A comparison between spectrophotometric and titrimetric methods of estimating trypsin¹

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SUMMARY The spectrophotometric method described is quick and effective and a reliable alternative to a titrimetric method for measuring trypsin activity in duodenal juice.

A spectrophotometric method for the determination of trypsin activity in duodenal juice was suggested by Schwert and Takenaka in 1955, and a modification of this method was later described by Lundh (1957). Other methods involve the use of a pH stat. automatic titrator (Haverback, Dyce, Gutentag, and Montgomery, 1963) and a pH meter (Wiggins, 1967). In this paper, the method of Schwert and Takanaka, as modified by Seravac Laboratories, is compared with the titrimetric (pH meter) method described by Wiggins (1967).

Materials

Over a period of two months, 50 consecutive samples of duodenal juice were analysed for trypsin activity by spectrophotometric and titrimetric methods. The samples were taken fresh and at random from patients referred for pancreatic function tests using the method of separate gastric and duodenal intubation and pancreatic stimulation by secretin followed by pancreozymin (Bank, Marks, Moshal, Efron, and Silber, 1963).

Methods

SPECTROPHOTOMETRIC METHOD

Principle

The specific substrate, N-benzoyl-L-arginine ethyl hydrochloride (BAEE) in phosphate buffer pH 8.0 is hydrolysed in the presence of trypsin at the ester linkage and this hydrolysis causes an increase in optical density at 253 M μ . One unit of activity is that activity which causes an increase in optical density at 253 M μ of 0.001 per minute under the standard conditions. Allowing for dilution factor,

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time of assay, and volume, results are expressed in BAEE units/ml juice/minute. For conversion to international units, ie, μ moles/min/ml duodenal juice, see the calculation below.

Apparatus

Beckman D.B. model spectrophotometer.

Reagents

1 BAEE

2 0.001 N HCl

3 0.05 M phosphate buffer, prepared as follows: (a) 7.8 g NaH₂PO₄ 2H₂O—1 litre and (b) 7.1 g Na₂HPO₄—1 litre. Mix (a) and (b) to pH 8.

4 Substrate is obtained by mixing 8.6 mg BAEE with 100 ml phosphate buffer, giving a 0.00025 M solution.

Procedure

Pancreatic juice and dilution tubes are always kept on ice.

Dilute pancreatic juice with 0.001 N HCl, initially 1 in 5, increasing to 1 in 40, if necessary, with separate samples. A dilution of 1 in 5 applies to low enzyme activity, 1 in 40 for normal.

Place the following in the cuvettes at 25°C:—

	Test	Control (S)	Reference (R)
Substrate (ml)	3.0	3.0	3.0
0.001 N HCl (ml)	—	0.2	—
Juice solution (ml)	0.2	—	—

Set machine at 253 M μ . If the double beam is used, place the reference cuvette in 'R' and leave it there during the test. With the control adjust the optical density to read 0.05. Set the recorder correspondingly.

Add enzyme to substrate and start the graph at once. Let the recorder run for five minutes, taking the reading over a three-minute slope.

The machine is reset at 0.05 with each assay and the graph is reset.

Each assay is repeated. A 5% error is arbitrarily accepted.

A standard with crystalline trypsin of known activity is assayed with each test.

Calculation

Let $1/D$ = dilution used

$\frac{E_{253}}{3}$ = change in optical density per minute at wavelength 253 m μ

$$\text{Result in BAEE units/min/ml} = \frac{5D \times E_{253}}{3 \times 0.001}$$

Result in μ moles/min/ml duodenal juice (international units) can be expressed: $\frac{5D \times E_{253}}{3} \times \frac{1}{359}$

according to the following data (Trautschold and Werle, 1957; Rick, 1956).

The optical density difference between 10^{-3} M solution of N-benzoyl-L-arginine and the ethyl ester is 1.15 at 25°C, pH 8.0, and with a 1 cm light path. With a volume of 1 ml an optical density change of + 1.15/min corresponds to the conversion of 1 μ -mole of substrate/minute. With a 3.2 ml assay volume the reaction of 1 μ mole of substrate/min is equivalent to an increase in optical density of 0.359/minute.

TITRIMETRIC METHOD

The method of Wiggins (1967) was used.

Results

Each result constitutes the mean of two readings. Figure 1 shows a comparison of the results by the two methods. Two points coincided on the computerized graph (Fig. 1), this accounting for the 48 marked points of the 50 results. The correlation coefficient was 0.84. The wider scatter at values greater than 11.2 μ moles/min/ml (4×10^3 BAEE units) and 30 μ equiv/min/ml is of minor importance as most of our abnormal results are less than 8.3 μ moles/min/ml (3×10^3 BAEE units), particularly in post-pancreozymin samples.

Discussion

The spectrophotometric method does not appear to be widely used. However, the statistically significant degree of correlation with the titrimetric method

Fig. 1 Graphical comparison of results.

shows that it is an effective alternative for trypsin estimation. In a laboratory such as ours, where the demand for this estimation is frequent, the spectrophotometric method permits a rapid yield of results for fresh specimens while the patient is having the pancreatic function test, it is technically simple to do, and economizes by using less of an expensive substrate. It must be stressed, however, that owing to the turbidity of samples obtained after a Lundh test meal, the spectrophotometric method is unsuitable.

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