does not fully deplete the enzyme of endogenous nucleotides. We agree with Garrett & Penefsky (1975) that the ATPase activity is constant for long periods (up to 3 weeks) when the enzyme is stored in 50% glycerol even in the absence of ATP. After treatment of ATPase by the Garrett & Penefsky (1975) procedure, reaction of the enzyme (in 50% glycerol/100nm-Tris/H$_2$SO$_4$, pH8.0) with 4-chloro-7-nitrobenzofurazan still led to inactivation of the enzyme after modification of one tyrosine group per enzyme molecule. This observation is of interest since our previous work on this reaction was done in the presence of 4nm added ATP. Clearly the added ATP that was necessary to stabilize the enzyme (Ferguson et al., 1975a) does not cause the asymmetry of the enzyme towards 4-chloro-7-nitrobenzofurazan.

The absorption spectrum of the ATPase in 50% glycerol at pH8.0 showed an unexpectedly strong $A_{240}$, which was absent when the ATPase was dissolved at pH8.0 in a buffer lacking glycerol. A similar increase in $A_{240}$ was observed when lysozyme and bovine serum albumin were transferred into buffers that contained 50% glycerol. These effects were not seen at pH7.5. The absorbance at 240nm can be attributed to ionized phenolic tyrosine groups, as N-acetyltyrosine ethyl ester showed very similar behaviour. Glycerol therefore seems to increase the ionization of the phenolic groups of tyrosines.

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The Nature of the Random Experimental Error Encountered when the Kinetics of Acetylcholine Hydrolase are Determined

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The question of how best to fit the Michaelis–Menten equation to data for the initial velocities of an enzymic reaction can only be answered satisfactorily if the nature and distribution of any experimental error are known (e.g. Eisenthal & Cornish-Bowden, 1974; Atkins & Nimmo, 1975). However, a survey of the literature shows that when most workers estimate Michaelis–Menten parameters they either completely ignore the nature of their experimental error or assume by default that it is normally distributed and of constant magnitude. In fact this is not altogether surprising, because so far as we are aware only Storer et al. (1975) have tried to find out how experimental error really is distributed. They studied two enzymes (glucokinase and x-chymotrypsin) and concluded that in both instances the S.D. of the initial velocity increased with its magnitude. They also concluded that the distribution of error might well be long-tailed (leptokurtic) rather than normal.

We are interested in the enzyme acetylcholine hydrolase (acetylcholinesterase; EC 3.1.1.7), whose kinetic parameters we wish to determine accurately (e.g. Cramb et al., 1974; Atkins & Nimmo, 1975). However, since there was no justification for assuming that the conclusions of Storer et al. (1975) could be extended to acetylcholinesterase as assayed by ourselves, and since our enzyme preparation is sufficiently stable for many replicate measurements to be made with it, we have determined and now describe how the error in our initial-velocity data is distributed.

The enzyme was assayed in haemolysates at 30°C with acetylthiocholine as substrate by using a spectrophotometer linked to a chart recorder, which produced a continuous trace of absorbance against time (Cramb et al., 1976); these traces were essentially

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Table 1. Statistical analysis of initial-velocity data

A total of 75 assays was run at each substrate concentration (see the text). The upper values in each row were obtained by one observer, and the lower ones by the other. The indices of skewness and kurtosis were calculated and their statistical significance was assessed as described by Pearson (1936) and Geary (1936) respectively.

<table>
<thead>
<tr>
<th>Substrate concn. (µM)</th>
<th>Mean angle</th>
<th>Initial velocity (arbitrary units)</th>
<th>Index of</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Skewness</td>
</tr>
<tr>
<td>12.5</td>
<td>28°</td>
<td>0.523</td>
<td>0.049</td>
<td>0.771†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.532</td>
<td>0.051</td>
<td>0.559§</td>
</tr>
<tr>
<td>40</td>
<td>51°</td>
<td>1.128</td>
<td>0.140</td>
<td>-0.138</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.222</td>
<td>0.194</td>
<td>-3.252†</td>
</tr>
<tr>
<td>80</td>
<td>25°</td>
<td>1.394</td>
<td>0.063</td>
<td>1.438†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.424</td>
<td>0.092</td>
<td>0.909†</td>
</tr>
<tr>
<td>200</td>
<td>36°</td>
<td>2.214</td>
<td>0.048</td>
<td>-0.530§</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.220</td>
<td>0.081</td>
<td>-0.033</td>
</tr>
</tbody>
</table>

*, †, ‡ and § denote $P < 0.01, 0.02, 0.05$ and 0.10 respectively that the distribution is normal.

Linear with time for at least 1 min. Initial velocities were determined by drawing straight lines through the traces by eye, measuring their inclination to the horizontal and computing the tangents of these angles. To minimize reading errors the chart speed was adjusted to give slopes of between 25° and 65°. All the assays were performed by the same operator and the traces read by the same two observers throughout. All the assays at a given concentration of substrate were run on a single day with a bulk preparation of enzyme. In no instance did the initial velocity vary with time in an obviously systematic manner.

The results are in Table 1. The following should be noted. (1) At three of the four concentrations of substrate the initial velocities had s.d. values that were similar in size, whereas at the fourth (40 µM) the s.d. value was appreciably larger. (2) One of the observers reading the traces consistently arrived at a slightly greater mean value and substantially greater s.d. than the other. (3) The underlying distributions were not usually normal (Gaussian) in shape and were often asymmetric. They tended to be skewed towards higher values when the angle was small and towards lower ones when it was large. (4) The data cannot be used to test whether or not the enzyme conforms to Michaelis-Menten kinetics because the activity of the enzyme preparation could have varied between days and hence substrate concentrations.

Our conclusions therefore differ from those of Storer et al. (1975) in that our s.d. values seem to be independent of initial velocity, whereas theirs tended to increase with it. On the other hand we agree that the initial velocities are unlikely to be normally distributed, and, moreover, that the shapes of the distributions they actually follow could vary with substrate concentration. These uncertainties underline the appeal of methods for fitting the Michaelis-Menten equation that are relatively insensitive to the way in which the error is distributed, such as the direct linear plot (Eisenthal & Cornish-Bowden, 1974). Finally our results make the eminently predictable point that individuals reading the same 'linear' trace are likely to arrive at slightly but systematically different answers.

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Pearson, E. S. (1936) *Biometrika* 28, 306-307

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