

## THE KINETICS OF PENETRATION

### VIII. TEMPORARY ACCUMULATION

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Potassium accumulates in many living cells, becoming much more concentrated inside than outside. This also happens in certain models<sup>1</sup> in which carbon dioxide is continuously supplied to the artificial sap to imitate its production by the living cell. In such models the concentration of potassium steadily increases up to a certain point and then remains stationary while the volume of the artificial sap continues to increase. This seems analogous to what happens in growing cells.

The nature of accumulation in such cases can be shown more clearly by setting up a system which is left to itself instead of being continually supplied with new material.

As an example we may cite a model in which 0.03 M KOH was placed in an outer aqueous phase *A* separated by a non-aqueous phase *B*, from an inner aqueous phase *C* containing 1.0 M HCl, which may be called artificial sap. Phase *B* consisted of 70 per cent guaiacol plus 30 per cent *p*-cresol which substances may be collectively designated HG. KOH reacts with HG to form KG and passes as such through *B* to *C* where it reacts with HCl to form KCl. The result is the same as though KOH passed as such, so that we may speak of KOH as passing through *B* to *C*.

A typical experiment (No. 123) was made as follows: Model I was used.<sup>1</sup> Phase *A* contained at the start 2 liters of 0.03 M KOH, phase *B* 1100 cc. of G. C. mixture (70 per cent guaiacol plus 30 per cent *p*-cresol) which was saturated with water, and phase *C* contained 100 cc. of 1.0 M HCl. Phases *A* and *B* were stirred mechanically, phase *C* by bubbling a stream of air. The temperature varied from 21–25°C. From time to time phase *C* was removed, its volume determined, and

<sup>1</sup> Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, 1931–32, 15, 667.

a sample taken for analysis. The total acidity was determined by titration with standard alkali, the concentration of chloride ion was determined by titration with standard silver nitrate, potassium thus being found by difference. Values for the potassium concentration so obtained checked satisfactorily with those found by our customary procedure.<sup>1</sup>

We have KOH on one side of the non-aqueous layer and HCl on the other. They tend to mix by passing through this layer and forming KCl in *A* and in *C*. But in a given time so much more KOH than

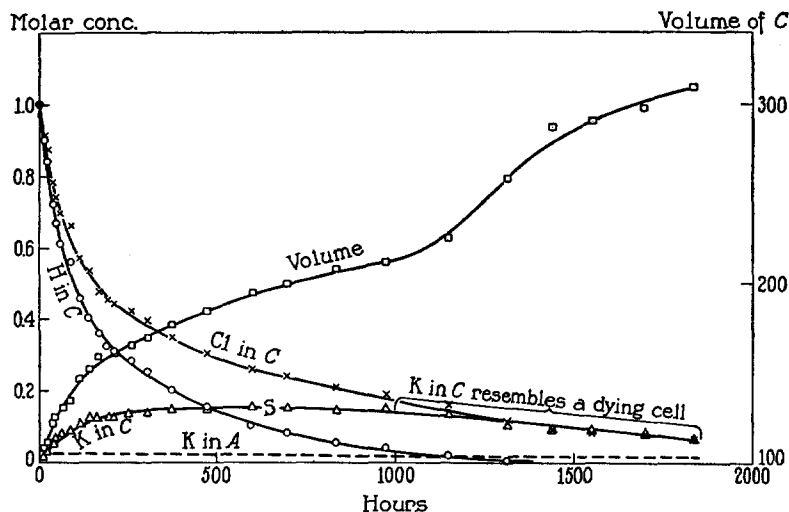


FIG. 1. Curves showing that the concentration of potassium in *C* reaches a maximum (at *S*) and then falls off. The concentration of KOH in *A*, and of HCl in *C*, falls from the start. In this experiment (No. 123) *A* contained at the start 0.03 *M* KOH, *B* was guaiacol plus *p*-cresol, and *C* contained 1.0 *M* HCl at the start. Volumes of *C* are given in cubic centimeters.

HCl passes through *B* that most of the KCl is formed in *C*, where the concentration of potassium becomes much greater than in *A*. This, however, is only temporary for when the system comes to equilibrium the composition of *A* and *C* will be identical since all the substances present can pass through the non-aqueous layer.

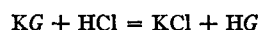
The behavior of potassium is seen in Fig. 1.<sup>2</sup> Its concentration in the artificial sap (in *C*) increases until it is much greater than in the

<sup>2</sup> In this, as in the other figures, the curves are drawn free-hand to give a rough fit.

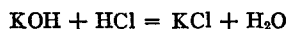
external solution (in *A*). It reaches a maximum at *S* and then declines.<sup>3</sup> At equilibrium it will be the same in *A* and *C*. The decline is analogous to what happens in a dying cell and equilibrium corresponds to death.<sup>4</sup>

The rapid inward movement of potassium at the start depends on two things, (1) KOH reacts with the guaiacol and *p*-cresol to form organic salts which may be collectively called KG and treated as one since their behavior is very similar. (2) KG has a relatively high partition coefficient<sup>5</sup> (defined as the concentration in the non-aqueous divided by that in the aqueous phase) and in consequence a relatively high concentration gradient in *B*, as the result of which its diffusion<sup>5,6</sup> through *B* is relatively rapid. (As explained in previous papers<sup>5,6</sup> it moves through *B* chiefly in molecular form.)

When KG reaches the artificial sap it is changed to KCl according to the scheme



and HG is retained in *B*. The result is the same as if KOH penetrated and reacted in the sap according to the scheme



We may use this scheme as more convenient and state that potassium will tend to enter as long as the ionic activity product (K)(OH) is greater in *A* than in *C*.

As KCl has a relatively low partition coefficient in *B* its concentra-

<sup>3</sup> The concentration declines for two reasons, (1) water moves from *A* to *C*, and (2) KCl moves from *C* to *A*: there is also a movement of HCl in the same direction which continues as long as the pH of *A* is greater than that of *C* (which is still the case at 1551 hours).

These movements, of course, influence the volumes. At the start the volume of *A* is 2 liters: this declines as water moves into *C*. The volume of *C* which starts at 100 cc. increases and at 1551 hours is 290 cc. This is to be expected since the osmotic pressure is higher in *C* than in *A*.

<sup>4</sup> This might mean a Donnan equilibrium in case the cell contained ions unable to pass out through the cell wall. Otherwise the internal and external solutions would eventually become identical.

<sup>5</sup> Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, 1933-34, **17**, 445, 469.

<sup>6</sup> Osterhout, W. J. V., *J. Gen. Physiol.*, 1932-33, **16**, 529.

tion gradient remains low and it moves outward very slowly. Eventually, however, as its concentration in *C* rises, its outward movement becomes great enough to offset the entrance of *KG* so that the concentration of potassium becomes practically stationary for the time being (*S*, Fig. 1).

This temporary stationary state corresponds to the permanent steady state in those models in which the concentration of *KG* in *A*

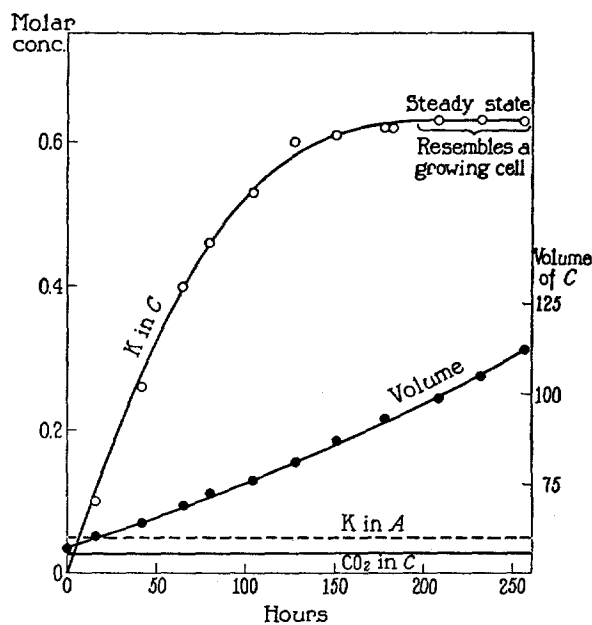


FIG. 2. The concentration of potassium rises to a fixed value; that of potassium in *A* and of  $\text{CO}_2$  in *C* remains constant. In this experiment (No. 64 of previous papers<sup>1,5,6</sup>) *A* contained at the start 0.05 *M* *KG* and this was kept approximately constant by a continuous flow, and *C* contained at the start distilled water;  $\text{CO}_2$  was bubbled through *C* during the entire experiment; *B* was guaiacol plus *p*-cresol. Volumes of *C* are given in cubic centimeters.

is kept constant<sup>5,6</sup> and  $\text{HCl}$  is replaced by  $\text{CO}_2$  in *C*, the concentration of the latter being kept constant. We then find (Fig. 2) that the accumulating substance (which is  $\text{KHCO}_3$  instead of  $\text{KCl}$ ) increases until its concentration is much higher in *C* than in *A*. In consequence water enters *C* and this continues until the steady state is reached in which

water and electrolyte enter in a fixed ratio and the volume of  $C$  continues to increase while its composition remains approximately constant.<sup>7</sup> Consequently the potassium curve rises to a certain level at which it remains. If the supply of  $\text{CO}_2$  falls off the pH of  $C$  rises and the curve declines.

The steady state appears to be analogous to what we find in growing cells. For example, when we add to the sea water in which *Valonia* is growing<sup>8</sup> a small amount of  $\text{NH}_3$  it enters and there is a rapid accumulation of  $\text{NH}_4\text{Cl}$  which reaches a steady state in which its concentration remains approximately constant.

It would seem that in the cell, as in the model, accumulation depends on the fact that the permeability to the accumulating substance is greater for the form in which it enters than for the form in which it goes out (*e.g.* greater for  $\text{NH}_3$  than for  $\text{NH}_4\text{Cl}$  and greater for  $\text{K}^+$  than for  $\text{KCl}$ ). This depends chiefly on the partition coefficients and diffusion constants.<sup>5,6,9</sup>

Before leaving this subject let us glance for a moment at the effect of injury. This may produce a twofold effect, (1) increase of permeability, (2) lessened accumulation. The total amount of electrolyte taken up in a given time will be influenced by these factors and in the injured cell may be greater than normal or less, depending somewhat on the length of the interval of time chosen. A dead *Valonia* cell might for a short time take up more electrolyte than a living one but in the end the living cell would take up more.

Let us now consider the energy relations. We may write

$$F_A = F_o + 2.3 RT \log (K_o) (\text{OH}_o)$$

and

$$F_C = C_o + 2.3 RT \log (K_i) (\text{OH}_i)$$

where  $F_o$  is the thermodynamic potential of 1 mole of KOH in the standard state,  $F_A$  and  $F_C$  are the thermodynamic potentials of KOH

<sup>7</sup> This is Experiment 64 of a preceding paper (see footnote 1). See also footnote 5.

<sup>8</sup> Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1930-31, **14**, 301. The ammonia appears to enter as  $\text{NH}_3$  rather than as  $\text{NH}_4^+$ .

<sup>9</sup> See Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 983, ff., 1002.

in *A* and *C* respectively:  $(K_o)(OH_o)$  and  $(K_i)(OH_i)$  are the ionic activity products outside (in *A*) and inside (in *C*) respectively.<sup>10,1</sup> Hence

$$\Delta_{KOH} = F_A - F_C = 2.3 RT \log \frac{(K_o)(OH_o)}{(K_i)(OH_i)}$$

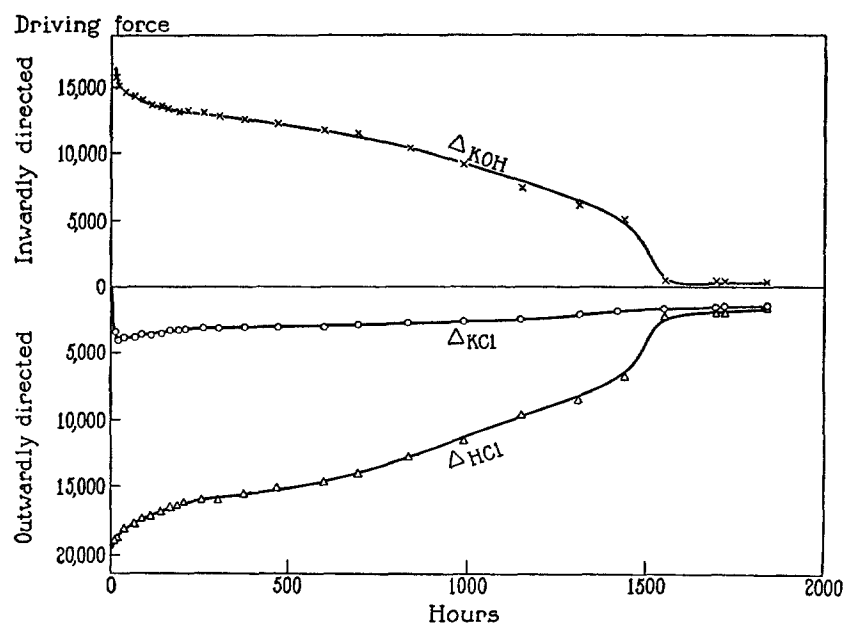


FIG. 3. Shows changes in the model (Experiment No. 123; see Fig. 1) in respect to the following:  $\Delta_{KOH}$ , the driving force which causes KOH to move inward (*i.e.* from *A* to *C*);  $\Delta_{HCl}$ , the driving force which causes HCl to move outward (*i.e.* from *C* to *A*); and  $\Delta_{KCl}$ , the driving force which causes KCl (the accumulating substance) to move outward (this passes through a maximum). The ordinates for  $\Delta_{KOH}$  represent the value of

$$\Delta_{KOH} = 2.3 RT \log \frac{(K_o)(OH_o)}{(K_i)(OH_i)}$$

the ordinates for  $\Delta_{HCl}$  represent the value of

$$\Delta_{HCl} = 2.3 RT \log \frac{(H_i)(Cl_i)}{(H_o)(Cl_o)}$$

(the equation for  $\Delta_{KCl}$  is similar). We put  $R = 1.988$  and  $T = 296^\circ$ .

<sup>10</sup> Cf. Lewis, G. N., and Randall, M., *Thermodynamics*, New York, McGraw-Hill Book Co., 1923.

It is evident that  $\Delta_{\text{KOH}}$  represents a driving force directed inward (*i.e.* causing KOH to move from *A* to *C*) and  $\Delta_{\text{HCl}}$  and  $\Delta_{\text{KCl}}$  represent driving forces directed outward. To make this evident in the graph (Fig. 3)  $\Delta_{\text{KOH}}$  and  $\Delta_{\text{HCl}}$  are plotted in opposite directions.

We see that  $\Delta_{\text{KCl}}$ , starting at zero, rises and then falls. The fall could be prevented if HCl were kept constant in *C* and if KOH in *A* were continuously renewed by a flow of solution which at the same time removed KCl from *A*.<sup>11</sup> This is evident from Fig. 4 which shows

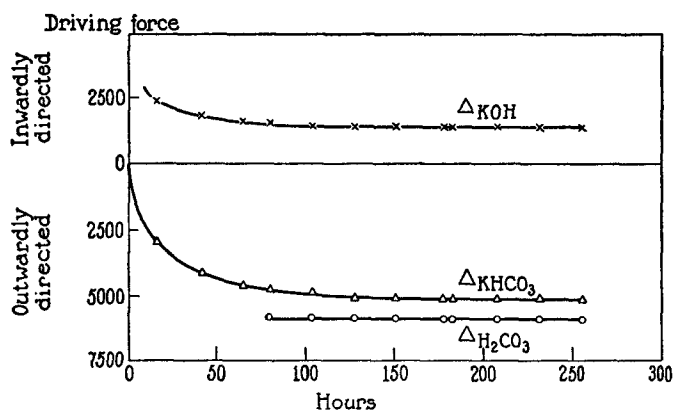


FIG. 4. Shows behavior in the model (Experiment No. 64; see Fig. 2) of the following:  $\Delta_{\text{KOH}}$ , the driving force which causes KOH to move inward (*i.e.*, from *A* to *C*);  $\Delta_{\text{H}_2\text{CO}_3}$ , the driving force which causes  $\text{H}_2\text{CO}_3$  to move outward (*i.e.*, from *C* to *A*); and  $\Delta_{\text{KHCO}_3}$ , the driving force which causes  $\text{KHCO}_3$  to move outward. After  $\Delta_{\text{KHCO}_3}$  becomes constant the volume of the artificial sap in *C* continues to increase (which seems to be analogous to what happens in growing cells).  $\Delta_{\text{KOH}}$  and  $\Delta_{\text{H}_2\text{CO}_3}$  would show more change if KOH and  $\text{CO}_2$  were not continuously renewed (this differs from the experiment shown in Fig. 3 in which KOH and HCl were not renewed). The ordinates for  $\Delta_{\text{KOH}}$  represent the value of

$$\Delta_{\text{KOH}} = 2.3 RT \log \frac{(\text{K}_o)(\text{OH}_o)}{(\text{K}_i)(\text{OH}_i)}$$

the ordinates for  $\Delta_{\text{H}_2\text{CO}_3}$  represent the value of

$$\Delta_{\text{H}_2\text{CO}_3} = 2.3 RT \log \frac{(\text{H}_i)(\text{HCO}_3)_i}{(\text{H}_o)(\text{HCO}_3)_o}$$

(the equation for  $\Delta_{\text{KHCO}_3}$  is similar). We put  $R = 1.988$  and  $T = 296^\circ$ .

<sup>11</sup> This case would differ from that illustrated in Fig. 1 in that HCl moving into *A* produces relatively little effect since the solution in *A* is continually renewed.

an experiment of this sort in which, however,  $\text{CO}_2$  was substituted<sup>12</sup> for  $\text{HCl}$  in  $C$ , its concentration being kept constant.

The calculation of  $\Delta$  made it necessary to take account of the activities of  $\text{K}^+$  and  $\text{Cl}^-$ . Although these are not known an attempt was made to approximate<sup>13</sup> them as follows: the activities of  $\text{K}^+$  and  $\text{Cl}^-$  were regarded in all cases as equivalent to those in a solution of  $\text{KCl}$  of the same ionic strength, on the assumption that in such a solution the activities of  $\text{K}^+$  and  $\text{Cl}^-$  are equal. The ionic strength in  $A$  is practically equivalent to the concentration of  $\text{K}^+$  and in  $C$  to the concentration of  $\text{Cl}^-$ .

The activity of  $\text{KCl}$  was taken from the tables of Harned.<sup>14</sup> The concentrations in  $A$  of  $\text{K}^+$  and  $\text{Cl}^-$  and the activities of  $\text{H}^+$  and  $\text{OH}^-$  were in some cases estimated by graphic interpolation.

In the experiment shown in Fig. 3 energy is furnished by the reactions  $\text{KOH} + \text{HG} = \text{KG} + \text{H}_2\text{O}$  and  $\text{KOH} + \text{HCl} = \text{KCl} + \text{H}_2\text{O}$ , and also by differences in osmotic pressure in  $A$  and  $C$ . In the experiment shown in Fig. 4 the chief sources of energy are the reaction  $\text{KG} + \text{H}_2\text{CO}_3 = \text{KHCO}_3 + \text{HG}$ , and likewise the continuous addition of  $\text{KG}$  and  $\text{CO}_2$  to the system. There are also differences in osmotic pressure.

#### DISCUSSION

During the first part of the experiment potassium tends to go in as  $\text{KOH}$  (since  $(\text{K}_o)(\text{OH}_o) > (\text{K}_i)(\text{OH}_i)$ ) and as  $\text{KG}$  (since  $(\text{K}_o)(\text{G}_o) > (\text{K}_i)(\text{G}_i)$ ), and at the same time to go out as  $\text{KCl}$  (since  $(\text{K}_i)(\text{Cl}_i) > (\text{K}_o)(\text{Cl}_o)$ ). These opposing tendencies do not balance until  $\text{K}_i$  becomes much greater than  $\text{K}_o$ : hence potassium accumulates. The reason is that much less  $\text{KCl}$  than  $\text{KG}$  moves through  $B$  in a given time because the partition coefficient of the latter is relatively high.

<sup>12</sup> The value of  $\Delta_{\text{H}_2\text{CO}_3}$  falls off somewhat because the concentration of  $\text{CO}_2$  increases somewhat in  $A$  because  $\text{CO}_2$  moves from  $C$  into  $A$  a little faster than it is removed by the flow of liquid in  $A$ .

It will be noticed that no points are given on the graph in Fig. 4 for  $\Delta_{\text{H}_2\text{CO}_3}$  in the early part of the experiment. This is because no determinations were made of  $\text{CO}_2$  in  $A$  during the early part of the experiment.

<sup>13</sup> A rough approximation is all that can be attempted as individual ion activities cannot be ascertained. See Guggenheim, E. A., *J. Phys. Chem.*, 1929, **33**, 842; MacInnes, D. A., in Cold Spring Harbor symposia on quantitative biology, 1933, **1**, 190.

<sup>14</sup> Harned, H. S., in Taylor, H. S., *A treatise on physical chemistry*, New York, D. Van Nostrand Company, Inc., 2nd edition, 1931, **1**, 769, 772.



This illustrates very clearly that in addition to the driving force and the diffusion constant we must also take account of the partition coefficients. Since  $KG$  and  $KCl$  are weak electrolytes in  $B$  it is the diffusion constants of the molecules with which we have to do. As  $KG$  has the larger molecule it probably has the smaller diffusion constant, yet moves more rapidly than  $KCl$  through  $B$  because its partition coefficient and in consequence its concentration gradient is greater. Similar principles apply to living cells (where potassium may unite with a constituent  $X$  of the protoplasm to form  $KX$ ).

It is evident that, other things being equal, accumulation will increase as the pH of the sap decreases, since the products<sup>15</sup>  $(K_+) (OH_-)$  and  $(K_+) (G_-)$  will be kept lower and consequently the driving force of  $KG$  and  $KOH$  will be greater. Hence anything that checks the production of acid by the cell may be expected to check accumulation and likewise growth. Conversely a rise of the external pH may be expected to have the opposite effect. This accords with experience, as discussed elsewhere.<sup>9</sup>

This model recalls the situation in *Valonia* (and many other living cells) where potassium accumulates chiefly as  $KCl$ . It has been suggested<sup>16,9</sup> that in this case potassium enters as  $KOH$  and forms  $KA$  in the sap (where  $A$  is an organic anion). In some plants it accumulates as  $KA$ <sup>9</sup> but when  $HCl$  exists in the external solution it will tend to enter and displace the weaker acid  $HA$  (if this be carbonic it can readily escape): hence potassium may accumulate to a greater or less extent as  $KCl$ .

#### SUMMARY

A model<sup>17</sup> is described which throws light on the mechanism of accumulation. In the model used an external aqueous phase  $A$  is separated

<sup>15</sup> What applies to one of these products applies also to the other since under the conditions of the experiment  $G^-$  stands in a constant relation to  $OH^-$  (Osterhout, W. J. V., *J. Gen. Physiol.*, 1932–33, **16**, 529. Similar considerations may apply to a considerable extent to living cells.

<sup>16</sup> Osterhout, W. J. V., *Proc. Soc. Exp. Biol. and Med.*, 1926–27, **24**, 234; *J. Gen. Physiol.*, 1930–31, **14**, 285; *Biol. Rev.*, 1931, **6**, 369.

<sup>17</sup> *Note Added to Proof*.—Our attention has been called to a paper by Teorell (Teorell, T., *Skand. Arch. Physiol.*, 1933, **66**, 225) describing temporary accumulation.

by a non-aqueous phase *B* (representing the protoplasm) from the artificial sap in *C*. *A* contains KOH and *C* contains HCl: they tend to mix by passing through the non-aqueous layer but much more KOH moves so that most of the KCl is formed in *C*, where the concentration of potassium becomes much greater than in *A*. This accumulation is only temporary for as the system approaches equilibrium the composition of *A* approaches identity with that of *C*, since all the substances present can pass through the non-aqueous layer. Such an approach to equilibrium may be compared to the death of the cell as the result of which accumulation disappears.

During the earlier stages of the experiment potassium tends to go in as KOH and at the same time to go out as KCl. These opposing tendencies do not balance until the concentration of potassium inside becomes much greater than outside (hence potassium accumulates). The reason is that KCl, although its driving force be great, moves very slowly in *B* because its partition coefficient is low and in consequence its concentration gradient in *B* is small. This illustrates the importance of partition coefficients for penetration in models and in living cells. It also indicates that accumulation depends on the fact that permeability is greater for the ingoing compound of the accumulating substance than for the outgoing compound.

Other things being equal, accumulation is increased by maintaining a low pH in *C*. Hence we may infer that anything which checks the production of acid in the living cell may be expected to check accumulation and growth.

This model recalls the situation in *Valonia* and in most living cells where potassium accumulates as KCl, perhaps because it enters as KOH and forms KA in the sap (where *A* is an organic anion). In some plants potassium accumulates as KA but when HCl exists in the external solution it will tend to enter and displace the weaker acid HA (if this be carbonic it can readily escape): hence potassium may accumulate to a greater or less extent as KCl.

Injury of the cell may produce a twofold effect, (1) increase of permeability, (2) lessened accumulation. The total amount of electrolyte taken up in a given time will be influenced by these factors and may be greater than normal in the injured cell or less, depending somewhat on the length of the interval of time chosen.