URETATE DISTRIBUTION IN BLOOD*

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In the study of the distribution of electrolytes between cells and plasma, Henderson (1), Van Slyke et al. (2), and others have dealt principally with ions occurring naturally in relatively large concentration; i.e., bicarbonate and chloride. Other ions, as lactate and urate, are present in normal blood in such low concentration that the determination of their distribution between cells and plasma is accompanied by particular technical difficulties. There are certain pathological conditions, however, of which gout is an example, in which the urate concentration in blood is increased sufficiently to reduce some of the technical difficulties. In the blood from such patients it is possible to measure the changes in distribution of this electrolyte between serum and cells with change in pH.

In this communication there will be described certain physicochemical properties of blood obtained from patients suffering from gout. Specifically, the applicability of the Gibbs-Donnan law of equilibrium to the distribution of urate ions in these bloods will be tested. A general description of the specimens of blood is unnecessary as no significant variation from normal human blood has been noted concerning chloride, bicarbonate, or hemoglobin content. Our present purpose is the consideration of the concentration of urate in cells and plasma as a function of hydrogen ion concentration and degree of oxygenation of hemoglobin.

Material and Methods—The three gouty patients in this study from whom blood was obtained were suffering from recurrent attacks of acute gout. The diagnosis in patients F. M. (3) and

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F. N. was based upon the recovery and identification of sodium urate crystals from cutaneous tophi. Patient M. T. had clinical gout, but at the time this work was done had developed no palpable tophi.

Venous blood was drawn from these patients in the basal state and immediately defibrinated. Approximately 15 cc. portions of this blood were added to tonometers at varying tensions of CO₂ and O₂ and were equilibrated in a water bath at 37.5° (3). After 20 minutes of equilibration, whole blood for measurement of oxygen content, carbon dioxide content, and urate concentration was removed. The remaining blood was transferred under oil to a graduated centrifuge tube and centrifuged for three 20 minute periods. Calculation of the cell volume at infinite time was made according to the method of Hirota (4). Values for pH₄ were derived from the equation:

\[ \text{pH}_4 = \text{pK}' + \log(\text{BHCO}_3) - \log(\text{H}_2\text{CO}_3) \]

Urates were determined in whole blood, serum, and cells according to the method of Benedict and Behre (5) or that of Folin (6). In the experiment on January 28, 1936, the urate concentrations were determined by both methods on aliquot portions of the same sample. The accuracy of the Benedict determination on aliquot portions of similar sera on subsequent days was investigated in seventeen samples obtained from as many patients with various pathological conditions. The urate concentration was determined first on each sample on the day when the blood was withdrawn. After the remainder of each sample had been kept at 4° for a period varying between 2 and 6 days, the determination was repeated. In twelve of the sera the maximum variation was less than 0.02 milli-equivalent per liter. In the remaining five samples the maximal variation was less than 0.04 milli-equivalent per liter.

Samples of sera measured volumetrically and weighed samples of cells were used for determination of urate and chloride. The distribution between serum and cells was calculated according to the equations:

\[
\frac{(U')_s/(H_2O)_s}{(U')_w/(H_2O)_w} \quad \text{and} \quad \frac{(Cl')_s/(H_2O)_s}{(Cl')_w/(H_2O)_w}
\]
In these equations the concentration of the urate ion (U') and of the chloride ion (Cl') are expressed in milli-equivalents per liter. H₂O was determined on weighed samples.

In certain experiments the blood was treated as described above and without the addition of any substance. In other experiments from 0.5 to 0.6 milli-equivalent per liter of unneutralized uric acid was added to the whole blood. All of the uric acid which was added did not go into solution; therefore, it was necessary to filter these bloods through several layers of fine gauze before equilibration.

Natural Occurrence of Urate within Cell—It is assumed generally that urates are present naturally in blood cells as well as serum (7, 8) and that the erythrocyte wall exercises a conditioned permeability in respect to the transfer of this ion (9). In view of Olmsted’s recent report (10) that glucose is not present in human blood cells immediately after withdrawal from the body, it was thought desirable to carry out similar experiments concerning the presence of urate within the cell. For this purpose blood was drawn from patient F. N., placed in a centrifuge tube under oil, and centrifuged at once. No anticoagulant or other substance was added. The elapsed time between the withdrawal of the blood and the precipitation of the serum protein was approximately 10 minutes. Urate was determined shortly after by the method of Benedict. Other samples from the same venesection were allowed to stand for 2 hours at 4° and 37.5° respectively, before analysis. In a similar fashion samples were kept at 4° and 37.5° respectively, for 6 hours before analysis. At the end of 2 hours there was a decrease in (U), from 0.50 to 0.49 milli-equivalent per liter and at the end of 6 hours it had decreased to 0.48. No difference in urate distribution was observed between the samples kept cold and those kept warm. In another experiment done on the same day, (U)c and (U)b were determined as well as (U)s. Blood from the same venesection as above was defibrinated under oil and portions were equilibrated at pH₀ = 7.30 for 15, 60, and 120 minutes respectively, in the water bath at 37.5°. There was a decrease in (U), from 0.50 to 0.48 milli-equivalent per liter over the 2 hour period. The (U)c remained unchanged at 0.21 and (U)b.

1 In this paper the subscripts c, s, and b refer to cells, serum, and whole blood respectively.
TABLE I
Experimental Observations on Oxygenated Blood

<table>
<thead>
<tr>
<th>Date</th>
<th>Subject</th>
<th>Uric acid method</th>
<th>Whole blood</th>
<th>Serum</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$pCO_2$ mm. Hg</td>
<td>Total CO$_2$ m.-eq. per l.</td>
<td>Oxygen capacity m.-eq. per l.</td>
</tr>
<tr>
<td>Jan., 1936</td>
<td></td>
<td></td>
<td>10.1</td>
<td>8.1</td>
<td>6.97</td>
</tr>
<tr>
<td>21</td>
<td>M. T.</td>
<td>Folin</td>
<td>181.7</td>
<td>28.5</td>
<td>41.5</td>
</tr>
<tr>
<td>23</td>
<td>&quot;</td>
<td>Benedict</td>
<td>8.4</td>
<td>7.0</td>
<td>7.17</td>
</tr>
<tr>
<td>28</td>
<td>F. N.</td>
<td>&quot; *</td>
<td>174.3</td>
<td>29.4</td>
<td>41.0</td>
</tr>
<tr>
<td>28</td>
<td>&quot;</td>
<td>Folin*</td>
<td>13.7</td>
<td>10.3</td>
<td>10.5</td>
</tr>
<tr>
<td>30</td>
<td>F. M.</td>
<td>Benedict</td>
<td>218.0</td>
<td>38.3</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.0</td>
<td>11.7</td>
<td>10.8</td>
</tr>
</tbody>
</table>

* Determinations were made on aliquot portions of the same filtrates.
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decreased from 0.32 to 0.30 milli-equivalent per liter during the same period. These data are interpreted as indicating that within the time limits of our experiment at a constant pH, there is no significant migration of urate from serum to cells and that urate does exist in cells in vivo.

**Change in \((U)_s\) and \((U)_c\) As a Function of \(pH\)**—In Table I are given data from four experiments on oxygenated blood to which no uric acid was added. In all experiments the reaction of the drawn blood was altered to give one set of determinations in the alkaline range near \(pH_s = 7.60\) and another set of determinations in the acid range near \(pH_s = 6.80\). This range of hydrogen ion concentration exceeds greatly the usual physiological variation. Associated with this change in \(pH_s\) were the well recognized changes in cell volume and in \((HCO_3^-)\) and \((Cl^-)\) distribution. The average change in \(pH_s\) for the four experiments shown in Table I was 0.81. The average decrease in \((Cl^-)_s\) was 9.6 and the average increase in \((Cl^-)_c\) was 8.8 milli-equivalents per liter. The average increase in cell volume percentage was 3.2. The change in \((U)_s\) was in the same direction as, and was roughly proportional to, the change in \((Cl^-)_s\). The data in Tables I and II are not corrected for the increase in free uric acid in the acid range. In an aqueous solution of \(pH 7.60\) about 99 per cent of the total urate in solution is in the form of sodium urate and only 1 per cent as free acid (11). At \(pH 6.80\) about 84 per cent is in the form of the sodium salt and 14 per cent as free acid. When these corrections are applied to the data the percentile shift is increased. Simultaneous with the decrease in \((U)_s\), there was an increase in \((U)_c\). This change is similar to that observed for \((Cl^-)_c\). There was no change in \((U)_c\) except in the experiment of January 23, 1936; the discrepancy of 0.12 milli-equivalent in that experiment presumably was due to an error in determination. It is evident from these data that there is a migration of the urate ion between serum and cells with varying pH and that this migration is like that observed for the chloride ion.

**Distribution of Urate between Cells and Plasma**—This has been a subject for dispute (12, 13) since the introduction into clinical chemistry of micromethods for the determination of this constituent in human blood. It is generally accepted that the concentration of urate in exudates and edema fluid is similar to the
<table>
<thead>
<tr>
<th>Date</th>
<th>Subject</th>
<th>Uric acid method</th>
<th>Whole blood</th>
<th>Serum</th>
<th>Cells</th>
<th>Chloride</th>
<th>Urate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pCO₂</td>
<td>Total CO₂</td>
<td>Cell volume</td>
<td>Urate</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Jan., 1936</td>
<td></td>
<td></td>
<td>mm. Hg</td>
<td>m.-eq. per l.</td>
<td>per cent</td>
<td>m.-eq. per l.</td>
<td>mm. Hg</td>
</tr>
<tr>
<td>21</td>
<td>M. T.</td>
<td>Folin</td>
<td>8.8</td>
<td>7.8</td>
<td>37.7</td>
<td>0.98</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benedict</td>
<td>207.5</td>
<td>30.3</td>
<td>42.5</td>
<td>1.08</td>
<td>26.2</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>Benedict</td>
<td>9.0</td>
<td>6.8</td>
<td>38.8</td>
<td>0.74</td>
<td>7.7</td>
</tr>
<tr>
<td>28</td>
<td>F. N.</td>
<td>&quot;</td>
<td>185.4</td>
<td>29.1</td>
<td>41.2</td>
<td>0.78</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;</td>
<td>11.9</td>
<td>9.5</td>
<td>51.5</td>
<td>0.87</td>
<td>11.9</td>
</tr>
<tr>
<td>28</td>
<td>Folin*</td>
<td></td>
<td>215.4</td>
<td>36.9</td>
<td>54.6</td>
<td>0.89</td>
<td>11.9</td>
</tr>
<tr>
<td>30</td>
<td>F. M.</td>
<td>Benedict</td>
<td>11.7</td>
<td>11.0</td>
<td>51.2</td>
<td>1.06</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>220.0</td>
<td>40.7</td>
<td>53.3</td>
<td>0.90</td>
<td>37.8</td>
</tr>
</tbody>
</table>

* Determinations were made on aliquot portions of the same filtrates.
concentration in serum (14), but no such agreement exists concerning the distribution in blood cells and serum. The ratio 23:100, not corrected for water content, given by Folin and Svedberg (15) is the lowest of any large series in the literature. When Benedict's ratio of 74:100 (5) is corrected for water content of serum and cells, a ratio of approximate unity is obtained.

In Table II are given the experimental data on blood to which uric acid was added. Except for the addition of this substance to the blood the experiments were performed in the same manner as those depicted in Table I. Our principal interest in these

![Fig. 1. Distribution ratio of chloride and urate as a function of pH.](http://www.jbc.org/)

experiments was the changing distribution ratio as a function of pH. In Tables I and II and Figs. 1 and 2 the values for \((U)\) are related to the pH of the serum and not of the cell. If the ratio is calculated for pH within the cell and the data corrected accordingly, the urate ratio in the acid range is slightly greater than shown.

In these experiments it is presumed that the added uric acid distributed itself between the serum and cells and behaved in a fashion similar to the urate already present. The percentile increase in concentration in serum and cells substantiates this presumption. With increasing acidity (Fig. 1) the urate distribution ratio increased at approximately the same rate as the chloride.
ratio. At \( \text{pH}_s = 7.40 \) the average chloride ratio of the three experiments was 0.68. At the same \( \text{pH}_s \) the average urate distribution ratio was 0.60. Van Slyke, Hastings, Murray, and Sendroy (16) have shown by electrometric measurements that the distribution of hydrogen ions between serum and cells is such that the ratio \((aH^+)_s/(aH^+)_c\) approximately equals 0.50 at \( \text{pH}_s = 7.40 \). The agreement of the urate ratio with the reciprocal of the hydrogen ion ratio is better than the ionic distribution of chloride and bicarbonate and suggests that the activity and the concentration of the urate ions are more nearly alike than in the case of chloride and bicarbonate.

Fig. 2. Distribution ratio of chloride and urate as a function of degree of oxygenation of hemoglobin and \( \text{pH}_s \).

**Effect of Oxygenation-Reduction and \( \text{pH}_s \) Change on Urate Distribution**—One experiment was performed to determine the effect of oxygenation and reduction of the blood in the acid and alkaline range on the distribution of urate between serum and cells. The data are shown graphically in Fig. 2. The anion distribution ratios predicted by Van Slyke, Wu, and McLean (2) were lower in oxygenated blood than reduced blood at the same \( \text{pH}_s \). In our experiments at \( \text{pH}_s = 7.80 \) the spread of the chloride ratio between oxygenated and reduced whole blood was 0.08. At the same \( \text{pH}_s \) the spread of the urate ratio was 0.10. With increasing acidity
the spread is diminished as observed by Dill (17) for the bicarbonate distribution ratio. At pH, = 6.80 the spread of the chloride ratio was 0.01 and the urate ratio was 0.02. At pH, of approximately 6.60 the reduced and oxygenated distribution ratios for chloride and urate, respectively, are apparently equal.

**Maximum Concentration of Urate in Serum**—In equilibration experiments of serum with sodium urate, Gudzent (18) approached a maximum solubility with a urate concentration of 0.5 milli-equivalent per liter. Our observations indicate that at least twice this concentration may be observed in the serum from gouty patients. The maximum concentration of 0.89 milli-equivalent per liter of serum in blood drawn from M. T. on January 30, 1936, is less than 1.3 milli-equivalents per liter, the maximum solubility observed by Bechhold and Ziegler (19). In the experiment of January 28, 1936, uric acid was added to the blood and a concentration of 1.63 milli-equivalents per liter of serum was obtained. These observations suggest that Gudzent's maximum values are too low, either for urate occurring in the serum of patients with gout or for *in vitro* experiments when uric acid is added to blood.

**SUMMARY**

1. The concentration of urate in serum and cells agrees approximately with the Gibbs-Donnan law of equilibrium as applied by Van Slyke and associates to blood. As the reaction of the serum becomes more acid, there is a migration of urate ions from the serum to the cells. A reverse migration takes place as the reaction becomes more alkaline.

2. When urate is added to blood it distributes itself between cells and serum in the same proportion as the naturally occurring urate.

3. At pH, = 7.40 the urate distribution ratio as defined by the relation

   \[
   \frac{\text{Cell urate}}{\text{Cell water}} + \frac{\text{serum urate}}{\text{serum water}}
   \]

   is approximately 0.60.

4. If blood containing added urate is oxygenated or reduced, the urate ratio changes in a manner similar to the chloride and bicarbonate ratios.
5. After the addition of uric acid the maximum concentration of serum urate observed by us in one instance was 1.63 milliequivalents per liter, a value which considerably exceeds the value previously ascribed to the solubility of urate in serum.

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BIBLIOGRAPHY


