any serrations along the down slope of the emptying curve.'

What Howard *et al* show in figure 1 of their study is an emptying artifact by ultrasound. A change in width and height of the gall bladder after fatty meal is no guarantee that gall bladder bile actually emptied. A tensed gall bladder may fail to empty despite the fact it looks smaller in diameter. In addition there is no way of ensuring that the slice chosen is identical in all of the measurements. The authors have correctly admitted that ultrasound often depends upon patient cooperation and the technologist's skill. A fully filled gall bladder volume is about 40–50 ml. Estimated ultrasound error in measuring the diameter of two large gall bladders was 0.77 mm with a standard deviation of 0.4 mm. What is the error when the volume is between the 0–20 ml range? In a study comparing simultaneous measurement of gall bladder emptying by cholescintigraphy and ultrasound a good correlation was found in the mid range of gall bladder ejection fraction between 20–50%.¹ In five patients, however, where gall bladder ejection fraction was between 0–20% by scintigraphy, the ultrasound overestimated gall bladder ejection fraction as 15–40%. In three of the patients with scintigraphic ejection fraction between 55–85%, the ultrasound underestimated the ejection fraction between 45–65%. Therefore, a geometric method that may be adequate to measure resting gall bladder volume may not be ideal to measure bile emptying especially when volume changes are small.

Figure 1 shows the typical pattern of bile emptying following fatty meal ingestion. Note that after oral ingestion, the gall bladder does not begin to empty for 16 minutes. After 16 minutes, it shows a sustained emptying without any refilling.

It seems more appropriate to combine ultrasound and scintigraphic techniques to assess gall bladder function. Ultrasound is appropriate to measure the resting gall bladder volume and scintigraphy is ideal to measure bile emptying following cholecystokinin or fatty meal stimulation. I suggest the authors use a non-geometric method before proposing a new theory for gall bladder emptying.

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on many points, there remain a few criticisms which we wish to refute. Krishnamurthy's main criticism is that we used ultrasound inappropriately to study gall bladder emptying. He argues that our technique relies on a geometric (sum of cylinders) method and that the changes we saw in gall bladder volume were artefactual.

In our paper, we went to considerable lengths to show that the changes seen in gall bladder volume were not as a result of measurement artefact. Firstly, we showed that the pattern of emptying was altered by changing the stimulus to contraction. Hence, the emptying pattern in response to a cholecystokinin infusion was qualitatively and quantitatively different to that seen in response to a physiological meal stimulus, indicating that the changes were because of an alteration in the behaviour of the gall bladder itself – rather than to errors in measuring gall bladder volume. After a cholecystokinin infusion, the minute-by-minute fluctuations in maximum length and width were increased compared with baseline despite an overall decrease in gall bladder volume. Secondly, by making repeated measurements of the diameters of large 'static' gall bladders in vivo we estimated that the accuracy of measuring gall bladder diameter was 0.77 mm (SD 0.44 mm). This effectively excludes measurement artefact as the explanation for changes in gall bladder volume, especially for small (5–10 ml) gall bladders, which are easier to image than large (40–50 ml) gall bladders. As the volume of a cylinder is proportional to the square of its radius, similar errors in width measurements will have less effect on calculated volume for small compared with large diameter gall bladders.

Dr Krishnamurthy rightly points out that ultrasound is an observer dependent technique requiring each individual gall bladder silhouette to be individually focused. This is an advantage, however, in that it allows the operator to compensate for changes in the position and orientation of the gall bladder as it contracts. We have previously used radionuclide scanning (cholescintigraphy) and ultrasonographic techniques to study gall bladder emptying.¹ Although both methods have their limitations, in our opinion ultrasound is more sensitive than cholescintigraphy, but in the absence of a 'gold standard' discrepancies between the results obtained with the two methods cannot, with validity, be ascribed to artefacts in ultrasonography. Cholescintigraphy, however, cannot be regarded as a 'non-geometric method'. Northfield and colleagues have shown that for accurate counts during cholescintigraphy, a depth correction is required.² Unless anterior and posterior cameras are used, there may be considerable errors in estimating counts over the gall bladder. Furthermore, his assertion that 'measurement of the absolute volume is not required' is based upon in vitro validation studies which may have little relevance to the situation in vivo. Krishnamurthy *et al* validated their method by measuring counts in a balloon filled with water containing ^{99m}Tc of constant specific activity.³ The balloon was moved 0.5 cm, to and fro, 30 times per minute at a constant distance from the gammacamera – hardly a fair representation of the movement of the human gall bladder with respiration.

The important question is whether the gall bladder refills during the postprandial period. This cannot be answered by cholescintigraphy using a single isotopic marker. During cholecintigraphy, the fasting gall bladder fills with isotopically labelled 'hot' bile following an intravenous injection of ^{99m}Tc HIDA. Isotope

counts rise to a maximum over the gall bladder which is then made to contract, after which isotope counts fall progressively (how could they do otherwise?). The so called 'gall bladder emptying' in the figure of Krishnamurthy's letter is, in fact, the change in isotope counts of the area of interest over the gall bladder. It only represents true emptying if there is no gall bladder refilling and the 'specific activity' of gall bladder bile (counts per unit vol of bile) remains constant. Any refilling of the gall bladder with 'cold' bile would lead to a progressive fall in 'specific activity' which would seriously underestimate the true gall bladder emptying rate. Cholescintigraphic 'emptying' curves, therefore, represent not only true emptying but also the displacement of 'washout' of 'hot' gall bladder bile by 'cold' hepatic bile and so are entirely consistent with intermittent filling and emptying of the gall bladder. Northfield and colleagues⁴ labelled hepatic bile with indocyanine green and gall bladder bile with ^{99m}Tc HIDA. Using this dual isotope technique, they measured absolute gall bladder storage and evacuation and also found that gall bladder filling and emptying is intermittent.

Dr Krishnamurthy proposes a combination of ultrasound and scintigraphic techniques to assess gall bladder function. Such a study has recently been reported by Jazrawi *et al*⁵ and showed evidence of postprandial gall bladder refilling as well as emptying in response to a meal. Physiological studies in both humans and animals^{6–8} have shown that gall bladder dynamics are more complex than those suggested by the conventional model. In contrast with Krishnamurthy's findings, but in agreement with evidence from several others, we found a cephalic phase to gall bladder emptying.^{9–11} It is time to reassess the complex neural and humoral factors involved rather than adhere dogmatically to old theories in the face of new evidence.

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Reply

EDITOR, — We thank Dr Krishnamurthy for the interest he has expressed in our paper and for his thoughtful comments. While we agree

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Acetorphan and diarrhoea

EDITOR, — We were interested to read the paper of Baumer (*Gut* 1992; 33: 753-8) regarding the antisecretory effects of enkephalinase (neutral endopeptidase 24.11) inhibition with acetorphan in cathartic and infectious diarrhoea in man and would like to stress another role for enkephalinase enzymes in down regulating the inflammatory response.¹

Enkephalinase (neutral endopeptidase 24.11) reduces the response of neutrophils to inflammatory peptide stimuli² including the bacterially derived F-met peptides. Peptidase enzymes are thought to protect the gut from the inflammation induced by bacterial peptides.³ We have studied enkephalinase activity in peripheral blood neutrophils in ulcerative colitis⁴: patients with ulcerative colitis showed enkephalinase activity of 1.07×10^{-6} M/30 min/ 10^5 neutrophils (geometric mean); in contrast healthy volunteers showed enkephalinase activity of 2-24 (difference from colitis patients $p=0.048$, confidence interval for ratio of means 0.23 to 0.98). These preliminary data suggest that peripheral blood neutrophils from patients with active ulcerative colitis may have a reduced ability to down regulate their responses to both bacterial F-met peptides and endogenous neuropeptides although it is not clear whether this is primary or secondary.

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Reply

EDITOR, — The proposal by Cole and Hawkey for a role of enkephalinase (membrane metalloendopeptidase, EC 3.4.24.11) in the degradation of bacterial F-Met-Phe-Leu and other pro-inflammatory peptides in patients with ulcerative colitis seems an interesting suggestion. We feel, however, that this idea is not supported by their data¹ and, furthermore, seems unlikely.

Firstly, the assay they used to establish a slight significant change ($p=0.048$) in a small sample of patients ($n=6$) is incorrect as thiorphan is used at a concentration 10 000 times higher than actually required for a specific assay.² We have also found that this

experimental condition leads to an overestimation of the specific enzyme activity of isolated human neutrophils by up to 50%. Secondly, among the six patients studied by Cole *et al.*,¹ 'two were receiving steroids, three steroid enema, five oral salicylates, and four azathioprine' and it would seem, therefore, rather premature to attribute any change in their neutrophils to the disease rather than to such treatments. Thirdly, it seems misleading to study inactivation of pro-inflammatory F-Met peptides by peptidases of neutrophils rather than by intestinal mucosa. Indeed, pro-inflammatory F-Met peptides generated by intestinal bacteria may play a part in intestinal inflammatory disorders if they cross the epithelial barrier in which local peptidases seem to play a critical role. Hence Chadwick *et al.*³ have identified the major mucosal peptidase responsible for F-Met-Leu-Phe hydrolysis as a carboxypeptidase which is down regulated in Crohn's disease and have also suggested a role for a F-Met deformylase and a peculiar F-Met aminopeptidase. By contrast, a role for enkephalinase, whose activity in mucosa is greater than in neutrophils, was not shown in this study.

In addition, it seems likely that bacterial colonisation and pullulation within the intestinal lumen facilitates the crossing of bacterial pro-inflammatory peptides. In this respect, the use of a purely antisecretory agent, such as the enkephalinase inhibitor acetorphan as an anti-diarrhoeal agent seems preferable to traditional opiate like antidiarrhoeals, such as loperamide, with an antitransit mode of action.^{4,5} In support of this idea, various opiates, given to patients with chronic ulcerative colitis to control debilitating diarrhoea, were found to induce, even after a few days and in a high percentage of subjects (17 of 18 in one study), a fulminating ulcerative colitis complicated by toxic megacolon.^{6,7} This effect is presumably a consequence of their action on colonic motility seen after a single small dose was given in 14 patients.⁸ Whereas, various enkephalinase inhibitors seem without the typical antitransit effects of μ opiate antidiarrhoeals,^{4,5} both in rodents⁹ and humans.¹⁰

The suggested role of enkephalinase in down regulating inflammatory response in airways, through degradation of tachykinins,¹⁰ has also been challenged recently as the inhibitor acetorphan given to volunteers with asthma had no deleterious effects.¹¹ Hence the anti-inflammatory role of the peptidase is far from established.

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NOTES

National Association for Colitis and Crohn's Disease

NACC is pleased to invite applications for the 1993 grant awards. Projects may address any aspect of inflammatory bowel disease. NACC is particularly keen to encourage not only high quality mainstream research, but also welcomes applications which may be considered as realistic 'what if' research projects. These are likely to be smaller, relatively inexpensive applications which might be viewed less favourably by larger, less specialised organisations. Although all applications are assessed together, NACC wishes to encourage projects which address social and quality of life issues of inflammatory bowel disease where methodology remains difficult.

The closing date for applications is Friday 30 April 1993. Further details are available from Dr P B McIntyre, Honorary Secretary, Medical Advisors NACC, Queen Elizabeth II Hospital, Howlands, Welwyn Garden City, Herts AL7 4HQ.

Barcelona '93 - II United European Gastroenterology Week

This will take place from 19 to 24 July 1993 in Barcelona, Spain. Further information from Prof J R Malagelada, c/o UNICONGRESS, Calle Calvet 55-57 (4th floor), 08021 Barcelona, Spain (Tel: 34 3 414 03 22; fax: 34 3 414 02 51).