THE VAPOUR PRESSURES OF AQUEOUS SOLUTIONS WITH SPECIAL REFERENCE TO THE PROBLEM OF THE STATE OF WATER IN BIOLOGICAL FLUIDS

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I

INTRODUCTION

The study of the physical properties of solutions of a number of organic and inorganic substances in water has led observers, from time to time, to assume the existence of combinations between the ions or molecules of the solute with the solvent. Thus the deviation of the colligative properties (freezing point depression, osmotic pressure, etc.) of certain solutions from the theoretically calculated values has been explained as due to such hydrate formation (14), although alternative hypotheses have always been possible, e.g., that the solute produces the observed deviations by shifting the equilibrium existing normally between the various states of aggregation of water. Transport experiments have likewise led to the view (22) that ions are hydrated but the results thus obtained are open to criticism (30) as are also the results obtained by the combination of transport numbers with Stokes’s law for the motion of a sphere in a viscous medium. The results obtained by such methods are, moreover, in marked disagreement with one another. Thus for the chloride ion, colligative properties give no indication of hydration; transport experiments (assuming the hydrogen ion to hold one molecule of water) indicate the chloride ion to be combined with four molecules of water; application of Stokes’s law (assuming the hydrogen ion not to be hydrated) gives the hydration number of the chloride ion as twenty; while application of the Stokes-Einstein formula for the motion of particles in a

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The solution of the state of water in colloidal solutions and biological fluids also has been a matter of controversy in recent years. It has often been argued that much of the water present in colloidal solutions is bound to the solute and hence differs in its properties from the remainder of the solvent which acts as a solvent for the solute-water combination. The question whether such combination actually occurs and, if so, how the water in such systems differs from the water in simple solutions is of fundamental importance. The existence of "bound" water differing in its reactions from that of the solvent might permit reactions impossible in simpler solutions. Indeed such possibilities have been advanced to explain certain biological phenomena, but the proof for the existence of such "bound" water has been based on rather dubious data. The freezing methods of Jensen and Fischer (13), Rubner (24), and Thoenes (32) are open to a number of objections. Moran (19, 20), by the use of a dilatometric method, has recently been unable to confirm the results so obtained. Another method which has been utilized for determining the state of water in solutions depends on the determination of some colligative property (freezing point or vapour pressure) of a substance in the solution under investigation and comparison of the result with that obtained when the same substance is dissolved in pure water. Gortner (6) and his collaborators, utilizing sucrose and freezing-point depression measurements, adduced evidence which they believed to indicate the existence of "bound" water in colloidal solutions. A. V. Hill (11), utilizing his thermal method for determining vapour pressure, demonstrated the absence of any appreciable amount of "bound" water in blood, muscle, etc. He added a number of substances (NaCl, KC1, sucrose, etc.) to the solutions investigated. Certain organic substances—urea, creatine, lactic acid, and succinic acid—gave inconsistent results which were attributed to adsorption or other possible interfering reactions.

The term "bound" water has been used by most authors in a vague sense, but Hill (11) has clearly defined the term to mean water which
is not capable of dissolving in a normal manner (with normal depression of vapour pressure) substances added to it. It was in the sense of this definition that the present investigation was undertaken to determine the state of water in solutions and the extent to which we may apply the colligative method described above for differentiating “free” and “bound” water. This method assumes that the colligative properties of a solution containing several solutes is an additive function of the properties of the individual constituents. Our knowledge of such solutions, however, is very limited, although of greatest importance in biological fluids which contain usually many solutes. The present study, therefore, was made to include measurements of the vapour pressure lowering of solutions containing several solutes as compared to the vapour pressure lowerings produced by the individual constituents.

II

Experimental Methods

The vapour pressure measurements recorded in the present paper were made by the thermal method devised by Hill (10) as described in the papers of Hill (10, 11) and Margaria (18). The determinations were carried out at 20.3°C. in a water bath maintained constant to ±0.001°C.

It was necessary in the present work to determine the vapour pressure depressions of dilute aqueous solutions (0.01 to 0.1 molar) for which previous methods (the static or dynamic) are quite unsuitable. Hill’s thermal method is accurate at such low concentrations and, in fact, yields results which are of greater absolute accuracy than for more concentrated solutions. The study of dilute solutions requires the use of highly sensitive galvanometer and thermopiles. The galvanometer used in the present work was the moving magnet type described by Downing (3) but modified by making the system sufficiently astatic to render shielding unnecessary (3). The instrument had an internal resistance of 50 ohms, with the coils in series, and its sensitivity was altered by manipulating an external magnet. The sensitivity was fixed so that the most dilute solutions used (0.01 molar) gave a deflection (against water) of about 100 mm. on a scale 2 metres distant from the galvanometer.

The sensitivity of the apparatus may also be increased by decreasing the distance between the walls of the tube (in which the thermopile is inserted) and the thermopile. Thus, reducing this distance to 1 cm. by the use of stationary parallel plates on which the solution, normally placed on the walls of the tube, was put, increased the sensitivity of a given thermopile 60 per cent. This phenomenon is due to the existence of a gradient of vapour pressure between the neutral solution.
on the wall and the solution on the thermopiles. Proximity increases this gradient and produces a more active exchange by evaporation or condensation at the thermopiles, and hence the development of a greater temperature difference between their two faces.

From the effect on the sensitivity of the instrument of the distance between thermopile and wall it is clear that it is essential for high accuracy to maintain the spatial relationship (between thermopile and wall) constant in successive experiments. This is best done by the use of a metal tube with a fixed arrangement for its attachment. The use of a metal tube, moreover, hastens the attainment of equilibrium particularly if it is short and closely fitted to the thermopile. By reducing the size of the air space in the tube in which the thermopile is placed, one not only increases the sensitivity of the instrument but also materially hastens the attainment of equilibrium. The smaller the air space, the less tendency there is for the development of convection currents due to the unavoidable temperature differences existing in such spaces and the more rapidly is a dynamic equilibrium established between the three solutions present in the chamber.

In the present work the method of reversing the solutions on the two faces of the thermopile, to avoid errors due to asymmetry of the instruments (18), was employed. Such reversal, however, is unnecessary if a series of determinations is made in which the solution used as the reference substance and the one placed on the wall of the tube are not altered in composition during the course of the determinations. If, for example, a solution whose vapour pressure is \( p_1 \) is to be studied, we need only place it on one face of the thermopile with another solution or water (whose vapour pressure we may call \( p_0 \)) on the opposite face and compare the reading so obtained with the reading from a similar arrangement in which a solution of known vapour pressure, \( p_0 \), replaces \( p_1 \). The excess temperature of the first face of the thermopile will be \( K_1(p_2 - p_0) \), where \( p_2 \) is the vapour pressure of the solution on the wall of the tube. The excess temperature on the other face will equal \( K_2(p_2 - p_0) \) and the final temperature read will equal \( K_1(p_2 - p_0) - K_2(p_2 - p_0) \). In the case of our second solution likewise the final temperature read will equal \( K_1(p_2 - p_0) - K_2(p_2 - p_0) \). The difference between the two sets of readings is \( K_1(p_2 - p_0) \). The last expression shows the difference between the observed readings to be directly proportional to the vapour pressure difference of the two solutions and to be unaffected by the asymmetry of the instrument.

The avoidance of reversal of the solutions on the thermopile is useful in cases where the fluid available is insufficient for duplicate experiments, or where a lesser degree of accuracy is acceptable in lieu of the extra labour involved in double determinations. Where the highest possible accuracy is required, however, it is desirable to reverse the solutions in successive determinations thus avoiding the possibility of an interference by extraneous electromotive forces in the external circuit. Such extraneous currents, although constant, will cause appreciable errors, except in cases where \( p_2 \) and \( p_0 \) (in the above example) are approximately equal.

The probable errors of the results for the dilute solutions recorded in the present
paper are approximately 0.5 per cent of the recorded reading. In the case of the more concentrated solutions studied, the probable error is about 1 per cent. Reversed readings were made with four thermopiles, the averages being recorded in the tables. Two sets of such readings (sixteen readings in all) were made for the dilute solutions of KCl and NaCl and a single set of reversed readings (eight in all) for the other results quoted. The probable error was found to decrease but slightly when more than four sets of determinations were made.

All the results given in the tables of the present paper are based on actual determinations which have been interpolated, where necessary, to the nearest 0.01 of a mole for convenience of recording.

### TABLE I

The Vapour Pressure Depressions of Dilute Aqueous Sodium Chloride Solutions at 20.3°C.

<table>
<thead>
<tr>
<th>Moles of NaCl per 1000 gm. of H₂O</th>
<th>0.03</th>
<th>0.04</th>
<th>0.05</th>
<th>0.06</th>
<th>0.07</th>
<th>0.08</th>
<th>0.09</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molal relative vapour pressure lowering* × 10⁻⁴</td>
<td>3.42</td>
<td>3.39</td>
<td>3.37</td>
<td>3.34</td>
<td>3.32</td>
<td>3.31</td>
<td>3.30</td>
<td>3.29</td>
</tr>
<tr>
<td>Probable error.....</td>
<td>±0.018</td>
<td>±0.010</td>
<td>±0.012</td>
<td>±0.014</td>
<td>±0.010</td>
<td>±0.007</td>
<td>±0.008</td>
<td>±0.007</td>
</tr>
</tbody>
</table>

* The molal relative vapour pressure lowering is defined by the expression \( \frac{P_s - P}{MP_s} \) in which \( P_s \) is the vapour pressure of the solvent; \( P \) that of the solution, and \( M \) is the number of gram formula weights of solute per 1000 gm. of solvent.

### III

The Vapour Pressures of Dilute Aqueous Sodium and Potassium Chloride Solutions

In determinations of vapour pressure by Hill's method comparison is made between the solution to be investigated and one whose vapour pressure is known (or water in the case of very dilute solutions). In the present work it was required to know the vapour pressure of very dilute solutions (less than 0.1 molar) in which range data are not available due to the errors in the usual methods for determining the vapour pressures of solutions of such low concentrations (8). Determinations, therefore, were carried out with dilute NaCl and KCl solutions, the results of which may be used as standards for vapour
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pressure studies involving other dilute solutions. The data for NaCl, given in Table I, are based on the value of $3.29 \times 10^{-2}$ as the molal relative lowering for a 0.1 molal solution as found by Norris (12). The data for KCl, given in Table II, are based on those of Table I. The value, $3.35 \times 10^{-2}$, for the 0.05 molal KCl solution is in good agreement with that of Frazer, Lovelace, and Sease (12) for this concentration, $3.36 \times 10^{-2}$, considering the probable error of the present results and those obtained by the static method for very dilute solutions (8, 16).

The molar vapour pressure lowerings of dilute sucrose solutions were found to be $1.78 \times 10^{-2}$ for a 0.1 molal solution and $1.77 \times 10^{-2}$ for a 0.05 molal solution—values which are in good agreement with those of Beard (12) for the 0.1 molal solution at which concentration only, data are available.

### Table II

<table>
<thead>
<tr>
<th>Moles of KCl per 1000 gm. of H₂O</th>
<th>0.03</th>
<th>0.04</th>
<th>0.05</th>
<th>0.06</th>
<th>0.07</th>
<th>0.08</th>
<th>0.09</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molal relative vapour pressure lowering $\times 10^{-2}$</td>
<td>3.41</td>
<td>3.38</td>
<td>3.35</td>
<td>3.33</td>
<td>3.31</td>
<td>3.30</td>
<td>3.29</td>
<td>3.28</td>
</tr>
<tr>
<td>Probable error</td>
<td>±0.015</td>
<td>±0.014</td>
<td>±0.012</td>
<td>±0.010</td>
<td>±0.013</td>
<td>±0.010</td>
<td>±0.008</td>
<td>±0.006</td>
</tr>
</tbody>
</table>

As stated above, urea when added to blood was found by Hill (11) to give anomalous results for its vapour pressure lowering as compared to its solution in 1 per cent NaCl. The wide distribution of urea in biological materials renders important a knowledge of its colligative properties. The vapour pressures of aqueous urea solutions as found in the present work are given in Table III.
It will be observed that the molar relative vapour pressure lowering of aqueous urea solutions is constant (within the limits of the experimental error) over the range of 0.05 to 0.5 molar. The average molar relative lowering for these dilute solutions, however, is $1.64 \times 10^{-2}$ which is appreciably lower than the theoretical value calculated on the assumption that urea exists in aqueous solution as a simple molecule. It would appear that urea is either associated in aqueous solution or affects the activity of the solvent in such a way as to lead to the abnormally low molal vapour pressure lowerings observed. Jones (14) also noted the abnormally low freezing point depressions of urea solutions which he attributed to association. The freezing point depression-concentration curve for urea as found by Jones differs from that for the other associated substances, which he studied, in the comparative constancy of the molal freezing point for dilute solutions. This observation is in accord with the constancy of the molal vapour pressure lowerings observed in the present work. Frankel (5) has recently observed the constancy of the molal refractive index of urea solutions. Such constancy, although an indication of an apparently constant molecular weight, is no criterion necessarily of the condition of the dissolved substance as is evidenced by the results of Jones and of the present study.

Dieterici (12) and Perman and Price (12) have also found abnormally low vapour pressures of urea solutions at $0^\circ$ and $70^\circ$, respectively. Dieterici's results lead to the same conclusion regarding the abnormality of urea solutions at $0^\circ$ as those deduced by Jones (14) from freezing point data as opposed to the normal freezing point lowering.

### Table III

The Vapour Pressure Depressions of Dilute Aqueous Urea Solutions at 20.3°C.

<table>
<thead>
<tr>
<th>Moles of urea per 1000 gm. of H₂O</th>
<th>0.05</th>
<th>0.10</th>
<th>0.15</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molal relative vapour pressure lowering $\times 10^{-2}$</td>
<td>1.66</td>
<td>1.64</td>
<td>1.65</td>
<td>1.63</td>
<td>1.65</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>Probable error</td>
<td>$\pm 0.017$</td>
<td>$\pm 0.013$</td>
<td>$\pm 0.012$</td>
<td>$\pm 0.010$</td>
<td>$\pm 0.008$</td>
<td>$\pm 0.007$</td>
<td>$\pm 0.007$</td>
</tr>
</tbody>
</table>
observed by Flügel (4). Hendricks (9) has also shown that crystalline urea consists of molecules having the formula \((\text{CONH}_2)_2\).

Abnormality of the colligative properties of aqueous solutions of organic compounds is much more common than is ordinarily supposed. A normal colligative property appears to be the exception rather than the rule among organic compounds. Such abnormalities have been observed by Jones (14) for the depression of the freezing points of succinic, oxalic, citric and acetic acids, ethyl alcohol, acetone, etc.; by Frankel (5) for the refractive indices of aniline, glycooll, phenol, formic and propionic acids, malonic ester, etc.; by Grollman (7) for the directly measured osmotic pressures of phenol; and by others, using various methods of experimentation, for a number of substances (12).

Comparison of the data of the present paper with those of Dieterici and Perman and Price, quoted above, would indicate that the association of urea (if such be assumed to occur) increases with increasing temperature. At the temperature of the mammalian body, one would therefore expect a greater abnormality of the colligative properties of urea than that observed at lower temperatures. This increase in the degree of association with increasing temperature is of frequent occurrence in the case of organic substances (12), although the reverse may be true in the case of some (e.g. phenol (7)). Consequently it is unjustifiable to assume a normal molecular weight in calculating the colligative properties of an organic substance, as is usually done, despite the fact that the substance manifests a normal depression of the freezing point.

In view of the abnormal behaviour of aqueous urea solutions described above, the effect of adding to such solutions other substances was investigated, for it is in such complex mixtures that urea occurs biologically. The method used in investigating these mixtures and those to be subsequently described was as follows. The solute (urea in the present case) was added to an aqueous solution of known composition. The resultant mixture was placed on one side of the thermopile while a portion of the original solution was placed on the opposite face. The resulting reading measured the vapour pressure lowering produced by adding urea to the solution in question. A second experiment was then performed in which the vapour pressure of an aqueous urea solution was determined. The ratio of the readings
obtained in the two experiments above described is equal to the ratio of the vapour pressure lowering produced by urea when added to the solution investigated to its lowering in aqueous solution. For example, the reading of a solution which was 0.1008 molar with respect to urea and 0.0272 molar with respect to NaCl was 548 (deflection in mm. on the galvanometric scale) when the solution was placed on one side of the thermopile with a 0.0272 molar NaCl solution on the other side. A second solution of urea (0.1012 molar in composition) when opposed by water on the second face of the thermopile gave a reading of 552. The ratio of the vapour pressure lowering of urea in a 0.0272 molar NaCl solution to its lowering in water is therefore

\[
\frac{548}{552} \times \frac{0.1012}{0.1008} = 1.00
\]

In other words, for the mixed solution of NaCl and urea in question, the vapour pressure lowering is an additive function of the lowering of the individual constituents.
Table IV gives the vapour pressure lowerings produced by urea when added to a number of solutions as compared to the vapour pressure depression of its aqueous solution. In certain cases, e.g. 1 per cent NaCl, 1 per cent gelatin (at pH 7.0) and Ringer's solution, urea behaves as anticipated, i.e., it produces the same depression of the vapour pressure of these solutions as it does when added in equal concentration to pure water. In many solutions, however, its effect on the vapour pressure is different from what would be anticipated from its behaviour in pure water. This anomalous behaviour may be assumed to result from a shift in the equilibrium existing in aqueous solutions by the addition of various substances to the solution. Acid and alkali apparently favour dissociation thus giving values greater than unity in the last column of Table IV. The maximum values obtained (1.09) correspond to the theoretically calculated values for urea. Substances such as sucrose or gelatin (pH 5.0), giving values (in the last column of Table IV) which are less than unity, may be considered as favouring association of the urea molecules or producing some other change leading to an increased vapour pressure of the solution. In blood also, as shown by Hill (11), urea shows an abnormally low ratio.
Other organic substances—creatine, lactic acid, and succinic acid—were also found by Hill (11) to give anomalous results when added to blood. In view of the abnormal behaviour of the colligative properties of these substances when dissolved in water, one might anticipate a similarity in their behaviour in complex solutions (i.e. those containing several solutes) to that found for urea. The results obtained with these substances in aqueous solution and in solutions of 1 per cent gelatin and molar sucrose are given in Table V. The marked effect of the latter substances on the apparent vapour pressure lowerings of lactic acid and succinic acid is shown in the last column of Table V. The same conclusions as were discussed above regarding urea, may be drawn concerning the behaviour of these substances.

V

**The Effect of Added Sucrose on the Vapour Pressures of Various Aqueous Solutions**

The preceding results, obtained with various organic compounds, made it obvious that substances of this class may deviate so markedly from the laws of solutions and be so easily affected when added to other solutions, that they are unsuited for determining the state of water in solution. Sucrose might be expected to behave equally anomalously and it is unfortunate that Newton and Gortner (23) adopted this substance for studying water binding. Because of the prominence which the results obtained by their method has received, a further study has been made of the vapour pressures of solutions, with and without the addition of sucrose, to determine to what extent the results are valid for interpreting the condition of the water in such solutions.

Sucrose is normally believed to be hydrated in solution (25) so that it is necessary to correct any observed readings of solutions to which sucrose is added for the water of hydration removed by the sucrose from the solution. Although Newton and Gortner were aware of this hydration and corrected for its effect on the freezing point depression of the sucrose, they failed to realize the equal effect of this hydration in increasing the freezing point depression of the substances in the solution being studied. This source of error in Newton and Gortner’s method has been recently pointed out by Moran and
Smith (21). The formula used by Newton and Gortner (6) for determining bound water is:

\[
\text{Percentage of bound water} = \frac{\Delta a - (\Delta + K_m)}{\Delta a - \Delta} \times 89.2
\]

in which \(\Delta a\) is the freezing point depression observed on adding 0.01 mole of sucrose to a quantity of the solution, which is being investigated, containing 10 gm. of water: \(\Delta\) is the freezing point depression of the solution investigated; \(K_m\) that of the sucrose solution added (corrected for its hydration) which in the case of the molar solutions used, equals 2.085°, and 89.2 is the percentage of "free" water in a molar solution of sucrose. The added sucrose should, if we assume it to be hydrated, remove six molecules (108 gm.) of water from each 1000 gm. of total water present in the solution, thereby increasing the freezing point depression of the solution, \(\Delta\), to \(\frac{1000}{892}\). The latter term should, therefore, be introduced, in place of \(\Delta\) into the above formula of Newton and Gortner, which now becomes

\[
\text{Percentage of "bound" water} = \frac{\Delta a - \left(\frac{1000}{892} \Delta + K_m\right)}{\Delta a - \frac{1000}{892} \Delta} \times 89.2
\]

This modification of Newton and Gortner's method of calculation will markedly affect results quoted by these authors in which \(\Delta\) is appreciable compared to \(\Delta a\) and \(K_m\) as is the case in practically all of the substances studied by these authors with the exception of gum acacia.

In the latter case, the correction is comparatively slight.

Newton and Gortner's results by the above method, as applied to the juices of the wheat plant (Triticum vulgare) led these authors to conclude that winter hardiness in such plants is related to the amount of bound water present in different varieties. If one corrects their results by the use of the modified formula described above, one finds very little evidence to substantiate this theory. Thus in the case of Triticum vulgare var. super, in which 4.4 per cent of the water was found to be bound, recalculation shows the results to indicate that all the water is actually free. Recalculation of the results for a second
species of the same plant shows—3.6 per cent of the total water to be bound (an obviously impossible result) instead of 0.9 per cent as given by Newton and Gortner. The above two varieties of wheat were non-hardy, but in the case of Triticum vulgare var. Minhardi, a hardy species, recalculation also shows a "negative" amount of "bound" water to be present instead of 2.2 per cent as claimed.

Obviously, then, the method employed by Newton and Gortner is unsuited for the determination of the amount of bound water in solutions and leads, in many cases, to the impossible conclusion that a negative amount of water is actually bound. It might be objected that

TABLE VI

<table>
<thead>
<tr>
<th>Substance added</th>
<th>Moality in final solution</th>
<th>Moality of sucrose in final solution</th>
<th>Ratio of vapour pressure lowering produced by substance added to that produced by adding same substance to H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.07</td>
<td>1.0</td>
<td>1.12</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.75</td>
<td>&quot;</td>
<td>1.00</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
<td>&quot;</td>
<td>1.05</td>
</tr>
<tr>
<td>KCl</td>
<td>0.093</td>
<td>&quot;</td>
<td>1.00</td>
</tr>
<tr>
<td>Urea</td>
<td>0.15</td>
<td>&quot;</td>
<td>0.90</td>
</tr>
<tr>
<td>Urea</td>
<td>0.3</td>
<td>&quot;</td>
<td>0.80</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.04</td>
<td>&quot;</td>
<td>1.18</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>0.1</td>
<td>&quot;</td>
<td>1.20</td>
</tr>
</tbody>
</table>

the results of Newton and Gortner are rendered invalid by the method employed by these authors (freezing point measurements) which may be subject to great error in the fluids studied. Application of vapour pressure determinations lead one, however, to the same conclusion, viz., that sucrose, like the other organic substances described in the preceding section, is unfit for determining the state of water in solutions, except possibly in certain unpredictable cases.

A series of results obtained during the present study is given in Table VI. They demonstrate the complexity of the conditions existent in solutions containing sucrose and the marked deviations from the
simple behaviour assumed by Newton and Gortner. Nor is there any apparent generalization by which one may predict the lowering obtained on adding sucrose (sufficient to make a molar solution) to simple solutions. In the case of more dilute solutions (0.05 molar sucrose), the conditions, as will be subsequently shown, are more easily interpreted.

The result (Table VI) obtained on adding NaCl (to make a 0.07 molar solution) to molar sucrose is compatible with the usually accepted view that sucrose is hydrated with the formation of a hexahydrate. When such a solution is 0.75 molar with respect to NaCl, on the other hand, the results would indicate either the complete absence of any water of hydration or the dissociation of part of such water from the sucrose hydrate. This dissociation would be only partial, for were it complete, the observed lowering of the NaCl-sucrose solution would have been less than the sum of the lowerings due to NaCl and sucrose when dissolved separately, since the liberated water of hydration by diluting the sucrose as well as acting as solvent for the NaCl would diminish the vapour pressure lowering exerted by the former.

In the case of KCl added to molar sucrose, one obtains results analogous to those with NaCl, but differing quantitatively from the latter. A 0.05 molar KCl solution indicates the presence of 5 per cent "bound" water which corresponds approximately to the formation of a sucrose trihydrate instead of the hexahydrate indicated by the stronger (0.07 molar) NaCl solution. The two salts obviously differ in their action in sucrose solutions. A 0.093 molar solution of KCl gave no indication (as neither did the 0.75 molar NaCl solution) of hydrate formation. The latter result is in accord with the electromotive force measurements of Corran and Lewis (2) who demonstrated that KCl is apparently soluble in the water of hydration of sucrose, which is tantamount to saying that the KCl-sucrose solution behaves as if no water of hydration existed. These authors did not investigate solutions less than 0.1 molar in respect to KCl in which, according to the results of Table VI, water of hydration is still apparent.

One can attribute the above results with NaCl and KCl-sucrose solutions to the formation of complexes of the type C12H22O11·NaCl·2H2O, such as have been demonstrated to occur by Schoorl (27).
The anomalous results shown by the organic substances, urea, lactic acid, and succinic acid, have been discussed in the preceding section. It is most probable that they undergo marked changes—dissociation or association—in the presence of sucrose and consequently give the results shown in Table VI. Any attempt to calculate the degree of hydration of sucrose by the use of these substances would lead to the view that either a decahydrate of sucrose exists (data of lactic and succinic acids) or that a "negative" quantity of water is bound. Conversely, using sucrose to determine the hydration of urea, lactic acid or succinic acid solutions would lead to similar conclusions regarding these substances. In the case of biological fluids we deal for the most part with solutions of organic substances of the nature of urea, lactic acid, succinic acid, etc., and in such cases the use of molar sucrose, as in the method of Newton and Gortner, leads to erroneous results. The "negative" values for the amount of water bound in the expressed juice of wheat, as calculated above from the data of Newton and Gortner, are quite explicable when we consider the results of Table VI which deal with organic substances dissolved in molar sucrose.

VI

The State of Water in Several Colloidal and Inorganic Solutions

In the view of the above findings, it appeared that the substances most likely to be suitable for determining hydration would be inorganic salts such as NaCl or KCl. These two substances are particularly appropriate for the investigation of biological material. Their natural presence in such material would make it probable that a further addition of a small quantity of them would not produce any marked effect on such biological systems. NaCl and KCl are also desirable because of the absence of any gross hydration of their ions. For the purpose of the present study, the following substances were selected—gelatin and gum acacia, as examples of hydrophilic colloids, which according to the claims of many "bind" water to a great extent; and LiCl and CaCl₂ whose cations are strongly hydrated judging by their hygroscopicity and the deviation of their colligative properties from the theoretically calculated values.

The terms "hydration" and "water binding" have been used inter-
changeably by many authors, the latter term being used perhaps in a
less well defined sense than the former. There seems to be no reason,
however, for such a division in regard to the manner in which water
may be combined with a solute. Whether such water be actually
united chemically with the formation of compounds amenable to
stoichiometric representation, or whether the water be merely ad-
sorbed in the sense in which a dye is adsorbed on proteins in solution,
such water will differ from the remainder of the solvent in its physical

TABLE VII
The Apparent Hydration of Several Hydrophilic Colloids as Determined by the
Colligative Method

<table>
<thead>
<tr>
<th>Substance added to solution</th>
<th>Molality of substance added in final mixture</th>
<th>Ratio of vapour pressure lowering produced by the added substance to</th>
<th>Apparent hydration of the colloid in the original solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>molal in 1.0 kg of H2O</td>
<td>ratio to its lowering when dissolved in pure water</td>
<td>gm of H2O per gm of colloid</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.05</td>
<td>1.01</td>
<td>1.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
<td>1.03</td>
<td>3.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.1</td>
<td>1.03</td>
<td>3.0</td>
</tr>
<tr>
<td>Gum acacia (10 per cent solution)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.05</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>KCl</td>
<td>0.07</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.043</td>
<td>1.005</td>
<td>0.5</td>
</tr>
<tr>
<td>“</td>
<td>0.09</td>
<td>1.05</td>
<td>0.7</td>
</tr>
<tr>
<td>“</td>
<td>0.18</td>
<td>1.07</td>
<td>0.7</td>
</tr>
</tbody>
</table>

and chemical properties. We shall therefore use the term “hydration”
in the subsequent part of this paper to include the usual implications
of the term “water binding” and vice versa.

Gelatin

The results obtained by the present method for gelatin are given
in Table VII. The results with KCl and sucrose agree in indicating
an hydration of gelatin of about 3 gm. of water per gram of gelatin.
The results for NaCl, however, indicate a lower hydration. Such
hydration of gelatin has been demonstrated by a number of previous observers (15, 28) but the magnitude of the hydration as found in the present study is comparatively small. The results are in closer agreement with those of Moran (19) and Adair and Callow (1) than they are with those of Kunitz (15). The method of Kunitz, however, differs from that used in the present work in that any water transported with the gelatin particles either by capillarity or other mechanical means would be determined as water of hydration. Such mechanically carried water would exert a normal vapour pressure and hence appear as normal solvent when investigated by the method used in the present study. The addition of salts affects the hydration of gelatin and the discrepancy between the results obtained with KCl and NaCl may be attributed to their difference in action in this respect or to a greater combination of the NaCl with gelatin (31) as compared to KCl.

The question of the effect of passage of a gelatin solution from the sol to the gel form on the state of water in such solutions is of interest. The hydration of a gel formed from a 5 per cent gelatin solution was found to be approximately that of the sol form calculated for the same concentration. This is in agreement with the conclusion of Svedberg (28). By saturating filter paper with the sol and placing it on the thermopiles where gelation occurred, it is possible to determine the vapour pressure of gels by Hill's method in a manner similar to that by which solutions are studied. A preliminary experiment on human blood also indicated that the process of clotting was not accompanied by any detectable change in vapour pressure. These results indicate that we cannot picture the process of gelation as involving any combination of colloid with water but that the latter is merely held, in its usual state, in the interstices of the gel fibrillae.

**Gum Acacia**

Gum acacia may be taken as a typical hydrophilic colloid. Its apparent hydration is given in Table VII. It will be observed that the experiments both with NaCl and with KCl fail to indicate any hydration of this substance. The results with sucrose (which as was shown above are of little value for determining hydration) vary considerably with change in the concentration of the sucrose. In a
0.18 molar sucrose solution, gum acacia apparently behaves as if each gram of the substance combined with 0.7 gm. of water, a value equal to that found by Newton and Gortner (6) for acacia at 0° when sucrose sufficient to make a molar solution was added. As we decrease the concentration of sucrose, the apparent degree of hydration rapidly diminishes; and only 0.05 gm. of water appears to be combined with each gram of acacia when we utilise a 0.043 molar sucrose solution for our determination. Obviously then the amount of sucrose added determines the result obtained, which is what might be expected from the anomalous behaviour of sucrose as described above. It is only with dilute solutions (0.05 molar) of sucrose that accurate results approximating those obtained by the use of other substances are obtained. Theoretically it is to be expected that the most accurate data (as regards furnishing information concerning the state of water) will be obtained when the least possible quantity of a substance is added to the solution in question. For in the case of infinitely dilute solutions the activities of substances in mixed solutions are most likely to be the same as their activities when present alone in solution (16). Unfortunately our knowledge concerning the behaviour of moderately concentrated mixtures is still too inadequate to permit the use of such mixtures for our present problem. It is obvious, however, that we must consider not only the possible interaction of electrolytes in mixed solutions but the interaction of electrolytes with non-electrolytes, as well, as has been pointed out by Scatchard (26).

The results obtained in the present work with gum acacia and gelatin, those of Hill (11) on blood, those of Svedberg (29) using his method of ultracentrifugalisation, those of Moran (19, 20) obtained by a dilatometric method, all give evidence for the view that hydrophilic colloids are not hydrated to a great extent as has been claimed by many authors. It is well known that certain solid colloidal substances (e.g. silica gel) are capable of adsorbing appreciable quantities of water and it is probable that certain hydrophilic colloids in solution combine with water as a result of the residual affinities which these substances possess. In the case of gelatin the amount so held is appreciable, but in the case of the blood proteins, gum acacia, etc., the amount is undetectable.
Lithium and Calcium Chlorides

It is generally agreed that the lithium ion is strongly hydrated and this hydration was therefore investigated by the present method. The results, as given in Table VIII, show variations which far exceed the experimental errors of the methods employed. Thus, whereas a mixture 0.05 molar with respect to NaCl would indicate that 13 per cent of the water in a 1.33 molal LiCl solution is bound to the solute, the result with KCl shows only 4 per cent to be bound. The results with sucrose, if corrected as shown in Section IV of this paper,

<table>
<thead>
<tr>
<th>Substance added to solution</th>
<th>Molarity of substance added in final mixture</th>
<th>Ratio of vapour pressure lowering produced by the added substance to its lowering when dissolved in pure water</th>
<th>Apparent hydration of the solute in the original solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LiCl (1.33 molar)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.05</td>
<td>1.13</td>
<td>5.4</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
<td>1.04</td>
<td>1.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.05</td>
<td>1.06</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CaCl₂ (0.236 molar)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.05</td>
<td>1.075</td>
<td>17</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.1</td>
<td>1.065</td>
<td>--</td>
</tr>
</tbody>
</table>

lead to values which depend upon the concentration of sucrose employed.

Results, equally dependent on the nature of the substance added to the solution investigated and its concentration, were obtained with CaCl₂ as shown in Table VIII. The results obtained with the three substances NaCl, KCl, and sucrose, differ markedly. The apparent absence of any hydration of CaCl₂ when KCl is added to CaCl₂ may be explained as due to a partial dissociation of the CaCl₂-H₂O complex, such as was described in Section IV in discussing sucrose-
NaCl mixtures. The results obtained not only vary greatly, as has been shown, with different concentrations of added substances, but equally divergent results are obtained when varying the concentration of the solution investigated. Thus when KCl, sufficient to make a 0.04 molar solution, was added to a 0.05 molar CaCl₂ solution (whose molal relative lowering at 20.3° was 4.52 × 10⁻⁵) it was found that the lowering produced by the added KCl was 3 per cent greater than that produced when KCl (in equal concentration) was added to water.

The results obtained for LiCl and CaCl₂ in Table VIII indicate that the colligative properties of these solutions and those of NaCl and KCl when added to them, are not additive. The activities of such mixed solutions have been discussed by Lewis and Randall (16) who have indicated some of the departures of the behaviour of such mixed solutions from that of their individual constituents. It is apparent from the results of the present paper that such departures from an additive or predictable relationship are too large to permit one to use the colligative method for the determination of hydration in solutions of inorganic substances. Biological systems contain such inorganic substances in at times fairly high concentration, and the question arises as to how far one may utilise the colligative method for determining water of hydration in such fluids.

The safest method for such determinations would appear to demand that one dissolve a minimal amount of a constituent (already present in a fairly high concentration), in the solution to be investigated, and compare the result with that obtained when the substance is added to its aqueous solution, isomolar with the solution to be investigated. Such a procedure would appear to be reliable when it can also be demonstrated that the substance is not altered (by complex formation or state of aggregation) in the solution investigated from its condition in water. Thus in the case of blood solutions, electromotive force and ultrafiltration measurements have demonstrated NaCl to be present as in a simple aqueous solution. If now a small quantity of NaCl be added to such a solution and the results compared with that obtained on adding the same amount of NaCl to an aqueous NaCl solution isomolar with blood (11), the result should give a measure of the state of water in blood.

It may be pointed out, in conclusion, that the difference in the
results of the present paper and those obtained by other workers using other methods may be attributed to differences in the methods employed for determining "water of hydration." Water held by mechanical occlusion in the interstices of an electrically charged particle will move with such a particle when subjected to the influence of an electric current, and transport experiments would indicate such water to be "bound." In measuring vapour pressure, on the other hand, only that portion of the solvent would be characterized as "water of hydration" which is incapable of entering freely into the equilibrium between the liquid and gaseous phases. The term "hydration" in its classical sense denotes molecular combination between solute and water. Such molecularly bound water would be expected to behave as part of the non-volatile solute and, therefore, be demonstrable as "bound" by vapour pressure or other colligative methods. The fugacity of water bound by mechanical occlusion, adsorption at an interphase, or the like, may, however, be sufficiently great to preclude the possibility of detecting such combinations by methods based on the measurement of colligative properties. Other methods, on the other hand, might show the existence of such loosely bound combinations, which might also be termed as hydrates. Our present knowledge concerning the fugacity of such combinations is, however, too limited to permit any definite conclusion as to the extent to which confusion in defining the term "hydration," has been responsible for the differences in the results of different authors.

VII

SUMMARY

Data for the depression of vapour pressure are presented for the following aqueous solutions: NaCl (0.03 to 0.1 molar), KCl (0.03 to 0.1 molar), urea (0.05 to 0.5 molar), sucrose (0.05 to 0.10 molar), lactic and succinic acids, creatine, CaCl₂ (0.05 molar), and mixtures of these substances with one another and with certain other solutions (gelatin, gum acacia, sea water, LiCl, etc.). The relation of the depression of vapour pressure of a mixed solution to that of solutions of the individual constituents was investigated in order to ascertain to what extent such studies may be used for the determina-
tion of the degree of hydration, or of the state of water, in solutions. Organic substances (urea, sucrose, etc.) showed anomalous results which were markedly affected and unpredictable in mixed solutions. They are, therefore, unsuited for the study of water binding. In the case of solutions of inorganic substances—LiCl and CaCl₂—the principle of the additive nature of colligative properties is also only approximately true—except perhaps in very dilute solutions. The limitations of the colligative method for determining the degree of hydration have been defined in accord with the above findings.

Studies of the vapour pressures of mixtures of gelatin or gum acacia with NaCl or KCl demonstrated that hydration in gelatin is relatively small at pH = 7 and undetectable in gum acacia solutions. The view, therefore, that hydrophilic colloids are strongly hydrated has not been substantiated. The passage from the sol to the gel state also was not accompanied in gelatin or in blood by any appreciable change in the degree of hydration of the hydrophilic colloids present in these substances.

Acknowledgment is due to Professor A. V. Hill for granting me the facilities of his laboratory and for his constant interest and advice. The present work was carried out during the tenure of a fellowship granted by the John Simon Guggenheim Memorial Foundation, whose generous assistance I wish to acknowledge. I am indebted to Mr. A. C. Downing for the installation of the galvanometer employed.

BIBLIOGRAPHY