Erythrocyte metabolism in patients on haemodialysis and continuous ambulatory peritoneal dialysis

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Summary

1. Erythrocyte metabolism was investigated and glycolytic intermediates were measured in nine patients with chronic renal failure who were subsequently treated with haemodialysis. The same investigations were performed in nine patients on continuous ambulatory peritoneal dialysis (CAPD) who had previously been treated with intermittent peritoneal dialysis (IPD) (eight patients) or dietary restriction (one patient).

2. The patients who received haemodialysis had a partially compensated metabolic acidosis before treatment. With haemodialysis, plasma phosphate (Pi) fell and base excess, erythrocyte 2,3-diphosphoglycerate (2,3-DPG), glucose consumption and lactate production rose significantly. In this group the most important influence on erythrocyte metabolism was base excess. The pattern of erythrocyte glycolytic intermediates showed that the rise in 2,3-DPG with haemodialysis was brought about within the Rapoport–Luebering shunt; there was no statistically significant decrease in haemoglobin–oxygen affinity.

3. The patients who received CAPD were not acidotic before starting this form of treatment. With CAPD, there was a significant increase in haemoglobin and fall in plasma phosphate, erythrocyte 2,3-DPG and glucose consumption. The major factors influencing erythrocyte metabolism in this group were plasma phosphate and haemoglobin concentration. The fall in 2,3-DPG was produced by inhibition of 6-phosphofructokinase (EC 2.7.1.11); despite this fall, haemoglobin–oxygen affinity was not affected.

Key words: chronic renal failure, continuous ambulatory peritoneal dialysis, 2,3-diphosphoglycerate, erythrocyte, haemodialysis, haemoglobin–oxygen affinity, kidney.

Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; 2,3-DPG, 2,3-diphosphoglycerate; Hb, haemoglobin; IPD, intermittent peritoneal dialysis; Pi, plasma inorganic phosphate.

Introduction

A variety of erythrocyte metabolic abnormalities have been reported in chronic renal failure [1]. The outcome of such abnormalities may be an alteration in the oxygen affinity of haemoglobin due to the effect of changes in the level of 2,3-diphosphoglycerate (2,3-DPG) combined with various degrees of acidosis. As patients with chronic renal failure are almost invariably anaemic, a change in the position of the haemoglobin–oxygen (Hb–O₂) dissociation curve due to altered Hb–O₂ affinity may have a significant effect on tissue oxygen delivery.

The oxygen affinity of blood as measured by P₅₀ (the PO₂ at which Hb is 50% saturated with O₂) is directly altered by pH (Bohr effect) and 2,3-DPG, whereas Hb concentration and plasma and erythrocyte inorganic phosphate (Pi) have an indirect effect via changes in 2,3-DPG. Plasma and erythrocyte pH, Hb and Pi are all frequently abnormal in renal failure and alter the 2,3-DPG
concentration by their modulating effect on erythrocyte glycolysis. Hence pH alone has both a direct and an indirect effect on \( P_{50} \).

In order to study glycolysis in chronic renal failure several authors have measured some of the erythrocyte phosphorylated glycolytic intermediates. Fructose diphosphate, glyceraldehyde 3-phosphate, 3-phosphoglycerate, phosphoenolpyruvate and pyruvate have been found to be raised \([2, 3]\). Other authors have reported 2,3-DPG to be raised \([2-5]\). However, there are other reports of normal \([6, 7]\) or low \([8]\) 2,3-DPG concentration. Previous studies of the glycolytic pathway have been only partial, so that a complete analysis of the glycolytic changes in chronic renal failure was not possible. \( P_{50} \) has been found to be raised in chronic renal failure \([4, 9, 10]\).

Concentrations of erythrocyte adenosine 5'-pyrophosphate (ADP) and adenosine 5'-triphosphate (ATP) are usually raised in chronic renal failure. The concentrations of both ATP and 2,3-DPG have been shown to correlate with \( P_i \) in a hypophosphataemic patient without renal disease and with presumably normal acid–base status \([11]\). A feature of many previous studies in chronic renal failure is that the acid–base status of the patient is unknown, but variation in pH may account for the discrepancies in 2,3-DPG concentrations reported.

Several studies of the effects of haemodialysis have been reported. Raidis et al. \([10]\) found a significant decrease in \( P_{50} \) after an 11 h session of haemodialysis in which the pH rose, but 2,3-DPG was not measured. Raich et al. \([5]\) reported that over a 6 h haemodialysis pH increased significantly, but unexpectedly 2,3-DPG fell, which related to a fall in \( P_i \). Although it was not measured, \( P_{50} \) would have been expected to fall. These authors therefore considered plasma \( P_i \) to be a more important influence than pH on 2,3-DPG concentration. However, Lichtman et al. \([12]\) found that 2,3-DPG, ATP and \( P_{50} \) were unchanged by 6 h of haemodialysis, although plasma pH and base excess rose during dialysis. Although plasma \( P_i \) fell by 45%, erythrocyte \( P_i \) was unchanged because of delayed equilibration between plasma and erythrocytes. The conclusions of such studies are therefore conflicting, owing to the variable duration of dialysis, the failure in some case to measure all known relevant variables and the metabolically dynamic nature of the situation studied.

We have attempted to build up a more complete picture of erythrocyte metabolism in chronic renal failure and the effect of long-term dialysis by measuring all the glycolytic intermediates (with the exception of 1,3-diphosphoglycerate and pyruvate) and simultaneously assessing the acid–base status. Owing to the complexity of the investigations, the groups studied were rather small but certain conclusions can be drawn from the data. One group was treated with haemodialysis and another with chronic ambulatory peritoneal dialysis (CAPD), a relatively recent form of treatment \([13]\) in which, because of the continuous nature of the technique, there are no gross fluctuations in plasma solute concentration or in acid–base balance. One reason for doing this is that changes in 2,3-DPG are delayed by several hours after acute changes in plasma pH and may continue for up to 24 h \([14]\). In order to measure the stable condition, we investigated patients in a steady state on long-term dialysis. Accordingly, samples were taken from the patients going on to haemodialysis just before they received their first-ever dialysis, and repeated just before a session of haemodialysis after they had received at least 2 months’ treatment. The patients treated with CAPD were studied just before they changed to this form of dialysis from intermittent peritoneal dialysis or dietary restriction. The samples were repeated after 6 months on CAPD.

**Methods**

**Patients**

The investigations described in this paper have complied with the recommendations of the Medical Research Council (1962/63). The approval of the Area Ethical Committee was obtained; subjects were fully informed of the nature of the investigations and gave their consent.

**Haemodialysis**

The nine patients treated with haemodialysis had chronic renal failure due to chronic glomerulonephritis (three), polycystic disease (three), hypertensive renal disease (two) or analgesic nephropathy (one). Before starting haemodialysis creatinine clearances ranged from 3 to 7.5 ml/min (mean 4.9; normal > 75), plasma creatinine from 672 to 1712 \( \mu \)mol/l (mean 1056; normal range 55–110) and urea from 18·4 to 59.8 mmol/l (mean 36·8; normal range 2·5–6·5). Haemodialysis was started when conservative measures including diet and medication were no longer able to support a satisfactory quality of life. Dialysis was performed three times a week in a hospital unit with Dylade haemodialysis machines and Gambro dialysis membranes, which
were re-used up to six times. The average period of haemodialysis was 18 h per week.

Continuous ambulatory peritoneal dialysis

The nine patients treated with CAPD had chronic renal failure due to chronic glomerulonephritis (three), hypertensive renal disease (three), nephrolithiasis (two) or polycystic disease (one). These patients were already on treatment with intermittent peritoneal dialysis or dietary restriction and were switched to CAPD when this form of treatment was introduced to the Unit. Therefore the clinical state before starting CAPD was approximately comparable with that of the haemodialysis group when established on dialysis. The patients in the two groups were entirely separate, however.

In these patients, creatinine clearances ranged from 1.2 to 5 ml/min (mean 2.4; normal >75), plasma creatinine on the day of starting CAPD ranged from 746 to 1782 μmol/l (mean 1072; normal range 55–110) and urea from 21 to 52.2 mmol/l (mean 30; normal range 2–5–6–5). CAPD was performed by using three 2 litre exchanges (eight patients) of peritoneal dialysate (Dianecol, Travenol) daily with approximate dwell times of 8 h, and 20 min drainage periods. One patient required three 3 litre exchanges per day [15]. A Silastic cannula was permanently introduced into the peritoneal cavity and all manipulations and bag changes were carried out with the strictest aseptic technique. The incidence of peritonitis varied from zero (six patients) to three (one patient) episodes in 6 months. No patient was withdrawn from the study because of infection.

General methods

Haemoglobin, reticulocyte count, plasma urea, creatinine and phosphate were measured by standard techniques [16, 17]. Arterial plasma pH (pHₐ) and erythrocyte intracellular pH (pHₐ) in heparinized blood from the femoral or radial artery or from the arterial side of an arteriovenous fistula were measured with the IL (Instrumentation Laboratory) 213 pH electrode. The intracellular pH was measured in a lysate of packed erythrocytes from the same arterial blood samples prepared by a freeze–thaw method [18]. Pₐ was derived from the Hb–O₂ dissociation curve drawn from points obtained by equilibrium of heparinized venous blood at various P₀₂ values and constant Pₐ in an IL 237 tonometer at 37°C. The P₀₂ of equilibrated blood samples mixed with potassium ferriyanide (2 mmol/l) in a ratio of 1:100 was measured with the IL O₂ electrode and the values were converted to percentage saturations of Hb with O₂ [19]. The Pₐ at pH in vivo (Pₐ(viv)) and the Pₐ at pH 7.4 (Pₐ(7.4)) were derived from the Pₐ at the pH of the equilibration by using a Bohr effect value of −0.48 [20].

Erythrocyte glycolytic intermediates were measured in extracts prepared from venous blood added immediately to ice-cold perchloric acid (0-6 mol/l) [21]. The blood/perchloric acid mixture was centrifuged at 4°C and the supernatant stored at −40°C before assay. Glycolytic intermediates were measured in the thawed and neutralized extract by coupling to reactions consuming NADH or generating NADPH and measuring the fluorescence in a Perkin–Elmer fluorescence spectrophotometer [22]. Erythrocyte 2,3-DPG was assayed spectrophotometrically in an extract of heparinized venous blood made in distilled water and NaCl solution (154 mol/l), followed by boiling for 10 min.

Erythrocyte ATP was measured by a bioluminescent technique with the LKB 1250 luminometer and luciferin–luciferase reagent [23].

Erythrocyte glucose utilization and lactate production were measured during a 2 h incubation of 4 ml of heparinized blood (with buffy coat removed and packed cell volume adjusted to approx. 40%) in an IL 237 tonometer at 37°C with 28 μmol of added glucose. Pₐ in the tonometer was maintained at 35 mmHg (4.67 kPa) throughout the incubation. Samples (0.5 ml) were withdrawn at 0, 1 and 2 h and extracted with 2 vol. of ice-cold perchloric acid (0-6 mol/l) before spectrophotometric assay of glucose and lactate [24]. Samples were also taken at these times for measurement of plasma pH and erythrocyte ATP. From the measured pH and known Pₐ the base excess was calculated with an IL blood acid–base calculator [20].

Calculations of the significance of differences between means were performed by paired t-tests and correlation coefficients were calculated by standard methods [25].

Results

The results of the investigations in the haemodialysis group are shown in Table 1 and those in the patients on continuous ambulatory peritoneal dialysis (CAPD) in Table 2.

There was a significant increase in haemoglobin in the CAPD group (P < 0.01) but not in the haemodialysis group. A reticulocyte response has been observed in individual patients starting on CAPD [26] but in this group of patients there
was no difference in the reticulocyte percentages before CAPD and after 6 months' treatment, when studied for the second time (P > 0.5). However, the reticulocyte counts do not permit conclusions to be drawn as to the mean age of the erythrocyte populations at each sampling point.

Plasma phosphate fell significantly in both groups (see Tables 1 and 2). Mean plasma pH (pH\textsubscript{r}) rose slightly in the haemodialysis group (P > 0.1) and remained very stable in the CAPD group. The base excess showed a significant increase in the haemodialysis group (P < 0.01) but no change in the CAPD group (P > 0.5).

Erythrocyte 2,3-DPG was low in the haemodialysis group before treatment, but rose to within the normal range with haemodialysis (P < 0.05). In the CAPD group, 2,3-DPG was raised before CAPD but fell with treatment to within the normal range (P < 0.01). Erythrocyte ATP was raised in the haemodialysis group but fell to normal with treatment (0.1 > P > 0.05), whereas the mean erythrocyte ATP remained in the normal range in the CAPD group throughout.

Erythrocyte glucose consumption was reduced and lactate production normal in the haemodialysis patients before treatment. After at least 2 months' treatment, both glucose consumption and lactate production increased significantly (P ≈ 0.02 and P < 0.05 respectively). In the CAPD group, glucose consumption and lactate production were both increased before treatment and lactate production increased significantly (P > 0.02). The levels of glycolytic intermediates are shown in Figs. 1 and 2, where they are expressed

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**Table 1. Results of investigations in patients receiving haemodialysis**

<table>
<thead>
<tr>
<th></th>
<th>Pre haemodialysis</th>
<th>On haemodialysis</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>7.5 ± 0.5</td>
<td>8.1 ± 0.9</td>
<td>0.5 &gt; P &gt; 0.1</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>2.3 ± 0.4</td>
<td>2.1 ± 0.5</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>P\textsubscript{i}  (mmol/l)</td>
<td>2.19 ± 0.18</td>
<td>1.40 ± 0.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma pH (pH\textsubscript{r})</td>
<td>7.324 ± 0.02</td>
<td>7.365 ± 0.03</td>
<td>0.5 &gt; P &gt; 0.1</td>
</tr>
<tr>
<td>Intracellular pH (pH\textsubscript{i})</td>
<td>7.208 ± 0.01</td>
<td>7.224 ± 0.002</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>pH\textsubscript{b}</td>
<td>0.172 ± 0.01</td>
<td>0.162 ± 0.02</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>p\textsubscript{a}CO\textsubscript{2}</td>
<td>7.222 ± 0.55</td>
<td>7.280 ± 0.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Base excess</td>
<td>-11.6 ± 2.3</td>
<td>-3.3 ± 0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>P\textsubscript{a}O\textsubscript{2} (mmHg)</td>
<td>29.3 ± 1.3</td>
<td>33.2 ± 1.4</td>
<td>P &gt; 0.1</td>
</tr>
<tr>
<td>P\textsubscript{a}CO\textsubscript{2} (mmHg)</td>
<td>26.9 ± 0.9</td>
<td>32.0 ± 1.5</td>
<td>0.1 &gt; P &gt; 0.05</td>
</tr>
<tr>
<td>Erythrocyte 2,3-DPG (mmol/l of cells) (4.55–5.65)</td>
<td>3.78 ± 0.70</td>
<td>5.51 ± 0.40</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Erythrocyte ATP (mmol/l of cells) (0.85–1.45)</td>
<td>1.64 ± 0.10</td>
<td>1.36 ± 0.20</td>
<td>0.1 &gt; P &gt; 0.05</td>
</tr>
<tr>
<td>Glucose consumption (mmol/l of cells) (3.80–4.50)</td>
<td>3.48 ± 0.49</td>
<td>4.57 ± 0.27</td>
<td>&gt;0.02</td>
</tr>
<tr>
<td>Lactate production (mmol/l of cells) (4.60–6.60)</td>
<td>6.26 ± 0.62</td>
<td>7.41 ± 0.59</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Table 2. Results of investigations in patients receiving continuous ambulatory peritoneal dialysis**

<table>
<thead>
<tr>
<th></th>
<th>Pre CAPD</th>
<th>On CAPD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>7.8 ± 0.6</td>
<td>10.2 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>2.7 ± 0.5</td>
<td>2.6 ± 0.4</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>P\textsubscript{i}  (mmol/l)</td>
<td>2.05 ± 0.13</td>
<td>1.41 ± 0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma pH (pH\textsubscript{r})</td>
<td>7.400 ± 0.02</td>
<td>7.412 ± 0.02</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Intracellular pH (pH\textsubscript{i})</td>
<td>7.204 ± 0.01</td>
<td>7.204 ± 0.02</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>pH\textsubscript{b}</td>
<td>0.185 ± 0.01</td>
<td>0.204 ± 0.02</td>
<td>0.5 &gt; P &gt; 0.1</td>
</tr>
<tr>
<td>pH\textsubscript{a}CO\textsubscript{2}</td>
<td>7.410 ± 0.05</td>
<td>7.375 ± 0.02</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Base excess</td>
<td>-2.8 ± 1.5</td>
<td>-3.5 ± 1.0</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>P\textsubscript{a}O\textsubscript{2} (mmHg)</td>
<td>29.9 ± 1.0</td>
<td>30.0 ± 1.3</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>P\textsubscript{a}CO\textsubscript{2} (mmHg)</td>
<td>30.1 ± 1.1</td>
<td>30.2 ± 1.1</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Erythrocyte 2,3-DPG (mmol/l of cells) (4.55–5.65)</td>
<td>6.84 ± 0.60</td>
<td>5.00 ± 0.20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Erythrocyte ATP (mmol/l of cells) (0.85–1.45)</td>
<td>1.18 ± 0.10</td>
<td>1.28 ± 0.12</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Glucose consumption (mmol/l of cells) (3.80–4.50)</td>
<td>4.78 ± 0.54</td>
<td>2.69 ± 0.76</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Lactate production (mmol/l of cells) (4.60–6.60)</td>
<td>8.72 ± 0.60</td>
<td>7.52 ± 0.74</td>
<td>0.5 &gt; P &gt; 0.1</td>
</tr>
</tbody>
</table>
Evthrocyte metabolism in chronic renal failure

Both groups of patients show the increased levels of intermediates before 2,3-DPG in the glycolytic pathway, which is typical of patients with severe anaemia [27]. The major changes in intermediates with dialysis were a rise in glyceraldehyde 3-phosphate ($P < 0.05$) and 2,3-DPG ($P < 0.05$) in the haemodialysis group and a fall in the CAPD group in fructose diphosphate ($P > 0.1$), glyceraldehyde 3-phosphate ($P < 0.01$) and 2,3-DPG ($P < 0.05$). The changes in intermediates in the two groups are shown in Fig. 3, where the changes are expressed as percentages of the initial concentrations of the intermediates. The changes in intermediates had an approximately reciprocal relationship in the two groups. Only 2,3-DPG changed significantly, which was largely due to the small scatter of the measurements and the fact that the changes in 2,3-DPG with dialysis were almost entirely in the same direction in each group.

In the haemodialysis group, there were significant correlations between base excess and glucose consumption ($r = 0.66, P < 0.01$) and lactate production and 2,3-DPG ($r = 0.67, P < 0.01$) (Fig. 4). There was a correlation between base excess and both lactate production ($r = 0.41, 0.1 > P > 0.05$) and 2,3-DPG ($r = 0.37, 0.5 > P > 0.1$). In the CAPD group, there were significant correlations between Hb and 2,3-DPG ($r = -0.75, P < 0.001$), $P_{50}$ and 2,3-DPG ($r = 0.52, P < 0.05$) (Fig. 5), glucose consumption and 2,3-DPG ($r = 0.52, P < 0.05$) and lactate production and 2,3-DPG ($r = 0.56, P < 0.02$) (Fig. 6). There was a correlation between $P_{50}$ and glucose consumption ($r = 0.40, 0.1 > P > 0.05$) and between $P_{50}$ and lactate production ($r = 0.39, P < 0.1$).

$P_{50}$ was increased in both groups before starting haemodialysis or CAPD [29.3 mmHg...
(3.9 kPa) and 29.9 mmHg (4.0 kPa) respectively; normal 26.7 mmHg (3.6 kPa)). $P_{50(\text{v.})}$ increased still further in the haemodialysis group despite the correction of acidosis (29.3–33.2 mmHg; 3.9–4.4 kPa) ($P \approx 0.1$). However, the $P_{50(\text{v.})}$ increased in the haemodialysis group from normal (26.9 mmHg; 3.6 kPa) to 32.0 mmHg (4.3 kPa) ($0.1 > P > 0.05$). The latter value is comparable with that in the CAPD group throughout the period of investigation. As there was no significant acidosis in the patients on CAPD, $P_{50(\text{v.})}$ and $P_{50(\text{v.})}$ were similar.

**Discussion**

In the normal subject without anaemia, there is a direct relationship between $P_i$ and erythrocyte glycolytic rate [28–30], so that erythrocyte ATP and 2,3-DPG are related to plasma and erythrocyte $P_i$ [11], as they are produced entirely by glycolysis. In the anaemic subject without renal disease (with normal acid–base status and $P_i$), 2,3-DPG levels are inversely related to Hb. This is due to the increased concentration of deoxyhaemoglobin in anaemia. Deoxyhaemoglobin is a
stronger base than oxyhaemoglobin and removes protons from the environment, which causes intracellular alkalosis and thereby stimulates glycolysis [7].

As is now established [31], the increase in 2,3-DPG in anaemia increases tissue oxygen delivery by shifting the Hb-O$_2$ dissociation curve to the right, as shown by an increased P$_{50}$. This mechanism maintains tissue oxygenation and provides some compensation for the reduced oxygen-carrying capacity of the blood in anaemia. Mitchell & Pegrum [9] estimated that in 30 anaemia patients with chronic renal failure there was a mean advantage due to the right shift of the curve of 0.5 g/dl above the measured Hb, which could result in a saving of as much as 25% in cardiac output in the resting subject.

In chronic renal failure, the simple relationships between P$_t$ and Hb and 2,3-DPG are complicated by a variable degree of metabolic acidosis. Erythrocyte glycolytic rate is greatly influenced by [H$^+$] as well as by P$_t$ and Hb [32]. In previous studies of erythrocyte metabolism in chronic renal failure, erythrocyte phosphorylated compounds including intermediates (2,3-DPG, fructose diphosphate and glyceraldehyde 3-phosphate) and adenine nucleotides (ADP and ATP) have been found to be raised or normal [2, 33–36]. Increased activities of some erythrocyte enzymes in a young cell population due to the increased rate of haemolysis in uraemia [35, 37] will lead to a change in the relative levels of glycolytic intermediates. A relationship between the levels of intermediates and plasma and/or intracellular P$_t$ has been reported in chronic renal failure by Lichtman and his colleagues [6, 38]. However, most workers have not measured all of the glycolytic intermediates, nor in many cases is the acid–base status of the patients known.

Our results show clear differences between the haemodialysis and CAPD groups. Before treatment the haemodialysis group, who had received no previous dialysis, had a partially compensated metabolic acidosis and low 2,3-DPG levels (Table 1). As the metabolic abnormality was corrected 2,3-DPG rose to within the normal range. This rise in 2,3-DPG occurred despite the fall in P$_t$ and there was no correlation between P$_t$ and 2,3-DPG. This contrasts with the CAPD group, in whom base excess was not significantly abnormal on either of the two occasions on which it was measured (Table 2). There was a significant increase in both glucose consumption and lactate production and a significant correlation between base excess and glucose consumption and between lactate production and 2,3-DPG. Taken together, these results suggest that the usual relationships between Hb and P$_t$ and 2,3-DPG were overshadowed in these patients by the profound effects of the metabolic acidosis on glycolytic rate (in spite of partial compensation) and thereby on 2,3-DPG and Hb–O$_2$ affinity. The net result of these changes in the haemodialysis group was an increase in both $P_{50(1,5)}$ ($P \approx 0.1$) and $P_{50(1,7,4)}$ ($0.1 > P > 0.05$). Although the increase in $P_{50(1,7,4)}$ did not reach statistical significance, this coupled with the small increase in Hb could lead to improved tissue oxygen delivery as a result of chronic haemodialysis. In view of the fact that there was no significant change in extracellular or intracellular pH (pH$_e$ or pH$_i$) with dialysis in the haemodialysis group but a significant rise in base excess, which correlated with erythrocyte glucose con-
It is an intriguing possibility that IHC03− concentration as well as [H+] has a direct effect on erythrocyte glycolysis. This possibility requires further investigation and clarification.

In the CAPD group, there was no metabolic acidosis as the patients had received intermittent peritoneal dialysis (or dietary restriction) and the base excess was around -3.0 on both occasions it was measured. Hb rose significantly on CAPD, which confirms the findings of others [39]. Erythrocyte 2,3-DPG fell during CAPD from an elevated level to within the normal range, which correlated with both the fall in plasma P50 and the increase in Hb. As would be expected in anaemia without acidosis or renal disease, there were significant correlations between 2,3-DPG and both glucose consumption and lactate production. Surprisingly, despite the fall in 2,3-DPG with constant pH and base excess, there was no change in P50. These results imply that patients on CAPD benefit not only from an increase in oxygen-carrying capacity due to increased Hb, but at the same time do not experience any deterioration in tissue oxygen delivery due to a fall in P50, which would be expected to occur owing to the fall in 2,3-DPG.

The factors responsible for the failure of P50 to fall in these patients remain to be elucidated. From the nomogram of Musetti et al. [40], which relates P50 to PCO2, pH and the molar ratio of 2,3-DPG to Hb, the CAPD patients would have been expected to have a mean P50(v.,) of 29.3 mmHg (3.91 kPa) before CAPD, compared with the closely corresponding observed mean of 29.9 mmHg (3.99 kPa). However, the nomogram would predict a mean P50(v.,) of 26.8 mmHg (3.57 kPa) after 6 months’ CAPD, whereas the observed value was 30.0 mmHg (4.0 kPa). Since acid–base status remained unchanged and 2,3-DPG fell, our results after CAPD are difficult to explain, but might suggest the existence of other factors which influence the position of the Hb–O2 dissociation curve. However, the numbers of patients studied were relatively small. In contrast, in the haemodialysis group P50 values are those expected from the measured values of pH, Hb and 2,3-DPG.

The major regulating steps for glycolysis are those controlled by hexokinase (EC 2.7.1.1), 6-phosphofructokinase (EC 2.7.1.40), pyruvate kinase (EC 2.7.1.11); DPGM, 2,3-diphosphoglycerate mutase (EC 2.7.5.4); DPGP, 2,3-diphosphoglycerate phosphatase (EC 3.1.3.13). Factors stimulating (†) and inhibiting (‡) enzyme activity are shown.

In the CAPD group, there was no metabolic acidosis as the patients had received intermittent peritoneal dialysis (or dietary restriction) and the base excess was around -3.0 on both occasions it was measured. Hb rose significantly on CAPD, which confirms the findings of others [39]. Erythrocyte 2,3-DPG fell during CAPD from an elevated level to within the normal range, which correlated with both the fall in plasma P50 and the increase in Hb. As would be expected in anaemia without acidosis or renal disease, there were significant correlations between 2,3-DPG and both glucose consumption and lactate production. Surprisingly, despite the fall in 2,3-DPG with constant pH and base excess, there was no change in P50. These results imply that patients on CAPD benefit not only from an increase in oxygen-carrying capacity due to increased Hb, but at the same time do not experience any deterioration in tissue oxygen delivery due to a fall in P50, which would be expected to occur owing to the fall in 2,3-DPG.

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The major regulating steps for glycolysis are those controlled by hexokinase (EC 2.7.1.1), 6-phosphofructokinase (EC 2.7.1.40), pyruvate kinase (EC 2.7.1.11) and 2,3-DPG mutase (diphosphoglyceromutase, EC 2.7.5.4) (see Fig. 7) [29, 32, 41]. The increase in glucose consumption with haemodialysis implies activation of hexokinase and phosphofructokinase, as suggested by Brewer et al. [27]. However, the relative activities of different enzymes will determine the levels of specific intermediates. From Fig. 1 it can be seen that whereas glyceraldehyde-3-phosphate and 2,3-DPG are increased by haemodialysis, 3-phosphoglycerate and 2-phosphoglycerate are reduced. This would suggest that 2,3-DPG concentration was increased primarily by changes within the Rapoport–Luebering shunt, with an increase in intermediates proximal to 2,3-DPG and a decrease in those distal to 2,3-DPG in the glycolytic pathway. 2,3-DPG mutase activity has been demonstrated to increase significantly with a small rise in pH [42] whereas 2,3-DPG phosphatase (diphosphoglycerate phosphatase, EC 3.1.3.13) activity is inhibited by a rise in pH and also by a fall in Pi [43]. Our results indicate that in the haemodialysis group the rise in 2,3-DPG was primarily mediated within the Rapoport–Luebering shunt owing to the combined effects of a rise in pH and a reduction of Pi (Table 1).

The pattern of intermediates in the CAPD group differs quite strikingly (Fig. 2). Apart from glucose 6-phosphate and fructose 6-phosphate, the levels of all intermediates including 2,3-DPG were reduced after 6 months of CAPD. The increase in the early intermediates with reduction of those distal to the phosphofructokinase step (see Fig. 3) would suggest that a decrease in phosphofructokinase activity was the major
factor in bringing about the fall in 2,3-DPG in these patients [28]. Tsuboi & Fukunaga [44] found that P1 stimulated phosphofructokinase activity, leading to enhanced glucose utilization but without increased lactate production, the latter being limited at a step subsequent to triose phosphate formation. The fall in P1 in the CAPD patients would account for our findings of a significant fall in glucose consumption without a significant fall in lactate production.

We conclude that where acidosis is marked in chronic renal failure, it is the predominant factor influencing erythrocyte metabolism and thereby Hb–O2 affinity, whereas in less acidotic patients P1 and Hb concentrations are more important. Despite the major metabolic changes in both groups, the erythrocyte adapted without significant change in $P_{50}$ (l.v.). However, there was a small increase in $P_{50}$ (l.v.) in the haemodialysis group as the acidosis was corrected. A similar increase 2,3-DPG with a resultant increase in P1, can be induced in patients with chronic renal failure by administration of daily oral sodium bicarbonate to prevent acidosis and increase Hb–O2 affinity, whereas in less acidotic patients the erythrocyte adapted without significant change in Hb–O2 affinity, whereas in less acidotic patients the erythrocyte adapted without significant change in $P_{50}$ (l.v.).

References


