% KCl.

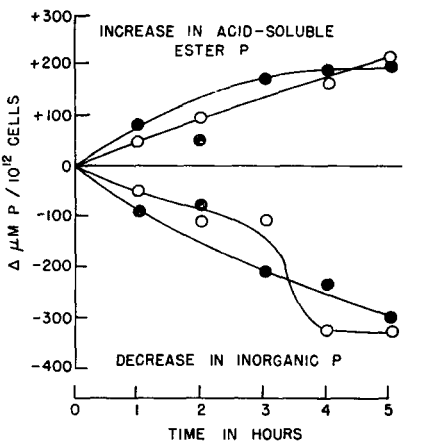

Fig. 2. Conversion of plasma inorganic phosphorus to cellular ester phosphorus by adenosine in ghost plasma suspensions. ○, ● Experiments 1 and 2. Adenosine 0.388 m*M*/50 ml suspension.

Addition to the ghost preparation of ribosides such as adenosine, guanosine, and inosine causes an appreciable conversion of plasma inorganic phosphorus to organic acid soluble phosphorus (Figs. 1 and 2). The amount of phosphate exchange is of a magnitude similar to that caused in intact erythrocytes by adenosine although the preliminary kinetics indicate the manner of transport not to be the same in each. Unfortified ghost suspensions are unable to promote phosphate esterification, in fact, after several hours the plasma inorganic phosphorus increases at the expense of the cellular ester pool. These observations agree qualitatively with published data where ribosides have been shown to cause esterification in whole cells and in frozen-thawed hemolysates[1,2]. Significant differences between stromal and intracellular phosphorylated intermediates have also been demonstrated[3] and an influence of adenosine upon the stromal fraction stated[4]. The pronounced and readily measured influence of nucleosides on the metabolic behavior

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of ghost plasma suspensions plus the inability of the latter to utilize glucose affords a convenient system in which to measure extra-glycolytic activities remaining in the post-hemolytic cell.

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Galacto-waldenase and the enzymic incorporation of galactose 1-phosphate in mammalian tissues

It has been found previously[1,2] that extracts of galactose-adapted *Saccharomyces fragilis* catalyze the incorporation of gal-1-P*, ** into uridylic-bound galactose according to the following equation:

$$\text{gal-1-P} + \text{UDPG} \rightleftharpoons \text{UDPgal} + \text{G-1-P} \qquad (1)$$

In the conversion of gal-1-P to G-1-P, UDPG acts catalytically since it can be regenerated by LELOIR's galacto-waldenase[4]:

$$\text{UDPgal} \rightleftharpoons \text{UDPG} \qquad (2)$$

Reaction (1) is a non-pyrophosphorolytic type of uridyl transfer catalyzed by an enzyme which we call GP uridyl transferase in contrast to PP uridyl transferase[3] which catalyzes the reversible pyrophosphorolysis of UDPG:

$$\text{UDPG} + \text{PP} \rightleftharpoons \text{UTP} + \text{G-1-P} \qquad (3)$$

We have now demonstrated the presence of large amounts of both GP uridyl transferase and galacto-waldenase in rat and calf liver. Moreover, since the transferase is able to resist heating to 50° centigrade at pH 5.9, it can be separated from the heat labile galacto-waldenase. The separation of the two enzymes makes it possible to prepare UDPgal of sufficient purity for use in a simple spectrophotometric assay for galacto-waldenase. UDPgal was formed from UDPG in the following system: liver GP uridyl transferase (10 mg protein), gal-1-P 28 μmoles, UDPG 10 μmoles, TPN 20 μmoles, glucose 1,6-diphosphate 0.01 μmoles, cysteine 300 μmoles, $MgCl_2$ 500 μmoles, phosphoglucomutase and glucose-6-phosphate dehydrogenase in 20 ml of 0.1 *M* tris buffer pH 8. G-1-P formed in this system (Equation 1) was removed by the successive action of phosphoglucomutase and glucose-6-phosphate dehydrogenase. The overall course of the reaction was followed by measuring TPN reduced. Four μmoles of UDPgal containing 8% impurity of UDPG was isolated from the reaction mixture by adsorption and elution from Darco[5] followed by paper chromatography in neutral ethanol ammonium acetate solvent[6]. UDPgal was assayed by conversion to UDPG which is able to reduce DPN in the presence of UDPG dehydrogenase[7] (see Fig. 1).

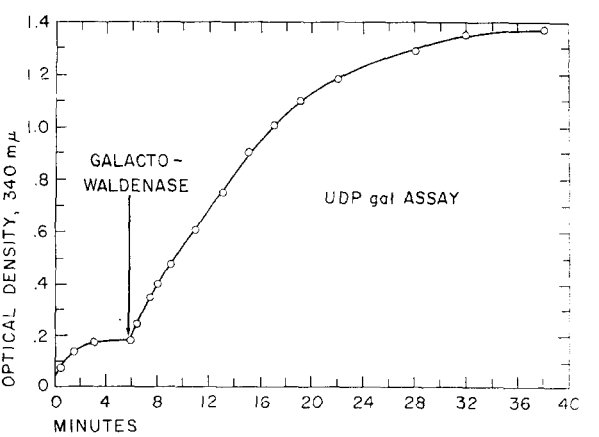

Fig. 1. Assay mixture contained: UDPgal, DPN 0.5 μmoles, and cysteine 5 μmoles in 0.5 ml 0.1 *M* glycine buffer at pH 8.6. UDPG dehydrogenase added at 0 time. Galacto-waldenase added at indicated time.