PostScript

LETTERS

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Hypomagnesaemia due to malabsorption is not always responsive to oral magnesium oxide supplementation alone

We read with interest the Gut File report by Dr Ross and colleagues of hypomagnesaemia due to malabsorption, eventually responding to oral magnesium oxide supplementation (*Gut* 2001;**48**:857–8). Our experience however has been different. For the past seven years we have managed a 65 year old woman with short bowel syndrome (right hemicolectomy for Duke's C colorectal carcinoma and thereafter a terminal ileal resection for abscess formation).

High ileostomy output was initially difficult to manage (after excluding infection, secreting gut hormone tumours by normal hormone levels, and despite dietary and pharmacological modifications). Clinical signs of hypomagnesaemia and hypocalcaemia ensued. An initial trial with magnesium glycerophosphate (September 1992 to December 1993) was insufficient to sustain her serum magnesium levels requiring frequent "top ups" of intravenous magnesium.[1] In December 1993, she was switched to magnesium oxide supplementation but despite this the frequency of intravenous magnesium "top ups" were not reduced. Compliance was not deemed to be an issue with our patient.

Since then we have managed this woman while still taking magnesium oxide supplements with almost 3–6 monthly (fig 1) intravenous magnesium replacement through a peripheral line and have avoided insertion of a Hickman line and all its associated complications.[2] While we agree that a trial of magnesium oxide is prudent and until the pharmacokinetics are better understood, this preparation may not be sufficient, especially in patients with extensive resection of the small bowel, as demonstrated in our patient.

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Regulation of gastric function by gastrin releasing peptide

Hildebrand *et al* reported data suggesting that gastrin releasing peptide (GRP) may be a physiological regulator of pre- and postprandial gastric acid secretion (*Gut* 2001;**49**:23–8). Interestingly, these effects were independent of gastrin and the authors appropriately questioned the physiological role of gastrin in regulating gastric secretion. Several aspects of the authors' conclusions deserve further clarification and discussion.

The authors concluded that alteration of somatostatin secretion is unlikely to explain the acid inhibitory action of BIM26226 because the GRP antagonist did not alter somatostatin mRNA levels. They also argued that the lack of change of gastrin mRNA supported the physiological data showing no alteration in gastrin secretion with BIM26226. In short term experiments such as these, it is incorrect to assume mRNA levels reflect peptide secretion rates. In longer term studies this may be true, but it is clear that neuropeptide translation, synthesis, and secretion are all regulated independently and changes may not occur in parallel.[1 2] Indeed, previous studies with GRP infusion in humans have shown that although secretion of gastrin peptide was enhanced, gastrin mRNA levels were actually decreased.[3] Thus it would be unwise to exclude the possibility of any peptide having a role in this dynamic system on the basis of unchanged mRNA levels.

The authors also state that muscarinic receptor activation inhibits somatostatin secretion from D cells. This is correct for fundic but incorrect for antral D cells. Muscarinic activation actually stimulates somatostatin release from antral D cells.[4–6] This would be more compatible with the mechanisms suggested by the authors: GRP enhanced neuronal acetylcholine release and this reduced fundic somatostatin mediated inhibition of histamine and acid secretion; in the antrum, stimulation of somatostatin release would then impair the gastrin response and could contribute to the lack of gastrin response seen in the current experiments.

The authors felt that previous in vitro studies of G cells were difficult to assess but they appear to have overlooked detailed studies of cultured human G cells. Squires *et al* demonstrated two receptors of the GRP family in antral tissue, namely GRPR (BB1) and BRS-3 (BB3).[7] BRS-3 is an orphan receptor that does not functionally respond to bombesin/GRP except at very high concentrations.[8] Single cell microfluorometry clearly showed antral G cells responding to bombesin with an increase in intracellular calcium, thus suggesting that human antral G cells express physiologically functional GRP receptors.[7] In the light of these data, the results of Hildebrand *et al* are very interesting and the current study should stimulate interest in the ever evolving understanding of gastric secretory function. Further studies with BIM26226 under different conditions, to more fully describe the pathophysiological role of GRP, are awaited with interest.

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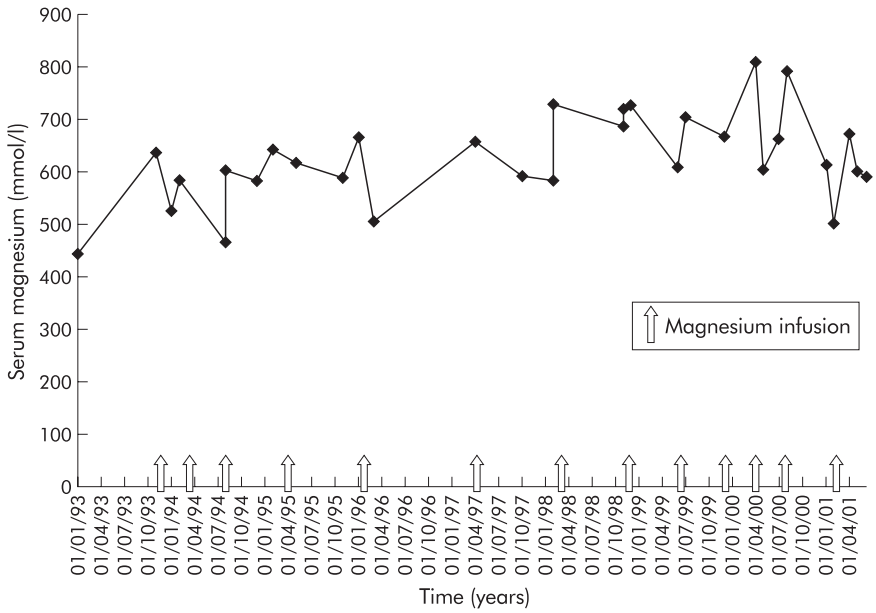

Figure 1 Serum magnesium levels in our patient over the course of treatment with magnesium oxide supplements and intravenous magnesium replacement.

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Authors' reply

We appreciate Dr Beales's interest in our study describing the effects of gastrin releasing peptide (GRP) and its antagonist BIM26226 on gastric acid secretion in healthy male subjects. One can only speculate on the mechanisms of GRP stimulated acid secretion. The points raised by Dr Beales are interesting and valid. However, a word of caution is necessary for some of his conclusions: data from rats and dogs cannot be used to explain the mechanisms of GRP on acid secretion as the effects of endogenous GRP on acid output were independent of plasma gastrin in humans. These results are clearly in contrast with findings in animals and suggest species differences. Thus the molecular mechanism of GRP stimulated acid production has not directly been substantiated in humans. This is not likely to be investigated in the near future because the antagonist BIM26226 is no longer available for human use.

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Osteoporosis is not a specific complication of primary biliary cirrhosis (PBC)

Newton *et al* (*Gut* 2001;**49**:282–7) described a retrospective study on bone mineral density (BMD) in a large cohort of patients with primary biliary cirrhosis (PBC). The authors concluded that osteoporosis is not a specific complication of PBC, but certain weaknesses in the study design do not support this conclusion.

(A) The authors did not include age and sex matched controls from the general population, or control groups with different types of liver disease.

(B) A proper methodological design comparing osteoporosis in PBC and in a normal population should calculate the standardised incidence ratio of osteoporosis for the two cohorts by comparing the observed incidence versus the expected incidence. The calculation should include 95% confidence intervals according to exact Poisson limits.[1]

(C) A logistic regression analysis including risk factors for osteoporosis (that is, age, menopausal status, smoking habits, alcohol consumption, etc) should have been performed.

The major drawback however is the lack of control populations with different types of liver disease. New data are emerging in the literature concerning this field. In particular, several recent studies (including one of our own)[2–4] have demonstrated that PBC in itself does not exert a negative influence on BMD. Thus we agree with Newton *et al* that osteoporosis in PBC should be revisited. In fact, analysis of the literature enables the following conclusions to be drawn. There are several osteoporosis risk factors common to liver disease, aging processes, or genetic variability, as well as cholestasis related risk factors, that are obviously not specific for PBC (table 1). The pathogenesis of osteoporosis is multifactorial, increasing with advancing age, and influenced by genetic factors, and it may be that liver disease accelerates bone resorption through various mechanisms.

Table 1 Risk factors for osteoporosis in liver diseases

Common risk factors	Cholestasis related risk factors
• Cirrhosis	• Calcium malabsorption
• Female sex	• Vit D malabsorption
• Old age	• Hyperbilirubinaemia
• Alcohol consumption	• Cholestyramine therapy
• Hypogonadism	
• Steroid therapy	
• Low BMI	
• VDR polymorphism	
• Impaired conversion to 25-OH vit D	
• Reduced osteocalcin activity	

BMI, body mass index; VDR, vitamin D receptor.

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Authors' reply

We read with interest the letter of Professor Floreani in which she agrees that it is timely to revisit the perceived dogma that patients with primary biliary cirrhosis (PBC) are predisposed to osteoporosis. It was pleasing to note that our findings compare favourably with work from her group. We would also like to take this opportunity to draw attention to two further studies which have been presented in abstract form since the submission of our manuscript, which confirm that there is no increase in prevalence of osteoporosis[1] and no increase in fracture risk in PBC populations taken as a whole compared with appropriately matched normal controls.[2] A further study has also been published recently[3] describing bone mineral density in a selected series of patients with PBC in whom the proportion with osteoporosis (defined by T score) was comparable with that seen in our series. We were pleased to note that in this prospective series other risk factors for osteoporosis were examined. They concur with our finding that increased age is an independent risk factor, although they do not present mean Z score data (bone mineral density data controlled for both sex and age). Their group, we would argue, was younger and had more severe disease than patients in our series, whom we would regard as more representative of the whole PBC population.

In our study we demonstrated that although 85/272 (31%) patients had osteoporosis, as defined by the WHO ($T < -2.5$) at the time of their first DEXA scan, mean Z score at the neck of femur was −0.1 and at the lumbar spine 0.1. As Z scores represent bone mineral density compared with an age and sex matched population, this suggests that the prevalence of osteoporosis seen in PBC is a reflection of the fact that this is predominantly a disease of postmenopausal women who show a generalised increased prevalence of osteoporosis. The use of Z scores implicitly controls our data for age and sex norms.

We agree with Professor Floreani that readdressing the question of osteoporosis prevalence in other chronic liver diseases (both cholestatic and non-cholestatic) would be of interest but we feel that this is not relevant to the current study.

Given the very real problems experienced by some PBC patients as a result of osteoporotic fracture (particularly in the early post-transplant period), further study of the aetiology is appropriate (although the retrospective nature of the current study makes the suggested logistic regression analysis inappropriate). The message that we (and more recently others) have been communicating is that the search for risk factors for osteoporosis should not be focused on liver disease specific factors but could more usefully be directed at more generalised population risk factors.

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Expression of isoforms of nitric oxide synthase in collagenous colitis

We read with interest the study by Perner *et al* (*Gut* 2001;**49**:387–94) investigating expression of various isoforms of nitric oxide synthase (iNOS, eNOS, and nNOS) in non-inflamed colon, collagenous colitis, and ulcerative colitis. Inducible NOS (iNOS) was identified by immunohistochemical analysis in the epithelium of patients with non-inflamed colon. The authors concluded that this might be a result of bowel preparation with bisacodyl.

Increased synthesis of nitric oxide has been detected by a number of different methods in patients with ulcerative colitis.[1 2] We have previously found physiological expression of iNOS in histologically normal colon using reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry, and immunoblotting.[3] Tissue from three different sources was studied. Surgical specimens were obtained from patients undergoing colectomy for colorectal cancer who had undergone bowel preparation with sodium picosulphate, colonoscopic biopsies from patients also prepared with sodium picosulphate, and rectal biopsies at sigmoidoscopy from patients who had received no bowel preparation. This last group of patients also underwent colonoscopy with sodium picosulphate preparation confirming the absence of colonic pathology. iNOS mRNA was identified in all samples by RT-PCR. iNOS protein was detected by immunoblotting in 77% of samples, by immunostaining in 80% of surgical specimens, and in 90% of biopsy specimens.[3] It is therefore possible that expression of iNOS in epithelial cells reported by Perner *et al* is, as we found, a result of physiological expression of iNOS rather than a secondary phenomenon as a result of bisacodyl bowel preparation.

The reason for iNOS expression in normal colonic epithelium is not currently clear. Nitric oxide production may aid maintenance of the epithelial barrier by preventing bacterial translocation or by inducing apoptosis. It is also possible that its presence represents a link between dietary or other luminal factors and the development of colorectal cancer.

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Authors' reply

We thank Cameron *et al* for their comment on our recent publication and important observations that suggest physiological expression of inducible nitric oxide synthase (iNOS) in histologically normal human colon. As we observed subtle iNOS labelling in colonic mucosal biopsies from our group of controls with non-inflamed bowel, we have subsequently studied whether bowel preparation with bisacodyl or polyethylene glycol prior to sigmoidoscopy might induce iNOS expression.

Ten healthy non-smoking male subjects were investigated. Mucosal biopsies were taken from the sigmoid colon prior to bowel preparation and again 12 hours after rectal administration of an enema consisting of bisacodyl (100 mg) or polyethylene glycol 3000 (6.4 g in 100 ml of water) in randomised order. Expression of iNOS protein was quantified by western blot analysis and localised by immunohistochemistry.

iNOS was expressed in the colonic mucosal biopsies from all subjects and localised in epithelial cells, particularly at the luminal border of the epithelial cells and more pronounced in the crypt epithelium. Expression of iNOS was unaffected by bowel preparation with bisacodyl or polyethylene glycol (fig 1).

Hence we agree with Cameron *et al* that expression of iNOS in epithelial cells is possibly a result of physiological expression of iNOS rather than a secondary phenomenon as a result of the bowel preparation per se or the effect of the secretagogue laxative bisacodyl. For the reasons given above, we also agree that nitric oxide may be important in maintaining the epithelial barrier and may represent a link between dietary or other luminal factors and the development of colorectal cancer, as hypothesised by Cameron *et al*, although high iNOS expression in collagenous colitis is not associated with an increased risk of malignancy.

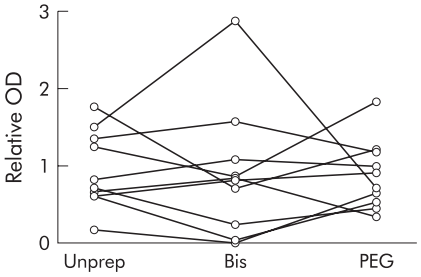

Figure 1 Expression of inducible nitric oxide synthase in mucosal biopsies from the unprepared (Unprep) sigmoid colon and 12 hours following bowel preparation with bisacodyl (Bis) or polyethylene glycol 3000 (PEG), analysed by western blotting and quantified by densitometry relative to a reference. Values in individual subjects are represented by circles and connected by lines. OD, optical density.

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CORRECTION

In the BSG abstract supplement published in April, abstract 402 by Davies *et al* (*Gut* 2002;**50**(Suppl II):A109) contained an error. The sensitivity and specificity for recurrence were given as 85.7% and 71.4% respectively. The correct result should be a reversal of these values, with sensitivity being 71.4% and specificity being 85.7%. The authors apologise for the error.

NOTICES

Broad Medical Research Program—Inflammatory Bowel Disease Grants

Funds for inflammatory bowel disease (IBD) research are available immediately from the Broad Medical Research Program of The Eli and Edythe L Broad Foundation for innovative projects regarding etiology, therapy, or prevention. Grants totalling approximately US$100,000 per year are available for basic or clinical projects. Larger erquests may be considered. Initial letter of interest (no submission deadline), simple application, rapid (60 day) peer review, and funding. Criteria for funding includes new ideas or directions, scientific excellence, and originality. Early exploratory projects, scientists not currently working in IBD, and/or interdisciplinary efforts are encouraged. Further information: Marciana Poland, Research Administrator, Broad Medical Research Program, 10900 Wilshire Blvd., 12th Floor, Los Angeles, CA 90024-6532, USA. Tel: +1 310 954 5091; email: info@broadmedical.org; website: www.broadmedical.org

Gastroenterology and Endotherapy European Workshop: XXth Anniversary

This course will be held on 17–19 June 2002 in Brussels, Belgium. Further information: Nancy Beauprez, Gastroenterology Department, Erasme Hospital, Route de Lennik 808, B-1070 Brussels, Belgium. Tel: +32 (0)20 555 49 00; fax: +32 (0)20 555 4901; email: beauprez@ulb.ac.be

EASL Monothematic Conference on Vascular Function in Liver Disease

This conference will take place on 30 June to 2 July 2002 in London, UK. Further information: Professor Jordi Bruix, EASL Liaison Bureau, c/o Kenes International, 17 rue du

Cendrier, PO Box 1726, CH-1211 Geneva, Switzerland. Tel: +41 22 908 0488; fax: +41 22 732 2850; email: info@easl.ch; www.easl-.com. Deadline for abstract submission **15 May 2002**. Further information: kmoore@rfc.ucl.ac.uk; tel: +44 (0)207 433 2876.

5th International Workshop on Pathogenesis and Host Response in *Helicobacter* Infections

This will be held on 4–7 July 2002 in Helsingør, Denmark. Further information: Dr Tina Ken Hansen, Department of Cardiology-Endocrinology E, Frederiksberg Hospital, Ndr. Fasanvej, DK-2000 Frederiksberg, Denmark. Fax: +45 3545 7708; email: helpatim@biobase.dk

ESPEN 2002

The European Society for Parenteral and Enteral Nutrition will be hosting its annual meeting on 31 August to 4 September 2002 in Glasgow, UK. The organisers anticipate 300 delegates, principally from Europe but also from the USA and the Far East. Further information: Mrs Pat Howard, Honorary Secretary, BAPEN, Head of Nutrition and Dietetic Services, Bristol Royal Infirmary, Bristol BS2 8HW. Tel: +44 (0)117 928 2049; Fax +44 (0)117 928 3005; email: pat.howard@ubht.swest.nhs.uk

Postgraduate Gastroenterology

This course will be held on 15–18 September 2002 in Oxford, UK. The course has been designed for consultants and registrars, including those who do not specialise in gastroenterology. Topics will include: Barrett's Oesophagus; The Case for Endoscopic Surveillance Debate; Liver Disease; Bacteria and the Gut; IBD Therapeutics, Gastrointestinal Bleeding, Endoscopic Training. Further information: Professor Derek P Jewell, University of Oxford, Nuffield Department of Medicine, Gastroenterology Unit, Gibson Laboratories, 2nd Floor, Radcliffe Infirmary, Block 21, Woodstock Road, Oxford OX2 6HE. Tel: +44 (0)1865 224829; fax: +44 (0)1865 790792; email: derek.jewell@ndm.ox.ac.uk; website: www.medicine.ox.ac.uk/gastro

3rd World Chinese Congress of Digestology

This congress will take place on 23–25 September 2002 in Beijing, China. Further information: Lian-Sheng Ma, President of WCCD, PO Box 2345 Beijing 100230, China. Fax: +86 6589 1893; email: wcjd@public.bta.net.cn