Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 967–971, February 1992
Medical Sciences

Preservation of metabolic activity in lyophilized human erythrocytes

(lyophilization/pentose phosphate shunt/methemoglobin/glycolytic enzymes)

RAYMOND P. GOODRICH*†, SAMUEL O. SOWEMIMO-COKER*, CHARLES R. ZEREZ‡, AND KOUICHI R. TANAKA‡

*Department of Basic Research, Cryopharm Corporation, Pasadena, CA 91107; and ‡Department of Medicine, Harbor-University of California Los Angeles Medical Center, University of California School of Medicine, Torrance, CA 90509

Communicated by John D. Baldeschweiler, October 10, 1991

ABSTRACT **Normal human erythrocytes (RBC) were freeze-dried under conditions that caused minimal modification in normal RBC metabolic activities. Because of the known effects of long-term storage on metabolic activities, we studied the effects of our lyophilization process on RBC metabolism. Of all the metabolic enzymes studied, only triosephosphate isomerase (D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1), enolase (2-phospho-D-glyceratehydro-lyase, EC 4.2.1.11), and pyruvate kinase (ATP:pyruvate O^2-phosphotransferase, EC 2.7.1.40) were decreased when compared with fresh control nonlyophilized RBC. The activities of these enzymes did not differ significantly from those of blood bank RBC. Concentrations of high-energy intermediates, ATP, and 2,3-diphosphoglycerate, along with lactate and ATP production were decreased in lyophilized RBC. No enzymes of the pentose phosphate shunt were altered during lyophilization. In addition, our data show that lyophilized RBC possess an intact capacity to (*i*) synthesize adenine nucleotides and (*ii*) reduce MetHb to Hb and, thus, maintain the Hb in a functional physiologic state similar to fresh nonlyophilized RBC. The present study demonstrates the possibility of lyophilizing RBC in a manner that maintains normal metabolic and enzymatic function upon rehydration.**

Maintenance of the metabolic functions of human erythrocytes (RBC) during long-term storage is crucial to their *in vivo* survival and physiologic functions. Several investigators have addressed this problem and have found that certain storage conditions can preserve the metabolic functions of RBC (1–4). Some of these storage conditions involve refrigeration in liquid media (1, 2), which allows for a 35- to 42-day shelf life, or frozen storage at −80°C, which permits long-term storage (up to 10 yr) but requires maintenance of low temperature (3, 4). The ability to freeze-dry RBC would eliminate this need for low storage temperatures. A successful process would require that damage to the cells normally induced by drying be avoided. Cells treated in this manner must retain normal cytoskeletal, metabolic, and oxygen-transporting functions. In this paper we examined the effects of freeze-drying on one of these characteristics—namely, metabolic function. Several previous studies have shown promise in this regard by demonstrating that certain protectants are effective in preserving the enzyme activities and functions of dry biological specimens (5). Until this time, however, no comparable demonstration has been provided due to limitations of available methods for protecting cells from damage during drying.

Normal adult human RBC generate energy almost exclusively through the metabolism of glucose primarily via the Embden–Meyerhof pathway and the pentose phosphate shunt (PPS) (Fig. 1). These pathways produce the cellular energy crucial to RBC survival and maintenance of proper cell functions (6, 7). In particular, two products of glycolysis, ATP and 2,3-diphosphoglycerate (2,3-DPG) act to regulate the oxygen affinity of Hb. The above glycolytic pathway requires proper functioning of the various glycolytic enzymes for the formation of the intermediates essential for the RBC to transport oxygen and to maintain those physical characteristics required for its *in vivo* survival in circulation. Abnormal metabolic activities have been seen in RBC stored for extended periods in either conventional liquid media or frozen in glycerol (8–10) and have been implicated in the mechanisms responsible for the rapid removal of transfused RBC.

Because maintenance of proper metabolic functions of RBC is so important to carrying out RBC physiologic functions, this study was designed to evaluate preservation of the key RBC glycolytic enzyme activities after lyophilization and rehydration of RBC. Although maintenance of such features does not guarantee *in vivo* survival, salient data from our studies show that metabolic functions of rehydrated lyophilized RBC stored at 22°C are comparable to that of blood bank RBC stored for equivalent periods in a hydrated state at 4°C.

MATERIALS AND METHODS

After informed consent was obtained, blood was drawn from six healthy adult individuals having no history of either hemoglobinopathy or abnormal RBC metabolism. Blood was withdrawn from each donor into plastic transfer bags (Fenwal Laboratories, Deerfield, IL) containing 63 ml of citrate phosphate dextrose-adenine anticoagulant by using conventional blood banking techniques. The blood units (500 ml each) were centrifuged at 1800 × *g* for 5 min at room temperature (22°C) to remove the buffy coat and plasma. The packed RBC were washed in isotonic dextrose saline, according to standard washing procedures (11), with an automated cell washer (model 2991, Cobe, Lakewood, CO). The cells were then lyophilized, according to procedures that have been described in detail (12). Samples prepared in this manner consistently exhibit moisture contents of 1–3%, as measured by the Karl Fischer method (13). At day 10, the dried RBC were rehydrated and reconstituted in phosphate-buffered rehydration buffers (360 mosmol, pH 7.4, Cryopharm, Pasadena, CA) at 22°C. Note, however, that with our current procedures 7 days is the time required to reduce the residual moisture content of the sample to a level (1–2%) that allows storage at ambient temperatures. Briefly, to rehydrate the RBC, 600 g of rehydration buffer was added to the dried RBC and then agitated on a wrist action shaker (Burrell, Pittsburgh) until the RBC were fully rehydrated (usually for 15 min). At the end of the rehydration step, the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TPI, triose phosphate isomerase; PK, pyruvate kinase; 2,3-DPG, 2,3-diphosphoglycerate; PPS, pentose phosphate shunt; RBC, erythrocyte(s).

†To whom reprint requests should be addressed.

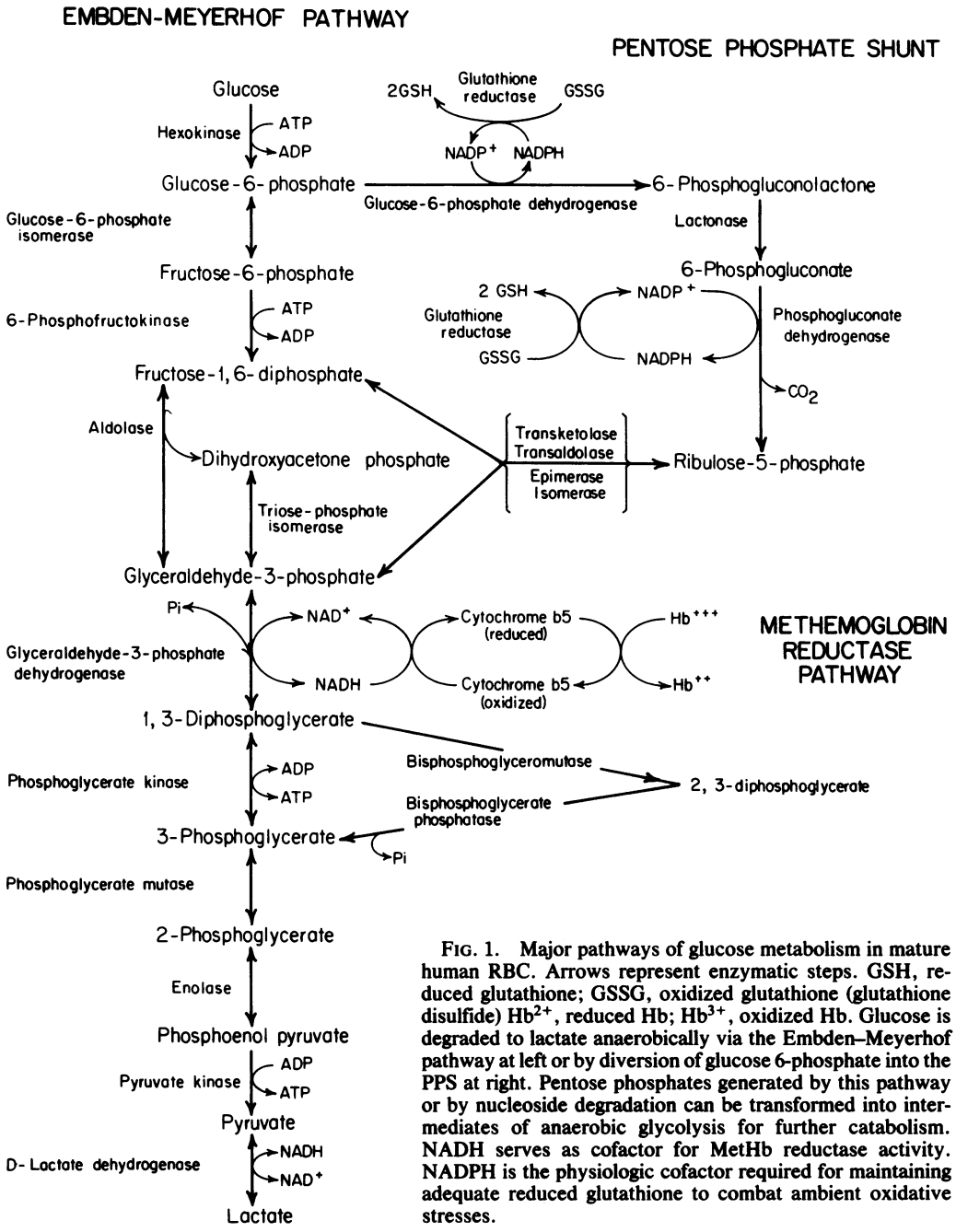

FIG. 1. Major pathways of glucose metabolism in mature human RBC. Arrows represent enzymatic steps. GSH, reduced glutathione; GSSG, oxidized glutathione (glutathione disulfide) Hb^{2+}, reduced Hb; Hb^{3+}, oxidized Hb. Glucose is degraded to lactate anaerobically via the Embden–Meyerhof pathway at left or by diversion of glucose 6-phosphate into the PPS at right. Pentose phosphates generated by this pathway or by nucleoside degradation can be transformed into intermediates of anaerobic glycolysis for further catabolism. NADH serves as cofactor for MetHb reductase activity. NADPH is the physiologic cofactor required for maintaining adequate reduced glutathione to combat ambient oxidative stresses.

RBC suspension was centrifuged at 1800 × *g* for 10 min at 22°C. The supernatant was removed, and the packed RBC were washed twice in special isotonic wash buffers (12) by centrifugation at 1800 × *g*, using a Cobe automatic cell washer. Reconstituted RBC were assayed for glycolytic enzyme activities and intermediates, according to described methods (14–19). A control blood sample was obtained from the autologous donor at the time of reconstitution of lyophilized RBC. Control RBC were treated similarly to reconstituted lyophilized RBC with respect to washing.

Rate of Adenine Nucleotide Synthesis. The rate of adenine nucleotide synthesis was measured by following the incorporation of [^{14}C]adenine into the adenine nucleotide pool in intact RBC with the method of Zerez *et al.* (20). Because all RBC samples had endogenous glucose and exhibited low rates of [^{14}C]adenine incorporation without exogenous glucose, a control was included in which incorporation was measured with 1.0 mM iodoacetate, an inhibitor of glycolysis. This step allowed the assessment of "background" [^{14}C]adenine incorporation (i.e., under conditions of total inhibition of glycolysis) by subtracting [^{14}C]adenine incorporation in the presence of iodoacetate from incorporation in its absence. Thus, reported rates of [^{14}C]adenine incorporation were corrected for this background incorporation.

Rate of MetHb Reduction. The rate of MetHb reduction in intact RBC was determined by using a described method (21).

Other Methods. Rates of ATP and lactate production were determined by the methods described by Beutler (22).

Statistical Analysis. Differences between lyophilized and nonlyophilized RBC were analyzed with a two-tailed Student's *t* test for paired data. Comparisons between lyophilized and blood bank-stored RBC were made by using the two-tailed Student's *t* test for independent data.

RESULTS

Activities of Glycolytic Enzymes. Results of the measurements of glycolytic enzyme activities for hemolysates from rehydrated lyophilized RBC and nonlyophilized fresh RBC

Table 1. Summary of activities of the glycolytic enzymes in hemolysates from rehydrated lyophilized and nonlyophilized RBC

Enzyme	Enzyme activity, μmol/min·g of Hb				
	Lyo	N-lyo	B-Bank	N-R	*P*
HX	1.26 ± 0.2	1.65 ± 0.1	1.20 ± 0.1	0.98–1.3	NS
PGI	44.7 ± 4.6	44.3 ± 2.7	48.3 ± 6.0	43.7–65.8	NS
PFK	12.1 ± 1.6	11.7 ± 1.0	9.73 ± 2.2	8.44–12.2	NS
Ald	3.59 ± 0.4	3.72 ± 0.5	2.39 ± 0.3	1.97–3.59	NS
TPI	1750 ± 460	2140 ± 490	2900 ± 777	2130–3340	<0.005
G3PD	318 ± 68	311 ± 43	244 ± 72	238–346	NS
DPGM	5.34 ± 0.7	4.64 ± 0.9	8.43 ± 2.2	3.93–5.9	<0.015
PGK	340 ± 147	340 ± 115	349 ± 47.7	212–341	NS
PGM	35.2 ± 5.1	38.1 ± 6.0	17.3 ± 6.7	13.9–38.0	NS
Eno	4.99 ± 1.0	7.60 ± 0.9	4.96 ± 0.9	4.2–6.6	<0.001
PK	18.9 ± 5.7	21.1 ± 5.4	15.0 ± 2.1	12.5–17.2	<0.032
LDH	231 ± 29	190 ± 19	141 ± 56	145–203	<0.001
G6PD*	12.4 ± 1.6	14.7 ± 1.8	ND	9.90–13.2	NS
6PGD*	11.1 ± 1.0	10.0 ± 1.1	ND	7.27–10.0	NS
TA*	0.97 ± 0.2	1.10 ± 0.3	ND	0.78–1.3	NS
TK*	0.68 ± 0.1	0.93 ± 0.7	ND	0.50–1.0	NS

Data represent the mean ± SD for six samples. Data from blood bank-stored RBC are included for comparison with rehydrated lyophilized RBC. Blood bank samples (total number = 3) were stored at 4°C for 10 days before analysis. Lyo, lyophilized, 10 days old; N-lyo, nonlyophilized freshly drawn blood; B-Bank, blood bank; N-R, normal range for freshly drawn blood; ND, not detected; NS, not significant (comparisons were made between lyophilized and nonlyophilized RBC); HX, hexokinase (ATP:D-hexose-6-phosphotransferase, EC 2.7.1.1); PGI, glucose-6-phosphate isomerase (D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9); PFK, 6-phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11); Ald, fructose-bisphosphate aldolase (D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13); G3PD, glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD^+ oxidoreductase (phosphorylating), EC 1.2.1.12); DPGM, bisphosphoglycerate mutase (3-phospho-D-glycerate 1,2-phosphomutase, EC 5.4.2.4); PGK, phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3); PGM, phosphoglycerate mutase (D-phosphoglycerate 2,3-phosphomutase, EC 5.4.2.1); Eno, enolase (2-phospho-D-glyceratehydro-lyase, EC 4.2.1.11); LDH, D-lactate dehydrogenase (lactate:NAD^+ oxidoreductase, EC 1.1.1.28); G6PD, glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase, EC 1.1.1.49); 6PGD, phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NAD(P)^+$ 2-oxidoreductase, EC 1.1.1.43); TA, transaldolase (sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glyceronetransferase, EC 2.2.1.2), TK, transketolase (sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycolaldehydetransferase, EC 2.2.1.1).
*Enzymes of PPS.

from autologous donors are summarized in Table 1. The range of enzyme activities for normal RBC and citrate phosphate dextrose-adenine-stored RBC are compared in Table 1. The activity of hexokinase in hemolysates was the same in rehydrated lyophilized RBC and nonlyophilized control RBC and was also similar to citrate phosphate dextrose-adenine-stored RBC. The activities of both triosephosphate isomerase (TPI; D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) and pyruvate kinase (PK; ATP: pyruvate O^2-phosphotransferase, EC 2.7.1.40, EC 2.7.1.40) were reduced in hemolysates from lyophilized RBC but were not different from blood bank-stored RBC (Table 1). Hemolysates from lyophilized RBC had TPI activity of 1750 μmol/min·g of Hb, which is significantly lower than that of control nonlyophilized RBC, 2140 μmol/min·g of Hb, $P < 0.005$. The activity of TPI in blood bank RBC is also higher than that of lyophilized RBC. However, TPI is not a rate-limiting enzyme because it is present in excess in RBC. The activity of PK in hemolysates of rehydrated lyophilized RBC was 18.9 μmol/min·g of Hb compared with 21.1 μmol/min·g of Hb in nonlyophilized control RBC, $P < 0.03$. The activity of PK in blood bank RBC was 15.0 ± 2.14 μmol/min·g of Hb, and this value is lower than that of lyophilized RBC. Because PK is a rate-limiting enzyme of glycolysis, the above result implies that enzyme function in lyophilized RBC is equal to or better than enzyme function in blood bank RBC. In contrast, the activities of D-lactate hydrogenase and bisphosphoglycerate mutase are significantly higher in lyophilized RBC than in fresh control nonlyophilized RBC, $P < 0.001$ and $P < 0.01$, respectively (Table 1). The activities of the remaining Embden–Meyerhof pathway enzymes other than the above are similar in lyophilized, nonlyophilized, and blood bank RBC (Table 1).

Levels of Glycolytic Intermediates. The levels of glycolytic intermediates in intact rehydrated lyophilized and control nonlyophilized RBC are compared and summarized in Table 2. Significantly higher concentrations of dihydroxyacetate phosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenol pyruvate, ADP, and AMP were found in intact lyophilized RBC compared with nonlyophilized control (Table 2). On the contrary, the concentrations of ATP and 2,3-DPG are lower than in control RBC. Note that the concentrations of intermediates for normal RBC in Table 2 are much lower than those concentrations for either lyophilized or nonlyophilized RBC. These values were from fresh blood drawn directly into perchloric acid and then processed immediately for glycolytic intermediates. In contrast, lyophilized and nonlyophilized control RBC were processed before the isolation of perchloric acid extracts for intermediates and, therefore, would be expected to differ from normal values.

The rate of lactate production expressed as μmol/g of Hb per hr is significantly reduced in lyophilized RBC, 6.60 ± 3.58 compared with 10.6 ± 2.86 in nonlyophilized control RBC, $P < 0.001$. Similarly, the rate of production of ATP by lyophilized RBC (0.368 ± 0.173 μmol/g of Hb per hr) is also significantly lower than control RBC (0.779 ± 0.305), $P < 0.01$. The total number of samples evaluated for lactate and ATP productions was six for both lyophilized and nonlyophilized RBC.

Table 2. Comparison of levels of glycolytic intermediates in rehydrated lyophilized and fresh nonlyophilized RBC

Intermediate	Glycolytic intermediate, nmol/g of Hb			P
	Lyo	N-lyo	NV	
G6P	49.8 ± 72.1	76.5 ± 102	100 ± 28.0	NS
F6P	0.92 ± 2.3	3.05 ± 7.5	15.6 ± 6.3	NS
FDP	760 ± 425	149 ± 179	4.70 ± 1.60	NS
DHAP	1770 ± 687	174 ± 147	37.5 ± 3.10	<0.012
GAP	112 ± 46.8	44.9 ± 43.5	9.38 ± 6.30	NS
2,3-DPG	3152 ± 938	9633 ± 2640	13,500 ± 2000	<0.004
3PG	611 ± 210	134 ± 56.1	122 ± 28.0	<0.006
2PG	338 ± 252	216 ± 165	31.3 ± 13.0	<0.046
PEP	216 ± 104	67.5 ± 50.8	50.0 ± 16.0	<0.01
Pyr	170 ± 52.2	193 ± 125	84.4 ± 25.0	NS
Lact	6032 ± 2730	9495 ± 3542	1140 ± 370	NS
ATP	1758 ± 392	3875 ± 780	3220 ± 280	<0.008
ADP	1743 ± 316	700 ± 133	409 ± 56.0	<0.003
AMP	2370 ± 343	204 ± 125	134 ± 25.0	<0.001

Data represent mean ± SD for six samples. Normal values are included for comparison with present data. Lyo, lyophilized, 10 days old; N-lyo, nonlyophilized freshly drawn blood; NV, normal values for freshly drawn blood; P, probability for comparisons between lyophilized and nonlyophilized RBC; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FDP, fructose 1,6-disphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenol pyruvate; Pyr, pyruvate; Lact, lactate.

MetHb Reduction and Level of Reduced Glutathione. The semilogarithmic plots of the residual MetHb and the incubation time are shown in Fig. 2. MetHb reduction in rehydrated lyophilized and in nonlyophilized RBC followed similar exponential decay (Fig. 2). The rates of MetHb reduction, expressed as the half-life for all samples tested, are shown in Fig. 3. The mean rate of MetHb reduction in lyophilized RBC was 17.9 ± 4.30 hr and was not significantly different from control nonlyophilized RBC, 17.4 ± 4.80 hr, $P > 0.05$ (Fig. 3). The concentration of reduced glutathione in lyophilized RBC was 689 ± 136 μg/g of Hb, which is significantly lower than that of nonlyophilized fresh RBC, 2150 ± 406 μg/g of Hb, $P < 0.001$. Although the concentration of reduced glutathione was lower in lyophilized RBC, preliminary data indicated that the activities of glutathione reductase [NAD(P)H] [NAD(P)H:oxidized-glutathione oxidoreductase, EC 1.6.4.2; lyophilized RBC = 6.86 and nonlyophilized RBC = 6.44 μmol/min·g of Hb] and glutathione peroxidase (glutathione:hydrogen-peroxide oxidoreductase, EC 1.11.1.9; lyophilized RBC = 19.9 and nonlyophilized RBC = 21.6 μmol/min·g of Hb) were not different from control RBC.

Rate of [^{14}C]Adenine Incorporation. The capacity of lyophilized RBC to synthesize adenine nucleotides (i.e., AMP, ADP, and ATP) was measured by following the incorporation of [^{14}C]adenine into adenine nucleotides. Nonlyophilized RBC had a rate of [^{14}C]adenine incorporation of 2.81 ± 0.40 nmol/min·ml of RBC (mean ± 1 SD, $n = 5$). In contrast, lyophilized RBC had a rate of incorporation of 0.407 ± 0.122 nmol/min·ml of RBC (mean ± 1 SD, $n = 5$), which decreased significantly ($P < 0.00005$) from nonlyophilized RBC. Although lyophilized RBC had a lower rate of [^{14}C]-adenine nucleotide incorporation, the ability of these cells to incorporate adenine into nucleotides is noteworthy because only freshly obtained RBC have this function.

DISCUSSION

A major problem in blood preservation concerns the metabolic and functional lesions occurring during long-term storage of RBC in conventional liquid media. Storage lesions ultimately result in shortened RBC survival after transfusion (23). For satisfactory preservation of RBC, it is, therefore, important that the metabolic functions of the cells are well preserved. Frozen storage of RBC reduces this problem but

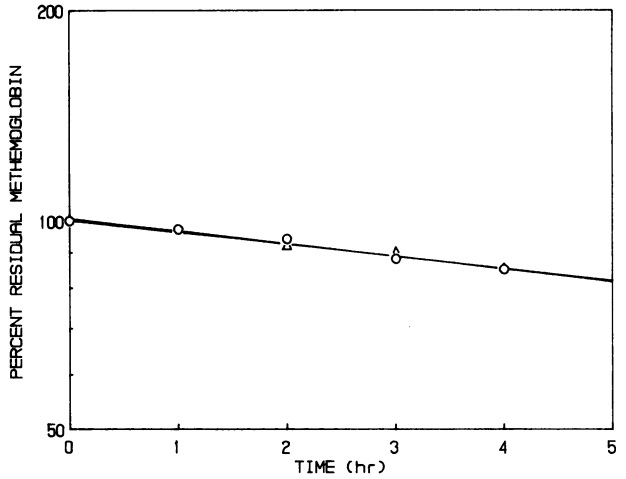

FIG. 2. Linear regression fit for semilogarithmic plots of percent residual MetHb vs. time of incubation for intact RBC from lyophilized (△) and nonlyophilized RBC (○). Data are means of six samples.

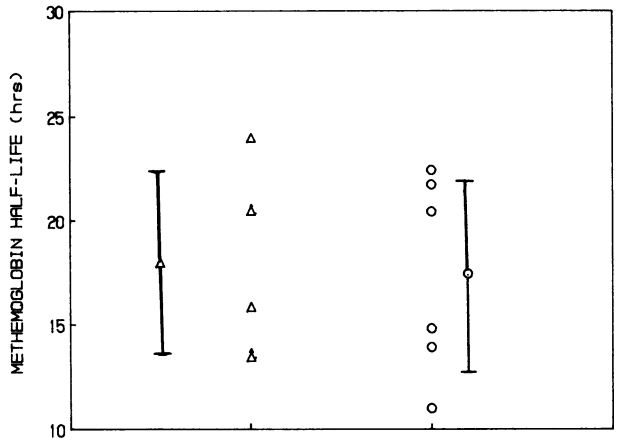

FIG. 3. Rate of MetHb reduction (expressed as half-life) in intact lyophilized (△) and nonlyophilized RBC (○). Mean ± SD MetHb half-life for both cell types are also shown.

requires the maintenance of low temperatures during storage and transport. Storage in the dry state requires that procedures be developed that protect the cells against dehydration-induced damage to enzymes, proteins, and membrane structure. The advantage of lyophilized RBC rests in the ability to store them for long periods under storage conditions that are far easier (i.e., room temperature, 22°C), making transport to sites of immediate need more feasible.

The relationship between glycolytic enzyme activities and freeze-dry processing of RBC has not been previously studied due to inabilities in producing intact and functional cells after drying. We have demonstrated that the activities of most enzymes involved in RBC metabolism are very well preserved in rehydrated lyophilized RBC prepared according to our procedures (12). Our data showed that of all enzymes studied, only TPI, enolase, and PK significantly decrease in lyophilized RBC when compared with fresh control nonlyophilized RBC. However, the activities of these enzymes do not significantly differ from those enzyme activities found in blood bank-stored RBC. TPI activity is the highest of any of the glycolytic enzymes by one or two orders of magnitude and may not be important in controlling glycolysis. That all major rate-limiting enzyme activities do not differ from these activities in fresh samples strongly suggests that the glycolytic pathway is functional in lyophilized RBC. It is interesting to also note that the activities of 6-phosphofructokinase and glucose-6-phosphate dehydrogenase have been reduced in RBC stored in conventional liquid media for an extended period (24). The impact of such changes in activities of 6-phosphofructokinase and glucose-6-phosphate dehydrogenase on the glycolytic pathway and posttransfusion survival remains to be determined. The activities of these enzymes are within the normal range in lyophilized RBC. In addition, none of the PPS enzymes were decreased by the lyophilization process. Because the major function of PPS is to produce NADPH and reduced glutathione, which together provide the main line of defense for RBC against oxidative injury, lyophilized RBC with intact PPS are not as likely to be susceptible to oxidant damage.

The MetHb reduction pathway is another important component of RBC metabolism. Decreased activity of this pathway may lead to accumulation of MetHb and loss of oxygen transport capabilities of RBC, in as much as the metheme portion cannot combine with oxygen. Our results show that lyophilized cells have intact capacity to reduce MetHb to functional Hb. The maintenance of both MetHb reduction and intact PPS suggests that lyophilized cells are not as likely to undergo Hb oxidation. Decreased antioxidant defenses in citrate phosphate dextrose-adenine-stored blood has been implicated as the mechanism responsible for the rapid removal of transfused RBC (26). That these defenses are still maintained in rehydrated lyophilized RBC further shows the efficiency of lyophilization technology in preserving the vital functions of RBC. Note also that PPS is important in the production of 5-phosphoribosylpyrophosphate, which is used by the RBC for synthesizing adenine nucleotides. These data also show that lyophilized RBC with their intact PPS can use an exogenous adenine source in the synthesis of adenine nucleotides.

Although concentrations of certain key glycolytic intermediates (ATP; 2,3-DPG) decrease in lyophilized cells, sufficient quantities of these intermediates remain for viability and continuation of the various ATP- and 2,3-DPG-dependent functions as before lyophilization. In any event, the concentrations of ATP and 2,3-DPG in lyophilized cells resemble those of RBC in blood banking during their current storage life.

These data show that a freeze-drying process can be done so that the RBC maintain their metabolic functions upon rehydration. The results indicate a successful step in the direction of a lyophilized RBC product for transfusion medicine.

We thank Ms. Susanne M. Savely and Ms. Nemone Schulz for expert technical assistance. We also acknowledge the help of Mrs. Sharon Crandall, Mr. Jon Olson, and the various blood donors for this study. This project was supported, in part, by a Basic Research Grant N00014-90-C-0053, from the United States Naval Medical Research and Development Command.

1. Strumia, M. M., Eusebi, A. J. & Strumia, P. V. (1967) *J. Lab. Clin. Med.* **71,** 138–147.
2. Beutler, E. (1974) in *The Human Red Cell in Vitro*, eds. Greenwalt, T. J. & Graham, A. J. (Grune & Stratton, New York), pp. 189–216.
3. Derrick, J. B., Lind, M. & Rowe, A. W. (1969) *Transfusion* **9,** 317–323.
4. Valeri, C. R. (1974) in *The Red Blood Cell*, ed. Surgenor, D. M. (Academic, New York), Vol. 1, pp. 511–574.
5. Crowe, J. H., Crowe, L. M. & Jackson, S. A. (1983) *Arch. Biochem. Biophys.* **220,** 477–484.
6. Brewer, G. J. (1974) in *The Red Blood Cell*, ed. Surgenor, D. M. (Academic, New York), Vol. 1, pp. 473–508.
7. Harmening-Pittiglio, D., ed. (1989) in *Modern Blood Banking and Transfusion Practices* (Davis, Philadelphia), 2nd Ed., pp. 1–23.
8. Dern, R. J., Brewer, G. J. & Wiorkowski, J. J. (1967) *J. Lab. Clin. Med.* **69,** 968–978.
9. Nakao, K., Wada, T., Kamiyama, T., Nakao, M. & Nagano, K. (1962) *Nature (London)* **194,** 877–878.
10. Jones, N. C. H., Mollison, P. L. & Robinson, M. A. (1957) *Proc. R. Soc. London Ser. B* **147,** 476–497.
11. Cobe (1985) in *Blood Cell Processor* (Cobe Lab., Lakewood, CO), pp. 1–3.
12. Goodrich, R. P. (1989) U.S. Patent 4,874,690.
13. May, J. C., Grim, E., Wheeler, R. M. & West, J. (1982) *J. Biol. Stand.* **10,** 249–259.
14. Beutler, E. (1984) in *Red Cell Metabolism: A Manual of Biochemical Methods* (Grune & Stratton, Orlando, FL), 3rd Ed., pp. 38–126.
15. Minakami, S., Suzuki, C., Saito, T. & Yoshikawa, H. (1965) *J. Biochem. (Tokyo)* **58,** 543–550.
16. Lowry, O. H., Passonneau, J. V., Hasselberger, F. X. & Schulz, D. W. (1964) *J. Biol. Chem.* **239,** 18–30.
17. Tanaka, K. R. (1969) in *Biochemical Methods in Red Cell Genetics*, ed. Yunis, J. J. (Academic, San Diego), pp. 167–181.
18. Matschinsky, F. M., Kaufman, F. C. & Ellerman, J. E. (1968) *Diabetes* **17,** 475–480.
19. Koutras, G. A., Hattori, M., Schneider, A. S., Ebaugh, F. & Valentine, W. N. (1964) *J. Clin. Invest.* **43,** 323–331.
20. Zerez, C. R., Lachant, N. A. & Tanaka, K. R. (1989) *J. Lab. Clin. Med.* **114,** 43–50.
21. Zerez, C. R., Lachant, N. A. & Tanaka, K. R. (1990) *Blood* **76,** 1008–1014.
22. Beutler, E. (1984) in *Red Cell Metabolism: A Manual of Biochemical Methods* (Grune & Stratton, Orlando, FL), 3rd Ed., pp. 122–124, 144–145.
23. Wolfe, L. C. (1985) *Transfusion* **25,** 185–203.
24. Noble, N. A., Tanaka, K. R. & Johnson, D. E. (1982) *Am. J. Hematol.* **13,** 1–8.