LETTERS TO THE EDITOR

Markers to study human colonic cell proliferation

EDITOR,—We noted with interest the paper by Kubben *et al* (*Gut* 1994; **35:** 530–5) on a comparison between proliferating cell nuclear antigen (PCNA) and ex vivo bromodeoxyuridine (BrdU) labelling. We have compared PCNA labelling in 86 human colorectal tumours to iododeoxyuridine (IudR) labelling after in vivo administration using both flow cytometric and immunohistochemical methods.[1]

In contrast with the authors' findings, we have not found a significant correlation between the two labels. This was despite correcting for the presence of IudR labelled daughter nuclei (a problem that has not been discussed in this paper) and using a variety of fixatives when assessing PCNA labelling. In our experience, the strongest correlation seen has been on comparison between IudR labelling assessed immunohistochemically and PCNA labelling after fixation in methanol ($r=0{\cdot}38$, $p=0{\cdot}015$). Fixation methods seem to affect the identification of PCNA in different parts of the cell cycle[2] and the apparently higher expression of PCNA than BrdU in Kubben's paper reflects this.

As we have stated before,[3] we feel that in comparisons such as this, it is necessary to analyse a much greater number of specimens from a greater number of subjects and attach less clinical significance to a weak correlation that is statistically significant.

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2 McCormick D, Hall PA. The complexities of proliferating cell nuclear antigen. *Histopathology* 1992; **21:** 591–4.
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Reply

EDITOR,—We are grateful to Wilson and Schofield for their comment on our study. Wilson and Schofield did not find a significant correlation between proliferating cell nuclear antigen (PCNA) and in vivo iododeoxyuridine (IudR) immunohistochemistry in 86 human colorectal tumours. The higher expression of PCNA than BrdU in our study they ascribe to the fixation method used.

Two populations of PCNA are present during S phase. One is nucleoplasmic, present in short term G_0 cells, and not apparent in cells fixed in organic solvents such as methanol or ethanol. The other form is associated with DNA replication sites and cannot be extracted with organic solvents.[1–3]

Our results are comparable with those of Weisgerber *et al*,[4] who used an organic solvent as fixative as well, and slightly lower of those of Risio *et al*,[5] who used formalin fixation (Table). Risio showed a decreasing correlation between PCNA and BrdU immunohistochemistry with increasing dysplasia of the tissue under investigation.

The progressive increase of PCNA expression with increasing dysplasia seems to be related to both hyperproliferation and neoplastic deregulation of PCNA synthesis. Although they do not provide sufficient technical details, the interesting results of Wilson and Schofield are in agreement with our study and the work of Weisgerber *et al* and Risio *et al.*

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Correlation of BrdU and PCNA immunohistochemistry on human colorectal tissue

Author	*Tissue*	*Subjects (n)*	r	*p Value*	*Mab*
Kubben	Normal	16	0·63	<0·05	19A2
Weisgerber[4]	Normal	17	0·6	0·011	19A2
Risio[5]	Normal	50	0·7	<0·001	PC10
	Low grade adenoma	59	0·61	<0·05	
	High grade adenoma	21	0·23	NS	
	Adenocarcinoma	20	0·15	NS	
Wilson[6]	Adenocarcinoma	86	0·38	0·015	PC10

Mab=monoclonal antibody against proliferating cell nuclear antigen; *r*=correlation coefficient.

Duodenal ulcer, gastric acid, and *Helicobacter pylori*

Editor,—Professor Hobsley's group (Gut 1994; 35: 1033–6) found significant decrease in maximal histamine stimulated gastric acid secretion corrected for pyloric loss, duodenogastric reflux, and stature in patients with duodenal ulcer or non-ulcer dyspepsia who were *H pylori* positive. We have four questions. (1) What were the results with the one hour basal acid output? (2) Why were only 68% (21 of 31) of the duodenal ulcer group *H pylori* positive with active chronic gastritis? The usual proportion of *H pylori* positivity in duodenal ulcer is 95%, and superficial or atrophic antral gastritis is almost invariable in duodenal ulcer. (3) The decrease in acid was significant only in the corrected data. Was either pyloric loss significantly reduced or duodenogastric reflux significantly increased in those infected with *H pylori*? (4) They speculate that the reduced acid in the *H pylori* positive duodenal ulcer group results from destruction of parietal cells: were body biopsy specimens taken to test this hypothesis? And have any of the patients had their *H pylori* eradicated, and did this increase their acid output?

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Reply

EDITOR,—We thank Drs Baron and Harris for their interest. We reply to their four questions. (1) The mean results of the second half hour (because the first half hour is not a reliable estimate of the basal[1]) were: *H pylori* positive (n=41), basal acid output 5·14 mmol/h, VG 111 ml/h; *H pylori* negative (n=21), basal acid output 4·97 mmol/h, VG 110 ml/h. (2) We do not know why 'only' 68% of our duodenal ulcer group were *H pylori* positive, although some evidence bearing on this point has been submitted for publication. We agree that 95% is commonly quoted, but in five recent publications the values were 67%,[2] 52·6%,[3] 66%,[4] 76%,[5] and 50%[6] (weighted average 65·9%). (3) The plateau/average values (SD) of duodenogastric reflux (VR) ml/min and pyloric loss ml/min were (*H pylori* positive first) −0·61 (2·6), 2·1 (3·2); 4·5 (6·8), 5·7 (6·7). The positive and negative patients did not differ significantly from each other. (4) Body biopsy specimens were not taken, hence the speculative nature of our suggestion. Some of the patients had their *H pylori* eradicated.[7] Acid output was not measured after eradication.

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