## Kinetics of histaminase

SIR,—Spencer (1963) outlined a method for the determination of relative concentrations of histaminase. In his experimental work he found that the plot of $\log_{10}(x_0/x_t)$ against t is not strictly linear ($x_0$ initial concentration of substrate, $x_t$ concentration at time t). If the reaction involved is of the form:

$$S + En \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S.En \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P.En \underset{k_6}{\overset{k_5}{\rightleftharpoons}} En + P$$

where S denotes substrate molecule, P product molecule and En enzyme molecule, it can be shown that $-1/e.d[S]/dt = (k_a[S]-k_b[P])/(k_c[S] + k_d + k_e[P])$ where $k_a$, $k_b$, $k_c$, $k_d$, and $k_e$ are each composites of some of the velocity constants above, square brackets denote molar concentrations and e is the concentration of enzyme. But $-1/e.d[S]/dt = 1/e.d[P]/dt$ and $[S] = [S_0]$ when $t = 0$, so $[P] = [S_0]-[S]$ and the rate equation takes the form $-1/e.d[S]/dt = (k[S]-k^1[S_0])/([S] + K)$ where $k + k^1$ are constants, and for a particular value of $[S_0]$, K also is constant. The reaction may be effectively irreversible if any of the backward velocity constants are sufficiently small since $k^1$ is directly proportional to $k_2.k_4.k_6$ and hence the rate equation becomes $-1/e.d[S]/dt = k[S]/([S] + K)$ which is formally the same as the Michaelis equation (1913) though the constants have different meanings. Integration and evaluation of the integration constant by insertion of $[S_0]$ for [S] when $t = 0$ gives the equation

$$k\,e\,t = ([S_0]-[S]) + 2{\cdot}303\ K \log_{10}([S_0]/[S]) \quad .. \quad .. \quad .. \quad .. \quad (1)$$

which differs from that used in the original paper solely in the presence of the term $([S_0]-[S])$ and thus accounts for the lack of linearity. The dependance of K on $[S_0]$ except when $k_6 = 0$ may account partially for dependance of the results on $[S_0]$ particularly after long times of incubation.

If $t_\alpha$ is the time at which $\alpha$ % of the substrate has been consumed

$$k\,e\,t-\alpha[S_0] = 2{\cdot}303\ K \log_{10}(1/1-\alpha) = \text{a constant for a given value of } \alpha \quad (2)$$

Thus if $t_{\alpha 1}$, and $t_{\alpha 2}$ are the corresponding times in incubation mixtures containing enzyme concentrations of $e_1$ and $e_2$ respectively but the same initial concentration $[S_0]$ of substrate

$$k\,e_1\,t_{\alpha 1}-\alpha[S_0] = k\,e_2\,t_{\alpha 2}-\alpha[S_0] \text{ or } e_1/e_2 = t_{\alpha 2}/t_{\alpha 1} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

and hence the method of determination in the original paper is theoretically sound despite the non-linearity of the plot used which is purely empirical.

The data of the paper provides a further test of the equations: if two different percentages of consumption are used the direct effect of variations of $[S_0]$ can be eliminated. From (2) for incubation mixtures containing initially $[S_1^0]$ of substrate, $e_1$ of enzyme; and $[S^0_2]$ of substrate and $e_2$ of enzyme.

$$\text{For percentage } \alpha \text{ consumption } k\,e_1\,t_{\alpha 1}-\alpha[S_1^0] = k\,e_2\,t_{\alpha 2}-\alpha[S_2^0] \quad .. \quad .. \quad (4)$$

$$\text{For percentage } \beta \text{ consumption } k\,e_1\,t_{\beta 1}-\beta[S_1^0] = k\,e_2\,t_{\beta 2}-\beta[S_2^0] \quad .. \quad .. \quad (5)$$

whence $\beta \times (4)-\alpha \times (5)$ gives

$$k\,e_1(\beta t_{\alpha 1}-\alpha t_{\beta 1}) = k\,e_2(\beta t_{\alpha 2}-\alpha t_{\beta 2})$$
$$\therefore e_1/e_2 = (\beta t_{\alpha 2}-\alpha t_{\beta 2})/(\beta t_{\alpha 1}-\alpha t_{\beta 1}) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Table 1 gives the application of this equation to the data of Fig. 4 of the original paper.

TABLE 1

| | | | |
|---|---|---|---|
| $[S_0]$, (μg/ml) .. .. .. | 1·53 | 2·01 | 2·49 |
| $\alpha = 50$, $t_\alpha$ (min.) .. .. | 25 | 26 | 27 |
| $\beta = 25$, $t_\beta$ (min.) .. .. | 10 | 10·5 | 11 |
| $(\beta t_\alpha - \alpha t_\beta)$ .. .. .. | 125 | 125 | 125 |
| Ratio of enzyme concentrations (calc.) .. .. .. .. | 1·00 | 1·00 | 1·00 |

Equation (6) may be used also for incubation mixtures containing the same $[S_0]$. Table 2 shows its application to the data in Fig. 3 of the original paper.

TABLE 2

| | | | | |
|---|---|---|---|---|
| Ratio of enzyme concentrations (pre-arranged) .. .. .. | 1·30 | 1·00 | 0·80 | 0·50 |
| $\alpha = 50$, $t_\alpha$ (min.) .. .. .. | 22 | 28 | 34 | 53 |
| $\beta = 25$, $t_\beta$ (min.) .. .. .. | 9 | 11·5 | 14 | 22 |
| $(\beta t_\alpha - \alpha t_\beta)$ .. .. .. .. | 100 | 125 | 150 | 225 |
| Ratio of enzyme concentrations (calc.) | 1·25 | 1·00 | 0·83 | 0·55 |

In practice using equation (6) to determine relative concentrations of enzyme would require greater experimental accuracy than keeping $[S_0]$ constant and using equation (3). There is a trend of deviation in the results of the calculations in Table 2 and in those of Table 1 of the original paper which may be due to a dependence of K on $[S_0]$ so that equation (2) is not strictly equal to a constant: the effect is rather greater when (6) is used. This can be allowed for by replacing K by $(K - \theta[S_0])$, where $\theta$ is a constant, in equation (2) with the result that equation (3) becomes $(e_1 + \psi[S_0])/(e_2 + \psi[S_0]) = t_{\alpha 2}/t_{\alpha 1}$, where $\psi$ is a constant. Substituting the data of Table 1 of the original paper for incubation mixtures A and B ($e_2 = 1{\cdot}30$ inits, $e_1 = 1{\cdot}00$ units, $t_{\alpha 2} = 22$, $t_{\alpha 1} = 28$, $\alpha = 50$) gives $\psi[S_0] = 0{\cdot}10$. Thus, for another incubation mixture using the same $[S_0]$, e may be calculated in the same units from $(e_1 + 0{\cdot}10)/(e + 0{\cdot}10) = t_\alpha/t_{\alpha 1}$ or, in this case $e = (30{\cdot}8/t_\alpha) - 0{\cdot}10$. This gives $e = 0{\cdot}80$ and $0{\cdot}48$ respectively for incubation mixtures C and D of the original paper: they were set up to be $0{\cdot}80$ and $0{\cdot}50$ respectively and the uncorrected calculation gives $0{\cdot}82$ and $0{\cdot}53$ respectively. Thus the accuracy of the results can be improved by using two control incubation mixtures in which the concentrations of the enzyme are in a known ratio.

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## References

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