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Abrupt transitions in kinetic plots: an artifact of plotting procedures

Kinetic data for enzymes that do not obey Michaelis–Menten kinetics have often been displayed in ways that suggest abrupt transitions between straight portions of double-reciprocal plots or other plots that are straight in the Michaelis–Menten case, a notable example being provided by glutamate dehydrogenase (EC 1.4.1.3) from cattle liver (Engel & Dalziel, 1969). Taken literally, such plots indicate that the first derivative of the rate with respect to the substrate concentration is a discontinuous function of the substrate concentration, an interpretation that raises serious difficulties for the underlying physical causes of the behaviour. It seems preferable, therefore, unless the existence of a saltus in the derivative is overwhelmingly demonstrated, to interpret the appearance of one as an optical illusion that is strongly reinforced by drawing a pair of straight lines (which are, of course, not data but an interpretation placed on data) on a kinetic plot [see Fig. 3 of Cornish-Bowden & Koshland (1975)].

Presenting new data for glutamate dehydrogenase of *Clostridium symbiosum*, Syed & Engel (1987) have recently reasserted the reality of saltuses in kinetic data, dismissing earlier doubts about them (Cornish-Bowden & Koshland, 1975; Bardsley, 1977) as being 'essentially on semantic grounds'. Semantic considerations are not, however, the primary source of difficulty in accepting the reality of saltuses; moreover, the data presented by Syed & Engel (1987) fit a single saltus-free model almost as well as they fit a pair of straight lines.

The relationships between different plots of kinetic data have been thoroughly explored by Bardsley and co-workers (e.g. Bardsley & Childs, 1975). Here it will be sufficient to note that the slope of an Eadie–Hofstee plot is defined if the slope of a plot of rate against substrate concentration is defined, and vice versa. A saltus in the slope of an Eadie–Hofstee plot therefore requires a saltus in the first derivative of rate with respect to substrate concentration, as assumed implicitly above. This is, however, impossible for any system in which the rate is a rational function of the substrate concentration with all denominator terms positive, i.e. for any rate equation that can be derived by the method of King & Altman (1956). It follows, therefore, that if a saltus exists the system cannot be in a true steady state or some other requirement for a valid steady-state analysis is not met.

This point is not semantic and needs to be discussed by anyone proposing the existence of a saltus in kinetic data. The only semantic aspect lies in the meaning attached to a word like 'simpler' as applied to kinetic behaviour: is behaviour 'simpler' if it can be explained by reference to a well-defined and plausible model but requires a curve for its expression on an Eadie–Hofstee plot, or is it 'simpler' if it is easy to represent by an exercise in ruler-and-pencil geometry but has no basis in the known laws of chemistry?

Data for glutamate dehydrogenase from *Clostridium symbiosum* were plotted in Fig. 1 of Syed & Engel (1987) assuming Michaelis–Menten kinetics with limiting rate $V = 19$ and Michaelis constant $K_m = 0.25$ at NAD^+ concentrations (s) less than 0.06, but with an abrupt change to $V = 5.5$, $K_m = 0.0289$ at s greater than 0.06. (Rates are in μM/min per μg enzyme, and concentrations are in mM, but as units complicate the discussion to no purpose they will be omitted.) Reading the co-ordinates of the 27 data points from their graph one can estimate a root-mean-square relative deviation of 4.6%. As plotted originally, there is an obvious saltus at $v/s = 60$, $v = 36$, i.e. at $s = 0.06$ (referred to twice in their paper as $s = 0.04$, but this appears to be a typographical error). However, there are very few observations in the vicinity of the saltus, which can thus readily be interpreted as an illusion created by one or two erratic points on the plot, and the data are consistent with a model generating a smooth curve. In Fig. 1, the curve was calculated as the sum of two Michaelis–Menten expressions with $(V, K_m) = (18.2, 0.333)$ and $(1.60, 0.000941)$. It does not

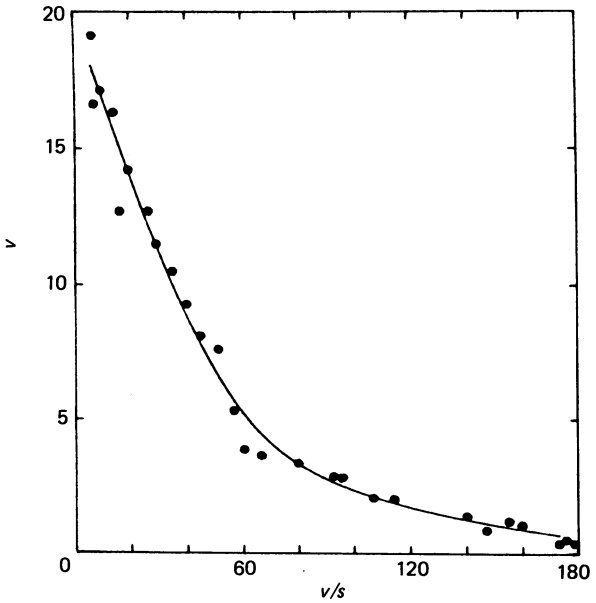

Fig. 1. Eadie–Hofstee plot for glutamate dehydrogenase

The experimental points are replotted from Syed & Engel (1987), but the curve is calculated by expressing the rate v as the sum of two Michaelis–Menten terms.

fit quite as well as the pair of straight lines, especially in the vicinity of $v/s = 60$, but with a root-mean-square relative deviation of 5.9% it is by no means poor. More important than the overall fit, however, there is no striking anomaly at $v/s = 60$; i.e. the deviations in this region are of similar magnitude to those found elsewhere in the data.

The simplest model giving a sum of two Michaelis–Menten terms is a mixture of two isoenzymes each obeying Michaelis–Menten kinetics. Here this explanation would be untenable, as the data refer to a purified enzyme available in crystalline form, but very similar behaviour can be obtained from models of negative co-operativity; for kinetic equations of enzymes with several interacting subunits these can be of much higher degree than the 2:2 equation obtained by combining two Michaelis–Menten terms into a single rational function. It would be surprising if higher-degree curves did not fit better than the one shown in Fig. 1, equalling or improving on the fit obtained with a pair of straight lines.

If I am wrong and the saltus in the data for glutamate dehydrogenase from *Clostridium symbiosum* is real it should be a simple matter to obtain much more convincing evidence for it. A set of 27 observations in the immediate vicinity of the postulated saltus (rather than scattered over a 1000-fold range of NAD^+ concentrations) ought to be sufficient.

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Abrupt transitions in kinetic plots: an empirical reality

It is necessary to respond to Cornish-Bowden's critique (1988) of our recent communication (Syed & Engel, 1987) on abrupt transitions in kinetic plots on two levels. First of all there is the specific question of the data for glutamate dehydrogenase from *Clostridium symbiosum*. Here there is apparent agreement that, in the limit, two different values are required both for K_m and V in order to explain the data. In physical terms, the consequent question is whether (*a*) there is a single population of enzyme molecules which together, over a range of [S], alter their kinetic properties, or (*b*) there are two coexisting populations with very different kinetic parameters. As Cornish-Bowden concedes, the latter postulate is in itself not a particularly obvious model for a homogeneous enzyme preparation consisting of only one type of polypeptide subunit. The crystallography has not as yet indicated any inherent asymmetry within the enzyme hexamer that could explain two apparently different kinetic populations (Baker *et al.*, 1987). Nevertheless, the enzyme does undergo a pH- and time-dependent conformational change (Syed & Engel, 1986), and conceivably a suitably poised slowly adjusting conformational equilibrium could provide a physical basis for the model used by Cornish-Bowden to fit our experimental points.

As to the fit itself, Cornish-Bowden concedes that the R.M.S. deviation is significantly poorer than for our own plot, but equally we must agree that our own case rests too heavily on a small cluster of critical data points. More detailed measurements over the crucial NAD^+ concentration range will be carried out.

Secondly, however, there is the general issue of abrupt transitions, and here we must insist that the disagreement is indeed semantic. It revolves around the meaning, not of the word 'simpler', as Cornish-Bowden suggests, but of the word 'abrupt'. Cornish-Bowden attributes to our use of the phrase 'abrupt transition' a meaning that was never intended, namely that the data show a total discontinuity, with a substantial change in $dv/d[S]$ in response to an infinitesimal change in [S]. Evidently, such a suggestion would violate stochastic principles. What we attempted to highlight (Engel & Dalziel, 1969; Syed & Engel, 1987) were shape features in kinetic plots (either Lineweaver–Burk or Eadie–Hofstee) for glutamate dehydrogenase that appeared to go beyond what most workers would accept as a 'smooth curve': to a first approximation these curves were made up of what we termed 'pseudo-linear sections'. These sections inevitably are linked by curved sections, but the essence of the abruptness lies in the fact that obvious curvature is confined to a relatively narrow range of [S].

In response to the comments about incompatibility with rational functions, and about "an exercise in ruler-and-pencil geometry", we can do no more than refer to the paper of Engel & Ferdinand (1973) which explored in some detail the mathematical (and, by implication, physical) conditions required to give rise to these curve shapes – without the need to invoke irrational functions. Cornish-Bowden makes the point for us admirably in asserting that higher-degree equations arising from negative co-operativity would probably give a better fit. This is no more than we have contended all along.

It is thus possible that more detailed experimentation will support Cornish-Bowden in the case of the bacterial glutamate dehydrogenase. Only, however, if he can provide an equally good 'smooth-curve' fit for the bovine enzyme (original data set available on request), together with a plausible physical interpretation, will we feel inclined to dismiss the reality of abrupt transitions – using the phrase in our intended sense rather than