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The Use of the Logarithmic Transformation in the Calculation of the Transport Parameters of a System that Obeys Michaelis-Menten Kinetics

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1. A logarithmic method is described for the calculation of the transport parameters, K_m and $V_{max.}$, of a biological system obeying Michaelis–Menten kinetics. 2. This logarithmic method leads to a way of estimating the transport parameters that has not apparently been used previously. It allows the separation of variance due to $V_{max.}$ from other variance, and so reduces the fiducial limits that can be placed on an estimation of K_m. 3. The results of studies on the transport of L-histidine and L-monoiodohistidine by rat intestinal sacs *in vitro* have been used to illustrate the application of the new method. Estimates of the transport parameters have also been made by two alternative procedures. The relative merits of the three methods are discussed.

The interaction of a substrate with any carrier or fixed site in a membrane can be described by an equation analogous to that derived by Michaelis & Menten (1913) for enzyme systems. In the simplest possible case the initial rate of transport of the substrate across the membrane, after a time lag, would be expected to be proportional to the number of carrier–substrate complexes. The transport system may then be said to obey 'Michaelis–Menten kinetics' (Wilbrandt & Rosenberg 1961). Such a system can be described by two parameters, K_m and $V_{max.}$. These parameters have usually been obtained by plotting $1/v$, the reciprocal of the initial transport rate, against $1/c$, the reciprocal of the substrate concentration, as in the method of Lineweaver & Burk (1934). The term 'substrate' is used here loosely to denote the compound transported.

However, in many transport studies it is found that the variance of $1/v$ increases markedly with increasing values of $1/c$. A modification of the Lineweaver–Burk method, employing weighting coefficients, was therefore used by Jervis & Smyth (1959), but this method is likely to be valid only when there is negligible variation in $V_{max.}$. The aim of the present paper is to describe a logarithmic method for the estimation of the transport parameters. This logarithmic method is likely to be especially useful in experiments in which the variation of the experimental values of v at any given value of c includes a large part due to the variation of $V_{max.}$ between individual specimens.

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This variation should be distinguished from the residual variation more properly described as true experimental error. The method retains the advantage of the Lineweaver–Burk plot that the independent and dependent variables are completely separated, but removes the very serious defect of the Lineweaver–Burk plot that necessitates the least-square line's being fitted to weighted data (Riggs, 1963). The theoretical basis of the new method is first discussed, and its application to studies of the transport of L-histidine and of L-monoiodohistidine by rat intestine is then described.

THEORY

General case. The basic equation for a transport system that obeys Michaelis–Menten kinetics may be written in the form (used by Lineweaver & Burk, 1934):

$$1/v = (1+K_m/c)/V_{max.} \quad (1)$$

where v is the initial rate of transport at substrate concentration c, $V_{max.}$ is the maximum initial rate of transport at very high substrate concentrations and K_m is the Michaelis constant for the system. Such an equation might be expected to apply to a single isolated intestinal sac. Other sacs might be expected to have different values of $V_{max.}$, but the carriers should have the same values of K_m. Then for a group of such sacs, and ignoring for the moment the residual experimental error, the equation corresponding to eqn. (1) is:

$$\mathrm{I}/v = (1+K_m/c)(\mathrm{I}/V_{max.}) \quad (2)$$

where I/v and $\mathrm{I}/V_{max.}$ are now the means of the reciprocal rates of transport. It can readily be seen from eqn. (2) that if there is no variation in K_m and if the residual experimental

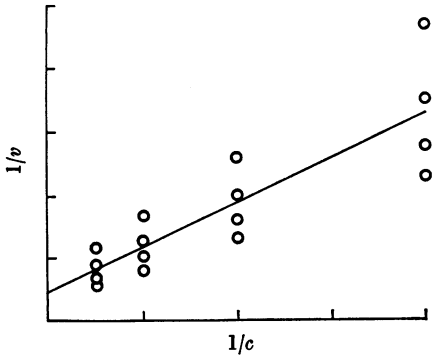

Fig. 1. Simulated Michaelis–Menten kinetic data plotted in the form used by Lineweaver & Burk (1934). K_m has been kept constant and $V_{max.}$ varied.

error were negligible then the variance of $1/v$ would be equal to $(1+K_m/c)^2$ times the variance in $1/V_{max.}$ at any given value of c. Therefore if simulated Michaelis–Menten-type kinetic data are plotted in the form used by Lineweaver & Burk (1934), K_m being kept constant but $V_{max.}$ being supposed to vary from specimen to specimen, the variation of the mean values of $1/v$ increases markedly with increasing values of $1/c$. The type of results expected under such conditions is shown in Fig. 1. The standard deviation of $1/v$ increases linearly with $1/c$.

Experimentally any variance due to errors in experimental technique will be superimposed on variance due to different values of $1/V_{max.}$. However, the variance due to $1/V_{max.}$ can be separated from any other variance and terms in c by taking logarithms of both sides of eqn. (1). Then:

$$\ln(1/v) = \ln(1/V_{max.}) + \ln(1+K_m/c) + z \qquad (3)$$

where z is the further 'error' term. From eqn. (3) it can be seen that:

$$\text{var}\ln(1/v) = \text{var}\ln(1/V_{max.}) + \text{var}\, z \qquad (4)$$

The logarithmic transformation thus displays the variance of the measurements $\ln(1/v)$ in two additive parts. The first part (and possibly the major part) clearly does not depend on c. The second part could possibly depend on c, but, as shown below, in the present studies it can be taken to be independent of c. Thus $\ln(1/v)$ is a variable that can for practical purposes be assumed to have constant standard deviation whatever may be the value of c.

To offset this advantage, however, we now have:

$$\ln(1/v) = \ln(1/V_{max.}) + \ln(1+K_m/c) \qquad (5)$$

a relationship between the expectation of $\ln(1/v)$ and $1/c$ that gives a curve instead of the straight-line relationship of eqn. (2). Suppose that K_0 is taken as a good initial estimate of K_m. Eqn. (5) may be written in the form:

$$\ln(1/v) = \ln(1/V_{max.}) + \ln[(1+K_0/c) + (K_m-K_0)/c] \qquad (6)$$

Application of Taylor's theorem (see, e.g., Kynch, 1955) to the last term of eqn. (6) gives:

$$\ln[(1+K_0/c)+(K_m-K_0)/e] \simeq \ln(1+K_0/c) + (K_m-K_0)/(c+K_0) \qquad (7)$$

when (K_m-K_0) is small. Then eqn. (6) becomes:

$$\ln(1/v) \simeq \ln(1/V_{max.}) + \ln(1+K_0/c) + (K_m-K_0)/(c+K_0)$$

Converting natural logarithms to logarithms to the base 10:

$$\log(1/v) - \log(1+K_0/c) \simeq \log(1/V_{max.}) + (K_m-K_0)/2{\cdot}303(c+K_0) \qquad (8)$$

Eqn. (8) is written in the form:

$$y = a+bx$$

where:

$$y = \log(1/v) - \log(1+K_0/c)$$
$$a = \log(1/V_{max.})$$
$$b = (K_m-K_0)/2{\cdot}303$$

and

$$x = 1/(c+K_0)$$

The best value of K_m to fit the experimental data can be found as follows. A value for K_0 is chosen (see below) and y is plotted against x. The slope of this line, fitted by the method of least squares, is determined and from this a value for K_m is obtained. This estimate of K_m becomes the value of K_0 for a second plot of y versus x. By repeating this process three or four times, the slope of the line is made smaller at each attempt until finally, when K_0 is equal to K_m, the slope of the line is zero.

The first estimate of K_0 can be made on the basis of the following considerations.

The basic equation is:

$$\log(1/v) = \log(1/V_{max.}) + \log(1+K_m/c) \qquad (9)$$

If $\log(1/v)$ is plotted against $1/c$ and a smooth curve is drawn through the points then the intercept on the $\log(1/v)$ axis is $\log(1/V_{max.})$. When:

$$\log(1/v) = 2[\log(1/V_{max.})]$$

then from eqn. (9):

$$\log(1/V_{max.}) = \log(1+K_m/c)$$

where the particular value of $1/c$ can be read from the graph. Then:

$$1+K_m/c = 1/V_{max.}$$

and so:

$$K_m = c(1/V_{max.}-1)$$

This value of K_m may be taken as a good initial value for K_0.

Then 95% confidence limits for the value of K_m, obtained by use of eqn. (8), are calculated from the equation:

$$K_m = K_0 + 2{\cdot}303(b \pm 1{\cdot}96 s_b)$$

where s_b is the standard deviation of the final slope of y versus x. Then 95% confidence limits for $\log(1/V_{max.})$ are calculated from:

$$\log(1/V_{max.}) = a \pm 1{\cdot}96 s_a$$

where s_a is the standard deviation of the intercept on the y axis. The standard deviations of the slope and intercept are calculated by the normal statistical procedures (see, e.g., Mather, 1951).

Low-saturation conditions. The basic equation is:

$$\log(1/v) = \log(1/V_{max.}) + \log(1+K_m/c)$$

At low saturation, i.e. when $c \ll K_m$, this equation becomes:

$$\log(1/v) = \log(1/V_{max.}) + \log(K_m/c)$$

Therefore:

$$\log(1/v) = \log(K_m/V_{max.}) + \log(1/c) \quad (10)$$

If $\log(1/v)$ is plotted against $\log(1/c)$ under low-saturation conditions a straight line is obtained having a slope of 1 and an intercept on the $\log(1/v)$ axis of $\log(K_m/V_{max.})$.

Competitive inhibition. The logarithmic method can readily be extended to obtain the parameters of the system when a competitive inhibitor is present. The basic equation for competitive inhibition can be written in the form:

$$\ln(1/v) = \ln(1/V_{max.}) + \ln(1+K'/c) \quad (11)$$

where:

$$K' = K_m(1+i/K_i)$$

K' is readily determined by the logarithmic method since eqn. (11) is of the same form as eqn. (5). Then, if the concentrations of the substrate c and of the inhibitor i are known, and K_m has been determined, the affinity constant K_i of the inhibitor for the carrier can be calculated from the equation:

$$K_i = K_m i/(K'-K_m)$$

It may be noted that K_i is calculated for two varying experimental functions, i.e. K_m and K'. Fiducial limits are therefore placed on the value of K_i by taking the two variables into account in accordance with the usual statistical practice (Saunders & Fleming, 1957).

EXPERIMENTAL

Materials. L-Histidine was obtained from L. Light and Co. Ltd., Colnbrook, Bucks. L-Monoiodohistidine was prepared by the method of Brunings (1947).

Methods. The velocity of transport of L-histidine and L-monoiodohistidine at various substrate concentrations was studied across the small intestine of male Wistar rats (weight approx. 150g.) by using an open-sac method based on that described by Crane & Wilson (1958). For each experiment four sacs, numbered I–IV, were prepared from each animal. The sacs were each 7cm. long. Sacs I and II were prepared from the small intestine just above its mid-point, and sacs III and IV from just below the mid-point. The four sacs from any one animal were filled with solutions of the substrate of different concentrations c_1–c_4. By using four animals the concentrations (c_1–c_4) were randomized with respect to position (I–IV) by using a Latin-square design. The mucosal and serosal solutions were initially of the same concentration and were prepared with Krebs & Henseleit (1932) bicarbonate saline, pH 7·2. The incubations were carried out at 37° and O_2+CO_2 (95+5) mixture was continuously bubbled through the mucosal solutions during the experiment.

The calculation of the rate of transport of the amino acids was complicated because fluid was transported from the mucosal to the serosal compartments during the experiment, and the volume of samples removed for analysis was not negligible compared with the total volume of the serosal solution.

The amount of fluid transported at any given time was estimated from a graph of fluid transport/g. initial wet wt. of tissue plotted against time. This graph was constructed from the results of separate experiments in which the weights of a large number of individual sacs plus their contents were measured as a function of time. The rate of fluid transport was found to be unaffected by the presence of L-histidine, and this was assumed to apply also to L-monoiodohistidine.

In studies of amino acid transport, four samples (each 0·05ml.) were withdrawn from the serosal solution at 10min. intervals after an initial 20min. incubation period during which no samples were taken. This period had been found sufficient to allow for the 'lag' that occurs in the transport of a solute from mucosal to serosal solution. The concentrations of histidine and monoiodohistidine in the samples were measured by a fluorimetric method similar to that described by Shore, Burkhalter & Cohn (1959) for the assay of histamine. Since the initial volume of serosal solution, the rate of fluid transport and the volume of solution removed for assay were known, the total amount of amino acid transported into the sac at the time of removal of each sample was estimated. The total amount transported was then plotted against time. The slope of the best straight line through these points gave the rate of transport in μmoles/min. This was divided by the initial wet weight of the sac to obtain the rate of transport in μmoles/g. initial wet wt. of tissue/min.

The volume of the mucosal solution used in these experiments was 50ml., so that the concentration of amino acid in the mucosal solution remained effectively constant during the experiments.

RESULTS AND DISCUSSION

The experimental results are summarized in Table 1. From eqn. (2), if $V_{max.}$ is the only source of variance then a plot of the standard deviation of $1/v$ against $1/c$ should give a straight line. Such a plot is shown in Fig. 2 for the initial velocities obtained experimentally with histidine. Eqn. (3) predicts that when the major variance is due to $V_{max.}$ then a plot of the standard deviation of $\log(1/v)$ against $1/c$ should give a straight line of zero slope and with an intercept equal to the standard deviation of $\log(1/V_{max.})$. Fig. 2 shows that for such a logarithmic plot of the same initial velocities of histidine

Table 1. *Velocity of transport for* L-*histidine and* L-*monoiodohistidine at different substrate concentrations*

Results are given as means ± S.E.M. The numbers of determinations are in parentheses.

Compound	Concn. (mM)	Velocity (μmole/g. initial wet wt./min.)
L-Histidine	0·25	0·025±0·002 (8)
	0·50	0·044±0·006 (8)
	1·00	0·070±0·004 (8)
	2·00	0·098±0·010 (8)
L-Monoiodohistidine monohydrochloride	0·25	0·062±0·008 (4)
	0·50	0·073±0·007 (4)
	1·00	0·287±0·033 (4)
	2·00	0·400±0·071 (4)

transport the points are scattered randomly about a straight line of zero slope. This random scatter represents the variation in the results due to errors in experimental technique and to the use of small samples. The results of both plots in Fig. 2 support the idea that the major variation in a determination of K_m, in which mean results are used that have been obtained from different pieces of tissue, is due to variations in $V_{max.}$. This variation is removed by the logarithmic method.

The results given in Table 1 were used to estimate the transport parameters of the system by using the Lineweaver–Burk method with and without weighting coefficients and the logarithmic method. With simulated results, in which only $V_{max.}$ is varied, the mean value for K_m calculated by each of the three methods is the same, but the fiducial limits on the value of K_m estimated by the logarithmic method are lower. However, with real experimental results this is not necessarily so, as is shown in Table 2 for L-monoiodohistidine. Here a value for K_m with quite narrow fiducial limits was obtained by using the Lineweaver–Burk plot with weighting coefficients, but a value of K_m with very wide limits was obtained by using the ordinary Lineweaver–Burk plot. No value for K_m could be obtained with the logarithmic plot. Application of weighting coefficients to the Lineweaver–Burk plot gives a value of K_m that appears better than is justified by the results. This happens because, in this case, when more importance is placed on the results obtained at high substrate concentrations, a straight line is positioned through the points that has a positive intercept on the ordinate. When, however, the results are plotted by the Lineweaver–Burk method, with equal weight on all points, then the increased importance of the results at low substrate concentrations allows the regression line to cut the ordinate at or below zero. This corresponds to a K_m value of infinite magnitude and means that the experiments were probably carried out under conditions of low saturation, so that the transport followed diffusion kinetics (see, e.g., Wilbrandt & Rosenberg, 1961). This is reflected in the failure of the logarithmic method to give a value of K_m under such conditions, although a value of $K_m/V_{max.}$ assuming low-saturation conditions can be estimated.

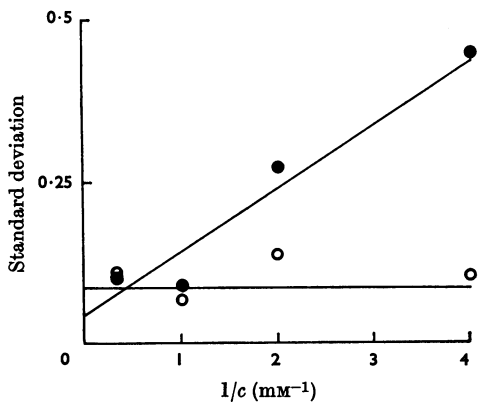

Fig. 2. Increase in standard deviation of $1/v$ with increasing $1/c$ (●). The standard deviation of $\log(1/v)$ plotted against $1/c$ remains constant (○). The points were obtained experimentally for L-histidine.

It is concluded that the logarithmic method for estimating transport parameters is generally applicable to systems obeying Michaelis–Menten kinetics. This method is particularly useful when transport rates are measured on different pieces of tissue, since $V_{max.}$ may then be the major source of variance.

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Table 2. *Kinetic parameters (with 95% confidence limits) for L-histidine and L-monoiodohistidine calculated by the 'logarithmic' method and the Lineweaver–Burk method applied with and without weighting coefficients*

	K_m (mM)	$V_{max.}$ (μmole/g. initial wet wt./min.)
(i) L-Histidine:		
log plot	1·50 (0·72–2·28)	0·169 (0·119–0·240)
Lineweaver–Burk (weighting)	1·55 (1·01–3·36)	0·171 (0·126–0·263)
Lineweaver–Burk (no weighting)	1·59 (0·88–8·50)	0·171 (0·103–0·502)
(ii) L-Monoiodohistidine:		
log plot	No value	No value
Lineweaver–Burk (weighting)	0·77 (0·46–2·39)	0·440 (0·328–0·668)
Lineweaver–Burk (no weighting)	3·79 (0·09–∞)	0·854 (0·217–∞)

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