that potent inhibitors of gastric acid secretion should be prescribed with caution in these patients, or in conjunction with fluconazole or nystatin.

The clinical relevance of oesophageal candidal growth after gastric acid inhibition in patients with systemic sclerosis is still unclear. Zamost *et al* studied two groups of such patients, one with erosive oesophagitis and impaired oesophageal peristalsis and one without oesophagitis but with impaired peristalsis in about half the cases.[1] The percentage of patients with positive fungal culture of oesophageal brushing was greater in the first group than in the second, although not significantly so. Moreover, positive smears with hyphae were found only in patients with erosive oesophagitis and oesophageal strictures. Thus, impaired oesophageal peristalsis, oesophagitis or oesophageal strictures may favour fungal growth in patients with systemic sclerosis. If this hypothesis is true, the increased frequency of positive cultures for *Candida albicans* reported by Hendel *et al* in their subgroup of systemic sclerosis patients treated with gastric secretion inhibitors may result not only from the effect of treatments but also from the higher frequency of severe oesophageal involvement in this group of patients in comparison with controls.[2] In fact, all patients receiving gastric secretion inhibitors had manometrically proved impaired oesophageal motility and abnormal gastro-oesophageal reflux whereas the control group consisted of consecutive patients with systemic sclerosis not requiring anti-reflux treatment in whom the frequency of oesophagitis and oesophageal motor dysfunction was not reported but was expected to be less than 60%.[1]

Whatever the cause may be that favours candidal growth in the oesophageal lumen of patients with systemic sclerosis, what is the clinical relevance of this growth? Hendel *et al* did not find mucosal invasive candidosis in any of their patients.[2] Eradication of candidal growth by nystatin or fluconazole did not influence the severity of oesophagitis,[1 2] and did not further relieve reflux symptoms previously improved by anti-reflux treatment.[2] On the other hand, gastric mucosal erosions and an increase in serum alkaline phosphatase were seen after fluconazole treatment.[2]

Patients with systemic sclerosis, impaired oesophageal peristalsis, and oesophagitis report reflux symptoms that are often severe, and oesophageal strictures and bleeding may complicate oesophagitis in some of them. Symptoms and endoscopic oesophagitis improve after gastric acid inhibition[3] and the risk of complications are possibly reduced by this treatment. On this basis we will continue to use potent gastric acid inhibitory drugs in patients with systemic sclerosis and oesophageal involvement.

The part played by *Candida albicans* in oesophagitis of these patients, the best level of gastric acid inhibition that should be reached to minimise adverse events and to ameliorate the symptoms and prognosis of oesophagitis, and finally the clinical usefulness of antimycotic treatments in patients with systemic sclerosis should be defined by appropriate controlled trials.

G BASILISCO
R BARBERA
M MOLGORA
M VANOLI
P A BIANCHI
Department of Gastroenterology, Institute of Scienze Mediche, Institute of Clinica Medica I, IRCCS, Università degli Studi di Milano, Via F Sforza 35, 20122 Milano, Italy

1 Zamost BJ, Hirschberg J, Ippoliti AF, Furst DE, Clements PJ, Weinstein WM. Esophagitis in scleroderma. Prevalence and risk factors. *Gastroenterology* 1987; **92:** 421–8.
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Tumour necrosis factor α in inflammatory bowel disease

EDITOR,—Murch *et al* (*Gut* 1993; **34:** 1705–9) show beautifully that tumour necrosis factor (TNF) containing cells, probably macrophages, are clustered in the upper mucosa in ulcerative colitis and are distributed more randomly, and apparently diffusely, in Crohn's disease. Unfortunately, the legends to their colour figures do not match what is illustrated by the figures. Nevertheless, their assertion that there is periarteriolar infiltration by TNF positive cells ('vasculopathy') may be true. In their discussion they review much evidence for why this could 'contribute powerfully towards thrombosis in this situation'.

The situation cannot, however, be simply explained in these terms. If there were such powerful promotion of thrombosis one should surely see this as a dominant feature of Crohn's disease. In practice, thrombosis of either small or large blood vessels is only rarely seen in Crohn's disease and what evidence there is for it depends on the use of special techniques.

The work of Murch *et al* is an important contribution to our knowledge of the pathogenesis of inflammatory bowel disease, but caution should be exercised in its interpretation.

I C TALBOT
St Mark's Hospital, London EC1V 2PS

Reply

EDITOR,—We thank Dr Talbot for his generous assessment of our paper. I must apologise for the mislabelling of our figures, which occurred in press.

Dr Talbot raises what may be a central point in the understanding of pathogenetic mechanisms in Crohn's disease: what is the extent of vascular thrombosis, and how much does it contribute towards physiological disturbance and tissue changes? I fully agree that vascular thrombosis is not commonly seen in routinely stained specimens, and that special techniques are required to give a true picture of the extent of vascular involvement. When these are used, a very different picture emerges, in which multiple microvascular events are clearly occurring. The extent of vascular abnormality in Crohn's disease has been incontrovertibly shown by Wakefield's elegant perfusion-fixation study[1]: the very clear message from this work is that most of the vascular abnormality occurs at a level too deep to be detected in a study of endoscopic biopsy specimens. While vascular abnormality has long been recognised in Crohn's disease, it is probable that only sizeable vessels will leave detectable remnants after thrombosis. When vascular endothelial remnants are specifically hunted they are numerous,[2] and we have additionally shown widespread attenuation of endothelial heparan sulphate in apparently healthy vessels.[3] We would thus contend that failure to detect microvascular abnormality represents limitation of standard techniques rather than vascular health.

This phenomenon is by no means restricted to Crohn's disease and occurs in probably all cell mediated immunopathologies. Early anatomical studies of allograft rejection showed perivascular macrophage accumulation with vasculopathy,[4] and severe acute vasculopathy has been found in a class II MHC restricted model of renal allograft rejection.[5]

Neovascularisation clearly must also occur, and it is clear from embryological studies that macrophages may induce this[6]: TNFα itself may contribute to new vessel formation as well as to the initial vasculopathy.[7] In this case, the ability to remodel tissue with production of appropriately normal extracellular matrix, rather than collagen, will determine outcome. The role of cytokines such as TNFα in the control of fibroblast function may thus be of greater importance than is currently recognised.

S H MURCH
Department of Paediatric Gastroenterology, St Bartholomew's Hospital, London EC1A 7BE

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Mycobacteria in the human intestine

EDITOR,—We read with much interest the article by Stainsby *et al* (*Gut* 1993; **34:** 371–4) about antibodies to mycobacteria in Crohn's disease and control subjects. They showed that a spectrum of antibodies binding to mycobacterial species was evident in control as well as patient populations, reflecting the ubiquitous nature of mycobacteria in the environment.

We also confirmed the ubiquitous nature of mycobacteria in the human intestine by polymerase chain reaction (PCR). The DNA extracted from the colonic tissues from patients with Crohn's disease, ulcerative colitis, and controls were subjected to PCR using TB1 and TB2 as primers to amplify the mycobacterial *groEL* gene.[1] Mycobacteria were detected in seven of 10 inflammatory bowel disease patients (3/5 with Crohn's disease and 4/5 with ulcerative colitis). Four of five control tissues were also positive for mycobacteria. These results suggested that some kinds of mycobacteria may be ubiquitously distributed in the human intestine or

simply pass through it as suggested previously.[2 3] Concerning *M paratuberculosis*, however, the conflicting results were reported by Elsaghier *et al.*[4] They showed significantly increased antibody concentrations to *M paratuberculosis* specific protein in Crohn's disease patients. This difference might result from the antigens used for their experiments. Stainsby *et al* used antigens that were filtered sonicate preparations of the mycobacterial species, and as they discussed in their article, the study of humoral immunity to *M paratuberculosis* in Crohn's disease should be devoid of the cross reactive nature of mycobacterial antigens. Furthermore, Sanderson *et al* reported that *M paratuberculosis* DNA was identified in 26 of 40 (65%) Crohn's disease, in one of 23 (4·3%) ulcerative colitis, and in five of 40 (12·5%) control tissues by PCR.[5] We agree with Sanderson *et al* that this high frequency of identification of *M paratuberculosis* in Crohn's disease could not be explained by secondary invasion of a previously damaged mucosa. Therefore, some kinds of mycobacteria may be ubiquitously distributed in the human intestine, but *M paratuberculosis* might participate in the pathogenesis of Crohn's disease.

Y YOKOYAMA
J KINO
K OKAZAKI
Y YAMAMOTO
First Department of Internal Medicine, Kochi Medical School, Nankoku Kochi, Japan

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Helicobacter pylori infection

EDITOR,—The EUROGAST Study[1] provided impressive confirmation of the geographical association between *Helicobacter pylori* infection and gastric carcinoma.[2]

The technique was serological, however, and necessarily considered geographically and ethnically disparate populations, so subgroup analysis for risk factors in *H pylori* infection may not be appropriate.[3]

It is known that serology does not always correlate well with active infection in apparently healthy subjects, and may merely provide a historical record.[4]

The 17 groups studied had between 132 and 229 subjects each, who presumably could have been from a variety of racial groups in the 13 different countries: these factors are well known to affect prevalence. The absence of a sex effect, and the increased frequency of infection at age 55–64 years compared with 25–34 years, harmonises well with the conclusions in other studies, and are easy to prove. But whether the technique is suitable to make statements about smoking and alcohol use is much more doubtful.

We used a reliable direct urease test (CLO test) for assessment of active *H pylori* infection in local British white patients to assess the effect of personal habits.[5] For the current cigarette smokers there was a clearly increased prevalence of *H pylori* infection (49·6% *v* 35·5% in non-smokers or those who had given up smoking at least a year before, $p<0·01$). This would be consistent with the known suppressive effects of smoking on immune defences; and also the association between peptic ulcer and smoking, as duodenal ulcer is uncontentiously very strongly associated with *H pylori*. Ours is the only study directly focused on this problem in a large homogeneous well defined population using an effective direct method for active *H pylori* infection.

I would like to persuade colleagues that this is indeed the correct answer and challenge doubters to produce a similarly coherent specific study devoted to this problem.

M C BATESON
Bishop Auckland General Hospital, Bishop Auckland, County Durham DL14 6AD

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Reply

EDITOR,—One aim of the EUROGAST study was to identify risk factors for *H pylori* seropositivity, using a common protocol to collect blood samples and questionnaire data from random samples of the general population in a wide range of different countries. Bateson criticises one conclusion from the study: that *H pylori* infection, as assessed by serology, is not associated with smoking.[1] He states that serology may be a poor indicator of current *H pylori* infection and that the use of different populations, with different prevalence rates, precludes general conclusions concerning risk factors for *H pylori* infection.

The lack of association between *H pylori* and smoking was seen in the whole EUROGAST population[1] and not in a subgroup analysis as indicated by Bateson. Furthermore, in none of the 17 individual centres was there a statistically significant association between smoking and *H pylori* seropositivity. The estimated odds ratio for smokers *v* non-smokers was 1·0 or higher in 10 study centres and was lower than 1·0 in seven centres (data available on request). This conclusion is consistent with the other large, population based studies that have investigated smoking in relation to *H pylori* infection, assessed by serology,[2] by serology and the urea breath test,[3] and by serology and histology.[4] The last two studies[3 4] used measures of current infection in addition to serology. Moreover, there is evidence suggesting that *H pylori* infection is most commonly acquired in early childhood[5 6] – that is, before most subjects take up smoking.

Those studies that have investigated the association between *H pylori* and smoking in patients undergoing endoscopy have variously reported a positive,[7–9] negative[10] or no[11 12] association.

The use of symptomatic patients may, however, lead to a spurious, non-causal relation between *H pylori* and smoking because both *H pylori* infection and smoking are independently related to gastric disease, especially peptic ulceration. The separate associations between *H pylori* and peptic ulceration and between smoking and peptic ulceration do not imply that there is an association between *H pylori* and smoking. Rather, it is plausible that smoking may increase the risk of disease in an *H pylori* infected subject.[13]

With regard to the use of serology to assess *H pylori* infection the evidence suggests that, in the absence of treatment, *H pylori* infections will persist for life.[14] The conclusion by Meyer *et al*, cited by Bateson, that spontaneous eradication of *H pylori* might commonly occur in healthy subjects,[15] was later retracted because of the low specificity of the serological test used in their study.[16] The only subjects likely to be seropositive in the absence of a current infection are those with severe gastric atrophy or intestinal metaplasia or both, as *H pylori* infection cannot persist in such conditions.[17] Such subjects would, however, be uncommon in the EUROGAST population where subjects were all aged under 65 years.

In conclusion, results from all of the population based studies weigh against the hypothesis that smokers are at an increased risk of *H pylori* infection. We would also suggest that patient groups may be an inappropriate population in which to study this relation.

P WEBB
D FORMAN
H MØLLER
D NEWELL
(on behalf of the EUROGAST Study Group)
Imperial Cancer Research Fund Cancer Epidemiology Unit, University of Oxford, Gibson Building, The Radcliffe Infirmary, Oxford OX2 6HE

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