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

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With the technical assistance of Marion Murphy

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 determinent 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-Leuberger 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. 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 pH and plasma inorganic phosphate concentration.

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
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, Tamme, 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 ± se) 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

Table 1

Patients Undergoing Cardiac Surgery

<table>
<thead>
<tr>
<th>No. pts</th>
<th>Diagnosis</th>
<th>Procedure (no.)</th>
</tr>
</thead>
<tbody>
<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.

Table 2

Frequency Distribution of Serum Inorganic Phosphorus before and after Cardiovascular Surgery

<table>
<thead>
<tr>
<th>Serum inorganic phosphorus (mg/100 ml)</th>
<th>Preop N</th>
<th>%</th>
<th>Third postop day N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 - 4.9</td>
<td>3</td>
<td>10.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4.0 - 4.4</td>
<td>4</td>
<td>14.3</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>3.5 - 3.9</td>
<td>11</td>
<td>39.3</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>3.0 - 3.4</td>
<td>7</td>
<td>35.0</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>2.5 - 2.9</td>
<td>3</td>
<td>10.7</td>
<td>6</td>
<td>22.0</td>
</tr>
<tr>
<td>2.0 - 2.4</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>25.9</td>
</tr>
<tr>
<td>1.5 - 1.9</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>25.9</td>
</tr>
<tr>
<td>1.0 - 1.4</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100.0</td>
<td>27*</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3.68 ± 0.10</td>
<td>2.41 ± 0.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

![Graphs showing changes in 2,3-DPG, P50, and ATP over days after surgery.](image)

**Figure 1**

Serum inorganic phosphorus, red cell ATP, and 2,3-DPG and hemoglobin-oxygen affinity (P50) in vivo. Values shown are the mean ± SD in 10 patients who underwent cardiac surgery. Day 0 refers to the day of operation.

In these patients, preoperatively, red cell 2,3-DPG was slightly elevated at 18.6 ± 2.32 μmole/g Hb (fig. 1) as compared to 14.6 ± 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 significantly reduced to 14.8 ± 2.43 μmole/g Hb. By the third day, 2,3-DPG content had returned to 17.6 ± 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 ± 0.16 μmole/ml red cells to 1.09 ± 0.18 on the first postoperative day and fell further to 1.04 ± 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 ± 0.10, still slightly below preoperative levels.

The mean in vivo P50 was 26.5 ± 2.2 mm Hg preoperatively and fell to 23.3 ± 2.9 on the first day (P < 0.05) (fig. 1). By the third postoperative day, P50 was similar to the preoperative level, and by the fifth postoperative day it had risen to 28.8 ± 2.3, slightly greater than the preoperative value.

The alteration in P50 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 ± 1.8g/100 ml), day 3 (13.4 ± 1.6), or day 5 (13.1 ± 1.7) as compared to the preoperative concentration (13.3 ± 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 concentration.
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, $P_{50}$, 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 metabolism$^8, 13$ 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-workers$^{15}$ 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

---

**Table 3**

**Units of Transfused Blood per Patient**

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Bypass patients</th>
<th>Closed commissurotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Stored ACD blood</td>
<td>18</td>
<td>9–30</td>
</tr>
<tr>
<td>Fresh ACD blood*</td>
<td>3</td>
<td>2–5</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td></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 $P_{50}$ in vivo in three patients undergoing closed mitral commissurotomy. Results for individual patients were very similar and therefore mean data are presented.

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pH
been
when
time
increased
an
received
alkalosis;19
respiratory
may
not
consequences,6'
phosphate resynthesis.
phosphoglycerate
kinase
in
part,
Since
the
conditions
that
erate mutase
6
nutrition,8'
14
phosphorus
Based on
the fall
in
plasma
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.18
Thus,
transfusion
of
whole
blood
results
in
the
infusion
of
significant
quantities
of
inorganic
phosphate
which
may
prevent
a
more
pronounced
fall
in
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phosphate
levels
during
organic
phosphate
resynthesis.
The
greater
magnitude
of
the
fall
in
inorganic
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in
the
three
patients
not
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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
antacids6
and
in
patients
receiving
parenteral
nutrition,8
14
bony
or
other
stores
of
phosphate
do
not
maintain
plasma
phosphate
concentration
when
exit
from
the
plasma
compartment
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
pCO2
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.8
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,
under
an
increased
Pso
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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with
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the
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in
the
preparation
of
this
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