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Cell Proliferation Kinetics in the Gastric Remnant*

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Abstract—*Biopsy specimens of the gastroenterostomal area in 60 long-standing asymptomatic postgastrectomy patients and of the mid-corpus region in five normal controls were cultured in [^{3}H]-TdR. Using radioautography, thymidine incorporation rate (TIR) and cell position of the labelled cells were scored. In the normal gastric mucosa cell proliferation was limited to the progenitor cell region. TIR of the stomal area was higher compared to the normal mucosa. In addition, an upward shift of the proliferative compartment towards the luminal surface was observed. In severe dysplasia an expanded pool of DNA-synthesizing cells was present in the uppermost layers of the gastric mucosa, but even in the surrounding non-dysplastic mucosa an upward shift of labelled cells could be demonstrated. These proliferative changes are considered to be compatible with increased cancer risk. The sequence of proliferative events suggests that early cancer formation in the gastric remnant takes place in the superficial mucosa and therefore is easily amenable to endoscopic biopsy.*

INTRODUCTION

PREMALIGNANT lesions manifest an abnormal proliferative profile. Lipkin *et al.* have demonstrated this for the colon [1] and there are some reports which suggest that the same is true for the premalignant gastric mucosa [2-8].

The operated stomach is considered a premalignant condition [9]. In order to learn more about the proliferative characteristics and the kinetic behaviour of the premalignant gastric mucosa, cell kinetics were studied in 60 long-standing asymptomatic postgastrectomy patients and five normal controls.

MATERIALS AND METHODS

In all the postgastrectomy patients a Billroth II resection had been performed for benign disease between 1931 and 1960, the mean postoperative interval being 24.1 yr (range 15-38 yr). After 8-12 hr of fasting 1-3 forceps biopsies were taken from the gastric side of the gastroenterostomal area in the 60 postgastrectomy patients and from corresponding levels of the mid-corpus region in the five normal controls during endoscopy with a forward viewing or 70° fibrescope. The specimens were immediately mounted on a piece of mesh [10] and the well-oriented biopsies were placed in a shaking waterbath at 37°C in flasks containing 3 ml of Hanks' Balanced Salts with 10% foetal calf serum and 25 μCi tritiated thymidine ([^{3}H]-TdR; Radiochemical Centre, Amersham, U.K.; sp. act. 29 Ci/mmol). The pH was adjusted to 7.2-7.4 with a 5% sodium bicarbonate solution and a constant carbogen flow (2 l/min; 95% O_2, 5% CO_2).

After 1 hr of incubation the tissue fragments were fixed in 10% buffered formalin for 24 hr, dehydrated and embedded in paraffin. Serial 4-μm sections were cut. The first four full-thickness sections were placed on a slide for radioautography; the following four sections were placed on a slide and stained with haematoxylin and eosin for histopathological examination and the next four sections again were used for radioautography. The same procedure was repeated half-way through the biopsy specimen. The slides used for radioautography were stained with periodic acid Schiff, dipped in Ilford G-5 emulsion and exposed for 4 weeks at 4°C. Finally the slides were developed with Kodak D19b, fixed with a 24% sodium thiosulphate solution and counterstained with haematoxylin. All the slides

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for histopathological examination were read blindly by the same experienced pathologist. The criteria for histological diagnosis and grading were derived from the definitions and descriptions of Oehlert [11].

For the radioautographic analysis only longitudinally sectioned gastric pits were used. The proliferative parameters were determined in each patient in at least 50 gastric pits at various sites of the biopsy specimen. All labelled cells per individual gastric pit were counted. The thymidine incorporation rate (TIR) was defined as the ratio between the number of labelled cells and the number of pits. The cell position of the labelled cells was determined taking as reference position ($R = 0$) the limit between the last foveolar cell and the first specialized glandular cell. Starting from this limit, the cell located just above this point was termed cell position +1, the next cell +2, etc. Downwards, the positions were termed -1, -2, etc. [12]. After subdividing the biopsies into various subclasses depending upon the dominant histological features, the individual cell position data were pooled and represented in a collective frequency histogram. In the collective frequency histogram the labelled cells were recorded as a function of their cell position, the spatial distribution of the labelled cells being expressed in percentages of the total number of labelled cells, while the TIR is the parameter for the mean number of labelled cells per gastric pit. All TIR results were expressed as mean ± S.D. The data were analysed by Student's t test. A P value of <0.05 was considered statistically significant.

RESULTS

In the five normal controls 421 labelled cells were counted in 569 gastric pits. The mean TIR was 0.74 ± 0.50. The spatial distribution of the labelled cells is represented in Figs 1-3.

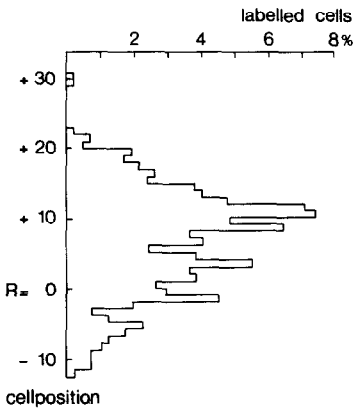

Fig. 2. Histogram of the spatial distribution of the labelled cells in the normal gastric mucosa.

In 23 postgastrectomy patients with gastritis 5563 labelled cells were counted in 1201 gastric pits. The mean TIR was 4.63 ± 3.24. The spatial distribution of the labelled cells is represented in Fig. 4.

In 26 postgastrectomy patients with foveolar hyperplasia, pseudopyloric metaplasia and cystic dilatation 12,332 labelled cells were counted in 1519 gastric pits. The mean TIR was 8.12 ± 5.46. The distribution of the labelled cells is represented in Fig. 5.

In two patients with severe dysplasia 1664 labelled cells were counted in 127 pits. The mean TIR was 13.12 ± 4.91. The distribution of the labelled cells is represented in Figs 6 and 7.

In the same two patients in 118 non-dysplastic pits surrounding the area of severe dysplasis 1416 labelled cells were counted. The mean TIR was 12.00 ± 2.47. The distribution of the labelled cells in these non-dysplastic pits is represented in Figs 8 and 9.

The differences between TIRs as measured in the various groups were significant (Student's t test; $P < 0.05$), the group of postgastrectomy patients with severe dysplasia being too small for statistical analysis.

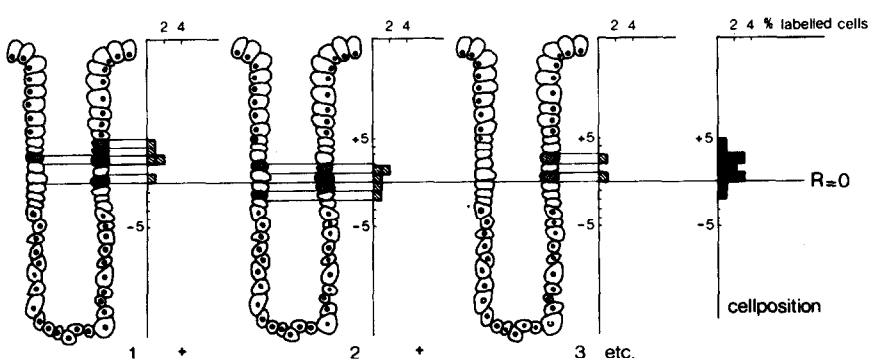

Fig. 1. Example of radioautographic analysis.

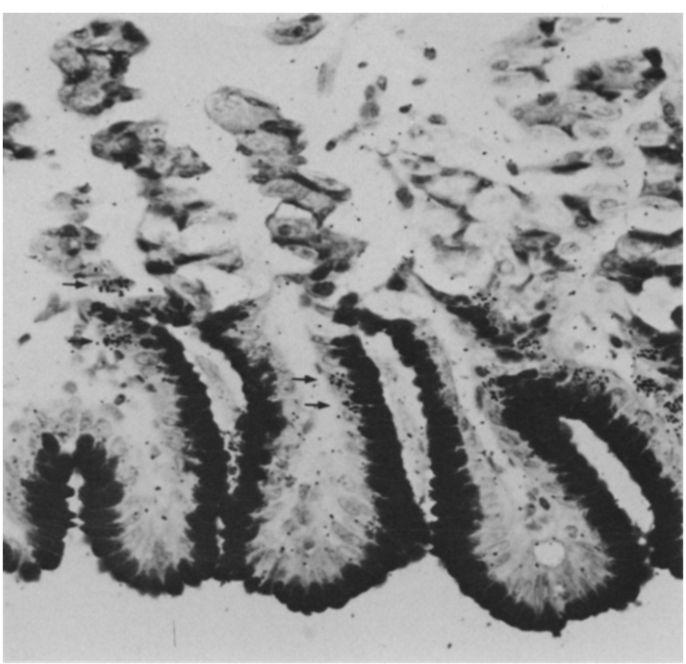

Fig. 3. Labelled cells in the normal gastric mucosa (radioautography; P.A.S.; × 350).

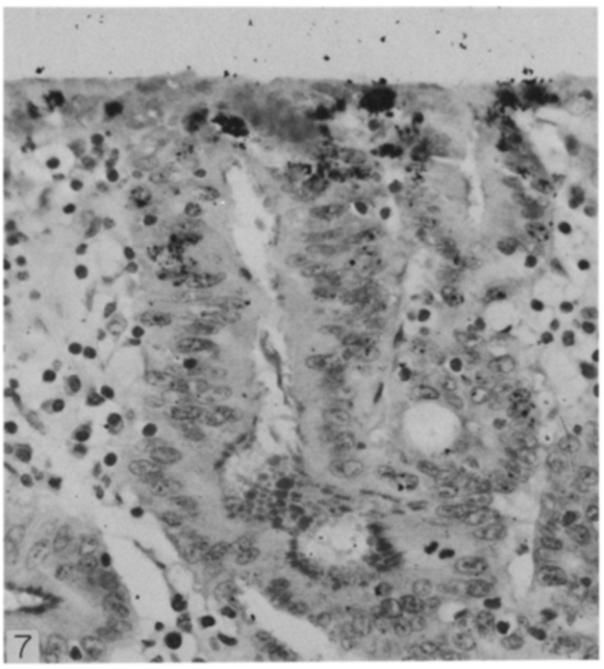

Fig. 7. Surface labelling in the dysplastic gastric mucosa (radioautography; P.A.S.; ×350).

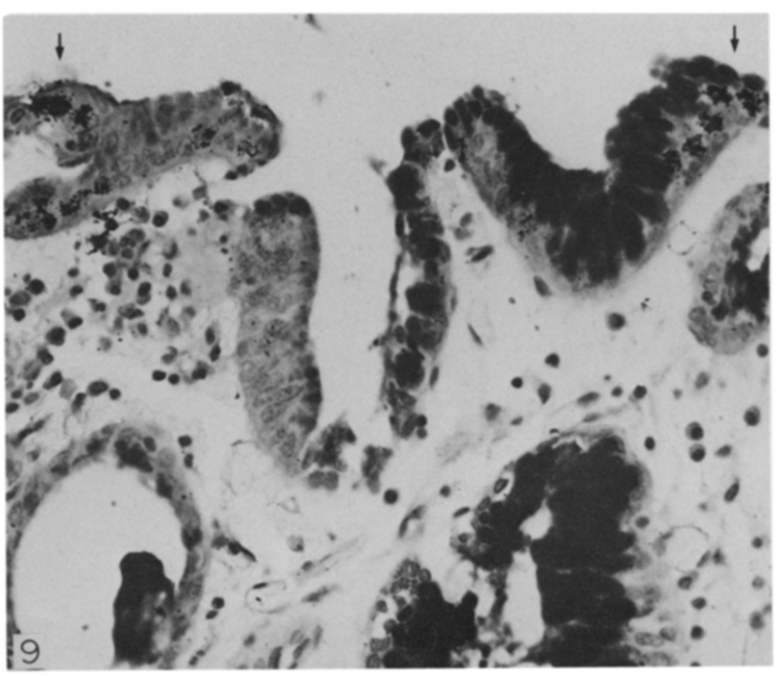

Fig. 9. Transition of dysplastic (right) to non-dysplastic (left) gastric mucosa with surface labelling at both sides (radioautography: P.A.S.; ×350).

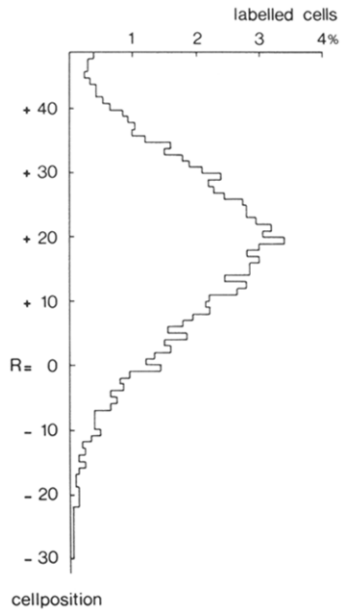

Fig. 4. Histogram of the spatial distribution of the labelled cells in the postgastrectomy patients with gastritis.

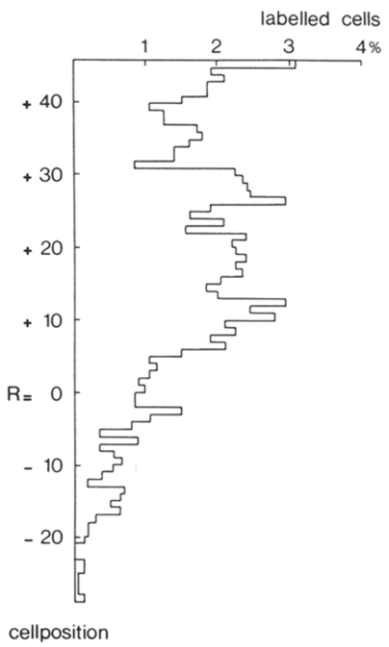

Fig. 5. Histogram of the spatial distribution of the labelled cells in the postgastrectomy patients with foveolar hyperplasia, pseudopyloric metaplasia and cystic dilatation.

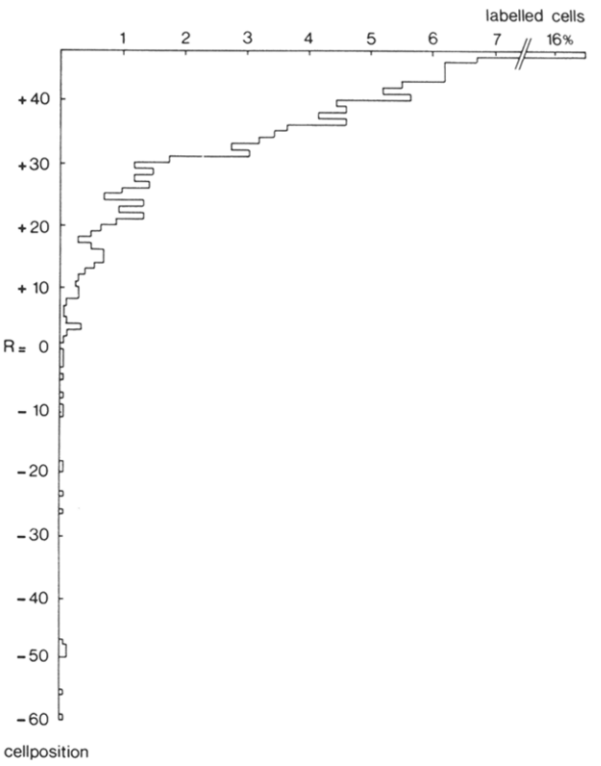

Fig. 6. Histogram of the spatial distribution of the labelled cells in severe dysplasia.

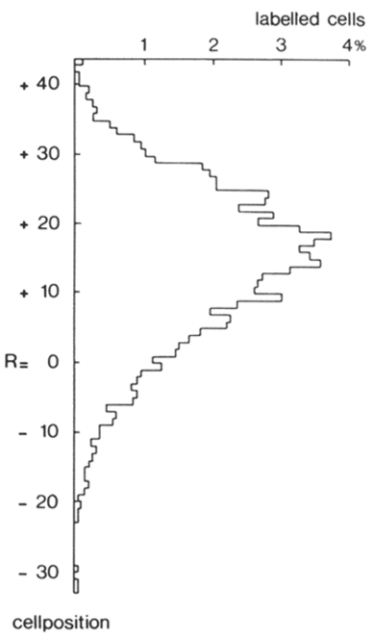

Fig. 8. Histogram of the spatial distribution of the labelled cells in the non-dysplastic gastric pits surrounding the area of severe dysplasia.

In two patients with a proven early stump carcinoma biopsy specimens could be taken for cell kinetics before a total gastrectomy was performed. In patient 1, in 289 non-suspicious gastric pits 3173 labelled cells were counted, the TIR being 10.98. In patient 2, in 75 non-suspicious pits 1044 labelled cells were counted, the TIR being 13.98. The histograms representing the localization of the labelled cells in both of these patients were comparable with Fig. 8 and showed an identical upward migration of the proliferative zone. Radioautography of a micro-invasive stump carcinoma in one of the incubated biopsies of another patient showed extensive surface labelling of the malignant cells. Exact recording of the labelled cells in this case was, of course, impossible, nor were the incubated tissue fragments suitable for adequate radioautographic analysis in six other patients.

DISCUSSION

The results of our study demonstrate that the number of labelled cells in the stomal area of the gastric remnant is higher than in the corresponding mid-corpus region of the normal gastric mucosa. The pool of DNA-synthesizing cells increases in parallel with the various histological alterations. In addition, we have shown an expansion and upward shift of the proliferative compartment. In severe dysplasia most DNA-synthesizing cells were encountered in the uppermost layers of the gastric mucosa.

Other authors have also observed an increased pool of labelled cells following gastric operation [2, 7]. Willems [13] reported DNA-derepression in B11-postgastrectomy patients and considered this to be related to increased cancer risk. Indeed, the malignant tendency of identical proliferative patterns in the premalignant colon has been clearly established [1].

Exogenous factors are probably of major importance in causing cancer in the gastric remnant [14]. The fact that most patients with cancer in the gastric remnant were operated for duodenal ulcer disease, a lesion that carries a low risk for gastric cancer when surgery is avoided [15], is a strong argument for the exogenous etiology of stump cancer. Reflux, achylia, abnormal bacterial flora, disturbed motility, lost antrum function, destruction of the gastric mucosal barrier and nitrosamines seem to play an important role [14, 16–20]. Nitroso compounds may cause a series of mutations and cell transformations which, after a latency of several years, ultimately lead to invasive malignancy in a number of cases [21, 22]. It is well known that especially DNA-synthesizing cells are susceptible for such mutations [21, 23]. In the operated stomach carcinogenic factors will impact most strongly upon the superficial gastric mucosa. Therefore it is reasonable to hypothesize that expansion and upward migration of the pool of DNA-synthesizing cells is attended with a considerable chance of relevant carcinogenic hits and reflects increased cancer risk.

Not only in severe dysplasia, but even in the surrounding histologically non-dysplastic-looking mucosa, could a remarkable upward shift of labelled cells be demonstrated. As in the colon, such observation might be helpful in predicting increased cancer risk [1, 13].

The results of this cell kinetic study also suggest that early cancer formation takes place in the surface epithelium. Presumably DNA-derepression starts in the progenitor cell region and extends from there to the luminal surface. Via successive histological abnormalities, it may result in the ultimate development of superficial spreading carcinoma (Fig. 10). Where the point of no return is located in this chain of events remains unknown. Because we never have observed regression of severe dysplasia, we consider this histological alteration as an irreversible premalignant lesion demanding close follow-up.

The above hypothetical model regarding the genesis of stump cancer is in fact comparable with the corresponding hypothesis related to the histogenesis of the intestinal type of gastric cancer in the non-operated stomach [23, 24]. In the etiology of this type of gastric cancer exogenous factors, especially nitroso compounds, also seem to play an essential role [22]. When, indeed, early cancer formation in the gastric remnant takes place in the superficial mucosa, this has also practical relevance. Surface carcinoma is easily amenable to endoscopic biopsy. Besides, it may remain limited to the superficial gastric layers for a rather long period of time [25]. Also, for this reason early detection would be possible with the existing endoscopic biopsy techniques.

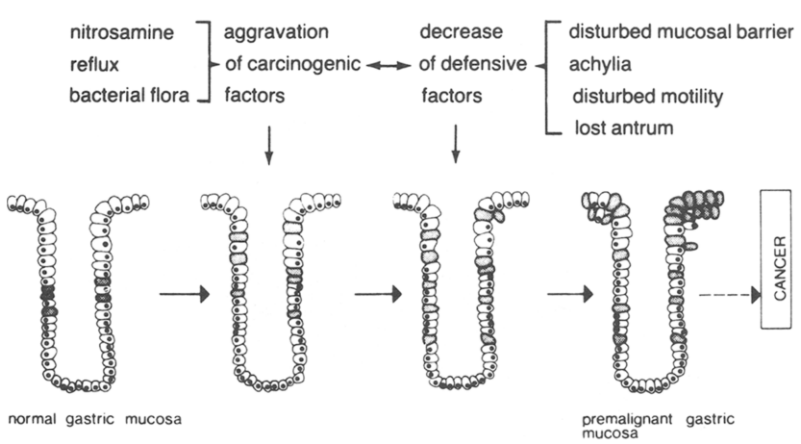

Fig. 10. Cell kinetic findings summarized in diagram; histogenesis of stump cancer (labelled cells are shaded).

REFERENCES

1. Lipkin M, Blattner WE, Fraumeni JF, Lynch HT, Deschner E, Winawer S. Tritiated thymidine labeling distribution as a marker for hereditary predisposition to colon cancer. *Cancer Res* 1983, **43**, 1899-1904.
2. Assad RT, Eastwood GL. Epithelial proliferation in human fundic mucosa after antrectomy and vagotomy. *Gastroenterology* 1980, **79**, 807-811.
3. Bell B, Almy TP, Lipkin M. Cell proliferation kinetic in the gastrointestinal tract of man. III. Cell renewal in esophagus, stomach and jejunum of a patient with treated pernicious anemia. *JNCI* 1967, **38**, 615-628.
4. Deschner EE, Winawer SJ, Lipkin M. Patterns of nucleic acid and protein synthesis in normal human gastric mucosa and atrophic gastritis. *JNCI* 1972, **48**, 1567-1574.
5. Deschner EE, Tamura K, Bralow SP. Early proliferative changes in rat pyloric mucosa induced with MNNG. *Front Gastrointest Res*, 1979, **4**, 25-31.
6. Deschner EE. Cell renewal in precancerous and tumour states of the gastrointestinal mucosa. XVIIth International Congress of the SMIER. *Acta Endosc* 1980, **X**, 9.
7. Hart Hansen O, Larsen JK, Svendsen LB. Changes in gastric mucosal cell proliferation after antrectomy or vagotomy in man. *Scand J Gastroenterol* 1978, **13**, 947-952.
8. Willems G, Bleiberg H. Proliferative changes in the gastric mucosa of patients with pernicious anemia. In: Gerard A, ed. *Gastrointestinal Tumors, a Clinical and Experimental Approach*. Oxford, Pergamon Press, 1979, 39.
9. Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T, SerckHanssen A. Precancerous conditions and epithelial dysplasia in the stomach. *J Clin Pathol* 1980, **33**, 711-721.
10. Rubin CE, Brandborg LL, Phelps PC, Tailor I HC. The apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. *Gastroenterology* 1960, **38**, 28-49.
11. Oehlert W. *Klinische Pathologie des Magen-Darm-Traktes. Histologische Diagnose und Differentialdiagnose am gastroenterologischen Biopsiematerial*. Stuttgart, Shattauer Verlag, 1978, 45-89.
12. Willems G, Vansteenkiste Y, Verbeustel S. Autoradiographic study of cell renewal in fundic mucosa of fasting dogs. *Acta Anat (Basel)* 1971, **80**, 23-32.
13. Willems G. Cell renewal in the alimentary tract. *Rev Esp Enferm Apar Digest* 1978, 135-154.
14. Dahm K, Rehner M. *Das Karzinom im operierten Magen*. Stuttgart. Thieme Verlag, 1975.
15. Burns GP, Taubman J. The association of gastric carcinoma with duodenal ulcer. *Br J Surg* 1967, **54**, 174-176.
16. van Heerden JA, Priestley JI, Farrow GM, Phillips SF. Postoperative alkaline reflux gastritis, surgical implications. *Am J Surg* 1969, **118**, 427-33.
17. Kowalewski K. Relationship between vagotomy, peptic ulcer and gastric adenocarcinoma in rats fed 2.7-diacetylaminofluorene. *Can J Surg* 1973, **16**, 210-217.
18. Du Plessis DJ. Gastric mucosal changes after operations on the stomach. *S Afr Med J* 1962, **36**, 471-478.
19. Schlag P, Meister H, Feyerabend G, Merkle P. Der Einfluss der duodeno-gastrischen Refluxes auf das Epithel an der gastroenteralen Anastomose. *Langenbecks Arch Chir* 1977, **344**, 207-17.
20. Schlag P, Ulrich H, Merkle P, Böckler R, Perer M, Herfath Ch. Are nitrite and *n*-nitroso compounds in gastric juice risk factors for carcinoma in the operated stomach. *Lancet* 1980, **ii**, 727-729.
21. Weisburger JH, Wynder EL, Horn CL. Nutritional factors and etiologic mechanisms in the causation of gastrointestinal cancers. *Cancer* 1982, **50**, 2541-2549.
22. Correa P. Precursors of gastric and esophageal cancer. *Cancer* 1982, **50**, 2554-2565.
23. Kunze E, Schauer A, Eder M, Seefeldt C. Early sequential lesions during development of experimental gastric cancer with special reference to dysplasias. *J Cancer Res Clin Oncol* 1979, **95**, 247-264.
24. Grundmann E. Histologic types and possible initial stages in early gastric cancer. *Beitr Pathol* 1975, **154**, 256-280.
25. Fujita S. Biology of early gastric cancer. *Pathol Res Pract* 1978, **163**, 297-309.