Red Cell Metabolism and Haemolysis in Patients on Dialysis

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A haemolytic component of nephrogenic anaemia has been repeatedly documented in patients treated conservatively (Desforges & Dawson, 1958; Stewart, 1967) as well as in those under maintenance dialysis. However, its exact pathogenetic mechanism has not been elucidated and it has not been established which metabolic alterations of the erythrocyte take place. In one hypothesis Bock et al (1962) postulated that the metabolic acidosis of renal insufficiency is responsible for the accelerated red cell destruction. The erythrocyte is able to metabolise glucose to lactate either directly via the so-called Embden-Meyerhof pathway or by the quantitatively less important pentose shunt. The first enzyme in this chain of reactions is hexokinase (HK). Because it is quite sensitive to a decrease in pH, it has been claimed that metabolic acidosis would induce a decrease in glycolysis with a decreased formation of ATP and also of the reduced form of glutathione (GSH) in the pentose shunt (Figure 1). Since GSH is of importance for the reduction of methaemoglobin (Met-Hb) to haemoglobin, Met-Hb concentration would increase. Both the slowing of glycolysis and the decrease in GSH-concentration with increased formation of Met-Hb could then result in decreased stability of haemoglobin, alterations of the cell membrane (Weed & Reed, 1966) and increased destruction of erythrocytes (Bock et al, 1962). In order to test this hypothesis a study of red cell metabolism was performed in twelve patients on maintenance dialysis.

METHODS

Twelve patients with irreversible chronic renal failure were investigated. They were dialysed for seven to ten hours twice weekly (except one patient with one dialysis per week) with coil dialysers in a recirculating-single-pass-system, using a dialysate acetate concentration of 38 mM/L. Blood was drawn immediately before dialysis.
Figure 1. Simplified scheme of glucose degradation in red cells (abbreviations see text)

The following erythrocyte enzymes were determined (methods in parentheses): Hexokinase (HK) (Grignani & Löhr, 1960); glucose-6-phosphate dehydrogenase (G-6-PD) (Deubelbeiss & Marti, 1965); 6-phosphogluconate dehydrogenase (6-PGD) (Jütting et al., 1965); pyruvatekinase (PK) (Tanaka et al., 1962); glutathionereductase (GR) (Beutler & Yeh, 1963). GSH was measured by the method of Stevenson et al., 1960 and Met-Hb by that of Evelyn and Malloy, 1938. ATP, 2, 3-diphosphoglycerate (2, 3-DPG) and whole blood pH were measured as described previously (Blumberg & Marti, 1972). For estimation of red cell half life the 51Cr method was used.

RESULTS

Predialysis whole blood pH was in the normal range in all patients except the one who was dialysed once weekly. The mean value of all patients was 7.37. Red cell half life on the other hand was frankly decreased with a mean of 19 days (normal 26 to 33 days) (Figure 2).

Of the five enzymes measured G-6-PD was normal, whereas the other four were elevated (Table I). GSH and Met-Hb were in the normal range, except in one patient who consumed phenacetin containing analgesics and who showed a slightly elevated Met-Hb-concentration. ATP and 2, 3-DPG on the other hand were significantly elevated (Table II).
Table I. Red cell enzymes (mean ± 2 SD)

<table>
<thead>
<tr>
<th></th>
<th>HK (IU/10^11)</th>
<th>PK (IU/10^11)</th>
<th>G-6-PD (erythrocytes)</th>
<th>6-PGD</th>
<th>GR (IU/10^11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>1.8* ± 1.2</td>
<td>64.8** ± 36.6</td>
<td>17.2 ± 7.8</td>
<td>18.3** ± 6.0</td>
<td>15.2** ± 7.8</td>
</tr>
<tr>
<td>Controls</td>
<td>1.4 ± 0.5</td>
<td>39.6 ± 15.7</td>
<td>16.2 ± 4.0</td>
<td>13.1 ± 3.0</td>
<td>10.4 ± 3.5</td>
</tr>
</tbody>
</table>

* Significant difference from normal, p < 0.05
** Significant difference from normal, p < 0.001

Table II. GSH, ATP and 2,3-DPG (mean ± 2 SD) and Met-Hb (range)

<table>
<thead>
<tr>
<th></th>
<th>GSH (mg/100 ml)</th>
<th>Met-Hb (%)</th>
<th>ATP (μmoles/10^11)</th>
<th>2,3-DPG (μmoles/10^11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>73.6 ± 5.0</td>
<td>0.18 (0-0.73)</td>
<td>27.9* ± 13.8</td>
<td>52.1* ± 21.4</td>
</tr>
<tr>
<td>Controls</td>
<td>73.9 ± 21.2</td>
<td>0-0.5</td>
<td>15.4 ± 7.1</td>
<td>33.9 ± 13.6</td>
</tr>
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* Significant difference from normal, p < 0.001

DISCUSSION

From our data it is evident that in patients on maintenance dialysis with a normal predialysis pH a moderate but definite degree of haemolysis with a decrease in red cell half life persists. In a previously published study (Eschbach et al, 1967) a similar degree of haemolysis has been found in dialysed patients. The estimation of the activity of five red cell enzymes revealed a significant elevation in four, among them hexokinase. It might be argued that enzyme activities as determined in vitro under optimal conditions do not reflect the in vivo situation. However, it must be pointed out that the formation of two products of glucose degradation proceeded at a
normal or accelerated pace: red cell ATP concentration was elevated and GSH was in the normal range (as well as Met-Hb concentration). Therefore, it can be assumed that the activity of enzymes involved in glucose metabolism was also normal or elevated in vivo. It seems likely that the increase in enzymatic activity was mostly due to a reduction in red cell age: a relationship between enzyme activity and red cell age has been demonstrated for HK, PK and 6-PGD (Brewer & Powell, 1963; Powell & DeGowin, 1965; Sass et al., 1964), but not for GR, which may have been elevated due to other factors (Bonsignore et al., 1964).

It is evident that the hypothesis mentioned above cannot be correct: in patients on maintenance dialysis the reduction in red cell lifespan cannot be due to a pH induced alteration of red cell metabolism, for pH as well as enzyme activities are not depressed in these patients. It is still possible that a disturbance in red cell membrane function might be present. For example, hypoactivity of the Na-K-ATPase (reversible upon dialysis) has been described (Welt, 1970). However, the exact mechanism of haemolysis remains to be established. It has been shown that an increase in red cell 2, 3-DPG (and to a lesser extent in ATP) concentration is of importance for tissue oxygenation, because it induces a shift of the O2-haemoglobin dissociation curve to the right; thereby the affinity of haemoglobin for oxygen is decreased and oxygen release in tissue is facilitated. This mechanism of adaptation has been described in several conditions with impaired tissue oxygenation, among them anaemia (Torrance et al., 1970).

In our patients a similar increase in the concentration of red cell organic phosphates was found as in a previous study (Blumberg & Marti, 1972); this is considered to represent an adaptation to anaemia.

**SUMMARY**

(1) Patients maintained on twice weekly dialysis using a dialysate acetate concentration of 38 mM/L revealed a moderate degree of haemolysis in the presence of a normal predialysis blood pH.

(2) Activities of several red cell enzymes of the glycolytic pathway and pentose shunt were generally elevated due in part to a reduction in mean erythrocyte age, while ATP concentration was elevated and GSH formation and Met-Hb reduction were normal.

(3) The increase in ATP and 2, 3-DPG-concentration represents a mechanism of adaptation to anaemia improving tissue oxygenation.

(4) The accelerated haemolysis of patients on maintenance dialysis cannot be explained by a pH induced alteration of red cell metabolism. Its exact mechanism remains to be elucidated.
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OPEN DISCUSSION

E RITZ (Heidelberg): I may have missed it, but how did you correct for blood
loss into the dialyser when you measured erythrocyte half lives?

BLUMBERG: This has been measured repeatedly in the literature. You can
measure (or at least estimate) blood loss and the blood losses you encounter
certainly could not account for the decrease in average red cell life span.

RITZ: Did you correct for it?

BLUMBERG: We did not correct for it, but even if you did, I believe you will
come out with approximately the same data.

A MEMBER (Germany): Did you measure your 2,3-DPG and ATP concentra-
tion before and after dialysis or only once in the patient? I ask this question
because we did studies on the sodium flux, on erythrocyte membranes and
found that there was a significant decrease in sodium exchange, especially
in sodium efflux before dialysis; but after a 10 hours' haemodialysis the
sodium efflux became normal.
BLUMBERG: The data I showed you for ATP and 2,3-DPG were measured pre-dialysis. In another study, not shown here, we measured DPG throughout a dialysis cycle. That is, pre-dialysis and post-dialysis after 24, 48, and 72 hours. We could not find a significant change in 2,3-DPG concentration in the red cells.

H J KRAMER (Homburg, Saar): Dr Blumberg, I would like to ask you about glucose dehydrogenase activity. We have found a significant increase in this activity, and since this enzyme initiates the pentose phosphate cycle this adds evidence to the suggestion that the pentose phosphate cycle is activated in blood cells in uraemia. I have two questions: did you correlate the ATP concentrations in your patients with the extra cellular phosphate concentration? It has been suggested that since extra cellular phosphate is increased this might stimulate the metabolism and synthesis of ATP. The second question is: did you measure the intracellular sodium and potassium concentration pre- and post-dialysis in red blood cells from your patients?

BLUMBERG: Can I answer the second question first? The answer is 'No'. To answer your first question, we did correlate ATP and DPG with plasma inorganic phosphate. ATP was very weakly correlated with a p of just less than 0.01. DPG did not correlate with plasma inorganic phosphate. The same has been found in dialysed patients by other authors. We don't know why this is so because in patients who are not maintained by dialysis, there is a close correlation between plasma inorganic phosphate and ATP, and rather poorer correlation between poorer correlation between DPG and inorganic phosphate. I don't know why dialysis patients behave differently.

C M KJELLSTRAND (Minneapolis): We have studied haemolysis in our patients too and found that we have a form of genetic phenomenon. Dialysate prepared with tap water will induce haemolysis in about one or two-thirds of our patients, and this haemolysis will increase to explosive proportions if you challenge them with an oxidative agent like (for example) sulphonamides or primaquine. My questions are: did you dialyse your patients against untreated tap water, or how did you purify your water? The second question is: were any of your patients on oxidative agents?

BLUMBERG: No, they were not on oxidative agents. The dialysate in our unit is prepared with softened water and not tap water: the content of calcium is much too high in our region to use untreated tap water.