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Cotransport of Organic Solutes and Sodium Ions in the Small Intestine: a General Model. Amino Acid Transport†

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ABSTRACT: A general, allosteric, noncompulsory model has been developed that may in principle be used to explain in a unified manner all organic solute and Na^+ cotransport systems in the small intestine, as well as some other carrier or enzyme systems, *e.g.,* brush border sucrase. This general model, simplified to ignore V (capacity) effects, is used to explain and fit new experimental results on cycloleucine (1-aminocyclopentanecarboxylic acid) transport in guinea pig jejunum and ileum, as well as published results (Curran *et al.,* 1967, *J. Gen. Physiol. 50,* 1261) on L-alanine transport in rabbit ileum. Equations have been developed to calculate the pertinent dissociation constants for both amino acids and Na^+. Quantitative comparisons between the results obtained indicate an essentially identical, noncompulsory mechanism for amino acid and Na^+ cotransport in the small intestine of guinea pig and rabbit.

Sodium ion is a specific activator of the transport of a variety of substances across several cell membranes (for review, see Schultz and Curran, 1970). A striking example is that of the small intestine, where at least ten different classes of solute transport mechanisms have been shown to require Na^+. The list includes the transport mechanism for sugars, amino acids (which in turn may be subdivided into four separate classes), bile salts, uracil, sulfate, phosphate, calcium, ascorbic acid, biotin (Berger *et al.,* 1972), and riboflavine (Rivier, 1973). There is also much evidence that the energy inherent in the existence of a Na^+ gradient across the brush border membrane can be used to produce an asymmetric (uphill) transport of organic solutes by virtue of the coupling between the two transports (for Na^+ and solute, respectively) at the level of a common carrier. The term, cotransport, specifically refers to this coupling.

Pros and cons of the so-called sodium gradient hypothesis have been amply discussed (Schultz and Curran, 1970; Heinz, 1972; Kimmich, 1973). In this series of papers, however, attention will be focused, not on the question of the source of energy for organic solute uphill transport, but rather on the mechanism of cotransport that implies a reciprocal activating relationship between Na^+ and organic solutes. As our theoretical basis we will use a general, allosteric,[1] noncompulsory model in which the carrier is defined as bifunctional, *i.e.,* as having two separate but functionally related binding sites, one for organic solute and one for Na^+. As first proposed by Alvarado (1966, 1967), the relationship between the two sites will be considered similar to that of an allosteric enzyme with one substrate and one allosteric (modifier) site. In fact, the model is suitable for application to some enzymes, *e.g.,* the case of Na^+ activation of brush-border sucrase (Mahmood and Alvarado, to be published). However, in this particular series dealing with transport carriers, and because of the nature of the cotransport concept, substrate and modifier are interchangeable, the model being entirely symmetrical. After defining the general model, we will show how it fits our experimental results on cycloleucine transport in guinea pig intestine, as well as those found by others using L-alanine and rabbit ileum (Curran *et al.,* 1967). Subsequent papers will be devoted to other cotransport systems, *e.g.,* that for sugars and Na^+.

Our ultimate goal in this project is: (1) to ascertain whether the same general model can accurately describe the cotransport of various organic solutes and Na^+ in the small intestine of mammals (and perhaps other vertebrates); and (2) if the above is found to be the case, to ascertain whether the Na-binding sites involved in amino acid and sugar transport activation are the same; *i.e.,* whether Na^+ acts a *general* rather than as an *individual* activator of solute transport in the intestine (Alvarado, 1972). An affirmative answer to this question would open

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[1] We use the term *allosteric* in its original sense, namely, "*indirect* interactions between *distinct* specific binding sites" (Monod *et al.,* 1965).

the way for an understanding of the brush-border membrane in terms of a mosaic or cooperative model.

Materials and Methods

Transport Measurements. Locally raised guinea pigs (300–500 g) were used. The entire small intestine from the ligament of Treitz was flushed out with saline and collected in phosphate buffer (Krebs and Henseleit, 1932) after eversion. The proximal one-third was considered as the jejunum and the distal one-third as the ileum. Transport was determined by the *tissue accumulation method* (Crane and Mandelstam, 1960) as described previously (Robinson and Alvarado, 1971). Briefly, 0.5-cm rings of either everted jejunum or ileum were incubated in buffers of the composition indicated below. The substrate was a mixture of tracer [^{14}C]cycloleucine (1-aminocyclopentanecarboxylic acid; Cal-Atomic) and cold cycloleucine (Sigma) to give the appropriate concentrations. When necessary, mannitol was added to maintain isoosmolarity. All incubations were for 5 min at 37°, in 5 ml of buffer saturated with 100% O_2, in 25-ml erlenmeyer flasks shaken in a New Brunswick gyrotory water bath shaker at about 150 rpm. After incubation, the tissues were gently blotted on filter paper and the radioactivity taken up was determined after tissue digestion with KOH (Robinson and Alvarado, 1971). Water content was 80% of the total net weight of tissue.

Buffer Solutions. All buffers used were of a composition similar to that of the Krebs and Henseleit (1932) phosphate (pH 7.2) with 300 mosmol/l. and 1.2 mequiv/l. each of Ca^{2+} and Mg^{2+}. In the experiments in Figure 2, the sodium phosphate was substituted with 10 mM Tris-chloride (pH 7.2). All the Na^+ and K^+ ions were replaced by Li^+ (chloride) and then either Na^+ or K^+ was added as indicated, maintaining the total Li^+ plus Na^+ (or Li^+ plus K^+) concentration constant at 140 mequiv/l. In the rest of the experiments the phosphate was in the K^+ form, at a fixed 8.5 mequiv of K^+/l., and Li^+ was used again as the Na^+-substituting cation.

Calculation of Results. Results are expressed as velocities (v), *i.e.*, as μmoles of substrate accumulated per ml of tissue water per min. The results were corrected for the extracellular space, measured with [^{3}H]mannitol in separate pieces of tissue. This space amounted to about 8% of the external substrate concentration, in 5 min. The corrected values are considered as a reliable index of the *initial velocity* of transport and equivalent to the *unidirectional influx* (J_{mc}). This relation has been verified in separate experiments where uptake by rings was compared with uptake by sacs in which the substrate in the medium does not have access to the serosal surface and there are no tissue cut ends. Similar results are found by both procedures (Alvarado and Mahmood, to be published). Use of rings, however, affords a rapid method which also permits better randomization of the tissues. As shown in Figure 3, for instance, one single experiment using one animal may yield quite satisfactory kinetic results.

Calculation of the Kinetic Parameters for Cycloleucine Transport. A standard procedure is used which allows for the determination of two saturation curves per experiment, using one animal. The randomized rings are rinsed for about 40 sec in Na^+-free buffer and then gently blotted on filter paper before incubation. Two series of eight flasks each are used. Since each flask has four intestinal rings which are separated into two groups for digestion and counting, each experiment provides eight points with four determinations per point.

For the calculation of the constants shown in Table I, the averaged raw transport data at each Na^+ concentration plus the initial and final (S) in the medium were fed to a Hewlett-Packard 9100B calculator programmed to correct for the extracellular space and time, and to print the results of (S)/v *vs.* (S) plus the corresponding straight line, calculated by the method of least squares.

TABLE I: Effect of [Na^+] on the Kinetic Parameters for Cycloleucine Transport in Guinea Pig Intestine.

n^a	[Na^+] (mM)	V_{max}	K_T
		Jejunum	
7	0	2.42	27.02 (16.8–45.0)[b]
2	23.9	2.26	12.25 (7.8–17.6)
2	55.35	2.74	10.33 (8.1–15.0)
2	96.2	2.12	7.27 (6.7–7.8)
2	101.33	2.69	6.00 (5.7–6.3)
3	129.5	2.00	5.55 (4.2–9.0)
7	133.9	2.50	6.89 (5.0–12.4)
		Mean = 2.39	
		Illeum	
6	0	4.00	29.40 (15.5–54.8)
3	28.9	4.29	15.46 (11.5–21.1)
5	57.9	4.50	11.43 (10.3–15.0)
5	101.3	3.88	6.29 (4.7–7.2)
5	133.9	3.79	7.33 (5.5–12.1)
		Mean = 4.09	

[a] Number of experiments. [b] Range.

We believe the variability encountered in our experiments is largely due to individual differences attributable to the lack of homogeneity of the guinea pig colony available to us.

Definition of the General Model

Organic Solute Transport Follows Michaelis–Menten Kinetics. It is a well-documented fact that many transport systems in the small intestine, particularly those for amino acids and sugars, conform quite closely to the equation

$$v = V_{max}(S)/[K_T + (S)] \quad (1)$$

where V_{max} is as usual and K_T is the equivalent of the Michaelis constant. Both parameters, however, may be complex functions of several possible factors that include temperature, pH, and the ionic composition of the medium. Among these factors, however, only the medium [Na^+] has been shown to have an overriding importance, and it is the only one that will be considered in the present study. K^+ has been shown to act in the hamster as an antagonist of Na^+ (Bosackova and Crane, 1965; Alvarado, 1965), although other workers found K^+ to be inert using the rabbit (Curran *et al.*, 1967). We will deal with K^+ in a separate publication and for the moment will consider that the small concentration of K^+ used in some of our experiments can be ignored.[2]

Effects of Na^+ on the Kinetic Parameters of Organic Solute Transport in the Small Intestine. Well-documented effects of Na^+ on intestinal transport systems include the following: (1) *velocity or V effects* (*e.g.*, sugar transport in rabbit ileum, Goldner *et al.*, 1969); (2) *affinity or K effects* (*e.g.*, amino acid transport in rabbit ileum (Curran *et al.*, 1967), sugar (Crane *et al.*, 1965) and biotin (Berger *et al.*, 1972) transport in hamster

[2] Use of a low (5–15 mM) K^+ concentration in intestinal transport experiments has become routine. It probably originates with an observation of Riklis and Quastel (1958) that such a trace of K^+ is required for optimal transport. This concept appears to be negated by the observation shown in Figure 2A, but this point we will discuss in detail elsewhere.

intestine); and (3) *mixed effects*, where Na^+ may modify both V_{max} and K_T, depending on the substrate used to test the system (*e.g.*, sugar transport in guinea pig intestine, Alvarado, 1972). As suggested earlier, the existence of mixed effects might prove to be widespread as the number of substrate analogs and animal species tested increases. As an example, sugar transport in the rabbit ileum (mentioned above) is in fact another case of mixed activation since it exhibits a definite K effect that cannot be ignored. Therefore, any model for Na^+ activation of organic solute transport must include an allowance for simultaneous K and V effects.

Noncompulsory Model. Let us assume that the carrier, C, has two distinct binding sites (represented respectively by the left and right side of the symbol) that may specifically and reversibly interact with either of the two cosubstrates, S and A, to respectively give the binary complexes S-C and C-A. Since the two sites are different, there being neither direct competition nor overlap (steric hindrance), formation of the ternary complex S-C-A is also possible; in fact, the very concept of cotransport implies the existence of such a ternary complex. It is necessary to postulate that the two binding sites for S and A be respectively specific and separate[3] to explain both cotransport and the possible transport of each substrate when alone (nonessential activation). On the other hand, and because of the specificity of the carrier, formation of complexes such as A-C, C-S, and A-C-S is considered impossible. Also, direct interactions between S and A are not considered. The model, illustrated in Figure 1, is defined by the following series of reactions:

$$S + C \rightleftharpoons S\text{-}C$$
$$K_s = (S)(C)/(SC) \quad (2)$$
$$A + C \rightleftharpoons C\text{-}A$$
$$K_a = (A)(C)/(CA) \quad (3)$$
$$S + C\text{-}A \rightleftharpoons S\text{-}C\text{-}A$$
$$K_s' = (S)(CA)/(SCA) \quad (4)$$
$$A + S\text{-}C \rightleftharpoons S\text{-}C\text{-}A$$
$$K_a' = (A)(SC)/(SCA) \quad (5)$$
$$S + A + C \rightleftharpoons S\text{-}C\text{-}A$$
$$K_{sa} = (S)(C)(A)/(SCA) \quad (6)$$
$$S + A \not\rightarrow \quad (7)$$

And, by definition

$$K_s/K_s' = K_a/K_a' = R \quad (8)$$

Reaction 6, although theoretically possible, is unlikely since it involves a triple collision. Therefore, formation of the ternary complex more likely occurs in a sequential manner, *i.e.*, by either of the two sequences of reactions, 2 + 5 or 3 + 4, as illustrated in Figure 1. Because we are in fact dealing with the same overall reaction through two different pathways, the following therefore becomes apparent

$$K_{sa} = K_sK_a' = K_aK_s' \quad (9)$$

which means that the two pathways in Figure 1 have exactly the same overall probability; hence the noncompulsory nature of the model.[4]

[3] The statement "specific and separate" does not necessarily imply that the two specificities must be different in all cases, the model being open for the possible consideration of homotropic cooperative effects.

[4] A random-order model similar to ours has recently been proposed by Thomas and Christensen (1971) for the cotransport of neutral amino acids and Na^+ in red cells. This model, however, differs from ours in at least two important respects: (1) amino acid and Na^+ are postulated to bind in juxtaposition and directly interact with one another; and (2) activation is essential; *i.e.*, there is no translocation when either of the two cosubstrates is alone.

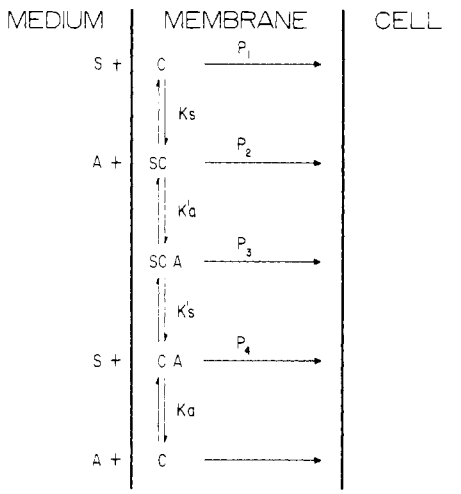

FIGURE 1: The general model for cotransport. This highly schematic diagram illustrates only the substrate-carrier interactions at the external side of the membrane and subsequent rate-limiting translocation step or unidirectional influx (left to right arrows). The reactions involved in the closing of the carrier cycle are not illustrated, but are incorporated into the coefficients, P_1 to P_4.

With regard to the translocation step, it is in principle assumed that all forms of the carrier, either free or combined, have mobility, although for the model to have generality we have to start from the assumption that each permeability constant may have a different numerical value

$$P_1 \neq P_2 \neq P_3 \neq P_4 \quad (10)$$

The transport velocity for either S or A will then be given by, respectively

$$v_s = P_2(SC) + P_3(SCA) \quad (11)$$

and

$$v_a = P_4(CA) + P_3(SCA) \quad (12)$$

Of course, equations for v_s and v_a will be entirely symmetrical and only one of them, that for v_s, will be considered. For this we only need further to define

$$(C_T) = (C) + (SC) + (CA) + (SCA) \quad (13)$$

where (C_T) is the total concentration of carrier. We make the simplification of considering only those substrate-carrier interactions taking place at the outer side of the membrane, as illustrated in Figure 1. This simplification is justified by the known irreversibility of the system (Finch, 1962) and lack of transconcentration effects (Curran *et al.*, 1967; Alvarado, unpublished). Left to right arrows in Figure 1 represent the unidirectional influx of the cosubstrates, the rate-limiting step. The assumption is made that the Michaelis-Menten equilibrium condition holds. The requirement (Kotyk, 1973) that the carrier cycle be closed is recognized but is not illustrated in the highly schematic Figure 1. Coefficients P_1 to P_4 are therefore meant to include: (1) the translocation from left to right of C, either alone or in combination with S and/or A; (2) the dissociation and release of either cosubstrate to the cell fluid; and (3) the return of the carrier. Our point is that, under the conditions of our experiments, the carrier returns empty, which would be equivalent to the regeneration of the free enzyme in traditional enzyme kinetics.

Substituting in eq 11 we obtain

$$v_s = \frac{P_2(C_T)[1 + [P_3(A)R/P_2K_a]]}{1 + [K_s/(S)] + [(A)/K_a][[K_s/(S)] + R]} \quad (14)$$

and

$$v_s = \frac{P_3(C_T)[1 + [P_2K_a/P_3(A)R]]}{1 + [K_s'/(S)] + [K_a/(A)][[K_s'/(S)] + (1/R)]} \quad (15)$$

Although the above two equations are equivalent, they are nevertheless both shown better to illustrate the full scope of the model, particularly in regard to the limiting values the two kinetic parameters, V_{max} and K_T, may attain at the two extremes of modifier concentration. A simple inspection of eq 14, for instance, readily reveals that, at (A) = 0, $V_{max} = P_2(C_T) = V_m$, and $K_T = K_s$. On the other hand, an inspection of eq 15 shows that at (A) = ∞, $V_{max} = P_3(C_T) = V_m'$, and $K_T = K_s'$. This, following current ideas about allosteric proteins (Koshland, 1970), can be interpreted in terms of an *allosteric transition* involving two different carrier forms

$$C \rightleftharpoons C' \quad (16)$$

The empty carrier predominates in the C form, but the binding to it of either allosteric modifier displaces the equilibrium of eq 16 to the right. Whether this transition results in activation or inhibition will depend on the relative values of the pertinent kinetic constants, as discussed in detail previously (Alvarado, 1967).

By introducing the appropriate values for the constants involved, eq 14 should in principle be able to explain all transport systems involving two cosubstrates (under the restrictions defined above). At each constant value of (A), eq 14 should simplify to give the equivalent of eq 1. The simplest imaginable solution when (A) > 0 is that $R = 1$ and $V_m/V_m' = 1$ in which case, although the modifier A is present, there will be no effect of any kind, *i.e.*, $V_{max} = V_m$ and $K_T = K_s$. This is the assumption made, for instance, when the carrier is in the presence of either Li^+ or choline$^+$.

Application of the Noncompulsory Model to Results on Cycloleucine Transport in Guinea Pig Intestine

Effect of Na^+ on the Initial Rate of Cycloleucine Transport in Guinea Pig Ileum. Figure 2A shows that the initial rate of cycloleucine transport in guinea pig ileum is a saturable function of the medium Na^+ concentration. Na^+ appears to be a nonessential activator since transport can take place in the absence of this cation. Both observations indicate that the experimental results may in principle be fitted by eq 14 which is one of a hyperbola concave downward, with a positive intercept (the assumption, however, must be made that $R > 1$ and/or $P_2 < P_3$). A plot of Dixon (Dixon and Webb, 1964) also indicates kinetics of the so-called *type b, i.e.*, a curve is obtained that appears to tend to a plateau or limiting v value at infinite Na^+ concentration (Figure 2B). Figure 2A also shows that K^+ does not replace Na^+; to the contrary, it has a definite inhibitory effect. This effect, however, will be discussed elsewhere.

Effect of Na^+ on the Kinetics of Cycloleucine Transport. An initial test of the effect of Na^+ on the kinetics of cycloleucine transport in guinea pig jejunum (Figure 3) and ileum (not shown) demonstrates that, at either of the two extremes of Na^+ concentration used, Michaelis–Menten kinetics are followed. Presence of Na^+ causes a decrease in K_T, leaving V_{max} essentially unchanged. This result suggests that the activation of cycloleucine transport by Na^+ may conform to a simplified form of eq 14 in which V_{max} is constant and independent of $[Na^+]$. By making $P_2 = P_3 = P$ [$V_{max} = P(C_T)$], and by substituting $[Na^+]$ for (A), eq 14 becomes

$$v_s = \frac{P(C_T)}{1 + [K_s/(S)][(K_a + [Na^+])/(K_a + [Na^+]R)]} \quad (17)$$

This equation, in which $R > 1$, is one of the so-called *type 1b* or *pseudocompetitive activation* (Alvarado, 1967). Of course, K_a represents the dissociation constant for Na^+ ($K_a = K_{Na^+}$).

Change in K_T as a Function of $[Na^+]$. A more complete study of the effect of $[Na^+]$ on the kinetics of cycloleucine transport was carried out using both jejunum and ileum and a series of Li^+-substituted phosphate buffers in which $[Na^+]$ ranged from 0 to 134 mM. The pooled results at each Na^+ concentration were analyzed according to Woolf's linear transformation where (S)/v is plotted against (S) (see Dowd and

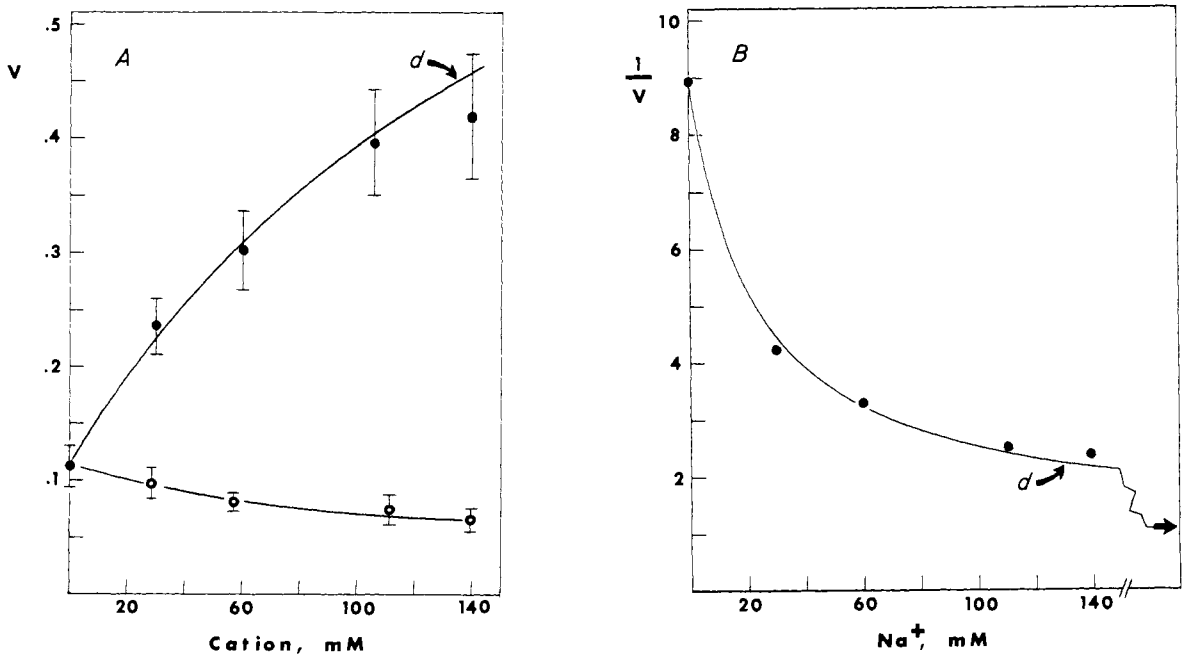

FIGURE 2: (A) The relative effects of Na^+ and K^+ on the initial velocity (v) of cycloleucine transport in guinea pig ileum. Transport was determined (5 min) in a Tris (pH 7.2) buffer containing 1 mM [^{14}C]cycloleucine and a mixture of Li^+ and either Na^+ (●) or K^+ (○) to give a total concentration of alkali metal ions of 139.5 mequiv/l. Each point is the mean of 16 determinations ± SD (two experiments for each Na^+ and K^+, respectively). The solid line d is a theoretical curve according to eq 1, using to solve it: $V_{max} = 3.370$, (S) = 0.97 mM, and the same values for K_T as a function of $[Na^+]$ as those shown in theoretical curve a. (B) The sodium data are plotted according to Dixon. The arrow indicates the extrapolation of $1/v$ to an infinite Na^+ concentration.

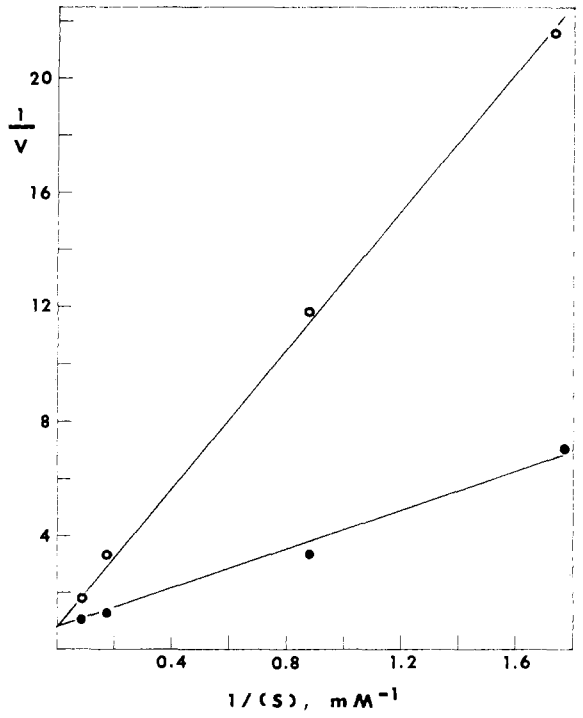

FIGURE 3: Linewaver-Burk plot of the kinetics of cycloleucine transport, in the presence (●) of 130 mM Na^+ or in the absence (○) of this cation. The results shown correspond to a single experiment using the jejunum of one animal. Each point is the mean of four separate determinations.

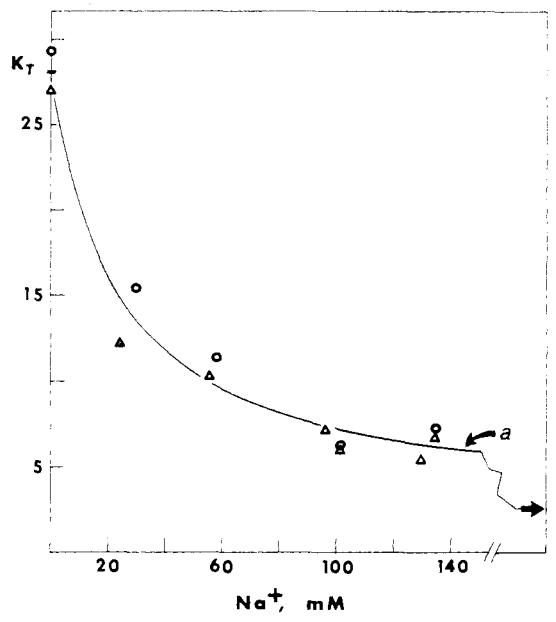

FIGURE 4: The effect of $[Na^+]$ on the K_T for cycloleucine transport in guinea pig jejunum (△) and ileum (○). Curve a shows the theoretical fit of the data according to eq 18. The arrow indicates the limiting value of K_T (K_s') at infinite $[Na^+]$.

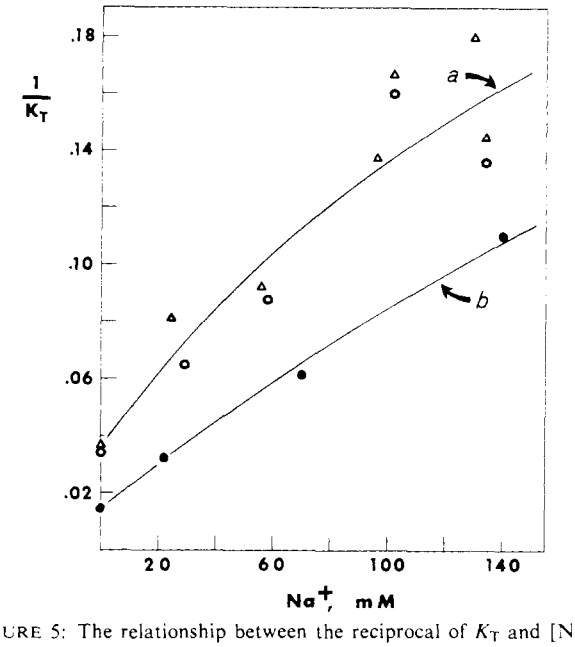

FIGURE 5: The relationship between the reciprocal of K_T and $[Na^+]$ for cycloleucine transport in guinea pig jejunum (△) and ileum (○); and for L-alanine transport in rabbit ileum (●). Curves a and b show the theoretical fit of the data according to eq 18.

Riggs, 1965). The results obtained (Table I) indicate that K_T is inversely related to $[Na^+]$, and ranges from a maximum of about 30 mM at $[Na^+] = 0$ to a value in the range of 5–6 mM at the highest $[Na^+]$ used. In confirmation of the results mentioned above, V_{max} seems to be constant and independent of $[Na^+]$, both in the jejunum and in the ileum. It appears that jejunum and ileum behave in a similar manner with regard to Na^+ activation of cycloleucine transport, a result not entirely surprising in view of the observations of Alvarez *et al.* (1969) with the rabbit. The surprising fact, however, is that K_T changes with $[Na^+]$ apparently identically in these two parts of the intestine, suggesting that the transport of some neutral amino acids is homogeneous along the entire length of the small intestine. This question, however, will be dealt with elsewhere. For our present purposes, K_T data from both jejunum and ileum will be grouped and used together for the calculation of the results. It will be shown that the same model explains both results, and identical theoretical curves can be used to fit the data of cycloleucine transport in guinea pig jejunum and ileum.

Kinetic Analysis of the Relationship between the K_T for Cycloleucine Transport and the Medium Na^+ Concentration. Since the effect of $[Na^+]$ on cycloleucine transport appears to be a pure case of K kinetics, attention will be directed from now on to the dependence of the value of K_T on $[Na^+]$. This is given by the equation

$$K_T = K_s'\{(K_a + [Na^+])/(K_a + [Na^+]R)\} \qquad (18)$$

that corresponds to a hyperbola, concave upwards. The data in Table I, plotted in this way (Figure 4), appear to confirm this prediction. In a similar manner, the data also seem to confirm that a plot of $1/K_T$ against $[Na^+]$ (Figure 5) should also give a hyperbola, but now concave downward.

That the experimental data appear to give such curve is interesting because it is at variance with the observations of Curran *et al.* (1967) using rabbit ileum. These workers found that, using L-alanine as substrate, a plot of $1/K_T$ against $[Na^+]$ gave a straight line (Figure 3 in the above reference). This is probably the main observation that led these workers to propose the compulsory model that we shall discuss in detail later.

It seems clear from eq 18 that K_s' could in theory be appraised by determining the value of K_T at saturating $[Na^+]$. Unfortunately, such appraisal cannot be made directly from the curves shown in Figures 4 and 5 since, obviously, we are far away from saturation with Na^+. It seemed therefore necessary to develop a linear transformation of eq 18 that would enable us to calculate all the constants involved. This has been achieved as follows. If we define the Na^+-dependent change in

K_T as $\Delta K = K_s - K_T$ and make the appropriate substitutions, then we obtain

$$\Delta K = (K_s - K_s')[Na^+]/(K_a' + [Na^+]) \quad (19)$$

This is an extremely useful transformation since it expresses ΔK as a function of $[Na^+]$ in the form of a hyperbola formally identical with the classical Michaelis–Menten equation if we consider the following equivalences: $\Delta K = v$; $(K_s - K_s') = V_{max}$, $K_a' = K_m$, and $[Na^+] = (S)$. In contrast with eq 18, therefore, eq 19 would be susceptible to several possible linear transformations, and if we choose that of Woolf, we obtain

$$[Na^+]/\Delta K = K_a'/(K_s - K_s') + [1/(K_s - K_s')][Na^+] \quad (20)$$

A plot of $[Na^+]/\Delta K$ *vs.* $[Na^+]$ should therefore give a straight line. From the slope (b) of this line, we should be able to calculate the value of K_s'

$$K_s' = (bK_s - 1)/b \quad (21)$$

and from the intercept (a) that of K_a'

$$K_a' = a(K_s - K_s') \quad (22)$$

Given these constants and the definition of R, the value of the remaining constant, K_a, can also be calculated.

As value of K_s for the calculation of ΔK from the data in Table I we used the average K_T for jejunum and ileum at $[Na^+] = 0$, based on the assumption that at $[Na^+] = 0$, $K_T = K_s$. For the treatment of the data according to Woolf's plot we used the same program as in the calculation of the transport results for the evaluation of V_{max} and K_T, making the appropriate substitutions as listed above. The results were indeed found to fit a straight line (line a, Figure 6) from which the pertinent kinetic parameters (listed in Table II, line a) were calculated.

Using this knowledge and making the appropriate substitutions in eq 18, theoretical curves were thus calculated for the value of K_T and its reciprocal as a function of $[Na^+]$. The agreement found between such theoretical curves and the experimental data (curve a in Figures 4 and 5, respectively) strongly supports the contention that the Na^+ activation of cycloleucine transport in guinea pig jejunum and ileum indeed conforms to eq 17 and can therefore be accurately described in terms of our noncompulsory model.

Although no measurement of amino acid dependent Na^+ fluxes has been undertaken at this time, the equations derived from our model and used to fit the experimental data with cycloleucine imply a 1:1 relationship between substrate (amino acid) and activator (Na^+) binding sites. It can be predicted, therefore, that in the guinea pig intestine, as is the case with rabbit ileum (Curran *et al.*, 1967), the *coupling coefficient* amino acid/Na^+ will range from 0 to a maximum of 1, depending on the degree of saturation of the system with the two respective modifiers.

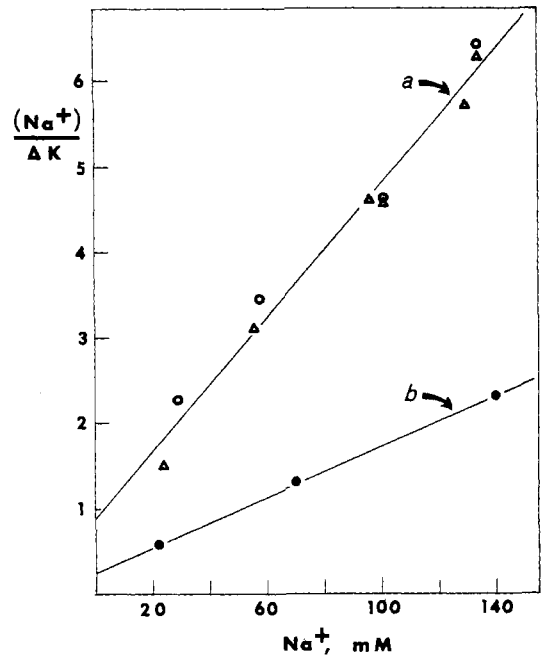

FIGURE 6: Plots for a linear transformation (eq 20) of transport data illustrating the relationship between ΔK and $[Na^+]$. The data correspond respectively to the transport of cycloleucine in guinea pig jejunum (Δ) and ileum (○), or to L-alanine transport in rabbit ileum (●).

Dependence of Transport Velocity on $[Na^+]$. As a final test of the noncompulsory model, theoretical values for K_T as a function of $[Na^+]$ were calculated according to eq 18 and substituted in eq 1. The agreement between such theoretical curves (d) and the experimental results shown in Figure 2 seems quite reasonable and lends further support to the model.

Application of the Noncompulsory Model to Published Results on Amino Acid Transport in Rabbit Intestine

L-Alanine Transport in Rabbit Ileum. Compulsory Model. A different model for amino acid and Na^+ cotransport in small intestine was proposed by Curran, Schultz, and colleagues. Using L-alanine and rabbit ileum, these workers postulated a *compulsory model* according to which (see Schultz and Curran, 1970, p 653) "There is a preferred pathway for formation of the ternary complex such that alanine must combine with the membrane component first and Na can only associate with the membrane component after the binary complex has been formed."

This model (Figure 4 in Curran *et al.*, 1967) may be considered therefore as a special solution of the general (noncompulsory) one in which the lower half is omitted (compare with our Figure 1). However, if the sites for S and A are separate, and there are no interactions between the two cosubstrates, there can be no "compulsory" pathway, as stated quite clearly in eq 9 above. Whether one pathway or the other is followed will depend on the relative concentrations and the affinity of each cosubstrate for its site. There is no basis for the claim that Na^+ cannot bind first.

Our objective in the following, therefore, is to show that the noncompulsory model fully explains the data on L-alanine transport in rabbit ileum, the compulsory model representing

TABLE II: Summary of Values Calculated for the Dissociation Constants of Substrate–Carrier or Activator–Carrier Complexes Involved in the Cotransport of Amino Acids and Na^+ in the Small Intestine.

Model[a]	Curve[b]	r	K_s	K_s'	K_a	K_a'
N–C	a	0.988	28.21	2.59	246.7	22.66
N–C	b	0.999	70	1.70	726.1	17.58
C	c		70			17.0
C	c′	0.999	65.23			22.76

[a] Results calculated according to the noncompulsory (N–C) or the compulsory (C) models, respectively. [b] The values given were used to calculate the theoretical curves plotted in some of the figures. Curve a refers to cycloleucine transport in the guinea pig; curves b, c, and c′ to L-alanine transport in rabbit ileum.

TABLE III: The Kinetic Meaning of the Complex Constant, K_T, or Its Reciprocal, According to the Two Models Discussed in the Text.

Compulsory Model[a]		Noncompulsory Model	
$K_T = K_s K_a'/[K_a' + (Na^+)]$	(I)	$K_T = K_s\{[K_a + (Na^+)]/[K_a + (Na^+)R]\}$	(II)
$1/K_T = 1/K_s + (1/K_s K_a')(Na^+)$	(III)	$1/K_T = [K_a + (Na^+)R]/\{K_s[K_a + (Na^+)]\}$	(IV)

[a] Taken from Curran *et al.* (1967) and translated to our notation ($K_s = K_1$ and $K_a' = K_2$).

an oversimplification of it, based on the not necessarily justified assumption that $K_a \gg [Na^+]$. To facilitate a direct comparison, equations for K_T according to each of the two models are shown in Table III. It is characteristic of the compulsory model that a plot of $1/K_T$ against $[Na^+]$ gives a straight line, a result apparently found in the rabbit by Curran *et al.* (1967; Figure 2). On the contrary, a similar plot according to the noncompulsory model would give a hyperbola, as shown above for the guinea pig.

It is theoretically possible, however, to transform the equations for the noncompulsory model (II and IV, Table III) into those of the compulsory model by assuming, as mentioned above, that $K_a \gg [Na^+]$. By making the appropriate substitutions, we obtain

$$K_T = K_s' K_a/(K_a + [Na^+]) = K_s K_a'/(K_a' + [Na^+]) \quad (23)$$

and

$$1/K_T = 1/K_s + (1/K_s' K_a)[Na^+] = 1/K_s + (1/K_s K_a')[Na^+] \quad (24)$$

These two equations are, of course, the same as I and III in Table III. Each one has been written intentionally in its two equivalent forms, to show that both pathways have equal weight, *i.e.*, the noncompulsory model may also yield a straight line in a plot of $1/K_T$ against $[Na^+]$, provided that we work well below saturation with Na^+. We will now recalculate the results of Curran and colleagues in the light of the above considerations.

Recalculation of the Data on Na^+ Activation of L-*Alanine Transport in Rabbit Ileum According to the Equations of the Noncompulsory Model.* As mentioned, Curran *et al.* (1967) found their results to fit a straight line when a plot of $1/K_T$ *vs.* $[Na^+]$ was made. Following their model, a calculation of K_s and K_a' was made from the intercept and slope of this line. The results given by these workers are included in Table II, line c. We have repeated this calculation by applying the method of least squares to the data of Curran *et al.* (1967; Table I). The results, shown in Table II, line c', are different from those published by Curran and colleagues. Particularly, the value of K_s (65.23 mM) differs appreciably from that expected from the relationship mentioned above that, when $[Na^+] = 0$, $K_T = K_s = 70$.

The same data were then recalculated according to the equations for the noncompulsory model. First, a plot of $[Na^+]/\Delta K$ against $[Na^+]$ was made and found to fit a straight line (Figure 6, line b), as dictated by eq 20. Using then eq 21 and 22, the full set of kinetic constants for L-alanine transport in rabbit ileum was calculated (line b, Table II). The agreement between the results found with this method and those given by Curran *et al.* (1967) is remarkable. But the noncompulsory model has allowed us to calculate all the constants involved, half of which the compulsory model ignores.

It seemed of interest to verify our hypothesis further by calculating theoretical curves and seeing which equations (*i.e.*, those for the compulsory model or those for the noncompulsory model) would better fit the results. Thus, two different theoretical curves were calculated for the value of K_T as a function of $[Na^+]$, using respectively for each (curves c and b, Figure 7) the values for the pertinent kinetic constants given in Table II and the appropriate equations (I and II in Table III, respectively). It seems clear that curve b fits the experimental results better. Since curve c was calculated using an equation based on the assumption that $K_a \gg [Na^+]$, it seems logical that at the lowest $[Na^+]$ used the fit is quite good, but the theoretical curve tends to deviate from the experimental results as $[Na^+]$ increases. A point of theoretical importance is that, according to the compulsory model, at infinite $[Na^+]$, K_T falls to zero, whereas, according to the noncompulsory model, a limiting value of K_T is reached (K_s') as illustrated quite clearly in Figure 7.

Further confirmation of our postulates for the noncompulsory model is found by plotting the theoretical curve of $1/K_T$ *vs.* $[Na^+]$ (Figure 5, line b) according to eq IV, Table III. It is seen that this curve, as predicted, is a hyperbola concave downward. The fit of this hyperbola to the results of Curran *et al.* (1967) seems beyond question. The fact that this hyperbola may be made almost coincident with a straight line (compare with Figure 3 in the above reference) derives from the prediction made before that K_a in rabbit ileum must be large. At the largest $[Na^+]$ used by Curran and colleagues (140 mM), the

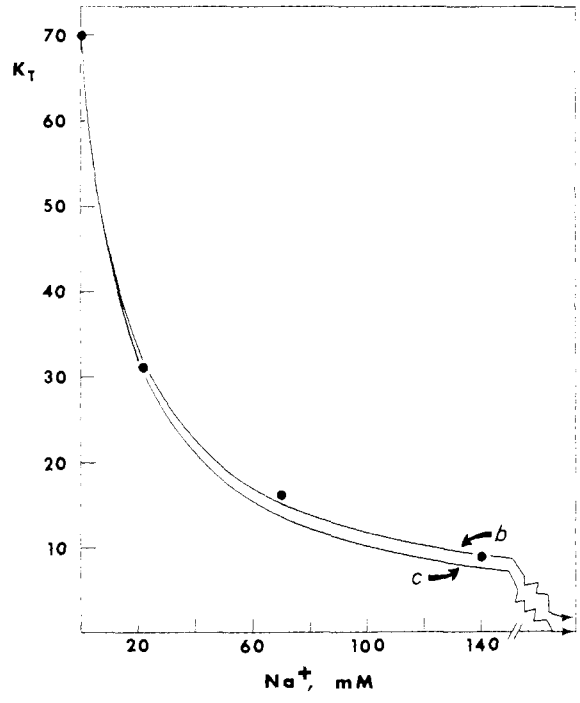

FIGURE 7: The effect of $[Na^+]$ on the K_T for L-alanine transport in rabbit ileum. Curves b and c correspond to theoretical fittings of the data according to the noncompulsory (b) or the compulsory (c) models, as explained in the text. The arrows indicate the limiting values of K_T at infinite $[Na^+]$. A χ-squared analysis of the data gave the following figures: curve b = 0.075; curve c = 0.488.

system was about five times below K_a. It must be emphasized, however, that although large, K_a does not approach infinity, as implied by the concept of the "compulsory" pathway.

Discussion

Rationale Behind the General Model for Cotransport and a New Look at the Polyfunctional Carrier Concept. The salient feature of our model is that it explains in a unifying manner all possible interactions in the intestine between two cosubstrates, including V (velocity) and K (affinity) effects as well as activation and inhibition. Thus, the model is not only one for cotransport but also one for reciprocal interactions and countertransport between two substrates binding to separate but functionally related sites. It is not a coincidence, therefore, that the present model is formally identical with those proposed earlier for the reciprocal interactions between sugars and amino acids at the level of a polyfunctional carrier (Alvarado, 1966, 1971), and for the effects of phloretin on sugar and on amino acid carrier transport (Alvarado, 1967, 1970).

In their thorough investigation of the mechanism of coupled transport between Na^+ and organic solutes, Schultz and Curran (1970) arrived at the conclusion that the two systems (for sugars and amino acids, respectively, in rabbit ileum) "obey entirely different kinetics" and therefore transport must occur through different, unrelated mechanisms.[5] The "entirely different kinetics," however, are only V and K kinetics which, we have seen, may be explained according to the same general model. It is becoming increasingly clear that essentially identical mechanisms underlie the operation of several Na^+-coupled transport systems in the small intestine, as first suggested when Alvarado (1966) pointed out that: "Allosteric enzyme inhibition as well as activation may conform to the same equation . . . This suggests that it might be possible to interpret in the same manner the activating effect of Na^+ on sugar and amino acid transport and the reciprocal effects of sugars and amino acids already discussed." What we want to emphasize now is that this similarity of mechanism may be rooted in some profound biochemical link between the various systems, common membrane Na^+-binding sites being perhaps the most obvious candidate for such a link. This idea appears to be supported by quantitative considerations made now and to be made in subsequent papers.

Low Affinity of the Carrier for Na^+. Perhaps one of the most striking findings in this study is the unexpectedly large value of the dissociation constant (K_a) for the Na^+-carrier complex, particularly in the rabbit, but also in the guinea pig (726 and 247 mM, respectively). This result would appear to suggest that the carrier probably does not have a strong negative charge that would require it to be bound, practically at all times, to some cation. Rather, it appears that a significant proportion of cation-free carrier is in operation in organic solute transport, although addition of the organic solute to the mucosal solution will tend rapidly to saturate the carrier with Na^+, as we shall discuss presently.

Before proceeding, however, it should be mentioned that neither our results nor those of Curran and colleagues have been corrected for a possible effect of unstirred layers (Winne, 1973) on the kinetics. For this reason, the quantitative data in Table II have to be considered with some caution. Nevertheless, the main purpose of this investigation was to ascertain whether a general agreement could be found between the experimental results and theoretical expectations derived from the allosteric model. In the following, we will draw some conclusions based, not on the absolute value calculated for each constant, but rather on their relative values, as shown in Table IV. A correction for unstirred layer effects would not change the relative values of the constants.

TABLE IV: Relative Value of the Dissociation Constants for Cotransport of Amino Acids and Na^+ in Small Intestine.[a]

	R	K_{sa}	K_s/K_{sa}	K_a/K_{sa}	K_a/K_s
Cycloleucine (guinea pig) (a)	10.89	0.64	44.15	386.	8.75
L-Alanine (rabbit ileum) (b)	41.3	1.23	56.9	590.	10.37

[a] From the data in Table II; lines a and b, respectively.

Quantification of the Reciprocal Activating Effects between Organic Solutes and Na^+. As defined above, the value of R (Table IV) may give an indication of the degree of activation caused by one modifier on the subsequent binding of the other. However, because it is a reflection of the redundancy of the system, the R parameter cannot be considered as satisfactory for the evaluation of the relative activating effectiveness of each modifier. Since R relates the values of the K and K' dissociation constants and is the same regardless whether one modifier or its opposite is considered, the erroneous impression may perhaps be acquired that both modifiers are equally effective in activating one another.

A truer index of the overall degree of activation achieved should be that given by the ratios between the dissociation constants for each of the two possible binary complexes and the overall dissociation constant of the ternary complex. The calculated values for these ratios (K_s/K_{sa}; K_a/K_{sa}) are given in Table IV. It is seen that they differ markedly from each other, both in the guinea pig (cycloleucine) and in the rabbit (alanine); but their values parallel each other quite closely in the two species. According to this definition, it seems readily apparent that Na^+ activates amino acid influx some 50 times (K_s/K_{sa} values of 44 and 57 for guinea pig and rabbit, respectively). On the other hand, amino acids activate Na^+ influx about 10 times over (K_a/K_{sa} ratios of 386 and 590, respectively). This difference in activating capacity is of course given by the ratios

$$[K_a/K_{sa}]/[K_s/K_{sa}] = K_a/K_s \qquad (25)$$

which is about 10 for both guinea pig and rabbit (8.75 and 10.37, respectively).

The interesting and inescapable conclusion seems to be that, in both guinea pig and rabbit intestine, amino acids are about ten times better as activators of Na^+ than Na^+ is of amino acid transport. A similar type of relationship appears to hold for sugar transport in several species (Alvarado and Mahmood, to be published) and for biotin transport in hamster intestine. In effect, from the data given by Berger *et al.* (1972), and making the appropriate changes in the notation, we have calculated that $K_a/K_s = 11.66$; *i.e.*, biotin is again some 10 times better an activator of Na^+ transport than is Na^+ reciprocally of biotin transport, in the hamster.

Such calculations indeed suggest a profound relationship

[5] In their review, Schultz and Curran (1970) considered the possibility of a general model (similar to the one proposed here) being capable of explaining in a unified way all Na^+-coupled transport processes. But in view of their own results on sugar and amino acid transport in rabbit ileum, they decided in favor of two separate, mechanistically quite different, models.

among the various Na^+-activated organic solute transport processes in the small intestine.

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