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Validity of the Jack-knife Technique for Analysing Enzyme Kinetic Data

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An observation by Duggleby [*Biochem. J.* (1979) **181**, 255–256] that estimates of kinetic parameters by the jack-knife technique [Cornish-Bowden & Wong (1978) *Biochem. J.* **175**, 969–976] are sometimes outside the range of estimates from which they are calculated has been examined. No significant correlation has been found between the occurrence of this behaviour and the actual quality of the estimates.

Duggleby (1979) has criticized our suggestion (Cornish-Bowden & Wong, 1978) that the jack-knife technique (Tukey, 1958; Miller, 1974) might provide a valuable statistical method for use in enzyme kinetics, because of its capacity to provide realistic estimates in circumstances where the more usual methods fail. In particular Duggleby (1979) drew attention to a property of the jack-knife that we shall refer to as 'out-of-range behaviour'. The relationship of this property to the reliability of jack-knifed estimates does not seem to have been previously studied, either analytically or by simulation, and we believe that it may be misleading to regard it as evidence of unsatisfactory estimation.

As the operation of the jack-knife has been described in detail both by Cornish-Bowden & Wong (1978) and by Duggleby (1979), we shall not repeat the description here. It will suffice to remark that in the process of estimating a parameter of interest β, an estimate $\hat{b}_0$ is first obtained by some appropriate method (e.g. least squares), using all of the data, together with g additional estimates $\hat{b}_{-i}$ obtained by the same method from the same data with groups of observations omitted in turn. These $(g+1)$ preliminary estimates are then transformed into g 'pseudo-values' $\tilde{b}_i$ and their geometric mean $\tilde{b}_0$, which is taken as the jack-knifed estimate of β. The essence of Duggleby's (1979) criticism is that sometimes the final value $\tilde{b}_0$ is outside the entire range of initial $\hat{b}_{-i}$ estimates. Although this out-of-range behaviour may be worrying, it is not clear that it provides an objective reason for regarding $\tilde{b}_0$ as an unreliable estimate of β.

To determine whether there is any relationship between out-of-range behaviour and the validity of the jack-knife, we have repeated the simulation described by Duggleby (1979). Methods were exactly as given in his paper, and apart from the slight sampling variations that one would expect from the use of a different random-number generator our results were exactly the same as his. We have amplified them, however, by examining whether there is any correspondence between the experiments in which the jack-knifed estimate of K_m was out-of-range and the experiments in which it was further from the true value of K_m than the corresponding least-squares estimate. The results are shown in Table 1 as a two-way classification table. In 215 out of 342 simulated experiments the jack-knifed estimate was further from the true value than the least-squares estimate, and in 73 (34%) of these 215 it was out-of-range; in the remaining 127 experiments in which the jack-knifed estimate was the

Table 1. *Out-of-range behaviour of the jack-knife*
In this study 342 experiments were simulated for an enzyme obeying the Michaelis–Menten equation with true parameter values $V = 1.0$, $K_m = 1.0$. In each experiment Gaussian errors with mean zero and standard deviation 0.03 were added to the rates calculated at substrate concentrations 0.25, 0.5, 1.0, 2.0 and 4.0. In each experiment a least-squares estimate of K_m was obtained by non-linear regression with equal weight assigned to each rate, and a corresponding jack-knifed estimate was obtained in each case as described by Duggleby (1979). Each experiment is classified by rows according to which method gave a K_m estimate closer to the true value of 1.0, and by columns according to whether the jack-knifed estimate of K_m was within the range of the preliminary estimates, less than the minimum of these, or greater than the maximum.

	Within range	Low	High	Total
Least-squares better	142	38	35	215
Jack-knife better	93	24	10	127
Total	235	62	45	342

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better, it was out-of-range in 34 (27%). Although the latter proportion is a little smaller than the former, the difference does not seem large enough to justify regarding out-of-range behaviour as a reason for rejecting the jack-knifed estimate: certainly it provides no guarantee that it is a worse estimate than the corresponding least-squares one. A more rigorous analysis of the results in Table 1 confirms that out-of-range behaviour provides no information about the relative qualities of the two estimates. A chi-square test carried out by standard methods (e.g. Ott, 1977) gives $\chi^2 = 4.952$, which is less than the tabulated value of 5.991 for $\alpha = 0.05$ with 2 degrees of freedom. One cannot therefore reject the hypothesis that the row and column variables are independent.

In 60% of our simulated experiments and those of Duggleby (1979), the jack-knife gave an estimate of K_m that was further from the correct value than the least-squares estimate. Thus the jack-knife is not on average a better method than least squares under the conditions defined by Duggleby (1979) and used also in the present study. This is not surprising, however, as the least-squares method is known to be optimal in the conditions used for the simulations. It is therefore perhaps more surprising that it should give better results in as few as 60% of experiments, a proportion similar to that seen in comparisons of the least-squares method with another sub-optimal method, the direct linear plot (Cornish-Bowden & Eisenthal, 1974).

An important advantage of the jack-knife in some circumstances is its capacity to eliminate or at least to decrease bias in fitted parameters. As Duggleby (1979) rightly remarks, however, this is not a persuasive reason for using it when there is no reason to expect the least-squares method to introduce perceptible bias. In fitting the Michaelis–Menten equation, for example, it is difficult to detect any bias introduced by the least-squares method. In our simulation, the means and standard deviations of the two K_m estimates were 1.007 ± 0.151 and 1.002 ± 0.191 for the least-squares and jack-knifed values respectively, the true values being 1.000: in neither case is there significant bias. Our original concern (Cornish-Bowden & Wong, 1978) was with a much more complicated model than the Michaelis–Menten equation, however, for which no thoroughly evaluated methods were or are available. In such cases the bias-decreasing power of the jack-knife may be at least as valuable as its ability to provide convenient and realistic precision estimates.

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