# LETTERS TO THE EDITOR

## 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; **38:** 211–5). Although they developed an exquisitely 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 with ischaemic times of 45 minutes to 1 hour 45 minutes compared with biopsy specimens frozen within 15 seconds.[1] Further degradation can also occur during prolonged storage at −70°C. The assay may be sufficiently sensitive to detect one viral genome, 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 numbers than β-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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1 Macpherson AJ, Chester KA, Robson L, Bjarnasson I, Malcolm AD, Peters TJ. Increased expression of c-myc proto-oncogene in biopsies of ulcerative colitis and Crohn's colitis. *Gut* 1992; **33:** 651–6.

## 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 transcription 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 paraffin wax 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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1 Wakefield AJ, Pittilo RM, Sim R, Cosby SL, Stephenson JR, Dhillon AP, *et al.* Evidence of persistent measles virus infection in Crohn's disease. *J Med Virol* 1993; **39:** 345–53.
2 Haase AT, Ventura P, Gibbs JR CJ, Tourtellotte WW. Measles virus nucleotide sequences: detection by hybridization in situ. *Science* 1981; **212:** 672–5.
3 Godec MS, Asher DM, Swoveland PT, Eldadah ZA, Feinstone SM, Goldfarb LG, *et al.* Detection of measles virus genomic sequences in SSPE brain tissue by polymerase chain reaction. *J Med Virol* 1990; **30:** 237–44.

EDITOR,—In their publication, Haga *et al* were unable to detect measles RNA in intestinal tissues of Crohn's disease (*Gut* 1996; **38:** 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 virion (one plaque forming unit of measles virus). However, Lund *et al*[1] 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 NASBA[2] (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,[3] 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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1 Lund G, Tyrrell D, Bradley R, Scraba G. The molecular length of measles RNA and the structural organization of measles nucleoscapsids. *J Gen Virol* 1984; **65:** 1535–42.
2 Compton J. Nucleic acid sequence-based amplification. *Nature* 1991; **350:** 91–2.
3 Macpherson A, Chester K, Robson L, Bjarnasson I, Malcolm A, Peters T. Increased expression of c-myc proto-oncogene in biopsies of ulcerative colitis and Crohn's colitis. *Gut* 1992; **33:** 651–6.

## Reply

EDITOR,—We reply to the claims of Chadwick and Wakefield. As Lund *et al*[1] described that one virion contains well in excess of one nucleocapsid and single measles virus particle contains several genomes, one plaque forming unit may not represent only one measles viral genome. With regard to the delay between resection and freezing of samples, I have already described the details in the reply to Dr Smith. Although we confirmed the absence of PCR inhibition by spiking the homogenate from 250 mg of control intestinal tissue with 5 pg of RNA from a measles infected cell culture, the spiking to postoperative control tissue might provide more exact evaluation for RNA degradation, even though the spikings before and after homogenisation might not make so much difference. Fundamentally we think that the most important strategy to seek measles virus is to choose the method that provides the highest sensitivity out of various methods available in the world.

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1 Lund GA, Tyrrell DLG, Bradley RD, Scraba DG. The molecular length of measles virus RNA and the structural organization of measles nucleocapsids. *J Gen Virol* 1984; **65:** 1535–42.

## 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 recording. The authors stated that this was consistent with the idea of oesophageal hypersensitivity to acid. This group of patients was very heterogenous, 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 up method, which is known to inaccurately locate the lower oesophageal sphincter in 58% of patients.[1] As reported by Anggiansah *et al*[2] 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 standardised[3] and severity of symptoms not assessed (for example, by visual analogue scales) it is difficult to consider a given pH monitoring as