THE KINETICS OF PENETRATION

VI. SOME FACTORS AFFECTING PENETRATION

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INTRODUCTION

Although sea water contains very much more sodium than potassium the latter predominates in the cells of many marine organisms. Presumably this is because it penetrates the protoplasm more rapidly. 1

A like situation in models affords a favorable opportunity to study certain variables which may be important in living cells.

In these models 2 electrolytes pass from an outer aqueous layer A (Fig. 1) through a non-aqueous layer B (representing the protoplasm) into an inner aqueous layer C (which may be called "artificial sap"); the latter consists at the start of distilled water and CO₂ bubbles in it throughout the experiment.

All three layers are stirred mechanically but at the phase boundaries are unstirred layers (A~B, B~B, and C~B) in which the movement of electrolytes depends on diffusion. The layers B, and B, in which diffusion is slowest, 3 regulate the process of penetration.

In these experiments A contained KOH and NaOH which had been previously shaken up with a non-aqueous mixture consisting of 70 per cent guaiacol + 30 per cent p-cresol (which will be called G. C. mixture). As a result KOH combined with the constituents of the non-aqueous phase to form organic salts which may be lumped together 4 and called collectively KG, the corresponding designation for

4 This introduces no serious error since the behavior of the organic salts seems to be very similar. Cf. footnote 2.
the sodium salts being NaG. We therefore have to do with the penetration of KG and NaG.

In the preceding paper\textsuperscript{4} it was assumed that the rate of penetration of potassium is proportional to the concentration gradient in the non-aqueous layer B. This gradient may be regarded as \( K_o' - K_i' \), where \( K_o \) represents the concentration of undissociated KG in the outer surface of \( B_o \), and \( K_i' \) that in the inner surface of \( B_i \) (the corresponding designations for sodium are \( N_a_o' \) and \( N_a_i' \)).

On reaching \( C \), KG and NaG come in contact with \( C \) and form \( KHCO_3 \) and \( NaHCO_3 \). This raises the osmotic pressure in \( C \) so that

![Diagram of layers in the model](image)

FIG. 1. Diagram of layers in the model. The aqueous phase A has an unstirred layer which is represented between \( d \) and \( e \); from \( e \) to \( f \) is the corresponding unstirred layer in the non-aqueous phase B. Similar layers are present at the boundary between the non-aqueous phase B and the aqueous phase C.

water enters from A. Eventually a steady state is reached in which water and salts enter in a fixed ratio and the volume of \( C \) increases while its composition remains constant. This appears to be analogous to what happens in living cells.

Previous studies\textsuperscript{6} indicate that the rate of entrance is proportional to \( K_o' - K_i' \); it is also proportional to the diffusion constant. Hence we may write as an approximation

\[
R_K = C_i D_K (K_o - K_i)
\]

\textsuperscript{4}Osterhout, W. J. V., \textit{J. Gen. Physiol.}, 1932-33, \textbf{16}, 529.

\textsuperscript{6}This equation takes no account of the entrance of water. Regarding the significance of the "constants" see Osterhout, W. J. V., \textit{J. Gen. Physiol.}, 1932-33, \textbf{16}, 529.
Here $R_X$ is the rate of entrance of moles of potassium into $C$ and $D_X$ the diffusion constant of undissociated $KG$ in $B$. Using a corresponding notation for sodium we have

$$\frac{R_X}{R_Na} = \frac{C_1D_X(K'O - K_i)}{C_1D_Na(Na'_o - Na_i)}$$

$C_1$ and $C_2$ are “constants” which depend on the rate of stirring, the surface area, and shape of $B$, etc., and when they are the same for $KG$ and $NaG$ cancel out.

This equation permits only qualitative predictions because the ratio $(K'_o - K'_i) + (Na'_o - Na'_i)$ changes during the process and several disturbing factors intervene (p. 455).

Let us now consider $K'_o$ and $K'_i$. We may make the usual assumption that on each side of the phase boundary there are very thin layers in approximate equilibrium with each other. Hence if the total concentrations of potassium ($KG + KOH + KHCO_3$) in the aqueous surface layers be $K_o$ and $K_i$ we may put

$$\frac{K'_o}{K_o} = S_{Ko} \quad \text{and} \quad \frac{K'_i}{K_i} = S_{Ki}$$

where $S_{Ko}$ and $S_{Ki}$ are the partition coefficients or absorption coefficients.

Hence we may put

$$\frac{R_X}{R_Na} = \left(\frac{D_X}{D_Na}\right) \frac{S_{Ko}K_o - S_{Ki}K_i}{S_{Na_o}Na_o - S_{Na_i}Na_i}$$

In view of this a knowledge of diffusion constants and partition coefficients becomes desirable.

**Diffusion Constants**

When only $KG$ is present the meaning of the diffusion constant $D_X$ requires no comment, but when we add $NaG$ it will affect the value of $D_X$.

$7$ The behavior of this ratio depends on the magnitudes of the partition coefficients as well as on their ratio. See p. 449.

$D_K$. The divergence will be small in guaiacol (in which $K_G$ and $Na_G$ are very weak electrolytes) but may be larger in water: this latter, however, does not concern us since for our purposes the diffusion in water may be regarded as negligible. Similar reasoning applies to the diffusion constant $D_{Na}$.

Let us now consider the ratio $D_K + D_{Na}$. In order to determine this the diffusion apparatus of Northrop and Anson was used. The upper part was filled with G. C. mixture containing equal concentrations of $K_G$ and $Na_G$ which were allowed to diffuse into the lower chamber containing G. C. mixture. The result showed that $D_K$ is so close to $D_{Na}$ that for our purposes they may be regarded as equal.

Similar tests in which water was employed as the solvent showed that $K_G$ and $Na_G$ diffuse ten to eleven times as fast in water as in G. C. mixture (this result is due in part to the higher viscosity of the G. C. mixture). Hence the diffusion through the unstirred aqueous layers $A_B$ and $C_B$ is so rapid that it may be neglected in the subsequent discussion. We need only consider the slow diffusion in the non-aqueous layers $B_o$ and $B_c$.

**Partition Coefficients**

Evidently the diffusion constants, $D_K$ and $D_{Na}$, are too similar to account for the experimental fact that potassium enters $C$ much faster than sodium. It would seem that the partition coefficients must be responsible for this difference.

In order to clarify the rôle of the partition coefficients let us first consider an hypothetical system in which $K_G$ and $Na_G$ are very weak electrolytes in $A$, $B$, and $C$, and in which $C$ contains no CO$_2$. Let the values of $K_o$ and $Na_o$ be constant at 0.05, the values of $S_{K_0}$ and $S_{Na_0}$ being 0.62, those of $S_{Na_c}$ and $S_{K_c}$ being 0.29, and those of $D_K$ and $D_{Na}$

9 This has been shown by a group of physical chemists whose results will shortly be published.


12 These vary with concentration but we may assume for purposes of calculation that $S_{K_0}$ is constant as well as $S_{Na_0}$.
being equal. A simple calculation\(^{13}\) indicates that in the early part of the experiment potassium should enter C faster than sodium and that \(K_+ + Na_+\) should be less than \(S_{K_+} + S_{Na_+}\). The experiments bear this out. The calculation is as follows:

Let us suppose that the unstirred layers are so thin that the concentration gradient in \(B\) is approximately linear and that \(C\) is a mere film, only a few molecules thick, and contains at the start only distilled water, the bubbling of \(CO_2\) being omitted. Let us now consider a layer in \(B\) of the same thickness as \(C\) adjoining the interface: this will be called \(B_i\). For convenience we suppose the total volume of each of these films to be 1 liter.

At the start of the experiment the concentration gradient of \(KG\) in \(B\), which will be called \(G'_{K}\), is \(0.031 - 0 = 0.031\), and the corresponding value for \(G'_{Na}\) is \(0.0145 - 0 = 0.0145\). Hence \(G'_{K} + G'_{Na} = 2.14\). We may assume that during the first small increment of time 1.62 millimoles of \(KG\) reach \(B_i\), and distribute themselves so that 1.0 goes into \(C\) and 0.62 remains in \(B_i\), giving a concentration of 0.00062 m in \(B_i\) and 0.001 m in \(C\) (the value of \(K_+\) remains constant at 0.031 since \(K_+\) is constant).

Since at the start \(G'_{Na}\) is 0.0145 and \(G'_{K}\) is 0.031 we suppose that the amount of \(NaG\) moving across \(B\) in the first small increment of time is approximately 1.62 (0.0145 + 0.031) = 0.756 millimole: of this 0.17 will remain in \(B_i\), and 0.586 will pass into \(C\) (so that \(S_{Na_+} = 0.17 + 0.586 = 0.29\); this is the value previously stated). At the end of the first increment of time \(G'_{K} = 0.031 - 0.00062 = 0.0304\) and \(G'_{Na} = 0.0145 - 0.00017 = 0.0143\). Hence \(G'_{K} + G'_{Na} = 0.0304 + 0.0143 = 2.1\) and \(K_+ + Na_+ = 0.001 + 0.000586 = 1.7\).\(^{14}\)

This calculation indicates that in the early part of the experiment the ratio \(K_+ + Na_+\) will be greater than unity and that as time goes

\(^{13}\) The significance of this method of calculation may seem doubtful, especially when we are not dealing with the earliest stages of the experiment, but it seems to be borne out by the results.

\(^{14}\) It is of interest to note that the ratio \(K_+ + Na_+\) in the early part of the experiment depends on the magnitudes of \(S_{K_+}\) and \(S_{Na_+}\) as well as on their ratio. Thus in the foregoing calculation we had \(S_{K_+} + S_{Na_+} = 0.62 + 0.29 = 2.14\) and the value \(K_+ + Na_+\) after the first increment of time was 1.7, but if we put \(S_{K_+} + S_{Na_+} = 6.2 + 2.9 = 2.14\) we get a different result for \(K_+ + Na_+\) since the value is 1.6. This may be shown as follows. Since \(G'_{K}\) and \(G'_{Na}\) are now ten times as great at the start we expect 16.2 millimoles of potassium and 7.56 of sodium to be moved. The concentrations in \(B_i\) are therefore \(KG = 0.0162 \text{ m}\) and \(NaG = 0.00756 \text{ m}\). These will distribute so that \(K_+ = 0.01395 \text{ m}, K_+ = 0.00225 \text{ m}, Na_+ = 0.00562 \text{ m}, \text{and } Na_+ = 0.00194 \text{ m}\). Hence \(K_+ + Na_+ = 0.00225 + 0.00194 = 1.16\).
on it will approach unity since the concentrations in C will approach those in A (i.e. 0.05 m KG + 0.05 m NaG).

The actual model (with no CO2 in C) differs from this hypothetical case since $S_{K}$ is not constant and is not equal to $S_{K}^0$: this applies also to sodium. Furthermore KG and NaG are strong electrolytes in A and C (though weak electrolytes in B) but this merely means that the diffusion of molecules of KG and NaG in C is replaced by the diffusion of the ions K+, Na+, and G-, thereby changing somewhat the diffusion constants.

Hence we expect that at the start potassium will enter C more rapidly than sodium and that both will reach the same concentration at equilibrium. This was found to be the case in earlier experiments (e.g. in Experiment 66 reported in a previous paper14). The ratios found in a later experiment (Experiment 112) are shown in Fig. 2 (in this, as in the other figures, the curves are drawn free-hand to give an approximate fit). In this case A contained 0.05 m KG + 0.05 m NaG and the ratio $S_{KG}^0/S_{NaG}^0$ was 2.14 (see page 452).

Model I was employed and a steady flow was maintained in A which kept its composition approximately constant: B contained G. C. mixture and C contained

14 Osterhout, W. J. V., and Stanley, W. M., J. Gen. Physiol., 1931–32, 15, 667, Fig. 6.
distilled water (no CO₂ bubbling), stirred with a stream of air. The volume of C at the start was 150 cc. The temperature was 21° ± 2°C. The concentration of potassium + sodium in C was determined by titration with standard acid and of sodium by the method previously described, potassium being determined by difference.

In most of our experiments CO₂ was present in C and in consequence KG and NaG reacted to form KHCO₃ and NaHCO₃ as soon as they reached the surface of C. Potassium and sodium then diffused through Cₜ in the form of bicarbonates. But since diffusion in this layer is very rapid as compared with that in B it may be left out of account. (An additional effect of the reaction with CO₂ is a great reduction of the ionic activity products (K)(G) and (Na)(G) in C.)

Before discussing such experiments it is desirable to consider more fully the equation for penetration. In doing this we may neglect Dₚ + Dₚ (since it is not far from unity) and write as a first approximation

$$\frac{K'}{K''} = \frac{K'_i - K'_o}{Na'_i - Na'_o}$$

The values of K'ₜ and Na'ₜ are kept approximately constant by continually renewing the solution in A. It has been shown elsewhere that the activity of K'ₜ is proportional to the ionic activity product (Kₜ)(OHₜ) in A. Hence we may put K'ₜ = Sₜₜ Kₜ = Cₜ(Kₜ)(OHₜ) and it is evident that the value of Sₜₜ will depend on that of OH⁻. We may treat K'ᵢ in the same way. All of this applies equally to sodium.

As already stated K'ₜ = Sₜₜ Kₜ: hence to approximate the value of K'ₜ a determination of Sₜₜ = (concentration of undissociated KG in

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16 This might not have much effect on the value of Kₜ + Naₜ as calculated by the method given on p. 449, since it might influence both of the diffusing substances in similar fashion.

17 Under the conditions of the experiment the activity of OH in C bears a constant relation to the activity of HCO₃⁻ and to that of the guaiacol ion G⁻.

We may write K'ₜ = Cₜ(Kₜ)(OHₜ) and K'ᵢ = Cᵢ(Kᵢ)(OHᵢ) where the subscripts o and i refer to the outside and inside solutions respectively. But Cₜ and Cᵢ vary with concentration because the non-aqueous phase changes.
the surface of $B_o$) $\div$ (concentration of $K^+$ in $A$) is desirable. This cannot be done directly but we may approximate it by determining\textsuperscript{18} $S_{K_0} = (\text{concentration of } KG \text{ in the surface of } B_o) \div (\text{concentration of } K^+ \text{ in } A)$. It seems highly probable that the ratio $S_{K_0} \div S_{Na_0}$ is approximately equal to $S_{K_G} \div S_{Na_G}$ and since we need comparative values only we may use $S_{K_0}$ and $S_{Na_0}$ in place of $S_{K_G}$ and $S_{Na_G}$.

The values of $S_{K_G}$ and $S_{Na_G}$ were determined as follows. An aqueous solution containing equal concentrations of $KG$ and $NaG$ was shaken with a relatively small volume of G. C. mixture on a shaking machine and was then allowed to stand for 24 hours or longer. The G. C. mixture was then shaken with half its volume of 0.08 $M$ HCl which removed practically all of the potassium and sodium. The determinations of potassium and of sodium were made as previously described.\textsuperscript{2} The temperature was 21° ± 2°C.

Since the aqueous phase loses more potassium than sodium it must be relatively large to keep its ratio $K_0 \div Na_0$ approximately constant or else it must contain more potassium at the start. As an illustration of the latter method we may cite the following: 104 cc. of aqueous solution of 0.222 $M$ KOH + 0.151 $M$ NaOH was shaken with 100 cc. G. C. mixture. After separation there was 98 cc. of the aqueous solution in which the concentrations were 0.107 $M$ potassium and 0.110 $M$ sodium. There was also 106 cc. G. C. mixture in which the concentrations were 0.116 $M$ potassium and 0.053 $M$ sodium. Hence we have $S_{K_G} = 0.116 \div 0.107 = 1.085$ and $S_{Na_G} = 0.053 \div 0.110 = 0.482$. Hence $S_{K_G} \div S_{Na_G} = 1.085 + 0.482 = 2.26$.

The situation is easily seen from the following considerations. For the aqueous phase let us put: volume = $V$, concentration of potassium = $C_K$, concentration of sodium = $C_Na$, moles of potassium = $M_K$, moles of sodium = $M_Na$, and designate the corresponding values in the non-aqueous mixture as $V'$, $C_K'$, etc. Assuming that $V = 1$ liter, $V' = 10$ liters, and that owing to the nature of the non-aqueous phase the partition coefficient, i.e. $C_K' \div C_K$ has a value of 0.4, and that at the start $C_K = C_Na = 1$, we have at equilibrium (providing no change in volume occurs and that potassium and sodium act independently)

\[
C_K = M_K = 1 - M_K'
\]

\[
\frac{M_K}{10} = C_K' = 0.4C_K = 0.4(1 - M_K')
\]

\textsuperscript{18} Strictly speaking we do not determine undisassociated $KG$ in $B_o$ but total (stoichiometric) concentration of potassium in $B_o$ which amounts to practically the same thing since the concentrations of KOH and KHCO$_3$ in $B_o$ are negligible in comparison with that of KG, and KG is a very weak electrolyte in $B$ (as shown by the unpublished work of physical chemists).
hence

$$M_K' = 0.8$$

$$C_K = M_K' = 1.0 - 0.8 = 0.2$$

$$C_K' = 0.8 + 10 = 0.08$$

Hence $$C_K' + C_K = 0.08 + 0.2 = 0.4$$: this is the value previously assumed for the partition coefficient.

Assuming that the partition coefficient for sodium, i.e. $$C_{Na'} + C_{Na} = 0.2$$ and proceeding in the same way we obtain for equilibrium $$C_{Na} = 0.335, C_K + C_{Na} = 0.2 + 0.335 = 0.594;$$ also $$C_{Na'} = 0.067$$ and $$C_K' + C_{Na'}' = 0.08 + 0.067 = 1.2.$$ This latter value changes to 1.02 when we put $$V = 1$$ and $$V' = 100$$ (instead of $$V' = 10$$), to 1.71 when we put $$V = 1$$ and $$V' = 1,$$ and to 1.95 when we put $$V = 1$$ and $$V' = 0.1.$$

We see that the greater the relative volume of the aqueous solution the nearer the ratio $$C_K' + C_{Na}$$ in the non-aqueous phase approaches to the ratio of partition coefficients which in this case is $$0.4 + 0.2 = 2.$$

A series of values of $$S_{KG}$$ and $$S_{NaG}$$ is shown in Fig. 3. They were determined when both KG and NaG were present in equal concentrations in the aqueous phase at equilibrium.\(^{19}\)

Let us now consider conditions at the inner phase boundary. Here the partition coefficients are $$S_K,$$ and $$S_{Na}$$ (where $$S_K = K_{K'} + K_{K}$$ and $$S_{Na} = Na'_{Na} + Na_Na$$). We may use $$S_{KG} = \text{(concentration of KG in the surface of B)} + \text{(concentration of K in C)}$$ in place of $$S_K.$$

The determinations are somewhat uncertain because on reaching $$C, \text{ KG is transformed to KHCO}_3, \text{ and we must therefore determine S}_{KG}, \text{ by shaking up an aqueous solution of KHCO}_3 \text{ with G. C. mixture so that not only KG but also KHCO}_3 \text{ is taken up. We can determine the total amount of potassium taken up but we do not know exactly what percentage of this is KG.}^{20} \text{ We can also deter-}

\(^{19}\) Adding NaG to KG affects $$S_{KG}$$ as follows: As stated elsewhere (footnote 5) $$K_{K'}$$ is proportional to the ionic product $$(K_K)(G_K)$$ when activities are taken. Using concentrations (and taking total potassium in B as approximately equal to $$K_{K'}$$) we may say that since $$S_{KG}$$ equals $$(KG \text{ in } B) + \text{(potassium in } A) \text{ the value of } KG \text{ in } B \text{ will be approximately doubled when we double } G, \text{ by adding NaG: this will double the value of } S_{KG} \text{ since the concentration of potassium in } A \text{ remains constant. This has been tested experimentally and is found to be approximately true.}

\(^{20}\) For example, when 0.63 M KHCO, at pH 7.5 was shaken up with G. C. mixture the concentration of potassium in the latter was 0.0062 M. The concentration of CO$_3$ + HCO$_3$ was 0.0025 M. Probably most of this was CO$_3$: hence if we put CO$_3$ = 0.002 M we have KG = 0.0062 -- 0.0005 = 0.0057 M. (It depends on the pH value of the aqueous solution.) Cf. footnote 5.
mine the total carbon taken up but we do not know exactly what per cent of this is $\text{HCO}_3$ and what is $\text{CO}_2$. We find that \((\text{concentration of potassium in G. C. mixture}) + (\text{concentration of potassium in aqueous phase})\) varies with concentration and hence we conclude that $S_{Ki}$ varies with concentration. This also applies to $S_{Na_i}$.

**Fig. 3.** Values of the partition coefficients $S_{KG}$ and $S_{NaG}$. These values were determined in the presence of equal concentrations of potassium and sodium. The total concentration of $G^-$, or guaiacol ion (taken as equal to $KG + NaG$), is plotted as abscissa. Thus the value for 0.05 means that an aqueous solution of 0.025 M $KG + 0.025$ M $NaG$ was shaken with a relatively small volume of G. C. mixture and the partition coefficients were calculated from the amounts of $KG$ and $NaG$ found in the G. C. mixture.

Since the ratio $S_{Ki} + S_{Na_i}$ varies with concentration (independently of pH) and since the concentration of $K_i + Na_i$ may rise during the experiment from zero to 1.2 M there is opportunity for a change in the ratio $K'_i + Na'_i$ which will affect the relative rates of entrance of potassium and sodium.

The values of $S_{Ki}$ and $S_{Na_i}$ also vary with pH (p. 451). Since the
pH value in C may rise from between 5 and 6 to the neighborhood of 7.6 during an experiment the values of \( S_{K'} \) and \( S_{Na} \) must increase accordingly. Variation in the flow of CO₂ will also affect the pH.

It should be remembered that the diffusion "constants" \( D_{K} \) and \( D_{Na} \) as here used are not constant since \( D_{K} \) varies with the concentration of NaG and vice versa (p. 447).

Other Factors

It thus appears that penetration depends largely on the diffusion constants and partition coefficients. But it is also affected by a number of other factors, among which are the following:

1. Even when the concentrations in the main body of A are kept constant those at the interface may vary for there is a concentration gradient in the unstirred layer \( A_B \) which determines the concentration at the outer surface of B. The more rapid the stirring the thinner this layer and consequently the nearer \( K_o \) and \( Na_o \) (i.e. the concentrations of \( K^+ \) and \( Na^+ \) at the inner surface of \( A_B \)) will approach the concentrations in the main body of A. When the concentrations in A are altered the value of \( K_o' + Na_o' \) may vary because the partition coefficients change. The ratio \( K_o' + Na_o' \) (and therefore of \( R_{K} \div R_{Na} \)) will therefore depend in part on the rate of stirring.

This ratio may also be influenced by temperature which alters the viscosity and hence the thickness of the layers (the viscosity is altered by the presence of electrolytes). Temperature may also affect the partition coefficients unequally.

If a model be used with diffusion in B more rapid than in A the ratio \( K_o' + Na_o' \) may vary because, with potassium moving into \( B_o \) more rapidly than sodium, its relative concentration in \( A_B \) must become steadily less, unless the rates of stirring and of diffusion be sufficient to renew the supply (see p. 452). As \( K_o \) decreases potassium will move more slowly into B.

2. In the stirred layers the forward movement of electrolyte will depend on such factors as rate of stirring, and viscosity.

3. There are concentration gradients in \( C_B \) which will depend on the factors already enumerated and which may change the ratio \( R_{K} \div R_{Na} \).

4. Surface forces may play a part; e.g., substances diminishing surface tension will tend to remain in the surface.
5. Reactions may occur at the outer surface and if slow enough may affect the result; e.g., loss of water by the penetrating substance or molecular association in the non-aqueous phase. At the inner surface the reverse processes will occur but here we may have in addition combinations with acids or other substances in C.

6. The penetration of electrolytes into C is accompanied by that of water. To understand its effect let us consider what happens when water is added suddenly to C. Thus if the concentration gradients in B be called \( G'_K \) and \( G'_Na \) and the ratio be

\[
\frac{G'_K}{G'_Na} = \frac{K' - K'_i}{Na' - Na'_i} = \frac{0.24 - 0.12}{0.10 - 0.04} = 2
\]

and water be added to C, doubling its volume, we have

\[
\frac{G'_K}{G'_Na} = \frac{0.24 - 0.06}{0.10 - 0.02} = 2.25
\]

In this case where the ratio \( K'_o + K'_i = 2 \) is less than \( Na'_o + Na'_i = 2.5 \) we observe that the ratio \( G'_K + G'_Na \) rises after the addition of water.

When \( K'_o + K'_i \) is greater than \( Na'_o + Na'_i \) the ratio falls. Thus if \( K'_o + K'_i = 0.24 + 0.02 = 12 \) and \( Na'_o + Na'_i = 0.13 + 0.02 = 6.5 \) we have before the addition of water

\[
\frac{G'_K}{G'_Na} = \frac{0.24 - 0.02}{0.13 - 0.02} = 2
\]

and afterwards

\[
\frac{G'_K}{G'_Na} = \frac{0.24 - 0.01}{0.13 - 0.01} = 1.92
\]

When \( K'_o + K'_i = Na'_o + Na'_i \) the addition of water does not change the ratio. Thus if \( K'_o + K'_i = 0.24 + 0.12 = 2 \) and \( Na'_o + Na'_i = 0.08 + 0.04 = 2 \) we have before the addition of water

\[
\frac{G'_K}{G'_Na} = \frac{0.24 - 0.04}{0.13 - 0.04} = 2
\]

\[22\] After the sudden addition of water the concentration gradients would no longer be linear.
and afterwards

\[ \frac{G_K}{G_{Na}} = \frac{0.24 - 0.06}{0.08 - 0.02} = 3 \]

Evaporation will, of course, produce the opposite effect.

The inward movement of water may have a different temperature coefficient from the movement of the penetrating substances: hence the composition of C may depend on temperature.\textsuperscript{21}

7. There is an outward movement of substances (from C to A); e.g., of KHCO\(_3\), NaHCO\(_3\), CO\(_2\), and H\(_2\)CO\(_3\). This may be neglected in the earlier part of the process of penetration but toward the end and in the steady state it may become more important. It is quite possible that the outward movement of KHCO\(_3\) may differ from that of NaHCO\(_3\) in such fashion as to produce a different ratio of K\(_+\) : Na\(_+\) from that which would otherwise occur.

In spite of the fact that penetration is influenced by so many factors the experiments indicate that the ratio of potassium to sodium in C depends chiefly on the partition coefficients.

EXPERIMENTS

(a) Diffusion from A to C

To illustrate this statement we may cite a series of experiments, in which A contained equal concentrations of KG and NaG, B contained G. C. mixture, and C contained distilled water in which CO\(_2\) was bubbling. The results are shown in Table I (p. 458). It is evident that potassium predominates in C in every case as would be expected in view of its higher partition coefficient. We see also that the average of the ratio K : Na in C does not differ greatly from \( S_{K_a} + S_{Na_a} \). There is considerable variation in the ratios of K : Na: changes during the progress of one experiment are shown in Fig. 4.

Models I, II, and III were used.\textsuperscript{2} A steady flow was maintained in A thus keeping its composition approximately constant. A, B, and C were stirred. The temperature varied between 20 and 25°C. The determinations of potassium and sodium were made as previously described.\textsuperscript{2}

\textsuperscript{22} At the start there may be an outward movement of water since the concentration of electrolytes is greater in A than in B.
TABLE I

Ratios of Potassium to Sodium in the Artificial Sap in C

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time of penetration</th>
<th>Ratio K + Na in C</th>
<th>Solution in A</th>
<th>Ratio of partition coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>109 a</td>
<td>days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>10</td>
<td>2.45</td>
<td>0.02 M KG</td>
<td>+</td>
</tr>
<tr>
<td>c</td>
<td>8</td>
<td>2.37</td>
<td>0.02 M NaG</td>
<td>+</td>
</tr>
<tr>
<td>58</td>
<td>11</td>
<td>2.28 steady state</td>
<td>0.1 M KG</td>
<td>+</td>
</tr>
<tr>
<td>63</td>
<td>9</td>
<td>1.4</td>
<td>0.1 M NaG</td>
<td>+</td>
</tr>
<tr>
<td>66</td>
<td>12</td>
<td>1.5</td>
<td>0.05 M KG</td>
<td>+</td>
</tr>
<tr>
<td>80</td>
<td>33</td>
<td>1.6</td>
<td>0.05 M NaG</td>
<td>+</td>
</tr>
<tr>
<td>81</td>
<td>4</td>
<td>2.7</td>
<td>In steady state</td>
<td>+</td>
</tr>
<tr>
<td>111</td>
<td>50</td>
<td>2.0</td>
<td>0.05 M NaG</td>
<td>+</td>
</tr>
<tr>
<td>Av. = 2.0</td>
<td></td>
<td></td>
<td>2.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Fig. 4. Ratios of potassium + sodium in C in a model in which A contained 0.05 M KG + 0.05 M NaG; B contained G. C. mixture, and C contained distilled water at the start but had CO₂ bubbling through it throughout the experiment (Experiment 111). The ratio of partition coefficients is $S_{KG} + S_{NaG} = 2.14$. The experimental errors are larger during the first part of the experiment when the concentrations in C are low.
It seemed desirable to examine the process of penetration at each interface separately. To examine the outer interface a model was constructed with A and B but without C. In A was placed 0.1 M KG + 0.1 M NaG, and in B was placed G. C. mixture. The results of a typical experiment (No. 83) are shown in Fig. 5.

In this case the concentration gradient of chief importance is that in the layer B0. Calling the concentration of undissociated KG at the outer surface of this layer $K'_{0}$ and that at the inner limit $29$ of this layer $K'_{o}$ we may write as an approximation

\[
\frac{R_{K}}{R_{Na}} = \frac{K'_{0} - K'_{o}}{Na'_{0} - Na'_{o}} = \frac{(SK_{Go}K_{o}) - K_{o}}{(SNa_{Go}Na_{o}) - Na_{o}}
\]

At the start, when $K'_{o}$ and $Na'_{o}$ are both equal to zero, we may write as an approximation

\[
\frac{R_{K}}{R_{Na}} = \frac{SK_{Go}}{SNa_{Go}Na_{o}}
\]

Since $K_{o} = Na_{o}$ we have

\[
\frac{R_{K}}{R_{Na}} = \frac{SK_{Go}}{SNa_{Go}}
\]

---

29 This is merely a convenient fiction since there is no definite limit at this spot.
We therefore expect at the start a correlation between the partition coefficients and the concentrations of potassium and sodium in $B$. This will also be true as the system approaches equilibrium. Hence it may well be true of the intermediate period and this appears to be the case since the ratio of $S_{K\text{gel}} / S_{Na\text{gel}}$ was found in shaking experiments (p. 452) to be $1.085 \div 0.48 = 2.26$ which is fairly close to the ratios observed during the progress of the experiment as shown in Fig. 5.

When approximate equilibrium was reached in this experiment the concentration of potassium in $B$ was 0.126 M and that of sodium 0.055 M. Hence we have $S_{K\text{gel}} = 1.26$ and $S_{Na\text{gel}} = 0.55$; these values are a little higher than those obtained in the shaking experiments$^{24}$ (where we found $S_{K\text{gel}} = 1.085$ and $S_{Na\text{gel}} = 0.48$). The ratio $S_{K\text{gel}} / S_{Na\text{gel}} = 2.29$ is also a little higher than the value of 2.26 found in shaking experiments.

Model III was employed.$^5$ Both $A$ and $B$ were stirred. A continuous flow in $A$ kept the composition constant to within 5 per cent. $B$ contained 500 cc. of G. C. mixture. Equilibrium was attained in about 52 hours. The temperature was $20^\circ \pm 2^\circ$C.

(c) Diffusion from $B$ to $C$

In order to examine the processes occurring at the interface between $B$ and $C$ a model was used in which $B$ was brought into equilibrium with $A$ (by shaking $B$ with a great excess of $A$) before the experiment was started (so that there was relatively little diffusion from $A$ to $B$ during the experiment): $C$ contained only distilled water at the start (no CO$_2$ was bubbled during the experiment). In this case the chief movement during the early part of the experiment was across the interface between $B$ and $C$ so that the layer of chief importance is $B_\phi$. Calling the concentration of undissociated KG at the outer limit $\phi$ of this layer $K_{\phi}$ and that at the other surface $K'_\phi$ (with similar designations for sodium) we may write as an approximation

$^{24}$ *I.e.* experiments in which a solution containing 0.107 M KOH + 0.110 M NaOH was shaken up with G. C. mixture as described on page 452. As shown in Fig. 3 the ratio $S_{K\text{gel}} / S_{Na\text{gel}}$ is very nearly the same at a concentration of 0.2 M $G^-$ as at a concentration of 0.217 M.

$^{25}$ This is, of course, a convenient fiction as there is no sharp limit at this spot.
A typical experiment (Experiment 86) was started with 0.1 M KG + 0.1 M NaG in A, distilled water saturated with guaiacol (no CO₂ bubbling) in C, and with B in equilibrium with A. From the very start B contained 0.1085 M KG and 0.048 M NaG. Since \( S_{KG_a} = 1.085 \) and \( S_{NaG_a} = 0.48 \) the ratio \( S_{KG_a} + S_{NaG_a} \) is 2.26. A simple calculation shows that soon after the start of the experiment \( K_t + Na_t \) might be in the neighborhood of 1.6 and this agrees fairly well (Fig. 6) with observation (although the validity of this method of calculation may be questioned: see page 449). As \( K_s = Na_s \) we expect that as equilibrium is approached \( K_t + Na_t \) will approach unity and this appears to be the case. The calculation is as follows:

Consider a thin layer of B, only a few molecules thick, adjoining the inner interface: we may call this \( B_i \) and the corresponding layer in C on the other side of the interface \( C_i \). For convenience we put the volume of each of these layers at 1 liter.

We see that \( B_i \) contains 0.1085 mole of KG and we may suppose that when water is brought in contact with it enough KG instantaneously moves into \( C_i \) to bring these two layers into approximate equilibrium. If during this process no more
KG moves into B, we may make the following calculation: After approximate equilibrium is reached we have

\[
\frac{K'_i - K_i}{K_i} = S_{KG_i}
\]

If for convenience we put \(S_{KG_i} = S_{KG} = 1.085\) we have

\[
\frac{0.1085 - K_i}{K_i} = 1.085
\]

whence \(K_i = 0.052\) and \(K'_i = 0.0565\). In the same way we have

\[
\frac{0.048 - Na_i}{Na_i} = 0.48
\]

whence \(Na_i = 0.0324\) and \(Na'_i = 0.0156\). Hence \(K_i + Na_i = 0.052 + 0.0324 = 1.6\). We then have for the ratio of gradients

\[
\frac{K'_i - K'_i}{Na'_i - Na_i} = \frac{0.1085 - 0.0565}{0.048 - 0.0156} = 1.6
\]

so that we might expect \(K'_i + Na'_i\) to be about 1.6 as indeed appears to be the case with the upper curve in Fig. 6 extrapolated to zero time.

Model III was employed. A constant flow in A kept its composition nearly constant: B was brought into equilibrium with A by shaking the two phases together before the experiment started: C contained at the start 75 cc. of distilled water saturated with G. C. mixture and was stirred by a stream of air. A and B were stirred mechanically. The temperature was 20° ± 2°C.

In another sort of experiment (Experiment 66) no CO₂ was bubbled during the first 236 hours and the concentrations in C reached the same level as in A, namely 0.05 m KG + 0.05 m NaG: B was then practically uniform throughout and contained 0.031 m KG + 0.0145 m NaG. The ratio \(S_{KG} + S_{NaG}\) was 0.62 + 0.29 = 2.14.

At 236 hours the bubbling of CO₂ began and 4 hours later the ratio \(K_i + Na_i\) in C was 1.65 (as shown at the ordinate marked 240 in Fig. 7). The ratio rose until the 264th hour after which it slowly fell and then gradually rose again.

\[26\] This makes the value of \(S_{KG}\) too large but as that of \(S_{NaG}\) will be too large by a corresponding amount the error in the ratios \(K'_i + Na'_i\) and \(K_i + Na_i\) will be small.

\[27\] Osterhout, W. J. V., and Stanley, W. M., J. Gen. Physiol., 1931–32, 15, 676, 678 (Experiment 66, Table 1, and Fig. 6).
In the steady state C contained 0.73 M KHCO₃ + 0.45 M NaHCO₃, giving a ratio of 1.62.

Model I was employed. A, B, and C were stirred as usual. A constant flow was maintained in A. The temperature varied from 20 to 25°C during the course of the experiment.

In concluding the experimental part we may say that in all cases (the diffusion constants being nearly equal) the ratio K / Na in C seems to depend chiefly on the partition coefficients. But other factors are sufficiently influential to produce considerable variation in these ratios.

**DISCUSSION**

It seems probable that many of the variables discussed in this paper are found in living cells such as those of *Nitella* and of *Valonia*.²⁸

²⁸ At this time A contained 0.043 M KG + 0.007 M KHCO₃ + 0.043 M NaG + 0.007 M NaHCO₃. The bicarbonate was due to diffusion of HCO₃⁻ and CO₂ from C into A.

²⁹ The models would resemble the living cells more closely if we employed KOH and NaOH in A and if KG and NaG, though soluble in the non-aqueous layer, were practically insoluble in water.
In *Valonia* and *Nitzula* we apparently have a continuous non-aqueous phase at the inner and outer protoplasmic surfaces. We may suppose that these surface layers correspond to the layers $B_2$ and $B_1$ in the model and that between them is an aqueous phase more or less stirred by protoplasmic movement or by convection currents. The external solution and the sap are well stirred by convection currents. We therefore seem to have opportunity for some of the variables that are found in models.

In these models the chief factors appear to be partition coefficients and diffusion constants. Is this true of living cells in general?

In seeking to answer this question we must remember that the importance of the partition coefficients depends on the speed of diffusion in the non-aqueous layers. When diffusion in these layers is slow enough to control penetration the partition coefficients become important. It would seem that this applies to most living cells since partition coefficients appear to play a highly important role. It is for this reason that Overton's theory is so useful, especially as amended by Irwin.

When the partition coefficients of two substances are not very different molecular size becomes important because it determines the diffusion constants. It may also be due in some cases to the fact that penetration is regulated more by the cell wall than by the protoplasm.

In many cases the non-aqueous layers are probably very thin. Hence the diffusion constants in these layers must be very small or the concentration gradients (due to the partition coefficients at the two surfaces of the layer) must be very gentle in order to make diffusion slow enough to have the process of penetration controlled by this layer.

The diffusion constants would, of course, be small if the viscosity were high. It is not necessary to suppose that the layers are solid since protoplasm in contact with water rounds up as though true surface tension existed.

It may be remarked in passing that the idea that protoplasm has a non-aqueous surface has been opposed on the ground that water and salts enter freely. But the model shows that this objection does not hold since water and salts freely pass through the non-aqueous layer.

---

Let us now consider the question of ionization. In our models KG penetrates very rapidly as compared with KCl although both are equally ionized in the external solution. But both are weak electrolytes in the non-aqueous phase and hence penetrate chiefly in molecular form. The difference lies in the fact that KG has a much higher partition coefficient than KCl.

There is another consideration which may well be mentioned here, namely, that partition coefficients are important in bioelectric effects. Assuming the latter to be chiefly due to diffusion potentials \(^{31}\) we may illustrate the situation by means of models. For example, when only KG is present the diffusion potential at the outer surface will depend on \(K'_o\) and at the inner surface on \(K'_i\). Now when \(S_{K_o} = S_{K_i} \) and \(K_o + K_i = 10\) then \(K'_o + K'_i = 10\). But when \(S_{K_o} = 1\) and \(S_{K_i} = 0.1\), and \(K_o + K_i = 10\) we have \(K'_o + K'_i = 100\) and the diffusion potential increases accordingly. Similar considerations would apply if KG were placed at the outer surface and NaG at the inner since the concentrations and consequently the potential in the non-aqueous phase would depend on the values of \(S_{K_o}\) and \(S_{Na}\).

Aside from the question of partition coefficients the outstanding fact brought out by these experiments is the large number of variables concerned. No attempt has been made to treat all of these quantitatively or to set up equations for the time curve of penetration. In a previous paper an empirical equation was given which fits the observations satisfactorily when potassium alone is present and a rigorous treatment has been formulated by L. G. Longsworth \(^{32}\) by means of which the time curve has been calculated. By means of other equations he has calculated the ratio of sodium to potassium in the steady state. But no attempt has been made as yet to include in this treatment all the variables mentioned in this paper.

The fact that with constant concentrations of potassium and sodium outside there is so much variation in their proportions in the artificial sap in C recalls the situation in Valonia where there is a considerable variation in the cell sap. It happens that the variation is similar in the two cases for if we divide the highest observed ratio of \(K + Na\) by


\(^{32}\) Longsworth, L. G., *J. Gen. Physiol.*, 1933–34, 17, 211.
the lowest we get in the model $2.7 \div 1.4 = 1.93$ and in the case of *Valonia* $5.72 \div 2.55 = 2.24$.

In conclusion it may be appropriate to repeat that when the living cell shows a great difference in the penetration of similar compounds of potassium and of sodium it seems safe to conclude that diffusion coefficients cannot be responsible since the molecular sizes cannot differ greatly. Hence the difference must lie in partition coefficients. This is a very important factor in dealing with living organisms.

**SUMMARY**

Some of the factors affecting penetration in living cells may be advantageously studied in models in which the organic salts KG and NaG diffuse from an aqueous solution $A$, through a non-aqueous layer $B$ (representing the protoplasmic surface) into an aqueous solution $C$ (representing the sap and hence called artificial sap) where they react with CO$_2$ to form KHCO$_3$ and NaHCO$_3$. Their relative proportions in $C$ depend chiefly on the partition coefficients and on the diffusion constants in the non-aqueous layer. But the ratio is also affected by other variables, among which are the following:

1. Temperature, affecting diffusion constants and partition coefficients and altering the thickness of the unstirred layers by changing viscosity.
2. Viscosity (especially in the non-aqueous layers) which depends on temperature and the presence of solutes.
3. Rate of stirring, which affects the thickness of the unstirred layers and the transport of electrolyte in those that are stirred.
4. Shape and surface area of the non-aqueous layer.
5. Surface forces.
6. Reactions occurring at the outer surface such as loss of water by the electrolyte or its molecular association in the non-aqueous phase. The reverse processes will occur at the inner surface and here also combinations with acids or other substances in the “artificial sap” may occur.
7. Outward diffusion from the artificial sap. The outward movement of KHCO$_3$ and NaHCO$_3$ is small compared with the inward movement of KG and NaG when the concentrations are equal. This
is because the partition coefficients of the bicarbonates are very low as compared with those of NaG and KG.

Since CO$_3^-$ and HCO$_3^-$ diffuse into $A$ and combine with KG and NaG the inward movement of potassium and sodium falls off in proportion as the concentration of KG and NaG is lessened.

8. Movement of water into the non-aqueous phase and into the artificial sap. This may have a higher temperature coefficient than the penetration of electrolytes.

9. Variation of the partition coefficients with concentration and pH.

Many of these variables may occur in living cells. (It happens that the range of variation in the ratio of potassium to sodium in the models resembles that found in Valonia.)