OBSERVATIONS ON A CHROMOGENIC AND A STARCH-IODINE METHOD FOR THE ROUTINE MEASUREMENT OF SERUM AMYLASE

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INTRODUCTION

THE starch-iodine method described by Wootton (1964) has long been in use in our laboratory for the measurement of serum amylase, primarily because of the simplicity of the procedure and its low cost. However, much objections have been raised (Rosalki and Tarlow, 1973) against starch-iodine methods, among which are: reaction not zero order kinetics (substrate concentration being sub-optimal), colour development interfered by serum proteins, different results obtained with starches of different origins, and the methods appeared less able to detect increased amylase activity in a number of sera shown to be definitely elevated by the saccharogenic and the chromogenic methods. Also precision was noted to be generally poor. With these drawbacks, consideration should be given to more satisfactory methods for the routine assay of serum amylase.

Chromogenic methods are more attractive than saccharogenic methods as the former are generally rapid and simple to perform, and hence, ideal for routine use. Several chromogenic methods have been assessed by Rosalki and Tarlow (1973), and found highly satisfactory for the determination of amylase. One of such methods is in the Amylochrome Kit (Roche Diagnostics, U.S.A.) which uses amylose-cibacron blue 3G-A as the substrate. A quick appraisal of this Kit was made in comparison with Wootton's method.

MATERIALS AND METHODS

Amylochrome Method

Amylase hydrolyses the water-insoluble amylose-cibacron blue 3G-A substrate, releasing the soluble dye complex. The unhydrolysed portion of the substrate is removed by centrifugation, and the absorbance of the blue dye complex, which is proportional to the amylase activity, is measured.

Reagents and Procedure: refer literature accompanying the Amylochrome Kit (Roche Diagnostics).

Wootton's Method

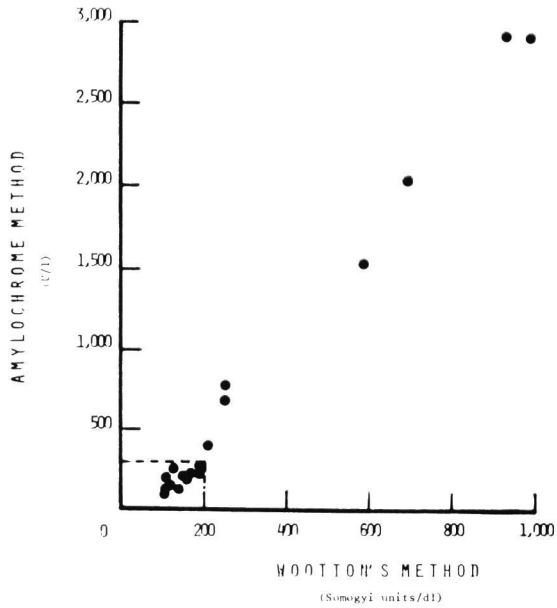
Starch is hydrolysed by amylase, and the remaining starch is reacted with iodine to form a blue colour which is measured. The decrease in colour, which gives a measure of the starch utilised, is proportional to the amylase activity.

Reagents and Procedure: refer Wootton (1964)

RESULTS AND DISCUSSION

Twenty patient samples were assayed by both methods, and the results shown in Fig. 1. Excellent correlation between the Amylochrome method and Wootton's method was noted, $r = 0.99$, $p<0.001$. This finding differs from that of Rosalki and Tarlow (1973) who showed that the starch-iodine method as developed by Caraway (1959) correlated poorly ($r = 0.10$ to 0.52) with both the dye methods studied and a saccharogenic method. We feel that Wootton's modification improved the performance of the starch-iodine method.

Serum samples were analysed in duplicates by the two methods. The precision of the Amylochrome method was found to be much better than that of Wootton's method (Table I). Greater sensitivity was also observed for the Amylochrome method;



Correlation between the Amylochrome and Wootton's method.

example, for the same enzyme value, the absorbance change in the method was twice that of Wootton's method. Further, the error contributed by instrument fluctuation was more pronounced in Wootton's method (Table II). An instrument fluctuation of 0.005 absorbance unit is not unusual, and while such a change means a 8 U/l amylase activity (a $\pm$ 2.7% error if activity is at 300 U/l) for the Amylochrome method, the same fluctuation causes a 15 Somogyi units /dl difference in Wootton's method ($\pm$7.5% error at 200 Somogyi units/dl). A larger error in Wootton's method, which is three times more than that for the Amylochrome method, can, therefore, easily arise from instrument fluctuation.

As documented in the literature accompanying the Amylochrome Kit, linearity is possible to 2,000 U/l. No dilution of a serum sample is therefore required for amylase activities up to about seven times the upper limit of normal. In Wootton's method, the test is performed on a 1 in 10 diluted serum (with normal saline), and the result holds good if activity is less than 400 Somogyi units/dl, which is only two times the upper normal limit. To avoid substrate exhaustion, further dilution is required if the activity exceeds this level.

Of the twenty human sera tested, two cases with 251 and 254 Somogyi units/dl (upper normal limit, 200 Somogyi units/dl) were found to have definite elevation of 769 and 683 U/l (against a normal upper limit of 300 U/l), respectively, by the Amylochrome method. This finding, also noted by Rosalki and Tarlow (1973), is thought to be due to inhibition in Wootton's method, probably by the

Table I
Precision data from the Amylochrome and Wootton's methods

	AMYLOCHROME METHOD U/l	WOOTTON'S METHOD Somogyi units/dl
Normal Range	upper limit 300	upper limit 200
Number of samples = 20 (in duplicates)		
Range of values obtained	122 to 2,920	98 to 980
Mean	852	343
Standard Deviation	22	18
Coefficient of Variation	2.6%	5.2%
Precision (95% limits) for the range of values obtained	$\pm$5.4%	$\pm$11.0%

Table II
Error due to instrument fluctuation, and sensitivity for the two methods

Method	Change in Absorbance	Calculated Amylase Activity	Error from instrument fluctuation
Amylochrome Method	0.185	300 U/l	
	$\pm$0.005*	8 U/l	300 $\pm$ 2.7%
Wootton's Method	0.065	200 Somogyi units/dl	
	$\pm$0.005*	15 Somogyi units/dl	200 $\pm$ 7.5%

* Absorbance fluctuation of instrument

benzoate which is added in the substrate preparation. Two very elevated cases gave 924 and 980 Somogyi units/dl by Wootton's method (about 5 times the upper limit of normal). The values obtained by the Amylochrome method were, 2,920 U/l for both cases (approximately 10 times the normal upper limit). Although both methods returned clearly elevated values, the Amylochrome method demonstrated greater capability for measuring high amylase activities.

In Wootton's method, the colour of the starch-iodine complex decreased rapidly on standing; the absorbances of the control and test fell disproportionately, resulting in false higher amylase activity being derived. It is, therefore, essential that the absorbances are read without delay. The colour obtained on the Amylochrome method was very stable, and remained unchanged up to more than 2 hours.

The many disadvantages and technical failings observed in the starch-iodine method point to the need for a more satisfactory method for the routine assay of serum amylase. The dye method examined here proved to be superior and robust, besides having a simple and rapid procedure, and can, therefore, be considered for replacing the less sensitive Wootton's method.

SUMMARY

A comparative study was made on the amylochrome method, marketed by Roche Diagnostics, U.S.A. against a starch-iodine method described by Wootton (1964), the latter presently used by our laboratory for the routine assay of serum amylase. Although both methods are rapid and simple, the dye method has the advantage of giving a stable colour, while the colour from the starch-iodine complex decreases rapidly on standing. The dye method also gave better precision, and demonstrated greater capability of measuring high amylase activities. Excellent correlation between the two methods was noted.

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