Erythrocyte adenosine triphosphate and 2,3-diphosphoglycerate after human renal transplantation: dissociation from hypophosphataemia

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Summary

1. Erythrocyte 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) were determined in normal individuals, uraemic patients on chronic haemodialysis and patients who underwent renal transplantation, and correlated with plasma phosphate and arterial blood pH.

2. Significant increases in the 2,3-DPG and ATP content were found in the uraemic patients and these persisted after transplantation in spite of marked hypophosphataemia.

3. No correlation was established with plasma phosphate for either of the compounds but 2,3-DPG had a significant correlation with arterial blood pH.

4. Normal values for ATP and 2,3-DPG were observed in post-transplant patients with normal haematological values. The high amounts of erythrocyte 2,3-DPG and ATP in the early post-transplant period are independent of the circulating concentration of inorganic phosphate, and might represent the response of erythrocyte glycolysis to changing arterial blood pH.

Key words: adenosine triphosphate, 2,3-diphosphoglycerate, hypophosphataemia, renal transplantation.

Introduction

Almost all patients with chronic renal failure are anaemic. Although their erythrocyte mass usually increases on chronic haemodialysis (Eschbach, Funk, Adamson, Juhn, Scribner & Finch, 1967), total correction only occurs after successful renal transplantation (Farooki & Kimber, 1971). Erythrocytes in uraemia display an increased rate of glycolysis and their amounts of 2,3-DPG and ATP have been found to be elevated (Lichtman & Miller, 1970; Blumberg & Marti, 1972). It has been postulated that the stimulus for glycolysis is the elevated serum phosphate, characteristic of patients with chronic renal failure (Lichtman & Miller, 1970).

Acute hypophosphataemia markedly reduces erythrocyte glycolysis in a variety of clinical states, with concomitant reductions in erythrocyte 2,3-DPG and ATP (Lichtman, Miller, Cohen & Waterhouse, 1971; Travis, Sugarman, Ruberg, Dudrick, Delivoria-Papadopoulos, Miller & Oski, 1971; Yawata, Hebbel, Silvis, Howe & Jacob, 1974). These alterations have been assumed to play a significant role in the development of erythrocyte destruction, leucocyte dysfunction and platelet abnormalities (Yawata, et al., 1974). After successful renal allotransplantation from living donors, elaboration of large urine volumes is commonly associated with pronounced reductions of serum phosphate concentration. This is usually the result of marked phosphaturia (Alfrey, Jenkins, Groth, Schorr, Gecelter & Ogden, 1968).

In view of the importance of an adequate oxygen delivery by erythrocytes and appro-
appropriate leucocyte function in patients receiving immunosuppressive agents, we studied the effects of the acute hypophosphataemia that develops after renal transplantation on the concentrations of erythrocyte 2,3-DPG and ATP. Such studies, conducted immediately after transplantation, also allowed a reassessment of the role of hyperphosphataemia in the elevated organic phosphates of erythrocytes.

Methods

After informed consent from all subjects was obtained, blood samples were drawn from patients with end-stage renal failure maintained on thrice-weekly haemodialysis. Each patient had been treated on a coil dialyser for longer than 6 months. Studies were also conducted on patients who had received a kidney transplant at least 4 months previously and whose packed cell volumes were stable on at least three separate determinations. Their results were compared with similar measurements on non-smoking, healthy volunteer subjects who had no evidence of renal, haematological or pulmonary disease. Eight patients were studied in the days immediately before and after they received a renal transplant from a related living donor (Table 1). They were receiving prednisolone (2 mg/kg body weight) and azathioprine (2.5 mg/kg body weight) during the days before the pre-transplant samples were obtained and through the period of observation. Five of the patients had received a blood transfusion 4 days before the renal transplant was performed and 2,3-DPG and ATP had returned to pre-transfusion values by the day of surgery. Two additional subjects with end-stage renal failure on haemodialysis were studied as outpatients. Both received a low-phosphate diet supplemented with phosphate binders (magnesium–aluminum hydroxide), to reduce the concentration of the serum phosphate to values comparable with those observed in the transplanted patients. Haemodialysis was performed for 6 h. When diluted, concentrations in the dialysis fluid were Na 130 mmol/l, Cl 104 mmol/l, K 2.5 mmol/l, acetate 37 mmol/l, Mg 1.5 mmol/l, Ca 2.5 mmol/l and glucose 11 mmol/l. Blood flow during dialysis was maintained at 200 ml/min. Dialysis fluid flow averaged 300 ml/min.

Blood samples were procured before haemodialysis in the uraemic patients, or in a fasting state after surgery either by puncture of the arterial end of a radial arteriovenous fistula or from the arterial side of an indwelling brachial arteriovenous cannula. All samples were drawn anaerobically in a disposable heparinized syringe and processed immediately.

Whole blood pH, $P_{CO_2}$, and $P_{O_2}$ were measured at 37°C in a pH/blood gas analyser (American Instruments, model 313). Packed cell volume was determined as the mean value of three measurements in a micro-haematocrit centrifuge. Haemoglobin was determined in a Coulter Counter. Serum phosphate was measured on a Technicon Auto-Analyzer by the method of Yee (1968). 2,3-DPG was measured spectrophotometrically on haemolysed whole blood collected in a 1:100 dilution with ice-cold water by an enzymatic method in which the change in absorbance related to the degradation

### Table 1. Clinical characteristics of the transplanted patients

All patients received a related living donor transplant. An asterisk identifies individuals who are smokers.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Time on chronic haemodialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.M.</td>
<td>16</td>
<td>M</td>
<td>Chronic glomerulonephritis</td>
<td>3 months</td>
</tr>
<tr>
<td>M.S.</td>
<td>29</td>
<td>F</td>
<td>Chronic glomerulonephritis</td>
<td>1 month</td>
</tr>
<tr>
<td>M.T.</td>
<td>41</td>
<td>F</td>
<td>Obstructive nephropathy</td>
<td>3 years</td>
</tr>
<tr>
<td>R.C.*</td>
<td>50</td>
<td>M</td>
<td>Chronic glomerulonephritis</td>
<td>3 months</td>
</tr>
<tr>
<td>W.W.</td>
<td>24</td>
<td>F</td>
<td>Malignant nephrosclerosis</td>
<td>8 months</td>
</tr>
<tr>
<td>M.S.</td>
<td>52</td>
<td>M</td>
<td>Chronic glomerulonephritis</td>
<td>2$\frac{1}{2}$ years</td>
</tr>
<tr>
<td>H.H.*</td>
<td>32</td>
<td>M</td>
<td>Bilateral vesico-ureteral reflux</td>
<td>7 months</td>
</tr>
<tr>
<td>J.T.*</td>
<td>46</td>
<td>M</td>
<td>Chronic glomerulonephritis</td>
<td>9 months</td>
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of 3-phosphoenolpyruvate in the presence of phosphoglycerate mutase, enolase and magnesium chloride was measured over 4 min at 30°C, and the concentration of 2,3-DPG read from a plotted standard graph (Calbiochem, 2,3-DPG Statpack). ATP was assayed spectrophotometrically in a neutralized perchloric acid (2 mol/l) extract of whole blood, by means of yeast hexokinase and glucose 6-phosphate dehydrogenase (Lamprecht & Trautschold, 1971). Results were expressed in mmol/l of erythrocytes. The extracts were stored frozen at −20°C until assayed, usually within a week. All samples were analysed in duplicate and all determinations in a single patient were processed at the same time. Statistical analysis was by Student's t-test.

Results are shown as mean values ± SD.

Results

Erythrocyte contents of ATP and 2,3-DPG and the serum phosphate concentration of the uraemic patients were significantly higher than those of the control group (P < 0.001). Their packed cell volumes and haemoglobin were substantially lower (Table 2). Comparative studies after renal transplantation showed that, on the fifth day, the serum phosphate had fallen to 0.14 ± 0.04 mmol/l but the 2,3-DPG and ATP remained elevated: 4.98 ± 0.8 and 1.91 ± 0.3 mmol/l of erythrocytes respectively. In the patients studied several months later, when the packed cell volume and haemoglobin values had become normal, the 2,3-DPG and ATP content of the erythrocytes returned to normal (3.91 ± 0.59 and 1.24 ± 0.28 mmol/l of erythrocytes).

In the eight patients studied prospectively (Fig. 1), the mean ATP concentrations before renal transplantation were 1.96 ± 0.56 and 2.07 ± 0.55 mmol/l of erythrocytes, on 2 separate days before surgery. 2,3-DPG values were 5.44 ± 1.7 and 5.06 ± 1.0 mmol/l. Haemoglobin was 8.1 ± 2.0 and 8.5 ± 1.4 g/dl and plasma phosphate concentration 0.53 ± 0.17 and 0.53 ± 0.21 mmol/l. Over the next 10 days, the concentration of the plasma phosphate fell to 0.14 ± 0.04 mmol/l as early as 3 days after transplantation. The haemoglobin concentration also showed a reduction to 6.07 ± 1.2 g/dl on the third day and both values stayed below the pre-transplant values as long as 15 days after the surgical intervention. Urinary excretion of phosphate was very high, 1.79 ± 0.36 mol (n = 4) in the first 24 h, decreasing to 0.66 ± 0.32 mol by the tenth day. By contrast, at no point during the period of observation did the 2,3-DPG and ATP concentrations fall significantly below the pre-transplant values. This was true for the mean values calculated from daily determinations on different patients as well as for the individual patients analysed separately, although some small day-to-day variations were observed.

Reticulocyte counts were done at different intervals in some of the patients. The results were variable, although reticulocytosis was observed in three patients (mean 2.6; range 0.1–11.0 reticulocytes %). In three other subjects, the number of cells stayed between 0.1 and 4.2% (mean 1.4) during the 10 days of daily determinations.

Arterial blood pH showed marked inter-patient variations in daily determinations, making statistical analysis very difficult (Table 3). Nevertheless, in individual patients the pH
FIG. 1. Sequential changes in haemoglobin, plasma phosphate, erythrocyte ATP and 2,3-diphosphoglycerate (2,3-DPG) in eight uraemic patients studied before and after renal transplantation. Values are mean ± sd. Statistical comparison was made with pre-transplant values as control: *P > 0.05; **P > 0.01; ***P > 0.005. No changes were observed in the organic phosphate compounds.

 됨을 기록한 후 1년 동안의 change in haemoglobin, plasma phosphate, erythrocyte ATP and 2,3-diphosphoglycerate (2,3-DPG)에 대한 그래프로 나타내었다. 값은 평균 ± 표준 오차이다. 전이 시점의 값으로 비교적으로 해석하였다: *P > 0.05; **P > 0.01; ***P > 0.005. 유산소산물의 변화는 관찰되지 않았다.

tended to remain in an alkaline range, with a low Pco₂, and a serum bicarbonate in the low normal range. There was a very significant correlation between arterial blood pH and erythrocyte 2,3-DPG (r = 0.52; P < 0.001), but no such correlation could be established for blood pH with erythrocyte ATP (Fig. 2). Comparison of plasma phosphate with 2,3-DPG and ATP (Fig. 3 and Fig. 4) did not show any correlation in spite of the wide variation of phosphate values (0.06–0.8 mmol/l).

In the two uraemic patients on chronic haemodialysis, in whom plasma phosphate concentration was reduced by the administration of antacids, erythrocyte ATP and 2,3-DPG decreased markedly when phosphate values were lowered to normal (Fig. 5). Values as low as 0.29 mmol of ATP/l of erythrocytes persisted in one
Table 3. Arterial blood pH, Pco₂ and bicarbonate in patients before and after renal transplantation

Values shown are mean ± SD with number of patients in parentheses. A respiratory alkalosis was commonly observed.

<table>
<thead>
<tr>
<th>Time from renal transplant (days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>-1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.05 (4)</td>
<td>7.46 ± 0.05 (5)</td>
<td>7.44 ± 0.03 (6)</td>
<td>7.45 ± 0.04 (5)</td>
<td>7.49 ± 0.01 (3)</td>
<td>7.46 ± 0.03 (4)</td>
<td>7.48 ± 0.04 (5)</td>
<td>7.50 ± 0.02 (5)</td>
<td>7.46 ± 0.11 (4)</td>
<td>7.50 ± 0.02 (2)</td>
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</tr>
<tr>
<td>Pco₂ (mmHg)</td>
<td>35.0 ± 7.4 (4)</td>
<td>31.1 ± 7.7 (5)</td>
<td>31.6 ± 6.1 (6)</td>
<td>28.2 ± 4.8 (5)</td>
<td>31.6 ± 6.5 (5)</td>
<td>32.3 ± 8.0 (3)</td>
<td>29.7 ± 10.2 (4)</td>
<td>30.7 ± 5.7 (4)</td>
<td>30.8 ± 2.0 (5)</td>
<td>28.8 ± 4.9 (2)</td>
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<tr>
<td>Bicarbonate (mmol/l)</td>
<td>24.6 ± 7.4 (4)</td>
<td>21.8 ± 6.5 (5)</td>
<td>20.45 ± 6.6 (6)</td>
<td>19.3 ± 3.6 (5)</td>
<td>21.1 ± 2.9 (3)</td>
<td>22.1 ± 4.9 (5)</td>
<td>23.0 ± 4.9 (4)</td>
<td>22.6 ± 3.0 (3)</td>
<td>21.7 ± 4.9 (4)</td>
<td>20.3 ± 4.4 (2)</td>
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</table>

A respiratory alkalosis was commonly observed.
FIG. 2. Correlation between arterial blood pH and erythrocyte 2,3-diphosphoglycerate (2,3-DPG) and ATP. The correlation was significant for 2,3-DPG ($r = 0.52, P < 0.001$), but no correlation was established for ATP. △, Values for a single patient on androgens.

FIG. 3. Correlation between erythrocyte ATP and plasma phosphate. No correlation was established. Solid symbols correspond to pre-transplant values. △, ▲ Values for a single patient who had been on androgens before transplantation. Shaded area is the range of normal values.

FIG. 4. Correlation between erythrocyte 2,3-diphosphoglycerate (2,3-DPG) and plasma phosphate. Symbols are as described in Fig. 3.
Erythrocyte ATP after renal transplantation

Sequential changes in plasma phosphate and erythrocyte ATP and 2,3-diphosphoglycerate (2,3-DPG) in two uraemic subjects (○, △) whose phosphate was corrected to low normal values. Significant decreases are shown for both patients. Arrows indicate day of commencement of phosphate depletion.

Discussion

Erythrocytes of uraemic patients display a variety of biochemical abnormalities. These include a high intracellular sodium, a decreased adenosine triphosphatase activity and other enzymatic abnormalities (Lichtman & Miller, 1970; Welt, Sachs & McManus, 1964). The glycolytic rate of erythrocytes of uraemic patients is normal (Morgan & Morgan, 1964) or elevated (Lichtman & Miller, 1970); this elevation presumably increases the concentrations of 2,3-DPG and ATP.

Figure 5. Sequential changes in plasma phosphate and erythrocyte ATP and 2,3-diphosphoglycerate (2,3-DPG) in two uraemic subjects (○, △) whose phosphate was corrected to low normal values. Significant decreases are shown for both patients. Arrows indicate day of commencement of phosphate depletion.

Patient for 7 days. The other subject also demonstrated a reduction in ATP to less than 0.5 mmol/l of erythrocytes. In one subject (L.G.), ATP and 2,3-DPG values returned to close to those present at the onset of the phosphate depletion, despite the maintenance of plasma phosphate concentrations markedly below those present originally. When the correlation between values for plasma phosphate and ATP (Fig. 6) and 2,3-DPG was analysed in these two patients, significant values were obtained: ATP: \( r = 0.69, P < 0.001 \); 2,3-DPG: \( r = 0.44, P < 0.05 \).
The increased erythrocyte 2,3-DPG and ATP in uraemia has been attributed to hyperphosphataemia. Decreases in the serum phosphate in uraemic patients have been correlated with marked falls in amounts of these metabolites within a few days of onset of hypophosphataemia. A slow return to the pre-existing values is observed after the phosphate increases again (Lichtman, Miller & Freeman, 1969). In contrast, this temporal relationship was not observed in our transplanted patients.

Our observations indicate that in the immediate post-renal transplant period, the maintenance of high erythrocyte ATP and 2,3-DPG concentrations is not dependent on the presence of hyperphosphataemia and that, in spite of a very low plasma phosphate concentration, the concentrations remain elevated. Hypophosphataemia may diminish the glycolytic rate of erythrocytes by decreasing the activity of glyceraldehyde 3-phosphate dehydrogenase (Travis et al., 1971). The phosphate concentrations at which this occurs are usually very low, but significant correlations have been established at all levels of serum phosphate. In the post-transplant patients, the majority of the determinations are within 0.1-0.2 mmol/l, with several values below 0.1 mmol/l. A correlation could not be established between ATP and 2,3-DPG values and plasma phosphate. In sharp contrast, therapeutic depression of plasma phosphate in two uraemic individuals caused dramatic depression of erythrocyte 2,3-DPG and ATP. These findings indicate that erythrocytes of successfully transplanted patients maintain high concentrations of 2,3-DPG and ATP despite hypophosphataemia, which in other circumstances could lead to their decline.

Besides serum phosphate concentration, other factors influence erythrocyte 2,3-DPG and ATP. These include pH, anoxia and certain drugs including androgens and thyroid hormones (Parker, Beirne, Desai, Raich & Shahidi, 1972). Thus acidosis decreases glycolysis and alkalosis stimulates it. A significant correlation between pH and erythrocyte 2,3-DPG has been reported with various acid-base disorders (Astrup, 1970), as well as in normal subjects during altitude-induced hypoxia and exogenously induced metabolic derangements (Billingham, Detter & Lenfant, 1971). In uraemic patients raised amounts of 2,3-DPG are found in spite of metabolic acidosis. It has been postulated, however, that because of the acidosis present they are inappropriately low for the degree of anaemia, and correction of the acidosis in four uraemic patients has resulted in increase of the 2,3-DPG content of the erythrocytes to values compatible with the haemoglobin deficit (Lichtman, Murphy, Whitbeck & Kearney, 1974). The exact inter-relations between blood pH, serum phosphate, erythrocyte 2,3-DPG and ATP in uraemia are not completely clear. Studies performed during haemodialysis have shown conflicting results: uniform falls in serum phosphate and increases in blood pH are reported; in some studies the erythrocyte ATP or the 2,3-DPG are found to fall concomitantly with serum phosphate (Raich, Rodriguez, Desai & Shahidi, 1973; Chollar & Desforges, 1974; Hirzel, Maher, Temple & Mengel, 1975), whereas other investigators have found no correlation with decrease in serum phosphate or increase in arterial blood pH (Miller, Zaroulis, Valeri & Stohlman, 1974; Lichtman, Murphy, Byer & Freeman, 1974). In our studies, in the immediate post-transplant period arterial blood pH was observed to rise, probably from rapid elimination of accumulated acid metabolites, restoration of the kidney's capability to regenerate bicarbonate and an unexplained respiratory alkalosis. Although this change toward an alkaline pH was significantly correlated with changes in 2,3-DPG concentration, the same correlation could not be established for ATP. Although no actual measurements
were done, an increase in pH would increase the enzymatic activity and the amounts of the compounds by accelerating the glycolytic rate and would tend to confirm the observations that blood pH is a more important regulator of erythrocyte glycolysis than the serum inorganic phosphate. Our studies in the post-transplant patient contrast with the observations of Ditzel (1973) of persistent decrease in erythrocyte 2,3-DPG in patients that have persistent hypophosphataemia after correction of diabetic ketoacidosis, and those of Young, Lichtman & Cohen (1973) which demonstrated a significant correlation between circulating serum phosphate concentrations and erythrocyte ATP and 2,3-DPG in patients studied immediately after cardiovascular surgery.

Young erythrocytes metabolize glucose at a more rapid rate than old erythrocytes. Their larger content of ATP and 2,3-DPG could explain some of the persistent elevations observed. However, this was not a consistent finding in all the patients, and no correlation could be established between reticulocyte counts and ATP or 2,3-DPG. Moreover, the reticulocytosis was not observed until several days after the transplantation, whereas ATP and 2,3-DPG values had already been high.

The findings by some investigators of reduced glycolysis in erythrocytes of uraemia is very likely attributable to acidosis (Morgan & Morgan, 1964). The addition of uraemia serum to erythrocytes and muscle cells depresses glucose consumption (Dzurik & Valovicova, 1970). The findings by Lichtmann & Miller (1970) that the hyperphosphataemia of uraemia is a major factor in elevation of erythrocyte ATP, help to explain some of these contradictions. If a uraemic toxin should depress glycolysis, the hyperphosphataemia would tend to over-ride the effect and stimulate glucose consumption. The lowering of the serum phosphate to normal values in the presence of uraemia and acidosis would decrease the concentrations of ATP and 2,3-DPG, as observed in our two uraemic patients. Our observations in post-transplant patients seem to indicate a less important role for phosphate. After renal transplantation, rapid removal of toxic metabolites, systemic alkalisos, and the presence of severe anaemia, would overcome the sudden hypophosphataemia that ensues after the regaining of renal function.

The persistent elevation of ATP and 2,3-DPG in spite of hypophosphataemia should maintain a shift in the oxyhaemoglobin dissociation curve to the right in the presence of low amounts of haemoglobin with its beneficial effects in tissue oxygen delivery. In the days immediately after renal transplantation, this would be a clear advantage for delivery of oxygen in these anemic individuals.

Acknowledgments

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