Interference by Gastrografin with a spectrophotometric trypsin assay

A. E. COWEN, HEATHER M. McGEARY, AND C. B. CAMPBELL

From the Departments of Gastroenterology and Biochemistry, Royal Brisbane Hospital, Brisbane, Australia

SUMMARY Small quantities of Gastrografin remaining in the intestinal tract some hours after introduction have been shown to cause falsely low trypsin values as determined by a spectrophotometric assay system. This interference is due first to the high absorbance of Gastrografin at 254 nm resulting in a falsely high background optical density. Secondly, Gastrografin inhibits esterase activity towards the synthetic substrate used in this assay. Gastrografin did not interfere with gelatin proteolysis by trypsin and did not affect amylase or lipase determination. Thus the instillation of Gastrografin into the duodenum before pancreatic function tests should be avoided when the trypsin content is to be determined spectrophotometrically.

The accurate placing of aspiration tubes is necessary for a variety of intestinal studies, including pancreatic function tests. Usually even non-radiopaque tubes are readily positioned using x-ray image intensification. However, difficulty is occasionally encountered in patients after gastric resections when the anatomy has been disturbed. Small quantities of radioopaque contrast material are frequently injected down the tube to assist in positioning of the tube.

This report shows that interference was encountered with an assay of intestinal trypsin content by small amounts of Gastrografin used to position a multipurpose intestinal tube (Cowen and Campbell, 1971) in a patient referred for pancreatic function studies following a Polya gastrectomy. Gastrografin (Schering) contains 76% (w/v) of sodium acid methyl glucamine salts of N, N-diacetyl-3, 5 diamino-2,4,6, tri-iodo benzoic acid. This substance appeared to interfere with the spectrophotometric assay system employed rather than with the activity of the trypsin present in the sample. There was no inhibition of the assay systems employed for lipase and amylase estimations.

Materials and Methods

DETERMINATION OF TRYPSIN ACTIVITY

Trypsin activity was measured by the method of Schwert and Takenaka (1955) as modified by

Bergmeyer (1965). Esterase activity towards N-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) as substrate was determined spectroscopically in thermostated quartz cells at 30°C in a Unicam SP 800 recording spectrophotometer. The increase in absorbance at 254 nm was followed over a one-minute period during the zero order phase of the reaction. Activities were expressed as μ moles BAEE hydrolysed per minute per ml of duodenal juice.

When inhibition of this method was suspected, proteolytic activity towards gelatin was measured semi-quantitatively as an estimation of trypsin content (Gordon, Levin, and Whitehead, 1952). Serial dilutions of duodenal juice with sodium carbonate (1%, w/v) were made, and the tryptic activity of these solutions was determined by their capacity to digest completely the gelatin layer of x-ray film at 37°C for 30 minutes. Results were expressed in terms of the highest dilution causing the appearance of a completely translucent area.

DETERMINATION OF AMYLASE ACTIVITY

Amylase activity was determined by following the hydrolysis of a dyed amylopectin substrate, DyAmyl¹, as described by Babson, Tenney, and Megraw (1970). After incubation at 37°C for 10 minutes, the concentration of released dye was measured at 540 nm in a Unicam SP 600. Activities were expressed as Somogyi units.

DETERMINATION OF LIPASE ACTIVITY

Lipase activity was determined by the rate of

Request for reprints to: A. E. Cowen, Gastroenterology Registrar, Royal Brisbane Hospital, Brisbane 4029, Australia.

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¹General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N.J. 07950.

liberation of fatty acids from an olive oil emulsion (2% w/v) essentially as described by Massion and Seligson (1967). This method was modified in that the fatty acids were extracted by the Dole procedure and titrated with a digital microtitrator¹ (Dole and Meinertz, 1960). Enzyme activities were expressed as μ -equiv fatty acids per litre duodenal juice per minute.

Experimental Procedure

CLINICAL STUDY

The patient, a 45-year-old woman, had previously undergone a Polya gastrectomy. She was referred for assessment of pancreatic function after presenting with diarrhoea, weight loss, and proven steatorrhoea. Investigations included pancreatic scan and assessment of pancreatic function using secretin and pancreozymin (Burton Evans, Harper, Howat, Oleesky, Scott, and Varley, 1960). The pancreatic uptake of seleno-methionine, the secretion volumes, bicarbonate concentration, and lipase and amylase concentrations were within normal limits. Values for the trypsin assay were abnormally low. This single abnormal result suggested some interference by the Gastrografin used to aid in the positioning of the tube. The study was repeated without the use of Gastrografin.

¹AMINCO American Instrument Company, Silver Spring, Maryland.

EFFECT OF GASTROGRAFIN

Using BAEE substrate, the tryptic activity of the duodenal juice was measured. The sample was then diluted with three concentrations of Gastrografin (1%, 1.5%, and 2.0% v/v), in a ratio of 2 volumes of sample to 1 volume of Gastrografin. As a control, 2 volumes of sample were diluted with 1 volume of normal saline. These prepared samples were then assayed using the BAEE substrate and gelatin proteolysis. The samples containing Gastrografin showed a high background OD compared with normal duodenal aspirates. The absorption curve of Gastrografin was therefore determined using a Unicam SP 800 recording spectrophotometer over the range 200 nm to 300 nm at a concentration of 0.005% (v/v) in isotonic saline.

Results

The results of trypsin assays in the duodenal aspirates taken during the two pancreatic function studies are shown in Figure 1. On the first occasion trypsin assays following pancreozymin stimulation were abnormally low. In the second study, without the use of Gastrografin, the values are normal.

The absorption curve of Gastrografin over the range 200 nm to 300 nm is shown in Figure 2. At this dilution (0.005%) an OD absorption of 1.0 at 254 nm was seen. This wavelength corresponded with that used to measure the hydrolysis rate of BAEE in

Fig. 1 Graph showing trypsin values in the two studies.

Fig. 2 Absorption curve of 0.005% solution of Gastrografin. Arrow indicates the absorption at 254 nm.

the routine trypsin assay of duodenal juice.

The results of trypsin assay in a single sample of duodenal juice, diluted with varying concentrations of Gastrografin, are shown in the Table. In the same samples no evidence of inhibition of gelatin proteolysis was seen.

	Trypsin Activity (μ moles/min/ml)	Inhibition ¹ (%)
Duodenal juice	15.9	—
Duodenal juice + 0.3% Gastrografin	7.4	30
Duodenal juice + 0.45% Gastrografin	4.0	61
Duodenal juice + 0.6% Gastrografin	1.0	90
Duodenal juice + saline	10.3	0

Table The effect of Gastrografin on trypsin assay of duodenal juice.

¹No inhibition of proteolytic activity towards gelatin was observed when these same samples were assayed by the method of Gordon (1952).

Discussion

These laboratory studies confirm the impression from

the serial tests of pancreatic function *in vivo* that small amounts of Gastrografin remaining in the intestinal tract some hours after introduction may interfere with the spectrophotometric assay of trypsin by the method of Bergmeyer. There is, however, no inhibition of proteolysis.

Interference with the spectrophotometric assay appears to occur by two mechanisms. First the high absorbance of Gastrografin at 254 nm results in a falsely high background optical density during the assay. Secondly Gastrografin inhibits esterase activity towards the synthetic substrate. Trypsin assay is commonly used in the assessment of pancreatic function and in some centres is the only enzyme estimation performed. Lipase estimation is technically more difficult and time consuming while amylase estimations may suffer from salivary amylase contamination. Although several methods to assay trypsin are in use, the spectrophotometric assay is technically simple, inexpensive, quick, and effective (Sacks, Bank, Kramer, Novis, and Marks, 1971). However, when this assay is to be employed, the instillation of Gastrografin into the duodenum of patients before the estimation of pancreatic function should be avoided.

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