LETTERS TO THE EDITOR

Nitric oxide synthase in gastric mucosa

EDITOR,—Rachmilewitz *et al* have reported (*Gut* 1994; **35:** 1394–7) increased nitric oxide synthase activity in the antral and fundal gastric mucosa from patients with duodenal ulceration and *Helicobacter pylori* infection. Nitric oxide synthase activity was estimated from citrulline production.

However, we have measured citrulline concentrations in gastric antral biopsy specimens (by LKB biochrom amino acid analyser) as an index of nitric oxide synthase activity[1] and found no differences before and after healing duodenal ulcers by eradication of *H pylori*.

In addition we estimated nitric oxide synthase activity in the cytosolic supernatants of antral mucosal biopsy specimens from the inhibition by monomethyl-L-arginine (L-NMMA) on the conversion of ^{14}C arginine to ^{14}C citrulline. Initially we used the same method as used by Rachmilewitz *et al*, and estimated ^{14}C citrulline concentrations using Dowex AG 50W-X8 (sodium form) columns. However, to validate this method we applied the fraction recovered from the column to thin layer chromotography. ^{14}C urea, ^{14}C ornithine, and other unidentified radiolabelled products were found in addition to ^{14}C citrulline. Therefore, we conducted the experiments using thin layer chromotography to avoid possible inaccuracies of the Dowex column method. Once again we did not find an increase in nitric oxide synthase activity in *H pylori* positive duodenal ulcer patients, but discovered that in this tissue L-NMMA significantly inhibits the formation of ^{14}C ornithine.

Demonstration of nitric oxide synthase activity in the Rachmilewitz study depends upon the inhibitory effect of monomethyl-L-arginine (incorrectly labelled L-NAME in their paper) on the formation of ^{14}C citrulline, which was estimated from the scintillant activity of the Dowex elute thought to arise from citrulline alone. As we have shown that this elute contains other radiolabelled metabolites of arginine, it is possible Rachmilewitz *et al* are measuring the inhibitory effect of L-NMMA on the formation of other labelled substances such as ^{14}C ornithine from ^{14}C arginine by glycine aminotransferase.

The Dowex AG 50W-X8 separation as a measurement of nitric oxide synthase activity originally described by Bredt and Snyder[2] was validated by thin layer chromotography for cerebellar tissue. It may not be accurate when applied to other tissues and, as suggested by them, should be validated by thin layer chromotography for each new tissue.

S J MIDDLETON
P D REYNOLDS
M SHORTHOUSE
J O HUNTER
Gastroenterology Research Unit, Addenbrooke's Hospital, Cambridge CB2 2QQ

S MOSS
Royal Postgraduate Medical School, Hammersmith Hospital, London

1 Middleton SJ, Shorthouse M, Hunter JO. Increased nitric oxide synthesis in ulcerative colitis. *Lancet* 1993; **341:** 465–6.

2 Bredt D, Snyder SH. Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci* 1990; **87:** 682–5.

Reply

EDITOR,—We read with interest the letter of Middleton *et al* casting doubts on the validity of the assay used by us for determination of gastric nitric oxide synthase activity.

We saw NADPH dependent L-NMMA inhibitable turnover of L-arginine in the gastric mucosa of patients with duodenal ulcer and *H pylori* infection. We attributed this activity to nitric oxide synthase. Middleton *et al* question the specificity of this assay, which is presumed to measure the conversion of L-arginine by citrulline (by nitric oxide synthase). They suggest that L-NMMA also inhibits the formation of ornithine in these tissues. If true, we might be measuring NADPH dependent L-NMMA-inhibitable conversion of L-arginine to ornithine. This would raise several possibilities: (a) that the assay and inhibitors are not specific for nitric oxide synthase – an important but, as yet, unprecedented finding; (b) that nitric oxide synthase activity results directly in formation of ornithine rather than citrulline, which is unlikely; or (c) that citrulline is indeed the primary product of nitric oxide synthase, but undergoes conversion to ornithine. For example, citrulline might be converted back to L-arginine by the actions of arginosuccinate synthetase/arginosuccinase; L-arginine could then be converted to ornithine by the actions of arginase.

We look forward to further studies on arginine metabolism in the gut of patients with *H pylori* infection, which are necessary to validate the concerns of the authors regarding the specificity of a commonly used assay.

J S STAMLER
Pulmonary and Cardiovascular Division, Duke University Medical Center, USA

D RACHMILEWITZ
Department of Medicine, Hadassah University Hospital, Jerusalem, Israel

Mycobacterium paratuberculosis and Crohn's disease

EDITOR,—We have studied the participation of mycobacteria in Crohn's disease and are interested in articles published on this topic. The paper of H M Fidler *et al* (*Gut* 1994; **35:** 506–10) clearly highlighted the ubiquitous nature of *Mycobacterium paratuberculosis* and its detection with the highly sensitive and specific tool of polymerase chain reaction in human tissue.

In our study (unpublished data) we were specifically interested in the group of patients with Crohn's disease with terminal ileum involvement, although three patients with classic Crohn's colitis alone on histological examination, were also included.

Polymerase chain reaction assays based on the direct amplification of 229 bp fragment and 400 bp of the repetitive mycobacterial insertion sequence IS900 present in the genome of *M paratuberculosis*, were applied to 73 sections of paraffin wax embedded tissue of 26 histologically confirmed Crohn's disease patients, all of which were negative on Ziehl-Neelsen staining for acid fast bacilli.[1–3]

These sections were divided into those with an area of minimal involvement and those with an area of well established ulceration. In 18 cases lymph nodes were also available for study. Granulomata were present in only one of 23 of 73 sections.

As the incidence of pulmonary tuberculosis in South Africa is among the highest in the world, the presence of *M tuberculosis* was also investigated, to exclude this organism as a complicating factor.[4]

Controls consisted of patients with colon cancer who had undergone a right hemicolectomy (n=34, 102 samples).

No positive amplification of the 400 bp sequence in any of the 26 patients after 40 cycles was found. In some of the 229 bp sequence amplification reactions, a slight product was found, pointing to the presence of *M paratuberculosis*. Reamplification of the 229 bp products was done and positive reamplification was detected in 10 of 26 (38%) of (Crohn's disease) patients and in four of 35 (11%) controls. There was a greater chance of detecting the organism in non-granulomatous sections of the disease 12 of 41 (29%) than in sections showing granulomata of one of 23 (4%).

There were no cases of Crohn's disease that were associated with tuberculosis. All eight terminal ileum samples with Johne's disease confirmed by culture and histological examination tested positive for *M paratuberculosis* DNA. The internal laboratory controls for each batch of samples repeatedly tested negative.

These initial results suggest that *M paratuberculosis* does not play a part in the pathogenesis of Crohn's disease. The detection of *M paratuberculosis* DNA in 38% of Crohn's disease patients on reamplification, however, seems to contradict this finding.

We find it interesting that in the study of Fidler *et al* none of the small bowel tissues (with or without granulomata) tested positive for IS 900 for *M paratuberculosis* with all the positive reactions being found in the large bowel specimens.

As two of three of our patients who had confirmed Crohn's colitis alone tested positive for *M paratuberculosis*, the question arises as to whether this organism is more likely to be associated with Crohn's disease activity of the large bowel rather than the terminal ileum.

Failure to amplify *M paratuberculosis* in Crohn's disease after 40 cycles combined with a low detection rate even after reamplifcation suggests that a possible association between *M paratuberculosis* and the pathogenesis of Crohn's disease remains unclear.

D L ERASMUS
T C VICTOR
P J VAN EEDEN
V FALCK
P VAN HELDEN
Gastroenterology Unit, Department of Internal Medicine, University of Stellenbosch, Medical School, PO Box 19063 Tygerberg, 7505, South Africa

1 Sanderson JD, Moss MT, Green EP, Tizard MV, Malik ZP, Hermon-Taylor J. Polymerase chain reaction (PCR) directly reports *M paratuberculosis* genomes in Crohns disease tissue DNA extracts. *Gastroneterology* 1991; **100:** A247.

2 Vary PH, Anderson PR, Green E, Hermon-Taylor J, McFadden JJ. Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. *J Clin Microbiol* 1990; **28:** 933–7.

3 Sanderson JD, Moss MT, Tizard MLV, Hermon-Taylor J. *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. *Gut* 1992; **33:** 890–6.

4 Victor T, Jordaan HF, van Niekerk DJT, Louw M, Jordaan A, van Helden PD. Papulonecrotic tuberculid: identification of *Mycobacterium tuberculosis* DNA by polymerase chain reaction. *Am J Dermatopathol* 1992; **14:** 491–5.