Hemoglobin Affinity for Oxygen in Chronic Renal Disease: The Effect of Hemodialysis

By Marshall A. Lichtman, Marion S. Murphy, Barbara J. Byer, and Richard B. Freeman

The affinity of hemoglobin for oxygen may increase significantly in subjects who are hypophosphatemic and alkalotic. We studied the organic phosphate content and oxygen binding by hemoglobin of red cells in subjects undergoing hemodialysis, during which time a decrease in plasma inorganic phosphate and an increase in blood pH may occur. Red cell 2,3-DPG was not correlated with plasma inorganic phosphorus, whereas red cell ATP was highly correlated with plasma inorganic phosphorus when analyses were made on predialysis samples. Predialysis red cell inorganic phosphorus was highly correlated with plasma inorganic phosphorus, supporting the concept that inorganic phosphorus is maintained by a gradient from plasma to cell. Plasma inorganic phosphorus decreased by 45% during the period of hemodialysis, whereas red cell inorganic phosphorus did not change. Red cell 2,3-DPG, ATP, and oxygen binding by hemoglobin at standard conditions of temperature, pH, and pCO₂ were not altered after 6 hr of hemodialysis. Plasma pH and base excess increased during dialysis. The increase in base excess, an estimate of the non-pH-dependent effect of CO₂ on oxygen binding by hemoglobin, counterbalanced a portion of the effect of elevated pH on hemoglobin-oxygen affinity under in vivo conditions. Hence, only a slight increase in oxygen binding by hemoglobin occurred. Moreover, late dialysis symptoms were not associated with the degree of alkalosis or with the extent of change in hemoglobin's affinity for oxygen. Red cell 2,3-DPG content was lower and hemoglobin's affinity for oxygen was higher in subjects with chronic renal disease than in nonazotemic subjects with similar hemoglobin deficits. Moreover, increased red cell ATP in chronic renal disease patients did not influence oxygen binding by hemoglobin.

Patients with severe chronic renal disease requiring hemodialysis are markedly anemic and would be expected to have an increase in red cell 2,3-diphosphoglycerate (2,3-DPG) and subsequently hydrogen ion leading to a decrease in hemoglobin's affinity for oxygen.

Extracellular factors are known to influence indirectly the affinity of hemo-
globin for oxygen. Plasma inorganic phosphate (P$_i$)\(^1\) and plasma pH\(^4\) may both affect the intracellular environment so as to change the ability of hemoglobin to bind oxygen. A reduction in plasma P$_i$, or an increase in plasma pH, both known to occur during hemodialysis, could act to increase hemoglobin–oxygen affinity and thereby reduce oxygen transport in patients compromised by a blood hemoglobin deficit. The following studies were conducted to assess hemoglobin function during hemodialysis.

**METHODS**

**Study Subjects**

Eighteen consecutive patients with chronic renal disease were studied immediately before and after 6 hr of hemodialysis using a hollow-fiber artificial kidney. These subjects had been receiving thrice-weekly hemodialysis for at least 3 mo prior to study. Fifteen milliliters of blood was obtained anaerobically before and after dialysis from the arterial side of the patient's fistula or shunt, anticoagulated with sodium heparin (15 \(\mu\)l/ml), chilled on ice, and studies made immediately thereafter. Three patients had been transfused 8-12 wk prior to study.

**Physicochemical Studies**

Blood hemoglobin was measured in duplicate by the cyanmethemoglobin method, and hematocrit was measured in triplicate in an International Equipment Company microhematocrit centrifuge at approximately 10,000g for 5 min. P$_i$ was measured in trichloracetic acid extracts of plasma and red cells, the latter previously washed three times with ice-cold 0.17 M NaCl.\(^5\) Tissue water content was measured on 1 ml of whole blood of known packed cell volume and 1 ml of autologous plasma that were weighed in duplicate before and after dessication at 80°C for 24 hr. Calculation of the water content per gram of cells or plasma could be made thereby. Red cell 2,3-DPG was measured by the method of Rose and Liebowitz\(^6\) and red cell adenosine triphosphate (ATP) by the luciferase method.\(^7\) Hemoglobin oxygen saturation was measured with an Instrumentation Laboratory (I.L.) Model 182 cooximeter. pH, oxygen tension (P$_o$), and carbon dioxide tension (P$_c$) were determined with I.L. Model 113 pH-gas analyzer. An I.L. Gas mixing module, Model No. 2081, Oxygen Monitor Model No. 2083, and Model 137 Tonometer were used to adjust P$_o$ to between 15 to 60 torr while CO$_2$ was maintained at 40 ± 0.2 torr. Each determination was made in duplicate. The P$_o$ at which hemoglobin was 50% saturated at 37°C, pH 7.4, P$_c$ = 40 torr (P$_c$ std.) was derived from a least-squares analysis of the experimental points. Base excess (BE) was calculated from the blood pH and P$_c$ as described by Severinghaus.\(^7\) The resultant P$_o$ at standard conditions was converted to P$_o$ in vivo by the formula: log P$_o$ (i.v.) = log P$_o$ std. + 0.0022 BE + 0.52 (7.40 pH) + 0.024 (T–37°C).\(^8\)

**RESULTS**

Plasma P$_i$ fell in each of the ten consecutive patients studied, and the mean concentration was reduced 400 (Table I). Four of the ten patients studied had normal or low plasma P$_i$ because of chronic administration of aluminum hydroxide gel (see Table I, patients F.W., D.M., M.R., and C.S.). The proportional reduction in plasma P$_i$, with dialysis was closely correlated with the height of the predialysis plasma P$_i$ values.

Red cell 2,3-DPG (16 ± 0.82 \(\mu\)moles/g Hb) was unchanged after 6 hr of dialysis (16.3 ± 0.82) (Table I), and although red cell 2,3-DPG was higher than that of healthy subjects (14.6 ± 0.39 \(\mu\)moles/g Hb) measured in our laboratory, it was below that expected for anemic subjects with a mean hemoglobin of 7.2 g/100 ml based on the studies of Torrance et al. (21 \(\mu\)moles/g Hb),\(^1\) and studies from our laboratory, in which the regression of 2,3-DPG on blood hemoglobin in anemic subjects without renal disease is Y = 27.7 – 0.88 X, where X = blood
Table 1. Blood Hemoglobin, pH, 2,3-DPG, ATP, and Hemoglobin–Oxygen Affinity Before and After Dialysis

| Study Subject | Blood Hb (g/100 ml) | MCHC (g/100 ml) | Blood pH | Base Excess (mMoles/liter) | Plasma P50 (pMoles/ml) | 2,3-DPG (pMoles/g Hb) | ATP (pMoles/g Hb) | P50 Standard (torr) | P50 (in vivo) (torr) | Δ P50 (in vivo) (torr) | Late Dialysis Symptoms |
|---------------|----------------------|------------------|----------|--------------------------|------------------------|-----------------------|---------------------|-------------------|-------------------|---------------------|------------------------|------------------------|
| J.W.          | 8.10                 | 33.1             | 7.37     | 7.46                     | 2.0                    | 1.86                  | 1.43                | 16.1              | 17.2              | 7.40                | 7.04                   | 26.5                   | 27.3                   | 27.7              | 25.7              | -2.0               | -                     |
| S.W.          | 6.97                 | 31.5             | 7.31     | 7.44                     | -3.5                   | 2.10                  | 1.22                | 17.7              | 16.1              | 6.50                | 7.05                   | 26.5                   | 26.5                   | 29.0              | 25.5              | -3.5               | -                     |
| G.F.          | 6.56                 | 32.9             | 7.36     | 7.44                     | -3.5                   | 2.35                  | 1.40                | 13.7              | 14.8              | 8.48                | 8.41                   | 23.7                   | 26.3                   | 24.4              | 24.5              | +0.1               | -                     |
| R.J.          | 9.40                 | 30.8             | 7.40     | 7.41                     | -3.0                   | -1.0                  | 1.54                | 14.7              | 14.2              | 5.42                | 7.05                   | 25.5                   | 26.0                   | 25.1              | 25.6              | +0.5               | +                     |
| M.Mc.         | 6.70                 | 31.2             | 7.37     | 7.47                     | -3.5                   | +4.0                  | 1.93                | 18.9              | 19.2              | 7.91                | 6.96                   | 25.5                   | 25.0                   | 25.9              | 23.5              | -2.4               | -                     |
| M.B.          | 8.86                 | 33.8             | 7.38     | 7.42                     | -2.5                   | -2.5                  | 2.95                | 13.3              | 13.9              | 7.13                | 6.75                   | 25.5                   | 25.0                   | 25.8              | 24.1              | -1.7               | -                     |
| F.W.          | 8.50                 | 30.9             | 7.48     | 7.46                     | +0.5                   | +0.5                  | 0.61                | 22.8              | 22.1              | 3.33                | 3.36                   | 27.0                   | 28.0                   | 24.6              | 26.1              | +1.5               | -                     |
| D.M.          | 6.79                 | 29.8             | 7.40     | 7.44                     | +0.5                   | +3.0                  | 0.55                | 15.8              | 15.5              | 3.83                | 4.47                   | 25.5                   | 25.3                   | 25.5              | 24.5              | -1.0               | +                     |
| M.R.          | 5.42                 | 30.4             | 7.43     | 7.44                     | +5.5                   | +4.5                  | 0.86                | 14.5              | 14.3              | 6.28                | 6.25                   | 24.3                   | 25.0                   | 24.1              | 24.4              | +0.3               | -                     |
| C.S.          | 6.97                 | 33.7             | 7.45     | 7.49                     | +1.5                   | +2.5                  | 1.05                | 14.9              | 15.6              | 4.95                | 5.45                   | 25.5                   | 25.7                   | 24.2              | 23.4              | -0.8               | -                     |
| Mean          | 7.23                 | 31.8             | 7.40     | 7.46                     | -0.6                   | +1.5                  | 1.58                | 16.2              | 16.3              | 6.11                | 6.30                   | 25.6                   | 26.0                   | 25.6              | 24.7              | -0.9               | -                     |
| SD            | 1.1                  | 1.4              | 0.050    | 0.025                    | 0.80                   | 0.37                  | 2.9                 | 2.6               | 1.7                 | 1.5                    | 0.99                   | 1.0                    | 1.6               | 0.96              | -                  | -                     |
| SE            | 0.36                 | 0.45             | 0.016    | 0.008                    | 0.25                   | 0.12                  | 0.82                | 0.82              | 0.54                | 0.46                   | 0.31                   | 0.32                   | 0.51              | 0.30              | -                  | -                     |

Ten healthy subjects

| Mean          | 14.6                 | 33.3             | 7.39     | 7.46                     | +2.9                   | 1.12                  | 14.6                | 3.48              | 26.1              | 26.7                   | -                     |
| SD            | 0.70                 | 0.63             | 0.026    | (1.0-5.0)* | 0.176                  | 1.2                   | 0.22                | 0.81              | 1.2               | -                     |

*Range.
†Antecubital vein blood.
hemoglobin in g/100 ml and \( Y = 2,3\text{-DPG} \) in \( \mu \)moles/g Hb.\(^9\) Hence, 2,3-DPG in this group of subjects would be expected to average 21.5 \( \mu \)moles/g Hb based on their blood hemoglobin concentration. Similarly, red cell ATP was not changed significantly over the period of dialysis (Table 1). Predialysis red cell ATP was 6.12 \( \pm \) 0.54 \( \mu \)moles/g Hb, and postdialysis red cell ATP was 6.30 \( \pm \) 0.46 \( \mu \)moles/g Hb, a cellular content considerably above that of healthy subjects (3.48 \( \pm \) 0.68 \( \mu \)moles/g Hb).

Hemoglobin's affinity for oxygen as indicated by \( P_{50} \) was virtually unchanged after dialysis, as would be anticipated from the unchanged red cell 2,3-DPG content (Table 1). Moreover, red cell ATP, although elevated markedly, had no influence on \( P_{50} \). Indeed, \( P_{50} \) was slightly lower in dialysis patients (25.6 \( \pm \) 0.31 torr) than in healthy subjects (26.4 \( \pm \) 0.27) despite a higher concentration of red cell ATP in the former. The slope of the regression of \( P_{50} \) on 2,3-DPG was less in dialysis subjects \([P_{50} = 3.81 (2,3\text{-DPG}/\text{Hb} \ \text{molar ratio}) + 21.8]\) than in healthy subjects \([P_{50} = 7.14 (2,3\text{-DPG}/\text{Hb} \ \text{molar ratio}) + 19.5]\).

In order to show the sensitivity of our instruments in identifying increased or decreased hemoglobin-oxygen affinity, we measured blood from subjects who were known to have such deviations. During the studies on patients undergoing hemodialysis, four samples of neonatal blood obtained from the "milked" umbilical cord immediately after delivery and found to contain 60\%-70\% alkali-resistant hemoglobin had a \( P_{50} \) of 19.5, 23.0, 23.5, and 21.5 torr, whereas three subjects with homozygous hemoglobin S were found to have a blood \( P_{50} \) of 40.8, 31.5, and 31.0 torr. Moreover, nonuremic anemic patients who were being studied throughout this time had appropriate elevations in 2,3-DPG, and \( P_{50} \) was strongly correlated with the 2,3-DPG concentration \((r = 0.85)\) (data not shown).\(^9\)

Blood pH increased in nine of ten subjects following dialysis (Fig. 1). However, base excess also increased from a mean of -0.6 mmole/liter to +2 mmole/liter (data summed algebraically). Therefore, mean \( P_{50} \) (in vivo) was barely altered from 25.6 torr before to 24.7 torr after dialysis (Table 1). Moreover, blood hemoglobin was slightly increased after dialysis (7.44 \( \pm \) 0.35 g/100 ml) as compared to the predialysis (7.25 \( \pm \) 0.36 g/100 ml) concentration, further compensating for the slight decrease in \( P_{50} \) (in vivo), such that at constant arterial and mixed venous \( P_{02} \), the A-V oxygen difference would be reduced 5\% Two of the ten subjects, R.J. and D.M., had late dialysis symptoms of headache and/or nausea. These two subjects had a +0.2\% and -4.0\% change

![Fig. 1. Correlation of red cell \( P_i \) and plasma \( P_i \) in predialysis blood samples from eight subjects.](image)
in $P_{50}$ (in vivo) with dialysis. D.M. had similar symptoms prior to onset of dialysis, making it difficult to associate them with the small changes in $P_{50}$ (in vivo). Two other subjects with relatively large decreases in $P_{50}$ (in vivo) were not symptomatic (e.g., J.W. and S.W.) (see Table 1).

Previous studies have indicated that red cell 2,3-DPG and ATP, primary determinants of oxygen binding by hemoglobin in vitro, were reduced when plasma inorganic phosphate was reduced, and hence a fall in red cell organic phosphates with dialysis was expected. However, the effect of plasma $P_i$ on red cell glycolytic rate and organic phosphates is due to the direct dependence of red cell $P_i$ concentration on extracellular $P_i$. It is known that elevation of extracellular $P_i$ results in an increase in red cell $P_i$; however, penetration of the red cell membrane by inorganic phosphate is relatively slow. Reduction of $P_i$ in the red cell may not occur during the period of hemodialysis when plasma $P_i$ is gradually reduced from high toward normal levels. Therefore, we studied eight additional subjects to examine red cell $P_i$ before and after dialysis. Again, red cell 2,3-DPG (14 ± 1.1 μmoles/g Hb) and ATP (6.25 ± 0.23 μmoles/g Hb) after 6 hr of hemodialysis were similar to predialysis values (2,3-DPG = 14.8 ± 0.91; ATP = 6.05 ± 0.28), and 2,3-DPG was again far below expected for the hemoglobin deficit. Predialysis red cell $P_i$ was highly and linearly correlated with plasma $P_i$ ($r = 0.95$) (Fig. 1). The regression equation describing this relationship was $Y = 0.28X + 0.01$, where $X$ is plasma $P_i$ in μmoles/ml and $Y$ is red cell $P_i$ in μmoles/ml RBC. This confirmed the dependency of red cell $P_i$ upon plasma $P_i$ over a broad range of plasma $P_i$ (0.7-3.0 μmoles/ml). Plasma $P_i$ fell 43% from 2.01 ± 0.32 to 1.14 ± 0.1 μmoles/ml after 6 hr of hemodialysis (Fig. 2). However, the marked reduction in plasma $P_i$ did not result in a change in red cell $P_i$, which was virtually un-
Red cell ATP was highly correlated ($r = 0.81$, $p < 0.01$) with plasma $P_i$ (Fig. 4L). The relationship of red cell ATP with plasma $P_i$ was curvilinear and reached a plateau at about 2.25 μmoles/ml plasma $P_i$. Red cell 2,3-DPG was not correlated with plasma $P_i$ in this sample of 18 chronic renal disease patients (Fig. 4U).

**DISCUSSION**

Previous studies have established the relationship of red cell glycolytic rate, 2,3-DPG, and adenosine triphosphate content with plasma $P_i$ concentration. Lowered plasma $P_i$ affects red cell glycolysis, 2,3-DPG, and ATP due to the dependence of red cell $P_i$ on diffusion from plasma. Intracellular $P_i$ is one of several modulators of red cell glycolytic rate. In addition, when severe limitation is placed on the intracellular $P_i$ pool, accumulation of organic phosphate compounds becomes impaired. Hence, failure of red cell $P_i$ to decrease during hemodialysis explains the inability of such an acute and transient reduction in plasma $P_i$ to influence the organic phosphate content of the red cell. The lack of effect of plasma $P_i$ on red cell $P_i$ is explicable in large part by (a) the failure of the gradient for $P_i$, expressed in terms of μmoles/kg of plasma and cell water to fall below 1.0 at the completion of dialysis in any subject, and (b) the extremely slow exodus of $P_i$ from red cells even into a medium free of $P_i$. Hence, even if a reversal of gradient occasionally occurred at the end of dialysis,
little change in red cell $P_i$ would be expected (11) since after termination of
dialysis plasma $P_i$ immediately begins its elevation to predialysis levels, excluding
a protracted effect of dialysis on red cell $P_i$.

Alkalosis during dialysis may increase hemoglobin's affinity for oxygen; however, the effect was (1) quantitatively small, (2) balanced in large part by a
comitant increase in base excess, and (3) not observed to be associated with late dialysis symptoms. We cannot exclude the possibility that alkalosis may be
one of several factors which interact to contribute to ill-feeling with dialysis, as
has been suggested by others,\(^\text{14}\) although it is unlikely that its effect is mediated
through altered hemoglobin oxygen affinity and impaired oxygen release.
Alkalosis will also result in an acceleration of red cell glycolytic rate and
2,3-DPG synthesis, although this requires longer periods of time.\(^\text{4}\) The elevation in blood pH seen at the termination of dialysis gradually returns to pre-
dialysis values.\(^\text{17}\) The pattern of change in blood pH with dialysis does not
appear adequate to elevate red cell 2,3-DPG in dialysis subjects unless elevated
pH is maintained. Sustained alkalosis between treatment periods requires adju-
tant treatment with oral NaHCO\(_3\).\(^\text{9}\)

Two previous studies have conflicted regarding the effect of hemodialysis on
red cell 2,3-DPG content, and studies of oxygen binding by hemoglobin were
not conducted.\(^\text{15,16}\) Our studies indicate that hemodialysis neither alters organic
phosphates nor significantly impairs the ability of the red cell to participate in
oxygen transport.

Of particular interest, although not central to this study, is the observation
that red cell 2,3-DPG and $P_50$ in patients with severe chronic renal disease are
less than expected for their hemoglobin deficit, confirming our previous findings
in this regard in nondialysed subjects with severe azotemia.\(^\text{18,19}\) The abnormality
is not corrected by hemodialysis as shown in the current report. Our findings
in this regard are in contrast to those of Blumberg and co-workers\(^\text{20}\) and
Mitchell and Pegrum,\(^\text{21}\) who have reported high 2,3-DPG content and $P_50$ in the
cells of most hemodialysis patients. In a separate report we will show that the
inability to develop alkalosis due to the large acid load is a central factor in the
failure to raise red cell 2,3-DPG levels in response to the anemia of chronic
renal disease. Red cell ATP rather than 2,3-DPG accumulates in the red cells
of anemic subjects in an azotemic environment. Although the sum of red cell
2,3-DPG and ATP in anemic subjects with chronic renal disease is significantly
greater than healthy nonazotemic subjects, ATP does not appear to contribute
to the affinity of hemoglobin for oxygen in vivo.\(^\text{22}\)

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