the effect of other degradative enzymes present in the samples. Hence, the results obtained by Markesich *et al* are not comparable to that of ours.^{1,2,4-6} Studies with gastric juice performed by others,³ however, showed lower viscosity values for *H. pylori* positive patients than that of *H. pylori* negative patients. Also hydrophobicity measurement data⁷ totally argue against the conclusions reached by Markesich *et al*.

There may be several reasons for the different results obtained by Markesich *et al*. The most important are the lack of control for the peptic activity during the processing and the mode of mucus gel sample preparation for the measurements. As such, the 'mucus gel' samples obtained after the centrifugation in both cases represent only the undegraded mucus, as the degraded one would remain in the supernatant. Hence, the viscosity measurements on the materials prepared from patients with and without *H. pylori* infection were carried out only on the undegraded 'insoluble mucus gel'. No reference of any sort is made as to the sample concentration (for example, mg protein/ml). The conditions used for 'mucus gel preparation' (for example, centrifugation 10000 rpm for 15 min) would result in the insoluble fraction consisting of some undegraded mucus, cell debris, and other particulate matter. Consequently, this kind of material would give very high and erroneous viscosity values, as is the case in this study.

We would also like to point out that the study by Markesich *et al*, in contrast with their contention, represents typical in vitro experiments, and unless a method is devised to take the viscosity measurements directly in the stomach, the claim is not valid.

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Reply

EDITOR,—Slomiany and coworkers not surprisingly are unhappy with the results of our evaluation of the viscosity of gastric mucus gel. We believe that we studied the real stuff. I am surprised that the objectors did not comment on, or attempt to deal with, the fact that data were internally consistent and changed with treatment. We apologise because upon

re-reading our manuscript it could be read that we implied that previous workers always purchased mucus in a bottle (*Gut* 1995; **36**: 327-9). We do contend that mucus obtained by instilling buffer into freshly dissected pig stomachs followed by filtering through a 0.45 µm filter, raising the pH to 9 for 30 minutes, dialysing it against distilled water, and then lyophilising it may not be comparable to the fresh mucus gel obtained from human beings.¹

Our goal was to try to test reality. Our detractors believed that we failed in that objective. Their approach has been to identify reality through careful reconstructive experiments using sophisticated biochemical techniques. Slomiany and coworkers are internationally recognised authorities in mucus biochemistry; our study was of mucus biology. Since our paper was published we have even found an earlier reference with similar findings that also give references to even earlier work.² Thus, as is often the case, we were not the first but instead extend previous findings in populations whose *H. pylori* status is defined. Time will tell whether God has been unkind to them or to Slomiany and coworker.

Finally, we would like to draw attention to the fact that the gastric mucus hydrophobicity story may be somewhat more complicated than it first seemed.^{3,4}

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Copper associated childhood cirrhosis

EDITOR,—The report of copper associated childhood cirrhosis by Horslen *et al* (*Gut* 1994; **35**: 1497-500) is of considerable interest. But I take issue with one small point.

The authors cite a case reported by Bartók *et al* in 1971¹ as an instance of idiopathic hepatic copper toxicosis. A glance at the

Figure 1: Edge of nodule. Granules resistant to distase digestion take the periodic acid-Schiff stain.

Figure 2: The granules mark for α_1 antitrypsin by immunostain.

Figure 3: At a similar site to Fig 2 rhodanine stainable copper is shown.

original publication arouses doubts whether another diagnosis might be correct. Early last year, through the courtesy of Professor J Ormos of the University of Szeged, I was sent a block of liver tissue from that case (see Figures). Stainable copper in large amounts was present, but other findings – using both immunohistochemical and standard techniques – confirmed my suspicion that the underlying disease was α_1 antitrypsin deficiency. This diagnosis had been recognised and published in the Hungarian literature²; I was not aware of this when I asked for tissue.

In any event, it is time that this patient's case no longer be invoked as an instance of non-Indian copper associated childhood cirrhosis or idiopathic hepatic copper toxicosis. Given parental consanguinity,^{1,2} she of course may have had both α_1 antitrypsin deficiency and another defect involving copper handling, but this is speculation.

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Reply

EDITOR,—We are extremely grateful to Dr Knisely for this further information on the case reported by Bartók *et al*,¹ and we agree that this case should not now be considered as a case of copper associated childhood cirrhosis (CACC). The postmortem liver copper content in this child, now known to have α_1 antitrypsin deficiency, was between 2947 and 3564 µg/g dry weight (normal <50 µg/g dry weight) thus emphasising the need to distinguish between secondary and primary causes of copper accumulation.

Evidence is now available to suggest that the remaining cases categorised as CACC type II do not all represent a single condition. The child described by Adamson *et al*² has

since been shown to have impaired synthesis of metallothionein in culture fibroblasts.³ In our case,⁴ however, basal and metal induced metallothionein synthesis in skin fibroblasts was normal,⁵ indicating that heterogeneity exists among cases of primary, non-environmental, non-Wilsonian copper poisoning.

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Eradication rate of *Helicobacter pylori*

EDITOR,—The paper by Moshkowitz *et al* (*Gut* 1995; 36: 845-7) suggesting that the pretreatment ¹⁴C-urea breath test results are related to the outcome of triple therapy consisting of colloidal bismuth, metronidazole, and amoxicillin raises several points. Unfortunately, the authors did not exclude confounding variables such as compliance, metronidazole resistance, smoking, age, and pretreatment with antibiotics and anti-secretory drugs, respectively, which may also have an important effect on the outcome of eradication therapy. Another weakness of this particular study is that the authors did not prove the correlation between the urea breath test and histology, although biopsy specimens were taken for histological examination in all patients. On the basis of previously published correlation studies between breath test results and histology, the authors concluded that it is the density of *H pylori* that predicts the outcome of eradication therapy. We cannot agree with this conclusion.

Firstly, the urea breath test, as an indirect measure, may reflect the total gastric urease activity, however, urease activities may vary considerably between *H pylori* strains.¹ Thus, in clinical practice the accurate prediction of the *H pylori* density by non-invasive breath tests will in all likelihood be the exception rather than the rule.

Several studies have shown that a significant correlation does exist between urea breath test results and both the density of *H pylori* infection and the severity of gastritis. But what does a significant correlation imply clinically? Because of the high sensibility of statistical tests in correlation analyses, rather weak correlations may result in statistical significance. It is, however, the determination coefficient (squared correlation coefficient) that reflects the proportion of results that would have been predicted properly when one of two correlated variables is known. For example, we have found highly significant correlations ($p < 0.0001$) between the ¹³CO₂ excess after intake of labelled urea and the histologically visible degree of

H pylori colonisation ($r = 0.81$), the degree ($r = 0.72$) and the activity of gastritis ($r = 0.69$) as well.² These correlations showed that the histological findings would have been predicted properly in 66%, 52%, and 48% of cases, respectively, which is of course clinically not very useful. It should also be mentioned that some of the studies quoted by Moshkowitz were published only as abstracts, which do not permit the estimation of the clinical relevance of the correlations, because the correlation coefficients are not given.

Previous studies have suggested that the density of *H pylori* determines both the grade and the activity of gastritis.^{3,4} Recent studies have also shown that the severity of gastritis predicts the success of eradication therapy with triple therapy⁵ as well as dual therapy consisting of omeprazole and amoxicillin⁶ in the sense that a more severe gastritis is associated with a higher likelihood of eradication success. Grade and activity of gastritis reflect the specific and non-specific immune response of the host to the infection with *H pylori*. Although scientifically unconfirmed up to now, it seems to be plausible that comparable to other infectious diseases it is the immune response that facilitates eradication of *H pylori* whatever treatment is used. Provided the urea breath test used by Moshkowitz *et al* does actually reflect the *H pylori* density and thus in turn the severity of gastritis, the finding of a higher eradication rate in patients with lower urea breath test values is in apparent contradiction to the studies quoted above.

In conclusion, despite a significant correlation the clinical usefulness of urea breath tests with respect to prediction of *H pylori* density and severity of gastritis remains yet unproved and such predictions should be used in clinical studies only. Also, without exclusion of confounding variables by means of multiple logistic regression analysis it is actually impossible to identify a single factor independently governing the success of eradication therapy.

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EDITOR,—We would like to comment on the article by Moshkowitz *et al* on the association between *H pylori* colonisation density and eradication rate with a triple therapy (*Gut* 1995; 36: 845-7). We agree that the bacterial density in the stomach is of considerable predictive value for eradication of the organism with triple therapy. We would suggest, however, that the treatment failure in the study was actually caused by metronidazole resistance, mainly acquired resistance that developed during the treatment.

In this study, unfortunately, the authors did not test the isolates for sensitivity to metronidazole, either before, or after the treatment. The authors referred to one of their previous studies showing a resistance rate of 6%, but the sample number was too small (only 18) to predict the prevalence of metronidazole resistant strains in their population. Moreover, even if this result were accurate, it could not exclude the possibility of acquired resistance in the strains that were not eradicated.

In an in vitro study, Berger *et al* assessed the effect of inoculum size on the susceptibility of 15 clinical isolates of *H pylori* to six antimicrobial agents by an agar dilution method. A considerable inoculum effect was found with metronidazole; the mean minimal inhibitory concentrations of the drug increased 21.2-fold (from 0.11 to 2.33 mg/l) as the inoculum size progressed from 10³ to 10⁷ cfu/spot.¹ Haas *et al* showed that the frequencies of spontaneous resistance for four isolates of *H pylori* to seven different classes of antimicrobial agents varied from $< 4.2 \times 10^{-9}$ to 5×10^{-5} . The highest rate of spontaneous resistance was to metronidazole, being from $< 3.5 \times 10^{-8}$ to 5×10^{-5} .² Furthermore, resistant mutants of *H pylori* to metronidazole, ciprofloxacin, and tobramycin could be easily selected in vitro, using the serial passage technique.²

In the authors' study, it is not surprising that they saw a poor eradication rate (64.4%) with their triple regimen, which incorporated a low daily dose of metronidazole (250 mg twice daily for two weeks). This dose was much lower than that in the regimens recommended by the Working Party (400 mg three times day for two weeks)³ and those currently used by others.^{4,5} Eradication rates of 72-95% were achieved with those regimens.³⁻⁵

The 'inoculum effect' and spontaneous resistance among *H pylori* strains in the stomach may account for the low eradication rate obtained in the study. It has been shown that, in untreated patients with duodenal ulcer or gastritis, the bacterial density of *H pylori* infection in the gastric antrum and body is as high as 1.3×10^8 to 6.9×10^8 cfu/g of mucosal tissue.⁶ After ineffective antimicrobial therapy, *H pylori* can still be cultured from antral biopsy specimens at concentrations of 10⁵ cfu/g of mucosa.⁷ Thus, metronidazole resistant mutants can be easily selected in the stomach when a low daily dose of metronidazole is given. In fact, even when higher doses of metronidazole are used, *H pylori* is not eradicated in all patients infected with sensitive strains. Strains cultured after triple therapy incorporating metronidazole are almost invariably resistant to metronidazole. Thus, primary and acquired metronidazole resistance is the major cause for the treatment failure even when good compliance is achieved.^{4,5} Moreover, poor compliance may lead to the emergence of metronidazole resistance because of incomplete or decreased dosage of the drug, resulting in low eradication rates.

What should also be emphasised in this study is that inclusion of a low daily dose of