The ADP and ATP Levels and the Phosphorylating Activity of Erythrocytes in Patients with Uremia Associated with Chronic Renal Failure

By

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The ADP and ATP levels of erythrocytes were determined by the column-chromatography in patients with uremia associated with chronic renal failure, in order to study the mechanism of the increased hemolysis in uremia. The in vitro incorporation of radiophosphorus in ADP and ATP of erythrocytes was also studied. The ADP and ATP levels of erythrocytes were slightly elevated in patients with uremia, averaging 11.9 mg/100 ml erythrocyte and 98.1 mg/100 ml erythrocytes, respectively. The ATP/ADP ratio was within normal limits. When normal erythrocytes were incubated with $^{32}$P in vitro for 15 minutes at 37°C, no radioactivity was demonstrated in the 5/1,000 N HO fraction containing inorganic phosphorus and AMP. However, when erythrocytes of patients with uremia were incubated with $^{32}$P for 15 minutes, a large amount of radioactivity was still present in the 5/1,000 HCl fraction. The in vitro incorporation of radiophosphorus in ADP and ATP of erythrocytes was markedly reduced in patients with uremia. These results demonstrate the decrease of the phosphorylating activity of erythrocytes in patients with uremia. When normal erythrocytes were suspended in uremic plasma and incubated with radiophosphorus, the in vitro incorporation of $^{32}$P was markedly reduced, suggesting the inhibitory effect of the increased non-protein nitrogen or toxic products in uremic plasma on the phosphorylating activity of erythrocytes. It seems likely that this decrease of the phosphorylating activity of erythrocytes might play some roles in the mechanism of the increased hemolysis in uremic patients.

It has been well known$^{1-13}$) that the life span of erythrocytes is markedly shortened in patients with uremia, indicating the presence of an increased hemolysis. However, little is yet known about the mechanism of the increased hemolysis in patients with uremia. Bock et al.$^{14}$) reported a decreased adenine-nucleotide content of erythrocytes and the inhibition of their hexokinase activity...
due to the acidosis, in seven cases of chronic renal failure. However, Pappenberg et al.\textsuperscript{15) could demonstrate no decrease of the adeninenucleotide level of erythrocytes in uremic patients. Guest and Rapoport\textsuperscript{16) reported an increase of the level of total acid soluble phosphates of erythrocytes in several patients with uremia. These facts suggest that the erythrocyte metabolism is impaired in patients with uremia and there may be some relationships between the impaired erythrocyte metabolism and the increased hemolysis.

Therefore, the level of ADP and ATP of erythrocytes and the \textit{in vitro} incorporation of \textsuperscript{32}P in the erythrocyte ADP and ATP were determined in patients with uremia associated with chronic renal failure by the column chromatography.

METHODS

1. Five patients with uremia associated with chronic renal failure were studied. Their serum non-protein nitrogen level was more than 130 mg/dl.

2. Twelve ml of heparinized blood were centrifuged for 30 minutes at 3,000 rpm, and the plasma and the buffy coat were removed separately. Erythrocytes was washed by the Krebs-Ringer solution three times and suspended in the above plasma. The above suspension was poured into the Warburg’s flask and incubated at 37°C for 5-10 minutes. Five ml of the suspension were examined for the determination of the level of ADP and ATP and another 5 ml of suspension were studied for the \textit{in vitro} incorporation of \textsuperscript{32}P. 100μ C of \textsuperscript{32}P-orthophosphate was added to 5 ml of the erythrocytes suspension in the Warburg’s flask. After 15 minutes incubation at 37°C, 5 ml of the suspension was poured into 10 ml of ice cold 8% HClO\textsubscript{4} solution and incubated for 30 minutes at 37°C. The above mixture was centrifuged for 15 minutes at 4,000 rpm at 5°C and then the supernatant was collected. 1N KOH was added to the supernatant drop by drop until the pH of the solution became BTB neutral. Then the precipitated HClO\textsubscript{4} was removed by the centrifugation and the supernatant was examined for the column chromatography using Dowex-1 X-2. The elution solvents were as follows: 1) 5/1,000 N HCl, 2) 1/100 N HCl, 3) M/50 NaCl in N/100 HCl, 4) M/25 NaCl in N/100 HCl and 5) M/15 NaCl in N/100 HCl.

RESULTS

1. The levels of ADP and ATP of erythrocytes of patients with uremia associated with chronic renal failure (Tables I and II)

The content of AMP, ADP, and ATP and the ATP/ADP ratio of normal erythrocytes averaged 0.6 mg/100 ml erythrocytes, 6.3 mg/100 ml erythrocytes, 55.3 mg/100 ml erythrocytes, and 9.9, respectively (Table I).

They averaged in erythrocytes of patients with uremia as follow: the AMP level averaged 0.9 mg/100 ml erythrocytes, ranging 0.3 to 1.4 mg. The ADP
TABLE I. ADP, ATP and ATP/ADP of Normal Red Blood Cells

<table>
<thead>
<tr>
<th>Name</th>
<th>AMP</th>
<th>ADP</th>
<th>ATP</th>
<th>ATP/ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>0.2</td>
<td>4.8</td>
<td>63.4</td>
<td>13.2</td>
</tr>
<tr>
<td>S</td>
<td>0.8</td>
<td>5.7</td>
<td>62.7</td>
<td>9.1</td>
</tr>
<tr>
<td>I</td>
<td>0.3</td>
<td>6.5</td>
<td>39.7</td>
<td>6.1</td>
</tr>
<tr>
<td>K</td>
<td>0.7</td>
<td>5.7</td>
<td>68.0</td>
<td>11.8</td>
</tr>
<tr>
<td>K</td>
<td>0.8</td>
<td>8.8</td>
<td>82.8</td>
<td>9.5</td>
</tr>
<tr>
<td>Mean</td>
<td>0.6±0.2</td>
<td>6.3±1.2</td>
<td>55.3±15.6</td>
<td>9.9±2.4</td>
</tr>
</tbody>
</table>

TABLE II. ADP, ATP and ATP/ADP of Uremic Red Blood Cells

<table>
<thead>
<tr>
<th>Name (NPN)</th>
<th>AMP</th>
<th>ADP</th>
<th>ATP</th>
<th>ATP/ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (171)</td>
<td>1.0</td>
<td>14.6</td>
<td>120.4</td>
<td>8.5</td>
</tr>
<tr>
<td>H (165)</td>
<td>0.4</td>
<td>9.6</td>
<td>78.1</td>
<td>9.2</td>
</tr>
<tr>
<td>K (178)</td>
<td>1.4</td>
<td>12.7</td>
<td>102.5</td>
<td>8.9</td>
</tr>
<tr>
<td>N (134)</td>
<td>0.5</td>
<td>9.4</td>
<td>69.1</td>
<td>7.4</td>
</tr>
<tr>
<td>O (148)</td>
<td>1.3</td>
<td>13.3</td>
<td>121.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Mean</td>
<td>0.9±0.2</td>
<td>11.9±2.4</td>
<td>98.1±24.2</td>
<td>8.6±0.2</td>
</tr>
</tbody>
</table>

TABLE III. Relative Specific Radioactivity of ADP and ATP of Normal and Uremic Erythrocytes Incubated with *32P-orthophosphate in vitro for 15 minutes at 37°C.

<table>
<thead>
<tr>
<th>Name</th>
<th>ADP</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>76200</td>
<td>2200</td>
</tr>
<tr>
<td>S</td>
<td>37800</td>
<td>4400</td>
</tr>
<tr>
<td>I</td>
<td>87500</td>
<td>5800</td>
</tr>
<tr>
<td>K</td>
<td>37800</td>
<td>1900</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>ADP</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>1900</td>
<td>960</td>
</tr>
<tr>
<td>H</td>
<td>7200</td>
<td>420</td>
</tr>
<tr>
<td>K</td>
<td>9800</td>
<td>300</td>
</tr>
<tr>
<td>N</td>
<td>10900</td>
<td>1000</td>
</tr>
<tr>
<td>O</td>
<td>9800</td>
<td>1000</td>
</tr>
</tbody>
</table>

N: Normal
U: Uremia

content ranged 9.4 to 14.6 mg, averaging 11.9 mg/100 ml erythrocytes. The level of ATP averaged 98.1 mg/100 ml erythrocytes, ranging 69.1 to 121.1 mg
ATP/ADP ratio averaged 8.6, ranging 7.4 to 9.2.

2. The in vitro incorporation of radioactive phosphorus in the ADP and ATP of erythrocytes (Table III; Figs. 1 and 2)

The relative specific radioactivity of ADP and ATP of normal erythrocytes incubated with $^{32}$P for 15 minutes at 37°C, ranged 37,600 to 87,500 cpm/μgP in ADP and 1,900 to 5,800 cpm/μgP. It ranged in erythrocytes of patients with uremia as follows: ADP, 1,900 to 10,900 cpm/μgP; and ATP, 300 to 1,000 cpm/μgP.

Fig. 1 shows the column chromatogram of normal erythrocytes incubated with $^{32}$P for 15 minutes at 37°C. Within 15 minutes incubation, radiophosphorous

![Normal Blood Column Chromatogram](chart.png)

Notes:
5/1,000 N HCl fraction: Inorganic phosphorous and AMP
1/100 N HCl fraction: ADP
1/50 M NaCl + 1/100 N HCl fraction: HDP
1/25 M NaCl + 1/100 N HCl fraction: 2,3-DPG
1/15 M NaCl + 1/100 N HCl fraction: ATP

Fig. 1. Column chromatogram of acid soluble phosphates of normal erythrocytes incubated with $^{32}$P-orthophosphates in vitro for 15 minutes at 37°C and the incorporation of $^{32}$P in them.
were incorporated in ADP, HDP, 2,3-DPG and ATP, leaving no radioactivity in the 5/1,000 N HCl fraction containing inorganic phosphorus and AMP.

Fig. 2 shows the columnchromatogram of erythrocytes of uremic patients incubated with radiophosphorus for 15 minutes at 37°C. In sharp contrast to normal erythrocytes, a large amount of $^{32}$P radioactivity was found in 5/1000 N HCl fraction.

3. The effect of uremic non-protein nitrogen of the phosphorylative activity of normal erythrocytes (Fig. 3).

Thoroughly washed normal erythrocytes were suspended in ABO compatible uremic plasma and the suspension was incubated with $^{32}$P for 15 minutes at 37°C. And then the changes of relative specific radioactivity in ADP and ATP were determined by the columnchromatography. The relative specific radioactivity of ADP and ATP of normal erythrocytes suspended in normal plasma was 400,000
DISCUSSION

The human erythrocytes have no microsomes, mitochondria and nucleus. There is a lot of evidence suggesting that their viability and integrity mainly depend upon the energy rich, acid soluble phosphates which are produced in the process of anaerobic glycolysis in them. Bartlet reported that the ATP level of erythrocytes stored in the ACD solution decreased markedly. Nakao and others labeled erythrocytes, which were stored in the ACD solution and showed a decrease of their ATP level with $^{51}$Cr, and transfused them into normal persons. Only 20% of the transfused labeled erythrocytes were present in the circulating blood 24 hours after the transfusion. However, when an adequate amount of inosine and adenine was added to the above stored erythrocytes suspension, the decreased ATP level of erythrocytes was restored markedly. When the above erythrocytes were labeled with $^{51}$Cr and transfused into normal persons, 80% of the transfused erythrocytes were detected in the circulating blood 24 hours after the transfusion. According to Nakao, the shape of erythrocytes stored in the ACD solution depends upon their ATP content. When the ATP level of stored erythrocytes decreases to less than 10% of the normal value, the shape of erythrocytes
be-spheres.

These facts strongly suggest that the ATP level of erythrocytes may have a significant role in the mechanism of hemolysis. Therefore studies on the content of ADP and ATP of erythrocytes of patients with uremia associated with chronic renal failure were undertaken, in order to find the mechanism of an increased hemolysis in uremic patients.

The ADP and ATP contents of erythrocytes of patients with uremia averaged 11.9 mg/100 ml erythrocytes and 98.1 mg/100 ml erythrocytes, respectively. The ATP/ADP ratio averaged 8.6. These results indicate that the levels of ADP and ATP of erythrocytes of uremic patients are not decreased but slightly increased and the ATP/ADP ratio is within a normal limit.

Bock et al. reported a decrease of the ATP level of erythrocytes in patients with uremia. However, according to Guest and Rapoport and Jahr, the contents of total acid-soluble phosphates and ATP are increased in erythrocytes of patients with uremia. Our results confirm their observation.

The halftime of the erythrocyte survival determined by the Cr method was markedly shortened in these patients with uremia associated with chronic renal failure, averaging 14 days. Therefore, it may be possible that erythrocytes of circulating blood were consisted mostly of the young erythrocyte population in these patients. It is well known that the ATP level of the young erythrocyte population is higher than that of the aged. Therefore, it seems likely that the elevation of the ATP level of erythrocytes in patients with uremia might be due to the presence of a lot of the young erythrocyte population.

Erythrocytes were incubated with 32P-orthophosphate in vitro for 15 minutes at 37°C, and the incorporation of 32P in ADP and ATP was determined by the column chromatography. The relative specific radioactivity in ADP and ATP was markedly decreased in erythrocytes of patients with uremia. When normal erythrocytes were incubated with radiophosphorus in vitro for 15 minutes at 37°C, no radioactivities were demonstrated in the 5/1,000 N HCl fraction containing inorganic phosphorus and AMP. However, erythrocytes of uremic patients were incubated with radiophosphorus in vitro for 15 minutes at 37°C, a large amount of radioactivities was still present in the 5/1,000 N HCl fraction. These results demonstrate that the phosphorylating activity is significantly reduced in erythrocytes of patients with uremia associated with chronic renal failure.

When normal erythrocytes were suspended in uremic plasma and incubated with radiophosphorus in vitro the incorporation of 32P in ADP and ATP was markedly reduced. These results indicate that the increased non-protein nitrogen or toxic substances in uremic plasma inhibit the phosphorylating activity of erythrocytes.

The decrease of the phosphorylating activity of erythrocytes may be induced
by the inhibition of either the stromal enzymes such as ATPase and 
glyceraldehyde-3-phosphate dehydrogenase or the intracellular soluble enzymes of the glycolytic 
pathway. It may be possible that toxic products in uremic plasma might exert 
some inhibitory effects on the stromal enzymes and induce the decrease of the 
phosphorylating activity. On the other hand it may be also possible that toxic 
substances in uremic plasma may enter the interior of erythrocytes and inhibit 
the intracellular enzyme activity. We cannot draw any conclusion about the 
exact mechanism of the inhibition of the phosphorylating activity of erythrocytes 
in patients with uremia.

References