Intestinal transfer mechanisms, measurements, and analogies

D. H. SMYTH

From the Department of Physiology, University of Sheffield

'The object of science', runs an old saying often attributed to Galileo, 'is to measure what can be measured and to make measurable what can be made measurable', and this is still a useful definition which covers much of our scientific activities. Lord Kelvin had a similar idea in mind when he said, 'When you can measure what you are talking about and express it in numbers, you know something about it'. The objects of our studies vary greatly in their susceptibility to expression in quantitative terms. The properties of a purified enzyme are more susceptible to measurement than the symptoms and signs of a sick child. This does not necessarily make the clinician less scientific, as much of his material may still be at the stage of being made measurable. The goal must, however, ultimately be to improve qualitative description by making it quantitative, and those who do not make the attempt to do this are likely to finish as second-class citizens in the world of science.

Intestinal absorption is a field where measurement and more precise quantitative description has made considerable strides in the last decade. This has been partly due to the stimulation of work on transport processes in other tissues, eg, nerve, muscle, red cell, etc, where more precise ways of measurement were already available. In these other tissues the concept of carriers has been extensively developed, and a good deal of work on intestinal absorption has depended on the application of the concept of carriers.

Analogy with Enzymes

When we start to measure something which has not been measured before we often find it convenient to make analogies with other and better known processes, and in the case of carrier transport one of the important analogies has been with enzymes. Hence many attempts at quantitative expression of intestinal absorption are based on terms drawn from enzymology. My assignment in this Symposium was to discuss mechanisms of intestinal transfer generally. It seemed that a useful approach to this would be to consider the analogy between enzymes and carriers, as I am sure that some subsequent speakers are going to use terms borrowed from enzymology. Enzymes have been very extensively studied now for about 50 years so it was natural that in trying to express the action of carriers quantitatively we should look to enzymology. I am going to discuss ways in which enzymes resemble carriers and ways in which they differ from them and in general terms the application of enzymology to carrier transport.

I have already committed myself to the importance of quantitative investigation and thinking. There is, however, a danger that by making something apparently quantitative we think that we have solved the problem. Mathematical terminology is not incompatible with loose thinking, and I am also going to discuss some of the pitfalls in applying enzyme concepts to carriers. It is easy to appear to understand how carriers function but to express these in quantitative terms which may be misleading. All is not gold that glitters, and few things glitter so dazzingly to some people as a few mathematical equations sprinkled through an article in a predominantly medical journal.

Carriers

In the first place what are carriers and why do we need to invoke them? The first problem of intestinal absorption is to get substances from the lumen of the intestine into the epithelial cell. The epithelial cell is bounded with a lipid membrane and all absorbed substances have to get through this. (We are ignoring the possibility that some substances may get between cells either through the tight junctions or through spaces where cells have been shed.) The three possible routes into the cell are shown in Figure 1. Some of the food substances are of a lipid nature and they can dissolve in the lipid membrane and diffuse through it. We can call this the 'lipid route'. Many of the others, including the end products of carbohydrate and protein, are insoluble in lipids and they must get through by some other route. The simplest concept is to postulate pores in the membrane filled with water, through which these substances can pass, and we can call this the 'aqueous route'. However, if
regulating problems: (1) how does the intestine admit these water-soluble substances, and (2) how does it select in doing so? The most satisfactory answer to this problem is by means of carriers, so that we have a third route, the 'carrier route'. We thus have three routes. I am not going to discuss further the lipid and the aqueous routes but only the carrier route, as most 'mechanisms' involve some kind of carrier.

Figure 2 shows the concept of the carrier. This is a hypothetical molecule in the membrane which is soluble in the membrane and can diffuse through it. At one point on the carrier there is an active site to which the substances may attach and the carrier with the attached substance is also lipid soluble. When the carrier passes through the membrane it can release the substance on the other side, so that the empty carrier can return to transfer more substance.

**Similarities and Differences in Enzymes and Carriers**

The concept of a membrane carrier has a good deal in common with that of an enzyme, although it has also some important differences. The feature most obviously in common is the active site or active centre. This is a chemical configuration at some part of the molecule which permits attachment of substances with particular chemical configurations. It is this which gives the specificity to the carrier or enzyme. In the case of enzymes we call the attached substances 'substrates'. In the case of intestinal transfer it is convenient to use the term 'absorbate'. Hence so far as attachment is concerned, substrate and absorbate are presumed to behave similarly. The big difference comes when we consider separation from the enzyme or carrier mechanism (Fig. 3). In the case of the enzyme the substrate leaves as a different substance or substances, for example, glucose attaches to hexokinase as glucose and leaves as glucose-6-phosphate, and sucrose attaches to invertase as sucrose and leaves as glucose and fructose. The products are found in the same compartment as the substrate, and the separation is chemical not spatial. In the case of a carrier the absorbate leaves the carrier in the same form in which it became attached (although we cannot exclude intermediary changes while it is in the transport mechanism). Furthermore, it leaves not in the same compartment but a spatial separation has been achieved. Carrier activity is only meaningful when the biological organization includes the possibility of compartmental separation.

Another important difference between enzymes and carriers is the experimental approach to defining their properties. The enzymologist often considers that the first problem is to purify and isolate the enzyme, and this has been achieved in
Intestinal transfer-mechanisms, measurements, and analogies

Fig. 3 Similarities and differences in enzymes and carriers. In both cases the substrate A attaches to the active site. In the case of the enzyme it leaves as a different substance or substances (B + C), but without spatial separation of A from B and C. In the case of the carrier it leaves as the same substance but spatial separation has been achieved.

over 600 cases. On the other hand, no carrier molecule has yet been isolated with any certainty, although many attempts have been made. There are several possible reasons for this. Perhaps only a very small amount of carrier is needed to transfer substances across the membrane, and the amount might be so small as to make identification and separation unlikely. On the other hand analogies with enzymes do not suggest that this is the cause of a failure, as we know also that enzymes can deal with a large amount of substrate. Perhaps another reason for failure to isolate the carrier is that it is a fundamental part of the cell structure and once we break the cell the carrier is changed into a form in which it can no longer be identified. Whatever the reason is, it is certain that this approach to the study of transfer mechanisms has up to now not been very successful, although perhaps it will be more successful in the future. It is interesting to remember that the isolation of urease by Sumner (1926) was severely criticized and even ridiculed, and perhaps the same rich harvest which followed the purification of enzymes awaits the isolation and purification of carriers. On account of this failure to isolate a pure carrier substance, the study of carrier processes is immensely complicated. With purified systems it is possible to carry out kinetic studies against a background of knowledge that is quite impossible with carriers, and a certain amount of the work on transport mechanisms is an almost lighthearted application to very complex systems of the ideas developed by enzymologists in much better controlled systems. This is not necessarily a bad thing (many advances in science are made by fools rushing in) but there is at least no harm in discussing with the hesitant angels the interpretation and significance of the results.

The common feature between enzymology and carrier transfer is basically the attachment of the substrate or absorbate to the active site. This results in the formation of a complex, and if we accept that the rate of the whole process (and this is a very big assumption to which I shall return) depends on the rate of formation of the complex both processes can be subjected to certain forms of kinetic analysis well defined by enzymologists.

Enzyme Kinetics

The amount of complex formed or the degree of saturation of the active sites depends on the concentration of the substrate or absorbate. At low concentrations when many sites are free, the degree of saturation is proportional to the amount of substrate or absorbate present. As this increases there is competition for free sites, so that the increase in saturation of sites falls off with increasing concentration and ultimately saturation is achieved. This is basically the process of adsorption, in which case the term ‘Langmuir adsorption isotherm’ is used. Michaelis and Menten (1913) applied this kind of analysis to the study of sucrose hydrolysis by invertase, and the term ‘Michaelis-Menten kinetics’ has long been used in enzymology. It has now spilled over into carrier transport, including intestinal absorption.

If v is the rate of the process, \( V_{\text{max}} \) the maximum rate possible, and C the concentration of substrate, the relation between these is given by the equation

\[
v = \frac{V_{\text{max}} C}{C + K_m}
\]

where \( K_m \) is a constant. The curve represented by this equation is shown in Figure 4. At the lower end of the curve the rate (v) is nearly proportional to the concentration (C), a relationship often called first-order kinetics. At the top of the curve the rate is becoming independent of the concentration, a relationship called ‘zero order kinetics’. Hence the relationship can be regarded as a gradual transition between first order kinetics at low concentration and zero order kinetics at high concentration. It is useful to think of the process in this way because it reminds us that diffusion kinetics, ie, the first-order kinetics, are not incompatible with carrier transfer. Another feature of the Michaelis-Menten curve is that it is possible to express the affinity quantitatively as the concentration (Km) which enables the process to proceed at half the maximum rate. These
two values, the $K_m$ and the $V_{\text{max}}$, are often used to characterize transport systems.

In order to determine the values of $K_m$ and $V_{\text{max}}$ a number of different plots are used of which the most popular is the plot of the reciprocal of rate and concentration introduced by Lineweaver and Burk (1934). This is obtained by a rearrangement of equation 1 into the form

$$\frac{1}{V} = \frac{K_m}{V_{\text{max}}} + \frac{1}{V_{\text{max}}}$$

which indicates the linear relation between $1/V$ and $1/C$. The negative intercept on the abscissa gives $1/K_m$ and the intercept on the ordinate $1/V$ (Fig. 5).

What is actually done in practice is to determine a series of values for $V$ and $C$, plot the reciprocals of these, and obtain $K_m$ and $V_{\text{max}}$ from the intercepts on the abscissa and ordinate.

**Inhibition and Competition**

Another common (in both senses of the word) practice of enzymological and transport workers is to use inhibition studies. Inhibition is of various kinds, but the most pertinent to transport mechanisms is competitive inhibition. This depends on the possibility of group specificity. The active site can combine not only with one substance but more often with a number of substances of related chemical structure. It has an order of preference for these, expressed quantitatively by the $K_m$ and all substances of the group compete with each other for attachment to the site. If we are interested in one member of the group we regard the others as competitive inhibitors. In general, a competitive inhibitor increases the $K_m$ of a substrate, ie, decreases the affinity for an enzyme without alteration of the $V_{\text{max}}$. Figure 6 shows the two plots (v against C and $1/v$ against $1/C$) for a substance in the presence and absence of an inhibitor. In this case the $K_m$ of the substance alone is 1 and in the presence of an inhibitor 2. The effect of the inhibitor can be worked out quantitatively, as its effect will be a change in the value of $K_m$ to a new value, $K_m/(1 + [I]/K_A)$, where $[I]$ is the concentration of the inhibitory substance, and $K_A$ its affinity constant. If A inhibits the activity of a particular enzyme on B, then B will inhibit the activity of the same enzyme on A. If the affinity constants are $K_A$ and $K_B$, then the affinity constant of A in the presence of B will be $K_A (1 + [B]/K_B)$ and the affinity constant of B in the presence of A is $K_B (1 + [A]/K_A)$. When the $K_m$s of A and B are determined separately, and in the presence of each other, the values all fit in with the expected changes, there is some reason to have confidence in the experimental procedures. Similar kinds of competitive studies can also be done in transfer or absorption studies, although the rigorous criterion described above is not often applied.
Allosteric Effects

One further common feature of enzymes and transport mechanisms is the possibility of allosteric effects. By this is meant the effect of a substrate attaching to a binding site on the affinity of an adjacent binding site for a quite different substrate. The important feature of this is that it enables substrates of quite different chemical structures to exert an effect on the handling of each other by an enzyme, whereas the concept of competitive inhibition discussed above would be limited to mutual effects of two substances of related chemical structure, eg, two hexoses affecting each other or two amino acids affecting each other. The concept of allosteric effects has also been applied to intestinal transfer, eg, the effect of hexoses on amino acids (Alvarado, 1966).

Intestinal Absorption Kinetics

With these facts in mind, let us look at the problem of characterizing transport mechanisms in the intestine by the techniques of enzymology, ie, in describing them in terms of a Km and V_{max}. The application of Michaelis-Menten kinetics to intestinal transport was first done by Fisher and Parsons (1953), and their paper still repays careful study. The usual method of applying Michaelis-Menten kinetics is to measure the rate of absorption or transfer at a number of different concentrations and to treat the data as in enzyme kinetics. Gastroenterologists are fairly loose in their idea of measuring rate. The rate may really be a rate, ie, μmoles absorbate/unit time, but frequently the concentrations achieved in some compartment in the system is used, or even the magnitude of an electrical potential. Let us assume that these are valid measurements of the rate and also ignore other problems, eg, whether we should use initial rate and concentration, or whether we can use average rates and concentration, or, as we more frequently do, average rate and initial concentration, and think only of the treatment of the data for rate and concentration. We will begin with the linearity of the plot, which is usually taken as evidence of Michaelis-Menten kinetics. It is not always appreciated how dangerously easy it is to get a straight line when we plot reciprocals, or at least something which will pass for a straight line to the eye of faith, which includes faith in the experimental error. (The faith is actually in the error, and not in the accuracy.) But it is not only the linearity of the plot that matters. If we take the case of diffusion kinetics, where rate is proportional to concentration, the reciprocal plot will also give a straight line. The difference is that in this case the straight line passes through the origin. It is therefore necessary in order to demonstrate Michaelis-Menten kinetics to show that there is a
negative intercept on the abscissa which is significantly different from zero (Fig. 7).

Two Carrier Systems

Suppose the data from a series of entirely unexceptionable experiments put in the form of a reciprocal plot give a straight line with a negative intercept on the abscissa which satisfies the most rigorous criteria, are we justified in characterizing the carrier involved by a Km and Vmax? To answer this question let us consider what would happen if we had a carrier-mediated system, but mediated by two carriers instead of one, and there is good evidence that this does happen in the intestine (Newey and Smyth, 1964). Consider first the case when the two carriers have the same Km but different values of Vmax, which we will call V1 and V2. If the rate by each carrier is v1 and v2 the total rate (v) will be the sum of these and hence

\[ v = \frac{V_1 C}{C + K} + \frac{V_2 C}{C + K} \]  

... (4)

which is equivalent to

\[ v = \frac{C(V_1 + V_2)}{C + K} \]  

... (5)

This is identical with a single carrier process with a Km the same as each of the two carriers, and a Vmax = V1 + V2, and the reciprocal plot of V and C would also give a straight line, with an intercept on the abscissa of -1/Km and on the ordinate of 1/V1 + V2. Unless we considered the possibility of two carriers we would draw the erroneous conclusion that there was one carrier. Hence Michaelis-Menten kinetics does not distinguish between one carrier and two carriers provided these have the same Km.

Supposing the two carriers had different values for both affinity and maximum rate, ie, K1, V1, K2 and V2. The rate of transport would be

\[ v = \frac{V_1 C}{C + K_1} + \frac{V_2 C}{C + K_2} \]  

... (6)

It is easy to show that the plot of 1/v against 1/C is no longer a straight line theoretically, but let us look at it in practice. Take a case where K2 = 2K1 and V2 = 2V1. Figure 8 shows the plot over a range of concentrations including both K1 and K2 and it is evident that this is so nearly a straight line that in most cases it would be taken for one. It is very easy to take arbitrary values of K1 and K2 and V1 and V2 and to show that over quite a range of differences in the characteristics of the two carriers the reciprocal plot gives something likely to be mistaken for a straight line. A Km will be determined which will lie between K1 and K2 and a Vmax which will approximate to V1 + V2. The same fallacy will appear if other plots are taken, eg, c/v against C, etc, although the likelihood of a mistake may vary. If three or more carriers are assumed the chances of error are just as great. We must therefore accept that on the evidence of a relationship between rate of concentration we cannot with any certainty characterize a carrier involved in a transfer process with a Km and Vmax. These considerations should make us extremely careful about defining a carrier in terms of its Km and Vmax, unless we are sure for other reasons that there is only one carrier.

What kind of reasons could we have for thinking that there is more than one carrier, if we get a reciprocal plot which looks reasonably like a straight line. One way of doing this is by the use of competition. If there is only one carrier, competition will always result in increase in Km and decrease in amount transferred for any particular concentration (Fig. 6). Supposing we have two carriers, one with a Km of 1 and one with a Km of 2. Figure 9 shows the plot for each of the carriers separately and for the sum of the two. If we inhibit one carrier completely all the movement goes by the other one, and the kinetics of the whole process become those of the uninhibited carrier. If we inhibit one partially the kinetics of the whole process becomes more like those of uninhibited carrier. The result of applying
Intestinal transfer-mechanisms, measurements, and analogies

![Graph showing plots of v against C for two carriers separately (curves I and II) and for the two carriers together (III).](image)

**Fig. 9** Plot of v against C for two carriers separately (curves I and II) and for the two carriers together (III). If the two carriers are functioning v is given by curve III. If carrier II is completely inhibited the process is now represented by curve I. In this case the rate is reduced, but the affinity is increased, i.e., the Km is reduced.

An inhibitor of one carrier to a process mediated by two carriers will always result in a decrease in the rate of transfer at all concentrations. But it will have very different effects in the Km depending on which carrier is inhibited. With inhibition of the carrier with the smaller Km the characteristics of the whole process will now approach the kinetics of the carrier with the large Km, and with the inhibition of the carrier with the larger Km the kinetic picture will approach the system with the small Km. In one case the Km will increase, in the other it will decrease. This is seen more easily from the Lineweaver-Burk plot in Figure 10. Hence we would get the anomalous result that an inhibitor could decrease transfer and at the same time apparently increase affinity. Daniels, Newey, and Smyth (1970) have in fact shown that this can happen in the case of intestinal transfer of amino acids. The only certain criterion of a one-carrier system is that all substances found to cause competitive inhibition of any substance transferred should produce increases in the affinity constant (Km) in such a way that the new value of Km is equal to Km (1 + 1/Ki), where I is the concentration of inhibitor and Ki the affinity constant of the competing substance. Even this strict criterion for the existence of one carrier is only valid if in all cases it is known that attachment to the carrier is the rate-limiting stage in the process, a problem discussed in the succeeding paragraphs.

It is also easy to show that a combination of a diffusion process with a carrier-mediated process will produce a kinetic picture, which, when studied by the Lineweaver-Burk plot, could very easily give rise to the impression that the process could be explained entirely by carrier-mediated transport, even when a substantial fraction, up to one quarter, of the transport depended on diffusion.

**Rate-limiting Stage**

Many processes involve a number of different stages each of which has its own characteristics, and it is sometimes important to consider how the characteristics of the process as a whole are related to those of its separate stages. Consider the analogy of a crowd of people going to a football match. They arrive by bus from distant areas, walk from the bus stop to the turnstiles, pass through the turnstiles and walk from there to their places in the ground. The rate at which the ground fills up must be dependent in some way on the rates of these processes, that is, (1) the speed of the buses, (2) the walking rate of pedestrians, (3) the rate at which people get through the turnstiles. If we measure the rate at which the ground is filling, which of these processes are we studying? This will depend on when the observation is made. If it is made at a time when there is a large crowd of people going in then it will almost certainly depend on the rate at which they can get through the turnstiles, because this is very much slower than the other two processes. This process is the 'rate-limiting stage' of the whole process of filling the ground. This could be verified if opening more turnstiles enabled the ground to fill up quickly and closing some turnstiles made it fill more slowly. The turnstiles would not, however, always be the rate
limiting complex is rate-limiting. In any case the characteristics of the whole process are likely to be related to those of whichever stage is rate-limiting. In studying the kinetics of any complex process it is important to consider whether some particular stage may be rate-limiting, if so what is that stage, and also whether the same stage is always rate-limiting in different conditions.

When we apply these considerations to enzymology the result is fairly simple. The breakdown of the enzyme substrate complex is the rate-limiting stage, and the rate of the whole process is thus proportional to the saturation of the enzyme. This is an inherent assumption in Michaelis-Menten kinetics. It is necessary to enquire if this assumption is correct in mediated transfer, because only if the rate of the whole process is proportional to the saturation of the carrier can we assume that the value of the Km determined experimentally is a property of the binding site. To do this we must examine some schemes of how the transfer mechanisms function.

There is first the attractive scheme formulated by Crane and his colleagues (see Crane, 1968), which includes the ternary carrier, the Na gradient, and differential affinity mechanisms. The hexose molecule can attach to a binding site on a mobile carrier, which can move across the membrane and equilibrate with the solution on each side. The saturation of the carrier at each side depends on the concentration of hexose at each side and the affinity of the carrier at each side. The affinity depends on the Na and K concentrations and these are such that the affinity inside the cell is smaller than outside. The mechanism depends essentially on an asymmetry of sodium concentration at the two sides of the membrane being maintained, and this asymmetry is maintained by the action of a Na pump extruding Na from the cell. In this scheme the basic mechanism of facilitated diffusion is being used to cause carrier-mediated transport against a gradient. The usual assumption in facilitated diffusion is that the rate of transport is proportional to the difference in saturation of the carrier at the two sides of the membrane. If the values of K inside and outside the cell are K₁ and K₄ and the concentrations of hexose C₁ and C₄ then the rate of transport (ν) is given by

\[ v = k \left( \frac{C_1}{C_1 + K_1} - \frac{C_2}{C_2 + K_2} \right) \]  

and provided that (C₁)/(C₁ + K₁) > (C₂)/(C₂ + K₂) movement of hexoses will take place against a concentration gradient. The important point here is that ν is not dependent on the attachment of the hexose to the carrier but on the more complex term inside the brackets in equation 7. Hence Michaelis-Menten kinetics will be expected only if (C₁)/(C₁ + K₁) = 0. Since C₂ > C₁ this can only happen if K₂ is infinitely high. Hence there must be some doubt if this attractive model could conform to Michaelis-Menten kinetics.

Let us examine another possible model of hexose transfer. Suppose the hexose attaches to a carrier and then enters into a transport process of undefined mechanism which can use metabolic energy. There are now two stages to consider, ie, the attachment and the transport, and the question must be asked which is the rate-limiting stage in the whole process. Is it attachment of hexose to the carrier, or is it the process requiring metabolic energy? The work of the Sheffield group (for references see Smyth, 1970) suggests that in vivo the availability of energy is not rate-limiting but in vitro it may be at least for galactose, Na, and a number of amino acids. This is shown by the facts (a) that the transfer of these substances is stimulated by the presence of metabolized hexoses and (b) that there may be competition for energy among different transport systems. If this is so then the rate-limiting stage in transfer may be availability of energy and when kinetic studies are made we may be investigating the Km, not of attachment of absorbate to the carrier site, but to some quite different process related to utilization of energy. Hence Michaelis-Menten analysis may not provide any information at all about the carrier. It is also possible that in different conditions different stages may be rate-limiting and this may explain the wide diversity of values of Kᵣ for the same substance obtained under different conditions. When we characterize the whole process in kinetic terms it is essential to ask which stage has been characterized. Only if attachment to the carrier is the rate-limiting stage are we describing the properties of the carrier.

The same kind of reasoning applies to competitive studies. If we study the movement of two substances and their mutual effects on each other, we may demonstrate, even by rigorous criteria, that they are competitors for the transfer process. But this does not necessarily mean that they are competitors for one specific transport site. Competitive kinetics are possible from competition for one site, from reciprocal allosteric effects of each substance on the other, or even from competition of two systems for the same source of energy.

**Conclusion**

In attempting to express quantitatively the role of carriers in intestinal transfer, the analogy of enzymes has been useful. The competition of two substances
for an enzyme can be used as a model for competition of substances in the intestinal lumen for a carrier, and studies of the rate of absorption at different concentrations may enable kinetic analysis to be made analogous to Michaelis-Menten kinetics with enzymes. In this way the absorptive process for a particular substance may be characterized by two constants, V\text{max} and K\text{m}.

Some care is needed in interpreting the results of this analysis and in particular in using these constants to define the properties of the carrier site. The characteristics determined by Michaelis-Menten kinetics are those of the rate-limiting stage in the whole process, and carrier transport in the intestine may be a good deal more complicated than the action of an enzyme on a substrate. There are likely to be a number of stages involved, and the rate-limiting stage may or may not be the attachment of the absorbate to the carrier site.

Even if attachment to the carrier is the rate-limiting stage it is still difficult to be certain whether only one carrier is involved, and this applies even with a very accurate kinetic analysis. It may be possible to get some information on the number of carriers involved by carrying out competitive studies with a number of different substances.

References


