LETTERS TO THE EDITOR

Phospholipase A₂ and acute pancreatitis in rats

EDITOR,—Phospholipase A₂ (PLA₂) is an enzyme that cleaves fatty acids from phospholipids, leading to the generation of inflammatory eicosanoids.¹ The group II isoenzyme of phospholipase A₂ (PLA₂-II) is raised in peripheral blood during various inflammatory states.² PLA₂ is induced in vitro and in vivo by the classic acute phase cytokine interleukin 6.^{3,4} Therefore PLA₂-II can be regarded as an acute phase protein. With great interest we read the well designed study of Furue *et al* (*Gut* 1997;41:826-31) who could clearly show that most PLA₂ activity during sodium deoxycholate induced acute pancreatitis in rats is due to the group II isoenzyme. Furue and colleagues suggested that the liver is the main source of this enzyme in their experiments because Nevalainen and coworkers⁵ and Crowl and coworkers⁶ described PLA₂-II activity in human hepatocytes. We agree with the suggestion of Furue *et al* that PLA₂-II is produced in human hepatocytes like classic acute phase proteins. This was also demonstrated by our group,⁷ but we think that the extrapolation of these findings from human tissue to animal experiments, as done by Furue *et al*, requires some clarification. Hatch *et al* were able to show that liver macrophages and not hepatocytes were the main source of PLA₂-II in rats during endotoxin induced acute phase reaction.⁷ As a consequence, liver macrophages but not hepatocytes could also be the main source of PLA₂-II in the animal experiments presented by Furue and collaborators. Therefore an alternative pathogenetic mechanism leading to liver damage in experimental induced acute pancreatitis should be taken into account. Our alternative pathogenetic sequence is that PLA₂-II released by liver macrophages attacks adjacent already damaged liver tissue, thus leading to complete destruction of hepatocytes with subsequent release of aminotransferases into the circulation, as shown by Furue *et al*. In conclusion, we agree that PLA₂-II could be an important mediator of liver failure in acute pancreatitis, but alternative pathomechanisms resulting from different production sites should be discussed.

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- 2 Prudzenski W, Vadas P. Phospholipase A₂: A mediator between proximal and distal effectors of inflammation. *Immunol Today* 1991;12:143-6.
- 3 Crowl RM, Stoller TJ, Conroy RR, *et al*. Induction of phospholipase A₂ gene expression in human hepatoma cells by mediators of the

acute phase response. *J Biol Chem* 1991;266:2647-51.

- 4 Bertsch T, Banks RE, Forbes MA, *et al*. Phospholipase A₂ activity is induced during treatment with recombinant interleukin-6 in patients with cancer. *Ann Clin Biochem* 1996;33:565-7.
- 5 Nevalainen TJ, Kallajoki M, Pesonen E, *et al*. Origin of circulating group II phospholipase A₂ in hepatocytes in a patient with epitheloid hemangioendothelioma of the liver. *Lab Invest* 1996;74:585-91.
- 6 Bertsch T, Banks RE, Forbes MA, *et al*. Phospholipase A₂ is induced during treatment with recombinant interleukin-6. In: Martin SM, Halloran SP, eds. *Proceedings of the XVI International Congress of Clinical Chemistry*. Cambridge: Pigott, 1996:62.
- 7 Hatch GM, Vance DE, Wilton DC. Rat liver phospholipase A₂ is an endotoxin stimulated membrane-associated enzyme of Kupffer cells which is released during liver perfusion. *Biochem J* 1993;293:143-50.

Reply

EDITOR,—We thank Drs Bertsch and Fischer for their interest in our paper. As group II PLA₂ (PLA₂-II) is known to be produced from a variety of cells upon stimulation with various stimuli, it is still difficult to ascertain the main source of increased levels of this enzyme in the circulation both in animal models of inflammation and in human patients.^{1,2} As Bertsch and Fischer comment, we have to be careful about transferring findings from human tissue to animal experiments. Also of note is that liver macrophages (Kupffer cells) and not hepatocytes could be the main source of PLA₂-II in our experiments on acute pancreatitis in rats. However, in contrast to the findings by Hatch and colleagues³ rat cultured hepatocytes can produce PLA₂-II upon stimulation via chemical hypoxic injury,⁴ suggesting that the production of PLA₂-II depends on both the type of cell and also the type of stimulus used. So we still think that hepatocytes are the main source of PLA₂-II in our experiments. In addition, vascular smooth muscle cells could also be a potential source of PLA₂-II as reported by Nakano and Arita⁵ who found high levels of PLA₂-II transcripts in aorta of endotoxaemic rats. Very recently high levels of PLA₂-II protein and overexpression of its mRNA in vascular tissue were also found in rat adjuvant arthritis.⁶ Therefore, the possible sources of PLA₂-II in our rat acute pancreatitis could be hepatocytes, Kupffer cells and smooth muscle cells, and this enzyme from different type of cells would work in concert to damage liver tissue. Of course, the immunohistochemical study to identify the cellular source of PLA₂-II in our experiments should be performed.

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- 1 Vadas P, Browning J, Edelson, *et al*. Extracellular phospholipase A₂ expression and inflammation: the relationship with associated disease states. *J Lipid Mediat* 1993;8:1-30.

2 Kallajoki M, Nevalainen TJ. Expression of group II phospholipase A₂ in human tissues. In: Uhl W, Nevalainen TJ, Büchler MW, eds. *Phospholipase A₂—Basic and clinical aspects in inflammatory diseases. Progress in Surgery*. Basel: Karger, 1997:8-16.

3 Hatch GM, Vance DE, Wilton DC. Rat liver mitochondrial phospholipase A₂ is an endotoxin-stimulated membrane-associated enzyme of Kupffer cells which is released during liver perfusion. *Biochem J* 1993;293:143-50.

4 Wang H, Harrison-Shostak DC, Lemasters JJ, *et al*. Contribution of pH-dependent group II phospholipase A₂ to chemical hypoxic injury in rat hepatocytes. *FASEB J* 1996;10:1318-25.

5 Nakano T, Arita H. Enhanced expression of group II phospholipase A₂ gene in the tissues of endotoxin shock rats and its suppression by glucocorticoid. *FEBS Lett* 1990;273:23-6.

6 Koo Seen Lin M, Katz A, Van Den Bosch H, *et al*. Induction of secretory phospholipase A₂ confirms the systemic inflammatory nature of adjuvant arthritis. *Inflammation* 1998;22:161-73.

Effect of gastric acid suppressants on human gastric motility

EDITOR,—I read with interest the recent article by Parkman *et al* (*Gut* 1998;42:243-50) who reported on the effects of H₂ receptor antagonists and omeprazole on interdigestive gastroduodenal motility and concluded that these drugs have a motor stimulant effect. This conclusion, however, could be invalidated by the unsatisfactory study design. The basal fasting period of 30 minutes prior to administration of the drug is very short when one considers that the mean duration of the cycle of the interdigestive migrating motor complex is at least three times as long¹; the 30 minute observation period after drug administration is also quite short. Parkman and colleagues could have recorded the phase III that frequently occurs spontaneously when the stress caused by the insertion of the probe has lessened. In addition, omeprazole was given before the insertion of the manometric probe so that no control period was recorded in these subjects. These biases could raise some perplexing questions about the real effectiveness of these acid suppressants in stimulating interdigestive gastroduodenal motility. However, this is a real effect as has been previously and repeatedly demonstrated for ranitidine,² famotidine,³ nizatidine,⁴ and particularly for omeprazole.⁵ Unfortunately these studies were not cited by Parkman *et al*.

The hypothesis of cholinergic activity put forward to explain the strange motor effects of these acid suppressants could be valid for only a few H₂ receptor antagonists. The most probable explanation is that acid suppression removes the inhibition exerted by acid on the initiation of phase III by motilin, as can be inferred from the close relation between the interdigestive motor and secretory activities of the stomach.⁶ However, as some authors have found an increase in plasma motilin after the administration of famotidine,³ I believe that a study on plasma motilin concentrations after the administration of these drugs, especially omeprazole, is desirable in an attempt to clarify the mechanism underlying this unexpected effect of these acid suppressants.

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- 1 Quigley EMM, Donovan JP, Lane MJ, *et al*. Antroduodenal manometry. Usefulness and limitations as an outpatient study. *Dig Dis Sci* 1992;37:20-8.

- 2 Bortolotti M. Interdigestive gastroduodenal motility in duodenal ulcer: role of gastric acid hypersecretion. *Am J Gastroenterol* 1989;84:17-21.
- 3 Sekiguchi T, Kusano M, Nishioka T, *et al*. Gastroduodenal motor dysfunction and plasma motilin concentration in patients with duodenal ulcers. In: Itoh Z, ed. *Motilin*. San Diego: Academic Press Inc, 1990:225-44.
- 4 Ueki S, Seiki M, Yoneta T. Gastroprokinetic activity of nizatidine, a new H_2 -receptor antagonist, and its possible mechanism of action in dogs and rats. *J Pharmacol Exp Ther* 1993;264:152-7.
- 5 Bortolotti M. Relationship between acid secretory activity and gastroduodenal migrating motor complex. *Hepatogastroenterology* 1996;43:1288-95.
- 6 Bortolotti M. Relationships between the interdigestive motor and secretory activities of the stomach. *Gastroenterol Int* 1996;9:139-54.

Reply

EDITOR,—We appreciate Professor Bortolotti's comments by our article. Our study showed that therapeutic doses of ranitidine, famotidine, and omeprazole, which suppress gastric acid secretion, affect both gastric motility and gastric emptying. Each agent delayed gastric emptying despite an increase in postprandial antral contractility as assessed using antral manometry, electrogastrography, and dynamic antral scintigraphy. Interestingly, these agents also stimulated antral phase III migrating motor complexes in the fasting state. In this letter, we address some of the comments raised by Professor Bortolotti.

The study was designed primarily to assess the effects of histamine type II receptor antagonists (H_2 RA) on postprandial gastric emptying. We also simultaneously recorded electrogastrography, antroduodenal manometry, and dynamic antral scintigraphy to determine the mechanisms of altered gastric emptying. The study design included a 30 minute period to observe the effects of the gastric acid suppressants on fasting gastric motility to use as appropriate reference values for the postprandial effects. We agree that the 30 minute periods were relatively short to study the fasting period fully, with its 90 minute recurrent fasting migrating motor complexes. As our study was a four way cross over study, the effects of gastric acid suppression were compared, not only with the time periods before the administration of the study drug, but also with appropriate placebo controlled arms in the same subject. Omeprazole was given orally as there is not an intravenous form in the United States. The effects of omeprazole were compared only with the placebo arm. Professor Bortolotti notes that our findings have been confirmed by several other investigators.

Our study was designed to see whether the cholinergic properties of ranitidine seen *in vitro*¹ could be seen *in vivo*. We compared the effects of ranitidine, an H_2 RA with cholinergic activity, with famotidine, an H_2 RA with little cholinergic activity, and with omeprazole, a proton pump inhibitor with no known cholinergic activity. We found that the effects of the gastric acid suppressants (ranitidine, famotidine, and omeprazole) were all similar, suggesting the effects seen were due to gastric acid suppression, rather than to selective properties of ranitidine. With the results of our studies, further investigation of the mechanism of gastric acid suppression on gastric motility can be undertaken. Professor Bortolotti suggests a study on plasma motilin concentrations after the administration of these drugs. We would also suggest measuring serum gastrin and somatostatin concen-

trations as these are also affected by gastric acid suppression and might mediate some of the gastric motor effects seen with gastric acid suppressants. Finally, one would want to monitor gastric pH during the study to help determine the degree of acid suppression with each of these agents. These points are addressed in the discussion section of our manuscript.

We thank Professor Bortolotti for his comments and the chance to address them. Professor Bortolotti has made many contributions to the relationship between gastric acid suppression and gastric motility, not only in normal subjects as our study, but also in gastric pathophysiology such as duodenal ulcer, gastric ulcer, and non-ulcer dyspepsia.

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- 1 Parkman HP, Pagano AP, Ryan JP. Ranitidine and nizatidine stimulate antral smooth muscle contractility via an excitatory cholinergic mechanism. *Dig Dis Sci* 1998;43:497-503.

Faecal elastase 1 in chronic pancreatitis

EDITOR,—I read with interest the recent paper by Lankisch *et al* (*Gut* 1998;42:551-4). The authors studied faecal elastase 1 and faecal chymotrypsin in 30 patients with chronic pancreatitis and concluded that the estimation of faecal elastase 1 is not helpful in diagnosing mild and moderate exocrine pancreatic insufficiency and that this enzyme is not superior to faecal chymotrypsin in the diagnosis of exocrine pancreatic insufficiency. As various studies have already been published on this subject, I believe that it is useful to report the results of these studies, mainly because they clearly show that Lankisch *et al*'s conclusions cannot be substantiated. Domínguez-Muñoz and colleagues,¹ in 20 patients with chronic pancreatitis (six mild, four moderate and 10 severe), found that the sensitivity of faecal elastase 1 was 0%, 100% and 100%, and that of faecal chymotrypsin 0%, 75% and 70%, in the three groups, respectively. Glasbrenner and colleagues² studied 63 patients with chronic pancreatitis (19 mild, 14 moderate and 30 severe) and found that faecal elastase 1 had a sensitivity of 47%, 79% and 100%, and faecal chymotrypsin a sensitivity of 32%, 36% and 63%, respectively. Löser *et al*,³ in 44 patients with chronic pancreatitis (eight mild, 14 moderate and 22 severe), found that faecal elastase 1 had a sensitivity of 63%, 100% and 100%, and faecal chymotrypsin a sensitivity of 25%, 50% and 86%, respectively. Finally, in 44 patients with chronic pancreatitis (nine mild, 13 moderate and 22 severe) we found⁴ a sensitivity of 22%, 77% and 100% for faecal elastase 1, and of 11%, 54% and 77% for faecal chymotrypsin. In the four studies combined, 171 patients with chronic pancreatitis were studied and the sensitivity of faecal elastase 1 was 38%, 87% and 100% in mild ($n=42$), moderate ($n=45$) and severe ($n=84$) chronic pancreatitis, respectively, whereas the sensitivity of faecal chymotrypsin was considerably lower, at 21%, 49% and 74% respectively. Based on these numbers, I believe that it can be clearly stated that faecal elastase 1 has a good sensitivity in patients with moderate and severe pancreatic insufficiency and that it is clearly superior to faecal chymotrypsin in the diagnosis of exocrine pancreatic insufficiency. Lankisch *et al*'s conclusion may

have resulted from the small number of patients included in their study.

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- 1 Domínguez-Muñoz JE, Hieronymus C, Sauerbruch T, *et al*. Faecal elastase test: evaluation of a new noninvasive pancreatic function test. *Am J Gastroenterol* 1995;90:1834-7.
- 2 Glasbrenner B, Schon A, Klatt S, *et al*. Clinical evaluation of the faecal elastase test in the diagnosis and staging of chronic pancreatitis. *Eur J Gastroenterol Hepatol* 1996;8:1117-20.
- 3 Löser C, Mollgaard A, Folsch UR. Faecal elastase 1: a novel, highly sensitive, and specific rubeles pancreatic function test. *Gut* 1996;39:580-6.
- 4 Gullo L, Ventrucci M, Tomassetti P, *et al*. Faecal elastase 1 determination in chronic pancreatitis. *Dig Dis Sci* (in press).

Reply

EDITOR,—We are grateful for the interest Professor Gullo has taken in our recently published paper. He disagrees with one of our conclusions, namely that the estimation of faecal elastase 1 is not distinctly superior to the usual faecal chymotrypsin estimation.

His disagreement is based on four different studies which he has combined for meta-analysis. We doubt whether these four studies may be used for a meta-analysis as faecal elastase 1 was studied using a variety of different methods. In Glasbrenner *et al*'s study¹ the faecal elastase test was done in patients in whom the diagnosis of chronic pancreatitis was based on endoscopic retrograde cholangiopancreatography (ERCP), and in the study by Domínguez-Muñoz and colleagues² the diagnosis was based on ERCP and computed tomography. Löser and colleagues³ tested, as we did, against the secretin-pancreozymin test, which is the gold standard for testing exocrine pancreatic function.^{4,5} The tests which Professor Gullo used are presently unknown as his study has yet to appear in print.

The upper limit of normal for a faecal chymotrypsin test is 3 U/g stool. Concentrations between 3 and 6 U/g stool usually raise a suspicion of exocrine pancreatic insufficiency. In our study, both concentrations were used. It would be interesting to know what the results of the other studies¹⁻³ would have been if they had used the upper level of normalcy. However, even if a correct analysis was to undermine in a larger number of patients our modest conclusion that faecal elastase 1 is not distinctly superior to the faecal chymotrypsin estimation, the criticism does not concern the main message of our paper.

We concluded that faecal elastase 1 is particularly helpful in detecting severe exocrine pancreatic insufficiency. Several studies, including ours, have shown that the severe form may be detected with a sensitivity of between 73 and 100%.^{1-3,6-8} However, a similar conclusion can be reached about various other indirect pancreatic function tests.^{4,5}

We concluded that the test was not helpful in the mild to moderate form where the sensitivity was 40% and 33%. This is in agreement with other studies scoring a sensitivity from 0 to 47%.^{1,2,8} for the mild form of chronic pancreatitis or exocrine pancreatic insufficiency. Only Löser and colleagues³ claim a sensitivity of 63% for mild and 100% for moderate cases. It should be noted that in these groups the incidence of patients with