CORRESPONDENCE

Standardisation of breast tissue fixation procedures

The Royal College of Pathologists Working Group have provided comprehensive guidelines for the reporting of histopathological specimens within the National Breast Screening Programme.¹ We are concerned, however, that tissue fixation procedures are not to be standardised among participating laboratories.

Most fixatives penetrate tissues slowly; for example, 4% formaldehyde solution penetrates by 3-8 mm in 24 hours,^{2,3} so the centre of small unsliced breast biopsy specimens will not have been fixed within that period. The guidelines provide detailed instructions in relation to mastectomy specimens, but overnight fixation is sanctioned for unsliced breast biopsy specimens.¹

We have recently shown that a delay in fixation of 24 hours produces a reduction in the number of observable mitotic figures in breast carcinomas of about 75%.^{4,5} In some tumours the decreased count after just six hours can result in a change in the overall modified Bloom and Richardson tumour grade and hence prognostic group.⁵

These findings have important implications for the accuracy and validity of any multi-centre study comparing breast carcinomas on the basis of a histological grading system that depends on the mitotic count. The inevitable variation in fixation delays of surgical specimens may be yet another factor which has contributed to the widely reported difficulties in consistency and reproducibility in breast carcinoma grading among centres.⁶

We believe that the failure to observe a defined system of tissue fixation may undermine any proposed pathological audit within the current National Breast Cancer Screening Programme.

RD START
SS CROSS
JHF SMITH
Department of Histopathology,
Northern General Hospital,
Merness Road,
Sheffield S5 7AV

- 1 Royal College of Pathologists Working Group. Pathology reporting in breast cancer screening. *J Clin Pathol* 1990;44:710-25.
- 2 Medawar PB. The rate of penetration of fixatives. *J R Microsc Soc* 1942;61:46-57.
- 3 Baker JR. Principles of biological microtechnique. London: Methuen, 1960:31-43.
- 4 Start RD, Flynn MS, Rogers K, Smith JHF. Delayed fixation significantly decreases observed mitotic figures in breast carcinoma. *J Pathol* 1991;163:154a.
- 5 Start RD, Flynn MS, Cross SS, Rogers K, Smith JHF. Is the grading of breast carcinoma affected by a delay in fixation? *Virchows Arch (Pathol Anat)* (In press).
- 6 Elston CW. Grading of invasive carcinoma of the breast. In: Page DL, Anderson TJ. *Diagnostic histopathology of the breast*. Edinburgh: Churchill Livingstone, 1987:300-11.

On behalf of RLP Advisory Group on Quality Assurance in Breast Screening Dr Sloane et al comment:

We note with interest the comments made by Drs Start, Cross, and Smith on the procedure for fixing breast biopsy specimens recom-

mended in the Pathology Guidelines for the National Breast Screening Programme.

We accept that in order to achieve uniformity of grading carcinomas specimens should ideally be sliced and immersed in fixative as soon as possible after excision. This may not only increase the number of mitotic figures but also the degree of morphological preservation. It may be impossible, however, for many pathologists to examine specimens within a short time of their removal with the consequent risk of leaving them unfixed for long periods. Incising and fixing them before thorough macroscopic inspection could lead to problems of subsequent orientation and interpretation, particularly if the incision is undertaken by the surgeon. In drawing up the Guidelines, the Working Group was very conscious of the need to strike a balance between the ideal and that which can practically be achieved. As most biopsy specimens from screened women are relatively small, we felt that recommending fixation in the intact state was acceptable and possibly safer than some of the possible alternatives.

Dr Start and colleagues report that delaying fixation of breast tumours reduces the observable number of mitoses and may change the modified Bloom and Richardson grade. As most of this work is still not published, we were obviously unable to take their observations into account when drawing up the Guidelines and are still not in a position to be able to evaluate in detail their relevance to the screening programme. A possible difficulty in this respect is that they have studied the effect of delaying the exposure of the tissue to fixative rather than comparing the effects of fixing intact or sliced specimens of specified size.

Although our Guidelines were published after extensive consultation, they were not intended to be sacrosanct but to be reviewed periodically in the light of experience and published data. We are about to begin such a review. We value the comments of Dr Start and colleagues and would be delighted to hear from anyone else who would like to express their views.

Gastric histamine concentration and IgE in *Helicobacter pylori* infection

Queiroz *et al* recently observed a significantly lower gastric histamine concentration in *Helicobacter pylori* positive patients than in *H. pylori* negative subjects and hypothesised that this might be due to increased histamine release with a subsequent depletion of stores.¹

We evaluated the immune response involving IgE in patients with chronic gastritis who were infected with *H. pylori*.² Of 26 patients with *H. pylori* infection, 22 (84%) tested positive for basophil bound specific IgE (determined by the histamine release test) and 18 (69%) for serum specific IgE (determined by an enzyme linked immunosorbent assay). In contrast, among 17 subjects in whom the bacterium was not detected, only one, with histologically confirmed gastritis,

had cell bound and serum specific IgE. In four subjects who tested positive by the histamine release test without detectable specific circulating IgE, acid elution experiments showed that histamine release from basophils occurred as a result of an immunological mechanism. To demonstrate further the class specificity of the response, the *H. pylori* antigen was used to challenge normal basophils passively sensitised with serum from IgE positive patients. The significant histamine release obtained on exposure to antigen excluded interference with antibodies other than IgE directed against the same antigen. Inhibition experiments with bacteria other than *H. pylori* showed that the IgE antibody was specifically directed against this organism.

Our data strongly suggest that an immunological response involving IgE is present in patients infected with *H. pylori*. It is therefore conceivable that IgE has a role in inducing histamine release in the stomach through the activation of mast cells. In fact, these cells, which store histamine in human gastric mucosa, express specific surface receptors with high affinity for IgE. On antigenic stimulation mast cells are activated, undergo degranulation, and release their mediators by a secretory, energy requiring process. Not only histamine, but also other preformed or newly generated mediators released by mast cells, may be implicated in tissue damage.

The ability of *H. pylori* to induce a specific IgE immune response could answer key questions regarding the mechanisms of gastroduodenal inflammation.

A ACETI
D CELESTINO
M CAFERRO
A GRILLI
A PENNICA
O LERI
A SEBASTIANI
Institute of the Clinic of
Tropical and Infectious Diseases,
La Sapienza University,
00161 Rome, Italy
V CASALE
F CITARDA
E M CONTI
A GRASSI
F SCARRETTA
F AMEGLIO
Regina Elena,
National Cancer Institute,
Rome, Italy

- 1 Queiroz DMM, Mendes EN, Rocha GA, *et al*. *Helicobacter pylori* and gastric histamine concentrations. *J Clin Pathol* 1991;44:612-3.
- 2 Aceti A, Celestino D, Caferro M. Basophil-bound and serum immunoglobulin E directed against *Helicobacter pylori* in patients with chronic gastritis. *Gastroenterology* 1991;101:131-7.

Dr Queiroz comments:

We have shown that *H. pylori* positive patients, both children¹ and adults,² have lower gastric histamine concentrations than *H. pylori* negative subjects. This might be secondary to an increased release of histamine from mast cells which store histamine in human gastric mucosa; and induced by some *H. pylori* by-product; or by gastrin which is increased in *H. pylori* positive patients.³

Aceti *et al*⁴ have shown that there is an immunological response involving IgE in *H. pylori* positive patients and they have formulated the attractive hypothesis that in these patients specific IgE could have a role in gastric histamine release through activation of mast cells which express specific surface receptor for IgE. According to these authors,