represents a particular substrate concentration. The displacements are indicated by the arrows in Fig. 2*A* and 2*B*, where the dotted lines show the zone in which the experimental points are found when subject to a maximum error of ±5 per cent. It may be noted that in the v versus v/S plot this zone runs parallel to the theoretical curve in contrast to the inverted plot.

In view of the reasons discussed above, it would seem then that there is no real basis for the continued use of the inverted plots.

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There seems to be some confusion here between two things which are in reality quite distinct, namely, (*a*) the choice of the series of substrate concentrations which will give the best results, and (*b*) the best method of plotting the results obtained. These are largely independent: one is free to select the best series of concentrations without even knowing which method of plotting will be used, and when the results have been obtained one is free to plot them by either method.

With regard to the choice of substrate concentrations, we have in fact made no recommendation that the series should give equally spaced points on the reciprocal plot (namely, "equal increments of $1/S$"), as Dr. Hofstee seems to imply, nor do we recall any such recommendation by others. On the contrary, our statement that it is advantageous to have a concentration of points near the left-hand side of this plot implies approval of some such series as "equal increments of pS."

With regard to the method of plotting, both methods are of course perfectly valid, and it is our belief, based on experience of plotting results in both ways, that there is not a great deal to choose between them. This is where we differ from Dr. Hofstee, who believes that plot *B* (Fig. 2) is so greatly superior to plot *A* that there is no reason for the continued use of the latter.

The purpose of plotting is twofold: (*a*) to determine K_M and V_m, and (*b*) to check that the system obeys the Michaelis equation ('linearity', that is, of the graphs). By actual use, we find that the two methods are about equally good in both respects; the accuracy of determination of the constants from a given set of results is about the same, and it seems to us that deviations from linearity are revealed almost equally well by the two methods. We venture to think that if the reader will plot a few cases in both ways he will come to the same conclusion.

A main argument for plot *B* seems to be that a series of concentrations of the kind commonly preferred will give a more uniform distribution of points along the straight line than in the case of plot *A*. It does not follow, however, that such a uniform distribution of points will give the most accurate results; for we would point out that the position of a straight line is determined much more precisely by points near its ends than by points near its centre.

Our main reason for preferring plot *A* is that one can readily identify the different points with particular substrate concentrations, and so see what is taking place. This is not the case with plot *B*, which has no scale of substrate concentrations; the quantity which is plotted depends both on the arbitrarily fixed concentration and the resulting observed velocity, so that it is necessary to perform a division sum to discover what substrate concentration corresponds to a given point. Any error in v affects both co-ordinates, displacing the point obliquely. Also rather more calculation is involved in the actual plotting by this method. We think that many workers will continue to use plot *A*, the inverted, or as we would prefer to call it, the reciprocal plot.

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TWO-DIMENSIONAL HIGH-VOLTAGE PAPER ELECTROPHORESIS OF AMINO- AND OTHER ORGANIC ACIDS

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Amino-Acids

It had been demonstrated before[1] that the application of high potential gradients to the electrophoresis of amino-acids leads to sharp separations after comparatively short running times. It was felt, however, that a higher degree of resolution and greater certainty of identification of the separated compounds could be attained by the adoption of a two-dimensional technique, that is, subjecting the sample to electrophoresis on the same sheet under two different *p*H conditions with consequently differing migration patterns.

A two-dimensional technique for the separation of amino-acids has been described by E. L. Durrum[2], who obtained encouraging results with mixtures of