

# Trypsin and chymotrypsin in duodenal aspirate and faeces in response to secretin and cholecystokinin-pancreozymin

J. K. SALE, D. M. GOLDBERG, B. THJODLEIFSSON, AND K. G. WORMSLEY<sup>1</sup>

*From the Department of Chemical Pathology, Royal Hospital, Sheffield, and The Department of Therapeutics, University of Dundee*

**SUMMARY** The secretion of trypsin and chymotrypsin into the duodenum in response to secretin and CCK-PZ has been compared with the faecal excretion of the enzymes following similar stimulation in 47 individuals undergoing clinical investigation. The faecal output of chymotrypsin correlated well, but trypsin less satisfactorily, with the secretion of the respective enzymes into the duodenum. The faecal excretion of chymotrypsin following stimulation with secretin and CCK-PZ can therefore be used as an index of pancreatic enzyme-secretory capacity.

The secretion of bicarbonate into the duodenum in response to secretin is a satisfactory and widely used criterion for assessing the secretory capacity of the exocrine pancreas (Wormsley, 1972a) despite the assumed origin of the bicarbonate from the biliary system and duodenal mucosa as well as the pancreas. Recently, the availability of cholecystokinin-pancreozymin (CCK-PZ) and derivatives or analogues has resulted in attempts to utilize the specific pancreatic secretion of enzymes into the duodenum to measure pancreatic exocrine function (Burton, Evans, Harper, Howat, Oleesky, Scott, and Varley, 1960; Sun, 1963; Banwell, Northam, and Cooke, 1967; Wormsley, 1969; Ribet, Duffaut, Vaysse, and Laval, 1972). However, for reasons ranging from psychological to anatomical, it is impossible to intubate the duodenum to obtain aspirate for analysis in some adults and children in whom investigation of pancreatic exocrine function is clinically necessary.

The present study has tested the feasibility of measuring pancreatic enzyme-secretory capacity, while avoiding the necessity for intubation, by comparing the amounts of trypsin and chymotrypsin excreted in the faeces with the amounts secreted into the duodenum following stimulation with secretin and CCK-PZ.

## Methods

All 47 subjects required assessment of pancreatic exocrine function during the clinical investigation of symptoms, or combinations of symptoms, such as abdominal pain, diabetes mellitus, or steatorrhoea (see table). Each individual underwent two tests, comprising intubation and faecal enzyme collection, in random order, with an interval of at least three days between tests. Informed consent was obtained for each procedure.

Techniques of gastric and duodenal intubation and hormonal stimulation have been described previously (Wormsley, 1969; Goldberg, Sale, Fawcett, and Wormsley, 1972). Each subject received an intraven-

<i>Diagnosis</i>	<i>No. of Cases</i>	<i>Confirmation of Diagnosis</i>
Duodenal ulcer	11	Laparotomy
Postacute pancreatitis	7	Laparotomy
Gastric ulcer	7	Laparotomy
Normal (adhesions, Mallory-Weiss)	6	Laparotomy or endoscopy
Diabetes mellitus	2	Biochemical evidence
Pancreatic carcinoma	2	Laparotomy
Chronic pancreatitis	2	Laparotomy
Crohn's disease	2	Radiology
Coeliac disease	2	Biopsy
Motor neurone disease	2	Electromyography etc
Peripheral neuritis	1	Electromyography etc
Gallstones	1	Laparotomy
Cushing's syndrome	1	Laparotomy
Immunoglobulin deficiency	1	Biochemical evidence

Table Final diagnostic status of 47 patients

<sup>1</sup>Address communications to KGW, Ninewells Hospital, Dundee DD2 1UB

Received for notification 28th November 1973.

ous infusion of 0.15 M sodium chloride containing secretin in a dose of 1.0 clinical unit per kg body weight each hour plus CCK-PZ in a dose of 1.0 Ivy unit per kg each hour, for 50 minutes. The total 50-minute outputs of trypsin and chymotrypsin were calculated for each subject and are referred to in the text as 'outputs (of the respective enzymes) in duodenal aspirate'. The hormones were obtained from the GIH Laboratory, Karolinska Institute, Stockholm.

On the evening before the faecal enzyme test, each subject was given a glycerol suppository (BP) (4.0 g) at 2000 hours. The treatment was repeated next morning at 0700 hours, in order to evacuate colonic faecal residues. At 0800 hours, each individual received 30 ml of a mixture containing 5.0 g sodium bicarbonate and 30 ml of mist magnesium sulphate (BP). The same infusion of hormones was then given as during duodenal intubation. After the infusion, each individual received a further 30 ml of each of the sodium bicarbonate and magnesium sulphate mixtures, together with a capsule containing 300 mg carmine (to act as faecal marker). Breakfast was deferred until 0930 hours and the remaining meals were taken at the normal times.

Faeces were passed directly into weighed 3.0 l polythene beakers, standing in a bowl filled with ice. The faeces were collected in two batches—from 0800 to 1400 hours and from 1400 to 0800 hours, respectively. The beakers containing faeces were weighed

on completion of the appropriate collection period and the contents were homogenized. Measured amounts of 0.15 M sodium chloride were added, as necessary. Aliquots were obtained from each homogenized sample and stored at  $-20^{\circ}\text{C}$  before analysis. The activities of trypsin and chymotrypsin were measured as described previously, using p-tosyl-arginine methyl ester and acetyl tyrosine ethyl ester as respective substrates (Goldberg *et al*, 1972; Goldberg and Wormsley, 1970).

One male patient with total pancreatic achylia (shown by laparotomy and biopsy to be due to fatty replacement of the pancreatic acinar tissue) underwent a further test. A polythene tube (1 mm d) was passed so that the tip lay in the jejunum, just beyond the duodeno-jejunal junction. Three-hundred mg purified trypsin (Sigma Chemical Co Ltd; cat. no. T8253) plus 100 mg carmine were infused into the jejunum and followed by magnesium sulphate in order to promote purgation. The faeces were collected as above.

## Results

### EFFECT OF PURGATIVE AND OF CARMINE ON ENZYMIC ACTIVITIES

Neither the magnesium sulphate nor the carmine affected the activities of trypsin and chymotrypsin in the doses used in the present study.

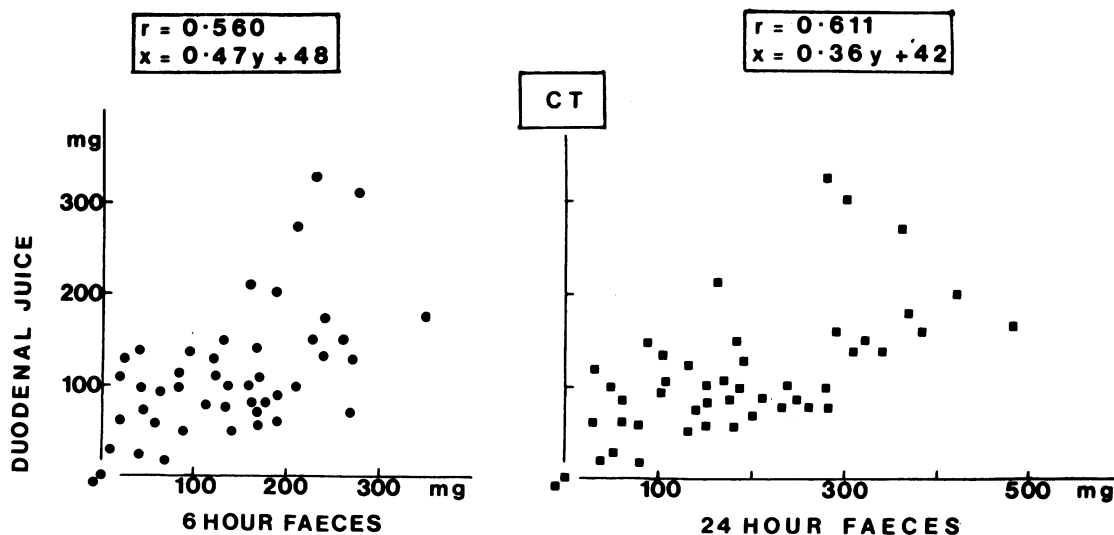


Fig 1 Comparison of outputs of chymotrypsin in duodenal aspirate and faeces.

Each point represents the outputs of chymotrypsin of one individual in duodenal aspirate compared with the faecal outputs in the six-hour and 24-hour collections after stimulation with secretin and CCK-PZ. The two points at the origin denote two subjects with no detectable output of enzymes.

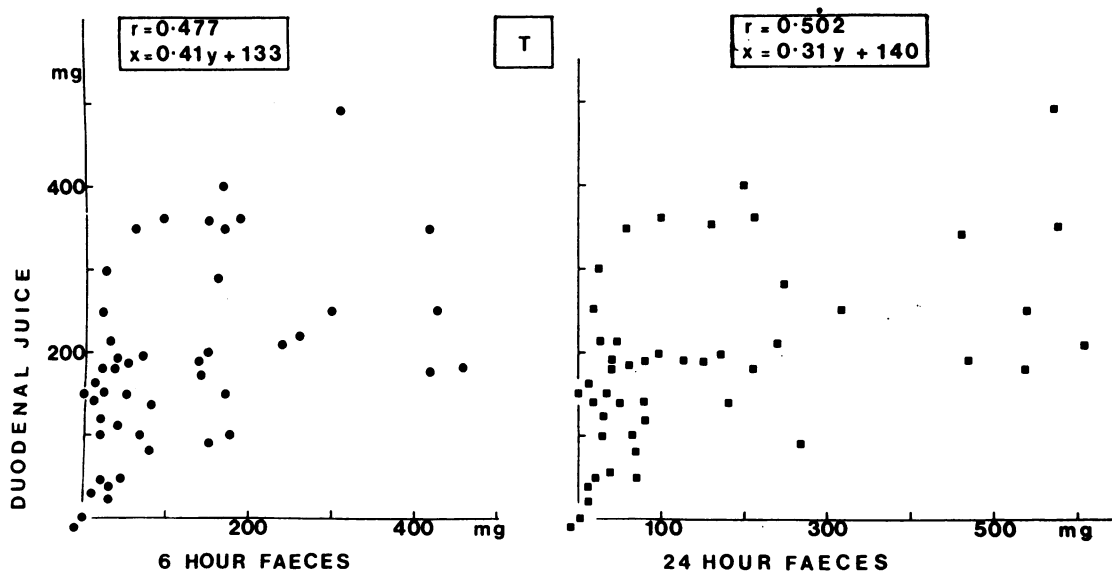


Fig 2 Comparison of outputs of trypsin in duodenal aspirate and faeces. Significance of symbols as in figure 1.

#### RECOVERY OF CARMINE

Carmine appeared in the faecal contents within six hours of administration in 80% of subjects and in all individuals during the second collection period.

#### RELATIONSHIP OF CHYMOTRYPSIN OUTPUTS IN DUODENAL ASPIRATE AND FAECES

There was significant correlation (fig 1) between the outputs of chymotrypsin in duodenal aspirate and faeces. In 53% of subjects, the faecal content of chymotrypsin during the first six-hour collection period was greater than the output in the duodenal aspirate and the proportion increased to 74% with the total 24-hour collection. The 24-hour faecal content of chymotrypsin was less than half the value of duodenal aspirate in only three individuals (one patient each with pancreatic carcinoma and Crohn's disease and one patient recovering from acute pancreatitis).

#### RELATIONSHIP OF TRYPSIN OUTPUTS IN DUODENAL ASPIRATE AND FAECES

The relationship between duodenal and faecal outputs of trypsin was not as good as for chymotrypsin (fig 2). In the majority of individuals, the faecal content of trypsin was less in the faeces than in duodenal aspirate, both in the six-hour (81% of patients) and in the 24-hour collections (72% of patients).

#### RELATIONSHIP OF SUM OF CHYMOTRYPSIN PLUS TRYPSIN IN DUODENAL ASPIRATE AND FAECES

The correlation between duodenal and faecal outputs

of the sum of both proteases was as good (fig 3) as the correlation between the duodenal and faecal outputs of chymotrypsin alone.

#### QUANTITATIVE CHANGE IN ACTIVITY OF TRYPSIN AND CHYMOTRYPSIN DURING INTESTINAL TRANSIT

Assuming that trypsin and chymotrypsin were secreted in the same ratio in response to magnesium sulphate and to food as into the duodenum in response to the exogenous hormones, it was calculated from the faecal outputs of chymotrypsin that the faecal excretion of trypsin during 24 hours represented losses during intestinal transit amounting to as much as four times the output of trypsin secreted in response to the 50-minute period of stimulation with secretin and CCK-PZ (fig 4).

#### RATIO OF CHYMOTRYPSIN TO TRYPSIN IN DUODENAL ASPIRATE AND FAECES

The ratio of chymotrypsin to trypsin increased in all except six subjects during transit through the intestine (fig 5). The two most significant decreases in ratio occurred in one patient with Cushing's syndrome and in one with pancreatic carcinoma, in whom the ratios had been unusually high in the duodenal aspirate (compared with the normal range, as described previously by Goldberg, Sale, and Wormsley, 1973).

#### FATE OF TRYPSIN INFUSED INTO THE JEJUNUM

Faecal output of trypsin during the 24 hours after

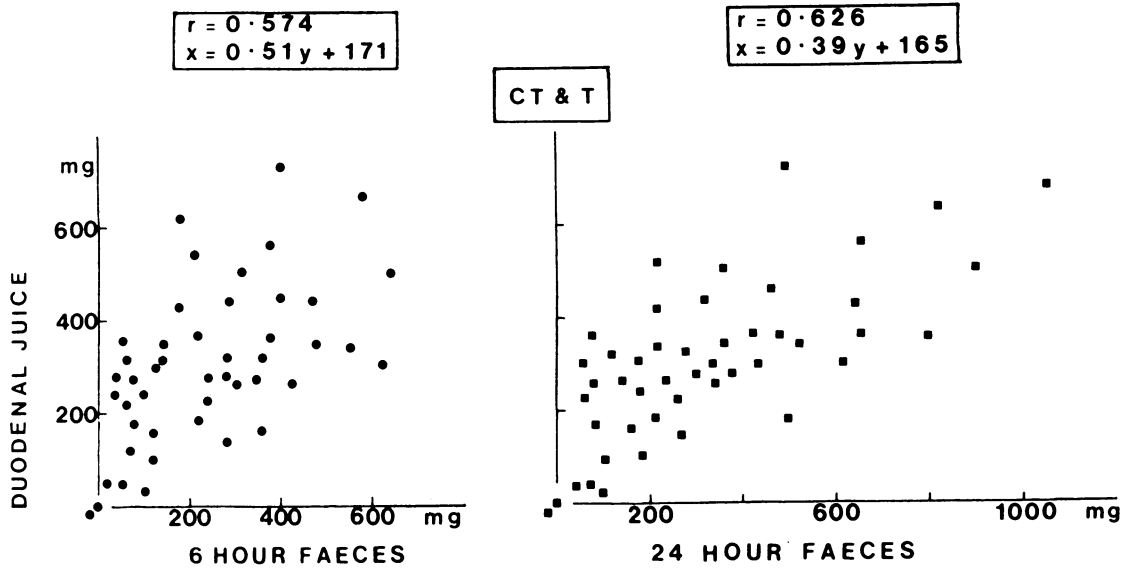


Fig 3

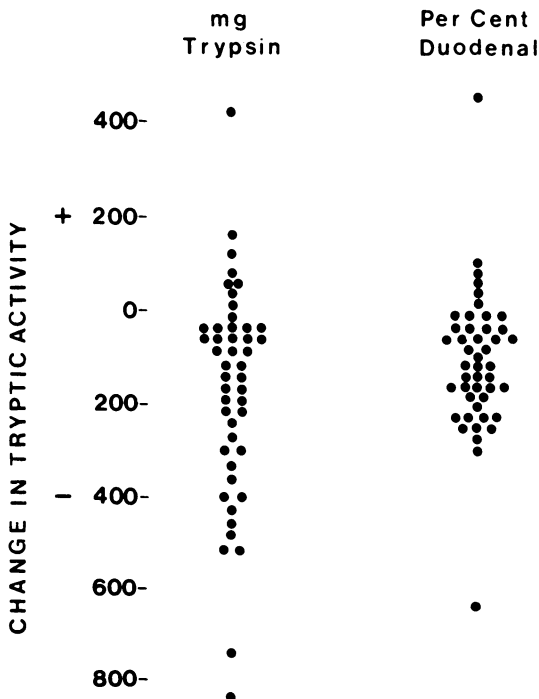


Fig 4

Fig 3 Comparison of sums of outputs of chymotrypsin plus trypsin in duodenal aspirate and faeces. Significance of symbols as in figure 1.

Fig 4 Calculated estimates of change in tryptic activity during intestinal transit. Each point represents the result from one individual, calculated from the 24-hour faecal outputs of trypsin and chymotrypsin as described in the text. The values represented by the ordinate refer to changes in tryptic activity depicted both in absolute terms (mg) or as a proportion (percentage duodenal) of the output of trypsin into the duodenum in response to stimulation with secretin and CCK-PZ. The minus sign indicates calculated loss of tryptic activity; the plus sign indicates calculated gain.

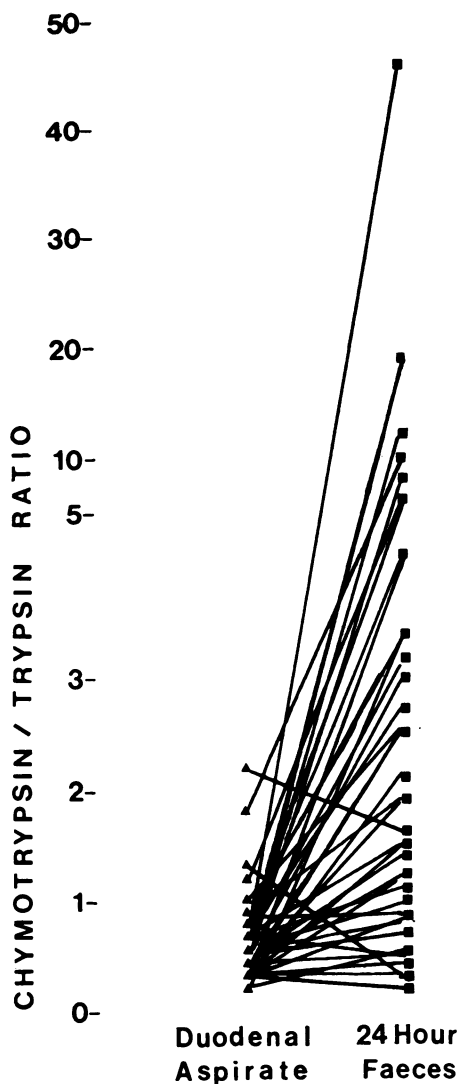


Fig 5 Ratio of chymotrypsin to trypsin in duodenal aspirate and 24-hour faecal collection.

Each pair of points represents results from one or more individuals. The scale is not continuous and is interrupted, as shown, as the ratio increases to greater than 3.

administration amounted to 23% of the amount infused into the jejunum of the patient with pancreatic achylia.

### Discussion

The present study is the first to compare the hormonally stimulated secretion of the pancreatic enzymes trypsin and chymotrypsin into the duodenum with

the faecal excretion of the enzymes after similar hormonal stimulation. The report describes the methodology and validation of the comparison.

### STIMULANT SCHEDULES

The dosage of stimulant hormones was selected both to accord with the specified stimulant schedule of a current international multicentre study of tests of pancreatic exocrine function and also in order to provide the highest rate of secretion into the duodenum together with the greatest degree of inhibition of gastric acid secretion compatible with satisfactory stimulation of pancreatic enzymes. Inhibition of gastric acid secretion, which results from the infusion of the combined hormones (Henriksen and Jørgensen, 1973), was sought because pancreatic proteases are rapidly and irreversibly inactivated by pepsin in the presence of acid (Heizer, Cleaveland, and Iber, 1965; Goldberg, Campbell, and Roy, 1969) and it therefore seemed desirable to suppress gastric acid secretion as much as possible, particularly because it had been shown that duodeno-gastric reflux sometimes occurred during pancreatic stimulation with exogenous hormones (Wormsley, 1972b). In order to minimize further destruction of pancreatic enzymes by gastric juice passing into the duodenum and to avoid destruction of enzymes regurgitated into the stomach, sodium bicarbonate was given before and after the period of pancreatic stimulation.

### ADMINISTRATION OF MAGNESIUM SULPHATE

Pancreatic enzymes are inactivated during intestinal transit, although the mechanisms are not known (Wormsley and Goldberg, 1972). The degree of inactivation appears to be lessened if the rate of intestinal transit is increased (Ammann, 1967). It therefore seemed appropriate to induce diarrhoea with a saline purgative (Martindale, 1972) in order to minimize inactivation of the exogenously stimulated pancreatic enzymes during passage through the intestine.

Magnesium sulphate in the duodenum also elicits secretion of pancreatic juice rich in enzymes (Hirschberg, 1928) and discharge of bile (Boyden, Bergh, and Layne, 1943) which may, in turn, stimulate the pancreatic secretion of enzymes (Forell and Stahlheber, 1966; Wormsley, 1970; Forell, Otte, Kohl, Lehnert, and Stahlheber, 1971) on contact with the duodenal mucosa. The use of magnesium sulphate may therefore prolong and increase the stimulus provided by the exogenous hormones to the pancreatic secretion of enzymes.

### INTERRELATIONSHIP OF THE ENZYMES

The present study has confirmed the reversal of the ratio of trypsin to chymotrypsin during intestinal



transit and the significantly greater recovery of chymotrypsin than trypsin in the faeces, which has been described previously (Haverback, Dyce, Gutentag, and Montgomery, 1963; Dyck and Ammann, 1965; Ammann, Tagwercher, Kashiwagi, and Rosenmund, 1968). The reason for the persistently high faecal content of trypsin in a few individuals is not clear, but high faecal outputs tend to occur in subjects who have high rates of secretion of trypsin into the duodenum.

Two hypotheses were considered in an attempt to explain the relatively greater reduction of tryptic than chymotryptic activity during intestinal transit. The less probable hypothesis—that both types of pancreatic protease were equally inactivated but that chymotryptic activity was derived from sources other than pancreas during passage through the intestine—was disproved, since the patient with pancreatic achylia did not excrete chymotrypsin. It seems likely, therefore, that the disappearance of tryptic activity is due to a greater degree of inactivation of trypsin than chymotrypsin during intestinal transit.

Nothing is known about the extent of inactivation of the human pancreatic proteases during intestinal transit, so that the absolute rates of degradation of trypsin and chymotrypsin cannot be accurately evaluated. However, an attempt has been made in the present report to calculate the approximate loss of tryptic activity during intestinal transit, based on the propositions that the two proteases have been secreted throughout the day in the same proportion as in hormonally stimulated duodenal aspirate and that chymotrypsin is not significantly degraded during intestinal transit. On the basis of these assumptions, it has been shown that the median daily loss of tryptic activity is equivalent to 150 mg of trypsin. The estimate must, however, be interpreted with due regard to two probable sources of error inherent in the underlying assumptions. In the first place, it is likely that some inactivation of chymotrypsin occurs, so that the calculated loss of trypsin is too low by a factor which depends on the degree of loss of chymotryptic activity during intestinal transit. No absolute values for loss of chymotryptic activity during passage through the intestine are available but comparison of the high average values for daily outputs of chymotrypsin in ileostomy effluent (Goldberg *et al*, 1972) with the faecal outputs of chymotrypsin in the present study indicates that some inactivation of chymotrypsin probably takes place in the colon. Secondly, evidence from two studies (Borgström, Dahlqvist, Lundh, and Sjövall, 1957; Worning and Müllertz, 1966) indicates that the concentration of chymotrypsin may be greater than trypsin in the duodenal contents after a (Lundh)

meal. If the ratio of chymotrypsin to trypsin in the pancreatic juice secreted in response to food is greater than in response to exogenous hormones, the calculated loss of trypsin during intestinal transit has been overestimated by a factor depending on the relative contribution of the food-stimulated secretion of proteases to the total output of the proteases in the faeces.

#### CORRELATION OF DUODENAL AND FAECAL ENZYME-SECRETORY RATES

Following stimulation with secretin plus CCK-PZ there is a very good correlation between the secretion of chymotrypsin into the duodenum during the 50-minute test period and the output of chymotrypsin in the faeces during the subsequent 24 hours, despite the fact that the 24-hour faecal collection contains not only the chymotrypsin secreted in response to the hormones, but also variable proportions of the pancreatic enzyme response to three meals and to the purgative. Faecal output of chymotrypsin less than half the duodenal output was found in only three subjects, so that the faecal content of chymotrypsin gave an excellent indication of the order of magnitude of the pancreatic enzyme-secretory capacity for chymotrypsin, as represented by the response to exogenous hormones. Correlation between the duodenal and faecal outputs of chymotrypsin during the first six hours after stimulation was not as good as for the 24-hour collection, indicating that the intestinal transit of the hormonally stimulated pancreatic enzymes, like carmine, was probably incomplete during the shorter collection period.

The faecal excretion of trypsin less accurately reflected the amount secreted into the duodenum in response to exogenous hormones. When faecal content of trypsin was high, the pancreatic capacity to secrete trypsin was also high, but the converse was not necessarily true, since very low levels of tryptic activity in the faeces were found in subjects whose capacity to secrete trypsin was quite normal. The faecal content of trypsin alone cannot therefore be used as a reliable index of pancreatic enzyme-secretory capacity although the combined outputs of chymotrypsin and trypsin correlated well with the combined outputs of the two proteases into the duodenum.

Measurement of the concentrations of chymotrypsin and, to a lesser extent, trypsin in random samples of faeces has been employed as a screening test for the detection of pancreatic disease for 10 years (Haverback *et al*, 1963; Dyck and Ammann, 1965; Ammann *et al*, 1968; Muller, Wisniewski, and Hansky, 1970) but the validity of using the concentration of pancreatic enzymes in 'spot' stool samples as an index of pancreatic function has been ques-

tioned (Banwell, Leonard, and Lobo, 1965; Wilding, Banwell, and Cooke, 1966; Smith, Ediss, Mullinger, and Bogoch, 1971). In the present study an attempt has been made to improve the correlation between pancreatic enzyme-secretory status and faecal excretion of the pancreatic enzymes by ensuring more strictly controlled stimulation of pancreatic enzyme secretion and quantitative determination of the resulting output of the pancreatic enzymes in the stool before assessing the use of the technique in confirming the clinical diagnosis and establishing the success of therapy in pancreatic disorders. The results of the present study indicate that the faecal excretion of chymotrypsin following stimulation with secretin and CCK-PZ can be used as an index of pancreatic enzyme-secretory capacity.

During the course of the study, KGW has received consecutive research grants from the Cystic Fibrosis Research Trust and the Scottish Hospital Endowments Research Trust, which are gratefully acknowledged. D.M.G. was generously supported by the Endowment Fund of the United Sheffield Hospitals.

#### References

- Ammann, R. W. (1967). *Fortschritte in der Pankreasfunktionsdiagnostik*. P. 27-30. Springer, Berlin.
- Ammann, R. W., Tagwercher, E., Kashiwagi, H., and Rosenmund, H. (1968). Diagnostic value of fecal chymotrypsin and trypsin assessment for detection of pancreatic disease. *Amer. J. dig. Dis.*, **13**, 123-146.
- Banwell, J. G., Leonard, P. J., and Lobo, R. M. F. (1965). Measurement of trypsin and chymotrypsin activity in stools to detect chronic pancreatic disease. *Gut*, **6**, 143-145.
- Banwell, J. G., Northam, B. E., and Cooke, W. T. (1967). Secretory response of the human pancreas to continuous intravenous infusion of pancreozymin-cholecystokinin (Cecekin). *Gut*, **8**, 380-387.
- Borgström, B., Dahlqvist, A., Lundh, G., and Sjövall, J. (1957). Studies of intestinal digestion and absorption in the human. *J. clin. Invest.*, **36**, 1521-1536.
- Boyden, E. A., Bergh, G. S., and Layne, J. A. (1943). An analysis of the reaction of the human gall bladder and sphincter of Oddi to magnesium sulphate. *Surgery*, **13**, 723-733.
- Burton, P., Evans, D. G., Harper, A. A., Howat, H. T., Oleesky, S., Scott, J. E., and Varley, H. (1960). A test of pancreatic function in man based on the analysis of duodenal contents after administration of secretin and pancreozymin. *Gut*, **1**, 111-124.
- Dyck, W., and Ammann, R. (1965). Quantitative determination of fecal chymotrypsin as a screening test for pancreatic exocrine insufficiency. *Amer. J. dig. Dis.*, **10**, 530-544.
- Forell, M. M., Otte, M., Kohl, H. J., Lehnert, P., and Stahlheber, H. P. (1971). The influence of bile and pure bile salts on pancreatic secretion in man. *Scand. J. Gastroent.*, **6**, 261-265.
- Forell, M. M., and Stahlheber, H. P. (1966). Gallefluss and Pankreassekretion. *Klin. Wschr.*, **44**, 1184-1189.
- Goldberg, D. M., Campbell, R., and Roy, A. D. (1969). Fate of trypsin and chymotrypsin in the human small intestine. *Gut*, **10**, 477-483.
- Goldberg, D. M., Campbell, R., and Roy, A. D. (1972). An investigation of the role of intraluminal proteolytic enzymes and intracellular enzyme inhibitors in ulcerative colitis. *Brit. J. exp. Path.*, **53**, 359-373.
- Goldberg, D. M., Sale, J. K., Fawcett, A. N., and Wormsley, K. G. (1972). Trypsin and chymotrypsin as aids in the diagnosis of pancreatic disease. *Amer. J. dig. Dis.*, **17**, 780-792.
- Goldberg, D. M., Sale, J. K., and Wormsley, K. G. (1973). Ratio of chymotrypsin to trypsin in human duodenal aspirate. *Digestion*, **8**, 101-109.
- Goldberg, D. M., and Wormsley, K. G. (1970). The interrelationships of pancreatic enzymes in human duodenal aspirate. *Gut*, **11**, 859-866.
- Haverback, B. J., Dyce, B. J., Gutentag, P. J., and Montgomery, D. W. (1963). Measurement of trypsin and chymotrypsin in stool: a diagnostic test for pancreatic exocrine insufficiency. *Gastroenterology*, **44**, 588-597.
- Heizer, W. D., Cleaveland, C. R., and Iber, F. L. (1965). Gastric inactivation of pancreatic supplements. *Johns Hopk. med. J.*, **116**, 261-270.
- Henriksen, F. W., and Jörgensen, S. P. (1973). Inhibition of gastric secretion by secretin and cholecystokinin. (Abstr.) *Acta endocr. (Kbh.)*, Suppl. 177, 332.
- Hirschberg, L. (1928). Sekretorische Funktion der Leber, des Pankreas und des Magens nach Einführung von MgSO<sub>4</sub> ins Duodenum. *Arch. Verdauungskr.*, **44**, 393-405.
- Martindale, W. (1972). *The Extra Pharmacopoeia*, p. 1048-1050, edited by N. W. Blacow, 26th ed. Pharmaceutical Press, London.
- Muller, L., Wisniewski, Z. S., and Hansky, J. (1970). The measurement of faecal chymotrypsin: a screening test for pancreatic exocrine insufficiency. *Aust. Ann. Med.*, **19**, 47-49.
- Ribet, A., Daffait, M., Vaysse, N., and Laval, J. (1972). Intérêt de la caeruleine en perfusion dans l'exploration fonctionnelle du pancréas exocrine. *Arch. Mal. Appar. dig.*, **61**, 541-548.
- Smith, J. S., Ediss, I., Mullinger, M. A., and Bogoch, A. (1971). Fecal chymotrypsin and trypsin determinations. *Canad. med. Ass. J.*, **104**, 691-697.
- Sun, D. C. H. (1963). Normal values for the pancreozymin-secretin test. *Gastroenterology*, **44**, 602-606.
- Wilding, P., Banwell, J. G., and Cooke, W. T. (1966). Fecal enzyme assay as an aid to the diagnosis of pancreatic exocrine insufficiency. *Amer. J. dig. Dis.*, **11**, 722-727.
- Wormsley, K. G. (1969). The response to infusion of a combination of secretin and pancreozymin in health and disease. *Scand. J. Gastroent.*, **4**, 623-632.
- Wormsley, K. G. (1970). Stimulation of pancreatic secretion by intraduodenal infusion of bile salts. *Lancet*, **2**, 586-588.
- Wormsley, K. G. (1972a). Pancreatic function tests. *Clin. Gastroent.*, **1**, 27-51.
- Wormsley, K. G. (1972b). Aspects of duodeno-gastric reflux in man. *Gut*, **13**, 243-250.
- Wormsley, K. G., and Goldberg, D. M. (1972). The interrelationships of the pancreatic enzymes. *Gut*, **13**, 398-412.
- Worning, H., and Mullertz, S. (1966). pH and pancreatic enzymes in the human duodenum during digestion of a standard meal. *Scand. J. Gastroent.*, **1**, 268-283.