Reduced Red Cell 2,3-Diphosphoglycerate and Adenosine Triphosphate, Hypophosphatemia, and Increased Hemoglobin-Oxygen Affinity after Cardiac Surgery

By Jerald A. Young, M.D., Marshall A. Lichtman, M.D., and Jules Cohen, M.D.

With the technical assistance of Marion Murph

SUMMARY

Serum inorganic phosphorous was decreased significantly on the third postoperative day following cardiac surgery in 18 patients initially studied. Reduced plasma inorganic phosphate has been shown to cause a reduced concentration of red cell organic phosphates, an important determinant of hemoglobin-oxygen affinity. Therefore, 10 consecutive patients were studied to determine if reduced 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) concentration and increased hemoglobin-oxygen affinity accompanied the fall in serum inorganic phosphate concentration.

A significant fall in 2,3-DPG and an increase in hemoglobin-oxygen affinity was present in red cells of patients studied on the first postoperative day. A reduction in red cell ATP was also present and persisted for 5 days during which time red cell 2,3-DPG returned to levels which were in excess of preoperative values. The reduction in serum inorganic phosphorous followed the reduction in red cell 2,3-DPG and correlated with the reduction in ATP. The latter changes may indicate the diversion of glucose and more specifically, 1,3-DPG into the Rapoport-Leubering pathway away from ATP generation at the phosphoglycerate kinase step and the utilization of plasma inorganic phosphate for 2,3-DPG resynthesis. Neither the transfusion of stored blood nor the effect of cardiopulmonary bypass fully explained the reduction in red cell 2,3-DPG and the inefficiency of hemoglobin function postoperatively. Further studies in postsurgical patients are needed to clarify the cause of the changes observed since they are potentially deleterious, especially in the subject with compromised cardiovascular and pulmonary function.

Additional Indexing Words:
Cardiopulmonary bypass Cardiac surgery Hemoglobin-oxygen affinity

RECENT STUDIES have demonstrated the important effect of erythrocytic 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) on hemoglobin function.1-3 The affinity of hemoglobin A for oxygen depends upon several factors. The most important of these are red cell hydrogen ion and organic phosphate concentrations, which are reciprocally related to oxygen-hemoglobin A affinity.

The concentrations of 2,3-DPG and ATP in the red cell are partly dependent, in turn, on the cell's rate of anaerobic glycolysis. Moreover, studies in man have demonstrated that red cell glycolytic rate and thereby the concentration of 2,3-DPG and ATP depend on both blood pH1,5 and plasma inorganic phosphate concentration.6-8

In the present study we have described a population of patients who developed hypophosphatemia following cardiac surgery. We have examined the temporal relationship between the decrease in serum inorganic phosphorus and

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concurrent changes in red cell 2,3-DPG, ATP, and hemoglobin-oxygen affinity. In these studies, plasma inorganic phosphorus appears to behave as a dependent variable, falling as a result of restoration of reduced red cell organic phosphate content postoperatively. The cause of the reduction in red cell organic phosphates and the clinical implications of these changes during the critical postoperative period are considered.

Methods

Study Subjects

Twenty-eight consecutive adult patients undergoing cardiac surgery were studied. Patients undergoing both open (utilizing cardiopulmonary bypass) and closed (without bypass) procedures were examined (table 1). Venous blood was collected in the absence of anticoagulants for measurement of serum phosphorus and with disodium ethylenediamine tetraacetic acid for measurement of hemoglobin and hematocrit. In 10 of these patients blood was collected in heparin for measurement of red cell 2,3-DPG, ATP, and hemoglobin affinity. Measurements were made preoperatively and on days 1, 3, and 5 postoperatively.

Physicochemical Studies

Serum inorganic phosphorus was determined by a modification of the technic of Dryer, Tammes, and Routh as described by Henry. Red cell 2,3-DPG was measured by the method of Rose and Liebowitz, and ATP by the luciferase method. An Instrumentation Laboratories model 137 tonometer was used to adjust pO₂ to 5 points between 15 and 80 mm Hg while CO₂ was kept constant at 40 mm Hg. Each determination of saturation was made in duplicate with an Instrumentation Laboratory model 182 cooximeter. pH and pO₂ were determined with an Instrumentation Laboratory model 113 pH-gas analyzer. The oxygen tension at which hemoglobin was 50% saturated (P₅₀) at 37°C, pH 7.4, pCO₂ 40 mm Hg, was calculated. The resultant P₅₀ at standard conditions was converted according to the method of Lenfant to an estimate of in vivo P₅₀. The latter value, P₅₀ (i.v.) is an estimate of hemoglobin-oxygen affinity under the conditions which prevailed in the patients at the time of study.

Blood hemoglobin and hematocrit were measured by standard technics. Mean corpuscular hemoglobin concentration was calculated from a standard formula. Correlation coefficients, linear regression parameters, and significance testing were performed using standard formulae.

Results

Preoperative serum inorganic phosphorus was 3.68 ± 0.55 mg/100 ml (mean ± s.d.) in all 28 subjects studied. There was no significant change on the first postoperative day, but by day 3, inorganic phosphorus had fallen significantly to 2.41 ± 0.81 (P < 0.01). Moreover, on day 3, serum inorganic phosphorus concentrations in 75% (20 of 27) patients was less than the lowest value observed preoperatively (table 2). By the fifth day, inorganic phosphorus had increased but was still significantly below preoperative concentrations. The course of these changes in serum phosphorus is shown in figure 1.

Because significant hypophosphatemia occurred postoperatively in the initial 18 subjects examined, an additional 10 patients were studied in greater

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Table 1

<table>
<thead>
<tr>
<th>No. pts</th>
<th>Diagnosis</th>
<th>Procedure (no.)</th>
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<tbody>
<tr>
<td>Patients in whom serum phosphorus was measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CAD</td>
<td>Aortocoronary bypass (5)</td>
</tr>
<tr>
<td>18</td>
<td>VHD</td>
<td>Valve replacements or open commissurotomy (13)</td>
</tr>
<tr>
<td>5</td>
<td>CHD</td>
<td>Closed mitral commissurotomy (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closure ostium secundum (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closure ostium primum (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closure ventricular septal defect and aortic valve replacement (1)</td>
</tr>
<tr>
<td>Patients in whom serum phosphorus red cell organic phosphates and oxyhemoglobin affinity were measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CAD</td>
<td>Aortocoronary bypass (2)</td>
</tr>
<tr>
<td>7</td>
<td>VHD</td>
<td>Valve replacements (4)</td>
</tr>
<tr>
<td>1</td>
<td>CHD</td>
<td>Closed mitral commissurotomy (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closure of ventricular septal defect and aortic valve replacement (1)</td>
</tr>
</tbody>
</table>

Abbreviations: CAD = coronary artery disease; VHD = valvular heart disease; CHD = congenital heart disease.

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Table 2

<table>
<thead>
<tr>
<th>Serum inorganic phosphorus (mg/100 ml)</th>
<th>Preop</th>
<th>Third postop day</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 - 4.9</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>4.0 - 4.4</td>
<td>4</td>
<td>14.3</td>
</tr>
<tr>
<td>3.5 - 3.9</td>
<td>11</td>
<td>39.3</td>
</tr>
<tr>
<td>3.0 - 3.4</td>
<td>7</td>
<td>35.0</td>
</tr>
<tr>
<td>2.5 - 2.9</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>2.0 - 2.4</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>1.5 - 1.9</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>1.0 - 1.4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3.68 ± 0.10</td>
<td>2.41 ± 0.16</td>
</tr>
</tbody>
</table>

*One subject did not have serum inorganic phosphorus measured on day 3.
HEMOGLOBIN-OXYGEN AFFINITY

was significantly reduced to $14.8 \pm 2.43 \mu \text{mole/g Hb}$. By the third day, 2,3-DPG content had returned to $17.6 \pm 3.71$, which was not statistically significantly different from preoperative values. By the fifth day, red cell 2,3-DPG content exceeded preoperative values (fig. 1).

Red cell ATP content fell from a preoperative value of $1.26 \pm 0.16 \mu \text{mole/ml}$ red cells to $1.09 \pm 0.18$ on the first postoperative day and fell further to $1.04 \pm 0.20$ ($P < 0.05$) on the third day after surgery (fig. 1). By the fifth postoperative day, red cell ATP had risen to $1.17 \pm 0.10$, still slightly below preoperative levels.

The mean in vivo $P_{50}$ was $26.5 \pm 2.2$ mm Hg preoperatively and fell to $23.3 \pm 2.9$ on the first day ($P < 0.05$) (fig. 1). By the third postoperative day, $P_{50}$ was similar to the preoperative level, and by the fifth postoperative day it had risen to $28.6 \pm 2.3$, slightly greater than the preoperative value.

The alteration in $P_{50}$ was highly correlated ($r = 0.83, P < 0.001$) with the content of red cell 2,3-DPG over the 5 days of study (fig. 2). Blood hemoglobin concentration was not significantly different on postoperative day 1 ($14.3 \pm 1.8 \text{g/100 ml}$), day 3 ($13.4 \pm 1.6$), or day 5 ($13.1 \pm 1.7$) as compared to the preoperative concentration ($13.3 \pm 1.3$).

A significant positive correlation was observed between red cell ATP and serum inorganic phosphorus ($r = 0.55, P < 0.05$) however, 2,3-DPG

**Figure 1**

Serum inorganic phosphorus, red cell ATP, and 2,3-DPG and hemoglobin-oxygen affinity ($P_{50}$) in vivo. Values shown are the mean ± so in 10 patients who underwent cardiac surgery. Day 0 refers to the day of operation.

detail. In these patients, preoperatively, red cell 2,3-DPG was slightly elevated at $18.6 \pm 2.32 \mu \text{mole/g Hb}$ (fig. 1) as compared to $14.6 \pm 1.2$ in normal subjects in our laboratory. On the first postoperative day, approximately 20 hours after surgery, 2,3-DPG content (when compared to preoperative values)
was not significantly correlated with serum inorganic phosphorus (r = 0.22). Indeed during the period day 1–5, mean red cell 2,3-DPG and serum inorganic phosphorus were varying reciprocally.

The seven patients who underwent cardiopulmonary bypass required an average of 21 units of blood, 18 of which had been stored 14–21 days, and three of which were less than 36 hours old (table 3). The blood administered on the day of surgery was infused during surgery and with few exceptions within 4 hours postoperatively. Hence, samples drawn on the morning of the first postoperative day were taken about 16 hours after completion of transfusion. In the case of the three patients in whom closed procedures were performed, no blood was administered after the operative period and three of the nine units administered were fresh (<36 hours) blood. The magnitude and direction of changes in red cell 2,3-DPG, ATP, P50, and serum inorganic phosphorus in these three patients were very similar to those of the entire group (fig. 3).

**Discussion**

Previous studies have indicated that hypophosphatemia could lead to deleterious changes in red cell metabolism6, 8, 13 and function.8, 14 Therefore, we examined the effect of the postoperative fall in serum inorganic phosphorus on the concentration of organic phosphate in the red cell. From the observed temporal sequence, the highly significant drop in red cell 2,3-DPG preceded the fall in serum inorganic phosphorus and may be ascribed in part to the transfusion of large quantities of banked blood.

The low 2,3-DPG and increased hemoglobin-oxygen affinity in our patients 16 hours after surgery is an underestimate of the magnitude of the changes at the termination of cardiopulmonary bypass since Bordiuk and co-workers15 have recently reported a more marked reduction of 2,3-DPG at the conclusion of cardiopulmonary bypass than 24 hours postoperatively. This is explicable in part by the restoration of 2,3-DPG concentration of stored red cells over 48 hours after transfusion.16, 17

Neither the data of Bordiuk et al.15 nor ours would support the conclusion that transfusion of stored blood or factors unique to cardiopulmonary

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**Table 3**

**Units of Transfused Blood per Patient**

<table>
<thead>
<tr>
<th></th>
<th>Bypass patients</th>
<th>Closed commissurotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored ACD blood</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Fresh ACD blood*</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>3</td>
</tr>
</tbody>
</table>

Abbreviations: ACD = acid-citrate-dextrose.  
*Fresh blood was stored <36 hours.

---

**Figure 3**

Mean serum inorganic phosphorus, red cell ATP, and 2,3-DPG and P50 in vivo in three patients undergoing closed mitral commissurotomy. Results for individual patients were very similar and therefore mean data are presented.

_Circulation, Volume XLVII, June 1973_
bypass, wholly explain the reported changes. Patients in our study who underwent closed mitral commissurotomy received very little transfused blood yet manifested the same changes in red cell organic phosphates, oxyhemoglobin dissociation, and serum phosphorus as those undergoing bypass procedures. The results in nonbypass patients suggest that similar changes may occur following noncardiac surgical procedures. However, to our knowledge, studies of the effect of noncardiac surgery on oxyhemoglobin affinity have not been reported.

The fall in serum phosphorus may be explained, in part, on the basis of its utilization for the resynthesis of 2,3-DPG. The degree to which phosphorus was utilized in 2,3-DPG regeneration is not accurately reflected in the serum concentration. A significant rise in inorganic phosphorus occurs in plasma of blood stored for several weeks.\(^6\) Thus, transfusion of whole blood results in the infusion of significant quantities of inorganic phosphate which may prevent a more pronounced fall in plasma phosphate levels during organic phosphate resynthesis. The greater magnitude of the fall in inorganic phosphorus in the three patients not undergoing cardiopulmonary bypass may be a reflection of this phenomenon since these patients had a similar reduction in red cell 2,3-DPG but received minimal amounts of transfused blood. Based on studies in patients on phosphate-binding antacids\(^6\) and in patients receiving parenteral nutrition,\(^8\) bony or other stores of phosphate do not maintain plasma phosphate concentration when exit from the plasma compartmen is accelerated. Also, the reduction in plasma inorganic phosphate could have been related to hyperventilation and respiratory alkalosis;\(^19\) however close monitoring of blood pH and pCO\(_2\) postoperatively maintained these variables in the normal range. Slight increases in pH were observed in some patients but did not correlate well with phosphorus level.

A possible explanation for the fall in ATP at a time when 2,3-DPG levels were rising rests in the competition for 1,3-DPG between diphosphoglycerate mutase which synthesizes 2,3-DPG and phosphoglycerate kinase which synthesizes ATP. If the mutase had an enhanced affinity for substrate or an increased rate of substrate utilization under the conditions that prevailed, 2,3-DPG synthesis would have been favored at the expense of ATP synthesis. Since the red cell can withstand enormous proportional decreases in ATP in vivo without significant deleterious consequences,\(^6,8\) whereas any reduction in 2,3-DPG impairs hemoglobin function, the preferential synthesis of 2,3-DPG under such conditions would be a reasonable expectation.

The postoperative reduction in 2,3-DPG and increase in hemoglobin-oxygen affinity that we observed must be considered potentially deleterious. Several factors contribute to oxygen delivery to the tissues and these may compensate for impaired oxygen release by hemoglobin.\(^9\) However, the postoperative cardiac patient may have compromised ventilation, cardiac function, and microvascular flow, the other systems required for compensation for inefficiency of hemoglobin function. Indeed, decreased hemoglobin-oxygen affinity appears to be a normal compensatory mechanism in low-output states.\(^20\) Our patients, 5 days postoperatively, in the absence of anemia, had an increased P\(_{oo}\) suggesting the need to facilitate oxygen transport during the postoperative period. Hence, limitation of this presumed compensatory change during the first few days after surgery may be an important factor compromising oxygen delivery.

It still remains to be determined whether a left-shifted oxyhemoglobin dissociation curve significantly impairs oxygen delivery. If so, the development and subsequent use of safe and effective technics for shifting the curve to the right might be important additions to therapy.

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