Gastric epithelial cell kinetics in the progression from normal mucosa to gastric carcinoma

R J Cahill, C Kilgallen, S Beattie, H Hamilton, C O'Morain

Abstract

Increased epithelial cell proliferation is associated with an increased risk of adenocarinoma and is associated with *Helicobacter pylori* infection. The aim of this study was to assess both gastric epithelial cell proliferation and the influence of *H pylori* infection on cell kinetics in the progression from normal mucosa to gastric carcinoma. One hundred and forty four subjects were assigned to study groups based on diagnosis and *H pylori* status: microscopically normal mucosa and *H pylori* negative (n=28); chronic active gastritis and *H pylori* positive (n=83); atrophic gastritis (n=9); intestinal metaplasia (n=19); gastric carcinoma (n=12). Gastric antral epithelial cell proliferation was assessed using the in vitro bromodeoxyuridine immunohistochemical technique and expressed as the labelling index per cent (LI%). Subjects with chronic atrophic gastritis, intestinal metaplasia or gastric cancer have increased gastric epithelial cell proliferation compared with normal mucosa (LI% mean (SEM): 5·14 (0·6), 4·68 (0·3), 6·50 (0·5) *v* 3·08 (0·2), p<0·001). This increase in gastric epithelial cell proliferation was not influenced by *H pylori* status. Gastritis associated with *H pylori* had an increased LI% compared with normal controls or subjects with *H pylori* negative gastritis (4·98 (0·2) *v* 3·08 (0·2), 3·83 (0·2), p<0·01). *H pylori* infection although associated with an increased epithelial cell proliferation in subjects with chronic gastritis, does not influence the increased epithelial cell proliferation seen in subjects with precancerous lesions or gastric carcinoma. This is further evidence that *H pylori* may be an initiating step in gastric carcinogenesis.

(*Gut* 1996; **38:** 177–181)

Keywords: gastric carcinogenesis, cell kinetics, *Helicobacter pylori*.

Department of Gastroenterology, Meath and Adelaide Hospitals, Trinity College, Dublin, Ireland
R J Cahill
S Beattie
H Hamilton
C O'Morain

Department of Histopathology, St James's Hospital, Dublin, Ireland
C Kilgallen

Correspondence to:
Professor C O'Morain, Department of Gastroenterology, Meath Hospital, Heytesbury Street, Dublin 8, Ireland.

Accepted for publication 24 July 1995

Although the overall incidence of gastric cancer has steadily declined during the past 50 years in the Western world, it is still a major health problem and it remains the second most common cancer in the world. Gastric cancer has a poor prognosis and in a recent British study the overall five year survival for subjects with resectable gastric cancer was 20%.[1]

The aetiology of gastric carcinoma has not been fully elucidated. In 1988 Correa and colleagues proposed a human model of gastric carcinogenesis based on epidemiological, pathological, and clinical findings. They postulated that gastric cancer develops through a complex sequence of events from normal mucosa to superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally to intestinal type gastric carcinoma. Correa suggested that an environmental agent induced chronic gastritis, the first step in the progression to malignancy.[2]

Helicobacter pylori is associated with a sixfold increased risk of gastric carcinoma.[3] *H pylori* is frequently found in association with gastric cancer and precancerous lesions.[4 5] Longterm studies of *H pylori* infection have provided evidence of a progression from *H pylori* gastritis to atrophic gastritis, intestinal metaplasia, and dysplasia.[6]

The mechanism by which *H pylori* participates in gastric carcinogenesis is largely unknown. We have identified an increased gastric epithelial cell proliferation associated with *H pylori* infection, which is reversed when the organism is eradicated.[7 8] Increased epithelial cell proliferation has been associated with an increased risk of adenocarcinoma.[9] The aim of this study is to investigate gastric epithelial cell kinetics at different stages in the progression from normal mucosa to gastric carcinoma and to assess the role of *H pylori* in this progression.

Methods

Biopsy specimens from 155 subjects, attending the endoscopy clinics of the Meath and Adelaide hospitals, Dublin, Ireland with dyspeptic symptoms were studied. Subjects with a history of gastrointestinal cancer excluding gastric cancer, adenomatous polyps, *H pylori* eradication therapy, non-steroidal anti-inflammatory drugs (NSAID) or antibiotic therapy in the previous three months were excluded from the study.

Diagnosis was made at endoscopy and confirmed by histological examination, CLO test, Gram stain, and culture. Subjects were assigned to one of five study groups based on histological findings. Group 1: macroscopically and microscopically normal mucosa, *H pylori* negative, group 2: type B gastritis, *H pylori* positive, group 3: atrophic gastritis, group 4: intestinal metaplasia, group 5: gastric adenocarcinoma. The diagnosis of gastric adenocarcinoma was further classified as intestinal or diffuse type according to the Lauren classification.[10] The gastric carcinomas were also classified according to the Goseki method of histological classification, which relates the histological type of gastric cancer to the mode of extension.[11]

TABLE I *Demographic profile of all study groups*

Study group	*No*	*Age mean (SD)*	*Age range (y)*	*Male:female*	H pylori *status pos:neg*
Normal	32	44 (16)	23–81	12:20	0:32
H pylori positive gastritis	83	45 (16)	17–84	41:42	83:0
Atrophic gastritis	9	72 (13)*	43–86	2:7	6:3
Intestinal metaplasia	19	59 (15)*	32–81	13:6	6:13
Gastric cancer	12	70 (9)*	53–82	10:2	3:9

*p<0·05 when compared with normal controls. The mean age of subjects with precancerous lesions and gastric carcinoma was significantly older than subjects with *H pylori* positive gastritis alone or normal mucosa.

Endoscopies were carried out between 9 am and 12 noon to control for diurnal variation. Seven antral mucosal pinch biopsy specimens were taken from each subject before and after treatment; one for microbiological assessment, one for CLO test (Delta-West, Bently, Australia), and one for histological assessment. Four antral mucosal biopsy specimens were taken for proliferation analysis from macroscopically normal mucosa away from any macroscopic lesions. These were immediately placed in modified Waymouths medium.

Cell proliferation technique

Specimens were cultured in Waymouths medium (Flow Labs, Hertfordshire) supplemented with 10% fetal calf serum, 1 mM glutamine and gentamicin, 50 μM bromodeoxyuridine (BrdU) (Sigma, Poole, UK), and 5 μM fluorodeoxyuridine (Sigma, Poole, UK). The specimens were incubated in the medium for one hour at 101·3 kPa of pressure in a sealed modular incubation chamber (Flow Labs, Hertfordshire) previously infused with 95% oxygen and 5% carbon dioxide at a thermoregulation temperature of 37°C. The specimens were fixed in Carnoys fixative overnight and embedded in paraffin wax. Immunohistochemical detection of the BrdU incorporation was carried out on 4 μm sections using the technique previously described[12] with a monoclonal antibody to BrdU (Dakopatts, Denmark). Labelled cells were visualised by the diaminobenzidine reaction and lightly counterstained with haematoxylin.

Immunohistological analysis

A mean of 20 sections were examined for each subject. Only entire gastric pits longitudinally sectioned, visible in their entire length, and greater than 100 cells were analysed. A mean number of 10 well orientated gastric pits were examined for each specimen.

Labelling index per cent (LI%) was measured by counting the number of BrdU positive cells and expressing the result as a percentage ratio of the total number of cells in a gastric pit. The relative positions of the positive cells in the pit were noted. For cell kinetic evaluation, each gland was divided into five compartments of equal size. The compartments were referred to by the ordinal numbers 1 (apex) to 5 (base) and the LI calculated for each compartment.

Statistical analysis

The total LI% and the LI% per compartment were compared between the study groups. Significance was analysed using Students' *t* test for unpaired data. p Values less than 0·05 were considered statistically significant.

Results

One hundred and fifty five subjects were recruited for the study. Thirty two subjects were included in the normal control group, 83 in the *H pylori* positive gastritis study group, nine subjects in the atrophic gastritis group, and 19 in the intestinal metaplasia study group. Twelve subjects were included in the gastric adenocarcinoma study group. Table I shows the demographic profile and *H pylori* status of the study groups. Subjects with type C gastritis without *H pylori* infection were included in this paper as a reference group, the demographic profile of these subjects is previously described.[7] A subgroup of the *H pylori* positive gastritis study group has also been previously reported.[7]

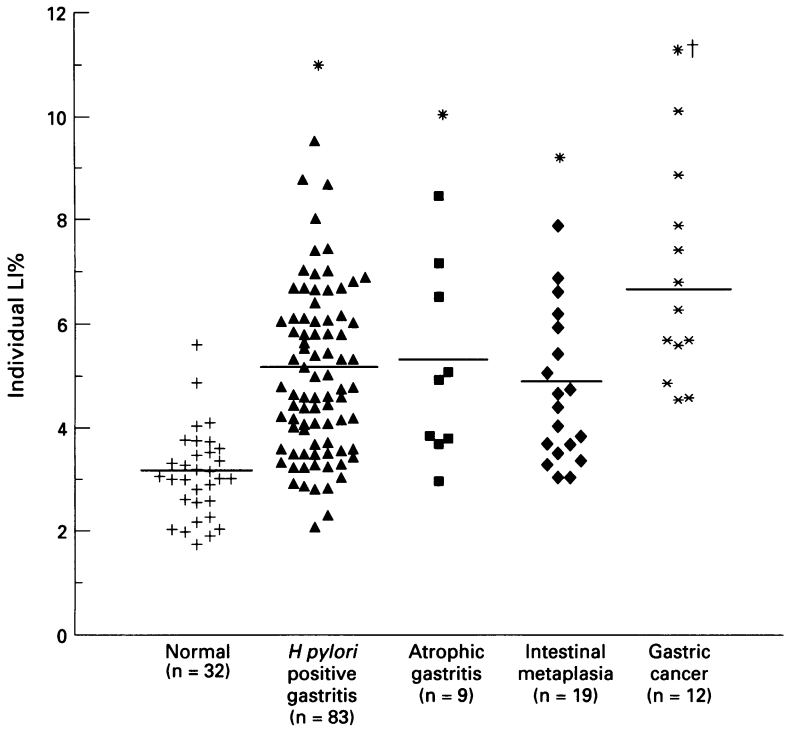
Subjects with chronic atrophic gastritis or intestinal metaplasia had an increased epithelial cell proliferation compared with normal mucosa (LI% mean (SEM): 5·14 (0·6), 4·68 (0·3) *v* 3·08 (0·2), p<0·01, Table II, Figure). The increased proliferation was seen throughout the entire gastric pit including the apex of the pit.

Tissue remote from gastric adenocarcinoma also had an increased epithelial cell proliferation compared with normal controls (LI%: 6·50 (0·5) *v* 3·08 (0·2), p<0·001, Table II,

TABLE II *Gastric epithelial cell kinetics of all study groups*

	Normal	H pylori *positive gastritis*	*Atrophic gastritis*	*Intestinal metaplasia*	*Gastric cancer*
No of subjects	32	83	9	19	12
Crypts per subject	10	10·1	10·3	10	10
Total cells counted per subject	1449 (29)	1445 (34)	1475 (70)	1505 (29)	1343 (62)
Total labelled cells per subject	44·2 (2·2)	47·6 (2·4)	74·4 (7·2)	69·4 (4·5)	87·2 (6·1)
Total cells per crypt	144·3 (3·2)	170·3 (2·3)	142·5 (5·3)	150·5 (2·6)	134·3 (5·6)
LI% total	3·08 (0·2)	4·84 (0·2)*	5·14 (0·6)*	4·68 (0·3)*	6·50 (0·5)*‡
LI% per compartment					
LI% 1	0·01 (0·01)	0·10 (0·03)	0·17 (0·1)	0·32 (0·11)*	0·57 (0·3)*
LI% 2	0·34 (0·08)	1·32 (0·1)†	1·68 (0·7)†	1·72 (0·4)†	2·84 (0·8)†
LI% 3	2·28 (0·28)	4·50 (0·3)†	4·42 (0·9)†	4·45 (0·5)†	5·82 (0·90)†
LI% 4	5·62 (0·44)	8·32 (0·3)†	9·70 (1·5)†	7·60 (0·8)†	10·20 (0·8)†
LI% 5	7·07 (0·44)	10·94 (0·7)†	10·18 (1·1)†	9·33 (0·8)†	13·18 (1·3)†

All results expressed as mean (SEM). *p<0·01, †p<0·005 when compared with normal controls and *H pylori* negative gastritis. ‡p<0·01 compared with all study groups except atrophic gastritis. All specimens were taken from the antrum remote from macroscopic lesions.



Epithelial cell kinetics of patients in all study groups (total LI%). Mean total LI% is also included. Mean LI% (SD) are as follows; normal=3·08 (0·2), H pylori *positive gastritis=4·84 (0·2), atrophic gastritis=5·14 (0·6), intestinal metaplasia=4·68 (0·3), gastric cancer=6·50 (0·5). *p<0·001 compared with normal controls, †p<0·01 compared with all study groups except atrophic gastritis.*

Figure). The increase of proliferation was seen in all compartments of the pit including the apex of the gastric pit. Four of the subjects were diagnosed with diffuse type adenocarcinoma and eight with intestinal type, there was no significant difference in the LI between both groups (LI%: 6·70 (1·03) *v* 6·36 (0·5)). Similarly there was no significant difference between groups classified according to the Goseki method although subjects with GI (n=4) had a lower proliferation rate compared with both GII (n=3) and GIII (n=5) with proliferation rates of 5·34% (0·4), 7·26% (1·5), and 7·1% (0·9) respectively.

The presence or absence of *H pylori* infection did not change the increased epithelial cell proliferation seen in subjects with atrophy, intestinal metaplasia or gastric carcinoma (Table III). *H pylori* positive gastritis had increased epithelial cell proliferation compared with normal controls and subjects with type C gastritis (*H pylori* negative) (Table III). This increased gastric epithelial cell proliferation was not significantly different from that associated with chronic atrophic gastritis, intestinal metaplasia or tissue remote from gastric carcinoma.

TABLE III *Influence of* H pylori *status on gastric epithelial cell proliferation of tissue remote from any macroscopic lesion in subjects with antral gastritis, atrophic gastritis, intestinal metaplasia or gastric cancer*

	Number	*LI%*	*p Value*
Antral gastritis			
H pylori negative	28	3·33 (0·2)	p=0·0014
H pylori positive	83	4·98 (0·2)	
Atrophy			
H pylori negative	3	4·98 (1·4)	p=0·519
H pylori positive	6	5·34 (1·8)	
Intestinal metaplasia			
H pylori negative	13	4·75 (1·6)	p=0·954
H pylori positive	6	4·60 (1·2)	
Gastric cancer			
H pylori negative	9	6·62 (2·1)	p=0·940
H pylori positive	3	6·44 (2·6)	

p Value compares subjects with *H pylori* infection with those without *H pylori* infection at the time of biopsy. Subjects with *H pylori* infection and antral gastritis had a significantly higher LI% compared with those with *H pylori* negative gastritis (reference group, 10). *H pylori* infection did not seen to influence the epithelial cell proliferation of subjects with precancerous lesions (atrophy, intestinal metaplasia) or gastric cancer. Data shown as mean (SEM).

Discussion

This study assessed gastric epithelial cell kinetics, using the BrdU immunohistochemical technique. Antral mucosal biopsy specimens remote from macroscopic lesions of subjects with *H pylori* positive gastritis, precancerous lesions (atrophy and intestinal metaplasia), and gastric carcinoma were studied. Subjects with *H pylori* gastritis, intestinal metaplasia or atrophic mucosa have an increased epithelial cell proliferation compared with normal mucosa. This increased epithelial cell proliferation is not significantly different from that associated with tissue remote from gastric carcinoma.

Most studies of gastric epithelial cell kinetics have used the tritiated thymidine technique. This technique is time consuming and entails the use of radioactive isotopes. The BrdU immunohistochemical technique has been found to be as accurate as the tritiated thymidine technique.[13]

Quantification of gastric epithelial cell proliferation can be carried out by assessing the number of proliferating cells in the entire gland or in the gastric pit. The assessment of epithelial cell proliferation in the gland entails quantifying proliferating cells in randomised fields. Assessment of gastric pit epithelial cell proliferation is the preferred method of assessment of gastric epithelial cell kinetics and it reflects overall gastric epithelial cell proliferation. In the assessment of epithelial cell kinetics of the gastric pit, the zone of maximum proliferation is in the base of the gastric pit. This has been shown in normal rat[14] and human mucosa[1] and has been confirmed by our study.

There is evidence that gastric cancer develops through the progression from normal mucosa to gastric atrophy, intestinal metaplasia to gastric carcinoma.[2] The pathogenesis of the sequence of events is largely unknown, however, there is a substantial amount of evidence supporting the role of *H pylori* in gastric carcinogenesis, which comes from many sources. Epidemiological studies have identified a high prevalence of serum antibodies against *H pylori* in areas of high incidence of gastric cancer.[3 15] Histological studies have also identified *H pylori* associated with precancerous lesions and gastric cancer, although *H pylori* cannot be found in intestinal metaplastic tissue or adenocarcinoma cells.[4 16] *H pylori* adheres to normal gastric mucosal cells and does not seem to adhere to intestinalised cells, therefore the incidence of *H pylori* infection as determined by biopsy methods decreases with progressing preneoplastic lesions.[17] This was seen in our study. Research

assessing serum antibody concentrations of subjects with these lesions would suggest that the subjects have probably been infected with *H pylori* at an earlier stage in the progression.[18]

Previous studies have assessed gastric epithelial cell proliferation in tissue adjacent to and remote from gastric carcinoma and found it increased compared with normal mucosa using the BrdU immunohistochemical technique.[19] Our study also identified an increased epithelial cell proliferation associated with gastric carcinoma. This increased epithelial cell proliferation was not related to the histological type of gastric carcinoma, intestinal or diffuse. Epidemiological studies have suggested that the intestinal type carcinoma is more influenced by environmental factors than diffuse type carcinoma, which is thought in part to be familial or genetic.[20 21] The role of *H pylori* in these types of gastric carcinoma is controversial, however, recent studies have identified *H pylori* as a risk factor for both types of gastric carcinoma.[18 22 23]

Few studies have assessed epithelial cell kinetics in the progression from normal mucosa to gastric carcinoma. Two studies have identified an increased epithelial cell proliferation associated with atrophic gastric mucosa using the tritiated thymidine technique.[24 25] These studies, however, did not assess the influence of *H pylori* infection on this changed epithelial cell proliferation. Our study assessed gastric epithelial cell kinetics at all stages in the progression from normal mucosa to gastric carcinoma in a large series of patients and identified an increased epithelial cell proliferation associated with gastric atrophy, intestinal metaplasia, and tissue remote from gastric carcinoma. This finding suggests that increased epithelial cell proliferation is one of the earliest identifiable abnormalities in the development of gastric carcinoma.

This study also showed increased epithelial cell proliferation associated with *H pylori* infection compared with normal mucosa, which our group has previously described.[7] This finding has been confirmed by other groups.[26–28] *H pylori* infection was associated with an increased total epithelial cell proliferation and an increased number of proliferating cells at the apex of the gastric pit. This is significant as a recent study has shown that cells proliferating near the luminal surface are more susceptible to carcinogens than those proliferating deeper in the gastric gland.[29] The increase in gastric epithelial cell proliferation associated with *H pylori* infection was not significantly different from that associated with gastric precancerous lesions. *H pylori* infection, however, did not seem to influence the changed gastric epithelial cell proliferation in subjects with precancerous lesions or gastric cancer, which suggests that although *H pylori* has a part to play in early gastric carcinogenesis, it may not have as strong an influence in the later stages of the disease.

In conclusion this study identified increased epithelial cell proliferation associated with gastric precancerous lesions and gastric carcinoma. This was also seen in subjects with *H pylori* infection in the absence of any precancerous change. *H pylori* may be the precipitating factor that triggers the genesis of events terminating in gastric carcinoma.

1 Hallissey M, Dunn JA, Ward LC, Allum WH for the British Stomach Cancer Group. The second British Stomach Cancer group trial of adjuvant radiotherapy or chemotherapy in resectable gastric cancer: five-year follow up. *Lancet* 1994; **43:** 1309–12.

2 Correa P. A human model of gastric carcinogenesis. *Cancer Res* 198; **48:** 3554–60.

3 Eurogast Study Group. An international association between Helicobacter pylori infection and gastric cancer. *Lancet* 1993; **341:** 1359–62.

4 Sasaki N, Momma K, Yamada Y, Tajima T, Shoji N, Handa N, *et al.* Helicobacter pylori and early gastric cancer: relation to atrophic gastritis in background gastric mucosa. *European Journal of Gastroenterology and Hepatology* 1993; **5:** S123–6.

5 Fiocca R, Luinetti O, Villani L, Chiaravalli A, Cornaggia M, Stella G, *et al.* High incidence of Helicobacter pylori colonisation in early gastric cancer and the possible relationship to carcinogenesis. *European Journal of Gastroenterology and Hepatology* 1993; 5 (suppl 2): S2–8.

6 Gilvarry J, Leen E, Sant S, Sweeney E, O'Morain C. The long-term effect of Helicobacter pylori on gastric mucosa. *European Journal of Gastroenterology and Hepatology* 1994; **6:** 43–5.

7 Cahill RJ, Sant S, Beattie S, Hamilton H, O'Morain C. Helicobacter pylori and increased epithelial cell proliferation: a risk factor for cancer. *European Journal of Gastroenterology and Hepatology* 1994; **6:** 1123–7.

8 Cahill RJ, Xia H, Kilgallen C, Beattie S, Hamilton H, O'Morian CA. Effect of eradication of Helicobacter pylori infection on gastric epithelial cell proliferation. *Dig Dis Sci* 1995; **40:** 1627–31.

9 Lipkin M. Biomarkers of increased susceptibility to gastrointestinal cancer: new application to studies of cancer prevention in human subjects. *Cancer Res* 1988; **48:** 235–45.

10 Lauren P. The two histological types of gastric carcinoma: diffuse and so-called intestinal type carcinoma. *Acta Pathol Microbiol Scand* 1965; **64:** 31–43.

11 Goseki N, Takizawa T, Koike M. Differences in the mode of the extension of gastric cancer classified by histological type: a new histological classification of gastric carcinoma. *Gut* 1992; **33:** 606–12.

12 Cahill R, O'Sullivan KR, Mathias PM, Beattie S, Hamilton H, O'Morain C. Effects of vitamin antioxidant supplementation on cell kinetics of patients with adenomatous polyps. *Gut* 1993; **34:** 963–7.

13 Lacy R, Kuwayama H, Cowart KS, King JS, Deutz AH, Sistrunk S. A rapid accurate immunohistochemical method to label proliferating cells in the digestive tract. A comparison with tritiated thymidine. *Gastroenterology* 1991; **100:** 259–62.

14 Tsujii M, Kawano S, Tsujii S, Ito T, Nagano K, Sasaki Y, *et al.* Cell kinetics of mucosal atrophy in rat stomach induced by long-term administration of ammonia. *Gastroenterology* 1993; **104:** 796–801.

15 Fox J, Correa P, Taylor NS, Zavala D, Fontham E, Janney F, *et al.* Campylobacter pylori-associated gastritis and immune response in a population at increased risk of gastric carcinoma. *Am J Gastroenterol* 1989; **84:** 775–81.

16 Clarkson KS, West KP. Gastric cancer and Helicobacter pylori infection. *J Clin Pathol* 1993; **46:** 997–9.

17 Loffield R, Willems L, Flendrig JA, Arends JW. Helicobacter pylori and gastric cancer. *Histopathology* 1990; **17:** 537–41.

18 Asaka M, Kimura T, Kato M, Kudo M, Miki K, Ogoshi K, *et al.* Possible role of Helicobacter pylori in early gastric cancer development. *Cancer* 1994; **73:** 2691–4.

19 Britio M, Filipe MI, Morris RW. Cell proliferation study on gastric carcinoma and non-involved gastric mucosa using a bromodeoxyuridine (BrdU) labelling technique. *Eur J Cancer Prev* 1992; **1:** 429–35.

20 Munoz N, Correa P, Cuello C, Duque E. Histologic types of gastric cancer in high- and low-risk area. *Int J Cancer* 1968; **3:** 809–18.

21 Correa P, Haenzel W. Epidemiology of gastric cancer. In: Correa P, Haenzel W, eds. *Epidemiology of cancer of the digestive tract.* The Hague, the Netherlands: Martinus Nijhoff, 1982: 58–84.

22 Parsonnett J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, *et al.* Helicobacter pylori infection and the risk of gastric cancer. *N Engl J Med* 1991; **325:** 1127–31.

23 Nomura A, Stemmermann GN, Heilbrun LK, Salkeld RM, Vuilleumier HP. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325:** 1132–6.

24 Deschner E, Winawer SJ, Lipkin HT. Patterns of nucleic acid and protein synthesis in normal human gastric mucosa and atrophic gastritis. *J Natl Cancer Inst USA* 1972; **48:** 1567–74.

25 Biasco G, Paganelli GM, Brillantti S, Lalli AA, Brandi G, Terranova A, *et al.* Cell renewal and cancer risk of the

stomach: analysis of cell proliferation kinetics in atrophic gastritis. *Acta Gastroenterolog Belg* 1989; **52:** 361–6.
26 Brenes F, Ruiz B, Correa P, Hunter F, Khamakrishnan T, Fontham E, *et al.* Helicobacter pylori causes hyperproliferation of the gastric epithelium: pre and post eradication indices of proliferating cell nuclear antigen. *Am J Gastroenterol* 1993; **88:** 1870–5.
27 Lynch DAF, Mapstone NP, Clarke AMT, Sobala GM, Jackson P, Morrison L, *et al.* Cell proliferation in Helicobacter pylori associated gastritis and the long-term effect of eradication therapy [Abstract]. *Gut* 1994; **35** (suppl 2): S4.
28 De Koster E, Buset M, Fernandes E, Martin N, De Reuck M, Deprez C, *et al.* Influence of Hp and gastritis on gastric antum and corpus mucosal cell proliferation [Abstract]. *Acta Gastroenterolog Belg* 1993; **56:** 61.
29 Sørbye H, Kvinnsland S, Svanes K. Effect of salt induced mucosal damage and healing on penetration of N-methyl-N-nitrosoguanidine to proliferative cells in the gastric mucosa of rats. *Carcinogenesis* 1994; **15:** 673–9.