THE EFFECT OF POTASSIUM OXALATE ON ELECTROLYTES OF BLOOD AND PLASMA.

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The common use of potassium oxalate as an anticoagulant and the probability that its use would affect the electrolyte distribution of blood led to a comparison of oxalated and defibrinated blood to find out if the use of this anticoagulant was justifiable in studies involving accurate analysis of electrolyte equilibria in blood. While few observers have had definitely as their object such a direct comparison of oxalated and defibrinated blood, a number have, in the course of other investigations, studied the two kinds of blood with respect to certain properties. The earlier work is concerned with the demonstration that the process of defibrination does not alter the electrolyte distribution.

Fredericq in 1878 (1) found this true of bicarbonate distribution. Hamburger in 1893 (2) extended the findings to the chloride and carbonate partition, and also determined that total solid and reaction of serum and "natural plasma" of both arterial and venous horse blood were identical. Christiansen, Douglas, and Haldane (3) claim, on the basis of indirect evidence, that the partial pressure of carbon dioxide of blood directly from the blood vessel must be distinctly lower than that of the same blood after defibrination. Their statement is based on the observation of a progressive fall in the carbon dioxide-combining power of defibrinated blood when exposed for a period of time (up to 5 hours) to a temperature of 37°C. They state that this difference between living and defibrinated blood is independent of the different degree of oxygen saturation of the two kinds of blood. Zuntz, whom they quote in substantiation of their statement, considers that the difference between living venous and defibrinated blood is due, at least in part, to the relative deoxygenation of the former. Warburg (4) found chloride and bicarbonate distribution in defibrinated and in hirudinized (horse) blood identical. The bulk of evidence indicates that defibrinated blood may be considered the same as the blood in the blood vessel.
Previous studies of the effects of potassium oxalate show no agreement among the investigators. Haggard and Henderson (5) stated that, although defibrinated blood has originally a higher carbon dioxide capacity than oxalated or untreated blood, exposure of defibrinated blood to low carbon dioxide tensions causes an irreversible loss of carbon dioxide capacity. Oxalated blood does not exhibit this decrease in power to bind carbon dioxide. This verified earlier work of Christiansen, Douglas, and Haldane (3). This was disproved by Austin, Cullen, and Peters (quoted in Peters et al. (6)).

In Joffe and Poulton's (7) work, the difference of carbon dioxide-combining power in oxalated and defibrinated blood, whether oxygenated or reduced, lies practically within the error of their method. But these authors show a distinctly higher carbon dioxide-combining power for oxalated plasma than for defibrinated serum. Their results, although remarkably consistent, are not decisive because they are derived from a statistical comparison of blood specimens analyzed under variable conditions and on widely separated occasions. Warburg (8) considers the addition of oxalate to blood as analogous to the addition of an equivalent amount of hypertonic saline solution. The serum of blood to which he had added hypertonic sodium chloride solution combined with slightly more carbon dioxide than the serum of blood treated with isotonic saline.

Experimental Procedure.

In the earlier studies on the effect of potassium oxalate, an attempt was made to use heparin (we were unable to secure hirudin) as an anticoagulant for comparison with potassium oxalate. We were forced to abandon the use of heparin, because the differences in oxalated and heparinized specimens were so marked that the reaction of the anticoagulants was suspected. The heparin was found to be quite acid and the potassium oxalate quite alkaline to phenol red. Recrystallization of potassium oxalate renders the salt neutral: less than 2 drops of 0.01 n NaOH are necessary to bring 10 cc. of a 1 per cent solution of the recrystallized salt to pH 7.35 to 7.4. Unfortunately the heparin could not be neutralized, so its use was replaced by defibrination of the blood. Since all the direct evidence quoted in the introduction goes to prove that electrolyte distribution is not influenced by defibrination, we feel justified in assuming that defibrinated blood is the same as the blood in the blood vessel provided the necessary precautions are observed in handling it.

Collection of Blood.—All blood was drawn from human subjects, patients on the Medical Wards of the New Haven Hospital. There
were two general types of experiments, "capacity" and "content" experiments. In "capacity" experiments, the blood was collected without precautions against contact and kept chilled until ready for use. It was then saturated with a definite tension (usually 40 mm.) of carbon dioxide in air. There were two types of "content" experiments. "Content" either means that the defibrinated blood was treated with oxalate directly after saturation with known carbon dioxide tension without exposure; or, that the blood was drawn from the subject directly into sampling tubes over mercury and defibrinated or oxalated without exposure. The latter type is a "true content" experiment.

Three different methods were used for the collection of the blood for capacity experiments:

Method A.—The total sample was withdrawn into an open vessel and defibrinated by stirring with a glass rod. Half of the defibrinated blood was added to enough solid neutral potassium oxalate to make a 0.2 per cent solution of the salt in the whole blood (Table II, Cases 12, 13, and 14).

Method B.—The blood sample was drawn from the vein through a three-way stop-cock, connected directly with the needle, alternately into two tubes, one of which contained enough neutral potassium oxalate to make an approximately 0.2 per cent solution of the salt in the blood. The stop-cock was turned every 8 or 10 seconds during the withdrawal of the blood, so as to insure original uniformity of the two samples. The blood in the tube containing no salt was defibrinated by stirring with a glass rod (Table I, except Case 9).

Method C.—The blood was withdrawn as in Method B, and then one-half of the defibrinated blood was mixed with enough potassium oxalate to make a 0.2 per cent solution. There were thus in this case three samples for comparison: oxalated blood, defibrinated blood, and defibrinated blood containing potassium oxalate (Case 9, Tables I and II).

For the content experiments blood was treated in two ways.

Method A.—A large amount of blood (usually 50 to 80 cc.) was defibrinated and then saturated at 40 mm. of carbon dioxide. Some of the saturated blood and serum separated from the blood was analyzed as defibrinated blood. The rest of the blood, after saturation, was delivered directly without air contact into a sampling bulb full of mercury and coated with enough potassium oxalate to make a 0.2 per cent solution. From a portion of this blood, serum was also separated. Whole blood and serum were analyzed as "defibrinated plus oxalate" blood. In two of the experiments, one-half of the oxalated blood was resaturated and analyzed (Table III).
Method B.—After the new technique (described in the succeeding article) (9) for defibrinating blood out of contact with air had been devised, the blood was drawn into a syringe under oil. One-half was delivered directly into a sampling bulb and defibrinated with mercury; the other half was delivered directly into a sampling bulb containing enough potassium oxalate to make a 0.2 per cent solution in the blood (Table III, Case 18).

Preparation and Use of Potassium Oxalate.—Technical potassium oxalate is recrystallized from hot water and washed twice with cold water. The recrystallized salt should be kept in Pyrex containers. In the course of months, it gradually becomes more alkaline, so it is well to recrystallize about every 6 weeks.

The introduction of the potassium oxalate into the blood is a matter of some importance. The desired amount of the neutral salt is made up in a fresh concentrated solution and introduced into the container which is to receive the blood. The mixture is dried by drawing a current of air through the vessel; frequent rotation of the vessel during the drying insures a uniform, thin coating of oxalate. When the blood is introduced it comes into contact with only a little oxalate at a time, which is immediately dissolved. This method obviates the danger of hemolysis which results when too great a concentration of oxalate comes into contact with only a small amount of blood, insures uniform rapid equilibrium conditions, and prevents the formation of any small clots.

Analytical Procedure.

Cell volume was determined by using a Daland hemocrit fitted to a No. 1 International Equipment Company, centrifuge. Duplicate determinations were carried out on each blood sample and always agreed within 1 volume per cent.

Oxygen capacity was determined in the Van Slyke constant pressure pipette by a method devised by Lundsgaard and Neill.1 Sometimes the blood cells from which the serum or plasma had been removed were used for oxygen capacity determinations as described in a previous communication (10).

Carbon dioxide was determined by the Van Slyke and Stadie method (11) in a calibrated constant pressure Van Slyke pipette.

Saturation of the blood with carbon dioxide was effected by the second saturation method described by Austin et al. (12).

Chlorides.—Until October 1, 1923, the chlorides were determined by the Austin and Van Slyke method (13) with the modification described by us elsewhere (10). Later determinations were done by the Van Slyke method (14).

Proteins.—Serum or plasma proteins were determined by macro-Kjeldahl procedure using 8 to 16 cc. samples.

1 Personal communication.
Experimental Results.

Capacity Experiments.—The results of the simplest experiments on comparison of oxalated and defibrinated blood are to be found in Table I. The blood was collected as described under Method B of the capacity methods. Oxygen capacity was done on both defibrinated and oxalated blood as an assurance that the blood samples had been well mixed, but no differences between the two greater than the error of the method were ever observed. Whole blood chlorides were determined on either specimen. The rest of the blood was saturated with 40 mm. of carbon dioxide in air. In two cases the samples were saturated at both 30 and 60 mm. of carbon dioxide, in order to give carbon dioxide absorption curves. Defibrinated and oxalated saturated whole bloods were analyzed for cell volume and carbon dioxide content. "True serum" and "true plasma" were analyzed for carbon dioxide content, sodium chloride content, and in some cases for protein concentration. Cell carbon dioxide and sodium chloride were calculated from whole blood and plasma or serum values, and cell volume.

There is no absolute consistency in the results. The greatest consistency is to be found in the cell volume changes. In all but one case (probably an error), oxalate caused contraction of the cells.

The carbon dioxide of whole blood and of plasma is very little changed in average experiments. Individual samples show considerable variations in both directions. The cell bicarbonate differences are, of course, greater than those of whole blood or serum; for, if the latter values change only slightly and the cell volume varies considerably, the cells must sustain great changes.

Average values for plasma and serum sodium chloride show little change, but individual samples show the same relatively large variations as do the bicarbonate values.

In Table II are given the data which were obtained from the comparison of defibrinated blood and defibrinated blood to which oxalate had been added. The cell volume shows an even greater tendency to lowering as a result of the addition of oxalate than in the experiments where oxalation was not complicated by defibrination. The electrolyte changes are, in general, the same as those demonstrated in Table I.
### TABLE I

**Comparison of Carbon Dioxide Capacity of Oxalated and Defibrinated Blood.**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Defibrinated.</td>
<td>None.</td>
<td>0.19 per cent oxalate.</td>
<td>4.5, 64, 20, 1.5 151.3</td>
<td>38.7 74.2 35.8 24.5</td>
<td>36.7 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>2</td>
<td>Defibrinated.</td>
<td>None.</td>
<td>0.23 per cent oxalate.</td>
<td>4.4, 64, 16, 151.45</td>
<td>36.5 20.3 29.2 34.5</td>
<td>36.7 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>3</td>
<td>Defibrinated.</td>
<td>None.</td>
<td>0.31 per cent oxalate.</td>
<td>3.4, 64, 16, 151.85</td>
<td>35.3 20.3 29.2 34.5</td>
<td>36.3 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>4</td>
<td>Defibrinated.</td>
<td>None.</td>
<td>0.4 per cent oxalate.</td>
<td>2.3, 64, 16, 151.75</td>
<td>35.3 20.3 29.2 34.5</td>
<td>36.3 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>5</td>
<td>Defibrinated.</td>
<td>None.</td>
<td>0.6 per cent oxalate.</td>
<td>1.4, 64, 16, 151.5</td>
<td>35.3 20.3 29.2 34.5</td>
<td>36.3 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>6</td>
<td>Defibrinated.</td>
<td>Moderate.</td>
<td>0.31 per cent oxalate.</td>
<td>4.5, 64, 20, 1.5 151.25</td>
<td>38.7 74.2 35.8 24.5</td>
<td>36.7 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>7</td>
<td>Defibrinated.</td>
<td>Slight.</td>
<td>0.4 per cent oxalate.</td>
<td>3.4, 64, 16, 151.75</td>
<td>35.3 20.3 29.2 34.5</td>
<td>36.3 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td>8</td>
<td>Defibrinated.</td>
<td>Very slight.</td>
<td>0.6 per cent oxalate.</td>
<td>2.3, 64, 16, 151.5</td>
<td>35.3 20.3 29.2 34.5</td>
<td>36.3 23.5 29.2 34.5</td>
<td>6.20 7.20 8.20 9.20</td>
<td>40 40 40 40</td>
</tr>
<tr>
<td></td>
<td>Defibrinated.</td>
<td>0.2 per cent oxalate.</td>
<td>0.2 per cent oxalate.</td>
<td>0.2 per cent oxalate.</td>
<td>Defibrinated.</td>
<td>0.2 per cent oxalate.</td>
<td>0.2 per cent oxalate.</td>
<td>Average.</td>
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<tr>
<td>9</td>
<td>40.75</td>
<td>17.35</td>
<td>46.6</td>
<td>36.2</td>
<td>53.75</td>
<td>4.52</td>
<td>2.35</td>
<td>6.01</td>
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<td>38.9</td>
<td>17.28</td>
<td>44.5</td>
<td>32.1</td>
<td>52.3</td>
<td>2.26</td>
<td>5.96</td>
<td>6.73</td>
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<td>10</td>
<td>42.6</td>
<td>10.08</td>
<td>39.0</td>
<td>29.8</td>
<td>47.25</td>
<td>5.35</td>
<td>4.40</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td>40.6</td>
<td>16.60</td>
<td>39.7</td>
<td>28.5</td>
<td>47.2</td>
<td>4.40</td>
<td>6.00</td>
<td>5.55</td>
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<td>43.5</td>
<td>16.68</td>
<td>52.7</td>
<td>40.2</td>
<td>62.3</td>
<td>5.35</td>
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<td>11</td>
<td>39.6</td>
<td>16.60</td>
<td>52.75</td>
<td>39.5</td>
<td>61.5</td>
<td>5.35</td>
<td>5.35</td>
<td>5.55</td>
</tr>
<tr>
<td></td>
<td>41.7</td>
<td>45.3</td>
<td>31.2</td>
<td>54.45</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>39.9</td>
<td>45.1</td>
<td>32.6</td>
<td>53.35</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>42.05</td>
<td>57.25</td>
<td>43.9</td>
<td>67.0</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>41.0</td>
<td>57.3</td>
<td>43.7</td>
<td>66.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

None. | Capacity, C.  
------ | ------------

Marked. | R.  
------ | ----

None. | R.  
------ | ----

Marked. | R.  
------ | ----

Very slight. | R.  
------ | ----

Slight. | R.  
------ | ----

Average. | Oxalated.  
--------- | ---------

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### TABLE II.

**Capacity Experiments. Comparison of Defibrinated Blood with Defibrinated plus Oxalated Blood and Also with Defibrinated plus Sodium Chloride Blood.**

<table>
<thead>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Defibrinated. plus 0.45 per cent oxalate.</td>
<td>12.0</td>
<td>22.48</td>
<td>44.55</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>Defibrinated. plus 0.22 cent oxalate.</td>
<td>40.75</td>
<td>17.35</td>
<td>46.6</td>
<td>36.2</td>
</tr>
<tr>
<td>13</td>
<td>Defibrinated. plus 0.2 per cent oxalate.</td>
<td>46.85</td>
<td>21.40</td>
<td>47.0</td>
<td>35.9</td>
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<tr>
<td></td>
<td>Defibrinated plus 0.07 per cent sodium chloride.</td>
<td>48.41</td>
<td>20.33</td>
<td>43.05</td>
<td>26.3</td>
</tr>
<tr>
<td>14</td>
<td>Defibrinated. plus 0.2 per cent oxalate.</td>
<td>48.41</td>
<td>20.33</td>
<td>43.05</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>Defibrinated plus oxalate.</td>
<td>46.00</td>
<td>20.41</td>
<td>44.73</td>
<td>30.9</td>
</tr>
<tr>
<td></td>
<td>Average.</td>
<td>41.68</td>
<td>20.19</td>
<td>45.57</td>
<td>32.3</td>
</tr>
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</table>
It should be emphasized that the bicarbonate and chloride differences observed here, though slight, represent real changes. While the chloride method is less delicate than the carbon dioxide method, it is accurate to within 0.1 gm. of sodium chloride per liter. Duplicate carbon dioxide analyses, even if the duplicates are separately saturated, regularly agree within 0.2 volume percent of carbon dioxide.

Plasma and serum proteins cannot be directly compared, because fibrin has been removed from the serum. In the only case where both plasma and serum proteins were determined the plasma value is higher than the serum value, probably because the dilution of the plasma by water derived from contraction of the cells more than compensated for the removal of the fibrin.

Content Experiments.—The results of the content experiments are given in Table III. The blood in Cases 15, 16, and 17 was treated as described under content collection Method A; that of Case 18 was collected as described under content Method B.

Considering Cases 15, 16, and 17, the cell volume shows the customary shrinkage on the addition of oxalate. The whole blood carbon dioxide content is the same in defibrinated and defibrinated plus oxalated blood. In one case (No. 16) serum carbon dioxide remains unaltered; in Cases 15 and 17 it drops in the oxalated specimen. There are two resaturation experiments, Cases 16 and 17. Although the defibrinated plus oxalated blood is resaturated at the tension used in the original saturation of the defibrinated blood, greater differences from both the defibrinated and defibrinated plus oxalated blood are observed in the resaturated sample. Even the whole blood carbon dioxide changes in Case 16. It is evident, therefore, that the addition of oxalate to blood as drawn (that is, at constant carbon dioxide content) alters the carbon dioxide tension, while oxalate added to blood at constant carbon dioxide tension, alters the carbon dioxide content.

The true content experiment (Case 18) shows about the same differences as do the others. A difference in the oxygen capacity was observed here. However, when the cells of the oxalated specimen were diluted, a viscous dark mixture resulted. Some of the hemoglobin may have been destroyed and it was difficult to measure the specimen for analysis. These considerations are probably responsible for the low oxygen capacity of the oxalated sample.
### TABLE III.
Content and Resaturation Experiments.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Method of treatment of blood</th>
<th>Carbon dioxide content</th>
<th>Sodium chloride content</th>
<th>Hemolysis</th>
<th>Method of treatment of blood</th>
<th>Carbon dioxide content</th>
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<tr>
<td></td>
<td></td>
<td>Cell volume.</td>
<td>Oxygen capacity.</td>
<td>Whole blood</td>
<td>Cells</td>
<td>Serum or plasma</td>
</tr>
<tr>
<td>15</td>
<td>Defibrinated, defibrinated plus oxalated, or oxalated blood.</td>
<td>45.55</td>
<td>19.80</td>
<td>45.55</td>
<td>35.1</td>
<td>54.3</td>
</tr>
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<td></td>
<td></td>
<td>43.55</td>
<td>19.73</td>
<td>45.6</td>
<td>35.1</td>
<td>53.7</td>
</tr>
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<td>16</td>
<td>Defibrinated, plus 0.2 per cent oxalate.</td>
<td>42.4</td>
<td>18.05</td>
<td>48.15</td>
<td>35.6</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.2</td>
<td>48.2</td>
<td>33.8</td>
<td>57.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.9</td>
<td>47.45</td>
<td>33.0</td>
<td>56.6</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Defibrinated, plus 0.2 per cent oxalate.</td>
<td>46.0</td>
<td>18.89</td>
<td>45.4</td>
<td>34.2</td>
<td>54.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41.1</td>
<td>45.3</td>
<td>32.0</td>
<td>53.95</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>43.4</td>
<td>45.2</td>
<td>34.9</td>
<td>53.05</td>
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<tr>
<td>Average</td>
<td>Defibrinated</td>
<td>' plus oxalate</td>
<td>' plus oxalate</td>
<td>' plus oxalate</td>
<td>' plus oxalate</td>
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</tr>
<tr>
<td></td>
<td>44.65</td>
<td>46.37</td>
<td>35.0 55.5</td>
<td>40.95</td>
<td>46.37</td>
<td>33.9 54.93</td>
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<tr>
<td>18 True content experiment</td>
<td>Defibrinated</td>
<td>51.7</td>
<td>21.36 46.65</td>
<td>36.9 57.15</td>
<td>4.23 2.78</td>
<td>5.87 None</td>
</tr>
<tr>
<td></td>
<td>0.2 per cent oxalate</td>
<td>45.9</td>
<td>20.47 46.55</td>
<td>35.0 56.3</td>
<td>4.34 2.70</td>
<td>5.73 Very slight</td>
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<td>Content, B.</td>
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</table>

A. J. Eisenman
Comparison of Addition of Osmotically Equivalent Amounts of Sodium Chloride and Potassium Oxalate to Defibrinated Blood.—Enough oxalate was added to defibrinated blood (Case 14, Table II) to make a 0.2 per cent solution. Enough sodium chloride was added to some of the same defibrinated blood to make a solution of sodium chloride in blood osmotically equivalent to a 0.2 per cent solution of oxalate.

The cell shrinkage is less, while the change in the whole blood bicarbonate is greater in the sodium chloride specimen; cell and serum bicarbonate are much the same in the chloride and oxalate samples, being greater in both than in defibrinated blood. The changes in serum protein concentration and cell volume in the three specimens are closely correlated.

Rate of Sedimentation of Cells in Oxalated and Defibrinated Blood.—Blood containing 0.2 per cent potassium oxalate and a sample of the same blood defibrinated were placed in narrow tubes and the time rate of settling of the cells was determined. The cells of the oxalated sample settled more rapidly. At the end of 2 hours the serum volume of the defibrinated specimen was 0.02 cc., that of the oxalated 0.18 cc. Complete sedimentation in the oxalated specimen was reached in 8 hours; complete sedimentation of cells in the defibrinated specimen was not attained at the end of 50 hours. The experiment was repeated, using for the comparison defibrinated blood and defibrinated blood containing oxalate. The serum volume of the two specimens after 2 hours, was approximately the same.

**DISCUSSION OF RESULTS.**

There seems to be no doubt that oxalate, in concentrations usually employed to prevent coagulation of blood, causes the blood cells to contract. The cell volume shrinkage is not regularly proportional to the oxalate concentration, although the average shrinkage is greater in those experiments in which high concentrations of oxalate were used. In nine specimens where the oxalate concentration is 0.17 to 0.2 per cent, the cell volume of the oxalated specimen varies from that of the defibrinated specimen by 1.15 to 3.60 volumes per cent, an absolute cell shrinkage of 2.5 to 9.0 per cent. However, if the concentration of oxalate is very excessive (0.4 to 0.6 per cent), there is a relatively larger absolute
shrinkage (14 to 15 per cent). It may be pointed out that no direct relationship is to be expected between cell volume changes and oxalate concentration in different bloods because of the effect of other variables; the direct relationship would presumably appear in comparisons on one sample of blood. Both the concentration of carbon dioxide and chlorides and their distribution also may be slightly but significantly altered by the addition of oxalate, but neither the magnitude nor direction of these changes in a given specimen of blood can be predicted.

Contraction of the blood cells under the influence of oxalate is what one would expect if the cells are impermeable to both potassium and oxalate ions, or unequally permeable to them. It has never been determined whether the oxalate ion penetrates the cell membrane. Information on other anions has, however, been secured. De Boer (15) showed that addition of carbon dioxide to blood caused a shift of sulfate ion across the cell membrane. Hamburger (16) and Van Slyke and Cullen (17) showed that transfer of chlorine from plasma to cells results if blood is treated with carbon dioxide. These results were confirmed qualitatively but not quantitatively by Fridericia (18). Buell (19) claims that inorganic phosphate is confined to the serum, which would indicate that the cell envelopes are impervious to phosphate. Buell’s observations have not been confirmed by other investigators. Rossdale (20), who limited his observations to the relative effects of citrate and oxalate on cell volume, found oxalate to have a greater effect than citrate in reducing cell volume. His results are, however, far from conclusive, since he did not weigh his salts.

The balance of evidence is against the permeability of the red cell membrane to cations. Gürber in 1895 (21) showed that alterations of carbon dioxide tension caused no transfer of sodium and potassium between cells and serum. This fact was subsequently verified by Hamburger. The latter later found that the addition of salts or water to blood did cause such a transfer (22). Recent unpublished studies from this laboratory (23) indicate that there is no transfer of sodium and potassium ions across the red cell membrane in response to additions of salts. The absence of calcium from the cells, pointed out by Halverson, Mohler, and Bergeim (24) and by Falta and Richter-Quittner (25) argues the impermeability of the membrane to calcium.
Ege (26) found that the rate of penetration of electrolytes into cells varies, and was not able to attain equilibrium short of hours with some salts. In the case of the experiments recorded here, time as a factor has been ruled out. Saturation of oxalated blood immediately after its withdrawal from the subject and saturation of a duplicate sample of the same oxalated blood 2 hours later gave identical values.

**Blood Saturated.**

<table>
<thead>
<tr>
<th></th>
<th>Immediately</th>
<th>After 2 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sols. per cent</td>
<td>sols. per cent</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>45.05</td>
<td>45.1</td>
</tr>
<tr>
<td>Oxygen capacity</td>
<td>20.8</td>
<td>20.9</td>
</tr>
<tr>
<td>Whole blood CO₂ capacity at 40 mm</td>
<td>45.65</td>
<td>45.6</td>
</tr>
<tr>
<td>True plasma “ “ 40 “</td>
<td>54.15</td>
<td>54.3</td>
</tr>
</tbody>
</table>

Earlier experiments reported from this laboratory (6) showed that oxalated blood, if kept cold, would not change its carbon dioxide-combining power for at least 3½ hours.

Record was kept of the apparent amount of hemolysis in each blood specimen. Degree of hemolysis does not seem to be correlated with the observed changes.

According to Cullen (27) and Hastings and Sendroy (28), the pH of serum and plasma is the same. It is hard to understand how this can be possible if cell volume and electrolyte distribution are as much affected as our experiments indicate. It seems more likely that the methods used by these observers were not sufficiently delicate to permit the detection of these differences. Colorimetric methods are accurate to only 0.02 pH, which corresponds to almost 2 volumes per cent of carbon dioxide on the average absorption curve. By the methods employed in our experiments changes of carbon dioxide of as little as 0.2 volume per cent can be detected with reasonable certainty.

In Cases 15, 16, and 17, we have, as in the later capacity experiments, the combined effects of defibrination and oxalate. The cell volume shrinkage is about the same as in the similar capacity experiments. As is to be expected, since the transfer of defibrinated blood to the oxalated chamber is accomplished anaerobically, the whole blood carbon dioxide content is not altered in the process. The drop in serum carbon dioxide content observed in two of the cases is to be explained, partly on the basis
of the dilution of the plasma with contraction of the cells, partly on the basis of ionic transfers across the cell membrane under the influence of potassium and oxalate ions.

It is clear then that the addition of oxalate to blood at a constant carbon dioxide tension (capacity experiments) alters the carbon dioxide content; while the same addition at constant carbon dioxide content (content experiments) alters the tension. This is emphasized in the resaturation experiment (Case 16, Table III) where both content and tension are independently varied, each variation causing distinct changes.

In Case 18 we have only the effects of potassium oxalate without the complication of defibrination. The blood here, although compared directly as drawn from the subject, without exposure to air and without resaturation with carbon dioxide, exhibits the same variations as the capacity experiments, and offers additional evidence that oxalate per se changes the carbon dioxide tension of the blood.

As pointed out above, Warburg claims that the action of potassium oxalate is similar to that of an equivalent amount of sodium chloride. Our failure to get comparable results from the addition of apparently osmotically equivalent amounts of potassium oxalate and sodium chloride may have been due to the fact that the amounts added were not isotonic. Sodium chloride and potassium oxalate are salts whose anions have different valencies, and their osmotic effects probably do not correspond with their ionic concentrations. When potassium oxalate is added to blood, some of the oxalate anion is precipitated by calcium, the calcium being replaced in its combinations by potassium. The calcium is confined to the serum, where there is always an excess of anion available. If calcium oxalate precipitation is taken into account in the calculations, the effect of the oxalate on cell volume and ionic distribution becomes no more consistent or predictable.

In two experiments (Table I, Cases 10 and 11) the carbon dioxide absorption curves of oxalated and defibrinated blood were compared. In both cases the whole blood carbon dioxide at 30 and at 60 mm. was the same in the oxalated and the defibrinated specimen. In Case 10 the plasma and serum carbon dioxide at 30 mm. were the same, but the 60 mm. serum was higher than the 60 mm. plasma. In Case 11 the 30 mm. serum was higher than
the 30 mm. plasma, and at 60 mm. plasma and serum were the same. If the plasma and serum curves are plotted on logarithmic paper (the logarithmic carbon dioxide absorption curve is a straight line (6)) it will be seen that they intersect (Fig. 1). In Case 10 they intersect at the 30 mm. point; in Case 11, they intersect at the 60 mm. point. The intersections in the processes occur in opposite directions. In Case 10 the serum curve is higher than the plasma at high carbon dioxide tensions; in Case 11 the serum curve is higher than the plasma at low tensions. If these were cases where defibrinated and oxalated whole blood differed at constant carbon dioxide tension, similar intersections of whole blood curves might be demonstrated. In other words, oxalate may influence both the height and the shape of the absorption curve. Such changes in the absorption curve under the influence of oxalate probably are the causes of the apparent inconsistency of the electrolyte disturbances. The effects of oxalate on bicarbonate and chloride distribution depend on whether the blood was studied at a carbon dioxide tension above or below the point of intersection of the defibrinated and oxalated absorption curves.

The rapid settling of blood cells in oxalated blood must be due to the removal of fibrin and not to the presence of oxalate, since

![Graph comparing CO₂ absorption curves of serum and oxalated plasma (Cases 10 and 11).](http://www.jbc.org/Downloadedfrom)
defibrinated blood to which oxalate has been added has the same cell sedimentation rate as defibrinated blood. This is an indication that oxalate definitely alters the physical properties of the blood. That the cell volume changes after oxalate are not due merely to physical alterations of this nature, which facilitate packing of the cells, is proved by the protein determinations in the oxalate-chloride comparison experiment. The serum and plasma protein values show evidence of a transfer of water from the cells that is in keeping with the cell volume findings. That this is true of electrolyte changes also is apparent from Experiment 18, in which the distribution of carbon dioxide in oxalated blood and defibrinated blood analyzed just as they were drawn, without air contact or saturation, differed.

**CONCLUSIONS.**

Since potassium oxalate has such varied and inconsistent influence on the electrolyte distribution in blood, it is impossible even to establish an average correction for its effects. Where only approximate accuracy is desired *(i.e. carbon dioxide values within 1 volume per cent, sodium chloride to within 0.2 gm. per liter, and cell volume to within 3 volumes per cent)*, the use of a concentration of oxalate not exceeding 0.2 per cent may be justified, because of the fact that it affords a simple means of preventing clotting. However, where the absolute accuracy attainable by the methods used for the determination of these constituents is desired, the use of potassium oxalate as an anticoagulant is not advisable and should be replaced by defibrination.

Although this study has not proved that defibrination itself is without effect on cell volume and electrolyte distribution, the bulk of evidence would indicate that this is the case.

The fact that the cells of defibrinated blood settle very slowly is an additional reason for the use of defibrinated blood. In certain pathological bloods, if oxalated specimens are used for study, the cells settle out so rapidly that it is almost impossible to obtain a good plasma-cell mixture and it is therefore difficult to get good check duplicate whole blood analyses.

The common use of sodium fluoride, which presumably acts like sodium chloride, is also to be deplored, because it affects the electrolyte distribution and because it is unnecessary.
SUMMARY.

1. Defibrinated and oxalated blood saturated with 40 mm. of carbon dioxide in air showed no consistent differences except as regards hematocrit, which was always lower in the oxalated specimen. The variations in electrolyte (bicarbonate and chloride) distribution were not consistent in direction or degree.

2. Defibrinated blood and defibrinated blood containing oxalate showed the same consistency as regards cell volume variations and the same inconsistencies as regards variations in the electrolyte distributions as did defibrinated and oxalated blood.

3. Blood drawn and defibrinated out of contact with air, when directly compared with blood drawn onto 0.2 per cent potassium oxalate out of contact with air, showed that oxalated and defibrinated blood as drawn directly from the body are no more similar than such bloods are after exposure to a new definite tension of carbon dioxide.

4. The carbon dioxide absorption curves of oxalated and defibrinated blood are not identical.

5. The action of a given concentration of oxalate is not the same as that of an equivalent amount of sodium chloride.

6. Time is not a factor. Equilibrium conditions are the same immediately after the addition of oxalate and 2 hours after its addition.

7. The use of defibrinated blood is advised for studies involving electrolyte distribution, because the effects of potassium oxalate are unpredictable.

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