

Short Communication**Anion Absorption by Plants**A UNARY INTERPRETATION OF "DUAL MECHANISMS"¹

Received for publication March 18, 1971

DONALD F. GERSON and RONALD J. POOLE

Department of Biology, McGill University, Montreal, Quebec, Canada

"Dual mechanisms" of ion uptake by higher plants are commonly observed while studying the kinetics of ion uptake over large concentration ranges (5, 7). They are characterized by a biphasic plot of initial uptake rate against concentration: the first phase (mechanism 1) approaching a limiting rate as the concentration increases between 0.01 mM and 1.0 mM; the second (mechanism 2), also apparently rate-limited, appearing between 1 mM and 100 mM. The experimental data generally approximate to the form of curves A, B and C in Figure 1, although minor fluctuations in slope may exist in the high concentration range.

Despite much study, the significance of the dual mechanisms is still in doubt, especially since the usual method of measuring influx kinetics may not give a good estimate of cell membrane influx as distinct from tonoplast influx (1, 2). What is not in dispute, however, is that influx at the cell membrane continues to increase with increasing concentration beyond the apparent saturation point of mechanism 1. At present, theoretical explanations for this phenomenon typically involve two distinct modes of ion movement across the cell membrane: either transport on two or more separate carriers or sites of differing affinities, or transport on a single carrier plus parallel free diffusion. (A recent explanation [17] in terms of a single mechanism is achieved only by abandoning the concept of carriers altogether.)

We propose that dual mechanisms could be attributed to a single uncharged carrier species at the cell membrane. The ion-carrier complex would then have the same charge as the carried ion, resulting in kinetics which are significantly different from the usual carrier model (an uncharged ion-carrier complex) but which are quite similar to the kinetics of dual mechanisms. Models involving charged ion-carrier complexes have been discussed by others (9, 11), but from points of view which are not applicable to this problem.

Dainty (3), among others, has pointed out that any explanation of the movements of ions across membranes must consider both the chemical and electrical gradients which are involved. If the ionic charge is not neutralized by the carrier, it is immediately obvious that an explanation of the kinetics of the carrier in terms of the Michaelis-Menten equation alone is inadequate: it has no term to account for the electrical gradient which is always present, and which is usually dependent on the external concentration of the ion. We may expect that the simultaneous alteration of membrane potential (E) and external concentration (C_0) would enhance or inhibit the rate of ion movement by the carrier. Since the membrane

potential is usually negative, and becomes more positive as a nearly linear function of the log of the external salt concentration, the influx of cations will be inhibited and the influx of anions will be stimulated as the external salt concentration increases. It is the application of this model to anions which is relevant to dual mechanisms. Placing emphasis on the behavior of an anion carrier is appropriate, since there is evidence that cation uptake, particularly at higher concentrations, is limited by the rate of uptake of anions (13, 14).

Derivation of the standard Goldman ionic flux equation,

$$\text{Flux}_{\text{in}} = -P \frac{zFE/RT}{1 - \exp(zFE/RT)} C_0 \quad (1)$$

for diffusion across a membrane depends on two assumptions (3): (a) that the electric field is constant (*i.e.*, that the voltage between the surface of the membrane and any point inside it is a linear function of their separation); also, (b) that the phase boundary potentials at the surfaces of the membrane either cancel or are zero (*i.e.*, that the phase boundary electric fields have no net effect on the flux of an ion). In addition to these, there is a necessary postulate that the concentration of the ion in the surface of the membrane is directly proportional to the concentration of the ion in the immediately adjacent solution.

However, for ions which move through the membrane exclusively on a carrier, such a proportionality does not exist. In this case, the concentration of the ion in the surface of the membrane, C' , has a hyperbolic relation to the solution concentration:

$$C' = \frac{\alpha C_0}{K + C_0} \quad (2)$$

K , in equation 2, has essentially the same meaning as the K_m of the Michaelis-Menten equation; it is the concentration at half-maximal saturation. The proportionality constant is α .

With some additional specifications, it becomes possible to re-derive the standard ionic flux equation for those situations in which ionic flux is carrier-mediated and is sensitive to the membrane potential. They are:

1. The carrier is in equilibrium with the adjacent solution at both surfaces of the membrane.
 2. K , *i.e.*, the affinity of the carrier for the ion in question, is identical at both surfaces of the membrane.
 3. The ionic species in question crosses the membrane only on the carrier.
 4. The movement of the ion-carrier complex is the rate limiting step of transport.
 5. The carrier does not neutralize the charge on the ion.
- Postulates 1, 3, and 4 are commonly assumed when applying

¹ This work was supported by a grant from the National Research Council of Canada.

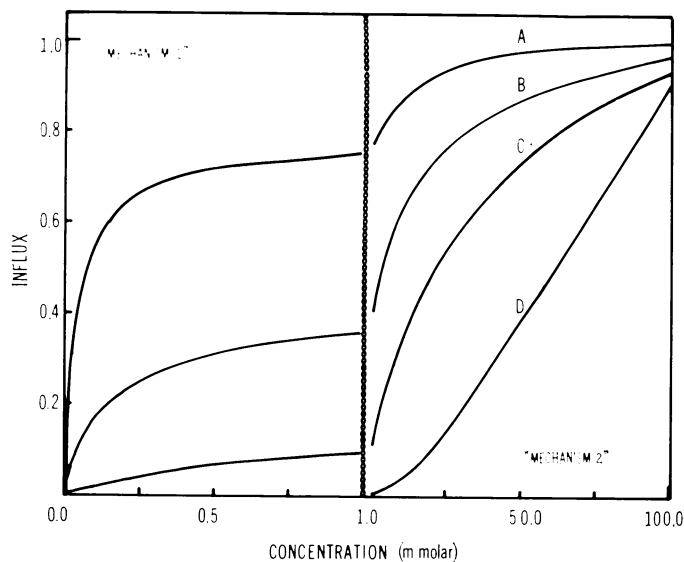


FIG. 1. Influx, in arbitrary units, as calculated from equation 3 over two concentration ranges. The parameters for the curves are (C_0 and K in mM, E in mV): A: $E = 5 \log C_0 - 60$, $K = 0.02$, $\alpha = 3.1$; B: $E = 20 \log C_0 - 60$, $K = 0.02$, $\alpha = 1.4$; C: $E = 58 \log C_0 - 60$, $K = 0.02$, $\alpha = 0.38$; D: $E = 58 \log C_0 - 60$, $K = 1.0$ M, $\alpha = 3.8$. The relationship of E to C_0 is arbitrarily set at values comparable to those in the literature. The value of K for curves A, B, and C is also close to those experimentally observed for "Mechanism 1." The value for K in curve D is arbitrarily set at a high value.

Michaelis-Menten kinetics. If postulate 2 does not apply, (e.g., during active transport) the influx kinetics will not necessarily be affected (see below). Thus the most significant assumption made here is postulate 5: that the carrier does not neutralize the charge on the ion.

Nearly all experimental data displaying dual mechanisms are from measurements of the initial uptake rate of isotopes, so the most relevant result derived from the above postulates is the influx equation:

$$\text{Flux}_{\text{in}} = -\alpha \frac{zFE/RT}{1 - \exp(zFE/RT)} \frac{C_0}{K + C_0} \quad (3)$$

This equation may be looked upon as the Michaelis-Menten equation with a variable V_{max} which depends upon the membrane potential. Thus, according to this equation, a high affinity carrier may become saturated at external salt concentrations approaching 1 mM, and yet its rate of operation may continue to increase above 1 mM, due to a progressive change in the membrane potential.

Figure 1 shows some curves generated by equation 3 for anion influx. The membrane potentials required to generate these curves were calculated from empirical equations based on measurements in the literature (see legend to Fig. 1). The origin of the membrane potential, whether from ion diffusion, electrogenic pumps, or indeed the operation of the ion-carrier presently under consideration, is irrelevant to its effect on the carrier. The effect of the membrane potential, as shown in Figure 1, is to produce influx kinetics resembling the dual mechanisms.

Data like curves A, B, and C in Figure 1 have been obtained for Cl^- uptake in beet root (12) and barley (4), and for Br^- uptake in barley (6). Data following any of these curves would usually be considered to be the result of two enzyme-like carriers with widely differing affinities (5, 7). In contrast, experi-

mental data following near-linear or concave curves such as curve D in Figure 1 have been considered to indicate passive diffusion across the cell membrane (7). Using solutions of NaCl or KCl in the concentration range of "Mechanism 2," Torii and Laties (18) noticed that the Cl^- uptake curves of corn root tips were linear or concave, respectively. They concluded that the influx was due to diffusion. Laties *et al.* (8) also invoked diffusion to explain similar concave curves for Cl^- uptake from potato disks at low temperatures.

However, an explanation of "Mechanism 2" in terms of diffusion is possible in these materials only under certain experimental conditions. Over the same Cl^- concentration range, the concave curve is replaced by a hyperbolic one when potato disks are aged, or when Cl^- uptake is measured in CaCl_2 solutions. In addition, Macklon and MacDonald (10) were able to measure the membrane potentials of potato and concluded that Cl^- uptake from CaCl_2 solutions was inexplicable in terms of free diffusion. These results point to the existence of a chloride carrier, and we feel that since a Cl^- carrier is implicated when the external solution contains Ca^{++} , then the carrier is probably still operative when the external solution contains K^+ . Thus, the concave curve obtained for Cl^- uptake from KCl solutions is not necessarily due to diffusion. The different uptake kinetics in the two situations may be caused by an increase in the affinity of the carrier for Cl^- in the presence of divalent as opposed to monovalent cations, in addition to the observed leveling of the E versus $\log C_0$ relation (10).

In summary, some possible effects of the membrane potential on an electrically-charged carrier have been investigated, and it is shown that such effects could account for a wide variety of observations on ion transport in plant cells, including both biphasic and concave curves for influx versus concentration. Moreover, although we have formulated a model in terms of a simple "passive" carrier, it may also be applicable to active transport, depending on the mechanism of energy coupling. For instance, the influx of an electrically-charged ion-carrier complex may be dependent on the membrane potential, even though, at the inside of the membrane, metabolic energy may be expended to dissociate the complex.

This model is, however, clearly inadequate to explain all aspects of the dual mechanisms. In particular, it is specifically concerned with anions. While total cation uptake is undoubtedly affected by anion uptake (e.g., 13, 14, 16) the nature of this interaction is not clear. Thus the model does not throw any light on features of cation uptake such as the difference in specificity sometimes observed (5, 7) between the two concentration ranges. Other outstanding questions include the significance of the multiple inflections frequently reported (e.g., 4, 5, 16, 18) for mechanism 2, and the effect of transport across the tonoplast (2).

In spite of these limitations, we feel that the value of this model is that it draws attention to the possibility that carrier transport, as well as passive diffusion, may be influenced by the membrane potential. To test this model, there is a need for more studies in which membrane potential can be varied independently of the concentration of the ion under consideration. In one such study on beet disks (15), it appears that a change in potential induced by bicarbonate does not affect the transport of Cl^- ; while in another on pigeon red blood cells (19), the co-transport of glycine with Na^+ does seem to depend on the potential. Much more data of this kind must be obtained in order to adequately test our model, and generally to assess the role of the membrane potential in the movements of ions across cell membranes.

Acknowledgement—We thank Dr. D. R. Firth and especially Dr. J. Dainty for their valuable comments and suggestions.

LITERATURE CITED

1. CRAM, W. J. 1969. Short term influx as a measure of influx across the plasma-lemma. *Plant Physiol.* 44: 1013-1015.
2. CRAM, W. J. AND G. G. LATIES. The use of short term and quasi-steady influx in estimating plasmalemma and tonoplast influx in barley root cells at various external and internal Cl concentrations. *Aust. J. Biol. Sci.* In press.
3. DAINTY, J. 1962. Ion transport and electrical potentials in plant cells. *Annu. Rev. Plant Physiol.* 13: 379-402.
4. ELZAM, O. E., D. W. RAINS, AND E. EPSTEIN. 1964. Ion transport kinetics in plant tissue: complexity of the chloride absorption isotherm. *Biochem. Biophys. Res. Commun.* 15: 273-276.
5. EPSTEIN, E. 1966. Dual pattern of ion absorption by plant cells and by plants. *Nature* 212: 1324-1327.
6. HOOYMANS, J. J. M. 1964. The role of calcium in the absorption of anions and cations by excised barley roots. *Acta Bot. Neer.* 13: 507-540.
7. LATIES, G. G. 1969. Dual mechanisms of salt uptake in relation to compartmentation and long-distance transport. *Annu. Rev. Plant Physiol.* 20: 89-116.
8. LATIES, G. G., I. R. MACDONALD, AND J. DAINTY. 1964. Influence of the counter-ion on the absorption isotherm for chloride at low temperature. *Plant Physiol.* 39: 254-262.
9. LAUGER, P. AND G. STARK. 1970. Kinetics of carrier-mediated ion transport across lipid bilayer membranes. *Biochim. Biophys. Acta* 211: 458-466.
10. MACKLON, A. E. S. AND I. R. MACDONALD. 1966. The role of transmembrane electrical potential in determining the absorption isotherm for chloride in potato. *J. Exp. Bot.* 17: 703-717.
11. MARKIN, V. S., L. I. KRISHTALIK, Y. A. LIBERMAN, AND V. P. TOPALY. 1967. Mechanism of conductivity of artificial phospholipid membranes in presence of ion carriers. *Biofizika* 14: 256-264.
12. OSMOND, C. B. AND G. G. LATIES. 1968. Interpretation of the dual isotherm for ion absorption in beet tissue. *Plant Physiol.* 43: 747-756.
13. PITMAN, M. G. 1964. The effect of divalent cations on the uptake of salt by beet root tissue. *J. Exp. Bot.* 15: 444-456.
14. PITMAN, M. G., A. C. COURTICE, AND B. LEE. 1968. Comparison of potassium and sodium uptake by barley roots at high and low salt status. *Aust. J. Biol. Sci.* 21: 871-881.
15. POOLE, R. J. 1966. The influence of the intracellular potential on potassium uptake by beetroot tissue. *J. Gen. Physiol.* 49: 551-563.
16. RAINS, D. W. AND EPSTEIN. 1967. Sodium absorption by barley roots: its mediation by mechanism 2 of alkali cation transport. *Plant Physiol.* 42: 319-323.
17. THELLIER, M. 1970. An electrokinetic interpretation of the functioning of biological systems and its application to the study of mineral salts absorption. *Ann. Bot.* 34: 983-1009.
18. TORII, K. AND G. G. LATIES. 1966. Dual mechanisms of ion uptake in relation to vacuolation in corn roots. *Plant. Physiol.* 41: 863-870.
19. VIDAVER, G. A. 1964. Some tests of the hypothesis that the sodium-ion gradient furnishes the energy for glycine active transport by pigeon red cells. *Biochemistry* 3: 803-807.