A Comment on the 'Jack-knife' Technique for Analysing Enzyme-Kinetic Data

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The use of the 'jack-knife' technique in the analysis of enzyme-kinetic data [Cornish-Bowden & Wong (1978) *Biochem. J.* 175, 969–976] is examined. The method can give parameter estimates that appear to be incorrect.

The analysis of enzyme-kinetic data usually involves fitting the data to some assumed rate equation by non-linear regression. When the Gauss–Newton method (Duggleby & Morrison, 1977), or a variant of it, is used to perform this analysis, then it is a straightforward task to obtain estimates of parameter values and their asymptotic standard errors. If the rate equation is complex, then two difficulties arise: (1) the computations may involve inversion of a large, ill-conditioned matrix with the result that arithmetic difficulties will dominate the calculation, and (2) there may be high correlations between parameters, and in such circumstances the standard error is not a good guide to the error in a parameter estimate.

In a study of alcohol dehydrogenase, Cornish-Bowden & Wong (1978) found that standard errors calculated by the matrix-inversion method were obviously wrong (such as imaginary values). This difficulty was overcome by using the 'jack-knife' technique (Tukey, 1958; Miller, 1974), and it was suggested that this method may be profitably applied to other enzyme-kinetic analyses. The present paper examines this proposition and demonstrates that the method published by Cornish-Bowden & Wong (1978) can give unlikely estimates of parameter values. Standard errors calculated by this method may be realistic.

Methods and Results

As described by Cornish-Bowden & Wong (1978), the 'jack-knife' technique consists of five stages. (1) The whole data set of n observations is fitted to a particular rate equation to obtain an estimate ($\hat{b}_0$) of each parameter. (2) The data set is divided into g subsets, each containing h observations ($n = gh$). (3) The i th subset of observations is omitted and the remaining data are fitted to obtain a new estimate ($\hat{b}_{-i}$) of each parameter. (4) The natural logarithm of $\hat{b}_{-i}$ is converted into a corresponding pseudo-value ($\tilde{b}_i$) by using the formula:

$$\ln \tilde{b}_i = g \ln \hat{b}_0 - (g-1) \ln \hat{b}_{-i}$$

That is, $\tilde{b}_i$ is chosen such that $\hat{b}_0$ is the weighted geometric mean of $\tilde{b}_i$ and $\hat{b}_{-i}$. (5) A 'jack-knifed' estimate ($\hat{b}_0$) is obtained by taking the geometric mean of the g values of $\tilde{b}_i$ (i.e. the arithmetic mean of $\ln \tilde{b}_i$).

This technique was applied to an artificial set of initial-velocity data ($n = 5$, $g = 5$, $h = 1$), which were fitted to the Michaelis–Menten equation (Table 1). It is difficult to accept that the final value of 0.932 represents the best estimate of the Michaelis constant. Not only does it differ considerably from the best-fit value to all the data (1.022), it is also less than all the estimates ($\hat{b}_{-i}$) obtained from subsets of the data. This result is not due to some peculiarity of the particular data set that is presented in Table 1. Of 342 sets of data that were simulated, the 'jack-knifed' value was outside the entire range of the $\hat{b}_{-i}$ values in 104 sets. Table 1 illustrates another

Table 1. Application of the 'jack-knife' technique to simulated enzyme-kinetic data

Theoretical velocities were calculated from the Michaelis–Menten equation ($V = 1.0$, $K_s = 1.0$) at each of five substrate concentrations (A_i) and a random error equal to 3% of V was applied to each theoretical velocity to give the simulated velocities (v_i). The data were fitted to the Michaelis–Menten equation by non-linear regression (Wilkinson, 1961) to give the least-squares estimate of the Michaelis constant ($\hat{b}_0$). Five subsets of data were formed by omitting each data point in turn, and each subset was analysed to give a new estimate of the Michaelis constant ($\hat{b}_{-i}$). The 'jack-knife' technique was applied to these values as described in the text to give the pseudo-values ($\tilde{b}_i$) and their geometric mean ($\hat{b}_0$). The results presented represent one of 342 such simulations.

Parameter	$i \dots$	0	1	2	3	4	5
A_i	—	0.25	0.5	1.0	2.0	4.0	
v_i	—	0.190	0.312	0.512	0.685	0.787	
$\hat{b}_{-i}$		1.022	0.999	0.946	1.066	1.019	1.219
$\tilde{b}_i$		0.932	1.119	1.392	0.863	1.034	0.505

shortcoming of the 'jack-knifed' estimate of the Michaelis constant: it is further from the true value of 1.0 than is the least-squares estimate. Again, this property is not confined to this particular set of data, as it was observed in 61% of the simulated experiments.

Discussion

The example presented in Table 1 was selected to demonstrate a result that is obviously incorrect, but similar results may be found in the data of Cornish-Bowden & Wong (1978). Thus the value that they calculate for the rate constant k_{-3} (4.044) is greater than any of the $\hat{b}_{-i}$ values from which it is derived ($\hat{b}_0 = 3.996$; $\hat{b}_{-1} = 4.003, 3.987, 3.911, 3.997, 4.031$ and 3.992). Similarly, the value for k_{-4} is less than any of the corresponding $\hat{b}_{-i}$ values. The original purpose of the 'jack-knife' technique was to eliminate bias in parameter estimation (Miller, 1974), but it is by no means obvious that there is bias in parameters that are estimated by non-linear regression. There would not appear to be any validity in introducing a technique to eliminate a bias for which there is no evidence.

The 'jack-knife' technique may be useful in problems of interval estimation, such as calculating standard errors of parameters. Cornish-Bowden & Wong (1978) found that the matrix-inversion method gave unlikely results and suggested that the 'jack-knife' technique is superior on the basis that it gave results that were felt to be reasonable. It is not clear whether the failure of the matrix-inversion method was due to arithmetic difficulties that surround the inversion of near-singular matrices, or to some more fundamental problem. In the few cases which have been examined in this laboratory, the 'jack-knife' technique gives values for standard errors that are comparable with those obtained by matrix inversion, but it would seem premature to abandon this latter method until the clear superiority of the 'jack-knife' technique is demonstrated.

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