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Erythrocyte Energy Metabolism in Hereditary Spherocytosis *

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Summary. The incorporation of extracellular orthophosphate-^{32}P into cellular ATP, 2,3-diphosphoglyceric acid, and inorganic phosphate has been measured over a period of 6 hours *in vitro* in red blood cells from normal subjects and from patients with hereditary spherocytosis who had undergone splenectomy. The pattern of labeling of the intracellular compounds was found to be the same in both types of red blood cells, as reported by other workers using much shorter periods of incubation. In addition, in the present study it was possible to compare the net flux of extracellular phosphate into ATP between the two groups of erythrocytes. These latter results suggest that the actual turnover rate of ATP was not abnormal in these patients with hereditary spherocytosis.

Introduction

Two current views of the primary defect in the red blood cells of patients with hereditary spherocytosis (HS RBC) invoke an abnormality in the cellular membrane (1–3). One of these views (2, 3) postulates a structural defect that leads to premature loss of membrane material and a reduction of the critical hemolytic volume of the RBC. This view is an extension of the proposal originally made by Selwyn and Dacie (4). The other current view (1) stresses the importance of swelling and colloid osmotic lysis of the RBC as the hemolytic mechanism. According to this latter view, the effect of greater permeability of the membrane to cations in HS RBC is partially compensated for by an increased rate of active cation transport; this is thought to result in a substantially greater rate of glycolysis and turnover of ATP in these erythrocytes. It has also been suggested in the past (5, 6) that the production of ATP is decreased in HS RBC. The purpose of the present study is to evaluate further the actual turnover of ATP phosphorus in normal and HS erythrocytes in view of these conflicting proposals.

Previous studies of glycolysis in HS RBC have included the following: measurements of the level of glycolytic intermediates and nucleotides in the fresh RBC (7–11), rates of glucose utilization, lactate production and changes in concentrations of ATP and glycolytic intermediates under various *in vitro* conditions (4, 8, 10–12), the activities of various enzymes in the Embden-Meyerhof Pathway and hexose-monophosphate shunt (13), and, finally, measurements of the incorporation of orthophosphate-^{32}P into phosphorylated compounds involved in the energy metabolism of the RBC (5–7, 9, 10, 14). Most, but not all, of these studies suggest that glycolysis in HS RBC does not differ substantially from normal.

Prankerd, Altman, and Young (5), using technics available in 1953 and 54, studied the incorporation of extracellular inorganic ^{32}P into cellular ATP, inorganic phosphate (P_i), and 2,3-diphosphoglyceric acid (2,3-DPG) in HS RBC.

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The process was investigated serially over 6 hours *in vitro*. Results obtained suggested that the partitioning of ^{32}P among ATP, 2,3-DPG, and P_i was abnormal in HS RBC and that this might reflect some defect in glycolysis in these cells. Motulsky, Gabrio, Burkhardt, and Finch (6), using similar technics, seemed to confirm this finding.

Subsequently, Shafer (7, 14), in a careful study using the newer ion exchange column chromatography, was unable to find any abnormality in the pattern of labeling of the phosphorylated glycolytic intermediates in HS RBC after 5 minutes of incubation with ^{32}P. Zipursky, Mayman, and Israels (9), using a 2-minute exposure to ^{32}P, and Keitt (10), using an incubation of 15 minutes with the isotope, also were unable to demonstrate a difference in the pattern of labeling when comparing HS and normal RBC. The longer period of incubation used by Prankerd and associates has not been tested again, however.

In all of the above studies, only the pattern of labeling of the compounds within each experiment was determined. Prankerd and associates and Zipursky and co-workers presented the actual specific activities obtained but pointed out the wide variability that existed from experiment to experiment. To facilitate comparison of the patterns, Shafer chose the specific activity of 2,3-DPG and Keitt that of ATP as unity in presenting results in each experiment. In these studies, therefore, it was not possible to compare the absolute net flux of ^{32}P into ATP in HS and normal RBC. Thus, estimates of the actual turnover rate of ATP phosphorus in the two kinds of RBC could not be made from these results.

In the present study, the incorporation of extracellular orthophosphate-^{32}P into ATP, 2,3-DPG, and P_i of HS and normal RBC has again been studied serially over 6 hours. Ion exchange column chromatography has been used to isolate the compounds studied. In addition, the actual specific activity of extracellular P_i has been determined at each point. With this value it has been possible to compare the actual fluxes of phosphate into ATP in HS and normal RBC. These fluxes are directly proportional to the actual turnover rate of ATP. The results indicate that the pattern of labeling is normal in HS RBC even when studied over 6 hours; more importantly, the results provide no evidence for an altered rate of ATP turnover in HS RBC compared with normal cells.

Methods

Five patients with HS who had undergone splenectomy from 13 to 20 years before the study were used as experimental subjects. All had been included as splenectomized patients in the study reported by Prankerd and associates (5). The diagnosis was established in each by evidence of spherocytosis in stained smears of peripheral blood, the characteristic patterns of autohemolysis and osmotic fragility, the expected genetic pattern of transmission, the presence of an enlarged spleen at operation with an abundance of red cells in the splenic pulp, and the correction of the hemolytic anemia after splenectomy. Hematocrits ranged from 38 to 44%, and reticulocyte counts were 3% or less in all patients at the time of this study. Hematologically normal donors were used as controls.

Blood was collected from the subjects into Na_2EDTA (1.25 mg per ml blood). Sterile techniques and glassware were used throughout. The blood was centrifuged at $2,000 \times g$ for 15 minutes, the plasma was removed and saved, and the buffy coat was removed as completely as possible. The packed RBC were then resuspended in their plasma (at a hematocrit of 35 to 40%), to which sufficient glucose had been added to achieve a final concentration of approximately 12 μmoles per ml whole blood, and to which orthophosphate-^{32}P had been added in an amount to provide approximately 10 μc radioactivity per ml whole blood. After mixing, a sample was taken and immediately centrifuged at $2,000 \times g$ for 15 minutes; this constituted the time zero or "pre" sample. The centrifugation of the blood was initiated within 1 minute of the time of exposure of the RBC to the plasma that contained orthophosphate-^{32}P. All of the above manipulations were carried out at 4° C.

Incubations of the blood were carried out in polyethylene flasks at 37° C with agitation in a Dubnoff metabolic shaker. Separate portions were incubated for 30 minutes and 1, 2, 4, and 6 hours. At the end of each period, samples were taken for determination of total hemoglobin, hematocrit, and RBC count; the latter determination was performed by a model B Coulter counter. The blood was then centrifuged at $2,000 \times g$ for 15 minutes, the plasma was removed, and the RBC were washed three times with 0.17 M NaCl; the buffy coat was removed after each centrifugation. After the final washing, the RBC were suspended in 0.17 M NaCl to achieve a hematocrit of about 40%. All of the above procedures were carried out at 4° C. Extraction of the washed RBC with trichloroacetic acid and chromatography of the neutralized extract on Dowex 1-X8 chloride resin were carried out essentially as described by Bartlett (15, 16). The neutral trichloroacetic acid extract was diluted with water, usually to 200 ml, to reduce the concentration of the suspending NaCl solution in the extract to 0.001 mole per L or less before it was applied to the column. This initial 200-ml fraction was collected and analyzed;

it contained no phosphorus, adenine, or radioactivity. The composition and volume of the eluting solvents and the results of the chromatography in a typical sample are shown in Figure 1. Cellular P_i was isolated by rechromatography on activated charcoal of the pooled fraction eluted with 0.01 N HCl from the Dowex 1-X8 chloride column, as described by Bartlett (17). ATP was identified by its elution position and by the adenine to phosphorus ratio of 1 : 3 in the 0.5 M NH_4Cl fraction. 2,3-DPG was identified by its elution position.

Radioactivity was measured with a low background, thin window gas flow counting system.[1] Sufficient counts were accumulated in each case so that the standard error of the net counting rate was 1% or less.

The peak tubes eluted from the Dowex 1-X8 chloride column with 0.003 N HCl had an adenine to phosphorus ratio of 1:1 (Figure 1). This was considered to be adenosine monophosphate (AMP). We calculated the specific activities of ATP reported here by assuming that the two terminal phosphates were equally labeled and that the first phosphate had the specific activity of AMP phosphorus. In fact, this result differed little from the usual mode of assuming that the first phosphate of ATP is unlabeled, since the specific activity of AMP phosphorus was essentially zero during the first 4 hours of incubation. In 2,3-DPG, the radioactivity was assumed to be equally divided between the two phosphates, as shown most definitely by Shafer (7).

Calculation of the net flux of extracellular phosphate into the cellular compounds requires the isolation of the actual precursor extracellular phosphate and the determination of its specific activity. In the present instance, this compound is the diffusible inorganic phosphate of the plasma. We isolated this phosphate, therefore, by preparing an ultrafiltrate of the plasma with the Toribara apparatus (18) for each sample.

[1] Model C110-B, Nuclear-Chicago Corp., Des Plaines, Ill.

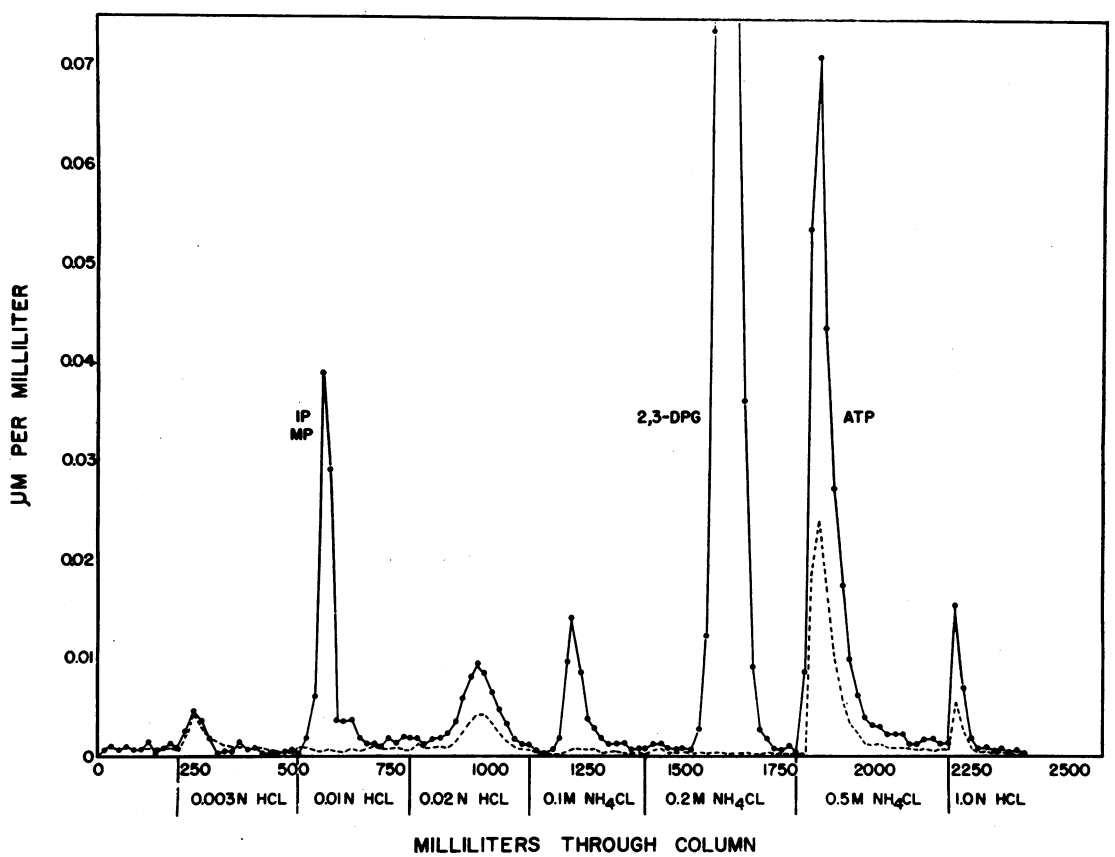

FIG. 1. DOWEX 1- × 8 CHLORIDE SEPARATION OF PHOSPHATE COMPOUNDS FROM 1.6 ML OF ERYTHROCYTES FROM A PATIENT WITH HEREDITARY SPHEROCYTOSIS (HS RBC). The neutral trichloroacetic acid extract was run through a 1.4- × 14-cm column of Dowex 1- × 8 chloride (200 to 400 wet mesh), and the absorbed compounds were eluted with the indicated eluents in 20-ml fractions at approximately 5 ml per minute. The values in the 2,3-diphosphoglyceric acid (2,3-DPG) peak, which is not shown, were 0.18, 0.19 and 0.10 μmole P per ml eluate at eluent volumes of 1,600, 1,620 and 1,640 ml, respectively. Solid line with solid circles = total P per milliliter eluate; dashed line = adenine (D, 260 mμ) per milliliter eluate. IP = inorganic phosphate; MP = monophosphate.

TABLE I

*RSA of erythrocyte phosphate compounds during in vitro incubation with ^{32}P in five normal subjects and five patients with HS**

Incubation time	ATP		P_i		2,3-DPG	
	Normal	HS	Normal	HS	Normal	HS
hours						
½	4.2 (3.1– 5.1)†	3.5 (2.9– 4.4)	4.0 (2.3–5.1)	2.8 (2.2–4.2)	0.9 (0.9–1.0)	1.0 (0.9–1.2)
1	5.6 (4.5– 6.6)	5.2 (4.1– 6.5)	3.4 (2.5–3.9)	3.2 (1.8–5.6)	2.1 (1.9–2.5)	2.3 (1.9–2.9)
2	7.5 (6.0– 9.3)	6.2 (4.6– 7.9)	5.6 (4.4–7.7)	4.1 (2.1–5.7)	4.5 (3.6–5.3)	4.4 (3.7–5.2)
4	9.3 (8.1–11.1)	8.7 (6.6–10.7)	7.5 (6.9–8.0)	5.8 (3.6–7.1)	7.2 (6.0–8.9)	6.6 (5.2–8.2)
6	9.5 (7.6–11.3)	9.5 (7.0–12.2)	7.9 (6.8–9.8)	6.8 (4.7–9.1)	8.2 (7.2–8.9)	7.9 (6.1–9.7)

* Abbreviations: RSA = relative specific activity; HS = hereditary spherocytosis; P_i = inorganic phosphorus; 2,3-DPG = 2,3-diphosphoglyceric acid. See text for definition and calculation of RSA as a per cent.
† Mean values, ranges in parentheses.

The incorporation of radioactivity into the cellular compounds is reported as their "relative specific activity" (RSA).[2] We obtained this value as a per cent by dividing the specific activity of the cellular compound by that of the corresponding plasma ultrafilterable phosphorus at time zero and multiplying the resulting quotient by 100.

$$RSA = \frac{SAc_{ti}}{SAp_{to}} \times 100, \qquad [1]$$

where c_{ti} = cellular compound at any time from zero to 6 hours, and
p_{to} = plasma ultrafilterable phosphate at time zero.

The specific activity of the plasma ultrafilterable phosphorus at time zero gives the exact measure of the concentration of radioactive phosphorus at the start; its use for determining the RSA of the compounds studied allows a precise comparison to be made of the plasma radioactivity incorporated intracellularly in each experiment. In addition, the RSA as used here gives a measure of the net accumulation, or flux, of plasma phosphate in the cellular compounds. Thus, the RSA of 9.5% in HS and nomal RBC ATP found at 6 hours (Table I) means that 9.5% of the phosphorus present in the two terminal phosphates is phosphorus that was originally present in the plasma at time zero. The RSA of the cellular compounds at time zero were measured and were uniformly less than 0.05%.

Autohemolysis was determined by the method of Young, Izzo, Altman, and Swisher (19), and was less than 1.5% in each instance at 6 hours. Glucose utilization was determined over 4 hours in both types of RBC with a modification of the anthrone method (15). The concentration of erythrocyte ATP was determined by an enzymatic method (20) that utilized the conversion of 3-phosphoglycerate to 1,3-diphosphoglycerate in the presence of ATP and the subsequent formation of glyceraldehyde 3-phosphate and P_i with the oxidation of NADH to NAD.[3]

The effects of the centrifugations and washing procedures used in this study on erythrocyte ATP were studied in four normal subjects. The concentration of ATP, expressed in micromoles per milliliter RBC, in whole fresh blood was (mean and range) 1.46 (1.37 to 1.56). After a single centrifugation with removal of the buffy coat and resuspension of the cells in their own plasma (the treatment given the "pre" samples), the concentration was 1.39 (1.32 to 1.55); after samples of the same cells had been washed three times in 0.17 M NaCl (as done before trichloroacetic acid extraction), the concentration was 1.43 (1.31 to 1.52). It can be seen that three washings in saline at 4° C cause no decrease in the amount of cellular ATP. The small decrease found between whole blood and the centrifuged RBC may be due to the removal of the buffy coat.

Blood pH was measured with a model G Beckman pH meter. Probabilities were calculated by using Student's *t* test.

Results

Figure 2 shows the pattern of incorporation of extracellular inorganic ^{32}P observed in a representative sample of HS and normal RBC; this pat-

[2] There is no generally accepted definition of "relative specific activity"; it is used in a variety of ways by various authors to indicate that the specific activity of a given compound is being compared to the specific activity of another compound or with the amount of radioactivity in a given system. The exact meaning, thus, varies from study to study.

[3] The reagents for this determination were obtained in kit form from Calbiochem, Los Angeles, Calif.

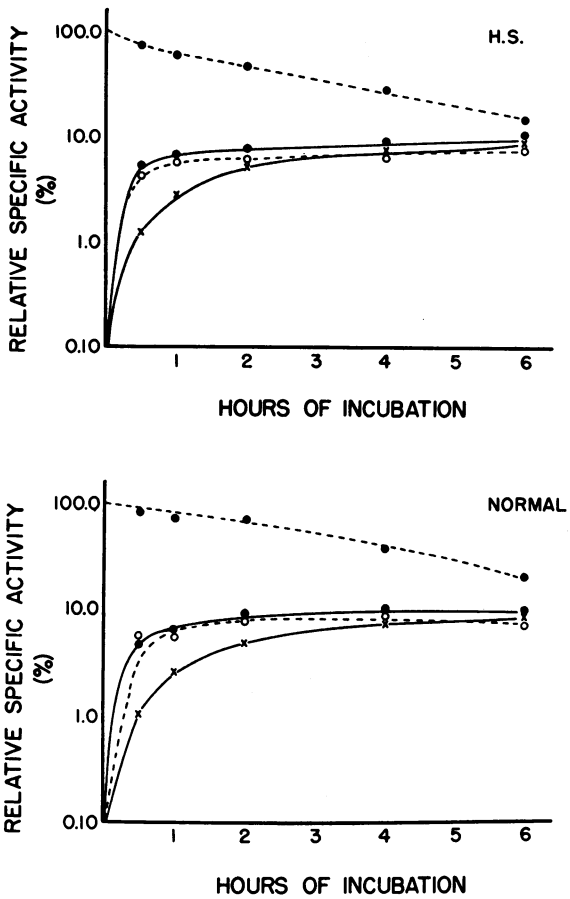

FIG. 2. RELATIVE SPECIFIC ACTIVITY (RSA) OF PLASMA AND RBC PHOSPHATE COMPOUNDS DURING INCUBATION WITH ^{32}P IN A PATIENT WITH HS AND IN A NORMAL SUBJECT. The ordinate has been compressed logarithmically. Solid circles and dashed line = plasma ultrafilterable inorganic phosphate (P_i); solid circles and solid line = cellular ATP; open circles and dashed line = cellular P_i; ×'s and solid line = cellular 2,3-DPG.

tern is the same in the two kinds of RBC, even over 6 hours. The rate of incorporation is rapid over the first hour, but even at 6 hours the specific activities of the cellular compounds are still increasing and are less than the specific activity of the ultimate precursor, extracellular phosphate.

Table I summarizes the results of the intracellular RSA in all of the subjects. It can be seen that 2,3-DPG is labeled more slowly than the other compounds. In both kinds of RBC, the RSA of ATP exceeds that of P_i at every point.

Figure 3 compares the RSA of ATP of normal and HS RBC. The means ± 2 SE coincide so closely that it is clear there is no difference between

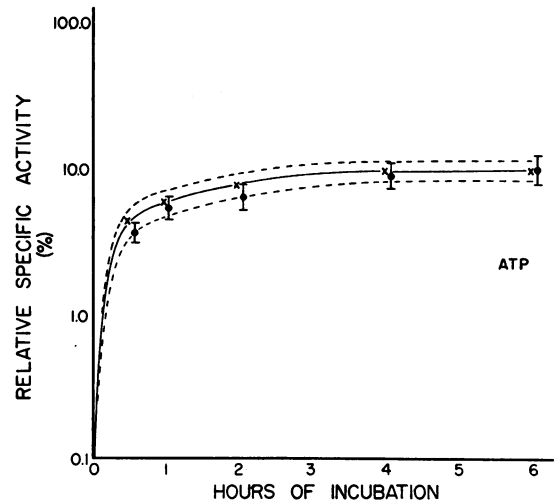

FIG. 3. COMPARISON OF THE RSA OF ERYTHROCYTE ATP IN FIVE NORMAL SUBJECTS AND FIVE PATIENTS WITH HS DURING INCUBATION WITH ^{32}P. The ordinate has been compressed logarithmically. Solid line with ×'s = mean ± 2 SE for normal subjects; bracketed solid circles = mean ± 2 SE for HS patients.

the two groups of RBC in this respect. Since the RSA of the extracellular diffusible phosphate is consistently higher than that of ATP, a significantly greater net flux of plasma phosphate into HS ATP (reflecting a greater turnover rate) would have been manifested by a significant increase in the RSA of this compound, at least at

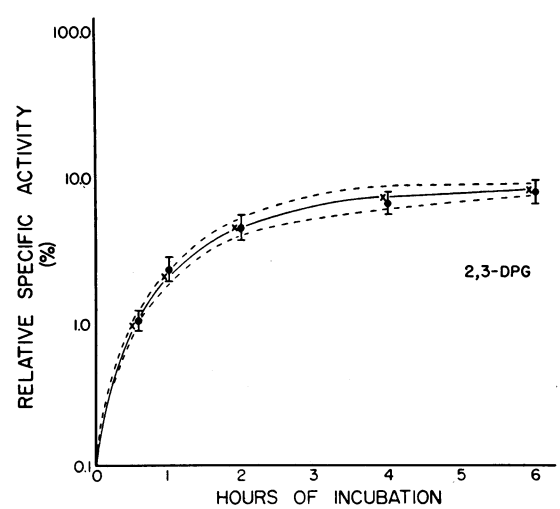

FIG. 4. COMPARISON OF THE RSA OF ERYTHROCYTE 2,3-DPG IN FIVE NORMAL SUBJECTS AND FIVE PATIENTS WITH HS DURING INCUBATION WITH ^{32}P. The ordinate has been compressed logarithmically. Symbols are as in Figure 3.

TABLE II

*Erythrocyte ATP, blood pH, and MCV in normal and HS RBC incubated for 6 hours with glucose**

Sample	ATP		pH		MCV	
	Normal (n = 7)	HS (n = 4)	Normal (n = 7)	HS (n = 5)	Normal (n = 7)	HS (n = 4)
	μmoles/ml RBC				*μ³/cell*	
"Pre"	1.43 (1.32–1.56)†	1.44 (1.27–1.52)	7.66 (7.52–7.85)	7.60 (7.52–7.85)	100 (94–104)	95 (89–101)
6-Hour	1.25 (1.03–1.37)	1.24 (1.11–1.38)	7.61 (7.40–7.71)	7.45 (7.40–7.70)	98 (94–102)	93 (85– 99)

* Abbreviations: MCV = mean corpuscular volume; RBC = erythrocytes.
† Mean values, range in parentheses.

some point. This assumes that the size of the ATP pool is the same in both kinds of RBC. As will be discussed below, this has been found to be the case in this and other studies (7–12).

Figure 4 shows the results of a similar comparison of the RSA of 2,3-DPG in the two kinds of cells. Again, no difference exists between the two kinds of erythrocytes.

Table II shows the values obtained for erythrocyte ATP concentration, pH, and mean corpuscular volume (MCV) under the circumstances of this study. The size of the ATP pool is the same in both types of erythrocytes. There was a modest decrease in the ATP content (about 13%) at the end of the 6-hour period in both groups.

The means ± 1 SE for glucose utilization (micromoles per milliliter RBC per hour), studied over 4 hours, were 2.24 ± 0.04, n = 7, in normal cells and 2.57 ± 0.12, n = 4, in HS erythrocytes. The difference between the two groups is significant ($p < 0.05$) but relatively small; the mean value in the normal RBC is 87% of the rate in HS erythrocytes. If glucose utilization is calculated on a per cell basis, which takes into account the MCV of 100 in normal cells and 95 in HS RBC (Table II), the difference between the two groups is smaller; the normal value becomes 92% of the HS rate and $0.1 < p < 0.05$. The residual glucose concentration ranged from 5 to 8 μmoles per ml blood.

Discussion

The following four primary technics are presently used to evaluate over-all erythrocyte glucose metabolism through the Embden-Meyerhof pathway in normal and pathological RBC: *1*) estimation of the rate of ATP phosphorus turnover with the use of ^{32}P (as in the present study); *2*) measurement of ATP content in fresh erythrocytes and the stability of ATP content in incubated RBC; *3*) *in vitro* measurement of the rate of glucose utilization and lactic acid production; *4*) determination of erythrocyte enzymatic activities for the various reactions of glycolysis, and the detection of abnormally high concentrations of glycolytic intermediates that reult from enzymatic blocks in the glycolytic sequence. In the findings presented here, the first three of the above methods have been used.

Estimation of the turnover rate of ATP phosphorus. According to the over-all glycolytic equation, inorganic phosphate is introduced into ATP in the following combined steps:[4]

$$\text{glyceraldehyde 3-phosphate} + P_i + \text{ADP} + \text{NAD} \rightarrow \text{3-phosphoglyceric acid} + \text{NADH} + \text{ATP}. \quad [2]$$

In the present study, the specific activity of ATP exceeded that of P_i at every point, although the difference was not large. Bartlett (22), Gerlach, Fleckenstein, and Gross (23), and Tatibana, Miyamoto, Odaka, and Nakao (24) have shown previously that this is the case from time zero to 30

[4] The phosphorylation of ADP in the step 2-phosphoenolypyruvate + ADP → pyruvic acid + ATP involves a phosphate radical introduced originally from ATP itself, either in the hexokinase reaction or in the phosphorylation of fructose 6-phosphate to fructose 1,6-diphosphate. The phosphorolysis of inosine by inorganic phosphate to produce hypoxanthine and ribose 1-phosphate and the subsequent metabolism of the latter compound may, in theory, provide another mode of entry for inorganic phosphate into the adenylate pool; this pathway may be disregarded as quantitatively insignificant unless nucleosides are specifically provided as substrates (21).

minutes in labeling experiments of the sort described here. Since the specific activity of a product cannot continually exceed that of its precursor, these authors have indicated that intracellular P_i cannot be the P_i in reaction 2.

Bartlett (22) has suggested that reaction 2 occurs as an intramembrane event and that P_i is extracellular phosphate itself. This would account for the fact that the specific activity of the beta and gamma phosphates of ATP exceeds that of cellular P_i, since the specific activity of the extracellular phosphate remains considerably higher than that of the cellular compounds for some time (Figure 2). Support for this view has been provided by Schauer and Hillmann (25), who have isolated a phosphate-acceptor complex from the membranes of erythrocytes that, in labeling experiments, achieved a specific activity which was much higher than that of ATP and which approximated that of extracellular phosphate. Additional evidence is given in the work of Schrier (26, 27), who found substantial activities of glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase (the enzymes mediating reaction 2) in hemoglobin-free erythrocyte membranes. The point, however, is not entirely settled, and a direct calculation of the turnover rate of ATP phosphorus from the present results is not, therefore, completely warranted. In any case, under steady state conditions of the glycolytic chemical reactions, the net flux of extracellular phosphate into ATP will be closely related to the actual turnover of this phosphorus in both types of erythrocytes. Any increase or decrease in the turnover of ATP phosphate would, thus, have been reflected by a corresponding change in the RSA of this compound over the time course used in the present study. The data given in Table I and Figure 3 show that the RSA of HS ATP is virtually identical to that found in the normal cells. These results strongly suggest, therefore, that the turnover rate of ATP phosphorus in HS erythrocytes is neither increased nor decreased.

It should be mentioned, however, that the turnover of ATP phosphorus may not coincide exactly with the rate of net synthesis of this compound. DeVerdier (28), for example, has suggested that in erythrocyte hemolyzates reaction 2 may also mediate the direct exchange of P_i and ATP phosphorus. (There are, however, several serious obstacles to accepting this author's conclusion; one of these is his failure to provide direct evidence that ADP was in fact absent from the test systems.) There is no reason to suppose that such an exchange, if it occurred, would differ substantially in normal and HS erythrocytes. Nonetheless, modest differences in the glycolytic turnover of ATP might be concealed by such a process. For this reason and because the number of patients studied was not large, the present results would not exclude the possibility that the net turnover of ATP differed by about 10% or less in the two kinds of erythrocytes.

Level and stability of ATP. In the present study, a normal level of ATP was found in the fresh erythrocytes of the four patients with HS studied; such normal levels have also been found by all other workers (7–12). After 6 hours of incubation with adequate glucose, there was a small and equal decrease of ATP in both types of cells. In the presence of sufficient substrate, normal ATP stability in HS erythrocytes has also been found in other studies (10–12, 29). Mohler (11), however, has indicated that, in the absence of glucose, ATP is depleted more rapidly in HS RBC than in normal cells and that the depletion is minimized in the presence of ouabain. He suggested that this finding reflects a greater rate of ATP turnover and catabolism in HS RBC, which results from increased active cation transport. The latter effect has been attributed to a greater permeability to Na ions, which has been postulated to exist in HS erythrocytes (1, 30, 31). The present results do not support the view that the turnover of ATP is increased in HS RBC and, as indicated by Mohler, some reservations about his interpretation are warranted because the rate of ATP depletion in his studies was greater in HS RBC only during the first hour of a 3-hour period.

Glucose utilization. A consistent, but small, difference was found in the rate of glucose utilization between the two types of erythrocytes in this study. The difference between the means of the two groups was less than 10% when glucose utilization per cell was calculated. A slight elevation of the reticulocyte count (2 to 3%) was also present in the patients. The small difference found in glucose utilization may, therefore, reflect in part the slightly younger mean age of the red blood cells in the patients with HS.

Dunn and his associates (8) found a normal rate of erythrocyte glucose utilization and lactic acid production in six splenectomized patients with HS who had normal reticulocyte counts. Selwyn and Dacie (4) have reported that the rate of glucose utilization and lactic acid production is normal in the nonreticulocytes of patients with HS whether or not splenectomy has been performed.

Jacob and Jandl (1), on the other hand, have suggested that glucose utilization in HS RBC from splenectomized patients may be increased over the normal rate by as much as 35%. The high initial glucose concentration used in these studies (0.022 M glucose added) would work to make the fractional change in the ambient glucose concentration quite small over 4 hours and, therefore, make a precise estimation of the rate of glucose utilization somewhat difficult. In addition, the majority of these studies were carried out in the presence of 40 mM phosphate buffer. These differences in experimental conditions may account in part for the quantitative discrepancy between the findings of Jacob and Jandl and the present results.

Mohler (11) has also suggested that the rate of glucose utilization is significantly increased in HS RBC from patients with normal reticulocyte counts. However, in this work, the experimental circumstances were such that the rate of erythrocyte glucose utilization was considerably lower than that found in the present and other studies (8, 15, 32, 33). It is, therefore, difficult to compare Mohler's findings with our own.

As mentioned previously, the actual concentrations of glycolytic intermediates have consistently been found to be normal (7–12) in HS RBC. The rates of those individual reactions of the glycolytic sequence that can be measured have also been found to be normal in these erythrocytes (8, 13).

The present results suggest that the turnover rate of ATP in HS RBC is identical to that in normal cells, and they exclude the possibility that such turnover differs by much more than 10% from the normal rate. The results are, thus, in agreement with the majority of the previous studies cited above, which have been carried out to assess the level of energy metabolism in HS RBC.

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