of cytokines such as transforming growth factor β_1 is likely to be an epiphenonemon of the inflammatory process.

Finally, we support the view of Cavallini *et al* that while calcification might contribute to the pathogenesis of chronic pancreatitis in its later stages, there is no well documented evidence fulfilling Koch's postulates that it is probably a significant aetiological factor in the disease. Hence, we also cannot support the hypothesis of the Marseilles school.[2]

We consider that the disease known as 'chronic pancreatitis' is not a single pathological entity but rather a group of different aetiologies and pathogenetic processes sharing a few common morphological end points.[3] Within this overall group, we anticipate that a predisposition based upon an identifiable genetic abnormality is the likely primary aetiological factor responsible for at least a proportion of cases of chronic pancreatitis. Whether this defect occurs within the pathway of alcohol metabolism or is responsible for promoting an inappropriate cell mediated cytotoxic response to some pancreatic cellular antigen is presently unknown. Nevertheless, answers to such questions are vital if biologically appropriate treatment regimens for different aetiologically and pathogenetically distinct types of 'chronic pancreatitis' are to be developed and affected patients treated more rationally than at present.

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Diagnosis of invasive amoebiasis: renaissance of the morphology era

EDITOR,—In his leading article entitled Diagnosis of invasive amoebiasis time to end the morphology era (*Gut* 1994; **35:** 1018–21), Professor Ravdin rightly emphasises that microscopy cannot differentiate between pathogenic and non-pathogenic strains of *Entamoeba histolytica*. This applies equally to the cysts, and cultured trophozoites. Professor Ravdin goes on to suggest that microscopy should be abandoned in the diagnosis of invasive amoebiasis. He has underestimated the diagnostic value of one important point, first described by Losch in 1875.[1] In clinical specimens from patients with amoebic dysentery trophozoites of *E histolytica* may be seen with ingested red cells. These erythrophagocytic trophozoites are a specific feature of infection with invasive strains of *E histolytica* and may be seen on microscopy of fresh faecal specimens or in fixed smears stained with Field's stain. The current recommendations for the diagnosis of amoebiasis are microscopical examination of fresh stools and stained fixed faecal smears, and amoebic cultures of stool specimens.[2 3] A study[4] of patients with dysentery, diarrhoea, and asymptomatic carriage of pathogenic and non-pathogenic *E histolytica* confirms that microscopy is a highly efficient diagnostic method for amoebic dysentery. The sensitivity and specificity of erythrophagocytic trophozoites, were 96% and 100% respectively, when compared with amoebic culture and subsequent zymodeme typing of the isolated strains to confirm pathogenicity. We agree with Professor Ravdin that there is a need for diagnostic methods that do not depend on the detection of intact parasites. Tests that detect faecal or circulating amoebic antigen, or both, would be suitable for this purpose. To validate such assays they must be compared with a gold standard. With the current state of knowledge the standard should include the finding of erythrophagocytic trophozoites. We do not agree that currently available antigen detection tests have supplanted microscopy in the diagnosis of amoebiasis. Many things have changed since 1875 but the findings of erythrophagocytic amoebic trophozoites in stool specimens remains the simplest, cheapest, and most reliable test in the diagnosis of amoebic dysentery. Welcome to the morphology era!

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Reply

EDITOR,—González-Ruiz *et al* have shown that in expert hands the detection of haematophagous trophozoites has a high predictive value in diagnosing infection by pathogenic *Entamoeba histolytica* strains.[1] This study is very helpful and reaffirms the value of microscopy of faecal samples when it is readily available and highly skilled. Unfortunately, in many areas of the world complete laboratory facilities are not readily accessible. In addition, clinical laboratories in community settings may vastly overdiagnose amoebiasis and report leucocytes in stool as *E histolytica*.[2] Lastly, stool culture for *E histolytica* has repeatedly shown that microscopy has a low sensitivity.[3 4]

Clearly, there is a consensus that better, quantitative methodology is needed that ideally can be brought directly to the field. I suggested that in the future microscopy will not be needed as serological studies for anti-amoebic antibodies and studies of antigen and DNA detection will be sufficiently developed and field tested.[5] The gold standard for comparison of these new methods is stool culture and zymodeme determination, not stool microscopy for erythrophagocytic trophozoites as suggested by González-Ruiz and Bendall. Simple agglutination tests, based upon current enzyme linked immunosorbent assay and hybridisation technology, will evaluate for infection by multiple enteric parasites. This will be the most cost effective, sensitive, and specific methodology available for diagnosis of infections by '*E dispar*' and *E histolytica*. Currently, we should use practical criteria as suggested by González-Ruiz and Bendall, but in the 21st century, let us advance beyond the technology of the 19th century.

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Relation of acupuncture and vagal gastric acid secretion

EDITOR,—We read with interest the paper of Lux *et al* (*Gut* 1994; **35:** 1026–9), which examines the effects of various forms of acupuncture on sham feeding stimulated acid output in healthy volunteers. These results are entirely in accord with our own findings, previously published both in abstract form (the first of which was in this journal six years ago!)[1 2] and in a peer reviewed journal,[3] that acupuncture produces a significant decrease in sham feeding stimulated acid output under randomised, placebo controlled conditions in humans.

In those studies we also showed that the effects of acupuncture decreased sham feeding stimulated acid output was through naloxone sensitive opioid mechanisms, involving vagal efferent pathways. Furthermore, acupuncture produced neither a decrease in gastrin release nor a diminished parietal cell sensitivity to gastrin. While we agree with Lux *et al* that the mechanism through which acupuncture exerts its effect is not fully elucidated, it seems to be at least in part through opioid pathways, which may be similar to the mechanisms participating in the analgesic properties of acupuncture.

In their report, Lux *et al* cite another of our publications as concluding that acupuncture accelerates peptic ulcer healing.[1] This is incorrect; the cited study was conducted in healthy volunteers to examine the effects of acupuncture on sham feeding stimulated acid output. In our comprehensive review of all the

published literature on the gastrointestinal effects of acupuncture, we were unable to find any controlled study showing the effect of acupuncture on ulcer healing.[4] There have been three reports, however, of uncontrolled studies[5–7] suggesting that acupuncture may be of therapeutic benefit in peptic ulcer disease and Lux *et al* cited one of these.

It is unfortunate that Lux *et al* failed to recognise our own work, but more so that they did not extend our initial studies into the mechanisms participating in the inhibition of acid secretion by acupuncture.

Clearly, we agree with them that further studies are needed to examine therapeutic efficacy.

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Reply

EDITOR,—We should like to thank Dr Tougas and Professor Hunt for their interest in our study. The aim of our investigation was to compare different techniques of acupuncture in terms of their effect on gastric acid secretion stimulated by sham feeding. The results showed that, under the conditions of our study, only certain forms of acupuncture, namely electroacupuncture and transcutaneous electrical nerve stimulation, but not laser acupuncture or the classic needle acupuncture led to a significant inhibition of gastric acid secretion. Regrettably, the very interesting investigations into the mechanism of action of acupuncture on gastric acid secretion carried out by Tougas *et al*[1] were unfamiliar to us at the time of completion of our own study because of publication date, and for this reason were not cited. Had we known of the study, we would, of course, certainly have made reference to it. Instead, we cited the earlier results of this group, which were available in the form of an abstract.[2] In our introduction, the work of Li[2] was cited not only (correctly) with respect to earlier results on human gastric acid secretion, but also – erroneously – as one of two studies concerned with ulcer healing. In the discussion, however, the results reported by Li *et al*[2] – as warranted by the importance of their study – were again cited in detail. Our views about ulcer healing are in complete accord with those of Tougas *et al*, as we too pointed out that the effect of acupuncture in this area has yet to be proved.

We are of the opinion that the results of the study performed by Tougas *et al*[1] together with our own results complement each other, and we hope that they will stimulate further studies on the mechanism of action of acupuncture on gastrointestinal functions, in particular on its effect on gastrointestinal diseases.

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Cancer surveillance in ulcerative colitis

EDITOR,—In re-reading the editorial I wrote (*Gut* 1994; **35:** 587–9) I have identified an error that I wish to correct.

Paragraphs six and seven state that the analysis of 11 prospective colonoscopic surveillance studies compared groups of patients with and without low grade dysplasia. This is incorrect, the two groups compared were all patients submitted for surveillance on the one hand and those found initially to have low grade dysplasia on the other.

Sentence two in paragraph six should have read 'In all, 73 cancers were found in 1656 patients (4·4%) whereas 26 cancers were found in the subgroup of 313 patients with low grade dysplasia (8·3%). If dysplasia associated lesions or masses are excluded this falls to 6·2%'.

A similar mistake occurs in paragraph seven. The second sentence of which should read 'In all, cancer was present in 93 of 2044 patients (4·5%) whereas 35 cancers were found in 101 patients with high grade dysplasia (35%)'.

I apologise for the inaccuracies detailed above.

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Colorectal tumorigenesis

EDITOR,—We noted with interest the paper by Mulder *et al* (*Gut* 1995; **36:** 76–80) on expression of mutant p53 protein and CD44 variant proteins in colorectal tumorigenesis. The authors in their report have shown that CD44 v6 expression is restricted to moderately and severely dysplastic adenomatous polyps and colorectal cancers, but that it is not expressed in normal colon and mildly dysplastic adenomas. They also suggested that CD44 v6 expression is associated with tumour progression. We have studied CD44 v6 in frozen and paraffin wax embedded tissue sections from 11 normal colons, eight adenomatous polyps, and in 18 colorectal adenocarcinomas, with immunohistochemistry using anti-CD44 v6 antibody.[1] In contrast with Mulder *et al* we found expression of this variant in normal colonic crypt epithelium, and similar expression was also seen by Fox *et al*.[1] We also detected CD44 v6 protein in all eight adenomatous polyps irrespective of the grade of dysplasia, and in 15 of 18 colorectal adenocarcinomas. In the positive colorectal cancers CD44 v6 expression was strong and homogeneous in three, and heterogeneous and weak in 12. Survival at five years was: 0 of 3 in patients with homogenous, 9 of 12 with heterogeneous CD44 v6 expression, and 3 of 3 in negative cases. In colorectal adenocarcinomas, Mulder *et al* saw a correlation with Dukes's stage and tumour progression. Our study shows no apparent correlation of CD44 v6 expression with tumour progression, there being no linear trend with Dukes's staging or differentiation. The decreased survival of patients with colorectal cancer who express CD44 v6 strongly and homogeneously, however, suggest that this expression may be an independent adverse prognostic marker rather than a determinant of tumour progression.

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Reply

EDITOR,—We appreciate the comments on our article concerning p53 and CD44 expression in the adenoma-carcinoma sequence. The authors point to some discrepancies with their own results and these differences are not easily explainable. We assume that they have used different antibodies, similar to the ones used by Fox *et al*. It is noteworthy that Fox *et al* found only weak positivity in the bottom of the crypts. The authors also mention the use of both paraffin wax embedded and fresh frozen tissue, but it is not clear from their writing from which of these two the presented numbers are derived. In our hands antibodies against CD44 v6 give only reliable results on fresh frozen tissue. Finally, their findings of prognostication are comparable with ours,[1] which we consider reassuring as far as the value as prognostic marker of CD44 is concerned.

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