DETERMINATION OF THE SERUM CHOLINESTERASE

BY

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SUMMARY

Some of the variables which influence the results of determination of serum cholinesterase are reviewed. These include substrate concentration, pH and temperature. There is also a considerable variation between different workers in methods of expression of results.

The importance of serum cholinesterase (pseudocholinesterase or acetylcholine acyl-hydrolase) in the elimination of suxamethonium is well known and when a patient develops suxamethonium apnoea his blood is sent to the Biochemistry Department for estimation of pseudocholinesterase. When the result from the laboratory comes back, however, the anaesthetist not infrequently has the greatest possible difficulty in interpreting it. This difficulty does not apply to the other investigations carried out on these patients, namely the determination of the dibucaine and fluoride number. The result in these instances can come in only one form, namely an estimate of percentage inhibition of the activity of the enzyme in hydrolyzing benzoyl choline by the dibucaine or sodium fluoride included in the reaction solution. (The results of both of these determinations are, however, dependent both on the substrate and temperature of the reaction (King and Dixon, 1970).

BASIC CONSIDERATIONS

The essential difficulty in the interpretation of a cholinesterase result springs partly from the nature of enzymatic reaction. The rate of breakdown of the substrate of an enzyme increases progressively to a maximum, as the concentration of the substrate increases. This maximum is temperature-dependent, generally increasing with rise in temperature (King, J., 1970, personal communication). Beyond this maximum further increase in substrate concentration does not increase the velocity of the reaction. (This property is in fact the basis of the Michaelis constant of an enzyme reaction. The Michaelis constant is half the concentration at which maximal acceleration of the reaction occurs.) In general this difficulty is eliminated in laboratory determinations of serum cholinesterase by using concentrations of the substrate, which is usually either acetyl choline or benzoyl choline, substantially in excess of those required to produce maximum activity.

pH effects.

The activity of serum cholinesterase is pH-dependent to some extent at least. (Where the substrate is benzoyl choline its rate of hydrolysis is independent of pH from 6.8 to 8.0 though beyond these limits pH will in fact affect the result. The enzyme has its optimum activity at a pH of 8.5 (King, 1965).)

One of the products of the hydrolysis of acetylcholine is acetic acid. The more this is formed the more the pH of the solution will be reduced and the more the activity of the enzyme will be modified. Some methods of determination of cholinesterase actually depend on assessment of rate of pH change (Biggs, Carey and Morrison, 1958; Johnson and Whitehead, 1965).

Temperature.

Some estimations are carried out at 37°C and others at 25°C. The activity of the enzyme at these two different temperatures is different, though it is possible to produce factors for the conversion of the results at one temperature to those at another. (As already indicated the activity of enzyme inhibitors is also temperature-dependent.)

It therefore follows that apart altogether from the inevitable differences between laboratories due to minor variations in technique, there is also a major source of difference in the results of
cholinesterase determination depending on the effects of pH, temperature of reaction and the substrate on which the enzyme acts.

**EXPRESSON OF RESULTS**

Biochemists do not seem at one about the methods of expression of results of cholinesterase activity. Some express activity in terms of 100 ml of serum; others express activity in terms of 1 ml of serum. Too often this difference is hidden by expressing the result in some form of unit. In the original McArdle (1940) method, for example, 1 unit of cholinesterase activity was represented by the liberation of 1 ml of carbon dioxide (at 37°C) in 1 minute in Warburg apparatus into an atmosphere saturated with water vapour whose total pressure was 760 mm Hg. More recently units have tended to indicate that number of micromoles of substrate hydrolyzed by 1 ml of serum in 1 hour. In other parts of the world the amount of hydrolysis has been measured in terms of a minute rather than an hour and this type of unit has acquired for itself the title of “International”. It is, however, important to appreciate that the establishment of an international unit has merely succeeded in stabilizing the time factor. The other variables—pH, temperature, conditions of reaction, nature of substrate—remain as variable as ever. In an endeavour to help anaesthetists faced with the problem of interpreting the results, table I shows, in summary, some of the methods of determination of cholinesterase and the normal range of results.

Finally, there is the question of the practical significance of results. It might seem reasonable to express these as a percentage of some accepted value taken as 100 (e.g., the upper limit of normal) and then to suggest that when activity is less than, say, 25 per cent of this figure, the patient is in danger of having a prolonged apnoea after the administration of suxamethonium. Normal values, however, have a wide variation as indeed can be seen from table I. Further, the degree of reduction in activity estimated by different methods by no means always corresponds. It would therefore seem as if the whole question of cholinesterase determination and its relationship to suxamethonium apnoea is still wide open.

**Table I**

<table>
<thead>
<tr>
<th>Method</th>
<th>Substrate</th>
<th>Principle</th>
<th>Temp. (°C)</th>
<th>Normal values</th>
<th>Units in which expressed</th>
<th>International units</th>
</tr>
</thead>
<tbody>
<tr>
<td>McArdle (1940)</td>
<td>Acetylcholine</td>
<td>Acetic acid liberates CO₃ from bicarbonate</td>
<td>37</td>
<td>60-120</td>
<td>Microlitres CO₃ at 760 mm Hg in 1 min by 1 ml serum</td>
<td>2.68-5.35</td>
</tr>
<tr>
<td>de la Huerga, Yesinick and</td>
<td>Acetylcholine</td>
<td>Estimation of acetyl choline unhydrolyzed</td>
<td>37</td>
<td>130-310</td>
<td>Micromoles acetyl-choline hydrolyzed in 1 hr by 1 ml serum</td>
<td>2.16-5.15</td>
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<td>Papper (1952)</td>
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<tr>
<td>Biggs, Carey and Morrison</td>
<td>Acetylcholine</td>
<td>Change in colour of bromthymol blue with change in pH</td>
<td>25 ± 1</td>
<td>54-126</td>
<td>Micromoles acetic acid released in 30 min by 1 ml serum</td>
<td>1.8-4.2</td>
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<td>(1958)</td>
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<tr>
<td>Kalow and Lindsay</td>
<td>Benzoylcholine</td>
<td>Spectrophotometric estimation of rate of hydrolysis</td>
<td>26 corrected to 37</td>
<td>65-145</td>
<td>Micromoles benzoyl choline hydrolyzed in 1 hr by 1 ml serum</td>
<td>0.62-1.37</td>
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<td>(1955)</td>
<td></td>
<td></td>
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<tr>
<td>Johnson and Whitehead</td>
<td>Acetylcholine</td>
<td>Time for pH change from 7.3 to 7.1 in Astrup cell</td>
<td>37</td>
<td>207-403</td>
<td>Micromoles of acetic acid liberated per minute by 100 ml serum</td>
<td>2.07-4.03</td>
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<td>(1965)</td>
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for investigation, although many of the genetic abnormalities responsible for variation in sensitivity to suxamethonium have been fairly fully worked out.

ACKNOWLEDGEMENT
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REFERENCES


DETERMINATION DE LA CHOLINESTERASE SERIQUE

Quelques-unes des variables, qui influencent le resultat de la determination de la cholinesterase s&egrave;rique sont revues. Parmi elles figurent la concentration du substrat, le pH et la temp&eacute;rature. Il existe &eacute;galement une variation consid&eacute;rable entre les diff&eacute;rents auteurs en ce qui concerne leur m&eacute;thode d'exprimer les r&eacute;sultats.

BESTIMMUNG DER SERUM CHOLINESTERASE

ZUSAMMENFASSUNG

Gründe für die Variabilität der Serum-Cholinesterase-Bestimmungen werden überprüft, diese sind die Konzentration des Substrats, der pH-Wert und die Temperatur. Die verschiedenen Untersucher geben verschiedene Auswertungsmethoden an.