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A SIMPLE PROCEDURE FOR THE ESTIMATION OF SERUM CHOLINESTERASE APPLICABLE TO ROUTINE CLINICAL BIOCHEMISTRY

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For the past ten years ademand for a simple procedure for determination of serum cholinesterase has become increasingly great, since its significance for the diagnosis of hepatobiliary diseases has been established by various workers.[1~8] Several methods,[9~11] different in principle, have been introduced, but they have not hitherto been popularized as a routine laboratory procedure, at least in this country, because they required special instruments (Warburg's manometric apparatus and glass electrode pH-meter) which were expensive in cost as well as somewhat cumbersome in manipulation.

Recently a hydrogen-ionometric method by means of comparator with phenol red as pH indicator was developed for estimation of serum cholinesterase.[12] In this method a mixture of acetylcholine chloride solution and barbital glycerophosphate buffer solution was introduced in an equal amount into two test tubes and used as buffered substrate. To one of these was added the serum (sample tube) and to the other distilled water in volume equal to that of the serum (blank tube), and after addition of phenol red solution by drops they were incubated at 37C. At the end of one hour the enzymatic activity was stopped by eserine salicylate, their pH was measured with the standard series of phenol red tubes in a comparator, and the activity of serum cholinesterase was computed in terms of the pH fall in the sample tube minus the non-enzymatic pH drop in the blank tube.

This procedure (phenol red method) proved to be convenient in the routine laboratory examination for the purpose of detecting the drop of serum cholinesterase in the hepatobiliary and other disorders because of its simplicity and inexpensiveness.[13)~14)] Its accuracy and reliability were

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checked by Ammon's manometric[9] and Michel's glass electrode pH-meter method[10] which were believed to give exact determination of the serum cholinesterase. The purpose of this paper is to present the data obtained by us.

METHODS

Reagents

(1) Buffer solution. An amount of 0.5 g. of sodium barbiturate and 20g. of sodium β-glycerophosphate is dissolved in 400 ml. of distilled water. This solution which has pH of 8.3 is preserved in a refrigerator after five to ten ml. of chloroform has been added. It keeps for at least two months.

(2) Acetylcholine solution. One ampule of Ovisot (acetylcholine chloride of Daiichi Seiyaku Co. Tokyo, contained in an ampule 0.1g. in amount) is dissolved in two ml. of distilled water immediately before use.

(3) Phenol red solution. An amount of 0.1g. of phenol red is pestled in an agate mortar with 2.85 ml. of 0.1 N sodium hydroxide solution and 7.5 ml. of distilled water, made to volume of 250 ml. with distilled water in a glass cylinder.

(4) 0.01 g./dl. aqueous solution of eserine salicylate.

Procedure

Into individual test tubes (cf the same size as the pH comparator) A (blank), B_1, B_2, ... are introduced the distilled water, the serum samples S_1, S_2, ... and the reagents in the order given below, left to right, mixed and incubated in a water bath at 37C for an hour.

Test tubes	Buffer solution (ml.)	Distilled water (ml.)	Phenol red solution (ml.)	Acetylcholine solution (ml.)	Serum S_1 (ml.)	Serum S_2 (ml.)	• •
A	1.5	3.1	0.2	0.5	—	—	—
B_1	1.5	3.0	0.2	0.5	0.1	—	—
B_2	1.5	3.0	0.2	0.5	—	0.1	—
•	•	•	•	•	—	—	•
•	•	•	•	•	—	—	—

At the end of the specified time a drop of eserine salicylate solution is added to each, mixed and the pH of the tubes a, b_1, b_2, ... (for the test tubes A, B_1, B_2, ..., respectively) is read in a comparator with the standard phenol red tubes. The activity of serum cholinesterrase for each sample ΔpH (S_n) is computed as follows.

$$\Delta pH(S_n) = a - b_n$$

The procedure was checked for its reliability by the following methods.

1) Ammon's manometric method. Tamai's modification [15] was employed with slight change. An aliquot of 1.6 ml. of sixtyfold dilution of serum with a solution which has been prepared with 100 ml. of 9.0 g./l. aqueous solution of sodium chloride, 2.0 ml. of 11.5 g./l. aqueous solution of potassium chloride, 20.0 ml. of 13.5 g./l. of aqueous sodium bicarbonate solution and 2.0 ml. of 12.2 g./l. aqueous calcium chloride solution was introduced into a flask of Warburg's instrument, while 0.4 ml. of Ovisot solution (one ampule was dissolved in 4.4 ml. of distilled water) was brought in its sidearm. The instrument was filled with nitrogen gas which has carbon dioxide to five per cent, shaken 85 to 90 times per minutes in a thermostat at 38C and the manometer was read at every ten minutes for the period of thirty minutes. The activity of the serum cholinesterase was expressed in the amount of carbon dioxide (μL CO_2) liberated by 0.1 ml. of the serum for thirty minutes.

One hundred and twenty six samples of the serum were determined for cholinestrase by Ammon's method concurrently with the phenol red procedure for comparison.

2) Michel's method by means of glass electrode pH-meter.[10] Venous blood specimens were taken from forty five persons including healthy and sick individuals to obtain heparinized plasmas and blood sera. The plasmas were subjected to Michel's procedure, while the sera underwent the phenol red method.

Results and Discussion

Figure 1 represents the collation of the phenol red procedure to Ammon's manometric method. In the former procedure the pH of samples and blank tubes was read to one place of decimals with an accuracy of 0.1. The scattered dots which fall in a fairly narrow rectilinear strip discloses an intimate correlationship between these methods (coefficient of correlation $r = +0.90$). The sera from healthy persons remained within 151.5 to 257.2 μL CO_2 in Ammon's method, while in the phenol red procedure they were found within the range of ΔpH 0.8 to 1.2. The scrutiny of the figure reveals that the strip is considerably narrower in the subnormal range of the serum cholinesterase activity than in the normal and the supernormal. This indicates that the phenol red method compares fairly well with Ammon's method in the estimation of the sera whose cholinesterase activity is decreased below the lower limit of normal range (coefficient of correlation

$r = +0.95$), whereas it is not sufficiently sensitive in the study of those of normal and supernormal activity because of its difficulty in revealing the delicate variation in the high activity region.

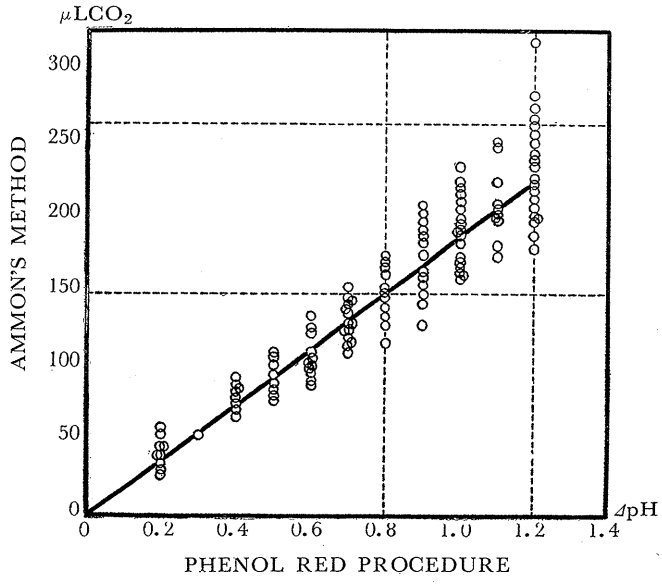

Fig. 1 Phenol red procedure as compared with Ammon's method

Figure 2 shows the results of comparison with Michel's procedure. In this case a special effort was made in the phenol red procedure to read pH to the second place of decimals (with significant level of 0.05) in a comparator. The correlation between them in the subnormal range (ΔpH below 0.68 in Michel's method and below 0.8 in the phenol red procedure) was as satisfactory as +0.98 was obtained for the coefficient of correlation, and +0.90 was given for the whole range of variation. However, the dots in the normal limits scattered in a considerably wide area, without lining up along a straight line.

The procedure failed to be improved to an appreciable extent in the sensitivity for the detection of the variation of serum cholinesterase in the normal and supernormal range, even when the comparator was substituted for a photoelectric colorimeter (light of 520 mμ wave length was selected) to read pH. This is illustrated in Figure 3, in which phenol red procedure adapted to photoelectric colorimetry is contrasted with Ammon's method.

It was accordingly thought that the buffered substrate employed in this procedure might be deficient in reflecting the exact amount of acetic

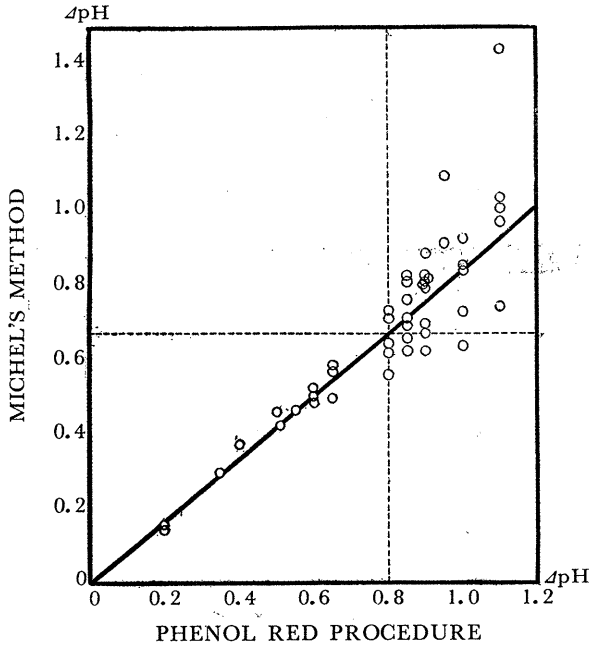

Fig. 2 Phenol red procedure as compared with Michel's method

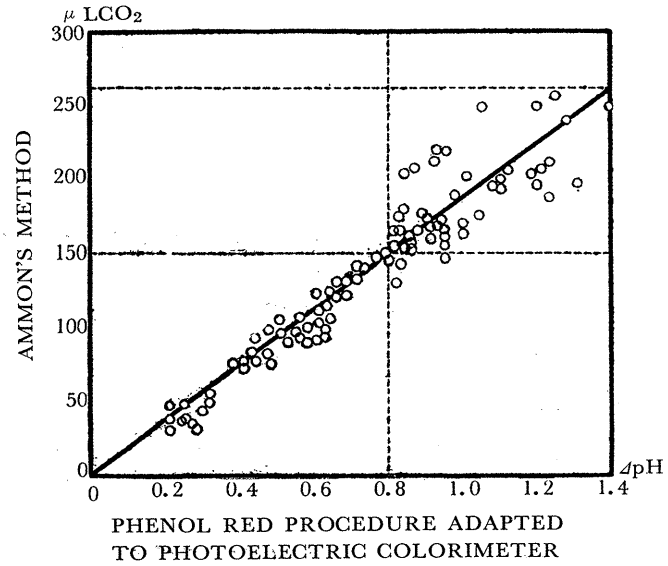

Fig. 3 Phenol red procedure adapted to photoelectric colorimetry as compared with Ammon's method

acid liberated by cholinesterase for the sera of high enzyme activity (normal and supernormal) despite the fact that it was sufficient for those of subnormal activity. Use of comparator would be rather advantageous for the procedure, because it contributed to the simplicity in manipulation without entailing so much impairment in accuracy, as seen from the comparison of the scatter of dots in Figure 1 with that in Figure 3.

At any rate the fact that the phenol red procedure is in close correlation to Ammon's and Michel's methods for the estimation of subnormal activity of an serum cholinesterase qualifies the procedure to be counted among the routine laboratory methods, since clinical biochemistry is chiefly concerned with the reduction in the activity of the serum cholinesterase which is common in the hepatobiliary diseases, cancer and ulcer of the gastrointestinal tract, and so forth, but not so much with the variation in the normal and the supernormal (nephrosis) activity.

Summary and Conclusion

A simple hydrogen-ionometric procedure for estimation of the serum cholinesterase by means of a comparator with phenol red as indicator is presented. This was compared with Ammon's and Mihel's methods to check its accuracy and reliability, and it was confirmed that the procedure gave estimations in the subnormal range of the serum cholinesterase activity with an accuracy satisfactory for clinical purposes, although it was a little insensitive to the detection of its variation in the normal and supernormal range.

The procedure was thought to be suitable for the clinical biochemistry that was obliged to deal with a large number of morbid materials within a short period of time, because it was simple in technique and required no expensive instruments.

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