Measles viral genomic sequences in intestinal tissue

EDITOR,—In a thorough investigation, Haga et al were unable to demonstrate the presence of measles virus RNA in intestinal specimens from patients with inflammatory bowel disease (Gut 1996; 36: 211–5). Although they developed an exquisite sensitive technique, there remains a fundamental flaw in their methodology. They described intestinal tissue postoperative resection times ranging from 20 to 90 minutes. Such prolonged ischaemic times would have reduced the sensitivity of their assay considerably, as substantial RNA degradation would have occurred. MacPherson et al reported a significantly lower yield of RNA in surgical intestinal specimens after resection than cell cultures of 45 minutes to 1 hour 45 minutes compared with biopsy specimens frozen within 15 minutes. Further degradation can also occur during prolonged storage at −70°C. The assay may be sufficiently insensitive that one viral RNA species is not detectable, but low copy number RNA species are likely to have been lost on the way. This could be tested by attempting to amplify an intestinal RNA species present in much lower copy number than B-actin from their extracted intestinal RNA samples.

The authors must apply their technique to freshly resected intestinal tissue before they can conclude that nested RT-PCR fails to detect measles, mumps or rubella viral genomes.

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Reply

EDITOR,—Dr Smith claims that there remains a flaw in the condition of intestinal tissue used to detect measles virus. It is partially acceptable that RNA degeneration occurs by ischaemia after surgery. Even so we need to use the resected specimen because the positive in situ hybridisation for measles virus was observed principally in the submucosa and serosa by Wakefield et al.1 It is important to seek measles viruses in various areas throughout the thickness of the intestine. To avoid the risk of failing to detect the measles virus in such a small sample as a biopsy specimen, the resected specimen must be more useful. It is impossible to freeze the intestinal specimens in such a short time as 45 seconds after resection. Concerning the ischaemic time of the specimens, some reports describe that generally measles virus is detectable if the autolysis time is less than six hours in the brain tissues of patients with subacute sclerosing panencephalitis (SSPE) by in situ hybridisation.2 Under −70°C, even after 20 years of storage, most cases of SSPE provide positive results for measles virus by nested reverse transcriptase polymerase chain reaction (RT-PCR).3 As a positive control in our study, the brain tissue of a SSPE patient had three hours of autolysis time and three years of storage time under −80°C. Even in parallelly embedded specimens, measles virus is detectable for immunohistochemical staining, in situ hybridisation, and RT-PCR. Generally, nested RT-PCR provides much higher sensitivity to detect viruses compared with immunohistochemical staining, electron microscopy, and in situ hybridisation. Although the conditions for RNA in the intestine might be different than in brain tissue, the most important strategy to seek measles virus is to choose the method that provides the highest sensitivity. Our methods satisfy these criteria.

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Editor.—In their publication, Haga et al were unable to detect measles RNA in intestinal tissue of Crohn’s disease (Gut 1996; 36: 211–5). Although an extremely sensitive technique was used in this study (nested PCR), their claim of being able to detect one measles genome may be unjustified. This claim was based on the assumption that one measles viral genome is present per measles virus (one plaque forming unit of measles virus).

However, Lund et al have shown that up to 1200 copies of the measles virus genome may be present in each measles virion. Experiments in our laboratory have shown that NASBA2 (nucleic acid sequence based amplification) is an order of magnitude more sensitive than reverse transcription followed by nested PCR (RT-PCR) for the detection of measles virus RNA.

The authors also reported a long delay (20 to 90 minutes) between resection and freezing of tissue samples. This delay could lead to significant RNA degradation,5 particularly in the case of low copy number RNA species. To detect significant RNA degradation, a low copy number RNA species should have been used as an internal control rather than B-actin, which may be detectable after degradation of low copy number RNA species.

These problems may be overcome by spiking postoperative control tissue with known numbers of a measles RNA transcript, extracting total RNA, and then performing RT-PCR (or NASBA) to evaluate the extent of RNA degradation and to quantify the sensitivity of these detection techniques.

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Oesophageal hypersensitivity

EDITOR,—We would like to comment on the paper by G Shi et al (Gut 1995; 37: 457–64). A statistically significant association of symptoms and reflux episodes was found in 96 patients with normal oesophageal exposure to acid during 24-hour pH monitoring. The authors stated that this was consistent with the idea of oesophageal hypersensitivity to acid. This group of patients was very heterogeneous, as 14 of 96 of these patients had reflux oesophagitis, 28 of 96 had hiatal hernia, and 22 of 96 were not endoscoped before pHmetry. Furthermore, in an unspecified number of patients the pH probe was positioned by the ph step method, which is known to accurately locate the lower oesophageal sphincter in 58% of patients.1 As reported by Anggiansah et al2 a placement of the probe at 10 cm instead of 5 cm above the lower oesophageal sphincter accounts for a change in diagnosis in 45% of patients. Furthermore, it is well known that patients often do not tolerate pHmetry and may diminish their food and beverage intake considerably. Underreporting of symptoms is common and severity of symptoms differs considerably among patients. As meal composition and timing were not standardised3 and severity of symptoms not measured (for example, by visual analogue scales) it is difficult to consider a given pH monitoring as

Letters
representative and even more to establish 
hypersensitivity to acid. Thus, these several 
difficulties in pH monitoring in a hetero-
genous patient group do, in our opinion, 
prevent a new classification of oesophageal 
hypersensitivity to acid. A prospective and 
standardised study in these patients is needed 
to better define whether the hypersensitive 
esophagus is a distinct clinical entity, or 
whether pH monitoring underdiagnoses 
gastro-oesophageal reflux disease.

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1 Marx HO, Richter JE, Sinclair JW, Price JE, 
Case LD. Gastrooesophageal pH step-up inac-
curately locates proximal border of lower 

2 Ajangkara A, Sumboonnanonda K, Wang J, 
Linsell J, Hale P, Owen WJ. Significantly 
reduced acid detection at 10 cm compared to 
5 cm below lower oesophageal sphincter in 
patients with acid reflux. Am J Gastroenterol 

3 Castiglione F, Erde C, Armstrong D, Bauerfeind 
ph-metry: should means be standardized. scand 

Reply

EDITOR,—We would like to thank Drs 
Borovicka and Michetti for their interest in 
our paper and appreciate their critical 
comments.

We would not say that our group of patients 
was 'very heterogeneous'. Indeed, almost all 
patients studied complained of 'typical' reflux 
symptoms although they did differ from a 
pathological point of view, that is presence 
(severity) or absence of mucosal lesions. 
The vast majority of them had a normal 
esophageal mucosa, a finding recently 
reported by Trimble et al 2 who studied 
patients with normal acid exposure and a 
convincing correlation between symptoms 
and reflux episodes. Therefore, in this patient 
population, endoscopy is often meaningless 
and this is why it was not performed in 23% of 
our patients.

Our study is a retrospective one with 
the inherent drawbacks, but it reports data from 
a large series of consecutive patients from one 
centre. It is therefore understandable that pH 
monitoring was performed under different 
conditions. Some had an oesophageal 
manometry because of their predominant 
symptoms (for example, dysphagia or 
ydophagia) and, in these patients, mano-
metric localisation of lower oesophageal 
sphincter (LOS) was used for positioning the 
PHe probe. In those with no previous mano-
metry, pH step up was used to localise LOS. 
Although manometric determination of LOS 
may be the 'gold standard' to position the pH 
probe, a close correlation was discovered 
between the manometrically localised LOS 
and the sudden pH change occurring when 
the electrode is moved from the stomach to 
the oesophagus.2,3 The mean difference 
found by Klausen et al 2 between manometric 
assessment and pH step up technique was less 
than 0.5 cm, which is in agreement with our 
own experience.

There are some physiological reasons to 
suggest that the type and amount of food 
ingested during the recording period as well 
as the degree of physical activity can actually 
affect the duration and the extent of 
esophageal acid exposure.3 In the early 
study performed in hospitalised patients and 
using stationary pHmetry, many authors 
including ourselves4 recommended to stan-
dardise diet and physical activity in the hope 
of reducing inter and intra-individual vari-
ability. Patients were told to avoid alcoholic 
beverages and foods to reduce artefacts and 
risks of confusion with reflux episodes. 
However, a free diet does not seem to change 
either the diagnostic value or the repro-
ducibility of the technique.4 In addition, 
Jamieson et al1 reported that there is no 
significant difference, with the exception 
of the number of reflux episodes, between 
esophageal pH recordings performed in 
an inpatient or outpatient environment. 
To identify the temporal relation between 
symptoms and reflux episodes, it becomes 
more and more important to emphasise the 
many advantages of ambulatory recording in 
patients engaged in everyday activities with 
no restriction regarding diet and exercise.5 
In contrast, standardisation may unnecessarily 
affect the patient’s regimen and reduce the 
ability of the test to detect a significant 
association between symptoms and reflux 
episodes.6

Although we did not assess the severity 
of symptoms in our patients, we correlated 
them with reflux episodes. When one or several 
symptoms occurred during reflux episodes or 
within two minutes of their end, and the pos-
sibility of this occurrence by chance was 
excluded by probability calculation, we 
assumed that refluxed acid had induced 
symptoms. As total acid exposure was within 
normal range and reflux episodes were 
actually shorter and less acidic than in 
patients with gastro-oesophageal reflux 
disease, an oesophageal hypersensitivity to 
acid could reasonably be hypothesised for 
this subgroup of patients. The presence of low 
grade oesophagitis in 19% of our patients 
indicates — as suggested by Heading’s term1 — 
that a greater mucosal sensitivity to damage 
can coexist with increased nociception in 
patients with ‘acid hypersensitive oeso-
phagus’.

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1 Trimble KC, Douglas S, Pryde A, Heading RC. 
Clinical characteristics and natural history of 
symptomatic but not excess gastrooesophageal 

2 Klausen AO, Schindbeck NE, Muller-Lissner 
SA. Esophageal 24-hour pH monitoring: is 
prior manometry necessary for correct position-
ing of the electrode? Am J Gastroenterol 1990; 
85: 1463–7.

3 Galmiche JP, Scarpignato C. Esophageal pH 
monitoring. Front Gastroent Res 1994; 22: 
71–108.

4 Galmiche JP, Denis P, Deschallier JP. Valeur 
diagnostique des examens complementsaires 
au cours du reflux gastro-oesophagien de l’adulte. 

5 Galmiche JP, Guillard EF, Denis P, Bouskak 
K, Lefrancos R, Colin R. Etude du pH 
oesophagien en periode post-prandiale chez le 
sujet normal et au cours du syndrome de reflux 
gastro-oesophagien. Intret diagnosique d’un 
score de reflux acide. Gastroentrol Clin Biol 

6 Shaker R, Helm JF, Dodds WJ, Hogan WJ. 
Relevations about ambulatory esophageal pH 

7 Jamieson JR, Hein JH, DeMeester TR, Bonavina 
24-H esophageal pH monitoring: normal val-
ues, optimal thresholds, specificity, sensitivity, 
and reproducibility. Am J Gastroenterol 1992; 
87: 1102–11.

8 Schlesinger PH, Donahue PE, Schmid B. 
Limitations of 24-hour intraesophageal pH 
monitoring in the hospital setting. 

NOTE

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