Influence of the Counter-ion on the Absorption Isotherm for Chloride at Low Temperature [1, 2]

George G. Laties, Ian R. MacDonald,[3] and Jack Dainty

Department of Plant Biochemistry, University of California, Los Angeles and Department of Biophysics, University of Edinburgh, Scotland

Ion uptake as a function of external concentration has generally been found to follow a hyperbolic relationship resembling the Freundlich or Langmuir adsorption isotherms in which uptake relative to concentration decreases as the external concentration rises (3). Deviations from this general rule can be caused by a variety of circumstances and range from a reported independence of concentration over a wide range for ion uptake by rye plants (10) to a direct proportionality to concentration for uptake of several ions by Chlorella (7). A unique anomaly in which chloride uptake by potato disks at 0°, resembled an exponential function of concentration was briefly noted by Laties (8) and has now been further investigated.

Materials and Methods

The procedure followed was essentially that previously outlined in greater detail (9). Potato (*Solanum tuberosum* L.) tubers used were of the Russet Burbank variety and one tuber generally yielded a sufficient number of disks (about 600) 1.0 mm thick and 9 mm in diameter for any one experiment. The disks were used either fresh, i.e. after 20 minutes washing in running distilled water, or after having been aged for 24 hours. Aging was carried out in a liter Erlenmeyer flask containing 150 ml of 0.1 mM $CaSO_4$ solution and 10 g of tissue, but nearly all the experiments reported here were repeated with disks aged in distilled water, and identical results were obtained. During aging the flasks were rotated on a New Brunswick Gyrotory shaker at room temperature.

A series of solutions covering a range from 1 mM to 100 mM was prepared by dilution from a stock solution. Before making up to volume a suitable amount of radioactive isotope was added to each solution, and the pH adjusted to 6.0 with the hydroxide of the cation under study. The solutions as used contained between 10 and 20 mμc/ml. Cl^{36} was supplied by Oak Ridge as 1.92 N HCl with a specific activity of 0.520 mc/g Cl. The amount of chloride added with the isotope was taken account of in preparing each solution. At the 1 mM concentration for instance, the Cl deriving from the radioactive solution was more than half the total Cl present. Cl^{36} uptake was followed from solutions of KCl, NaCl, and $CaCl_2$. The molar concentration of the $CaCl_2$ solutions was half that of the univalent solutions so that the amount of Cl supplied with each salt was the same.

Cl^{36} uptake was followed as described earlier. At the end of a 3 hour absorption period the disks were given a 10 minute wash with distilled water, thus ensuring the removal of all free space salt (9). The disks were then dried on planchets and their activity determined with a gas-flow Micromil window detector (Nuclear Chicago). Correction was made for self absorption which was approximately 20%.

Results

The uptake of Cl^{36} by aged tissue from KCl solutions containing from 5 to 100 mmole Cl/l was followed at 30° and proved to be a hyperbolic function of concentration reflecting the classical relationship of uptake to external concentration (fig 1). On the other hand with fresh tissue uptake at 30° more closely resembled an exponential followed by a linear function of concentration, and at 0° with both fresh and aged tissue the absorption isotherm was distinctly concave (fig 1) at the lower concentrations. Since the exponential effect was most prominently manifested at concentrations up to 40 mmole/liter most of the remaining experiments were carried out with a concentration range from 1 mM to 40 mM Cl.

The linear relationship between external concentration and Cl^{36} absorbed, over a part of the absorption isotherm (fig 1) is not to be ascribed to simple diffusive permeation of the free space resulting in passive equilibration with the external concentration. As stated earlier the Cl^{36} absorbed is that remaining after all the free space salt is removed. After a 60 minute absorption period in $CaCl_2$ at 0°, the Cl^{36} remaining in the tissue following only light blotting without any washing is shown in figure 2, and this linear relationship between external concentration and total uptake is contrasted in the same figure with the Cl^{36} remaining in the tissue following washing.

Figure 3 illustrates the absorption isotherm for

[1] Revised manuscript received July 22, 1963.

[2] Supported by grants from the Atomic Energy Commission and the Fulbright Travel Fund.

[3] On leave of absence from the Macaulay Institute for Soil Research, Aberdeen, Scotland.

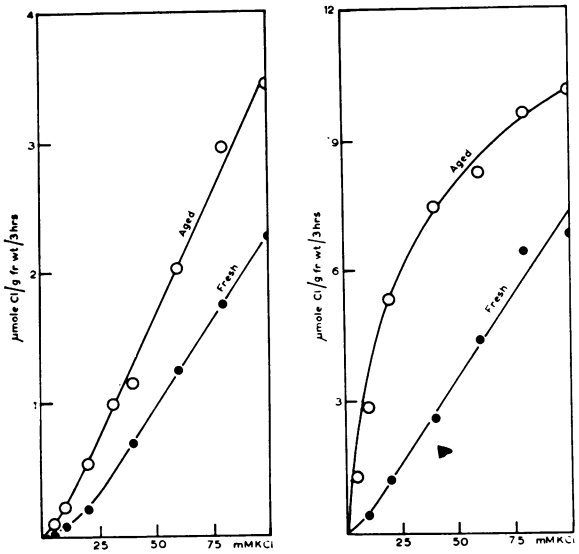


FIG. 1. Cl^{36} absorption from KCl by potato disks as a function of concentration. *Left.* Absorption by fresh and aged disks at 0°. *Right.* Absorption by fresh and aged disks at 30°.

Cl^{36} from different salt solutions at 0°. A concave uptake pattern is obtained with both fresh and aged tissue from KCl and NaCl but a significantly different pattern holds for Cl^{36} uptake from $CaCl_2$. With fresh tissue in $CaCl_2$ the isotherm is more nearly hyperbolic, and with aged tissue the classical hyperbolic relation to concentration is manifested. The difference in uptake pattern was maintained irrespective of whether the tissue was aged in distilled water or 0.1 mM $CaSO_4$. The difference in Cl absorption isotherms over an extended range of concentrations of KCl and $CaCl_2$ is shown in figure 4.

The exponential character of the absorption curve is not peculiar to a particular aspect of uptake, as for instance the initial rapid phase. This can be demonstrated by plotting the Cl^{36} values obtained after 30 minutes absorption and 24 hours absorption (fig 5). Apart from a difference in scale (indicating incidentally that the absorption rate is not constant over the 24 hour period) the absorption isotherms are identical and this is equally true of measurements taken at intermediate time intervals.

Figure 6 demonstrates that the concave pattern of Cl^{36} uptake from KCl^{36} by both fresh and aged tissue at 0° can be altered by adding K_2SO_4 to the KCl^{36} solutions such that the K^+ concentration is maintained at the same level (40 mM K^+) throughout the entire range of chloride concentrations. At the lower concentrations this additional K^+ can increase Cl^{36} uptake tenfold, e.g. 1 g fresh tissue absorbs 0.0012 μmole Cl^{36} from mM KCl in 3 hours but 0.012 μmole Cl^{36} in the presence of 40 mM K^+. Similarly aged tissue absorbs 0.012 μmole Cl^{36} and 0.07 μmole Cl^{36} in the absence and presence respectively of additional K^+ (40 mM).

Discussion

The rate of ion absorption has commonly been regarded as a hyperbolic function of external concentration and for the most part absorption experiments at normal temperatures have lent credence to this view. Uptake by aged disks at 30° (fig 1) typifies this relationship which is consistent with the hypothesis advanced by Epstein and Hagen (4), that ion uptake is mediated by a carrier substance in accordance with Michaelis-Menten enzyme kinetics.

Of more particular interest, however, is the absorption isotherm at 0°. Two features in particular call for explanation viz., the concave-upward nature of the KCl absorption isotherm (fig 1) and the effect of Ca^{++} on the Cl absorption isotherm (fig 3). These isotherms are analyzed below in terms of the physical forces acting on passive ion transport as set out by Dainty (1).

Cl^- Uptake from Increasing KCl Concentration. The passive flux of any ion is determined by both the chemical and the electrical potential gradients across the membrane. Using the simple Goldman theory, the net influx, J_j, of an ion j is given by

$$J_j = \frac{-P_j z_j\, FE/RT}{1 - \exp\,(z_j FE/RT)} \cdot \left[[C_j^o] - [C_j^i]\, \exp\,(z_j FE/RT)\right] \qquad \text{I}$$

where C_j^o and C_j^i are the external and internal concentrations of the ion j in mole × cm^{-3}, E is the electrical potential difference in volts between the inside and outside media, and z is the algebraic valency for the ion j. P_j is a permeability coefficient for the ion j as defined by equation I. F, R, and T have the usual significance.

The net influxes of K^+ and Cl^- from a KCl solution of known molarity will therefore be

$$J_K = \frac{-P_K\, FE/RT}{1 - \exp\,(FE/RT)} \cdot \left[[K_o] - [K_i]\, \exp\,(FE/RT)\right] \qquad \text{II}$$

and

$$J_{Cl} = \frac{+P_{Cl}\, FE/RT}{1 - \exp\,(-FE/RT)} \cdot \left[[Cl_o] - [Cl_i]\, \exp\,(-FE/RT)\right] \qquad \text{III}$$

The electrical potential, E, appearing in the above equations is a diffusion potential. It arises because of the tendency for different ions to move passively across the plasma membrane at unequal rates and it has the effect of adjusting the rates of ion movement so as to ensure that no net charge flows across the membrane. If the major passively moving ions are K^+, Cl^- and possibly HCO_3^-, the equation for E on the Goldman assumption of a uniform electrical field in the membrane assumed in the flux equations I, II, and III, is given by

$$E = \frac{RT}{F} \ln \frac{P_K\,[K_o] + P_{Cl}\,[Cl_i] + P_{HCO_3}\,[HCO_{3i}]}{P_K\,[K_i] + P_{Cl}\,[Cl_o]} \qquad \text{IV}$$

Figures 2 - 4

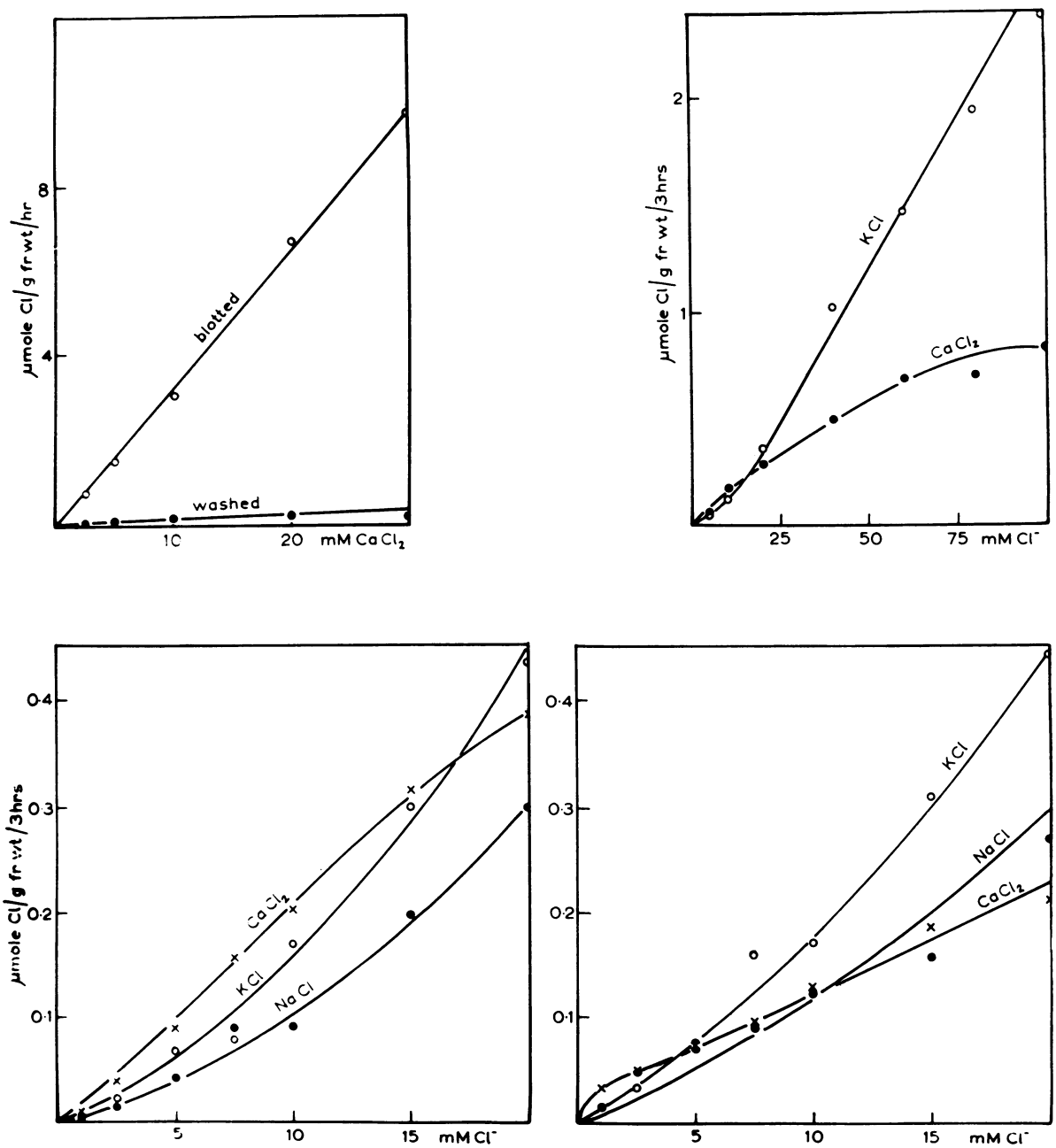
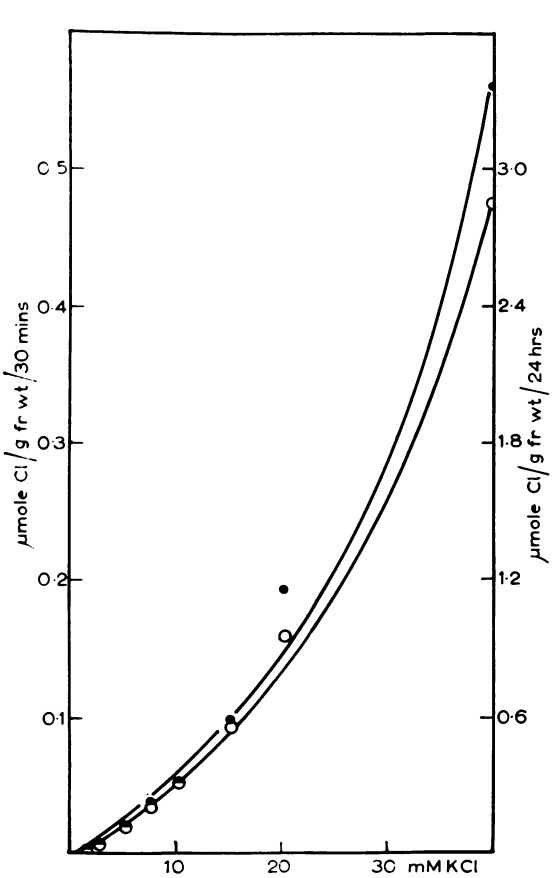


FIG. 2. (*upper left*). Cl^{36} absorption by aged potato disks at 0° as a function of concentration showing the effect of removing the free space Cl^{36} by thorough washing as compared with light blotting.

FIG. 3 (*lower left and right*). The effect of the counter-ion on the Cl^{36} absorption isotherm for potato disks at 0°. (*lower left*) Fresh disks, (*lower right*) Aged disks.

FIG. 4 (*upper right*). The effect of the counter-ion on the Cl^{36} absorption isotherm for fresh potato disks at 0° over an extended concentration range.



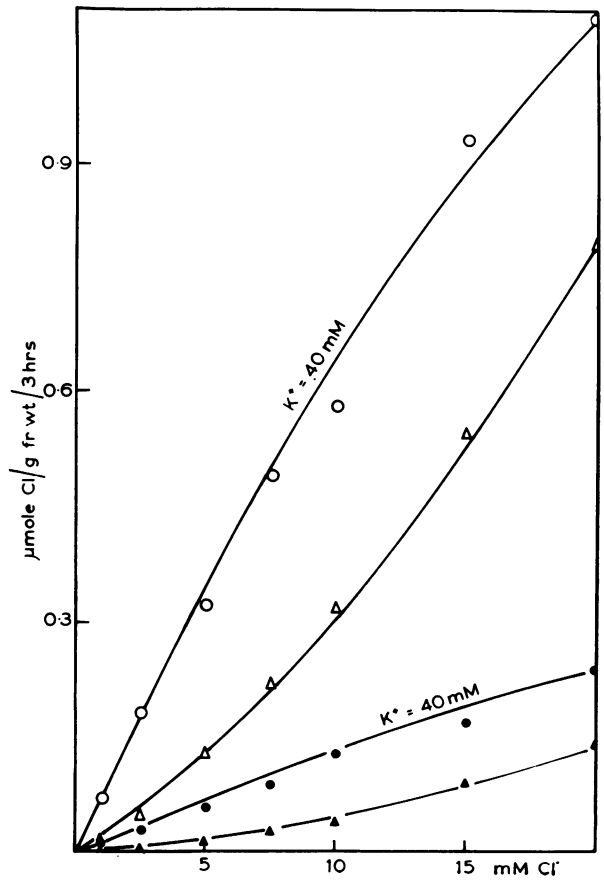


FIG. 5 (*left*). The effect of time on the Cl^{36} absorption isotherm for fresh disks at 0°. ○ absorption values at 30 mins. ● absorption values at 24 hours.

FIG. 6 (*right*). The effect of constant K^+ concentration on the Cl^{36} absorption isotherm for fresh and aged disks at 0°. Closed symbols: fresh tissue. Open symbols: aged tissue. Triangles: control uptake from KCl. Circles: uptake from KCl solutions to which K_2SO_4 was added to give 40 mM K^+ in each treatment. In each KCl solution the K^+ concentration was brought to 40 mM with K_2SO_4.

It is possible, although at present generally considered unlikely, that an actively transported ion, e.g. K^+, could contribute to the electrical potential if the pump was a so-called electrogenic one, i.e. if the moving ion-carrier complex was charged on one or other of its journeys to and fro across the membrane. This could be taken into account in IV by adding another term, which in this instance would be some function of $[K_o]$ and the cellular metabolism, to the numerator of the equation. The movement of other ions could also be allowed for in the equation. For example an outward movement of H^+ would necessitate introducing a term $P_H[H_i]$ in the denominator. In the following discussion we shall for the most part use equation IV as written, but these other possible amendments will be kept in mind.

Equation IV can be used to determine E at any given external concentration of KCl if the internal concentrations are known. Hence the net influxes of K^+ and Cl^- can be calculated from equations II and III if reasonable assumptions are made about the relative values of the permeability coefficients and the internal concentrations. $[K_i]$ for potato tuber tissue may be taken as 50 mM and it can be assumed that there is little or no Cl^- or HCO_3^- diffusing outwards from the protoplasm. On this basis and making the further assumption that $P_K = 2\ P_{Cl}$ the diffusion potential (E) across the plasma membrane can be calculated for a concentration range extending from 1 mM KCl to 40 mM KCl (table Ia) and for the same range of Cl^- concentrations but with K^+ constant at 40 mM (table Ib). Using these values for E the passive Cl^- fluxes (or strictly J_{Cl}/P_{Cl}) in the presence of these solutions can be calculated from equation III and are shown in shadow in figure 7a. The observed Cl^- uptake values by fresh tissue at 0° from these solutions (i.e. the values in fig 6) are shown in bold face in figure 7a. A substantial similarity can be observed between the experimental and calculated patterns of uptake. Equation III of course refers to net influx while the uptake values reported are for total influx but in this instance these can be

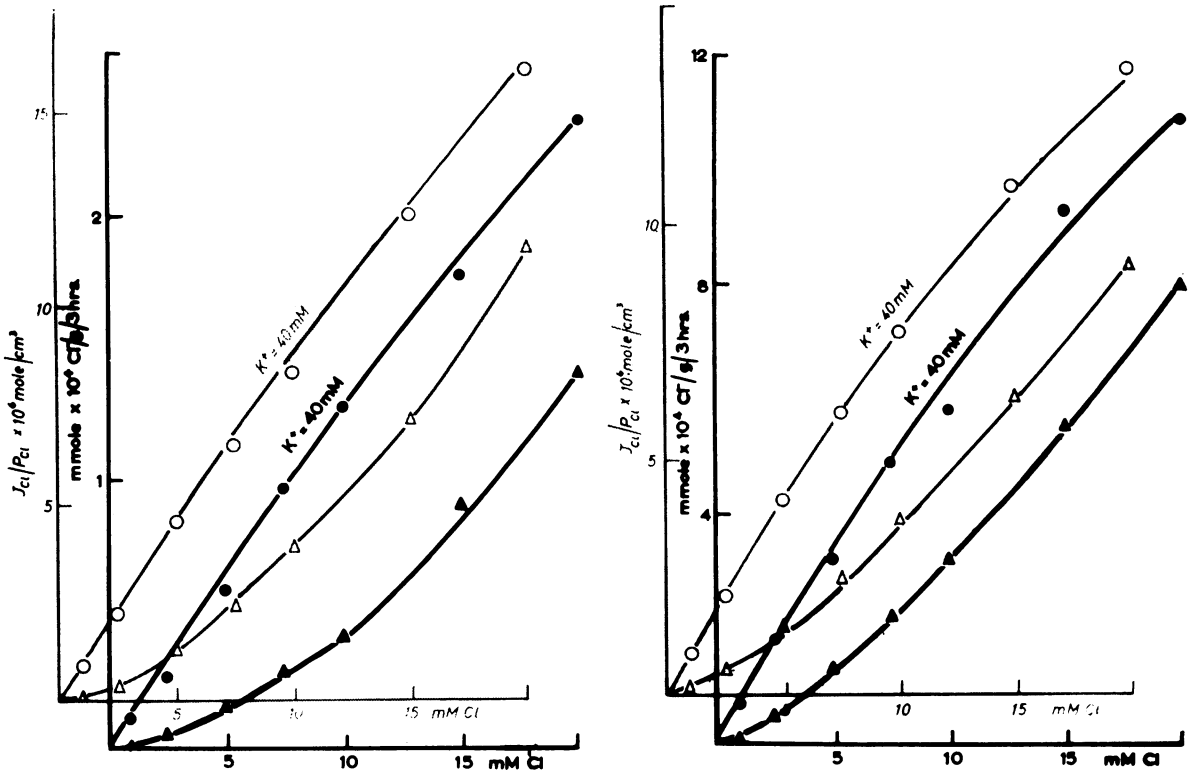


FIG. 7. A comparison between the observed uptake of Cl^- by fresh and aged tissue at 0° and the calculated passive influx of Cl^-. The experimental values are shown in bold face type. The calculated values are shown in shadow. (a) *left.* Observed values for fresh tissue. Theoretical values as calculated in tables Ia and Ib. (b) *right.* Observed values for aged tissue. Theoretical values as calculated in tables Ic and Id.

regarded as net influx values since we are assuming no internal chloride.

The Cl^- absorption isotherms for aged disks under the same conditions are shown in bold face in figure 7b (again the data from fig 6). The isotherms for aged tissue differ from fresh tissue in that the control isotherm is rather less concave and the constant K^+ isotherm slightly more convex. The calculated curves can be modified in order to approximate more closely to the experimental curves for aged tissue by assuming either an increased permeability to Cl^- or some small contribution to the diffusion potential from outwardly moving Cl^- or HCO_3^-. Calculated isotherms for influx (not net influx in this case) assuming $P_K = 0.5\ P_{Cl}$ and $[Cl_i] = 1$ mM are shown in shadow in figure 7b (the values are given in tables Ic and Id) and good agreement between observed and calculated patterns of uptake is obtained on this basis.

It is not unreasonable to assume an increase in P_{Cl} with aging and in any event any justifiable modifications to the calculated values would leave unaltered the basic similarity between the calculated and observed curves. The significance of this conformity is simply this: increasing the external KCl concentration decreases the absolute value of the potential difference across the plasma membrane (tables Ia and Ic) [although theoretically derived here this decrease in potential has been experimentally proven (5)] and this decrease in electrical potential with increasing chemical potential means that the real driving force on chloride increases more rapidly than the external concentration, hence the concave-upward curve. Since the observed curve follows the theoretical prediction we suggest that the uptake of chloride is rate-controlled, in these conditions, by passive movement across the plasma membrane.

Cl^- Uptake from Constant K^+ Solutions. The net influxes of Cl^- from solutions of increasing Cl^- concentration but constant K^+ (at 40 mM) can be calculated by the same method and the values shown in tables Ib and Id obtained. In this situation the potential difference across the membrane gets somewhat more negative and thus the inward driving force on Cl^- increases more slowly than the external concentration. But the change in electrical potential over the concentration range is small (< 10 mv in table Ib) and therefore the departure from linearity in the Cl^- influx curve is slight (fig 7a). The increased convexity of the calculated constant K^+ isotherm for aged tissue (fig 7b) is due to a somewhat larger increase in negative potential (table Id). Both the prediction of a slightly hyperbolic curve with constant K^+ and the observed correspondence

Table I

Theoretical Values of the Electrical Potential (E) and Cl^- Flux (J_{Cl}/P_{Cl}) in Varying Concentrations of KCl

Table Ia, calculation at varying external [KCl] of E from equation IV and J_{Cl}/P_{Cl} from equation III assuming $[K_i] = 50$ mM, $[Cl_i] = 0$ mM, $[HCO_{3i}] = 0$ mM, and $P_K = 2\ P_{Cl}$. Table Ib, as Ia but $[K_o] = 40$ mM throughout. Table Ic, as Ia but $[Cl_i] = 1$ mM and $P_K = 0.5\ P_{Cl}$. Table Id, as Ic but $[K_o] = 40$ mM throughout.

Table Ia

mM KCl conc	FE/RT	Emv	$J_{Cl}/P_{Cl} \times 10^6$
1.0	—3.92	—92.1	0.08
2.5	—3.02	—71.0	0.29
5.0	—2.35	—55.2	1.24
7.5	—1.97	—46.3	2.39
10.0	—1.71	—40.2	3.79
15.0	—1.34	—31.5	7.05
20.0	—1.10	—25.9	10.98
40.0	—0.56	—13.2	30.00

Table Ib

mM Cl^- conc	FE/RT	Emv	$J_{Cl}/P_{Cl} \times 10^6$
1.0	—0.23	— 5.48	0.90
2.5	—0.25	— 5.83	2.26
5.0	—0.27	— 6.39	4.52
7.5	—0.30	— 6.96	6.46
10.0	—0.32	— 7.50	8.43
15.0	—0.36	— 8.53	12.48
20.0	—0.41	— 9.52	16.10
40.0	—0.56	—13.20	30.00

Table Ic

mM KCl conc	FE/RT	Emv	$J_{Cl}/P_{Cl} \times 10^6$
1.0	—2.85	—67.0	0.17
2.5	—2.50	—58.8	0.54
5.0	—2.15	—50.5	1.42
7.5	—1.92	—45.1	2.47
10.0	—1.76	—41.4	3.65
15.0	—1.55	—36.4	6.27
20.0	—1.41	—33.1	9.11
40.0	—1.13	—26.6	21.56

Table Id

mM Cl^- conc	FE/RT	Emv	$J_{Cl}/P_{Cl} \times 10^6$
1.0	—0.21	— 5.03	0.89
2.5	—0.27	— 6.32	2.16
5.0	—0.36	— 8.39	4.17
7.5	—0.44	—10.27	5.99
10.0	—0.51	—12.01	7.68
15.0	—0.63	—14.71	10.82
20.0	—0.76	—17.95	13.32
40.0	—1.13	—26.56	21.56

to the theoretical line confirm the basic soundness of analyzing the data on electrochemical principles.

The Effect of Ca^{++} on the Cl^- Absorption Isotherm. In figure 3 the Cl^- uptake curve from $CaCl_2$ is shown to differ fundamentally from that from KCl or NaCl. Whereas with the univalent salts the Cl^- absorption isotherm is concave-upward, with Ca^{++} as the counter-ion the isotherm is convex-upward particularly with aged tissue. This change in shape can be accounted for if, with increasing external concentration of $CaCl_2$, the potential difference becomes more negative, i.e. the absolute value of the potential difference increases, so that, the overall driving force on Cl^- increases more slowly than the external concentration. Such a situation would be similar to the conditions obtaining under constant K^+ only the increase in negative potential would have to be more substantial to account for the distinctly convex-upward shape found experimentally. There is, however, good experimental evidence to support the view that with increasing concentrations of $CaCl_2$ (in contradistinction to KCl or NaCl) the potential becomes more negative (Higinbotham, Etherton, and Foster, personal communication). Moreover this situation can be reasoned to from a theoretical consideration of the factors involved.

Table II

Theoretical Values of the Electrical Potential (E) and Cl^- Flux (J_{Cl}/P_{Cl}) in Varying Concentrations of $CaCl_2$

Calculation at varying external [$CaCl_2$] of E from equation IV and J_{Cl}/P_{Cl} from equation V assuming $[K_i] = 50$ mM; $[Cl_i] = 1$ mM; $P_K = 0.1\ P_{Cl}$ and $2\ P_{Ca} = 0.01\ P_{Cl}$.

$CaCl_2$ mM	FE/RT	Emv	$J_{Cl}/P_{Cl} \times 10^6$
1.0	—1.78	—41.83	0.36
2.5	—1.99	—46.84	0.79
5.0	—2.26	—53.08	1.32
7.5	—2.46	—57.76	1.73
10.0	—2.62	—61.50	2.06
15.0	—2.87	—67.33	2.60
20.0	—3.04	—71.44	3.05
40.0	—3.48	—81.78	4.42

With $CaCl_2$ outside and K^+, Cl^-, and HCO_3^- inside the membrane the equation for the electrical potential is derived from the following quadratic: if $\chi = \exp(FE/RT)$ then

$$\chi^2 + \chi\left[1 - \frac{P_{Cl}[Cl_i] + P_{HCO_3}[HCO_{3i}]}{P_K[K_i] + P_{Cl}[Cl_o]}\right] = \frac{4P_{Ca}[Ca_o] + P_{Cl}[Cl_i] + P_{HCO_3}[HCO_{3i}]}{P_K[K_i] + P_{Cl}[Cl_o]} \qquad V$$

Ignoring the $[HCO_3^-]$ term (which may actually be more important than the $[Cl_i^-]$ term but doesn't affect the argument at this level), it can be shown that the condition for getting a more negative potential (i.e. a decreasing χ) as $CaCl_2$ increases is that

$$\frac{2P_{Ca}}{P_{Cl}} < \frac{P_{Cl}[Cl_i]}{P_K[K_i]} \qquad VI$$

This condition can be achieved either by having $P_{Ca} <<< P_{Cl}$ or by the Ca^{++} so affecting the membrane as to make P_K for the outgoing K^+ very much reduced, or by a combination of both. This latter assumption is not altogether unreasonable as there

is evidence in muscle (6) that the outward P_K can be very small.

E has been calculated for a range of external $CaCl_2$ concentrations assuming $[Cl_i]$ = 1 mM, $[K_i]$ = 50 mM, P_K = 0.1 P_{Cl} and 2 P_{Ca} = 0.01 P_{Cl}. On calculating the influx of Cl^- from these values (table II) the passive influx of Cl^- is found to be as shown in figure 8. It can be concluded therefore that the apparently aberrant character of the Cl^- absorption isotherm from $CaCl_2$ is in agreement with what might reasonably be predicted and once more suggests passive influx.

The higher Cl^- uptake from $CaCl_2$ compared with KCl at low concentrations is less easily explained. Since very little Ca^{++} should enter the cell it cannot be thought of as accompanying Ca^{++} influx. It may be that Ca^{++} facilitates the exchange of Cl^- with anions, possibly HCO_3^-, in the protoplasm. In this connection Elgabaly (2) showed that uptake of Cl^- by roots of 4 species investigated was invariably higher from $CaCl_2$ (1 meq Cl^-/liter) than from NaCl.

Cl^- Fluxes and Permeability Coefficients. Taking the average cell diameter to be 100 μ the Cl^- uptake values can be expressed as fluxes. Flux values ranging from 0.02 × 10^{-14} mole × cm^{-2} × sec^{-1} in 1 mM KCl to 7.0 × 10^{-14} in 40 mM KCl with fresh tissue at 0°, and corresponding values of 0.2 and 28.0 for aged tissue are then obtained. Taking the J_{Cl}/P_{Cl} values derived from equation III on the assumption that P_K = 2 P_{Cl} (table Ia) a value for P_{Cl} ≏ 0.24 × 10^{-8} cm × sec^{-1} is obtained for fresh tissue and about 10 times this value for aged tissue.

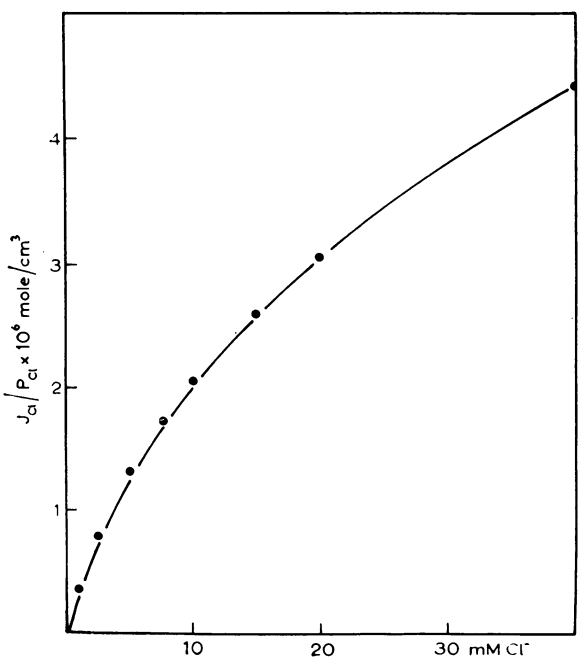


FIG. 8. Calculated passive influx of Cl^- from an increasing concentration of $CaCl_2$ as derived in table II.

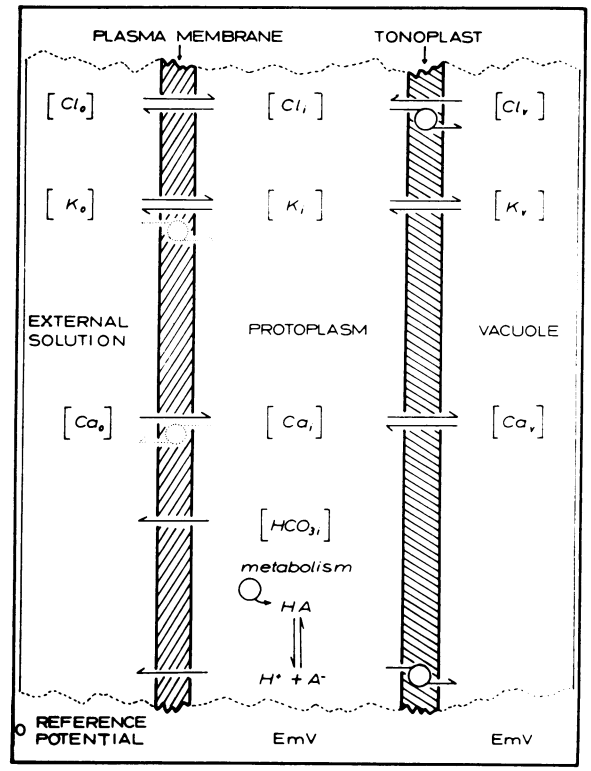


FIG. 9. Postulated ionic situation across cell membranes. Passive movements contributing to the diffusion potential are indicated by arrows. —○→ indicates pumps which do not contribute to the potential.

The Role of Metabolism in Cl^- Uptake. By way of interpreting the absorptive process at the cellular level the different ionic movements across the cell membranes may be envisaged as shown in figure 9. Passive movements contributing to the diffusion potential are indicated by arrows. The electrical potential difference between the cell and the external solution can be considered to be located in its entirety across the plasma membrane since evidence by Walker (11) and Etherton and Higinbotham (5) indicates that the potential across the tonoplast is small.

A model situation may be envisaged in which the rate-limiting step is across the plasma membrane. With fresh tissue at 0° about 0.2 μmole Cl^-/g is absorbed from 20 mM KCl over 3 hours. According to our model this represents passive movement into the protoplasm as determined by the electrochemical potential gradient. Once in the protoplasm, however, the possibility remains that the Cl^- is then actively transported into the vacuole. In this event transfer into the vacuole will determine the extent of observable uptake despite the fact that movement across the plasma membrane is rate-determining. It is significant that following a period of uptake there is no leakage from the cell on transference to distilled water. If Cl^- is transported into the vacuole,

transport is in proportion to movement across the plasma membrane and it would seem that the chloride pumps have not reached saturation at the concentrations used although approaching it in the case of aged tissue at 30°. In this view while the absorption kinetics are determined by diffusive processes the inhibitory effect of DNP on Cl^- uptake at 0° (9) can be accounted for because active transport into the vacuole will be DNP sensitive, and therefore in the presence of DNP movement across the tonoplast becomes rate-limiting.

With fresh tissue at 30° Cl^- uptake is of the order of 1.25 μmole/g from 20 mM KCl over 3 hours. Under these conditions the absorption isotherm remains concave again suggesting that passive ion movement across the plasma membrane is still rate-limiting. The increased uptake over that at 0° probably results from an increase in P_{Cl} at the higher temperature. Aged tissue at 0° absorbs about 3 times as much Cl^- as fresh tissue under the same conditions (fig 1). Again this increase would be due, on our model, to an increased permeability to Cl^-. An increased P_{Cl} is entirely reasonable and is in fact suggested by the experimental evidence since a closer approximation to the absorption isotherm is obtained by assuming an increase in P_{Cl} (fig 7b). In addition there is evidence (unpublished data) that after 48 hours aging, the Cl^- absorption isotherm at 0° in the presence of univalent counter-ions is convex. This would result from a further increase in P_{Cl} between 24 and 48 hours. Similarly an increase in P_{Cl} would account for the increasing convexity of the $CaCl_2$ isotherm between fresh and 24 hour aged tissue.

At 30° uptake by aged tissue is considerably higher (5.5 μmole/g/3 hours) and the absorption isotherm follows the classical hyperbolic pattern. In this situation the plasma membrane can no longer be rate-limiting and rate control may be located at the tonoplast with the pumps exerting a determining influence.

An additional factor which could contribute to the hyperbolic character of the absorption isotherm at 30° is the increased production of organic acid in aged tissue at higher temperatures. If organic acid synthesis and dissociation should result in an increased concentration of H^+ in the cytoplasm (A^- ions having moved into the vacuole), a term $P_H[H_i]$ must be included in the denominator of the Goldman equation (IV) for the membrane potential and this, the outward diffusion of H^+ would tend to minimize the change in electrical potential due to increasing KCl concentration especially if $[H_i]$ increased with KCl concentration. Some evidence indicating an increase in organic acid production with increasing KCl concentration is available. This situation in which the membrane potential remains relatively unaltered with increasing salt concentration could provide an alternative explanation of the lack of concavity of the 30° aged tissue absorption isotherm if penetration of the plasma membrane were the rate-limiting step rather than the carrier-mediated transport across the tonoplast.

Conclusion

We have put forward in this paper a hypothesis (or model), discussed above and summarized in figure 9, which largely explains on the basis of well-known physico-chemical principles backed by experimental evidence, our findings on the uptake of Cl^- by potato slices. We think that this model gives a substantially true picture of the situation although refinements have to be and can be made. For example the inter-connection between passive movement and metabolism, both as affecting pumping activity and the production of ions such as H^+ and organic acid anions the movement of which will influence the membrane potential and hence the fluxes of all other ions, must be elaborated. We think it is a good hypothesis in the sense that it makes a number of verifiable predictions. Some, for instance the effect of constant K^+ on the Cl^- absorption isotherm, have been confirmed in this paper. Others such as the magnitude of the relative permeability coefficients and the effect of aging, temperature, Ca, etc., on them can be checked by further experimentation and the hypothesis confirmed or rejected.

Summary

The influence of the counter-ion on the Cl^- absorption isotherm for potato (*Solanum tuberosum* L.) slices has been studied with the use of Cl^{36}. Whereas at 30° the Cl^- absorption isotherm in KCl for slices aged 24 hours is hyperbolic, with freshly cut slices at this temperature and with both fresh and aged slices at 0°, Cl^{36} absorption resembles an exponential function of external concentration. In the presence of $CaCl_2$, however, the absorption isotherm is hyperbolic under all conditions. Maintaining K^+ at constant concentration (40 mM) while varying Cl^- from 1 mM to 20 mM has the effect of altering the Cl^- absorption isotherm from a concave-upwards to a convex-upwards pattern.

These results are shown to be consistent with a straightforward electrochemical model (or theory) of the transport of ions across plant cell membranes. This model is discussed in detail and particular attention is given to the assumptions inherent in the model and to the predictions arising from it. Two predictions, viz., the effect of Ca^{++} and the effect of constant K^+ on the Cl^- absorption isotherm are substantiated in this paper.

Literature Cited

1. Dainty, J. 1962. Ion transport and electrical potentials in plant cells. Ann. Rev. Plant Physiol. 13: 379–402.
2. Elgabaly, M. M. 1962. On the mechanism of anion uptake by plant roots. 2. Effect of the cation-exchange capacity of plant roots on Cl^- uptake. Soil Science 93: 350–52.
3. Epstein, E. 1956. Mineral nutrition of plants: Mechanisms of uptake and transport. Ann. Rev. Plant Physiol. 7: 1–24.
4. Epstein, E. and C. E. Hagen. 1952. A kinetic study of the absorption of alkali cations of barley roots. Plant Physiol. 27: 457–74.

5. ETHERTON, B. AND N. HIGINBOTHAM. 1960. Transmembrane potential measurements of cells of higher plants as related to salt uptake. Science 131: 409–10.
6. HODGKIN, A. L. AND P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of simple muscle fibres. J. Physiol. 148: 127–60.
7. KNAUSS, H. J. AND J. W. PORTER. 1954. The absorption of inorganic ions by *Chlorella pyrenoidosa*. Plant Physiol. 29: 229–34.
8. LATIES, G. G. 1959. The generation of latent-ion-transport capacity. Proc. Natl. Acad. Sci. U.S. 45: 163–72.
9. MACDONALD, I. R. AND G. G. LATIES. 1963. Kinetic studies of anion absorption by potato slices at 0°. Plant Physiol. 38: 38–44.
10. OLSEN, C. 1950. The significance of concentration for the rate of ion absorption by higher plants in water culture. Physiol. Plant. 3: 152–64.
11. WALKER, N. A. 1955. Microelectrode experiments on Nitella. Australian J. Biol. Sci. 8: 476–89.

Relationship between the Physical Nature of Mitochondrial Membranes and Chilling Sensitivity in Plants [1, 2]

James M. Lyons,[3] T. A. Wheaton,[4] and Harlan K. Pratt

Department of Vegetable Crops, University of California, Davis

Chilling injury in plants—the metabolic injury that occurs at low (0–10°) but not freezing temperatures—has been treated in descriptive studies (3, 9, 10, 11, 28), but little evidence has been developed on the basic nature of the injury. Lieberman et al. (14) examined the oxidative and phosphorylative activities (at 25°) of mitochondria derived from sweet potato roots which had been stored at 7.5 and 15°. Both oxidative and phosphorylative activities of the mitochondria from roots stored at the chilling temperature (7.5°) began to decline by the fifth week of storage, and the particles were completely inactive by the tenth week, but the activity of particles from nonchilled roots (15°) showed little change during this period. In contrast, Minamikawa et al. (19) reported that, while oxidative activity was less in mitochondria from chilled than from nonchilled sweet potato roots, there was no difference in the P/O ratios. It is difficult to reconcile these conflicting reports; however, in each case the highest P/O ratio of the control was only 1.0 to 1.5 (using α-ketoglutarate as substrate), indicating severe damage to the mitochondria during isolation procedures. Shichi and Uritani (25) worked with intact tissue and observed that sweet potato disks would not respond to dinitrophenol after the roots had been stored for 8 to 10 days at 0°. Furthermore, Lewis (11) found that chilling tomato fruit tissues for a short time at 0° decreased their subsequent incorporation of P^{32} at 20°, demonstrating an effect of chilling on phosphorylation.

Richardson and Tappel (23) recently demonstrated a difference in flexibility of mitochondrial membranes from warm-blooded animals as compared to those from cold-blooded animals. Using light-scattering technique to follow swelling, they showed that mitochondria of fish liver had the ability to swell at a rapid rate over a wide range of temperature down to 0°, but mitochondria from rat liver did not have this ability at the lower temperatures. They also showed that a correlation existed between membrane flexibility, as evidenced by mitochondrial swelling, and fatty acid composition of the mitochondrial membrane: the more flexible membranes from cold-blooded animals had a higher proportion of unsaturation in their fatty acids than did the less flexible membranes of the warm-blooded animals. The authors suggested that the increased flexibility made it possible for the mitochondria to carry on metabolic functions at lower temperatures.

Since metabolic studies with chilling-sensitive plant tissue have indicated that mitochondrial activity is affected by chilling temperatures, several plant species were examined to see whether a relationship between chilling sensitivity and membrane flexibility

[1] Revised manuscript received July 22, 1963.
[2] Based on a portion of a dissertation submitted by James M. Lyons to the Graduate Division, University of California, Davis, in partial satisfaction of the requirements for the Ph.D. degree, 1962. This investigation was supported in part by a research grant (EF-156) from the Division of Environmental Engineering and Food Protection, United States Public Health Service.
[3] Present address: Department of Vegetable Crops, University of California, Riverside.
[4] Present address: Citrus Experiment Station, University of Florida, Lake Alfred.