simple rules. These rules, which are identical in form with those already worked out for oxido-reductions and phosphorylations (Dixon, 1949), enable deductions about the nature of the enzyme-substrate or enzyme-inhibitor combination and the ionization constants of the groups involved to be drawn from the effect of pH upon the affinities.

I am grateful to Dr R. K. Morton for allowing me to quote his unpublished experiments on phosphatase.

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The Determination of Enzyme Inhibitor Constants

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The Michaelis constant, K_m, that is to say the equilibrium constant of the reversible combination of an enzyme with its substrate, is most conveniently determined by Lineweaver & Burk's (1934) method of plotting. The great advantage of this method is that by plotting $1/v$ against $1/s$ it makes it possible to represent the Michaelis equation

$$v=\frac{Vs}{K_m+s} \qquad (1)$$

by a straight line. Here v is the reaction velocity with the substrate concentration s, and V is the maximum velocity obtained at high substrate concentrations. Lineweaver & Burk calculate K_m from the slope of the line and its intercept on the vertical axis, but there is a simpler method which they do not mention, namely to produce the line to the left of the vertical axis, when it will cut the base-line at a point giving $-1/K_m$, as shown in Fig. 1. This is easily shown by putting $1/v=0$ in the reciprocal form of equation (1), namely

$$\frac{1}{v}=\frac{K_m}{V}\frac{1}{s}+\frac{1}{V},$$

which then gives $1/s=-1/K_m$.

The inhibitor constant, K_i, that is to say the equilibrium constant of the reversible combination of the enzyme with a competitive inhibitor, has hitherto usually been obtained by calculation from the Michaelis equation for a competitive system

$$v=\frac{Vs}{K_m\left(1+\frac{i}{K_i}\right)+s}, \qquad (2)$$

where i is the inhibitor concentration. For this calculation the effect on the velocity of varying independently both s and i must be determined.

There is, however, a simple graphical method of determining K_i which, as far as the writer is aware, has not been described previously. If $1/v$ is plotted against i, keeping s constant, a straight line will be obtained, and if this is done at two different substrate concentrations s_1 and s_2 the lines will cut one another at a point on the left of the vertical axis, as shown in Fig. 2. This point lies at $-K_i$, which can therefore be read off directly.

The proof is as follows. Each line represents the reciprocal form of equation (2), namely

$$\frac{1}{v}=\frac{K_m}{Vs}+\frac{1}{V}+\frac{K_m}{Vs}\frac{i}{K_i}. \qquad (3)$$

At the point of intersection $1/v$ and i will be the same for both lines, as also will V since the inhibition is competitive. Therefore

$$\frac{K_m}{s_1}+1+\frac{K_m}{s_1}\frac{i}{K_i}=\frac{K_m}{s_2}+1+\frac{K_m}{s_2}\frac{i}{K_i},$$

or

$$\frac{1}{s_1}\left(1+\frac{i}{K_i}\right)=\frac{1}{s_2}\left(1+\frac{i}{K_i}\right).$$

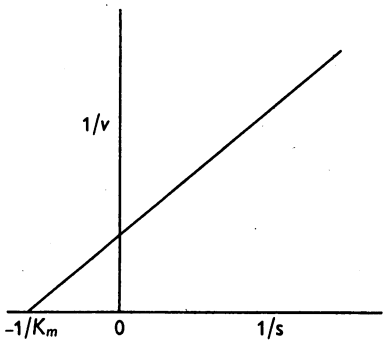

Fig. 1. Determination of K_m.

This can only be true if either $s_1=s_2$ or $i=-K_i$.

If desired, K_m can also be determined from the same plot when K_i has been found, for each line cuts the base-line at a value of i equal to $-K_i(s/K_m+1)$.

Alternatively, if K_m has already been determined by a Lineweaver plot in the absence of inhibitor, it is only necessary to carry out inhibition experiments at one substrate concentration. The intersection point giving K_i lies at a height of $1/V$, as may be seen by putting $i=-K_i$ in equation (3), and this quantity will already have been given by the Lineweaver plot (intersection with the axis for $1/s=0$). It is therefore only necessary to draw a horizontal line at a height of $1/V$, and the point where it intersects the inhibitor line will give $-K_i$. This procedure, however, must only be used for competitive cases.

The case of a non-competitive inhibitor is shown in Fig. 3. Here the lines do not cross, but they meet at a point on the base line which again gives $-K_i$. This is easily seen on putting $1/v=0$ in the reciprocal non-competitive equation

$$\frac{1}{v}=\frac{1}{V}\left(1+\frac{K_m}{s}\right)\left(1+\frac{i}{K_i}\right). \qquad (4)$$

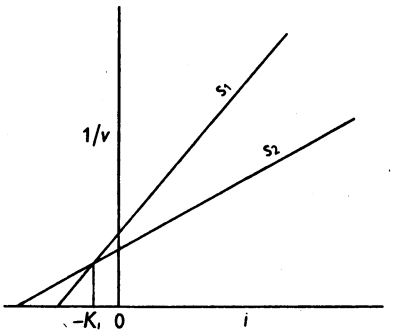

Fig. 2. Determination of K_i (competitive inhibition).

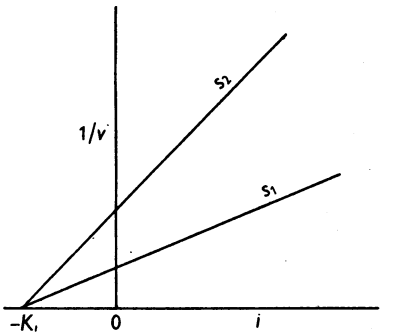

Fig. 3. Determination of K_i (non-competitive inhibition).

SUMMARY

A simple graphical method for determining enzyme inhibitor constants is described.

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