# THE KINETICS OF PENETRATION

## IX. Models of Mature Cells

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Models have been constructed[1] which in certain respects resemble living cells. In such models potassium salts pass from an aqueous solution *A* through a non-aqueous layer *B* into the artificial sap *C* where they react with $CO_2$ to form $KHCO_3$. Potassium reaches a much higher concentration in *C* than in *A*: in consequence water enters *C*. Eventually a steady state is reached in which water and electrolyte enter in a constant ratio and the volume of *C* increases while its composition remains approximately constant. This seems to be analogous to what happens in actively growing cells.

Can we also imitate mature cells in which growth has ceased? This involves some interesting problems. In so far as the cessation of growth is due to a decrease in metabolism it would seem possible to imitate it in the model by decreasing the amount of $CO_2$ in the artificial sap (in *C*).

To test this we set up a model in which *B* consisted of 70 per cent of guaiacol plus 30 per cent *p*-cresol (called G.C. mixture). By shaking G.C. mixture with 0.05 M KOH we obtained organic potassium salts called collectively K*G*: this solution flowed through *A* (during the entire experiment), keeping its composition approximately constant.[2] *A* and *B* were stirred mechanically.

In previous experiments, where *C* consisted at the start of distilled

[1] Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, 1931–32, **15,** 667. Osterhout, W. J. V., and Kamerling, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 507. Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, 1933–34, **17,** 445, 469.

[2] Some of the K*G* was converted to $KHCO_3$: see Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, 1931–32, **15,** 667.

water through which $CO_2$ was bubbled, it was found that the concentration of $KHCO_3$ in *C* increased to about 0.63 M. In the present experiment 0.49 M $KHCO_3$ was placed in *C* at the start to save time and the accumulation of potassium proceeded from that point. Pure $CO_2$ was bubbled through *C* at the start: this procedure was changed later as described in subsequent pages.

Model I[1] was used (Experiment 129). *B* consisted of 1100 cc. G. C. mixture. *C* contained at the start 68 cc. The $CO_2$ was passed through a solution of approximately the same osmotic pressure as that in *C* so as not to add or subtract much water from *C*. When it was desired to reduce the percentage of $CO_2$ nitrogen was added to it and the bubbling was continued at the usual rate.

The concentration of potassium in *C* rose steadily until it became about 0.63 M where it remained approximately stationary although the volume continued to increase. This is due to the fact that potassium and water enter in a fixed ratio.

The $CO_2$ supply was then decreased (at 480 hours, as shown in Fig. 1) to about one-fourth:[3] this caused the concentration of potassium to fall off, but it subsequently rose again. Air was then substituted for $CO_2$ and the concentration of potassium fell abruptly. This was due in part to the entrance of water (as shown by the volume curve) and in part to the exit of potassium (as shown by the curve of millimoles). Potassium probably passed out[4] as K*G*, KOH, and $KHCO_3$.

Beginning at 2282 hours, 1.7 per cent of $CO_2$ (by volume) was bubbled through *C*. The concentration of potassium began to increase and the increase became more rapid when 5 per cent $CO_2$ was used. On changing to 1 per cent (followed by 0.8 and 0.5 per cent $CO_2$) the concentration of potassium fell off (but the volume continued to increase).

These results show that the increase in volume of the artificial sap depends on the supply of $CO_2$ and it seems probable that this is also true of the living cell.

[3] This was done by mixing nitrogen with the $CO_2$ and bubbling at the same rate as before.

[4] Since the partition coefficient of $KHCO_2$ is very small as compared with that of K*G* its concentration gradient in *B* and rate of movement in *B* is doubtless correspondingly low.

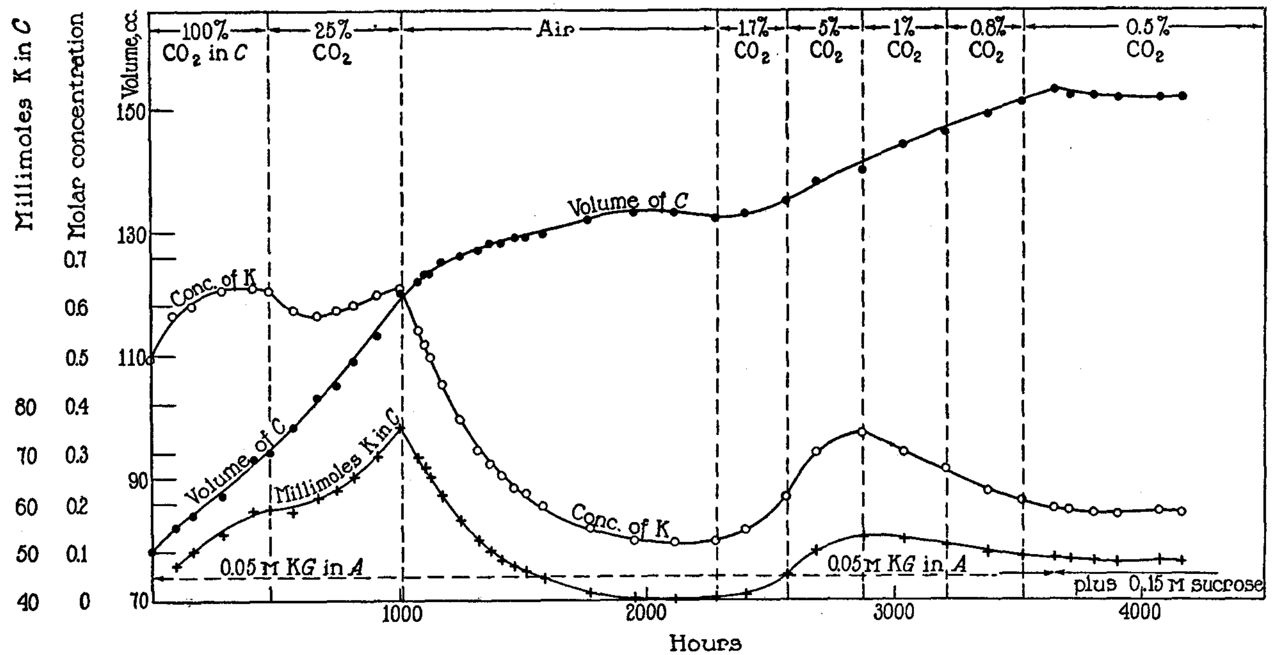


FIG. 1. Shows the volume of the artificial sap and its content of potassium expressed as molar concentration or as total millimoles present. At the start pure $CO_2$ was bubbled through $C$: at 480 hours this was replaced by a mixture of 75 per cent nitrogen + 25 per cent $CO_2$ (by volume): at 1010 hours this was replaced by air. Subsequently $CO_2$ was mixed with nitrogen to give the following percentages (by volume): at 2282 hours 1.7 per cent; at 2566 hours 5 per cent; at 2876 hours 1 per cent; at 3210 hours 0.8 per cent; at 3250 hours 0.5 per cent. At 3646 hours enough sucrose was added to $A$ to make its concentration 0.15 M.

In order to imitate mature cells which have ceased to grow we must stop the increase in the volume of *C*. Three factors may be considered in this connection.

1. Adjusting the concentration of electrolyte in *C* until the amount going out is equal to that going in. This can be controlled in part by varying the supply of $CO_2$ in *C* and the concentration of the penetrating electrolyte in *A*.

2. Imposing a mechanical restraint, such as is found in the cell wall of *Nitella,* which checks the entrance of water and the increase in volume in spite of the relatively high osmotic pressure in the sap. This cannot be done in the model.

3. Placing in the external solution a substance which enters slowly and so helps to counterbalance the osmotic pressure of the artificial sap and retard the entrance of water, as happens with NaCl in the case of *Valonia*. We might employ an electrolyte in the model for this purpose, but it seemed more convenient to use sucrose. This was added in sufficient amount to make its concentration 0.15 M, thus making the osmotic pressure in *A* approach that in *C* (this imitates the condition found in *Valonia* where the osmotic pressure in the sap is only slightly higher than in the sea water).

As a result of this procedure the volume remained constant within 1 cc. for about 2 weeks (the concentration of potassium was 0.18 M or 3.6 times that in the external solution). The experiment was then discontinued.

We may conclude that it is possible by means of a model to imitate certain important features of mature cells; *i.e.*, to produce a steady state in which the volume and composition of the artificial sap remain approximately constant, the concentration of potassium being higher inside than outside. This is quite different from the steady state described in previous papers in which the composition of *C* remained constant while its volume increased (this steady state apparently resembles that in the growing cell).

## DISCUSSION

The temporary nature of accumulation is clearly brought out by this experiment[5] for as soon as we stop bubbling $CO_2$ in *C* the con-

[5] Osterhout, W. J. V., and Kamerling, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 507.

centration of potassium in $C$ falls off. Eventually $A$ and $C$ will become identical since all the substances present can move through $B$.

In previous experiments with models a steady state was achieved in which the composition of $C$ remained constant while its volume increased. This would correspond to the condition of an actively growing cell.

In the present case both the composition and the volume of $C$ were kept approximately constant in the final phase of the experiment. The concentration of potassium was considerably higher than in $A$. This would correspond to the condition of a cell which had ceased to grow.

Under these conditions the entrance of potassium is equal to its exit. The entrance of potassium will continue as long as the external activity product [K] [$G$] is greater outside than inside. The relation of this product in $C$ to that in $A$ depends chiefly on the following:

1. On entering $C$, K$G$ reacts with $H_2CO_3$ to form $KHCO_3$ and H$G$ (guaiacol). Hence the more rapidly $CO_2$ is bubbled through $C$ the lower the product [K] [$G$] and the greater the rate of entrance of potassium (since its concentration gradient in $B$ will be larger[6]).

2. The movement of $KHCO_3$ from $C$ to $A$.

3. The formation of $KHCO_3$ in $C$ raises the osmotic pressure, causing water to enter and lowering the concentration of potassium. This can be controlled by adding to $A$ substances which enter slowly (or not at all) by which the entrance of water is checked. Hence the concentration of potassium in $C$ can be raised by adding such substances to $A$.[7]

In the living cell the entrance of water can be hindered by mechanical restraint such as the cell wall of plant cells or by the pressure of surrounding tissues. It can also be brought about by the presence in the external solution of a substance which penetrates slowly (or not at all); in *Valonia* the NaCl of the sea water acts in this way.

In addition to these factors there are doubtless others, for example, in mature cells the protoplasm may become less permeable to certain

[6] Osterhout, W. J. V., *J. Gen. Physiol.*, 1932–33, **16**, 529. Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, 1933–34, **17**, 445.

[7] This statement relates to substances which do not greatly lower the value of the product [K][$G$]: for example, it would not apply to the addition of HCl.

electrolytes or to water. Certain erythrocytes which are said to be impermeable to potassium must have taken it up in their earlier stages of development.


## SUMMARY

To imitate cells which have ceased to grow we have made models in which artificial sap is separated from the external solution by a non-aqueous layer (representing the protoplasm). A stream of $CO_2$ is bubbled through the artificial sap to imitate its production by the living cell.

Potassium passes from the external solution through the non-aqueous layer into the artificial sap and there reacts with $CO_2$ to form $KHCO_3$: its rate of entrance depends on the supply of $CO_2$. Hence the increase of volume depends on the supply of $CO_2$ (as is probably true of the living cell).

By regulating the supply of $CO_2$ and the osmotic pressure we are able to keep the volume and composition of the artificial sap approximately constant while maintaining a higher concentration of potassium than in the external solution. In these respects the model resembles certain mature cells which have ceased to grow.