The mammalian erythrocyte is a unique cell, characteristically devoid of a functional tricarboxylic acid cycle (1), and thus presumably dependent on the Embden-Meyerhof pathway exclusively for adenosine triphosphate energy production. Although the erythrocyte additionally retains an active hexosemonophosphate shunt system yielding reduced triphosphopyridine nucleotide, it is not evident that conversion of this energy to ATP occurs in these cells. In view of these considerations, it is reasonable to assume that the survival of the erythrocyte is fundamentally dependent on the functional state of the glycolytic pathway. Further, since the mature erythrocyte retains little synthetic capacity, including an inability to carry out protein (2, 3) and nucleotide (4, 5) biosynthesis de novo, the rate of loss of the single most labile, endowed component of the glycolytic apparatus could effectively limit the life span of these cells.

The present report summarizes experimental evidence that indicates a limiting role which the adenine nucleotides assume, as essential cofactor components, in the glycolysis (and thus perhaps the ultimate survival) of the erythrocyte. Indirect support of the present findings is contained in several recent reports (6-8).

EXPERIMENTAL PROCEDURE

Erythrocytes—Human blood samples drawn in either acid citrate-dextrose (NIH Formula B) or disodium EDTA (1 mg per ml) were employed. Samples drawn in acid citrate-dextrose included outdated blood (obtained through the courtesy of the Peninsula Memorial Blood Bank, Burlingame, California). Erythrocytes were separated by centrifugation and washed three times with 10 volumes of 0.9 % NaCl solution, and the leukocyte-rich top layers were discarded.

Measure of Erythrocyte Glycolysis—The glycolytic capacity of erythrocyte samples was determined under standard, optimal conditions of glucose utilization. A 10 % suspension of cells was incubated at 37° in an isotonic medium containing excess glucose (12 μmole per ml of cells), 0.025 M Na2HPO4, 0.05 M glycylglycine buffer at pH 7.8, and NaCl. n-Glucose disappearance (and occasionally lactate production) was determined hourly, and the average hourly utilization was computed per unit of hemoglobin as a measure of the glycolytic capacity. Glucose was determined (9) by glucose oxidase (Worthington Biochemical Corporation); lactate by lactic dehydrogenase (Boehringer and Sons) with a fluorometric (Turner fluorometer) adaptation of an existing procedure (10); and hemoglobin as acid hematin (11).

Measure of Erythrocyte Purine Nucleotides—Total purine nucleotides as well as the DPN and ATP content of erythrocytes were determined in the following manner. The washed erythrocytes were extracted with 9 volumes of cold 5 % trichloroacetic acid. Following removal of the acid in ether, aliquots were assayed directly for DPN (12) and ATP (13) by means of fluorometric adaptations of published enzymatic procedures, which increased the sensitivity greater than 100-fold. The presence of glucose-6-P in the extract was corrected for in the ATP assays. Total purine nucleotides were determined by quantitative procedures involving isolation of the nucleotide bases. The following two methods were employed.

Procedure A involved preliminary separation of nucleosides and bases from nucleotides when necessary (i.e. only following incubation of erythrocytes in the presence of added nucleosides and bases) by a chromatographic technique (14), with the use of a solvent system in which nucleosides and bases are freely mobile, leaving nucleotides remaining at the origin as a group. The nucleotides were hydrolyzed and the purine bases were isolated by paper chromatography. Over-all less accompanying the isolation procedure was corrected on the basis of adenine-14C recovery following addition of a trace level of the corresponding base-labeled nucleotide to the original cell extract. Loss was conveniently assessed by the fractional recovery of added radioactivity.

Procedure B was employed primarily in the measure of adenine nucleotide formation and involved the use of labeled precursor (adenine-8-14C). The nucleotides were preliminarily separated from residual nucleoside and base, and the nucleotide purine bases were isolated as described in Procedure A. The ratio of the specific activity of precursor adenine to that of nucleotide adenine was taken as a measure of newly synthesized nucleotide. Thus, a relative specific activity of 0.5 was assumed to represent a doubling of the initial adenine nucleotide pool. The initial nucleotide pool was first determined on an aliquot of the cells according to Procedure A.

Hydrolysis leading to liberation of nucleotide purine bases involved heating a 1-ml volume of sample in 0.5 N HCl for 1 hour at 100°. Samples following hydrolysis were flash-evaporated to dryness, redissolved in a small volume of water, and chromatographed. Chromatography was carried out with Whatman No.
3MM paper sheets and an overnight developing period; the solvent was water-saturated butanol to which excess solid NH₄HCO₃ had been added (14). This solvent readily separates adenine, hypoxanthine, and guanine in that order from the mixture and leaves nucleotides, when present, remaining at the origin as a group. The same solvent was employed in the preliminary separation of nucleosides and bases from erythrocyte nucleotides. The resolved purine bases were eluted from paper in 0.1 N HCl, and concentrations were determined by their specific ultraviolet absorption. Radioactivity was determined by spotting 100-μl aliquots of sample on a strip (2.5 X 3.5 cm) of Whatman No. 3MM paper which was placed upright in a counting vial containing 15 ml of scintillation medium (0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl)benzene in toluene) and counted with a Tri-Carb liquid scintillation counter (Packard Instruments).

Purine Compounds—Purine nucleosides and bases employed in the present study were of commercial origin, with the labeled compounds containing 14C in the ring carbon atom 8. The relative purity of each compound was assessed by paper chromatography.

RESULTS

Erythrocyte Glycolysis—Preliminary studies included a systematic examination of the glycolytic characteristics of the intact erythrocyte in general. Glucose (glucose-14C) was found to penetrate readily into the erythrocyte, in accord with the results obtained by Murphy (15). Glucose utilization by the erythrocyte occurred at a rate independent of glucose concentration until a lower limit of about 1 μmole per ml of cells was exceeded (an upper limit of 20 μmoles of glucose per ml of cells was tested).

Erythrocyte suspensions prepared in either fresh normal serum or synthetic salt medium utilized glucose at nearly equivalent rates provided that identical pH (16), comparable ionic strength, and excess inorganic phosphate (17, 18) were maintained in the incubation medium. Information concerning the effect of pH, buffer composition, and phosphate on glucose utilization is summarized in Table I. A lack of specific buffer ion effect, and the magnitude of pH and phosphate effects, are indicated by the results. The effect of phosphate concentration on glucose utilization rate is further illustrated in Fig. 1. Enhanced glucose utilization induced by phosphate did not result in a correspond-

<table>
<thead>
<tr>
<th>Incubation buffer*</th>
<th>Incubation pH†</th>
<th>Glucose utilized μmoles/hr/g Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycylglycine + phosphate</td>
<td>7.2, 7.4, 7.6</td>
<td>3.1, 6.1, 7.0, 7.9, 9.9</td>
</tr>
<tr>
<td>Glycylglycine</td>
<td>7.4, 7.8, 8.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Tris</td>
<td>7.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Phosphate</td>
<td>7.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Tris + phosphate</td>
<td>7.4</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* Incubation mixtures contained 5% erythrocytes, the designated buffers in a final concentration of 0.05 M, and excess glucose (10 μmoles per ml of cells) in approximately isotonic media; NaCl additions were used as necessary.
† pH was maintained within 0.1 unit in all cases.

Fig. 1. Stimulation of glucose utilization by the intact erythrocyte by phosphate. Approximately 10% erythrocyte suspensions were incubated in isotonic media containing excess glucose (12 μmoles per ml of cells), 0.05 M glycylglycine buffer at pH 7.8, and added phosphate at (A) 0 M, (B) 0.005 M, (C) 0.01 M, and (D) 0.025 M.

Reduced Glycolytic Capacity and Relationship to Cofactors—Storage of blood is known to result in progressive loss of erythrocyte viability and a decreased capacity of the cells to utilize glucose. The pattern of glycolytic failure was examined under various incubation conditions, and the results of two such studies are illustrated in Fig. 2. The patterns were found to be generally qualitatively similar, with loss of cellular glycolytic capacity proceeding at greater or lesser rates depending upon the composition of the suspending medium and the temperature employed. Glycolytic failure accompanying the incubations was examined further in relation to the relative maintenance of the essential
ATP was found to reflect a generally corresponding depletion of dium at 30°, is shown in Fig. 3. Incubation under these conditions resulted in a marked reduction of the glycolytic capacity of the cells with concomitant decreases in cellular ATP within the 4-day period in which the cells were examined. The decrease in ATP was found to reflect a generally corresponding depletion of the total cellular adenine nucleotide pool as indicated, rather than its conversion to a lesser phosphorylated state. The DPN content of the cells was found to be only slightly reduced over the incubation period examined. The dependence of erythrocyte glycolysis on adenine nucleotide concentration was strongly suggested on the basis of these initial observations. 

Elevation of Erythrocyte Nucleotide Pools—The suggested role of adenine nucleotides as limiting agents of erythrocyte glycolysis was further examined by experimentally inducing elevation of the existing cellular nucleotide pools. Although purine nucleotide synthesis de novo does not occur (4, 5) in the erythrocyte, evidence for incorporation of the preformed bases of the natural purines and of their nucleoside derivatives (5, 19, 20), as well as nicotinic acid (19, 21), into nucleotides has been obtained. Further, net adenine nucleotide formation in erythrocytes has been reported (6, 22). Since only limited information was available concerning purine nucleotide synthesis leading to net accumulation in the erythrocyte, the question of synthesis was further investigated in this regard.

Incubation of erythrocytes in the presence of 14C-labeled purine precursors resulted in the incorporation and distribution of the label in the cellular purine nucleotides as shown in Table II. Incorporation of label did not occur in adenine nucleotides from sources other than adenine. In separate experiments which are not shown, labeled adenine was found to be equally distributed among AMP, ADP, and ATP (but not DPN), in support of their existence in a single metabolic pool. Incubation of cells with labeled adenosine and inosine resulted in patterns of similar label distribution with incorporation occurring primarily in hypoxanthine ribonucleotide (IMP), detectable amounts in guanine nucleotides (quantity not measured), and an essential absence in the adenine nucleotides. The inability of adenosine to serve as a precursor for adenine nucleotide formation was attributed to its rapid deamination to inosine as well as its known resistance to phosphorolysis by erythrocyte purine nucleotide phosphorolase (23). In a single experiment (not shown), incubation with labeled inosine at high concentration resulted in the appearance of labeled IDP and ITP as well as the expected IMP. Labeled guanosine incubation led to considerable label incorporation in the guanine nucleotides and to further label distribution in IMP. Extensive synthesis of each of the purine nucleotides could be assumed on the basis of the relative specific activities recorded. The extent to which the indicated active syntheses did in fact result in net accumulation with corresponding elevation of the cellular purine nucleotide pools is revealed by the experiments shown in Table III. Extensive elevation of the adenine, guanine, and hypoxanthine ribonucleotide pools was obtained following incubation in the presence of appropriate precursors. In the presence of adenine, syntheses of guanine and hypoxanthine ribonucleotides from the corresponding nucleosides were suppressed in favor of adenine nucleotide formation as illustrated. On the other hand, elevation of both adenine and guanine nucleotide pools could be induced in the same cells by separate, successive incubations with appropriate precursors (see blood Sample I, incubation sequence f). Elevation of adenine nucleotide pools could be readily induced in both fresh as well as aged erythrocytes. Nucleotide formation in the erythrocyte from purine precursors was found to be markedly stimulated in the presence of inorganic phosphate. The observed stimulation was presumed to reflect an accelerated rate of nucleoside phosphorylation (23). The phosphorylation of nucleosides results in the liberation of purine base and formation of ribose 1-phosphate, which can undergo successive conversion to ribose 5-phosphate and then to 5-phosphoribosyl 1-pyrophosphate, which can, in turn, react with the liberated free base (or added adenine) to form the corresponding nucleotide (19).

Glycolytic Response Accompanying Adenine Nucleotide Formation in Aged Erythrocytes—The effect of increasing cellular concentrations of adenine as well as the other purine nucleotides in relation to erythrocyte glycolysis was variously examined. The results of an experiment in which glucose utilization was simultaneously followed in the course of adenine nucleotide formation

**TABLE II**

<table>
<thead>
<tr>
<th>Precursor compounds*</th>
<th>Specific activity of isolated compounds relative to labeled precursor†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nucleotide adenine</td>
</tr>
<tr>
<td>Adenine-8-14C + inosine</td>
<td>0.48</td>
</tr>
<tr>
<td>Adenosine-8-14C</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Inosine-8-14C</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Guanosine-8-14C</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Cells incubated for 90 minutes at 37° in 4 volumes of isotonic media containing 0.05 m phosphate at pH 7.5 and precursor compounds at 4 μmoles per ml of cells with radioactivity at 10,000 to 20,000 c.p.m. per μmole. Cells were prepared from a blood sample stored in acid citrate-dextrose medium for 25 days at refrigerated temperatures.

† Nucleotide purine bases were isolated according to Procedure B (see “Experimental Procedure”).
Selective elevation of purine nucleotide pools in intact erythrocytes

<table>
<thead>
<tr>
<th>Added precursors*</th>
<th>Nucleotide purine†</th>
<th>μmoles/g Ebb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenine</td>
<td>Guanine</td>
</tr>
<tr>
<td>I. Acid citrate-dextrose blood, 26 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. None</td>
<td>2.3</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>b. Adenine + inosine</td>
<td>5.0</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>c. Inosine</td>
<td>2.4</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>d. Adenosine</td>
<td>2.4</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>e. Adenine + guanosine</td>
<td>4.6</td>
<td>0.6</td>
</tr>
<tr>
<td>f. Guanosine</td>
<td>2.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Adenine + inosine†</td>
<td>4.1</td>
<td>3.0</td>
</tr>
<tr>
<td>II. EDTA blood, 1 day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. None</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>b. Adenine + inosine, 30 minutes</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Adenine + inosine, 60 minutes</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Adenine + inosine, 90 minutes</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

* Cells were incubated for 60 minutes (unless otherwise specified) at 37° in 4 volumes of isotonic medium containing 0.05 M phosphate at pH 7.5 and designated precursors at 5 μmoles per ml of cells.
† Nucleotide base was measured according to Procedure A with blood Sample I and according to Procedures A and B with blood Sample II.
‡ Cells were washed prior to the second incubation.

in erythrocytes aged by prolonged storage is summarized in Fig. 4. As illustrated in the curves designated A, cellular accumulation of adenine nucleotides was accompanied by a simultaneous increase in the rate of glucose metabolism. The control level of glucose utilized by these cells is shown in Curve B. The results support a direct relationship between glucose metabolism and the adenine nucleotide content of the cells, in further evidence of the limiting nature of this cofactor pool.

Specificity Studies—The specificity of adenine nucleotide restoration in effecting glycolytic recovery in aged erythrocytes is illustrated by the experiments summarized in Table IV. A number of compounds, many of which have not been listed, were tested in an attempt to induce glycolytic recovery in aged erythrocytes. As evidenced from the results which are shown, glycolytic recovery was achieved only in cells incubated in the presence of those compounds known to be capable of inducing adenine nucleotide formation (i.e., adenine + inosine). Restoration of cell potassium and provision of intermediates for DPN synthesis did not add to the glycolytic recovery induced by adenine nucleotide accumulation alone. Elevation of guanine and hypoxanthine ribonucleotide pools had no obvious effect on erythrocyte glycolysis.

Associated Loss and Recovery of Adenine Nucleotides and Glycolysis—The correlative relationship between the adenine nucleotides and erythrocyte glycolysis is further illustrated in Fig. 5. In this study, the concentration of ATP was determined as a relative measure of the adenine nucleotide content of the cells (see Fig. 8). Incubation of a blood sample over a 4-day period at 30° resulted in an associated loss of cellular ATP and glycolysis in the manner shown in the curves designated A. Daily removal of aliquots of the incubating blood sample, followed by a further brief incubation of the erythrocytes with adenine and inosine, resulted in an associated recovery of cellular ATP and glycolysis through the first 3 days of incubation as shown (see curves designated B). Glycolytic recovery did not occur, however, after 4 days of incubation in spite of successful cellular ATP restoration. Of additional interest in these

![Figure 4](http://www.jbc.org/)

**Figure 4.** Concomitant restoration of adenine nucleotides and glycolytic capacity of aged erythrocytes accompanying incubation in the presence of glucose plus adenine and inosine (A). Glycolytic utilization accompanying incubation in the absence of adenine and inosine is illustrated in Curve B. Incubation mixtures contained 10% suspensions of erythrocytes (prepared from refrigerated blood stored for 33 days in acid citrate-dextrose medium) in isotonic media containing excess glucose (10 μmoles per ml of cells), 0.05 M glycylglycine buffer at pH 7.8, 0.025 M Na₃HPO₄, and adenine and inosine (5 μmoles of each per ml of cells) where indicated. Glucose utilization and cellular adenine nucleotide concentrations were followed over the intervals shown.

<table>
<thead>
<tr>
<th>Compound(s) tested</th>
<th>Resulting glycolytic capacity μmoles glucose utilized/hr/g Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, inosine, adenine, adenine + glucose, guanosine, adenosine. ATP, ADP, AMP, inosine + K⁺, niacin, niacin + glutamine .</td>
<td>1.9-2.3</td>
</tr>
<tr>
<td>Inosine + adenine .</td>
<td>7.2</td>
</tr>
<tr>
<td>Inosine + adenine + K⁺ .</td>
<td>7.1</td>
</tr>
<tr>
<td>Inosine +adenine + K⁺ + niacin + glutamine .</td>
<td>7.5</td>
</tr>
<tr>
<td>None .</td>
<td>2.3</td>
</tr>
</tbody>
</table>

TABLE IV
Specificity of adenine nucleotide restoration in effecting glycolytic recovery in aged erythrocytes

Each compound was tested at 5 μmoles per ml of cells except glucose, which was tested at 10 μmoles, and K⁺ at 80 μmoles. Aged cells (from refrigerated blood stored for 33 days in acid citrate-dextrose medium) were incubated for 60 minutes at 37° in a 10% suspension in isotonic medium containing 0.025 M phosphate at pH 7.5 in the presence of the compounds listed below. The cells were subsequently thoroughly washed and tested for glycolytic capacity.
Correlative Relationship between Adenine Nucleotides and studies was the finding that glycolysis could be enhanced in fresh cells when the ATP content of these cells was elevated (compare zero incubation time values).

**Correlative Relationship between Adenine Nucleotides and Glycolytic Capacity**

**Table V**

<table>
<thead>
<tr>
<th>Storage of blood samples</th>
<th>Initial*</th>
<th>Following elevation of adenine nucleotides</th>
<th>Glycolytic capacity</th>
<th>Adenine nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Glycolytic capacity</td>
<td>Adenine nucleotides</td>
<td>Glycolytic capacity</td>
<td>Adenine nucleotides</td>
</tr>
<tr>
<td>EDTA</td>
<td>days</td>
<td>µmoles glucose utilized/hr/g Hb</td>
<td>µmoles/g Hb</td>
<td>µmoles glucose utilized/hr/g Hb</td>
</tr>
<tr>
<td>&lt;1</td>
<td>7.4</td>
<td>5.4</td>
<td>10.5</td>
<td>7.7</td>
</tr>
<tr>
<td>&lt;1</td>
<td>7.6</td>
<td>5.8</td>
<td>8.7</td>
<td>7.5</td>
</tr>
<tr>
<td>&lt;1</td>
<td>5.8</td>
<td>4.9</td>
<td>8.7</td>
<td>7.0</td>
</tr>
<tr>
<td>&lt;1</td>
<td>5.6</td>
<td>3.8</td>
<td>9.0</td>
<td>6.5</td>
</tr>
<tr>
<td>ACD†</td>
<td>28</td>
<td>4.1</td>
<td>3.0</td>
<td>7.5</td>
</tr>
<tr>
<td>30</td>
<td>2.1</td>
<td>2.4</td>
<td>5.2</td>
<td>5.8</td>
</tr>
<tr>
<td>39</td>
<td>0.9</td>
<td>0.7</td>
<td>6.6</td>
<td>3.9</td>
</tr>
<tr>
<td>55</td>
<td>0.2</td>
<td>0.6</td>
<td>4.8</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* Washed cells were tested directly for glycolytic capacity and total adenine nucleotide content, the latter according to Procedure A (see the text).
† Washed cells were incubated in isotonic media for 60 minutes at 37° in a final 20% suspension of the presence of adenine and inosine. Restoration of adenine nucleotides was induced by incubating 20% suspensions of cells for 60 minutes at 37° in an isotonic medium containing 0.05 m phosphate at pH 7.5 and adenine + inosine. Restoration of adenine nucleotides in the fresh cells when the ATP content of these cells was elevated (compare zero incubation time values).

**Table VI**

<table>
<thead>
<tr>
<th>Erythrocytes</th>
<th>ATP µmoles/g Hb</th>
<th>Glucose µmoles lactate produced hr/g Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepared from blood stored for 48 days in acid citrate-dextrose near 0°</td>
<td>0.63</td>
<td>6.1</td>
</tr>
<tr>
<td>Above, after incubation with adenine + inosine f</td>
<td>4.7</td>
<td>15.3</td>
</tr>
</tbody>
</table>

* A 10% suspension of cells was incubated at 37° in isotonic media containing each of the sugars at 50 µmoles per ml of cells, Na₂HPO₄ at 0.025 m, and glycylglycine buffer at 0.05 m, pH 7.8. Lactate was determined over a 3-hour incubation period.
† A 20% suspension of washed cells was incubated for 60 minutes at 37° in isotonic media containing 0.05 m phosphate at pH 7.5 and adenine + inosine (each at 5 µmoles per ml of cells). The cells were rewashed prior to reincubation with the various sugars.

Glycolysis of Fresh Erythrocytes--The extent to which adenine nucleotides determine the glycolytic capacity of erythrocytes prepared from freshly drawn blood was further examined in the experiment summarized in Table V. In each of four fresh blood samples examined, the glycolytic capacity of the erythrocytes could be enhanced on elevation of the adenine nucleotide content of the cells as illustrated. For comparative purposes, the results of similar determinations carried out on outdated blood stored in acid citrate-dextrose have also been included in the table. These results establish an over-all correlative relationship between glycolysis and adenine nucleotide pool size in both fresh as well as aged erythrocytes, over a wide range of cellular adenine nucleotide levels.

Correlative Relationship between Adenine Nucleotides and Hexose Metabolism--Since the degradation of other hexoses also proceeds mainly to lactic acid in the erythrocyte, it was of interest to examine their comparative utilization in relation to the adenine nucleotide content of the cells. In Table VI are shown the results of such a comparative study. Degradation of fructose and mannose was found to be similarly dependent on the adenine nucleotide content (ATP only determined) of the erythrocyte as in the case of glucose. Restoration of adenine nucleotides in the aged erythrocytes resulted in a corresponding enhanced metabolism of each of the sugars to lactic acid, in accord with their common dependence on the adenine nucleotides as essential cofactors in their degradation.

**Discussion**

In the present paper, evidence has been provided indicating that the adenine nucleotides assume a limiting role in the glycolysis of the human erythrocyte. Since the mature erythrocyte neither synthesizes purine nucleotides de novo (4, 5) nor is capable of resynthesizing adenine nucleotide from likely products accompanying its metabolic degradation (i.e. adenosine, inosine, or hypoxanthine; see Table II), a continual depletion of the cellular adenine nucleotide pool size would perhaps be unexpected. Although adenine nucleotide can be formed in the erythrocyte in vivo when adenine is supplied (representing the only known means of synthesis), it is not evident that adenine is normally

**Figure 5**

Concomitant loss and restoration of glycolytic capacity and ATP content of erythrocytes accompanying (A) loss of glycolytic capacity (△―△) and ATP (●―●) on extended incubation of a fresh blood sample in acid citrate-dextrose medium and (B) restoration of glycolytic capacity (△ ―△) and ATP (● –●) following incubation in the presence of adenine and inosine. Restoration of adenine nucleotides was induced by incubating 20% suspensions of cells for 60 minutes at 37° in an isotonic medium containing 0.05 m phosphate at pH 7.5 and adenine + inosine (5 pmoles of each per ml of cells).
available to the cells \textit{in vivo} (4). In this regard, the erythrocyte has been reported to contain several times less ATP than the younger reticulocyte, from which it is derived (24). Whether the observed cellular loss of adenine nucleotides accompanying aging of erythrocytes \textit{in vitro} represents a continuum of an existing deterioration process occurring \textit{in vivo} (albeit at an accelerated rate) remains to be determined. In this regard, the adenine nucleotides assume a similar limiting role in the glycolysis of both fresh erythrocytes and erythrocytes aged \textit{in vitro}, differing only in a matter presumably of degree.

\section*{SUMMARY}

The present report summarizes evidence for a limiting role of adenine nucleotides in the glycolysis of the erythrocyte. Loss of adenine nucleotides accompanying aging of blood \textit{in vitro} was accompanied by corresponding reductions in the glycolytic capacity of the cells. Restoration of adenine nucleotides in the depleted, aged cell by incubation with adenine plus inosine resulted in an associated recovery of the glycolytic capacity. Adenine nucleotides were also found to be limiting in the glycolysis of fresh erythrocytes. The implication of these findings was considered in relation to erythrocyte survival.

\section*{Acknowledgments}

The author is indebted to Miss June F. Allan for invaluable technical assistance, and to the personnel of the Peninsula Memorial Blood Bank for generous contributions of outdated human blood samples.

\section*{REFERENCES}


Limiting Role of Adenine Nucleotide in the Glycolysis of the Human Erythrocyte
K. K. Tsuboi


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