

SWELLING OF ERYTHROCYTES IN SOLUTIONS OF AMMONIUM SALTS

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Besides the hydrogen ion, as is well known, the ammonium ion is the only cation capable of passing through the membrane of erythrocytes. Possibly the mechanism of this is a simple diffusion; possibly, as assumed by Jacobs (1926-27), it consists in a diffusion of NH_3 into the erythrocyte, followed by an exchange of hydroxyl ions from the corpuscle for the anion with which the ammonium ion was combined in the surrounding fluid.

If the former possibility holds good, the rate of diffusion of ammonium salts into the red corpuscles may be estimated by means of the coefficient of diffusion of a single salt through the membrane:

$$\frac{2 U V}{U + V}$$

If the diffusion of the salt is based exclusively upon an exchange of anions, the question becomes more complicated, as there is a definable coefficient of diffusion only when there is the same fall in the concentration of the two anions.

Taking the simple diffusion of the ammonium ion for a working hypothesis, I have tried to work out an equation from which the rate of the swelling of erythrocytes in solutions of ammonium salts may be expressed by a single numerical quantity, which is to be regarded as a relative diffusion constant for the salt in question. Thus it becomes practicable to compare directly the rates of diffusion of various salts and to study the influence of different factors upon the diffusion rates of individual salts.

Equation for Swelling of Erythrocytes in Solutions of Ammonium Salts

Such an expression can be derived from Fick's law of diffusion:

$$(1) \quad \frac{dm}{dt} = Kq \frac{C_v - C_i}{l}$$

(dm is the amount of salt passing through the membrane in the time dt . C_v is the concentration of the salt in the surrounding fluid; C_i is the concentration of the salt within the erythrocytes. q is the area of diffusion (surface of the corpuscle); l is the distance of diffusion (*i.e.*, the thickness of the membrane).)

This law applies to gases and to solutions complying with the laws of gases.

In applying this law to the present condition, it is necessary to express dm , C_v , and C_i entirely by means of the erythrocyte volume and the initial concentration of the surrounding fluid, as for the sake of simplification it is desirable to use volumetric determinations exclusively.

We start with the assumption that the laws of gases apply to this condition, and that there is no restriction to the applicability of the van't Hoff-Boyle-Mariotte law: $p_o(v_o - x) = p_1(v_1 - x)$. (x is the dispersion phase of the erythrocytes, which takes no part in swelling or shrinking. v_o and v_1 are the volumes of the blood corpuscles in solutions with the osmotic pressures p_o and p_1 respectively.)

We further take it for granted that the surface of the erythrocytes does not change during the swelling and, consequently, that q and l can be put down as constant and can be included in K , a diffusion constant. By this method, K is only of relative value, and one does not claim it to be more.

Fick's law is then expressed according to the formula,

$$(2) \quad \frac{dm}{dt} = K (C_v - C_i).$$

First we shall study the conditions in a pure solution of an ammonium salt. Here the volume of the erythrocytes is v_o at the beginning of the experiment (after a possible instantaneous change in the water content if the solution is anisotonic). As there is no ammonium salt within the corpuscles, it will enter them by diffusion. This diffusion of the salt is followed by an absorption of water, so that C_v remains constant, as the amount of water absorbed is exactly the same as contained m mol salt when m is the total amount of diffused salt. We shall let v represent the volume of the erythrocytes at the time t . During the time dt there is a diffusion of dm mol salt into the erythro-

cytes, together with that amount of water which corresponds to dm mol in the surrounding fluid; $dv = \frac{dm}{C_v}$ liter, as the surrounding fluid contains C_v mol salt per liter of solution. Then we have:

$$(3) \quad C_v \frac{dv}{dt} = K(C_v - C_i).$$

C_i is determined partly by the equation $C_i = \frac{m}{v-x}$ (in which x is the dispersion phase of the erythrocytes), and partly by the equation $v - v_0 = \frac{m}{C_v}$; that is, $C_i = C_v \frac{v - v_0}{v - x}$. Thus the differential equation for the diffusion is:

$$\frac{dv}{dt} = K \left(1 - \frac{v - v_0}{v - x} \right), \quad K dt = \frac{v - x}{v_0 - x} dv.$$

By integration this gives:

$$KT = \frac{1}{2} \frac{(v - x)^2}{v_0 - x} + C.$$

C can be eliminated; $v = v_0$ when $T = 0$. This gives:

$$(4) \quad KT = \frac{1}{2} \frac{(v - x)^2 - (v_0 - x)^2}{v_0 - x}.$$

To test the validity of the expression experimentally it is sufficient merely to place the corpuscles in a solution of an ammonium salt and measure their volume from time to time.

As is evident from the equation—and from experiments—the corpuscles will keep on swelling till they have absorbed all the surrounding fluid. As a rule, however, they undergo hemolysis before this is accomplished.

The hemolysis may be prevented by the addition of a suitable amount of a non-permeating salt or non-electrolyte. In that case another expression is derived from Fick's law:

As before, the osmotic pressure will remain the same on both sides of the membrane only if it stays constant both outside and inside. This requires that the salt diffusing into the erythrocytes — dm mol — should take along an amount of water — dv liter — such that the

solution, dm mol salt in dv liter solution, has the constant osmotic pressure: $p = p_i + p^*_i = p_v + p^*_v$. p_i and p_v are due to the permeating salt, p^*_i and p^*_v depend on the non-permeating substance. $p_v = K^*C_v$, $p_i = K^*C_i$.

$$K^* \frac{dm}{dv} = p, \text{ or } \frac{dm}{dv} = C_v \frac{p}{p_v}.$$

When substituted in (2), it gives:

$$(5) \quad C_v \frac{p}{p_v} \frac{dv}{dt} = K (C_v - C_i).$$

Correspondingly, the amount of salt (m) diffusing into the corpuscles, together with the absorption of the amount of water in $(v - v_0)$ liter, is given as:

$$(5a) \quad \frac{m}{v - v_0} = \frac{p}{p_v} C_v.$$

The m mol salt is distributed in $(v - x)$ liter solution of the inner fluid, giving this the concentration C_i .

$$(6) \quad C_i = \frac{m}{v - x} = C_v \frac{p}{p_v} \frac{v - v_0}{v - x}.$$

Substituting this value for C_i in (5) gives:

$$(7) \quad \frac{p}{p_v} \frac{dv}{dt} = K \left(1 - \frac{p}{p_v} \frac{v - v_0}{v - x} \right);$$

$$(8) \quad \frac{dv}{dt} = K \left(\frac{p_v}{p} - \frac{v - v_0}{v - x} \right).$$

$\frac{p_v}{p}$ decreases with an increase in t , whereas $\frac{v - v_0}{v - x}$ increases, as $x < v_0$.

Therefore, diffusion and swelling will go on, until $\frac{p_v}{p} = \frac{v_0 - v_0}{v_0 - x}$; this means, as is also evident from the connection between C_i and C_v in (6), that $C_i = C_v$.

If we conceive the volume of the surrounding fluid as very large in proportion to that of the corpuscles, p_v may be taken as a constant.

If this be the case, then we have: $\frac{p_y}{p} = \frac{v_s - v_o}{v_s - x}$. When this expression is substituted in (8), we have:

$$(9) \quad \frac{dv}{dt} = K \left(\frac{v_s - v_o}{v_s - x} - \frac{v - v_o}{v - x} \right) = K \left(\frac{v_s - v}{v - x} \right) \left(\frac{v_o - x}{v_s - x} \right).$$

By integration, this gives:

$$(10) \quad KT = \frac{v_s - x}{v_o - x} \{ -(v_s - x) \ln (v_s - v) + v_s - v \} + C.$$

The value of C may be calculated, as $v = v_o$ when $T = 0$. We then have:

$$(11) \quad KT = \frac{v_s - x}{v_o - x} \left\{ (v_s - x) \ln \left(\frac{v_s - v_o}{v_s - v} \right) + v_s - v \right\}.$$

EXPERIMENTAL

A great number of experiments has been performed. As permeating salt ammonium bromide has been used. As non-permeating substance either Brinkman fluid or Christensen-Warburg fluid has been added to the system.

Brinkman fluid consists of:

Primary phosphate of potassium (Sørensen)	2.18 gm.
Secondary phosphate of sodium (Sørensen)	16.3 gm.
Distilled water, free of carbon dioxide, up to 1 liter.	
Δ 0.458. pH 7.53.	

Christensen-Warburg fluid consists of:

Oxalate of sodium (Sørensen)	11.3 gm.
Primary phosphate of potassium (Sørensen)	0.315 gm.
Secondary phosphate of sodium (Sørensen)	1.365 gm.
Boiled distilled water up to 1 liter.	
Δ 0.425. pH 7.35.	

The blood in all experiments was fresh human blood with sodium citrate as an anticoagulant. The temperature was kept constant by means of a water thermostat, in which the flasks with the salt solutions and the blood were immersed. At certain times after the beginning of an experiment, specimens were extracted and centrifugated at a rate of 3500 r.p.m. in Hamburger's cono hematocrit tubes. All volumes are expressed as per cent of a standard volume; namely, the volume of the blood corpuscles in their own plasma.

Calculation of the Diffusion Constant

Application of Equations 4 and 11 to the $v - t$ curves obtained offers several advantages. For one thing, it makes practicable a direct comparison of the results from experiments with different con-

centrations of permeating or non-permeating substance. Next, it allows the summing up of the results of an experiment with 10–20 volume determinations into a single value: K , supplemented by v_0 (in Equation 11, by v_s too).

There is a little difference in the calculation of the constant according to whether Equation 4 is used or Equation 11.

Equation 4 reads:

$$KT = \frac{1}{2} \frac{(v-x)^2 - (v_0-x)^2}{v_0-x},$$

and it is to be employed when the surrounding fluid consists only of permeating substance. It includes three unknowns: x , v_0 , and K . v and v_0 are calculated in per cent of the volume which the same amount of corpuscles would have in plasma.

The value of x —dispersion phase of the corpuscles—is set at 50 per cent of the volume in plasma. As all the v values are estimated in the same unit, x may as a rule be set at 50 (provided that x does not change).

The value of 50 for x is derived empirically. It is the value most fitting for human blood corpuscles in the fluids employed in the present work; that is, when all the values obtained have to be adapted to the expressions found for Boyle-Mariotte's law and Fick's law.

In the application of Equation 4 it is theoretically possible to find the value of v_0 by mathematical deduction from several determinations of v , as $(v-x)^2 = 2(v_0-x)KT + (v_0-x)^2$, but the estimations are not accurate enough for this. Therefore, v_0 has to be determined by extrapolation or by calculation according to Boyle-Mariotte's law. The derivation of K is the same in both equations; it will be mentioned later.

Equation 11 reads:

$$KT = \frac{v_s - x}{v_0 - x} \left\{ (v_s - x) \ln \left(\frac{v_s - v_0}{v_s - v} \right) + v_0 - v \right\},$$

and it is employed when the surrounding fluid contains non-permeating substance as well as permeating substance.

With Equation 11 the derivation of K is more difficult. Here we have four unknowns: v_s , v_0 , x , and K . Here too x is set at 50. As above, v_0 may be derived mathematically in several ways, but estimations of v as a rule are not accurate enough for this purpose.

So v_0 has to be determined by extrapolation; and this is practicable in particular if the same system is examined at different temperatures, as this gives a greater number of curves for the extrapolation, and v_0 is not affected by changes in the temperature. At low temperatures the swelling of the erythrocytes is slow, at high temperatures it proceeds rapidly. Thus it is practicable in this way to get a set of curves which all converge towards v_0 .

Finally, v_0 can be calculated approximately when the osmotic pressure of the surrounding fluid is known; this value is substituted in Boyle-Mariotte's law, and v_0 is calculated from this, Δ of plasma being reckoned as 0.56° . v_0 is more easy to obtain. The curve will approach asymptotically a finite quantity we here call v_s , although it really ought to be designated by v_∞ , as theoretically it is never reached. However, the corpuscles will soon attain to a size which differs from the theoretical v_∞ only so little that this deviation is far within the limit of mean error. Measuring the volume several times after this value is reached will therefore give constant values for the volume, and this will practically be equal to v_∞ . So when the same volume is obtained a few times in succession, it may be counted as v_s . Theoretically it would be practicable also to calculate v_s after Boyle-Mariotte's law when the osmotic pressure of the non-permeating substance in the surrounding fluid is known. It happens not infrequently, especially in protracted experiments, that the values for v_s fail to be altogether constant, as hemolysis, agglutination, and similar processes may prevent an accurate reading. When, on the other hand, the end value is reached early, v_s proves to be a constant value (Experiment 1).

EXPERIMENT 1

15 cc. Brinkman fluid + 10 cc. 2.2 per cent NH_4Br + 0.5 cc. blood, 1 hour old

Time	Volume of corpuscles in per cent of their volume in plasma
5 min.	113
11½ "	115
21 "	117
48½ "	116
19 hrs.	117
24 "	117

The value of v_0 is determined for each concentration of permeating salt, and v_0 and v_s are determined for each combination of permeating and non-permeating salts.

In each experiment a series of determinations of v are made at different points of time (t). It is now attempted to find a value for K such that the $v - t$ curve resulting from the substitution of the values obtained for v_0 , v_s , and x in the equation corresponds as well as possible to the $v - t$ values obtained.

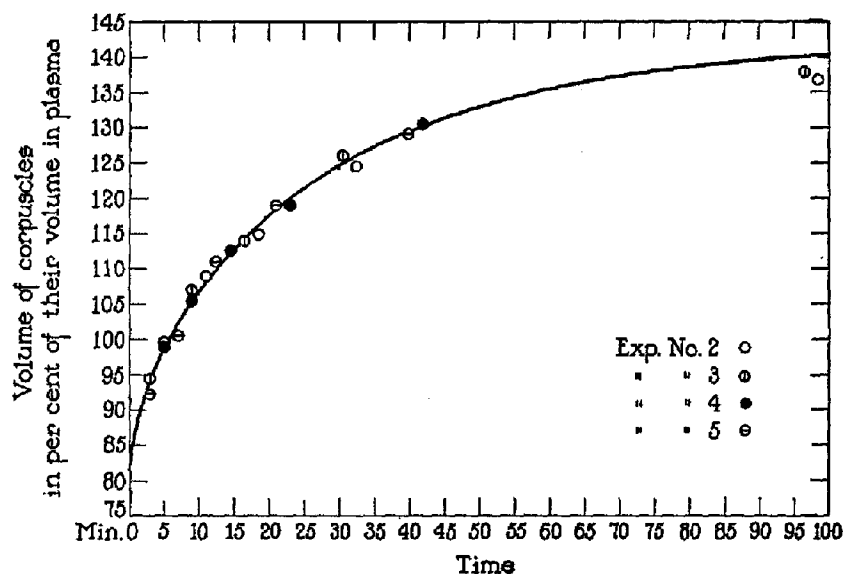


FIG. 1. Experiments 2-5.—15 cc. Warburg-Christensen fluid + 10 cc. 4.41 per cent NH_4Br + 0.5 cc. blood. Temperature 20° . Curve drawn: calculated values (v_0 82; v_s 144; K 6.5). Points plotted: observed values.

As the procedure is the same no matter which equation is employed, it suffices for illustration to show its application to the more complicated of the two equations (11).

Four experiments in the same system gave the results presented in Fig. 1.

v_0 and v_s cannot be estimated with sufficient accuracy from these four experiments alone, but summing up the results of all the experiments in this system (a total of 100) gives 144 as the average value for v_s in

132 determinations, and 82 as the average for v_0 . The latter value is the extrapolation value which fits in the best with the 100 experiments in the system, in which the K value varies from 1.0 to 30.

A trial is then made with substitution of some values for K in the equation, with selection of the value that gives a curve covering the greatest number of points—in this example K was 6.5.

In another series of experiments, one of the systems comprises seven separate experiments carried out at different times. The findings are presented in Fig. 2.

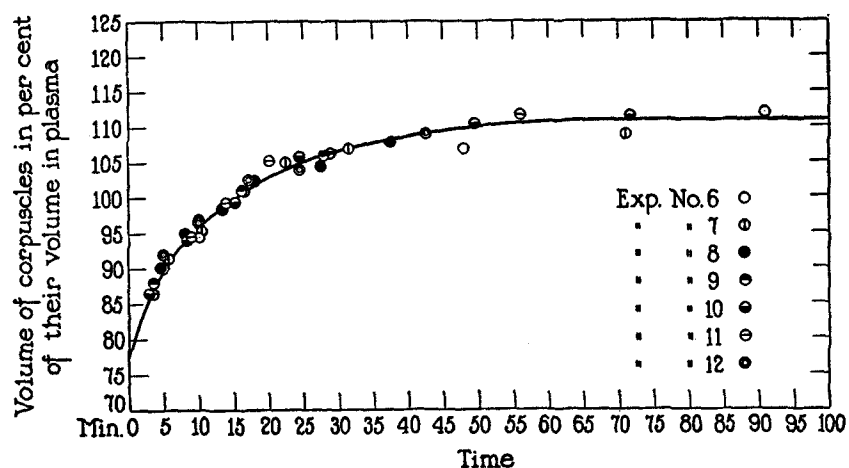


FIG. 2. Experiments 6-12.—15 cc. Brinkman fluid + 10 cc. 4.41 per cent NH_4Br + 0.5 cc. blood. Temperature 20° . Curve drawn: calculated values (v_0 77.5; v_∞ 112; K 6.5). Points plotted: observed values.

Here too K 6.5 was found to give the most consistent curve with v_0 77.5 and v_∞ 112. All the experiments agree; no other value for K would apply to any of them.

K may be calculated for every point and an average taken, but the "best possible" curve is almost as exact and much easier to do. Thus, a curve calculated on a formula derived from Fick's law very closely covers a set of points obtained by experiment. I do not claim that this fact has any value in proving the assumptions that we made in deducting the formula. Nevertheless, the size of the surface area of the blood corpuscles may very well be constant within the limits here

adhered to. Jacobs in working out a formula very like mine makes the same assumption.

In numerous experiments with different concentrations of permeating and non-permeating substances, the curve always has covered the points. Of course, the values of v_o and v_s have to be altered with the system. All values of v_o in the different systems follow the van't Hoff-Boyle-Mariotte law closely. The values of v_s do not always correspond to those expected from this law but there is a certain conformity in the variations in the different systems.

I think then, that these formulae may be used in testing the influences of certain external factors, *i.e.* temperature and hydrogen ion concentration—and the effect of certain substances upon the permeability of the blood corpuscle membrane. For if we carry on our investigations in the same system of permeating and non-permeating salts, any alteration in the size of the diffusion constant K may be taken as a proof of an alteration in the permeability of the corpuscle membrane.

SUMMARY

Two rather simple equations have been derived, which make it possible to express in a single number the result of a series of determinations of the volume of erythrocytes swelling in solutions of ammonium salts.

In all experiments made with several combinations of different concentrations of permeating and non-permeating salts, the curves calculated from the equations have covered the points found by experiment.

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