Methods and techniques

Simple method for estimating trypsin

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The estimation of trypsin in duodenal contents following the feeding of a standard test meal has been shown to give useful information in the diagnosis of pancreatic disease (Lundh, 1962; Cook, Lennard-Jones, Sherif, and Wiggins, 1967). The methods available for estimating trypsin are either complex (Lundh, 1957) or require a pH stat automatic titrator (Haverback, Dyce, Gutentag, and Montgomery, 1963). Since the demand for this estimation is relatively infrequent, the former will not find favour in a busy routine laboratory and it is difficult to justify the expenditure for an automatic titrator. The method described here is simple to do, has been found suitable for occasional use, requires only normal laboratory equipment, and gives the results directly in international units ($\mu\text{Eq. H}^+$ released/min./ml.).

PRINCIPLE

The rate at which H^+ is liberated by the hydrolysis of the specific substrate N benzoyl-L-arginine ethyl ester hydrochloride (B.A.E.E.) is measured. This is done by finding the time taken to neutralize a known amount of alkali. The conditions of the reaction have been set so that the pH stays within the range 7.9-8.4 where changes in enzyme activity with pH are very small.

REAGENTS

- 1 0.04 N NaOH
- 2 1% Sodium barbiturate
- 3 B.A.E.E.
- 4 0.05 Molar acetate buffer pH 5.8 containing 0.5 g. CaCl_2 per litre.

APPARATUS

Stopclock

pH meter with small electrodes preferably with expanded scale, e.g., E.I.L. 32A

Vials approximately 1 in. in diameter and 3 in. high.

MAGNETIC STIRRER The reaction should be stirred and carried out at 25°C. A satisfactory arrangement for occasional use can be set up with a suitable vessel placed on top of a magnetic stirrer. The water in this outer vessel can be periodically adjusted to 25°C. and a small stirrer bar operated in the reaction vial. Many other arrangements are possible depending on the equipment available.

PROCEDURE

SUBSTRATE SOLUTION Dissolve 0.5 g. of B.A.E.E. in 100 ml. of a 1:10 dilution of 1% sodium barbiturate. Adjust the pH to 9 and bring to 25°C. before use. (Sometimes this substrate solution becomes more acid on standing, if so readjust pH before use.)

SAMPLE Mix 1 ml. of intestinal contents with 9 ml. of 0.05M acetate buffer and bring to 25°C. One millilitre of the diluted intestinal contents is mixed with 5 ml. of substrate in the reaction vial and placed on the stirrer with electrodes immersed. The initial pH should be about 8.5, the reading falling continuously. When the pH is reading 8 the stopclock is started and then 0.1 ml. of 0.04 N NaOH added to the reaction mixture; the pH will rise to about 8.4 and is followed until it again reaches 8 when the stopclock is stopped. The time shown is that during which 4 $\mu\text{Eq. of H}^+$ is released. Samples with normal tryptic activity will take less than four minutes. If the time interval is greater than 10 minutes the reaction should be repeated with 1:5 dilution of intestinal contents. If the reaction still takes more than 10 minutes the result should be reported as less than 2 $\mu\text{Eq./ml./min.}$

CALCULATION

$$\frac{\mu\text{Eq. NaOH added}}{\text{Time}} \times \text{dilution of intestinal contents} \\ = \mu\text{Eq. H}^+ \text{ released per min. per ml. intestinal contents.}$$

RELIABILITY OF METHOD COMPARED WITH RESULTS WITH RADIOMETER

The results obtained by this method were compared with those obtained on the same specimens using a Radiometer titrator 11 with Autoburette ABU 1 b in a room maintained at 25°C. The conditions were as follows.

REAGENTS

0.5% B.A.E.E. in distilled water 0.1 N NaOH.

TITRATOR

Burette 2.5 ml., filled with 0.1 N NaOH speed 1
End point, pH 8.25
Titration, upscale
Proportional band, 0.1
Stop, manual.

Intestinal contents (0.5 ml.) were pipetted into 1 in. $\times$ 3 in. glass vials. The substrate (5 ml.) was pipetted into the vial which was immediately placed on the titration assembly and the titration started. When the end point is reached there is a slight overshoot. When the titration restarts, a period of 30 seconds is timed, at the end of which the burette register and the stopclock are returned to zero, while the titration continues. The titration is stopped at the end of two minutes and the burette register read. If these times are strictly followed, the results are reliable up to 40 $\mu\text{Eq./ml./min.}$ If higher activities are

FIG. 1. Tryptic activities: comparison of results between the two methods. The line represents perfect agreement.

found then substrate concentration becomes limiting and the estimation should be repeated using a smaller aliquot of intestinal contents.

CALCULATION

Millilitres 0.1 N NaOH consumed $\times 100 = \mu\text{Eq./min./ml.}$ of intestinal contents.

RESULTS COMPARED

Figure 1 shows a comparison of the results by the two methods. The determinations were performed over a period of approximately three months. The points plotted are the means of duplicate determinations by both methods.

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