agglutination provided that the precautions mentioned by Bridges and Taylor (1944) are observed; it is obviously best to confirm the findings by tube agglutination if possible when an unusual organism has been found. Whether the method will be of universal application in the field can only be discovered by trial. There are no obvious pitfalls except that a minor antigen in the organism may cause cross reactions, but this seems unlikely if dilute sera are used. Although the concentration of each individual serum in any mixture is low the total quantity of serum is rather large, and it is possible that this may occasionally give rise to non-specific effects.

An objection that might be raised from the point of view of serum production is that there is some waste of sera against rare antigens since these are used almost as often as commoner ones.

This does not appear to be a very serious fault when dealing with H- antisera, which are usually of high titre and therefore economical to produce.

Extension of this method to *Salmonella* O antisera is not very practical owing to their low titre and to the overlapping of antigens between O groups.

I am very grateful to Dr. Joan Taylor for her co-operation in testing this method and to her and Dr. G. S. Wilson for their comments on the manuscript. I must also thank Mr. T. Nash for a suggestion that initiated the work.

The polyvalent sera mentioned in this paper are available in limited quantities from the Standards Laboratory, Central Public Health Laboratory, Colindale, London, N.W.9.

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Third International Congress of Clinical Pathologists

The third international congress of clinical pathologists will be held in Brussels from July 14 to 20, 1957, preceded by a symposium to be held at Liège under the auspices of the C.I.O.M.S. The subject of the symposium is "Sensitivity to Drugs." Those who wish to attend all or part of the conference are asked to register as soon as possible with Dr. W. H. McMenemey, Maida Vale Hospital for Nervous Diseases, London, W.9.

The Estimation of True Lipase in Small Intestinal Contents

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An estimation of lipolytic activity in specimens of duodenal juice obtained by intubation is a necessary step in the assessment of intestinal function in relation to fat absorption.

In this method conditions *in vitro* were made to resemble as closely as possible those found *in vivo* during normal fat absorption. Therefore, neither preformed emulsion (Palmer, 1922), short-chain glycerides (Lagerlöf, 1942), or polyoxyethylene esters (Archibald, 1946) were used as substrates, but one of the common dietary long-chain triglycerides. A similar substrate was used by Willstätter, Waldschmidt-Leitz, and Memmen (1923). The mixing properties of intestinal motility were reproduced by agitation on a mechanical shaker. Bile was added to give the concentration normally found in the intestine.

Experimental

Special Apparatus.—A microid flask shaker (Griffin and Tatlock Ltd.), which carries two flasks each held in clamps 9 cm. from the fulcrum, is used. It was calibrated by recording vibrations on a smoked drum. This can also be done roughly by clamping a pencil in one arm and counting the number of dots on a paper held near during a period of 15 seconds.

Reagents.—The following are required:

Pancreatin U.S.P. XI, Philip Harris Ltd., Birmingham, 5%, w/v in water.

Bile salts, Difco No. 3, 25% w/v in water.

Tetramethyl ammonium hydroxide, B.D.H., 25%, diluted with ethanol to give an N/20 solution.

Olive oil, B.P., rendered acid-free and almost colourless by passage through an alumina column.

Thymol blue, 0.1% in ethanol.

Alumina, Hopkin and Williams, "chromatographic grade."

Method.—Acid-free olive oil, 1 ml., was added by syringe to 5 ml. phosphate buffer, pH 7.8, containing 0.5% bile salts in a 50 ml. stoppered conical flask. Duodenal juice, 0.1 ml., or 1 ml. pancreatin solution,

was added and the mixture shaken at a speed of 750 vibrations per min. for 30 min. at 37° C. The flask was held in the shaker arm so that the lower half of the flask dipped into water at constant temperature. The mixture was acidified with 5 drops of concentrated HCl and extracted by shaking by hand for 1 min. with 20 ml. of benzene. A 10 ml. sample of the upper benzene layer, to which was added 1 ml. ethanol, was titrated with N/20 tetramethyl ammonium hydroxide in ethanol between the yellow and the green-blue end-points, using thymol blue as indicator. This figure is a measure of the concentration of lipase in the sample of duodenal juice examined. Under these conditions, therefore, a unit of lipase can be expressed as the number of millilitres of N/10 alkali used to neutralize the fatty acids liberated from 1 ml. of acid-free olive oil in 30 min. at 37° C.

Results

In Fig. 1 units of lipase are plotted against time for various strengths of pancreatin. The pancreatin was dissolved in water, as completely as possible, then centrifuged and 1 ml. of the supernatant used for the determination. The reproducibility of the method is shown by 10 repeat estimations, using a 5% solution of pancreatin and shaking for 30 minutes, all of which fell between 5.10 and 5.20 units.

The influence of shaker speed is shown in Fig. 2, using a 5% solution of pancreatin and shaking for 30 minutes.

Comment

It will be seen from Fig. 1 that 30 minutes is an optimum time for determination of the enzyme activity.

FIG. 1.—Influence of enzyme concentration on the amount of fatty acid liberated in 30 minutes.

FIG. 2.—The effect of shaker speed on the amount of fatty acid liberated in 30 minutes.

It must be emphasized that lipase is unstable in the presence of trypsin, but this instability is reduced to a minimum at 0° C. Specimens should therefore be kept at this temperature while awaiting analysis.

Above 700 vibrations per minute shaker speed has very little influence on lipolysis but is very marked below this figure. The shaker must always be given time to warm up, otherwise the results are erratic.

Tetramethyl ammonium hydroxide is used because the carbonates are soluble in alkali and do not deposit in the burette tip (Schmidt-Nielsen, 1942).

This method has been used for the analysis of duodenal contents in children with coeliac disease (Anderson, Frazer, French, Gerrard, Sammons, and Smellie, 1952), in the sprue syndrome (Frazer, 1952), and in kwashiorkor (Thompson and Trowell, 1952), and for the estimation of faecal lipase in pancreatic fibrosis (Ross and Sammons, 1955). It has been adapted as a manometric method using the Warburg apparatus (Bueding, 1955).

The normal values are 2–6 units per 0.1 ml. duodenal juice, a figure which does not appear to vary with age.

Summary

A method is described for the estimation of lipolytic activity in small intestinal contents, using olive oil as substrate, and under conditions which correspond to those found in the intestine.

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