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SIMPLE TEST-PAPER METHOD FOR THE CLINICAL DETERMINATION OF PLASMA PSEUDACHOLINESTERASE

BY

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Pseudocholinesterase (ChE) is produced in the liver and is normally present in the plasma, though its precise function is unknown. A simple method for the estimation of this enzyme, requiring no elaborate apparatus, would have certain practical advantages. The ChE level of the plasma falls in certain diseases of the liver and has been advocated as a measure of the damage to this organ; it is also of importance in the control of anticholinesterase therapy in myasthenia gravis. It is, however, in connexion with the use of short-acting muscle relaxants such as suxamethonium, which are normally destroyed *in vivo* by ChE, that a knowledge of the enzyme activity of the plasma is particularly desirable. A small proportion of the population, who are otherwise in good health, are deficient in plasma ChE, and if suxamethonium is used on these patients the time of paralysis may be greatly prolonged. This situation may also arise in patients with severe liver damage undergoing general anaesthesia. Preliminary estimation of the plasma ChE in such subjects would reveal this defect.

Methods for the estimation of ChE are based on the amount of acetic acid liberated from acetylcholine either by causing it to release carbon dioxide from a bicarbonate buffer, as in the manometric technique of Ammon (1933), or by observing the fall in pH of a barbitone/phosphate buffered system in a given time (Michel, 1949; Aldridge and Davies, 1952). These methods are accurate but require special apparatus and are laborious and unsuited to clinical work.

More recently, simpler methods based on the colour change of suitable indicators have been published (Caraway, 1956; Rappaport *et al.*, 1959; Smith *et al.*, 1959). Limperos and Ranta (1953) described a method using bromothymol blue in which visual inspection of the mixture of blood and reagents after a short incubation gave an approximate value for the ChE. Herzfeld and Stumpf (1955) described a further simplification of this technique in which filter paper impregnated with a choline ester and bromothymol blue is used. The paper is applied to the plasma or serum

to be tested and the time required for the colour of the indicator to change to that of a standard tint, corresponding to the liberation of a definite amount of acetic acid, is a measure of the ChE activity. These test-papers are now commercially available, and we have compared the results given by them with those obtained by the method of Michel (1949).

Material and Method

The subjects were mostly patients undergoing general anaesthesia. In some patients blood was drawn before and after injection of 2.5 or 5 mg. of the anticholinesterase drug neostigmine methylsulphate; in this way widely differing ChE levels were obtained. Samples from a few patients with jaundice were also included.

The test-papers are yellow strips, 4 by 1 cm., which, when cut in half, provide two pieces 2 cm. long and sufficient for two estimations. Exactly 0.05 ml. of serum or plasma from heparinized blood, free from red cells and haemolysis, is placed at the centre of a perfectly clean microscope slide—that is, one that is free from acid or alkali. The test-paper is applied to the drop of serum so that this is completely absorbed. (N.B. The serum must not be applied to the paper.) The colour of the paper then changes to a dark bluish green. At the same time a stopwatch is started. The test-paper is covered with another clean slide, using gentle pressure and taking care to avoid the inclusion of air bubbles between the slide and paper and to secure even distribution of the fluid. As the action of the ChE proceeds the colour of the paper changes through yellowish green tones to the original yellow. When it matches the colour standard supplied with the test-papers (olive green) the time is noted.

The time between contact of the paper with the serum and matching with the colour standard is inversely proportional to the ChE activity of the serum: thus if the time taken is less than 12 minutes a high plasma ChE level is present, whereas if it is 12 to 20 minutes a normal level exists. Times of 20 to 35 minutes or longer denote a low ChE activity. In practice, observation of the test-paper at 12, 20, and 35 minutes is sufficient to identify the grade of ChE activity.* It is recommended that the strip of standard colour should also be included between two slides. Observations should be carried out in a constant light and at average room temperature (20° C.). Serum or plasma may be kept for a day or two in the refrigerator before estimation and the papers must be protected from moisture, ammonia and the fingers.

Results

There was good agreement between the results obtained with the test-papers and those obtained with Michel's method, except at very low levels (see Chart). The initial ChE values of those subjects who received neostigmine were normal by both methods.

Herzfeld and Stumpf (1955) made a comparison of their method with that of Ammon (1933). They did not claim great accuracy for the filter-paper technique (they were using test-papers prepared in their laboratory) but thought it suitable for routine work: we are in agreement with this. They observed that strongly icteric sera gave results somewhat higher than the true value, presumably because the bilirubin alters the tint of the

*In more recent batches of test-paper these figures have been slightly modified, 7 to 18 minutes being given as normal; the reaction should then be inspected at 5, 18, and 35 minutes.

bromothymol blue. We have confirmed this effect, which is small and most apparent at low levels of ChE, but it does not detract from the practical value of the test.

A disadvantage of the method is that the colour of the end-point (an olive green) is difficult to identify with precision, and in the case of low values the time at which matching with the standard tint occurs cannot be determined to within 5 or even 10 minutes; for clinical work this is not a serious drawback. The effect of variations of room temperature is small.

The test-paper method has the advantage that the plasma is undiluted, therefore if reversible inhibitors are present their effect is not diminished, as compared with *in vivo* conditions, as would be the case if the plasma were diluted as in other methods.

Ideally the ChE level of every patient receiving an injection of the short-acting muscle relaxant suxamethonium should be tested before the induction of anaesthesia. As the incidence of a low enzyme activity is only about 1 in 2,000 normal patients such a procedure would be impracticable. A familial incidence of this condition has also been noted (Lehmann and Ryan, 1956). Nevertheless, in the presence of liver disease and nutritional deficiencies (such as carcinoma of the oesophagus) a prolonged reaction to the relaxant occurs much more often; it is in such cases that the test will be found of greatest value.

Results with test-paper and with Michel's method. Normal values should fall within hatched area.

Summary

The test-paper offers a simple method for the estimation of plasma pseudocholinesterase, in which the plasma is allowed to act upon a substrate containing acetylcholine and bromothymol blue. The results with this method are in reasonably good agreement with those obtained from Michel's method, and although it is not a method of great precision it is suitable for routine clinical work. The value of this simple test in cases of liver disease and also in patients undergoing clinical anaesthesia with the use of the muscle relaxant suxamethonium is also emphasized.

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MARSUPIALIZATION OF BARTHOLIN'S CYSTS

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Bartholin's cysts have been treated for many years by total excision and are still so dealt with in many clinics. This procedure is sometimes regarded as a minor gynaecological operation and simple to perform, but difficulties are often encountered in practice. Troublesome haemorrhage may arise; the cyst may be ruptured, causing difficulty in finding the correct layer of cleavage. The latter situation necessitates removal of the cyst and gland *in toto*. Obliteration of the remaining cavity is not always an easy task, and failure to do this can result in post-operative haemorrhage and delayed healing by second intention. The patient may suffer from painful scarring and discomfort. Loss of secretion may cause trouble should the opposite gland be malfunctioning—artificial lubricants are not always acceptable to the patient. When the cyst has been excised the recurrence rate is high and subsequent attempts to operate in a fibrosed area present considerable difficulties.

In the presence of infection, oedema of the surrounding tissues renders the dissection of the thick and friable cyst wall almost impossible, and if the surgeon enters the corpus cavernosum haemorrhage can be very severe indeed.

Excision of an abscess is therefore seldom undertaken while active infection is present; the delay in operating adds to the anxiety and discomfort of the patient.

Davies (1948) proposed a simple method of treatment of Bartholin's cysts aiming at restoration of the function of the gland. He treated 25 cases by incision close to the hymen in the region of the opening of the duct. The cavity of the cyst was then packed with iodoform gauze, which was removed and replaced twice weekly for three weeks. The packing was to prevent the small incision from closing and to permit epithelization of the new ostium with squamous epithelium. The method was not always successful, and there was difficulty in maintaining a stoma even with continuous drainage for several weeks, because retraction of the wall of the cyst occurred as the inflammation subsided, permitting union across the opening.

Jacobson (1950) described the simple operation of marsupialization with construction of a new mucocutaneous junction, and reported 19 cases showing good results. A second series of 140 patients who had 152 cysts with only four recurrences was reported later