BEHAVIOR OF WATER IN CERTAIN HETEROGENEOUS SYSTEMS*

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In various models, designed to imitate living cells, the behavior of water presents interesting features. If these occur in vivo, as appears possible, they may help to explain some of the puzzling aspects of water relations in the living organism.

The living cell is a heterogeneous system since the protoplasm is essentially an aqueous sol or gel with a surface layer which is non-aqueous.¹ In the models the non-aqueous surface layer is represented by guaiacol or by some other liquid immiscible with water.

The experiments fall into 2 groups.

1. Diffusion Experiments.—Solutes diffuse from an aqueous solution A through a non-aqueous liquid B, into C which consists at the start of distilled water. Under certain conditions water moves from A to C. In some cases the non-aqueous layer, B, is stirred mechanically but this does not eliminate diffusion since on each side of each interface there is an unstirred layer through which substances must pass by diffusion.² The mechanical stirring of B hastens the process by transporting water and solutes rapidly from one of these unstirred layers to the other and presumably affects all the diffusing species alike.

The following types will be described.

Type I: no mechanical stirring (p. 369). In a U-tube (Fig. 1) a non-aqueous liquid, B, saturated with water is in contact at one side with an aqueous solution, A, and at the other with distilled water, C.

Type II (p. 371). As in Type I except that B is anhydrous at the start and is stirred mechanically.


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Type III (p. 372). As in Type II but A and B are shaken together and then placed in contact with C (distilled water).

2. Shaking Experiments.—The movement of water is hastened by shaking together the aqueous and non-aqueous phases until equilibrium is attained. (Diffusion would, of course, accomplish the same thing if sufficient time were allowed.)

The activity of the water was permanently raised by shaking A and B together. In certain cases where A was shaken with B and B was then shaken with C, water passed from A to C where its activity became higher.

The following types will be described.

Type IV (p. 374). A non-aqueous liquid, B, is shaken with an aqueous solution, A. After equilibrium is attained the non-aqueous phase is shaken with distilled water, C.

Type V (p. 376). A non-aqueous liquid, B, is shaken with distilled water, C. After equilibrium is attained the non-aqueous liquid is shaken with an aqueous solution.

Methods

Materials.—The materials employed were as follows: Merck's reagent trichloroacetic acid purified by vacuum distillation, Kahlbaum's c.p. guaiacol, Baker's c.p. acetone, Eastman's ethylene chloride, Rossville's absolute ethyl alcohol (gold shield).

Analyses.—In experiments with trichloroacetic acid the total acid was determined by titration with CO₂-free sodium hydroxide, using methyl red as an indicator. When sulfuric acid was present it was determined by the benzidine hydrochloride method of Raschig and the trichloroacetic acid was obtained as the difference. Guaiacol was determined photometrically, using the phenol method of Theis and Benedict with slight modification. The solutions were made up in 10 ml. volumetric flasks and kept at 25° for 30 minutes before measuring. The solutions were measured with the photoelectric densitometer described by Longsworth. A green filter transmitting a narrow band at

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5600 Å was used to increase the sensitivity and eliminate errors due to change in the yellow color of the reagent. The filter was made of Schott and Gen filter glasses (2 mm. BG-18, 8 mm. BG-11, and 2 mm. OG-1). A calibration curve was constructed, giving the amount of guaiacol as a function of the optical density using a standard solution containing trichloroacetic acid and guaiacol.

The specific gravity of the solutions was measured with a small picnometer. The water content was calculated by difference.

To obtain the data given in Fig. 7 guaiacol was placed in a weighed and calibrated graduated cylinder and shaken with water. Successive amounts of trichloroacetic acid were added. After each addition, the cylinder was shaken in a thermostat and the two conjugate liquid phases were allowed to separate. The weights and volumes were recorded and measurements were made of the density and concentration of guaiacol and trichloroacetic acid in the aqueous solution. From these data the volume and composition of both phases were computed.

To obtain the data shown in Fig. 4 conjugate solutions were prepared with water, guaiacol, and trichloroacetic acid in equilibrium at 25°C. Portions of about 50 ml. of the guaiacol phase (B) were placed in Babcock centrifuge tubes and shaken with about 0.50 ml. of water. The mixtures were brought into equilibrium and allowed to separate at 25°C. The volumes were then measured and the concentrations of trichloroacetic acid determined by titration.

Apparatus.—In addition to the U-tube shown in Fig. 1 the following are the types of apparatus employed. All were kept in a water thermostat at 25 ± 0.02°C.

Apparatus No. 2 (Fig. 2) is a U-tube modified to permit greater precision in measuring the volumes of A and C and to permit the use of larger volumes of A. Calibrated tubes (D) of 4 mm. bore are attached to the arms and two bulbs of about 10 ml. capacity are inserted in the A arm. A capillary stop-cock E with a tube F for the introduction of mercury is attached to the bottom of the U-tube in order that the aqueous solution may be pushed up into the calibrated tubes D.

Apparatus No. 3 (Fig. 3) is essentially a modified U-tube of about 20 mm. bore. To the arms A and C are attached tubes of about 8 mm. bore containing bulbs of about 12 ml. capacity. Marks D are placed on the narrow tubes below the bulbs, and at the 15 and 20 ml. levels above the bulbs are placed marks E, measuring from D. The bottom part of the U-tube is replaced by a tube of 3 mm. bore in order to reduce the volume of the B solution. This tube bears a side arm F which is connected to a mercury reservoir G permitting the solution in the arms to be pushed up into the narrower graduated tubes for volume measurement. This reservoir is connected to the vacuum line through a 3-way stop-cock to facilitate manipulation. The B solution is circulated between the A and C compartments without breaking the interface by an all glass pump H. This pump is actuated by alternate pressure and suction produced by intermittent squeezing of a rubber tube attached to the pump at one end and having the other end plugged. This pump is connected to the lower parts of A and C through capillary stop-cocks J which permit regulation of the rate of circulation and prevent mercury from entering the pump while making volume measurements. The A and C solutions are stirred by bubbling air through fine capillaries K. The air is previously bubbled from a sintered Pyrex disk through a 2 m KCl solution to reduce the volume change from evaporation or condensation. The levels may be regulated by applying back pressure by means of the air trap L which may be attached to either arm.
Formation of drops may be seen in $B$ and some of these arrive at the $B$-$C$ interface: they are not detached from the $A$-$B$ interface but are formed in the body of $B$ by the same process that causes water to pass from $B$ to $C$ at the interface.

To ascertain whether stirring could detach minute drops of $A$ from the $A$-$B$ interface and carry them over to $C$ the following test was made. At the start $A$, $B$, and $C$ were brought into equilibrium so that on standing no change occurred in their volumes. Stirring was then started and carried on longer and much more vigorously than in actual experiments. Passage of drops from $A$ to $C$ would be shown by an increase in the volume of $C$. No such increase was observed although a change of 0.2 per cent could be readily detected.
In some experiments in which \( A \) contained sulfuric as well as trichloroacetic acid the sulfuric acid entering \( C \) was so much less than the entering water that if the entire movement of sulfuric acid were attributed to the formation of droplets at the \( A-B \) interface and their transport in drop form to \( C \) we could say that a considerable part of the water entering \( C \) did not move in the form of droplets. This is easily shown by calculating the amount of water which would be contained in the supposed droplets. (Regarding the partition coefficient of sulfuric acid see Fig. 5.)

It might be suggested that there is some diffusion in an aqueous film between the guaiacol and the glass. If such a film exists its cross-section must be very small and in consequence diffusion through it must be very inconsiderable.

Losses due to sampling in the shaking experiments are not shown in the tables because the reported figures are adjusted to compensate for them. Thus if one tenth of the solution is taken for analysis the subsequent values for volumes and for millimoles are multiplied by \( 10 \div 9 \).

Unless otherwise stated the experimental errors are approximately as follows. For density 0.2 per cent; for volume 0.2 to 1 per cent; for concentrations: trichloroacetic acid 1 per cent, guaiacol 3 per cent, acetone 1 per cent, water 1 per cent.

In stating the results the figure following the sign \( \pm \) refers to this experimental error; it is not the statistical probable error.

In all cases the temperature was \( 25 \pm 0.02^\circ C \).

In the following experiments guaiacol was employed as the non-aqueous liquid.

Type I. In a U-tube (Fig. 1) we place guaiacol at \( B \). Resting on the guaiacol at \( A \) and \( C \) are aqueous solutions which form separate phases in contact with the guaiacol.

The guaiacol is shaken with distilled water until equilibrium is attained and we place in \( A \) and \( C \) equal amounts of the water which has been shaken with the guaiacol: the system is in equilibrium throughout and no movement of water occurs.

If we now lower the activity of the water in \( A \) by adding trichloroacetic acid we might expect water to move from \( C \) to \( A \). If this occurs it is presumably only at the start because we find as a rule that after standing for some time water and acid have moved from \( A \) to \( B \) and from \( B \) to \( C \). The higher the concentration of acid in \( A \) the greater the movement of water into \( C \).

In order to analyze the solutions we remove all of \( A \) and all of \( C \) thus

6 Figures in parentheses in the tables refer to those cases in the non-aqueous phases where the composition was determined by subtracting the amount of each constituent in the corresponding aqueous phase or phases from the total amount of that constituent in the system. Figures in square brackets refer to water which was estimated by subtracting the weights of other constituents from the total weight of the phase.

7 Unless the volumes are identical the upper surfaces of \( A \) and \( C \) will not be at the same height and consequently there will be a difference of vapor pressure.

8 See p. 370.
destroying any gradients which exist in them. We then find that the vapor pressure of water in C is higher than in A. The gradients in A are such that at the upper surface water will have a somewhat lower activity than at the bottom because at the bottom acid is leaving A, thereby increasing the activity of the water (see p. 375). The gradients in C are such that at the upper surface the activity of the water is greater than at the bottom because acid is entering at the bottom and thereby decreasing the activity of the water (see p. 375). Hence if we compare the upper surfaces of A and C we see that the difference in the vapor pressure of the water may be higher than the analyses indicate and that in any case the movement of water vapor through the air above the solutions must be from C to A.

The movement of water in the apparatus can continue for months, ceasing only when A and C become identical in volume and in composition.

Presumably water moves at first into A and then in the reverse direction. Hence the per cent of increase of water in C depends on when the experiment is stopped. This may explain the great variation we find in this and other types of experiment and the fact that in some cases there is a loss of water from C. (We cannot very well get time curves by taking samples at intervals from C since this would stir the contents of C and so disarrange the system. We therefore take out all of C at the end of each experiment and make analyses.)

To save space we have reported only those experiments which show relatively large gains of water in C and it should be understood that in every case a range of smaller values was obtained.

Table I shows the result of an experiment in which A consisted of an aqueous solution of trichloroacetic acid, B consisted of guaiacol saturated with water, and C consisted of water saturated with guaiacol. As B and C were in equilibrium at the start there was no reason for water to move from B to C unless forced to do so by the acid diffusing from A. This type of experiment will be called Type I a.

It is evident that water moved from A to B and from B to C: the increase of water in C amounted to 44 per cent. Here the volume of B was 5 ml. and that of C was 1 ml. When the relative volume of C was larger the gain

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9 This was tested by means of Barger's method. Barger, G., Tr. Chem. Soc., 1904, 85, 286. When this shows an increase in the vapor pressure of water in the aqueous phase we conclude that there is an increase in the activity of water in this phase (see also footnote 15, p. 382).

10 But as the acid leaves the lower surface of A the solution in this region becomes lighter and tends to rise.
was less striking. When the volume of B was 8 ml. and that of C was 20 ml. the highest gain was about 6 per cent.

Type II was set up because it seemed desirable to start with anhydrous guaiacol in B so that all the water entering C would have to come from A.

**TABLE I**

An Experiment (Type I, Unstirred) Showing Movement of Trichloroacetic Acid and of Water from A through B (Guaiacol) into C (Water)

In 66 hours 23.8 millimoles of water entered C and thereby gained in respect to concentration, mole fraction, and activity. The increase of water in C was 44 ± 1.1 per cent (Apparatus No. 2).

<table>
<thead>
<tr>
<th>Volume, ml</th>
<th>Trichloroacetic acid, concn., M</th>
<th>Millimoles</th>
<th>Water, concn., M</th>
<th>Millimoles</th>
<th>Guaiacol, concn., M</th>
<th>Millimoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>1.64</td>
<td>16.4</td>
<td>47.8</td>
<td>478</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>5.8</td>
<td>50.4</td>
<td>398</td>
<td>0.226</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>-0.90</td>
<td>-10.6</td>
<td>+2.6</td>
<td>-80.0</td>
<td>+0.226</td>
<td>+1.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47.3</td>
<td>43.3</td>
</tr>
</tbody>
</table>

**TABLE II**

An Experiment (Type II, Stirred) Showing Movement of Trichloroacetic Acid and of Water from A through B (Anhydrous Guaiacol) into C (Water)

In 22.5 hours 116 millimoles of water entered C and thereby gained in respect to concentration, mole fraction, and activity. The increase of water in C was 10.5 ± 0.6 per cent (Apparatus No. 3).

<table>
<thead>
<tr>
<th>Volume, ml</th>
<th>Trichloroacetic acid, concn., M</th>
<th>Millimoles</th>
<th>Water, concn., M</th>
<th>Millimoles</th>
<th>Guaiacol, concn., M</th>
<th>Millimoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>2.41</td>
<td>48.2</td>
<td>[43.6]</td>
<td>[871]</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>0.90</td>
<td>12.0</td>
<td>[49.8]</td>
<td>[665]</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>-1.31</td>
<td>-36.2</td>
<td>[+6.2]</td>
<td>[-200]</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td>(17.9)</td>
<td>(90.0)</td>
<td>(1107)</td>
<td>(0.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td></td>
<td>0.0</td>
<td>0.19</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td></td>
<td>0.0</td>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

(C consisting of distilled water at the start). When this was tried diffusion was so slow that it became necessary to hasten the process by stirring B (see p. 365). Table II shows a result obtained in this way: in 22.5 hours C gained 10.5 per cent water, all of which came from A.
Type III. In order to minimize changes at the \( A-B \) interface, \( A \) (trichloroacetic acid in water) is shaken with \( B \) (guaiacol) and after equilibrium is attained the non-aqueous phase, now called \( B' \), is brought into contact with \( C \) (distilled water). Water moves into \( C \), producing a large gain. This should be considered in connection with experiments described later (Figs. 4 and 8).

The discussion of these results will be deferred until after the description of the shaking experiments to which we may now turn.
FIG. 5. The partition coefficient, $S$, equals concentration in the guaiacol phase + concentration in the aqueous phase. As the concentration of trichloroacetic acid in the aqueous phase increases the partition coefficients of water and of sulfuric acid increase up to the plait point ($P$) where they become unity. The partition coefficient of trichloroacetic acid passes through a maximum and then approaches unity. That of guaiacol decreases to unity at the plait point.

FIG. 6. The partition coefficient $S'$ equals concentration in the aqueous phase + concentration in the guaiacol phase. As the concentration of trichloroacetic acid in the aqueous phase increases the partition coefficient, $S'$, of guaiacol increases up to the plait point ($P$) where it becomes unity.
Shaking Experiments

These were in all cases carried to equilibrium at 25 ± 0.02°C.

Type IV. We shake A (trichloroacetic acid solution) with B (anhydrous guaiacol) and call the resulting phases A' and B'. After equilibrium is attained we shake B' with C (distilled water) and call the resulting phases B'' (non-aqueous) and C' (aqueous). We then find an increase of water in C' (Fig. 4).

The acid passing from A to B increases the solubility of water in the guaiacol phase (Fig. 5) so that water moves with the acid into B'. When B' is subsequently shaken with distilled water, C, acid passes from B' into C, leaving B' supersaturated with respect to water and the resulting excess of water in B' is taken up by C'. (For the behavior of guaiacol see Fig. 6.)

In such experiments water passes from an aqueous phase (A) where its vapor pressure is relatively low to another aqueous phase (C') where its vapor pressure becomes much higher. This means that the water entering C gains in activity. The activity is regarded as proportional to the vapor pressure at very low concentrations. At higher concentrations we merely assume that as the activity decreases the vapor pressure also decreases without, however, being directly proportional to it.

As the result of the two shakings the original aqueous solutions, A and C, become more nearly alike. There is a net loss of free energy for the system as a whole although there is a gain in free energy on the part of the water which moves from A to C. There is a loss in free energy on the part of the trichloroacetic acid and of the guaiacol.

These statements may be illustrated by Table III. We see that A, consisting of 3.47 M trichloroacetic acid and 37.8 M water, was shaken with anhydrous guaiacol (B) until equilibrium was attained. The resulting aqueous phase, which may be called A', consisted of 1.29 M trichloroacetic acid, 46.5 M water, and 0.44 M guaiacol; the non-aqueous phase, which will be called B', consisted of 2.46 M acid, 19.3 M water, and 3.85 M guaiacol.

Next B' was shaken with distilled water (C) until equilibrium was attained. The

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11 The heat relations are affected by the presence of guaiacol, as is shown by the following experiment.

A solution, A, consisting of 70 ml. of 2.37 M trichloroacetic acid in water was mixed with a stirring rod in a Dewar flask with C, consisting of 16 ml. of distilled water. Both A and C were originally at 25.2°C. On mixing the temperature rose to 25.8°C.

Another portion of A, 70 ml. at 24.9°C, was mixed with B, consisting of 16 ml. anhydrous guaiacol at 25.0°C. On mixing the temperature fell to 23.9°C. The resulting mixture was near the plait point and gave two phases nearly identical in composition. The experiment was repeated with 1 ml. less guaiacol giving a single phase. The fall in temperature was practically the same.
resulting aqueous solution, which will be called $C'$, contained 1.22 M acid, 47.0 M water, and 0.40 M guaiacol. We see that $(700 - 432 =) 268$ millimoles of water moved from $A$ to $C'$ and in so doing its activity was raised since the vapor pressure of water in $C'$ was higher than in $A$.

The changes in the activity of the water can be qualitatively described on the basis that increase in concentration, in mole fraction, and in vapor pressure of water in the aqueous solution indicates increase in activity.

On shaking $A$ with $B$, thereby changing $A$ to $A'$ and $B$ to $B'$, acid passes from $A$ to $B$ and the concentration of water in $A$ rises from 37.8 M to 46.5 M (the mole fraction rises from 0.915 to 0.964). Hence the vapor pressure and activity of the water rise and this applies also to the water which passes into the guaiacol phase, $B'$, since the vapor pressure of the water in $B'$ is equal to that in the aqueous phase, $A'$, in equilibrium with $B'$.

### Table III

<table>
<thead>
<tr>
<th></th>
<th>$A$</th>
<th>$B$</th>
<th>$A'$</th>
<th>$B'$</th>
<th>$C$</th>
<th>$B''$</th>
<th>$C'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, ml.</td>
<td>60.75</td>
<td>31.3</td>
<td>15.1</td>
<td>77.4</td>
<td>7.80</td>
<td>70.1</td>
<td>14.88</td>
</tr>
<tr>
<td>Density, gm./ml.</td>
<td>1.248</td>
<td>1.127</td>
<td>1.102</td>
<td>1.226</td>
<td>0.997</td>
<td>1.226</td>
<td>1.097</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>3.47 M</td>
<td>0.0 M</td>
<td>1.29 M</td>
<td>2.46 M</td>
<td>0.0 M</td>
<td>2.45 M</td>
<td>1.22 M</td>
</tr>
<tr>
<td>Millimoles</td>
<td>211</td>
<td>0.0</td>
<td>19.0</td>
<td>190</td>
<td>0.0</td>
<td>(172)</td>
<td>18.2</td>
</tr>
<tr>
<td>Water</td>
<td>37.8 M</td>
<td>0.0 M</td>
<td>[46.5] M</td>
<td>(19.1) M</td>
<td>55.4 M</td>
<td>(17.7) M</td>
<td>[47.0] M</td>
</tr>
<tr>
<td>Millimoles</td>
<td>2294</td>
<td>0.0</td>
<td>[702]</td>
<td>[1480]</td>
<td>432</td>
<td>[1240]</td>
<td>[700]</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>0.0 M</td>
<td>9.1 M</td>
<td>0.44 M</td>
<td>3.85 M</td>
<td>0.0 M</td>
<td>4.08 M</td>
<td>0.40 M</td>
</tr>
<tr>
<td>Millimoles</td>
<td>0.00</td>
<td>285</td>
<td>6.7</td>
<td>298</td>
<td>0.0</td>
<td>(286)</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Note: Small discrepancies occur because the values are in some cases obtained by analysis and in others by synthesis or by graphical estimation from the solubility curves.

While the activity of the water rises that of the acid and of the guaiacol falls. As $A$ changes to $A'$ the mole fraction of acid is lowered from 0.086 to 0.027 so that its activity decreases (the vapor pressure of acid in $A'$ and $B'$ must be the same at equilibrium). The activity of the guaiacol is also lowered since its mole fraction in $B'$ (0.15) is much less than in $B$ (1.0).

When $B'$ is shaken with distilled water, $C$, it might be expected that water would move from $C$ to $B'$; this may happen at the start but as acid moves from $B'$ into $C$ it leaves $B'$ supersaturated with respect to water. The excess water moves from $B'$ into $C$.

According to Table III the concentration of water becomes higher in $C'$ than in $A'$ (i.e. 47.0 M instead of 46.5 M, and the mole fraction becomes 0.966 instead of 0.964). Whether the difference in the concentration of water in $A'$ and $C'$ is exactly 0.5 M is of no importance. It is evident that $C'$ must have a higher percentage of water than $A'$ because shaking $B'$ with water lowers the percentage of acid in $B'$, thereby changing it to $B''$ which is nearer the base of the triangle on the curve in Fig. 7 than $B'$ is. Hence
$B''$ is in equilibrium with an aqueous phase $C'$ which has a lower position on the curve than $A'$ and contains more water than $A'$ because it is nearer to the apex of the triangle marked $H_2O$. This follows from the fact that the tie lines (e.g. $mn$ and $m'n'$, Fig. 7) never cross.

**Type V.** When anhydrous guaiacol ($B$) is shaken with distilled water ($C$) and then with trichloroacetic acid solution ($A$) no water passes from
C to A. Although the guaiacol phase is saturated with water after shaking with C it takes up more water when brought into contact with A because it is altered by the entrance of acid from A.

For example, water and guaiacol are shaken together until equilibrium is attained and the resulting phases are called B_w and C_w (Table IV). The vapor pressure of water, which is the same in both phases, will be called a.

We now lower the vapor pressure of water in C_w from a to a (x), where x < 1, by adding trichloroacetic acid until the concentration of acid is 2.04 M and call the resulting solution C_{GA}. We then shake C_{GA} with B_w (guaiacol saturated with water) and call the resulting phases B'_w and C'_{GA}: the vapor pressure of water in each at equilibrium may be called y.

The vapor pressure of water in B_w is a, which is greater than the vapor pressure of water in C_{GA} which is a (x). Hence we might expect water to move from B_w to C_{GA}. No doubt this happens at the start but it escapes detection because as acid moves from C_{GA} to B_w it raises the vapor pressure of the water in C_{GA} and lowers it in B_w. When the vapor pressure of the water in C_{GA} becomes higher than in B_w water moves into B_w until at equilibrium the vapor pressure of the water becomes the same in both phases: calling this vapor pressure y we find that the vapor pressure of the water decreases in the order a > y > a (x). Hence the net result is a move-

### Table IV

Guaiacol (B) was shaken with water (C) giving the non-aqueous phase B_w and the aqueous phase C_w. To the latter trichloroacetic acid is added, changing it to C_{GA}: this was then shaken with B_w giving B'_w and C'_{GA}. Water passed from C_{GA} to B_w, i.e., from a region where its activity was relatively low to one where its activity was higher.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>C</th>
<th>B_w</th>
<th>C_G</th>
<th>C_{GA}</th>
<th>B'_w</th>
<th>C'_{GA}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, ml.</td>
<td>13.7</td>
<td>10.8</td>
<td>14.3</td>
<td>10.3</td>
<td>12.5</td>
<td>18.1</td>
<td>8.8</td>
</tr>
<tr>
<td>Density, gm/ml</td>
<td>1.129</td>
<td>0.997</td>
<td>1.126</td>
<td>0.997</td>
<td>1.158</td>
<td>1.185</td>
<td>1.046</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>Concentr., M</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.04</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Millimoles</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.5</td>
<td>21.4</td>
</tr>
<tr>
<td>Water</td>
<td>Concentr., M</td>
<td>0</td>
<td>55.4</td>
<td>2.57</td>
<td>54.2</td>
<td>[44.6]</td>
<td>[9.0]</td>
</tr>
<tr>
<td></td>
<td>Millimoles</td>
<td>0</td>
<td>599</td>
<td>41.0</td>
<td>558</td>
<td>[558]</td>
<td>[163]</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>Concentr., M</td>
<td>9.10</td>
<td>0</td>
<td>8.65</td>
<td>0.185</td>
<td>0.153</td>
<td>7.19</td>
</tr>
<tr>
<td></td>
<td>Millimoles</td>
<td>125</td>
<td>0</td>
<td>124</td>
<td>1.91</td>
<td>1.91</td>
<td>130</td>
</tr>
</tbody>
</table>

Note: Small discrepancies occur because the values are in some cases obtained by analysis and in others by synthesis or by graphical estimation from the solubility curves.
ment of water from $C_{GA}$ to $B_a$ where the vapor pressure and the activity of the water are higher than in $C_{GA}$.

Let us now consider the results of other experiments which have yielded the data of Figs. 4–9. The partition coefficient of trichloroacetic acid (Fig. 5) is of interest in that it passes through a maximum. In such ex-

![Diagram](image)

**Fig. 8.** A solution of trichloroacetic acid (A) is shaken with anhydrous guaiacol (B); when equilibrium is attained we call the resulting phases $A'$ and $B'$. When $B'$ is shaken with distilled water (C), acid, guaiacol, and water pass from $B'$ into C, changing C to $C'$. The graph shows calculated increase of the ratio, moles of water entering $C'$ + moles of acid entering $C'$, as the concentration of acid in $A'$ is increased. The graph is calculated from Fig. 7, for the ratio $B' + C = 10$ and the observed points are shown as circles (O). The observed points for the ratio $B' + C = 100$ are shown as squares (□). See p. 385. At the left of the minimum there is a difference between observation and calculation, due, in part at least, to the fact that the calculation is made on the basis of $B' + C = 10$ and the observations are for $B' + C = 100$.

**Fig. 9.** The concentration of water in the guaiacol phase increases as that of acid increases (while the concentration of acid in the aqueous phase is rising from zero to 1.3 m). The curve is derived by calculation from Fig. 7 hence no experimental points are shown (but it has been checked by preliminary experiments).

experiments the addition of sufficient acid causes the two phases to fuse into one. If this happens to occur at the plait point we have the following situation: the successive additions of acid bring the two phases nearer and nearer together in composition until they become identical and all partition coefficients become unity. The plait point ($P$) is shown\(^\text{13}\) in Fig. 7. When

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\(^\text{13}\) Regarding the plait point, and the curved vertical line (conjugation curve) which enables us to find any conjugate phase when the phase in equilibrium with it is known,
the plait point is reached at 25°C. A contains about 2 $m$ trichloroacetic acid: the weight percentages are, water 55.3 per cent, guaiacol 17.2 per cent, and trichloroacetic acid 27.5 per cent.

It is evident that Fig. 7 enables us to calculate the values in Figs. 4, 5, 6, 8, and 9.

This may be illustrated by an example. In Fig. 7 an aqueous phase $m$ is joined by a tie line to a guaiacol phase $n$. The position of $n$ in the figure shows that it has the following weight percentages: water 29.5 per cent, guaiacol 37.7 per cent, trichloroacetic acid 32.8 per cent. We now shake 72.4 gm. of $n$ (containing 21.4 gm. water) with 6.0 gm. of distilled water thereby increasing the per cent of water in the mixture produced by shaking so that the mixture is nearer to that at the apex of the triangle which is marked $H_2O$. The mixture is on the tie line connecting $m'$ and $n'$, so that on standing the mixture will separate into two phases, $m'$ and $n'$. The position of the aqueous phase, $m'$, in the figure shows that it has the following weight percentages: water 76.7 per cent, guaiacol 4.9 per cent, trichloroacetic acid 18.4 per cent. The total weight of $m' + n'$ is 72.4 + 6.0 = 78.4 gm. and $n'$ contains 26.8 per cent water by weight.

We now set up the following equation

$$0.767x + 0.268(78.4 - x) = 27.4$$

where $x = \text{the weight of } m'$, whence $x = 12.8$ gm. This is the weight of $m'$ and since $m'$ contains 76.7 per cent by weight of water its weight content is 9.83 gm. Since 6.0 gm. of water was used for shaking with $n$ it is evident that this 6.0 gm. of water has increased to 9.83 gm., an increase of 64 per cent.

Let us now return to the diffusion experiments and consider the effect of substituting various substances.

When we substitute acetic or monochloroacetic acid for trichloroacetic in Types I and II (p. 365) we find little or no increase of water in $C$.

Results like those observed with trichloroacetic acid, but much less striking, were obtained with non-electrolytes; e.g., with acetone. For example, in Type II (p. 365) when $A$ at the start contained 20 ml. of 5.8 $m$ acetone, $B$ 8 ml. anhydrous guaiacol, and $C$ 20 ml. distilled water it was found after 24 hours that the increase in water in $C$ was 2.1 ± 0.1 per cent, all of which must have come from $A$. The ratio, moles of water entering $C$ + moles of acetone entering $C$, was 0.6.

In Type III (p. 366) larger increases were obtained. For example, after shaking $A$ with $B$, $A$ contained 20 ml. of 2.18 $m$ acetone + 3.4 $m$ guaiacol, $B$ contained 8 ml. guaiacol with 4.18 $m$ acetone and 3.8 $m$ water, $C$ contained $\ldots$


The plait point is the point at which fusion of the two phases occurs without changing the composition of either; at the plait point all partition coefficients become unity. Fusion elsewhere than at the plait point involves changes in composition and the partition coefficients are not unity.
2 ml. distilled water. After 1.4 hours (stirred) the increase in C was 8.0 ± 0.2 per cent. The ratio, moles of water entering C + moles of acetone entering C, was 1.8.

When water is made to pass from A to C by shaking A with B, thus changing B to B' and then shaken with C (distilled water) increase of water in C may be obtained, amounting to over 400 per cent. Such an experiment showing a gain of 84 per cent of water in C is illustrated in Table V.

The mutual solubilities of water, acetone, and guaiacol are shown in Fig. 10.

### Table V

<table>
<thead>
<tr>
<th>Volume, ml</th>
<th>Density, gm./ml.</th>
<th>Conc., M.</th>
<th>Millimoles</th>
<th>Conc., M.</th>
<th>Millimoles</th>
<th>Conc., M.</th>
<th>Millimoles</th>
<th>Conc., M.</th>
<th>Millimoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A'</td>
<td>B'</td>
<td>C</td>
<td>B''</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.0</td>
<td>0.907</td>
<td>13.8</td>
<td>3.7</td>
<td>43.9</td>
<td>5.96</td>
<td>32.5</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
<td>23.2</td>
<td>0</td>
<td>4.65</td>
<td>6.11</td>
<td>0</td>
<td>6.02</td>
<td>4.51</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>790</td>
<td>0</td>
<td>17.2</td>
<td>268</td>
<td>0</td>
<td>196</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Guaiacol</td>
<td></td>
<td>0</td>
<td>9.1</td>
<td>1.09</td>
<td>2.68</td>
<td>0</td>
<td>3.38</td>
<td>0.723</td>
<td></td>
</tr>
</tbody>
</table>

Note: The values for guaiacol in A', B', B'', and C were obtained by analysis and the corresponding values for water and acetone were then obtained from Fig. 10.

When ethanol was substituted for acetone and ethylene chloride for guaiacol substantial gains in water were found in C in Types II and III (pp. 365–366).

In one case of Type II there were in A 20 ml. of 6.5 M ethanol in water, in B 8 ml. of anhydrous ethylene chloride, and in C 2 ml. of distilled water. After 22.5 hours the increase in water in C was 5.0 ± 0.1 per cent, all of which came from A. The ratio, moles of water entering C + moles of ethanol entering C, was 0.35.

Similarly in a case of Type III (p. 366) after shaking A and B together A contained 20 ml. 9.1 M ethanol in water, B 8 ml. of 4.3 M ethanol plus 2.5 M water, and C 2 ml. distilled water. After about 4 hours the increase in water in C was 23.5 ± 0.5 per cent. The ratio, moles of water entering C + moles of ethanol entering C, was 0.51.
It is well known that ethanol increases the solubility of water in ethylene chloride.\(^\text{14}\)

**DISCUSSION**

As the concentration and mole fraction of water increase in the guaiacol phase the vapor pressure, and the activity, of water in the guaiacol phase decrease. This is evident from Table VI and Fig. 11 which show that as the mole fraction of water in the guaiacol phase increases the mole fraction of water in the corresponding aqueous phase decreases. It is also evident from Fig. 7 which shows that as the guaiacol phase takes up more water

and acid and moves along the curve toward the plait point it comes into equilibrium with aqueous phases containing less and less water (i.e. further from the H₂O apex of the triangle).

As the concentration and mole fraction of water in the aqueous phase fall off the vapor pressure of water in the aqueous phase falls off. Hence the vapor pressure, and consequently the activity of water in the corresponding non-aqueous phase must also fall off because at equilibrium the vapor pressure of water must be the same in both phases.

A similar result is seen when we substitute acetone for trichloroacetic acid as is evident in comparing Fig. 10 with Fig. 7. The corresponding figure for ethanol, ethylene chloride, and water shows that similar relations obtain here.

### TABLE VI

Showing that as trichloroacetic acid is added to the system the mole fraction of water in the guaiacol phase increases and the mole fraction of water in the conjugate aqueous phase decreases. See Fig. 11.

<table>
<thead>
<tr>
<th>Mole fraction of water</th>
<th>In aqueous phase</th>
<th>In guaiacol phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.996</td>
<td>0.250 (no acid)</td>
<td></td>
</tr>
<tr>
<td>0.984</td>
<td>0.318</td>
<td></td>
</tr>
<tr>
<td>0.977</td>
<td>0.611</td>
<td></td>
</tr>
<tr>
<td>0.966</td>
<td>0.731</td>
<td></td>
</tr>
<tr>
<td>0.964</td>
<td>0.754</td>
<td></td>
</tr>
<tr>
<td>0.950</td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>0.939</td>
<td>0.863</td>
<td></td>
</tr>
<tr>
<td>0.909</td>
<td>0.909 (plait point)</td>
<td></td>
</tr>
</tbody>
</table>

As the addition of trichloroacetic acid to the water-guaiacol system causes the activity of water in the guaiacol phase to fall off we may say that it falls from \( a_w \) to \( a_w \alpha \), where \( \alpha < 1 \). Calling the activity coefficient before the addition of acid \( f_w \) we have \( f_w = a_w = c_w \), where \( c_w \) is the concentration

\[ f_w = a_w = c_w \]

At very low concentrations the activity of water in the aqueous phase is approximately proportional to its vapor pressure. At higher concentrations we merely assume that when the vapor pressure falls the activity also falls without being directly proportional.

Before the addition of acid the aqueous phase contains 0.185 M guaiacol and the guaiacol phase contains 2.87 M water. Hence if we adopt the same standard state for both phases the activity coefficient of water in the guaiacol phase is much greater than unity and the same may be said of the activity coefficient of the guaiacol in the aqueous phase.
of water in the guaiacol phase. When enough acid is added to bring the system close to the plait point the concentration of water in the guaiacol phase rises from 2.87 m (the value before acid is added) to 36.5 m, an increase of 12.7 times. Since, as already noted, \( a_w \) has fallen to \( a_wx \) (where \( x < 1 \)) we now have (calling the present activity of water \( f'_w \))

\[
\frac{f'_w}{f_w} = \frac{a_w}{a_wx} \times 12.7.
\]

Fig. 11. Shows that as trichloroacetic acid is added to the system the mole fraction of water increases in the guaiacol phase and decreases in the aqueous phase in equilibrium with the guaiacol phase. Hence as the mole fraction of water increases in the guaiacol phase the vapor pressure and the activity of water in the guaiacol phase decrease. See Table VI.

Fig. 12. Hypothetical diagram to show molar concentrations of trichloroacetic acid and of water in an unstirred layer of B, when a steady state of diffusion of acid has been set up under the conditions of Fig. 9. Concentrations and activities in B are plotted as ordinates (which are on a smaller scale for water than for acid). The activity of water in B falls off as the concentration of water increases (see p. 375).

This drastic reduction in the activity coefficient of water indicates an attraction\(^{17}\) of the acid for the water.

We may also infer an attraction\(^{17}\) of the acid for guaiacol. Fig. 7 shows that as the aqueous phase takes up more guaiacol it comes into equilibrium with guaiacol phases containing less and less guaiacol. Presumably the

\(^{17}\) To what extent formation of definite compounds occurs must be left open.
activity of guaiacol in the guaiacol phase falls off as its mole fraction falls off, just as happens with water in the aqueous phase. As the activity of guaiacol in the guaiacol phase falls off so does the activity of guaiacol in the conjugate aqueous phase in equilibrium with it. On this basis the activity of guaiacol in the aqueous phase falls off as the concentration and mole fraction of guaiacol increase in this phase. When the concentration has increased from 0.185 M (the value before acid is added) to 1.65 M (the value at the plait point)—an increase of 8.92 times—the value of the activity coefficient of guaiacol in the aqueous phase has decreased to less than 1 ÷ 8.92 of what it was before acid was added.

It is the ability to attract both guaiacol and water which enables the acid to bring about the fusion of the two phases when enough acid is added.

Somewhat similar results were obtained when acetone was substituted for trichloroacetic acid (Fig. 10). The corresponding diagram for ethanol, ethylene chloride, and water shows that similar relations obtain here. A number of systems follow this pattern as is evident from the diagrams in the International Critical Tables. Hence we may conclude that a variety of substances act like trichloroacetic acid in attracting water as well as less polar compounds.

The results of the shaking experiments may now be considered in relation to the diffusion experiments.

Let us commence with Type I where a solution of trichloroacetic acid in water, A, is placed in contact with guaiacol saturated with water, B. The shaking experiments show that when these solutions are brought into contact acid passes from A to B (Figs. 4, 7, and 8) and as a result water moves from A to B.

Does this depend on the formation of hydrates? This question arose at the outset but it seemed doubtful that all of the movement of water could be explained in this way. This question was recently taken up by Bent who presented evidence of hydrate formation, but it should be noted that he worked with acetic acid which in our experiments produced little or no movement of water from A to C (see p. 379).

To explain the entrance of water into C as wholly due to definite hydrates

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18 These show that as the non-aqueous phase takes up more water it comes into equilibrium with phases containing less and less water, just as in the guaiacol-water-trichloroacetic acid system. Cf. International Critical Tables, New York, McGraw-Hill Book Co., Inc., 1928, 3, 398 ff.

19 E.g., various organic acids, lower alcohols, acetone, phenols, aniline hydrochloride, and pyridine.

would require the assumption that in the shaking experiments and in the
diffusion experiments more than 25 molecules of water are combined in
some cases with 1 molecule of acid (Fig. 8).

Since Fig. 8 shows that the ratio, moles of water entering C + moles of
acid entering C, is not constant it would be necessary to assume that the
degree of hydration is variable. In that case we might expect the degree
of hydration to fall off as the proportion of available water decreased; i.e.,
as the concentration of acid increased. This is not what occurs as is evident
from Fig. 8. Although the curve falls at first it soon passes through a
minimum. It then rises which differs from what might be expected if all
the water entering C were water of hydration.

The curve, as drawn, was computed from the solubility data given in Fig. 7. At low
concentrations it differs from the observed values shown as squares. This is due, in
part at least, to the fact that it is made on a different basis. The reason is that more
accurate predictions can be made when the volume ratio of B' + C is taken as 10 but
the observation is more accurate when this ratio is taken as 100.

It is evident that at zero concentration of acid no acid can pass into C but some
water can do so since the guaiacol is saturated with water and when some of this guaiacol
passes into C some water must accompany it: hence the ratio will be infinite. As the
concentration of acid increases the curve will pass through a minimum as shown.

The effect of trichloroacetic acid on the activity coefficient of water
(p. 382) in the guaiacol phase indicates an attraction between the acid and
the water but does not necessarily imply the formation of definite hydrates.
A loose association between the water and the acid is all that need be in-
ferred.

Let us now consider the situation at the interface in the diffusion experi-
ments. Although there is always some agitation due to mechanical stirring
or to convection there is an unstirred layer on each side of each interface
(p. 365). Let us consider such a layer in B adjacent to the A-B
interface when a steady state of diffusion of acid has been reached so that the con-
centration gradient of acid in this layer is linear, as shown in Fig. 12. There
will then be a concentration gradient of water if we have the conditions\(^22\) of
Fig. 9 for in B there is a definite concentration of water corresponding to
each concentration of acid. Although the concentration gradient of acid
is due to the flux of acid the concentration gradient of water need not be

\(^22\) At concentrations higher than those shown in Fig. 10 complications ensue as is
evident from Fig. 5 which shows that the partition coefficient of trichloroacetic acid passes
through a maximum. Above this maximum the addition of trichloroacetic acid to the
system causes trichloroacetic acid to move from the guaiacol phase to the aqueous phase.
due to flux of water: there might conceivably be a flux of water in the opposite direction under certain conditions.

If, as already stated (p. 382) the activity of water in B falls off as its concentration increases we may represent the activity of water in B by the broken line in Fig. 12.

If the conditions of Fig. 9 obtain at the B-C interface, water may pass under suitable conditions from B to C if the concentration of acid falls off in this region of B, for this region will be left in a supersaturated state with respect to water and since it is at the B-C interface some of the excess water may pass into C.

Such a falling off in the concentration of acid might occur if a portion of B were transported by convection or stirring from a region nearer A (a region richer in acid) to the unstirred layer at the B-C interface (p. 365). Acid would then diffuse from B into C and water would follow it if the activity of water in the unstirred layer of C were sufficiently lowered by the entrance of acid. The process might resemble that discussed in connection with Type III (p. 372).

The process would be favored by the fact that the acid diffusing into C would tend to remain at the bottom of C on account of its higher specific gravity. (When C is analyzed at the end of the experiment the whole of it is taken so that the concentration of acid at the B-C interface is not known.)

The steady state has been discussed because it is useful in clarifying the situation although it does not occur in our experiments. In these the concentration of acid in the unstirred layer of A steadily falls (with a consequent rise in the activity of the water). In the unstirred layer of C it steadily rises (with a consequent fall in the activity of the water). It might be suggested that this could continue until the activity of water in the latter becomes less than in the former so that water would move from A to C. In that case analysis might still indicate a lower activity of water in A than in C since the analysis takes no account of the concentration at the interface because it is made by removing the whole of A or of C as the case may be. Thus on the basis of the analyses we should say that the water moves from a region of low to one of high activity.

But in order to bring about such a movement of water from A to C it
would seem that the concentration of acid would have to become greater in
the unstirred layer of C than in that of A.

Of course, if hydrates are formed water may enter C as water of hydration
whenever acid enters C.

It is not possible to give a complete picture of the process of diffusion
without knowing the concentrations and activities of the diffusing sub-
stances in all parts of the system. The difficulties of obtaining this are
obvious.

The movement of water in the diffusion experiments recalls the so called
"anomalous osmosis" observed with solid membranes. But the latter
phenomenon, in the opinion of many investigators, requires the diffusion
of electrolytes through pores. In that case the movement of water ob-
served by us would seem to belong to a different category and a different
name will therefore be convenient. As the water has the appearance of
being carried from a region of lower to one of higher activity by the diffusing
substance we suggest the term anaphoresis, from ἀνά (up) and φόρης (a
being borne).

Since in some respects guaiacol acts like certain protoplasmic surfaces it
seems possible that similar phenomena may occur in living cells. If so
these results have an obvious bearing on the movement of water in the
organism and on methods of studying permeability. It becomes necessary
to know to what extent a substance entering or leaving the cell carries water
with it in the manner here indicated.

In comparing the models with living cells and tissues we should bear in
mind that in the organism there is both convection and mechanical agitation
(the latter may be due to active or passive motion including protoplasmic
streaming).

In some cases the movement of substances in the organism (e.g. auxins)
appears to present unexpected features. The question may be raised
whether these substances are carried along by some other substance as the
water in our experiments appears to be carried along by trichloroacetic acid.

Certain puzzling cases of water movement in the organism appear to
present some analogy with these phenomena. One of the most striking
things about the kidney is the fact that the water moves in a circular
path; i.e., most of the water passing from the blood into glomerulus and
tubules moves back again into the blood. The analogy with Fig. 1 would
be closer if we substituted for the air in the model a membrane more per-

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Fig. 13. We assume that the model shown in Fig. 1 is modified by substituting for the air above the solutions a membrane more permeable to liquid water than to acid and to guaiacol; this membrane is called 1. The guaiacol is called membrane 2. The water would then take a circular path, passing through both membranes and if the trichloroacetic acid in A were renewed from time to time this would go on indefinitely.

In the kidney the water also takes a circular path. Corresponding to membrane 1 in the kidney is the wall or “membrane” between the blood and the glomerulus; corresponding to membrane 2 is the wall or “membrane” between the tubules and the blood.
between the blood on one side and glomerulus and tubule on the other: corresponding to 2 is that between the tubule and the blood. In both cases the water pursues a circular path, passing first through one membrane and then through the other.

There are, of course, great differences between the kidney and the model, e.g., the kidney does work and requires energy and the passage of water from the blood to the glomerulus is believed to depend largely on hydrostatic pressure. But the kidney resembles the model in that the circular path of the water is a striking feature.

**SUMMARY**

In various models designed to imitate living cells the surface of the protoplasm is represented by guaiacol which acts in some respects like certain protoplasmic surfaces. The behavior of water in these models presents interesting features and if these occur in vivo, as appears possible, they may help to explain some of the puzzling aspects of water relations in the living organism.

When sufficient trichloroacetic acid is added to a two-phase system of water and guaiacol the two phases fuse into one. The effect of the acid is due to its attraction for water and for guaiacol. This is shown by the following facts.

During the addition of the acid the mole fraction of water in the guaiacol phase increases but the activity of water in the guaiacol phase falls off. The activity coefficient of water may fall to less than one twelfth the value it had before acid was added.

The behavior of guaiacol presents a similar picture. During the addition of acid the mole fraction of guaiacol in the aqueous phase increases but the activity of the guaiacol in the aqueous phase presumably decreases. Its activity coefficient calculated on this basis may fall to about one ninth of the value it had before the acid was added.

Somewhat similar results are obtained when acetone is substituted for trichloroacetic acid or when ethanol is substituted for trichloroacetic acid and ethylene chloride for guaiacol.

As trichloroacetic acid increases the mutual solubility of guaiacol and water we find that guaiacol saturated with water and having a high vapor pressure of water can take up water from an aqueous solution of trichloroacetic acid with a low vapor pressure of water: acid passes from the aqueous to the guaiacol phase, thus raising the vapor pressure of water in the aqueous phase and lowering it in the guaiacol phase.

Diffusion experiments present some interesting features. When an aque-
ous solution, $A$, of trichloroacetic acid is separated by a layer of guaiacol, $B$, from distilled water, $C$, under certain conditions water moves from $A$ to $C$. This depends on the fact that acid moves in the same direction and appears to carry water with it. Similar but less striking results were obtained with acetone diffusing through guaiacol and with ethanol diffusing through ethylene chloride.

These phenomena differ from "anomalous osmosis" through solid membranes if it depends, as many suppose, on the diffusion of electrolytes through pores. We therefore suggest the term "anaphoresis" for the phenomena described here.

Measurements of the mutual solubilities of water, guaiacol, and trichloroacetic acid and of water, guaiacol, and acetone are given and are discussed in relation to the diffusion experiments. To give a complete picture of the process of diffusion we need to know the activities and concentrations in all parts of the system. The difficulties of achieving this are obvious.

The solubility relations are such that a concentration gradient of trichloroacetic acid in guaiacol produces a concentration gradient of water in the same direction, but the activity gradient of water is in the opposite direction.

Since in certain respects guaiacol acts like some protoplasmic surfaces it seems possible that similar phenomena may occur in living cells. If so these results have an obvious bearing on the movement of water in the organism and on methods of studying permeability. It becomes necessary to know to what extent a substance entering or leaving the cell appears to carry water with it in the manner here indicated.

In certain of the diffusion experiments the water takes a circular path, passing out of the dilute solution at one point and back into it (as vapor) at another. This circular path recalls the situation in the kidney where the water continually passes out of the blood into the glomerulus and tubule and then back into the blood from the tubule (where the solution is more concentrated). In both cases the circular path of the water is an essential feature.