THE KINETICS OF PENETRATION

IV. DIFFUSION AGAINST A GROWING POTENTIAL GRADIENT IN MODELS

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Cells of Nitella in tap water show an outwardly directed E.M.F. of from 50 to 300 millivolts. Previous experiments\(^1\) suggest that this is largely due to potassium ions whose entrance into the vacuole produces an outwardly directed E.M.F. This tends to oppose their further penetration but they nevertheless continue to enter.

Something of this sort seems to happen in certain models recently described.\(^2\) An example is seen in an experiment in which 0.05 m KOH is shaken with a small excess of a mixture of 70 per cent guaiacol and 30 per cent p-cresol (which may be called G.C. mixture) which converts most of the KOH to organic salts of potassium. For convenience these organic potassium salts may all be regarded as K-guaiacolate and called KG.\(^3\)

The resulting solution is allowed to flow in a steady stream over a layer of G.C. mixture. We may then say that the KG passes from the outer solution (A) through the non-aqueous layer of G.C. mixture (B) and penetrates into the inner solution (C), which at the start consisted of distilled water in which CO\(_2\) was bubbling. The latter may be regarded as analogous to the sap of Nitella and designated as "artificial sap."\(^4\) B will then correspond to the non-aqueous protoplasmic layer and A to the external solution bathing the Nitella cell.


\(^3\) This involves no serious error as the K-p-cresolate seems to behave like the K-guaiacolate.
On reaching C, KG reacts to form KHCO₃ which may accumulate until its concentration reaches 0.63 m. Hence the osmotic pressure in C becomes much greater than in A. The energy required to produce this result comes from the reaction of KG with CO₂. In part this energy finds expression in the form of E.M.F.'s.

In order to measure the E.M.F.'s produced in the model the apparatus shown in Fig. 1 was employed. With this arrangement we may suppose that a thin layer of II, which we may call L, (perhaps only a few molecules thick) will come at once into equilibrium with III and will in consequence have a different composition from the rest of II (which may be called I).

![Diagram of experimental setup](image)

**Fig. 1.** Aqueous solutions are placed in I and III and a non-aqueous liquid in II. The salt bridges, S.B. (consisting of glass tubes filled with agar made up in saturated KCl solution) were placed at the sides in order that KCl sinking downward might not contaminate the interfaces.

Presumably we have the three potentials shown below, namely,

<table>
<thead>
<tr>
<th>Aqueous (I)</th>
<th>Non-aqueous (II)</th>
<th>Aqueous (III)</th>
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<tbody>
<tr>
<td>P₁</td>
<td>l</td>
<td>P₃</td>
</tr>
<tr>
<td>I</td>
<td>lᵦ</td>
<td>P₂</td>
</tr>
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the phase boundary potentials $P₁$ and $P₃$, and the diffusion potential $P₂$ in the non-aqueous layer.

Unless otherwise stated the procedure was as follows. The aqueous solution in I (Fig. 1) and the non-aqueous solution in II were shaken together and left in
contact for 24 hours or more before they were placed in the apparatus. The aqueous solution in III was shaken with a small excess of the non-aqueous solution in II, placed in a separatory funnel and freed from the non-aqueous portion which was discarded. Hence when the solutions were placed in the apparatus there was presumably no exchange of ions between I and II, but ions moved from III into II and set up a diffusion potential in II. If there be any diffusion potential in III due to exit of ions from II it must be small.

To imitate electrical conditions in the model near the start of the experiment aqueous solution from A (consisting of 0.05 M KG) is placed in I. Non-aqueous solution from B (consisting of G.C. mixture containing KG) is placed in II. In III we might place distilled water, but as this gives irregular results we prefer to use 0.001 M KHCO₃ (which would represent the condition of C in Model III a short time after starting the experiment).

This gives a potential of 45 millivolts, I being negative to III in the external circuit (hence A is negative to C in the model). On bubbling CO₂ in III (without disturbing the interface unduly) KG in II begins to combine with CO₂ at the interface to form KHCO₃ (which is much more soluble in III than in II). Hence the concentration of KHCO₃ in III increases and that of H⁺ decreases.

This is illustrated by an experiment in which solution from A in a model which had been running for some time was placed in I (this consisted of 0.03 M KG + 0.02 M KHCO₃). Solution from B was placed in II and 0.0001 M KHCO₃ in III. At the start the P.D. was 33 millivolts (I being negative to III in the external circuit): this gradually rose to 35 millivolts. Then CO₂ was bubbled in III and in the course of 70 minutes (shown in Fig. 2) III became negative to I in the external circuit so that positive current tended to flow from III through II into I. Stirring II facilitated this change by bringing more KG to the interface between II and III. In an experiment in which II was stirred occasionally, III became 24 millivolts negative to I (in the external circuit) in the course of 55 minutes. (When III

As a rule the diffusion potentials appear to be much greater in G. C. mixture than in water.

\(^{1}\) I.e., before much KHCO₃ entered A.

\(^{2}\) Average of 43, 48, 35, and 54.

\(^{3}\) This was done in a U-tube having an upright tube in the center through which a stirrer was inserted in II.
is stirred for a moment, it becomes temporarily less negative to I because stirring carries KHCO₃ away from the interface between II and III.)

These experiments show that in the model the positive current at the start tends to flow from A through B into C but that later this is reversed so that C becomes negative to A in the external circuit. This negativity continues to increase until the steady state is reached. To ascertain the negativity in the steady state solutions taken from the model in the steady state were placed in the apparatus (solution from A was placed in I, from B in II, and from C in III). This gave 48 millivolts, III being negative to I in the external circuit (hence

8 This is the average of 5 determinations giving 48, 49, 51, 37, and 53 respectively.

Fig. 2. At the start liquid from A in the model is placed in I (Fig. 1) and is 33 millivolts negative (in the external circuit) to 0.001 x KHCO₃ which is placed in III. Liquid from B is placed in II. When CO₂ is bubbled through III it reacts with KG in B to form KHCO₃ which dissolves in C and reverses the p.d. in the course of 70 minutes.
in the model C is negative to A). Enough KCl was then added to I to make the concentration of potassium equal to that in III and the reading became practically zero\(^{10}\) despite the fact that I was more alkaline than III (this will be discussed later).

The measurements were made by means of a Compton electrometer (Cambridge Instrument Co.) the use of which was kindly loaned by Dr. Irwin. The shielding and grounding were so carefully carried out that steady readings could be made even with extremely high resistances in the circuit.

Unless the measurements became fairly steady after the first 2 minutes the experiment was rejected (a drift of more than 10 per cent between the 2nd and 10th minute not being acceptable).

It is of interest to know whether the negativity of C is due to increase of K\(^+\) or to decrease of H\(^+\). At the start the activity of H\(^+\) in C was between 10\(^{-5}\) and 10\(^{-6}\) but at the steady state it was 10\(^{-7.5}\): hence there is about a hundredfold decrease which might account for the change in potential of (45 + 48 =) 93 millivolts were it not for the fact that the activity of K\(^+\) in the meantime increased from zero to (0.63)\(^{11}\) = 0.40.

In the presence of so much K\(^+\) it would seem that these variations in H\(^+\) do not cause much change of potential as was indicated by the fact that when the concentration of potassium in I and III was equalized (as just described) the p.d. was practically zero despite the difference in pH value.

In order to test this further a solution of 0.6 M KCl was placed in I and in III: in II was placed G.C. mixture shaken with 0.6 M KCl. As expected this gave zero. Enough HCl was now added to III to make the difference of pH value on the two sides more than 2 pH units; i.e., greater than in the model (in the model the pH value of A is 9.1 and that of C is 7.5, a difference of 1.6). The reading was still zero or so near it as to be within the limits of experimental error.\(^{12}\)

\(^{9}\) KCl has about the same effect on potential as KHCO\(_3\).

\(^{10}\) Repeated four times.

\(^{11}\) The activity coefficient 0.63 is obtained by interpolation from the data of Harned (Harned, H. S., J. Am. Chem. Soc., 1929, 51, 416) on the assumption that it is the same as in a solution of 0.63 M KCl and that the activities of K\(^+\) and Cl\(^-\) in such a solution are equal.

\(^{12}\) The pH values were determined colorimetrically as described in a former paper.\(^{2}\)

\(^{13}\) Repeated three times.
It is therefore clear that in the presence of so much K\(^+\) the observed variations in pH value are negligible as far as effect on potentials is concerned.

We may therefore conclude that in the model the penetration of potassium creates an outwardly directed potential against which it continues to diffuse inward, thereby increasing the outward potential. This continues until the steady state is reached.

It is evident that this potential may be due to phase boundary potential or to diffusion potential.\(^{14}\) In the latter case we must take into account the inward diffusion of KG, creating an inwardly directed potential (due to the greater mobility\(^{15}\) of K\(^+\) as compared with G\(^-\)) and the outward diffusion of KHCO\(_3\), producing an outwardly directed potential (due to the greater mobility\(^{15}\) of K\(^+\) as compared with HCO\(_3^-\)).

In the case of living cells a similar situation might exist. It has been suggested\(^1,2\) that potassium may form in the non-aqueous layers of the protoplasm a compound, KX, which (like KG in the model) reacts in the sap with an acid HA to form KA: subsequently HA is exchanged for HCl existing in the external solution. If in the non-aqueous layers of the protoplasm KX and HCl pass inward practically undissociated, and KCl outward, more slowly\(^{16}\) but with a higher degree of dissociation, the net movement of potassium ions and of chloride ions in the non-aqueous layer will be an outward one so that there might be an outwardly directed diffusion potential\(^{17}\) due to KCl. This might account in part for the outwardly directed potential observed in *Nitella* when the sap contains 0.025 M KCl + 0.025 M NaCl and\(^{18}\) the outside

\(^{14}\) Donnan potential may be ruled out because there are no ions which cannot pass through the non-aqueous phase.\(^5\)

\(^{15}\) This is indicated by measurements of the concentration effect.

\(^{16}\) Just as in the model KHCO\(_3\) passes outward more slowly than KG passes inward. It is supposed that KG is very little dissociated in the G. C. mixture.\(^9\)

\(^{17}\) This would be aided by the higher concentration of KCl and possibly by a greater difference between the mobility of anions and cations in the non-aqueous layers.

\(^{18}\) Since the protoplasmic surfaces are not identical an organic electrolyte formed between these surfaces may set up an outwardly directed potential which is greater than the corresponding inwardly directed potential. This would account for the outwardly directed potential observed when sap is placed on both sides of the protoplasm (cf. Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, 11, 391).
of the protoplasm is in contact with tap water. (The higher concentration of KCl inside the cell would be due to the energy of metabolism.)

It is of interest to note that if we regard the P.D. as due to diffusion potential we may say that in the model, as well as in Valonia, the mobility of the cations appears to be greater than that of the corresponding anions.

SUMMARY

In a model consisting of a non-aqueous layer (representing the protoplasm) placed between an inner, more acid, aqueous layer (representing the sap) and an outer, more alkaline, aqueous solution (representing the external solution bathing a living cell) the penetration of potassium creates an outwardly directed potential against which potassium continues to diffuse inward, thereby increasing the outward potential. This continues until the steady state is reached. The potassium sets up less potential in entering than in escaping and the net result is an outwardly directed potential. A similar process appears to take place in certain living cells.