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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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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1 Faber RG, Hobsley M. Basal gastric secretion: reproducibility and relationship with duodenal ulcers. *Gut* 1977; **18:** 57–63.
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Cancer of the oesophagus

EDITOR,—In the absence of controlled trials of the treatment of oesophageal cancer several authors have turned to descriptive studies to investigate the survival of patients with this condition.[1–4] The most recent of these was reported by Sagar *et al* (*Gut* 1994; **35:** 941–5). While agreeing with several points made in this paper, we were disappointed that it failed to set their results, based on an audit of patients treated at the General Infirmary at Leeds, in the context of other published studies.

The Table summarises the results of those descriptive studies based in the United Kingdom and published since 1980. Although varying in location and time, each has attempted to avoid the biases of selective case series by reporting the results, after treatment, of all cases diagnosed within a given period from a defined population.

The results of these studies show considerable consistency, and three important points regarding surgery are apparent: surgical resection has poor longterm survival, high early mortality, and is performed in only a few of all patients presenting with oesophageal cancer. Surgery offers the prospect of 'cure' (if taken as five year survival) to less than 3% of all patients presenting and is irrelevant to those 60–80% of patients felt unsuitable for operation. Any argument that surgery should be considered in more patients must face up to postoperative death figures that can amount to a third of patients.

Taken together we feel these studies strongly reinforce the 'nihilistic' view of surgery for oesophageal cancer referred to by Sagar *et al*. If resection is being performed chiefly for symptomatic palliation then it must prove itself on those terms against cheaper and less invasive techniques, such as endoscopic intubation or laser recanalisation.

Surgery can offer cure for oesophageal cancer, but we should recognise that, with the current pattern of disease staging at presentation, this applies to a much smaller proportion of patients than the 20–40% currently being offered surgical resection. We support the call by Sagar *et al* (also voiced in the 1993 NCEPOD report[5]) for oesophagectomy to be carried out by specialist surgeons, however we feel their priority must be the better selection of cases not the treatment of more patients. Equal priority must be given to setting up proper trials of the increasing number of palliative treatments offered to those unsuitable for surgery, who are currently the majority in the United Kingdom.

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Reply

EDITOR,—I read with interest the comments of Drs Oliver and Logan and am grateful for the opportunity to reply.

Their Table shows the similarity in outcome of four United Kingdom studies. We chose not to set our results in the context of United Kingdom studies alone but referred instead to the excellent review of 130 series in the world medical publications by Dr Muller.[1]

We did not advocate a need to simply increase the numbers of patients undergoing oesophagectomy but rather implied a need for more patients with cancer of the oesophagus to be adequately considered for surgical resection and agree that careful selection of cases is essential.

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1 Muller JM, Erasmi H, Stelzner M, Zieran U, Pichlmaier H. Surgical therapy of oesophageal carcinoma. *Br J Surg* 1990; **77:** 845–57.

Current concepts in metastasis

EDITOR,—The progress report (*Gut* 1994; **35:** 996–1000) provides a concise and instructive overview of the role of adhesion molecules in invasion and metastasis. Metastasis is viewed as an evolutionary process entailing the sequential selection of subclones with increasingly aggressive characteristics. This concept has been promulgated mainly on the basis of in vitro and animal models utilising cell lines. Extrapolating these experimental findings to the clinic may lead to conceptual inaccuracies. For example, taking colorectal cancer as the model, is it certain that metastases represent subclones that can be distinguished genetically from the primary tumour? Colorectal cancers show remarkable stability in their morphological and phenotypic characteristics with time. Liver metastases appearing years after removal of the primary may be indistinguishable from the primary. Indeed the same heterogeneity that is seen in the primary may be echoed in the secondary deposits.[1] Are there really multiple cell populations or are we overinterpreting examples of transient epigenetic modulation?

The period in the evolution of colorectal cancer in which there is undoubted subclonal selection is in the stepwise conversion of a normal cell to a cancerous cell through the intermediate stage of an adenoma. The outcome may be a well, moderately or poorly differentiated cancer with a metastatic potential that can be correlated with the grade of differentiation. However, evidence for the further selection of metastatic subclones, at least with respect to colorectal cancer, is lacking.

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1 Jass JR, Mukawa K, Richman PI, Hall PA. Do aggressive subclones within primary colorectal cancer give rise to liver metastases? *Int J Colorect Dis* 1989; **4:** 109–17.

NSAIDs and the chemoprevention of colon and oesophageal cancer

EDITOR,—I read with interest two articles in *Gut*. Drs Choi and Zelig (1994; **35:** 950–4) show that ulcerative colitis and Crohn's disease are pre-neoplastic colorectal lesions with similar clinicopathological features while Manzano *et al* (*Gut* 1994; **35:** 955–60) illustrate that ulcerative colitis is associated with T lymphocyte immunosuppression. Choi and Zelig suggest a potential for non-steroidal anti-inflammatory drugs (NSAIDs) as a chemopreventive measure in patients with inflammatory bowel disease on the basis that chronic inflammation is carcinogenic. I write to expand on this suggestion given the data of Manzano *et al*.

Epidemiological studies have shown that the regular consumption of the NSAID aspirin is associated with a reduced risk of

Descriptive studies of oesophageal cancer treatment (in the UK) published since 1980

Study details			Surgical resection				Radiotherapy		Intubation		No treatment	
				Survival rate* (%)		30 Day mortality		Median survival		Median survival		Median survival
Time period covered	Location	No	No (%)	1 Year	5 Year	(%)	No (%)	(day)	No (%)	(day)	No (%)	(day)
1956–1976	W Midlands[1]	4680	1104 (24)	36	11	32	725 (16)	NA	1002 (21)	NA	1581 (34)	NA
1975–1988	Leeds[2]	316	134 (42)	40	7	27	29 (9)	175	64 (20)	106	82 (26)	91
1976–1986	N Tees[3]	120	21 (18)	38	10	14	19 (16)	NA	57 (48)	167	16 (13)	NA
1982–1985	Nottingham[4]	268	92 (35)	41	2	9†	35 (13)	190	106 (40)	100	28 (10)	21

*Crude survival rates, †mortality during first hospital admission. NA = not available.