ENDOGENOUS URIC ACID AND HEMATOPOIESIS.

II. URIC ACID, RETICULOCYTES, AND ERYTHROCYTES AFTER HEMOLYSIS BY PHENYLHYDRAZINE HYDROCHLORIDE.

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The rôle of uric acid in the economy of the body has been the subject of many and varied investigations. The author has recently developed the hypothesis that this metabolite is produced from the nucleic acid resulting from the destruction of the nuclei extruded in the process of maturation of the erythrocytes. A series of determinations of the daily outputs of uric acid before and after severe hemorrhage showed a doubling for the period of regeneration followed by a gradual return to the basal level as the normal blood count was reached.

The present study was undertaken to test this hypothesis under the conditions of hemolysis and subsequent regeneration. Since the reticulated erythrocyte is considered to be the youngest red blood cell to be recognized in the peripheral circulation, a correlation of the uric acid outputs and the reticulocyte counts is to be expected. These counts were made daily, and several significant facts are established which tend to confirm the general hypothesis.

EXPERIMENTAL.

The daily uric acid outputs, erythrocyte counts, and reticulocyte counts were established for Dal Dale Doc, a thoroughbred Dalmatian coach dog, that was kept in a small experimental cage on a purine-free diet. After a control period of 21 days, the dog was given a capsule containing 50 mg. of phenylhydrazine HCl (Kahlbaum). During the next 10 days, this single dose reduced the red blood cell count from 5,709,000 to 3,320,000. Recovery
was rapid; the normal blood level was reached in 9 days and surpassed on the 10th day by a count of 6,260,000. During the period of active regeneration, as indicated by a rise in the red blood count.

**TABLE I.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Red blood cell count</th>
<th>Reticulocytes</th>
<th>Uric acid</th>
<th>Date</th>
<th>Red blood cell count</th>
<th>Reticulocytes</th>
<th>Uric acid</th>
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<td>Nov. 5</td>
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<td>266</td>
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<tr>
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<td>640</td>
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<td>500</td>
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<td>120,000</td>
<td>232</td>
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<td>50,400</td>
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<td>47,040</td>
<td>571</td>
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<td>1.2</td>
<td>59,040</td>
<td>727</td>
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<td>1.0</td>
<td>62,600</td>
<td>328</td>
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</table>

*50 mg. phenylhydrazine HCl by mouth.
† Maximum hemolysis.
‡ Maximum regeneration.
cell count, the uric acid increased from a basal level of 265 mg. to 481 mg., or 181 per cent.

A high uric acid level was also shown during the period of hemolysis. The marked marrow activity necessary to account for this output (432 mg.) is shown by the reticulocyte count which rose from a prehemolytic level of 22,836 per c.mm. to 69,990. Destruction and regeneration may thus go on side by side although one process may completely mask the end results of the other.

The data for the 21 days before and the 29 days after hemolysis are given in Table I.

**TABLE II.**

Erythrocyte Counts and Daily Uric Acid Outputs for a Dalmatian Coach Dog after Hemorrhage and after Hemolysis.

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg.)</th>
<th>Lowest red blood cell count (mill.)</th>
<th>Highest red blood cell count (mill.)</th>
<th>Days to complete regeneration</th>
<th>Average daily uric acid before hemorrhage (mg.)</th>
<th>Average daily uric acid after hemorrhage (mg.)</th>
<th>Uric acid increase per cent.</th>
<th>Total excess of uric acid above basal level (mg.)</th>
<th>Basal level reached (days)</th>
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</thead>
<tbody>
<tr>
<td>After 350 cc. hemorrhage</td>
<td>15</td>
<td>3.30</td>
<td>6.36</td>
<td>11</td>
<td>278,100</td>
<td>154</td>
<td>302</td>
<td>196</td>
<td>1738</td>
</tr>
<tr>
<td>After hemolysis by 50 mg. phenylhydrazine HCl</td>
<td>20</td>
<td>3.32</td>
<td>6.26</td>
<td>10</td>
<td>294,000</td>
<td>265</td>
<td>481</td>
<td>181</td>
<td>2160</td>
</tr>
</tbody>
</table>

**DISCUSSION.**

A. **Comparison of Uric Acid Output after Hemorrhage and after Hemolysis.**

In the previously published experiment, the red blood cell count after hemorrhage was 3,300,000, while after hemolysis the low level was 3,320,000. Regeneration was accomplished in approximately the same time in both instances, that is, in 11 days after hemorrhage and 10 days after hemolysis. The same high level was reached in each case; the count going to 6,360,000 after hemorrhage and 6,260,000 after hemolysis. The uric acid output above the basal level for the period of regeneration after hemorrhage was 1738 mg., and for the same period after hemolysis it was 2160.
mg. The average daily output was increased 190 per cent after hemorrhage and 181 per cent after hemolysis. The daily uric acid output returned to the basal level 21 days after hemorrhage and 18 days after regeneration was started in hemolysis. The shorter interval in the second case may be due to the hyperplasia established during the period of regenerative activity masked by the red blood cell destruction.

The data for comparisons are given in Table II.

It will be noted that while the same dog was used in the two tests, the basal uric acid output was 154 mg. before hemorrhage and 265 mg. before hemolysis. In the interval between the two tests, i.e. from April, 1929 to October, 1929, the dog increased in weight from 15 to 20.5 kilos, the output being thus roughly proportional to the weight.

**B. Action of Phenylhydrazine HCl.**

A single dose of phenylhydrazine HCl administered orally continued to act as a hemolytic agent for a period of 10 days as shown by a falling red blood cell count. A similar action is reported by Jones (1911) for a period of 6 days following injection of phenylhydrazine in the rabbit. Whether the action was due to slow absorption or slow elimination was not determined. The blood was dark on the first 2 days, but did not show the brown color characteristic of phenylhydrazine injection. The dog seemed to suffer no embarrassment except on the 8th day which was characterized by loss of appetite and general depression. The urine was dark on the first 2 days but showed a normal color thereafter.

The sharp drop of the red blood cell count on the 1st day from 5,890,000 to 4,840,000 followed by a rapid decrease in destruction and a small compensatory increase, indicates a strong and immediate stimulus to the erythropoietic system, involving both the liberation factor (as shown by the reticulocyte count rise from 0.5 to 2.2 per cent) and the maturation factor (as shown by the rise in uric acid from 265 to 624 mg.).

That the subsequent drop to the low level was in part due to bone marrow injury is shown by the appearance of a few normoblasts in the peripheral circulation on the 3rd day. Their number increased and represented from 1 to 2 per cent of the total leucocyte count from the 7th to the 11th day. After this they gradually disappeared.
C. Correlation of the Uric Acid Outputs and the Reticulocyte Counts.

It will be noted that both uric acid outputs and reticulocyte counts varied markedly for successive days after hemolysis. They present a series of peaks, which however are not simultaneous, but show an interval of lag varying from 3 to 4 days. The first peak, Peak A, for uric acid occurred on the 6th, while the first reticulocyte peak, Peak A', came on the 9th day. Peaks B, B'; C, C'; D, D'; E, E'; and F, F' follow in succession. The data are presented in Table III. The relative height of the peaks is also roughly proportional, as shown by the coefficient $\frac{R}{U}$ or

\[
\begin{array}{c|c|c|c|c|c|c}
\hline
\text{Peak} & \text{Date} & \text{Output} & \text{Peak} & \text{Date} & \text{Count} & \text{Days lag between peaks} \\
\hline
A & \text{Nov. 6} & 527 & A' & \text{Nov. 9} & 108,460 & 3 \\
B & " 8 & 624 & B' & " 12 & 102,900 & 4 \\
C & " 11 & 640 & C' & " 15 & 133,920 & 4 \\
D & " 15 & 512 & D' & " 17-19 & 120,900 & (3) \\
E & " 19 & 500 & E' & " 22 & 194,000 & 3 \\
F & " 26 & 570 & F' & " 28 & 60,660 & 2 \\
\hline
\end{array}
\]

\[R = \frac{\text{reticulocyte number}}{\text{mg. of uric acid}}.\]

The values for Peaks F and F' are relatively low and may represent a maturation of a reserve set of reticulocytes that are retained longer than those delivered to the peripheral circulation under the stress of a low red blood cell count.

D. Consideration of Reticulocyte Counts and Total Erythrocyte Regeneration.

If the total reticulocyte count in this series be compared to the total erythrocyte increase during the same period, it will be seen that the summation for the successive days from November 14 to
24 is far short of expectations. During this interval the total number of reticulocytes was 987,530 while the erythrocyte count increased by 2,940,000 giving an approximate ratio of 1:3. Examination of other series of reticulocytes and erythrocyte increases shows similar discrepancies. A consideration of the relative length of the reticulocyte period may account for these relationships.

On the assumption of a daily 1.5 per cent erythrocyte destruction, a like regeneration must also be postulated. Since the reticulocytes are identified as this young group of cells, they should be present in a concentration of 1.5 per cent. Since their normal value rarely exceeds 0.5 per cent, it follows that three generations must be born every 24 hours. The probability of the correctness of a short reticulocyte period is shown by the fluctuations occurring at 3 hour intervals in the work of Porter and Irving (1929) on liver therapy in pernicious anemia. On this hypothesis, the reticulocyte and erythrocyte values become consistent.

Calculations based on the reticulocyte percentages and the erythrocyte counts in the work of Minot, Murphy, and Stetson (1928) show reticulocyte outputs far in excess of the erythrocyte increase in given periods. In one case, the red blood cell count increased 200,000; the summation of the reticulocyte counts in the same time interval gives a value of 846,000. In a second case, the red blood cells increased 500,000 while the reticulocyte output was 1,424,000. These extremely high values may be interpreted as due to an unusually active marrow, the result of which is masked by a simultaneous hemolysis. The first 10 days after hemolysis in these experiments demonstrate such a relationship.

Another interpretation however may be put on the facts of high reticulocyte counts in cases of long standing. Eaton has shown that a marked hemorrhage is followed by a marked reticulocytosis. At a time interval corresponding to the length of life of the red blood cell, this crop of reticulocytes reaches senescence and is simultaneously destroyed, producing a "spontaneous anemia." This is followed by a second crop of reticulocytes, which in turn produces a second spontaneous anemia at their normal destruction. Thus a series of anemias is induced by a single hemorrhage. On this interpretation it follows that even a slight but continuous hemolysis will in time produce an extremely high proportional reticulocytosis, as 80 per cent reported by
Sappington (1918), after the use of small daily doses of phenylhydrazine; or 56 per cent reported by Minot, Murphy, and Stetson (1928) in pernicious anemia.

**E. Regeneration Rates after Hemorrhage and after Hemolysis.**

It is generally stated that regeneration after hemolysis is more rapid than after hemorrhage because of the retention of hemoglobin which may be reutilized in the maturation of the erythrocyte. The results obtained in these two tests show little or no difference in regeneration rate. After hemorrhage the average daily increase was 278,100; after hemolysis the rate was 294,000. These rates may seem to be high, but are well within the range of similar values given by other investigators. Muir's data (1911) show increases of 420,000 after hemolytic serums in the rabbit; Jones (1911) reports rates of 460,000 after phenylhydrazine in the rabbit; Dawson's cases (1901) give 433,000 after hemorrhage in the dog; Starr (1928) reports increases of 170,000 per day after liver therapy in pernicious anemia.

**F. Consideration of the Source of Endogenous Uric Acid.**

The foregoing data on uric acid after hemolysis are highly confirmatory of the hypothesis that endogenous uric acid is produced in the bone marrow. This hypothesis has been independently developed and considered by Riddle (1929) following his work on uric acid output after the administration of liver extract in cases of pernicious anemia. Potent extracts increased the output; non-potent extracts had no effect.

Investigations by Emmel (1914) on the histology of the marrow of the pig indicate that the erythrocyte may become an enucleated cell by a process other than nuclear extrusion. In these investigations and in many others of a similar type, free nuclei enter the peripheral circulation and may be identified as such (Bunting (1906) and Jones (1911)).

That the marrow constitutes the principal organ of uric acid formation is shown indirectly by the work of Bollman, Mann, and Magath (1925), in their effort to show the seat of destruction of this metabolite in the common dog. They have shown a high and constant production of uric acid in dogs from which the entire livers have been removed, and show extremely high blood uric acid levels in dogs from which all abdominal viscera were taken.
Since the work of Zwarenstein (1928) on uric acid after exercise precludes the muscular system, and Peck and Thompson (1908) have shown very low outputs in neurasthenia, the marrow remains the only nuclear tissue sufficient in amount to account for the observed variations.

SUMMARY.

1. The average daily output of uric acid for a period of 21 days for a thoroughbred Dalmatian coach dog was 265 mg.; the reticulocyte count was 22,836 and the red blood cell count 5,709,000.

2. The average daily output of uric acid for 29 days after hemolysis with phenylhydrazine HCl was 436 mg.; the reticulocytes 66,377.

3. Regeneration rates and uric acid outputs after hemorrhage and after hemolysis are very similar.

4. Correlation of uric acid output and reticulocytes is shown and indicates a lag of from 3 to 4 days between maturation and extrusion.

5. The reticulocyte counts and total erythrocyte regeneration are considered on the hypothesis of an 8 hour span for the reticulocyte stage.

6. Additional confirmatory data are represented to support the hypothesis that endogenous uric acid arises from the extruded nuclei of the normoblast at the maturation of the erythrocyte.

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Muir, R., *J. Path. and Bact.*, 16, 410 (1911).
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