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ESTIMATION OF MICHAELIS CONSTANT AND MAXIMUM VELOCITY FROM THE DIRECT LINEAR PLOT

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Summary

When estimates of Michaelis-Menten parameters are obtained from kinetic observations taken in pairs, as in the direct linear plot, bias can arise in the final estimates if any pairs lead to negative values of the maximum velocity V. This bias can be removed by treating such negative values as if they were large and positive, and by treating the corresponding values of K_m in the same way.

The direct linear plot [1] provides a method of estimating the parameters of the Michaelis-Menten equation that not only avoids all calculation but is also only weakly dependent on implied statistical assumptions [2,3]. It has been found useful in widely different branches of biochemistry [4—8], and is capable of being applied to models more complex than simple saturation phenomena [9]. However, it has become clear that appreciable improvement on the original method is possible without loss of its essential simplicity. In practice intersections sometimes occur in the third quadrant of the direct linear plot, i.e. they give negative estimates of both K_m and V, and these can lead to bias in the final estimates if taken at face value. This bias can be removed by treating $1/V$ and K_m/V as the primary parameters of the Michaelis-Menten equation, as we shall show in this paper.

Theory

Consider two observations (s_i, v_i) and (s_j, v_j) such that s_i, s_j, v_i and v_j are all positive and s_j is greater than s_i, and assume that apart from experimental error each observation obeys the Michaelis-Menten equation, $v = Vs/(K_m + s)$, with true parameter values K_m and V that are both positive. There is a single pair of estimates of K_m and V that satisfies both observations exactly [1], given by

$V_{(ij)} = (s_j - s_i)/((s_j/v_j) - (s_i/v_i))$ and

$K_{m(ij)} = s_i((V_{(ij)}/v_i) - 1)$.

With error-free data both expressions would necessarily be both finite and positive, but in practice they need not be either.

The dependence of $V_{(ij)}$ on v_j, for constant s_i, s_j and v_i, is shown in Fig. 1. Initially $V_{(ij)} = 0$ for $v_j = 0$, and it increases smoothly with v_j until $v_j = s_j v_i/s_i$, whereupon it 'flips' from large positive to large negative values and then approaches $-v_i(1 - s_i/s_j)$ smoothly as v_j increases further. Provided that our original assumption is correct, i.e. that the Michaelis-Menten equation is the correct model, and provided that s_i and s_j are sufficiently different that a meaningful intersection point can reasonably be expected, v_j is likely to approach or exceed $s_j v_i/s_i$ only if s_i and s_j are both much smaller than V. In this case the true values of s_i/v_i and s_j/v_j are almost equal and the observed values may be in fact equal or incorrectly ranked (s_i/v_i greater than s_j/v_j). For example, if s_i and s_j are $0.05 \times K_m$ and $0.1 \times K_m$ respectively, $V_{(ij)}$ will be negative if v_i is 2.5% too small and v_j is 2.5% too big. It follows that, although $K_{m(ij)}$ and $V_{(ij)}$ may be poor estimates of K_m and V, they are not

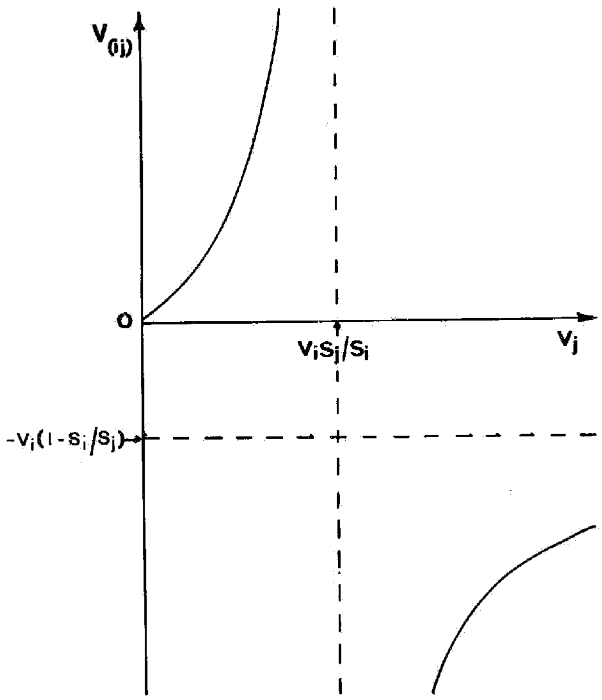

Fig. 1. Dependence of $V_{(ij)}$ on v_j. The estimate $V_{(ij)}$ is defined as $V_{(ij)} = (s_j - s_i)/((s_j/v_j) - (s_i/v_i))$, and is plotted against v_j for arbitrary constant values of s_i, s_j and v_i.

empty of information as they indicate that K_m is much larger than s_j and that V is much larger than v_i and v_j. So in finding the median estimate of V one should treat negative $V_{(ij)}$ values as very large positive estimates of V. The interpretation of negative $K_{m(ij)}$ values is a little more complex.

If $V_{(ij)}$ is negative, $K_{m(ij)}$ must also be negative. So, for the reason we have just considered, a negative $K_{m(ij)}$ value should be treated as a very large positive estimate of K_m if the corresponding $V_{(ij)}$ value is also negative. However, $K_{m(ij)}$ can also be negative in quite different circumstances when the corresponding $V_{(ij)}$ is positive, and in this case $K_{m(ij)}$ should be treated as a small or negative value of K_m. This is because negative $K_{m(ij)}$ values can occur if s_i and s_j are both large compared with the true value of K_m; then the true values of v_i and v_j are almost equal and the observed values may be in fact equal or incorrectly ranked (v_i greater than v_j). So a negative $K_{m(ij)}$ value with a corresponding positive $V_{(ij)}$ value indicates that the true value of K_m is much smaller than s_i and that the true value of V is of the same order of magnitude as $V_{(ij)}$.

These complexities may be avoided entirely by replacing K_m and V as primary parameters of the Michaelis-Menten equation with $1/V$ and K_m/V, because $1/V_{(ij)}$ and $K_{m(ij)}/V_{(ij)}$ show no discontinuities when plotted against positive v_i or v_j but are instead smooth monotonic functions over the whole physically meaningful range. Accordingly, if the direct linear plot is drawn as a plot of $1/V$ against K_m/V the interpretation is simple and no special rules are required. In such a plot, which is illustrated in Fig. 2, each observation is represented as a straight line with intercepts $1/v$ on the $1/V$ axis and s/v on the K_m/V axis.

One can arrive at essentially the same conclusion in a different way by considering the effects of errors in v_i and v_j on the values of $1/V_{(ij)}$ and $K_{m(ij)}/V_{(ij)}$. To simplify the discussion we shall use the term "median-unbiased" [11] to describe any quantity for which the experimental error is as likely to be positive as to be negative. If v_i and v_j are both median-unbiased, with errors that are not so large as to make either of the observed values negative, and if s_i and s_j are error-free, then both s_i/v_i and s_j/v_j are median-unbiased. So also is their difference and hence also $1/V_{(ij)}$, which is simply $((s_j/v_j) - (s_i/v_i))$ divided by a known quantity, $(s_j - s_i)$. Thus, by adding the very weak assumption that the absolute errors in v_i and v_j do not exceed 100% to the ordinary non-parametric assumption of median-unbiased observations, one can show that each $1/V_{(ij)}$ value is median-unbiased. Consequently the median of all the $1/V_{(ij)}$ must be a median-unbiased estimator of $1/V$. One can apply a similar argument to show that the median of all the $K_{m(ij)}/V_{(ij)}$ is median-unbiased. One cannot, however, apply similar arguments to $V_{(ij)}$, $K_{m(ij)}$, $V_{(ij)}/K_{m(ij)}$ or $1/K_{m(ij)}$, because v_i and v_j appear in the denominators of the expressions for all of these parameters; and, in fact, none of them is median-unbiased.

Dr. I.A. Nimmo (personal communication) and Porter and Trager [10] have pointed out an omission from our original description of the direct linear plot [1,2], in that we offered no guidance as to the treatment of replicate observations, with two or more values of v at one value of s. The values of $1/V_{(ij)}$ and $K_{m(ij)}/V_{(ij)}$ obtained from duplicate observations are infinite (or

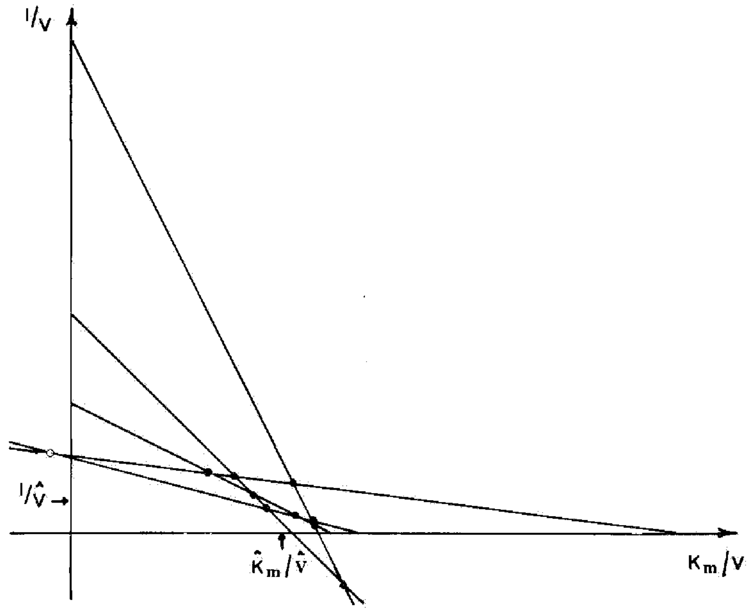

Fig. 2. Direct linear plot of $1/V$ against K_m/V. Each observation is plotted as a straight line with intercept $1/v$ on the $1/V$ axis and s/v on the K_m/V axis. Each intersection point provides an estimate of K_m/V and an estimate of $1/V$. The best-fit values $\hat{K}_m/\hat{V}$ and $1/\hat{V}$ are taken as the medians of the two series respectively, and are indicated on the axes. Intersection points that do not occur in the first quadrant (○ ▼) require no special treatment.

indefinite if the v values happen to be equal). They therefore have no sign and provide no information about the magnitudes of $1/V$ and K_m/V. This is entirely reasonable, as one cannot estimate the parameters of a two-parameter equation from observations at a single value of the independent variable. However, each $K_{m(ij)}$ obtained from duplicate observations has the value $-s_i$, and each corresponding $V_{(ij)}$ has the value zero. Thus although they purport to provide information about K_m and V the information is spurious and leads to bias if accepted at face value. In practice we have always omitted such estimates from the determination of the medians, and this is also true of the computer program that we have circulated [2].

The main conclusion to be drawn from this analysis is that better estimates of the Michaelis-Menten parameters can be expected if $1/V$ and K_m/V are estimated first, and other parameters, such as V and K_m, are calculated from them. The most direct way of achieving this is by the method shown in Fig. 2, in which each observation is drawn as a straight line with intercepts $1/v$ on the $1/V$ axis and s/v on the K_m/V axis. The median estimates of $1/V$ and K_m/V can then be found as described previously for K_m and V [1]. This plot has the added advantage over the original direct linear plot that most of the intersection points appear without extrapolation of the lines representing the observations.

The plot of $1/V$ against K_m/V requires a small amount of calculation, but this can be avoided by using the original plot of V against K_m [1], with a slight modification to the interpretation of intersection points in the third quadrant: these should be regarded as giving very large positive estimates of both V and K_m, not negative estimates. In effect one must regard the co-ordinates of points in the third quadrant as 'beyond infinity' rather than as 'below zero'. Intersections in the second quadrant should be taken at face value. The procedure is easier to apply than to describe in the abstract, and is illustrated in Fig. 3.

The proposal that negative numbers should in some contexts be treated as 'beyond infinity' rather than as 'below zero' may seen novel and objectionable. However, there is a well-established precedent in thermodyanics: when negative absolute temperatures occur in nuclear spin systems they are not cold but very hot, in the sense that they lose heat to systems at positive absolute temperatures [12,13]. For example, Purcell and Pound [12] observed such a system cooling from −350 K to +300 K, via infinite temperature, not via absolute zero.

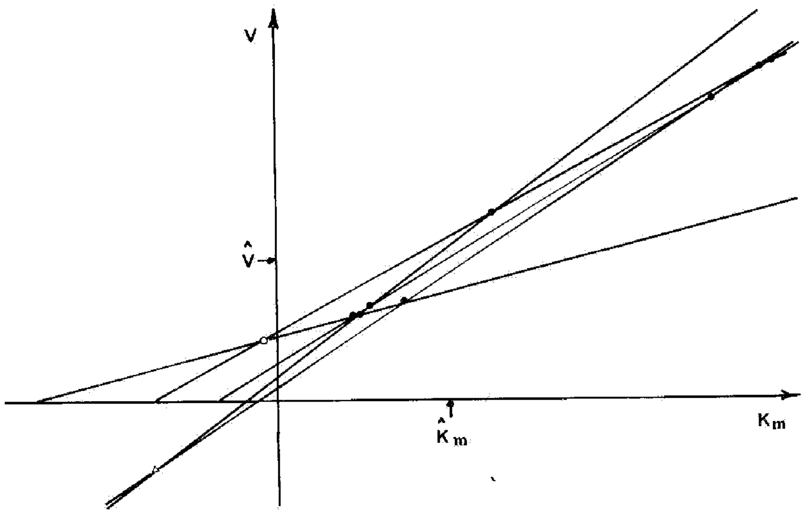

Fig. 3. Direct linear plot of V against K_m. The same observations as in Fig. 2 are shown but are plotted according to the procedure of Eisenthal and Cornish-Bowden [1]. In finding the median estimates the intersection point in the second quadrant (○) is taken at face value, but the intersection point in the third quadrant (▽) is treated as giving very large positive estimates of both K_m and V. The medians found with this convention are indicated on the axes as $\hat{K}_m$ and $\hat{V}$.

References

1 Eisenthal, R. and Cornish-Bowden, A. (1974) Biochem. J. 139, 715—720
2 Cornish-Bowden, A. and Eisenthal, R. (1974) Biochem. J. 139, 721—730
3 Atkins, G.L. and Nimmo, I.A. (1975) Biochem. J. 149, 775—777
4 Ljones, T. and Flatmark, T. (1974) FEBS Lett. 49, 49—52
5 Brooks, C.J.W. and Smith, A.G. (1975) J. Chromatogr. 112, 499—511
6 Debnam, E.S. and Levin, R.J. (1975) J. Physiol. 252, 681—700
7 Simon, J.R., Atweh, S. and Kumar, M.J. (1976) J. Neurochem. 26, 909—922
8 Browne, C.A., Campbell, I.D., Kiener, P.A., Phillips, D.C., Waley, S.G. and Wilson, I.A. (1976) J. Mol. Biol. 100, 319—343
9 Lenk, W. (1976) Biochem. Pharmacol. 25, 997—1005
10 Porter, W.R. and Trager, W.F. (1977) Biochem. J. 161, 293—302
11 Bradley, J.V. (1968) Distribution-free Statistical Tests, pp. 20—22, Prentice-Hall, Englewood Cliffs, N.J.
12 Purcell, E.M. and Pound, R.V. (1951) Phys. Rev. 81, 279—280
13 Hecht, C.E. (1967) J. Chem. Educ. 44, 124—127