

Trypsin and lactoferrin levels in pure pancreatic juice in patients with pancreatic disease

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SUMMARY Levels of immunoreactive trypsin were measured in pure pancreatic juice obtained endoscopically from 44 patients with suspected pancreatic disease. Patients with pancreatic cancer all had low trypsin concentrations (median 3.6 $\mu\text{g/ml}$, range 0.6-12.0), but those with chronic pancreatitis had very variable levels (median 14.2 $\mu\text{g/ml}$, range 3.2-76.8), showing a considerable overlap with patients without pancreatic disease (median 37.1 $\mu\text{g/ml}$, range 10.4-66.0). When levels of lactoferrin in pancreatic juice were measured, all patients with chronic pancreatitis were found to have much higher levels (all greater than 900 ng/ml) than control subjects or patients with pancreatic cancer (all less than 400 ng/ml). The combined measurement of trypsin and lactoferrin in pure pancreatic juice appeared to be more promising than any other currently available test for the separation of patients with pancreatic cancer from those with chronic pancreatitis.

The initial hope that the measurement of pancreatic enzymes in duodenal juice after secretin stimulation would provide a useful indicator of pancreatic function has not been realised. Enzyme estimation appears to add little to the diagnostic value of the secretin test (Brooks, 1972; Wormsley, 1972; Gyr *et al.*, 1975). With the advent of fiberoptic endoscopy secretin became the standard stimulus for collecting pure pancreatic juice endoscopically. We therefore thought it would be worth re-evaluating the value of enzyme estimation in pure pancreatic juice, as all the previous studies were carried out on duodenal juice.

The measurement of some individual enzymes may be more useful in differentiating the normal from abnormal pancreas. Choi and co-workers (1967) and Goldberg and Wormsley (1970) showed that proteolytic enzymes were better than amylase as an index of pancreatic secretory capacity. Sarles and co-workers (1966) reported that lipase disappeared completely from duodenal contents during secretin infusion, while the level of other enzymes did not change. It seems from this that proteolytic enzymes are probably the best as a diagnostic measure, and of the proteolytic enzymes trypsin was shown to be more sensitive than chymotrypsin in the assessment of pancreatic function (Goldberg *et al.*, 1972).

There is now an immunological method for measuring trypsin which measures trypsin-like immunoreactivity rather than catalytic activity. It can therefore measure both trypsin and trypsinogen, and is not affected by the presence of trypsin inhibitors. It is also more sensitive than the catalytic methods used before (Temler and Felber, 1974).

We have previously shown that the measurement in pure pancreatic juice of the iron binding protein lactoferrin is useful in the diagnosis of pancreatic diseases (Fedail *et al.*, 1978). Lactoferrin estimation can differentiate patients with chronic pancreatitis from controls and patients with pancreatic cancer. In the search for another parameter to help to differentiate cancer from controls we measured trypsin, as previous workers have shown that in pancreatic cancer enzyme output is usually more affected than in chronic pancreatitis (Burton *et al.*, 1960; Wormsley, 1969).

Methods

Trypsin assay was performed using the Hoechst Pharmaceutical/Behring Institute radioimmunoassay kit, which is now commercially available in the UK and Europe. With this kit the standards and radioiodinated trypsin are prepared from highly purified human pancreatic tissue and the antiserum raised in rabbits. A double antibody method is used to separate free from antibody-bound trypsin. The

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method measures both trypsin and trypsinogen, so is suitable for use with pure (inactivated) pancreatic juice. The performance of the kit has been assessed by Elias *et al.* (1977). It was found to be reliable and the results were reproducible.

Our findings as regards reproducibility were similar to theirs, with a within-assay variation of 4.6% and between-assay variation of 10.4%. Storage of samples of pure pancreatic juice at -20°C for periods of up to three months did not appear to affect measured trypsin levels, and no preservatives or enzyme inhibitors—for example, aprotinin—were added to stored samples.

The radioimmunoassay for lactoferrin was as previously described (Fedail *et al.*, 1978). Pure pancreatic juice was collected after selective pancreatic duct cannulation, 5–10 ml usually being obtained over two minutes after intravenous injection of 1 clinical unit/kg body weight of secretin (Boots, Nottingham, England). Because of the high trypsin content of pancreatic juice, samples were diluted 1:100 in phosphate buffered saline before assay. Forty-seven patients who had been referred for the investigation of clinically suspected pancreatic disease were studied. Cannulation of the pancreatic duct was impossible in three patients (two later shown to have a normal pancreas, one to have

chronic pancreatitis), leaving 44 for study. Twenty-four were eventually shown to have chronic pancreatitis, the diagnosis being established by two or more of the following criteria: abnormal endoscopic retrograde pancreatography (ERP) (Kasugai *et al.*, 1972), abnormal secretin test (Dreiling and Janowitz, 1962), and abnormal Lundh test meal (Mottaleb *et al.*, 1973). ERP was abnormal in all but two of these patients, and both of these had abnormal secretin and Lundh tests. There were 10 patients with cancer of the head of the pancreas, the diagnosis in all cases being confirmed by cytology, histology, or both. Ten subjects who had no radiological or biochemical evidence of pancreatic disease were regarded as a control group.

Results

The median trypsin concentrations in controls were 37.05 $\mu\text{g/ml}$ (range 10.4–66.0), patients with chronic pancreatitis 14.2 $\mu\text{g/ml}$ (range 3.2–76.8), and in patients with pancreatic cancer 3.6 $\mu\text{g/ml}$ (range 0.6–12.0) (Fig. 1)

Using the Wilcoxon rank sum test for unpaired samples, the difference between controls and chronic pancreatitis was barely significant ($P < 0.05$), and there was a considerable overlap in trypsin levels. Trypsin concentration was also significantly lower in cancer than in pancreatitis ($P < 0.01$). When lactoferrin and trypsin measurements were combined, an interesting pattern emerged (Fig. 2). The lactoferrin level effectively separated patients with chronic pancreatitis (all of whom had levels of 900 ng/ml or more) from those with pancreatic cancer or controls (all of whom had levels less than 400 ng/ml). Of those who did not have raised lactoferrin levels, the trypsin estimation separated all but one of the 'control' subjects from those with pancreatic cancer. As previously reported, one subject with low levels of trypsin and raised levels of lactoferrin had developed carcinoma of the pancreas after having had chronic pancreatitis for many years.

Discussion

There was considerable overlap in the pancreatic juice trypsin levels in the three groups, but patients with pancreatic cancer had uniformly low levels of trypsin.

The reason for the low trypsin in pancreatic cancer is not clear. It is not due to a smaller volume of secretion, as in this series no differences were noted between patients with cancer and pancreatitis in the volumes collected in the first two minutes after secretin stimulation. The pancreatic duct was outlined in all cases of pancreatic cancer. Four of them

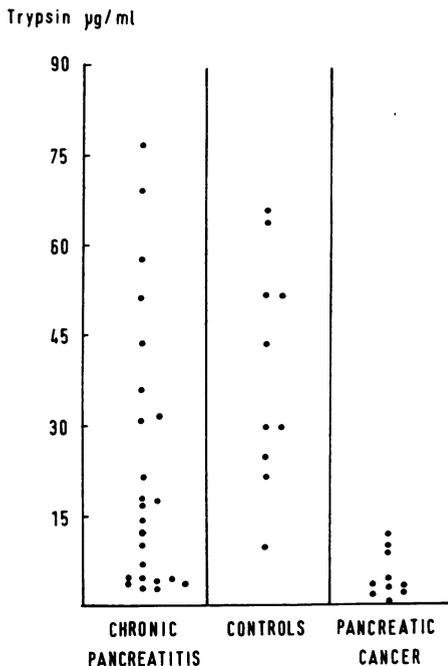


Fig 1 Immunoreactive trypsin levels in pure pancreatic juice.

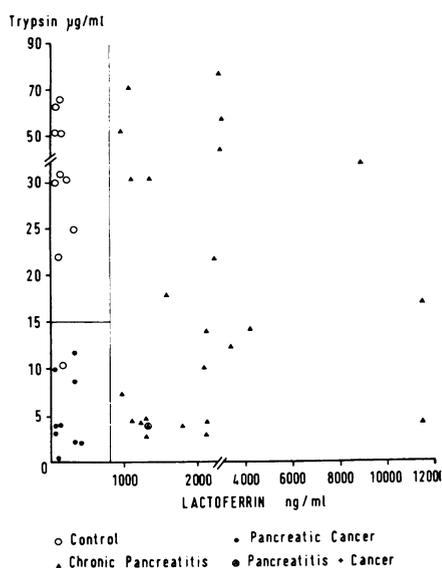


Fig. 2 Relationship between immunoreactive trypsin and lactoferrin levels in pancreatic juice in different groups of patients.

had a stricture in the region of the head of the pancreas; some patients with chronic pancreatitis, however, also had strictures but did not have a low trypsin concentration. The low trypsin may in part be due to malnutrition as a result of the severe anorexia from which most of these patients suffer, or else to a non-specific effect of malignant disease on protein metabolism.

None of the currently available tests can reliably differentiate pancreatic cancer from chronic pancreatitis. This is true of endoscopic pancreatography (Salmon, 1978), ultrasound examination (DiMagna *et al.*, 1977), computer-assisted tomography (Go *et al.*, 1978), and even laparotomy (Gambil, 1971; Trapnell, 1971). The results of the present study indicate that the estimation of lactoferrin in pure pancreatic juice is an excellent test for chronic pancreatitis, and that the combined measurement of lactoferrin and trypsin may be of additional diagnostic value in suspected pancreatic disease.

The most obvious limitation to the use of this test as a diagnostic aid is the need to obtain pure pancreatic juice by endoscopic cannulation. Studies are currently in progress in our laboratory to determine whether measurements of lactoferrin and trypsin in duodenal juice give similar diagnostic information. If so, as duodenal juice is much easier for most people to obtain, this might considerably increase the value of such measurements.

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References

- Brooks, F. P. (1972). Testing pancreatic function. *New England Journal of Medicine*, **286**, 300-303.
- 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.
- Choi, H. J., Goldstein, F., Wirtz, C. W., and Menduke, H. (1967). Normal duodenal trypsin values in response to secretin-pancreozymin stimulation and preliminary data in patients with pancreatic disease. *Gastroenterology*, **53**, 397-402.
- DiMagna, E. P., Malagelada, J. R., Taylor, W. F., Go, V. L. W. (1977). A prospective comparison of current diagnostic tests for pancreatic cancer. *New England Journal of Medicine*, **297**, 737-742.
- Dreiling, D. A., and Janowitz, H. D. (1962). The measurement of pancreatic secretory function. In *CIBA Foundation Symposium on the Exocrine Pancreas*, pp. 225-252. Edited by A. V. S. Rouck and De M. P. Cameron. London. Churchill.
- Elias, E., Redshaw, M., and Wood, T. (1977). Diagnostic importance of changes in circulating concentrations of immunoreactive trypsin. *Lancet*, **2**, 66-68.
- Fedail, S. S., Harvey, R. F., Salmon, P. R., and Read, A. E. (1978). Radioimmunoassay of lactoferrin in pancreatic juice as test for pancreatic disease. *Lancet*, **1**, 181-182.
- Gambil, E. E. (1971). Pancreatitis associated with pancreatic carcinoma. A study of 26 cases. *Proceedings of the Mayo Clinic*, **46**, 174-177.
- Go, V. L. W., and Sheedy, P. F. (1978). Ultrasonography, computed tomography, endoscopic retrograde cholangiography and angiography in the diagnosis of pancreatic cancer. *Medical Clinics of N. America*, **62**, 129-140.
- Goldberg, D. M., Sale, J. K., Fawcett, N., and Wormsley, K. G. (1972). Trypsin and chymotrypsin as aids in the diagnosis of pancreatic disease. *American Journal of Digestive Diseases*, **17**, 780-791.
- Goldberg, D. M., and Wormsley, K. G. (1970). The interrelationships of pancreatic enzymes in human duodenal aspirate. *Gut*, **11**, 859-866.
- Gyr, K., Agrawal, N. M., Felsenfeld, O., and Font, R. G. (1975). Comparative study of secretin and Lundh tests. *American Journal of Digestive Diseases*, **20**, 506-512.
- Kasugai, T., Kuno, N., Kizu, M., Kobayashi, S., and Hattori, K. (1972). Endoscopic pancreaticocholangiography. 2. The pathological endoscopic pancreato-

- cholangiogram. *Gastroenterology*, **63**, 227–234.
- Mottaleb, A., Kapp, F., Noguera, E. C. A., Kellock, T. D., Wiggins, H. S., and Waller, S. L. (1975). The Lundh test in the diagnosis of pancreatic disease. A review of 5 years' experience. *Gut*, **14**, 835–841.
- Salmon, P. R. (1978). Re-evaluation of endoscopic retrograde cholangiopancreatography as a diagnostic method. In *Clinics in Gastroenterology*, volume 7, pp. 651–666. Edited by K. F. R. Schiller. Saunders: London.
- Sarles, H., Figarella, C., Prezelin, G., and Souville, C. (1966). Comportement différent de la lipase, de l'amylase et des enzymes proteolytiques pancréatiques après différents modes d'excitation du pancréas humaine. *Bulletin Société Chimie et Biologie*, **48**, 951–957.
- Temler, R. S., and Felber, J. P. (1974). Radioimmunoassay of enzymes of the exocrine pancreas. *Radioimmunoassay: Methodology and Applications in Physiology and in Clinical Studies*, pp. 17–25. Edited by R. Levine and E. F. Pfeiffer. George Thieme: Stuttgart.
- Trapnell, J. (1971). The operative diagnosis of carcinoma of the pancreas. *British Journal of Surgery*, **58**, 849–851.
- Wormsley, K. G. (1969). The response to infusion of a combination of secretin and pancreozymin in health and disease. *Scandinavian Journal of Gastroenterology*, **4**, 623–632.
- Wormsley, K. G. (1972). Pancreatic function tests. *Clinical Gastroenterology*, **1**, 27–51.