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THE CARRIER THEORY OF ION TRANSPORT: A RECONSIDERATION

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Although the chemical nature of the ion carriers is still obscure, the carrier theory of ion transport remains a most useful working hypothesis in this field, and no evidence has yet been presented which conflicts with its basic concepts. Nevertheless, as will be discussed elsewhere (TROMP, 1962; BANGE and TROMP, in preparation), in recent years experimental facts, especially in the domain of ion competition, have become available in this laboratory which are not readily explained by the carrier theory in its conventional formulation. The time therefore seems ripe to reconsider some of its assumptions.

It seems hardly necessary to repeat here that in the conventional concept the ions are assumed to be bound reversibly to more or less specific ion carriers. The ion-carrier complex thus formed is assumed to pass through a barrier not permeable for the free ions and to be subsequently chemically transformed in an irreversible reaction in which the ions are released again. Schematically:

$$M + R \underset{k_1}{\overset{k_2}{\rightleftharpoons}} MR \xrightarrow{k_3} M + R'$$

in which M = ion, R = carrier, MR = ion-carrier complex and R′ = chemically transformed carrier. During steady-state absorption the total amount of carrier ($= [R_t]$) involved in the transport is assumed to be constant by synthesis of new carrier.

The usual kinetic treatment of this reaction scheme leads to the following formula for the rate of absorption ($= v$):

$$v = \frac{[M] \cdot V_{max}}{[M] + K_m} \tag{1}$$

in which

$$V_{max} = k_3 \cdot [R_t] \tag{1a}$$

and

$$K_m = (k_2 + k_3)/k_1 \tag{1b}$$

An important feature is that the amount of ion transported is assumed to be directly proportional to the amount of ion-carrier complex present:

$$v = k_3 \cdot [MR]$$

This would be obvious if the disintegration of the complex MR to M and R' were a spontaneous monomolecular reaction. However,

we fail to see why the complex MR should be stable at one side of the barrier and labile at the other. On the other hand, if in the transformation of R to R' another substance is assumed to be involved, e.g. an enzyme E, the implication is that either the amount of enzyme is large as compared to the amount of substrate ($= [MR]$) or that the affinity between substrate and enzyme is so low as compared to the amount of substrate available that the reaction proceeds in the region of porportionality. It will be clear that both assumptions are completely arbitrary.

Thus, to state the general case it would be better not to make any assumptions as to the amount of enzyme available. Unfortunately, this leads to very complicated equations. The alternative is to start from the usual situation in which the amount of enzyme is small as compared to the amount of substrate or, in our case, that in the reaction scheme:

$$M + R \underset{k_1}{\overset{k_2}{\leftrightharpoons}} MR + E \underset{k_4}{\overset{k_5}{\leftrightharpoons}} MRE \xrightarrow{k_6} M + R' + E$$

the amount of enzyme ($= [E_t]$) combining with the substrate MR according to the Michaelis-Menten theory is much smaller than the total amount of carrier involved ($= [R_t]$) which again will be supposed to be constant.

For the rate of this reaction ($= v$) we have:

$$v = k_6 \cdot [MRE] = \frac{k_6 \cdot [E_t] \cdot [MR]}{K_2 + [MR]}$$

in which

$$K_2 = (k_5 + k_6)/k_4$$

Assuming the equilibration between M and R to be rapid in comparison with the removal of MR from the outside of the barrier and no difference in the concentration of MR worth mentioning to exist between the outside and the inside of the barrier we have:

$$[MR] = \frac{k_1}{k_2} \cdot [M] \cdot [R]$$

$$= \frac{k_1 \cdot [M] \cdot ([R_t] - [MRE])}{k_1 \cdot [M] + k_2}$$

$$= \frac{k_1 \cdot [M] \cdot [R_t]}{k_1 \cdot [M] + k_2}$$

Substitution in the expression for v leads to the formula:

$$v = \frac{[M] \cdot V_{max}}{[M] + H} \qquad (2)$$

in which

$$V_{max} = \frac{k_3 \cdot [R_t] \cdot [E_t]}{[R_t] + K_2} \tag{2a}$$

and

$$H = \frac{K_1 \cdot K_2}{[R_t] + K_2} \tag{2b}$$

($K_1 = k_2/k_1$).

So it appears that our assumptions lead to the same type of relation between rate of absorption and concentration as the conventional kinetic treatment. However, in the author's view an essential advantage has been obtained. In the conventional theory the quantity K_m is a real constant, its value being determined solely by the velocity constants k_1, k_2 and k_3. On the other hand, the new expression for the half-value H contains in addition to a number of velocity constants the quantity $[R_t]$ which may be supposed to vary widely in different plant material. So the half-value is no longer a constant but varies with the amount of carrier available.

The experimental variability of the value of K_m was mentioned in the work of EPSTEIN and HAGEN (1952). In this laboratory BECKING (1956) and VAN DEN HONERT and HOOIJMANS (1961) made independent determinations of the half-value of NH_4-absorption in full-grown maize plants (single cross hybrid D × 9). The plant material used for these experiments had not been grown under constant conditions of light, temperature, etc. nor was their nutritional history very well defined. Becking found a half-value of about 0.013 milliequivalents/litre and Van den Honert and Hooijmans of 0.11 me/l. There is no evidence whatsoever that in maize more than one absorption mechanism is involved in NH_4-uptake. Therefore, the effect cannot be attributed to a difference in the ratio in which the different mechanisms participate in the absorption process.

Another example may be drawn from work done in this laboratory by LYCKLAMA on NH_4-absorption by *Lolium perenne*, the details of which will be reported elsewhere. In this case for greenhouse material the half-value appeared to vary with the season, a larger value being associated with a lower rate of NH_4-uptake during the winter. In our conception the phenomenon is readily explained by a lowering of the amount of available carrier under conditions of low photosynthesis. The same explanation may apply to differences reported by HELDER (1952) in the relation between external phosphate concentration and rate of phosphate absorption by maize plants under different experimental conditions of light, glucose supply and salt status.

Another and still more important advantage of the new formulation becomes evident when we turn our attention to phenomena of ion competition. The usual concept is that competition between ions occurs in the very first phase of the absorption process, i.e. during the binding of the ions to the carrier substances. Actually no other

type of competition is feasible within the framework of the usual formulation.

In the light of the extension of the theory advanced in these pages another type of competition may be visualized. Different ions may be transported each by their own carrier. If these carriers are chemically-related substances they may make use of the same enzyme for the chemical transformation which leads to the release of the bound ions. Where the amount of enzyme has been assumed to be small in comparison with the amount of carrier substances such a situation must of necessity lead to a competition between the different ion-carrier complexes for the enzyme in question. Schematically:

$$\begin{array}{ccccccccccc} & & \overset{k_2}{} & & & \overset{k_5}{} & & & & & \\ M_1 & + & R_1 \underset{k_1}{\leftrightharpoons} & M_1R_1 & + & E \leftrightharpoons & M_1R_1E & \rightarrow & M_1 & + R'_1 & + E \\ & & \overset{k_{22}}{} & & & \uparrow k_4 \quad \downarrow k_{55} & & k_6 & & & | \\ M_{11} & + & R_{11} \underset{k_{11}}{\leftrightharpoons} & M_{11}R_{11} & + & E \leftrightharpoons & M_{11}R_{11}E & \rightarrow & M_{11} & + R'_{11} & + E \\ & & & & & k_{44} & & k_{66} & & & \end{array}$$

This is the place to point out the fundamental resemblance which this scheme bears to the sequence of reactions leading to ion absorption proposed by Hokin and Hokin (1959). Put briefly, lipophilic phosphatidic acid would bind the ions at the outside of the membrane and transport them to the inside where the carrier would be decomposed by the enzyme phosphatidic acid phosphatase, releasing the ions into the internal water phase. Resynthesis of the carrier would occur from the decomposition product diglycerol and ATP with the aid of the enzyme diglycerolkinase. Specificity of the phosphatidic acid carrier would arise from a loose binding to some protein. This scheme was conceived on the basis of biochemical arguments. The identity with our scheme, the need for which originated from purely kinetic evidence, is striking.

This kinetic evidence was furnished by elaborate experiments on the competition of monovalent cations in wheat and barley performed in this laboratory by Tromp, a detailed account of which will be given elsewhere (TROMP, 1962; BANGE AND TROMP, in preparation). One of the most salient observations was that the inhibition of one ion by another was never complete, i.e. part of the absorption of the inhibited ion was insensitive to the inhibitor. The effect could not be ascribed to the participation of more than one mechanism in the absorption of the ions in question. This phenomenon suggested that the amount of competing material was restricted, and led us to assume that not the ions themselves but their carriers were involved in the competition reaction.

The kinetics of this type of competition may be derived as follows. In the steady state we have:

$$k_4 \cdot [M_1R_1] \cdot [E] = (k_5 + k_6) \cdot [M_1R_1E]$$

and $$k_{44} \cdot [M_{11}R_{11}][E] = (k_{55} + k_{66}) \cdot [M_{11}R_{11}E]$$

from which

$$[M_1R_1E] = \frac{k_4 \cdot (k_{55} + k_{66})}{k_{44} \cdot (k_5 + k_6)} \cdot \frac{[M_1R_1]}{[M_{11}R_{11}]} \cdot [M_{11}R_{11}E]$$

By substitution in the first steady-state expression and transformation we get:

$$[M_1R_1E] = \frac{k_4 \cdot [M_1R_1] \cdot [E_t]}{k_4 \cdot [M_1R_1] + k_{44} \cdot \frac{k_5 + k_6}{k_{55} + k_{66}} \cdot [M_{11}R_{11}] + k_5 + k_6}$$

For $[M_1R_1]$ and $[M_{11}R_{11}]$ we have (cf. page 140):

$$[M_1R_1] = \frac{k_1 \cdot [M_1] \cdot [R_{1t}]}{k_1 \cdot [M_1] + k_2}$$

and

$$[M_{11}R_{11}] = \frac{k_{11} \cdot [M_{11}] \cdot [R_{11t}]}{k_{11} \cdot [M_{11}] + k_{22}}$$

Substitution and reduction leads to the following expression for $[M_1R_1E]$:

$$[M_1R_1E] = \frac{[M_1] \cdot [R_{1t}] \cdot [E_t]}{[M_1] \cdot [R_{1t}] + K_2 \cdot ([M_1] + K_1) \cdot \left(\frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)}$$

in which

$$K_1 = k_2/k_1 \qquad K_2 = (k_5 + k_6)/k_4 \qquad K_{11} = k_{22}/k_{11}$$

and $$K_{22} = (k_{55} + k_{66})/k_{44}$$

Finally:

$$v_1^{11} = k_6 \cdot [M_1R_1E] = \frac{[M_1] \cdot V_{max1}^{11}}{[M_1] + H_1^{11}} \tag{3}$$

in which

$$V_{max1}^{11} = \frac{k_6 \cdot [R_{1t}] \cdot [E_t]}{[R_{1t}] + K_2 \cdot \left(\frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)} \tag{3a}$$

and

$$H_1^{11} = \frac{K_1 \cdot K_2 \cdot \left(\frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)}{[R_{1t}] + K_2 \cdot \left(\frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)} \tag{3b}$$

(v_1^{11} = rate of uptake of M_1 in the presence of a constant amount of M_{11} and conformable meanings of V_{max1}^{11} and H_1^{11}).

An analogous expression applies to the rate of absorption of M_{11} in the presence of a constant amount of M_1 ($= v_{11}^1$).

It appears from these formulae that the type of relation between rate of absorption and concentration of M_1 is not affected by the addition of a constant amount of M_{11} to the solution. Only the value of the quantities V_{max1} and H_1 has changed. These changes are characterized as follows:

1. The presence of M_{11} always entails a decrease in the value of V_{max1} but not even after the addition of excess M_{11} does V^{11}_{max1} fall below a certain limiting value.
2. The value of H_1 is always increased by the addition of M_{11} but again not even in the presence of excess M_{11} does H_1^{11} exceed a certain limit.
3. The magnitude of the effects obtained is determined by the value of R_{1t} relative to K_2 and of R_{11t} relative to K_{22}.

For the sake of comparison we have listed in Table I the characteristics of the three types of competition conceivable within the framework of the conventional theory (EPSTEIN and HAGEN, 1952). It should be noted, though, that in the author's view the experimental evidence for the existence of an uncompetitive and non-competitive type of inhibition in ion absorption is not abundant.

The comparison brings out the unequivocal differences between the features of "carrier competition" as conceived here on the one hand and "ion competition" on the other. It is especially the limited nature of the former that cannot be reconciled with the mechanism of ion competition unless more than one absorption site were involved. Whether or not this is the case will appear from whether or not the nature of the relation between the concentration and rate of absorption in the absence of the inhibitor is simple or complex. In a special case, another test has been used with success to elucidate this point (TROMP, 1962).

One feature is common to carrier competition and to the competitive

TABLE I

Properties of the competitive, uncompetitive and non-competitive type of inhibition (cf. EPSTEIN and HAGEN, 1952).

Type of competition	Rate of absorption ($= v_1$) of inhibited ion	Maximal rate of absorption in the presence of inhibitor	Half-value in the presence of inhibitor
Competitive . . .	$v_1 = \frac{c_1 . V_{max}}{c_1 + K_1 + c_2 . K_1/K_2}$	Unaffected	Unlimited increase with c_2
Uncompetitive . .	$v_1 = \frac{c_1 . V_{max} . K_2/(c_2+K_2)}{c_1 + K_1 . K_2/(c_2+K_2)}$	Unlimited decrease with c_2	Unlimited decrease with c_2
Non-competitive .	$v_1 = \frac{c_1 . V_{max} . K_2/(c_2+K_2)}{c_1 + K_1}$	Unlimited decrease with c_2	Unaffected

Denotations: c_1 and c_2 = concentration of inhibited ion and inhibitor respectively
K_1 and K_2 = Michaelis-Menten constant of inhibited ion and inhibitor respectively
V_{max} = maximal rate of absorption in the absence of inhibitor

type of ion competition. When the rate of absorption of ion A ($= M_1$) is studied at rising concentrations in the presence of a constant concentration of ion B ($= M_{11}$), the decrease in the rate of absorption for ion B can be shown to be proportional to the increase for ion A. The reverse applies in the same sense.

In the case of competitive inhibition this is clear from a simple transformation of the competition formula:

$$v_B = \frac{c_B \cdot V_{maxB}}{c_B + K_B + c_A \cdot K_B/K_A}$$

to:

$$v_B = \frac{c_B \cdot V_{maxB}}{c_B + K_B} \cdot \left(1 - \frac{c_A}{c_A + K_A + c_B \cdot K_A/K_B}\right)$$

(v = rate of absorption, V_{max} = maximal rate of absorption, c = concentration, K = Michaelis-Menten constant). So the ratio:

$$\frac{\text{decrease absorption rate ion B}}{\text{increase absorption rate ion A}} = \frac{c_B}{c_B + K_B} \cdot \frac{V_{maxB}}{V_{maxA}} = \text{constant.}$$

For carrier competition a more circumstantial derivation leads to the formula:

$$\frac{\text{decrease absorption rate ion } M_{11}}{\text{increase absorption rate ion } M_1} = \frac{[M_{11}] \cdot [R_{11t}]}{[M_{11}] \cdot [R_{11t}] + ([M_{11}] + K_{11}) \cdot K_{22}} = \text{constant.}$$

The procedure adopted for the study of competition phenomena in this laboratory includes the following series of experiments. The relation between the rate of absorption and concentration is determined for ion M_1 in the absence and presence of a constant concentration of ion M_{11}, and the same is done for ion M_{11} in the absence and presence of a constant concentration of ion M_1. The same experiments allow the determination of the rate of absorption of ions M_1 and M_{11} from a constant concentration but in the presence of increasing concentrations of ions M_{11} and M_1 respectively. The reason for this procedure will be discussed elsewhere (TROMP, 1962).

Theory can be shown to require that if carrier competition is involved, the H- and V_{max}-values within such a cycle should be linked by the following formulae:

$$\frac{H_1}{H_{11}} \cdot \frac{(c_{11} + H_{11})}{(c_1 + H_1)} = \frac{H_1^{11}}{H_{11}^1} \cdot \frac{(c_{11} + H_{11}^1)}{(c_1 + H_1^{11})} \tag{4}$$

and

$$\frac{V_{max1}}{V_{max1}^{11}} \cdot H_1^{11} \cdot H_{11} + \frac{V_{max11}}{V_{max11}^1} \cdot H_1 \cdot H_{11}^1 = \frac{V_{max1} \cdot V_{max11}}{V_{max1}^{11} \cdot V_{max11}^1} \cdot H_1^{11} \cdot H_{11} \cdot \frac{c_{11} + H_{11}^1}{c_{11} + H_{11}} + H_1 \cdot H_{11} \tag{5}$$

in which

H_1 = half-value for ion M_1 in the absence of ion M_{11},
H_1^{11} = half-value for ion M_1 in the presence of a constant concentration ($=c_{11}$) of ion M_{11}

and analogous meanings for the other quantities.

In this way the supposition of carrier competition may be put to a quantitative test for any set of experimental data. Such an analysis will shortly be given for a series of experiments on the competition of monovalent cations (BANGE and TROMP, in preparation).

As stated above, our point of departure was that the amount of enzyme E is small as compared to the amount of carrier R. When this condition is not satisfied and $[E_t]$ is of the same order of magnitude as $[R_{1t}]$ and $[R_{11t}]$, the intensity of the competition of the carriers for the enzyme E will diminish. As a result, the phenomena may be expected to be qualitatively of the same nature, although less pronounced than in the case considered here. As stated above (cf. page 142) no carrier competition is feasible in the extreme case that the amount of enzyme is very large relative to the amount of carrier substances.

A necessary condition for carrier competition is that the carriers are chemically-related substances. Therefore experimental evidence pointing to this type of competition may contribute indirectly to the final chemical identification of these still mysterious substances.

SUMMARY

The conventional kinetic treatment of the carrier theory of ion transport is subjected to a critical consideration. Elimination of an arbitrary assumption appears to lead not only to a better understanding of some phenomena but also to open the way to the formulation of a new type of competition. This type of competition is called "carrier competition" as opposed to "ion competition", and is based on the assumption that the separate ion-carrier complexes may compete for the same enzymatic breakdown reaction. The implication of such a concept is that the carriers are chemically-related substances.

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