Br. J. exp. Path. (1978) **59**, 229

EPITHELIAL REPAIR IN CHRONIC GASTRIC ULCERS

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Received for publication December 6, 1977

Summary.—Features of epithelial repair, namely mitotic activity, cell migration, and cell differentiation have been investigated in 17 mucosal biopsy samples from the edges of chronic gastric ulcers in 16 patients, using light and electron microscopy. Results have shown that mitosis persists at the margins of these lesions and that mitotic figures are significantly more numerous in the region of chronic ulcers than in normal mucosa. Cells with the ultrastructural features of migratory cells are present but adjacent epithelium shows abnormalities of differentiation. Lack of cell production does not appear to be the limiting factor in epithelialization and some other cause such as an abnormality of cell adhesion or re-establishment must be sought.

CHRONIC GASTRIC ULCERS remain a source of mortality and morbidity. The true incidence is difficult to assess accurately and most figures reflect the frequency of lethal complications. The Registrar General's Statistical Survey for England and Wales (1973) gives mortality figures of 937 males and 803 females. The incidence of complications has been estimated at 20% and although medical treatment may induce healing, subsequent recurrence rates may be as high as 80% (Kukral, 1968).

Although relatively uncommon before the 19th century, gastric ulcers were recognized by the Greek physician Diocles Carystius as long ago as 350 B.C. Marcellus Donatus of Mantua gave the first necropsy description in 1586 and Matthew Baillie is credited with the first accurate anatomical and pathological account in 1799 (Olch and Harkins, 1969). The 19th century was notable for the relative frequency of gastric ulcers in young women and for some of the early theories of aetiology, *e.g.* the role of erosions, of gastritis, of vascular embolism and infarction, of infection and of hyperchlorhydria (Cook, 1946). Current ideas are based on the two concepts of the ulcerogenic role of acid and pepsin and the protective mucosal mechanisms (du Plessis, 1975). The 25% of gastric ulcers which are either prepyloric or associated with pyloric stenosis of duodenal ulcer origin are associated with hyperacidity (Johnson, 1957). Some claim that the remaining 75% are also related to hypersecretion of gastric juice, due in this case to gastric stasis and consequent increased gastrin release (Dragstedt, 1969). However, gastric acidity is usually low in these patients and others suggest that a primary abnormality in mucosal defence exists. This defect has been attributed to duodenal reflux of bile (Capper, 1967) which reduces mucus viscosity, alters ionic movement across the epithelium (Davenport, 1968), and leads to epithelial damage with changes of chronic gastritis. Oi *et al.* (1969) have related the characteristic lesser curve site of ulcers to mucosal stresses set up by underyling muscle bundles and to change in mucosal type. The role of gastric antibodies is uncertain (Irvine, 1968) and the part played by gastric fibrinolysins speculative (Cox, Poller and Thomson, 1967).

Epithelial renewal plays an important part in maintaining mucosal integrity, and once ulceration has occurred local mitotic

activity, cell migration and cell re-establishment with differentiation are key events in epithelial repair. These processes have been inadequately investigated in chronic gastric ulcers and since a theoretical cause of indolence lies in their failure, they have been further studied with the aid of light and electron microscopy.

MATERIALS AND METHODS

Fresh biopsy samples were taken either at gastroscopy or at operation from 16 patients with chronic gastric ulcers, one patient having 2 biopsy samples taken at an interval of 2 weeks. The clinical details of these patients are shown in the table. In the absence of histological material from the ulcer bed, chronicity was judged on the gastroscopic or naked-eye appearances of a circular clear-cut lesion with sloughing base and a diameter of more than 0·5 cm. Most patients had a clinical history of more than 3 months and no ulcer was obviously in a healing phase as judged by history and appearances. Gastroscopic specimens were obtained under direct vision with an Olympus G1FK end-oblique viewing endoscope and all biopsy samples were taken from the ulcer edge, preferably with target-type forceps. These specimens were immediately orientated mucosa uppermost on ground-glass slides and placed in 4% methanol-free buffered formalin at 4°. Surgical specimens were taken by removing a 4 mm cube of tissue from the ulcer edge once the gastrectomy specimen had been removed and fixing this within 2 min of the resected area being clamped. Control material was taken from the pyloric regions of 7 patients with upper abdominal symptoms but normal oesophagus, stomach and duodenum at endoscopy, negative barium meal X-rays, and no histological abnormality on section. Biopsy samples were taken between 1200 and 1600 h.

After 24 h fixation, blocks were removed for electron microscopy by bisecting the gastroscopy specimens and cutting a 1 mm thick slice from surgical biopsy samples to include the ulcer edge. These specimens were transferred to buffer wash (sodium cacodylate) before post-fixation in osmium tetroxide, embedding in Araldite with the aid of propylene oxide as link reagent, and sectioning on an LKB Ultratome. Thin sections were stained with uranyl acetate and lead citrate and examined in an AEI Corinth 275 transmission electron microscope. The remaining material was embedded in paraffin wax and serially sectioned at 7 μm before staining with haematoxylin and eosin (H. &. E) or PAS/Alcian Blue.

Mitotic counts were performed on well orientated sections, leaving at least 2 sections between those counted. In such sections the glands could be followed from the muscularis mucosa to the surface and pits were cut longitudinally. All 4 phases of mitosis (prophase, metaphase, anaphase and telophase) were counted and plotted as to their exact position in the gland, in a total of 48 glands in each biopsy sample. The results were expressed as mitoses per 1000 total epithelial cells and statistical analysis was made by means of the chi-squared test based on median values of counts and corrected for small samples.

TABLE.—*Clinical Details of* 16 *Patients with Chronic Gastric Ulcers*

Patient	Age	Sex	Site of ulcer	Approximate diameter in cm	Length of history	Mitoses per 1000 cells
J.L.	56	M	Prepyloric	0·5	6 months	5·6
E.N.	54	M	Prepyloric	0·6	1 month	4·1
C.D.	30	F	Prepyloric	0·75	2½ years	2·8
S.W.	45	F	Lesser curve	1·0	10 years	4·2
S.W.	45	F	Lesser curve	1·0	10 years	5·5
L.F.	82	F	Lesser curve	1·5	4 months	4·7
J.G.	78	M	Lesser curve	1·5	"some years"	11·8
L.L.	58	M	Lesser curve	1·5	4 months	9·3
A.C.	67	F	Lesser curve	3·0	6 months	4·1
A.B.	71	M	Lesser curve	1·0	1 year	3·2
C.B.	56	M	Lesser curve	5·0	1 year	3·3
M.M.	57	F	Lesser curve	1·0	6 months	3·9
C.J.	77	F	Anterior lesser curve	1·0	"some years"	6·3
E.W.	77	F	Anterior lesser curve	1·0	"months"	6·9
R.D.	50	M	Posterior lesser curve	0·75	3 years	3·7
S.S.	65	M	Posterior lesser curve	1·0	2 years	5·8
D.G.	63	F	Greater curve	1·0	3 years	7·0

Mean age of ulcer patients 62 years.
Mean age of control patients 42 years.

RESULTS

All biopsy samples were characterized by an increase in gland height as judged by cell population and a fibro-purulent exudate overlying necrotic debris and mature granulation tissue on the ulcer surface. Migrating cells were usually difficult to identify with the light microscope. Two specimens showed foci of intestinal metaplasia and these were excluded from statistical analysis.

Mitotic activity

Mitotic counts for ulcer biopsy specimens were in general higher than those from normal stomachs (Fig. 1) and the median value of counts from ulcer specimens was significantly higher than that from normal mucosa ($P < 0{\cdot}005$). Mitoses were commonly seen in the glands immediately adjacent to the ulcer margin (Figs. 2 and 3). In normal mucosa mitoses were predominantly seen in the base of the gastric pits, with some activity in the pyloric glands but rare mitotic figures in surface cells. In ulcer specimens mitoses were seen in similar positions but dividing cells were common in surface epithelium. Mitoses were not seen in migrating cells.

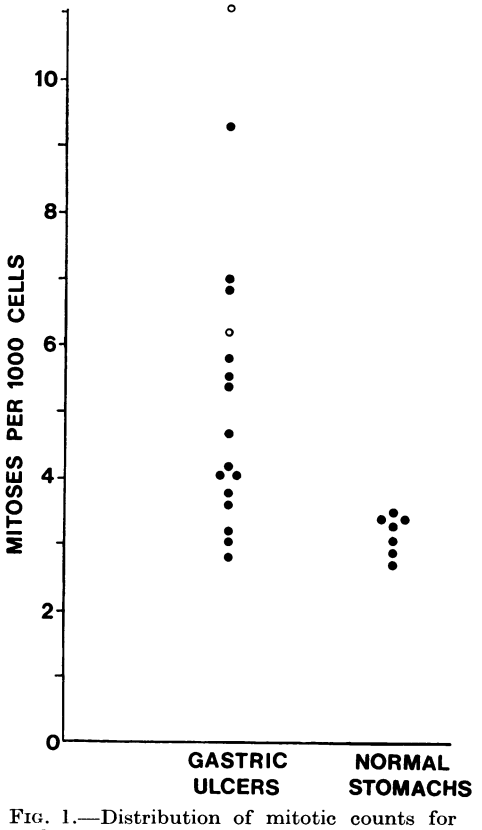

FIG. 1.—Distribution of mitotic counts for ulcer biopsy samples and normal gastric mucosa. The median value of counts for ulcer specimens is significantly higher than that for normal stomachs ($P < 0{\cdot}005$). ○ = biopsy samples showing intestinal metaplasia.

Cell migration and other features

Although evidence of cell migration was not usually obvious with the light microscope, electron microscopy confirmed the presence of such cells. These were recognized by absence of adjacent basal lamina, change in cell shape from being generally columnar to cuboidal and flat, a less complex plasma membrane, and relative decrease or absence of mucin granules (Fig. 4). Cells at the tip of the migrating layer often showed evidence of damage with mitochondrial swelling, vacuole formation, and myelin bodies. Tight junctions were seen between the epithelial cells at the surface of the migrating sheet and desmosomes were present between deeper parts of the plasma membranes (Fig. 5). The cells contained mitochondria, scanty rough endoplasmic reticulum, some Golgi complexes, microfilaments (Fig. 6) and occasional heterolysosomes. Polymorphs were seen within the migrating layer. The substrate for cell migration was in the form of amorphous material and debris overlying granulation tissue containing fibroblasts, inflammatory cells, capillaries and normal collagen fibril bundles. Specific cell-substrate contacts were not identified.

The epithelium at the edges of chronic ulcers was invariably of pyloric type. Chronic gastritis of varying degree as judged by the criteria of Whitehead (1973) was seen in all biopsy samples and in 2 cases foci of intestinal metaplasia were present. PAS/Alcian-Blue staining showed PAS-positive (neutral) mucins in both

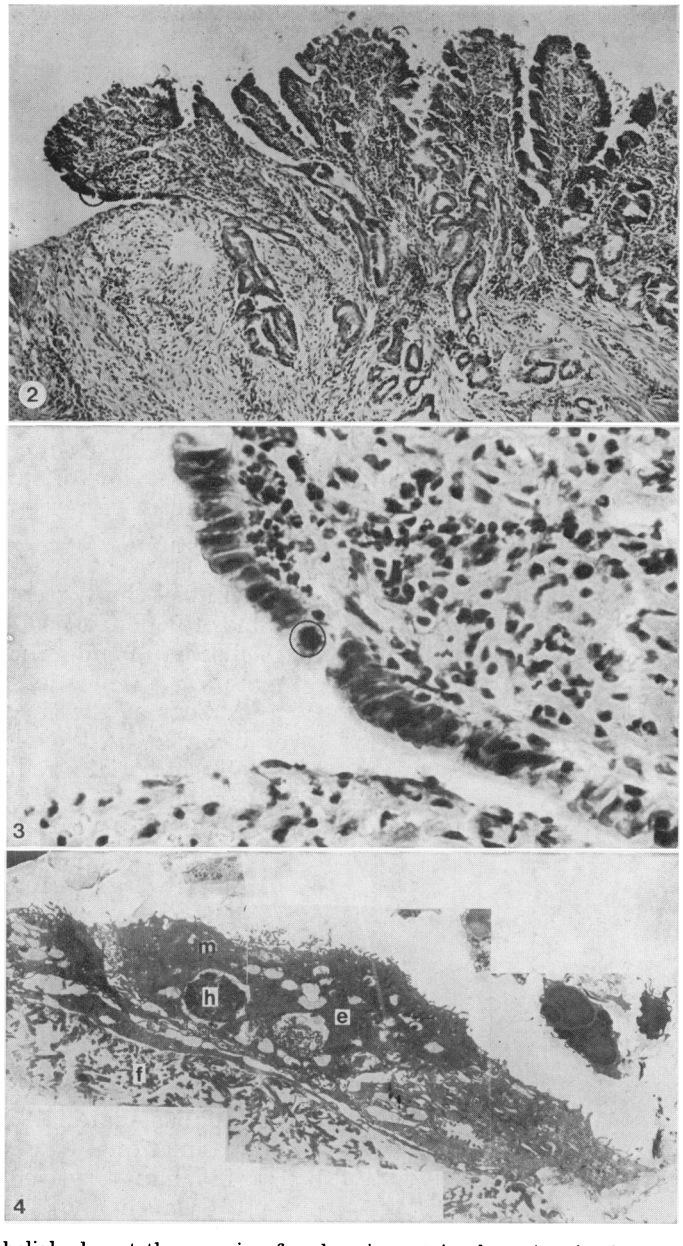

FIG. 2.—The epithelial edge at the margin of a chronic gastric ulcer. A mitotic figure in adjacent surface epithelium is encircled. H. & E. ×17.

FIG. 3.—Higher power view of the mitotic figure shown in Fig. 2. H. & E. ×146.

FIG. 4.—Electron micrograph montage of migrating epithelial cells (e) from the margin of an ulcer. Note the sparse mucin granules (m), heterolysosomes (h), and fibrin substrate (f). ×666.

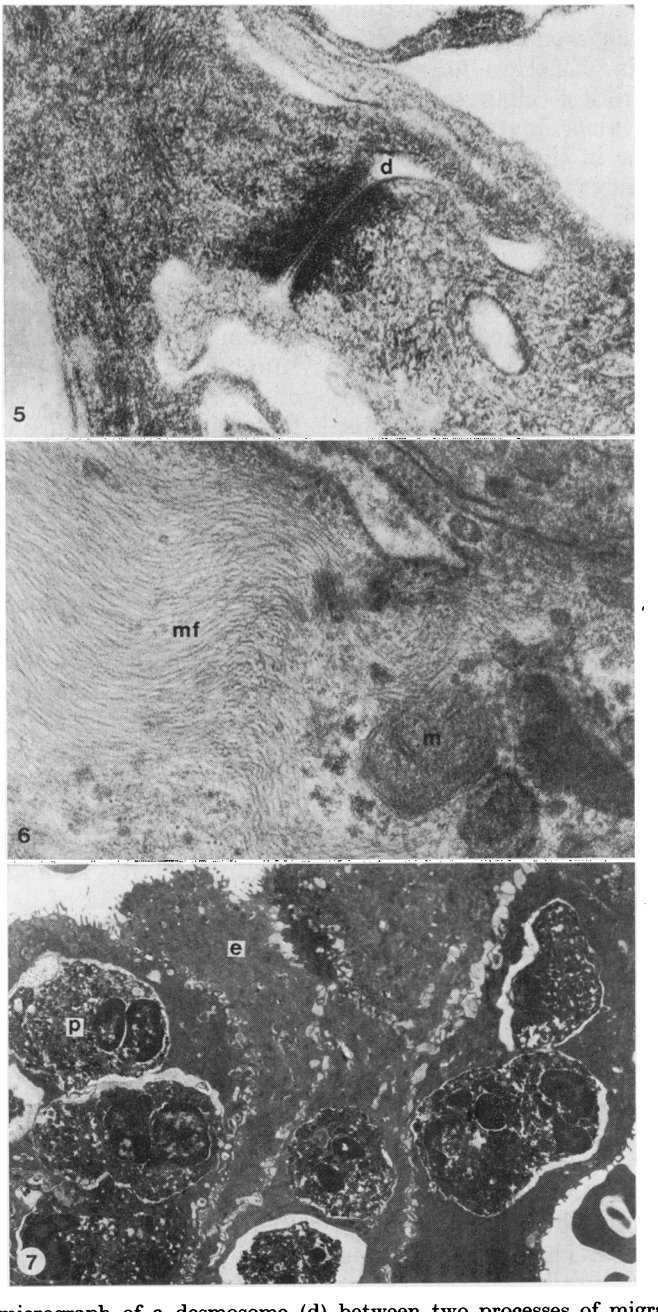

FIG. 5.—Electron micrograph of a desmosome (d) between two processes of migrating cells. The characteristic laminated structure is well shown. × 50,000.

FIG. 6.—Electron micrograph showing bundles of microfilaments (mf) within a migrating cell. A mitochondrion (m) is also visible. × 50,000.

FIG. 7.—Electron micrograph of an area of surface epithelium (e) adjacent to an ulcer margin showing many intraepithelial polymorphs (p). × 3000.

surface and gland cells. Alcian-Blue-positive mucin was seen in areas of intestinal metaplasia. Electron microscopy showed some striking changes in cell ultrastructure at ulcer margins. Surface cells were irregular in shape and size and degenerative changes were common as shown by loss of electron density and fragmentation of organelles. Desmosomes and surface junctional complexes were preserved. Mucin granules were fewer in number or absent and varied greatly in electron density. Surface microvilli were variable in size: in some cells they were stunted or absent with loss of associated glycocalyx; in other cells they were increased in height and number and the glycocalyx was preserved These latter changes were seen in cells having features of both gastric surface and intestinal cells. Polymorphs were common within the surface epithelium and were sometimes seen to be intracellular (Fig. 7). Mesenchymal abnormalities were present in the lamina propria. There was infiltration with acute and chronic inflammatory cells and a relative loss of collagen fibrils, intercellular spaces being occupied by amorphous, electron-dense granular material.

DISCUSSION

Epithelial regeneration is one of the fundamental processes in repair of gastric wounds and has been extensively studied in experimental animals in the acute situation (McMinn, 1969) but not in human chronic ulcers.

Mitotic activity

Details of mitotic activity in normal gastric mucosa have been recorded although not with particular reference to pyloric-type epithelium where ulcers invariably occur (Dawson and Morson, 1972). Lipkin and Bell (1968) quote a cell-cycle time of approximately 2 days and Willems (1972) gives a cell-cycle time of 30 h with a turnover time of 2 days, thus giving one of the most rapid renewal times of any tissue. These figures were based on labelling with ^{3}H-thymidine and therefore make comparison with the present series difficult. However, the mean mitotic index for normal mucosa of 3·2/1000 cells found here is very similar to that in the rat (Teir, Schaumann and Sundell, 1952), in which turnover time is of the same order as that described for man (Bertalanffy and Lau, 1962). Animal experiments have shown that mitotic activity is influenced by circadian rhythm, age, sex, stress, starvation and various hormonal changes. In the human stomach there appears to be a slight decrease in activity with age but no appreciable difference with sex (Suzaki, 1966).

The mitotic response to wounding has been investigated in the later stages of epithelialization in the rat's stomach (Adair, 1978) and the results have shown that mitotic activity is capable of being maintained at an increased level until epithelial continuity is re-established, even for as long as 6 weeks after wounding. Similarly Reeve (1974) has shown that in experimental colonic ulcers mitotic activity persists at the epithelial margin up to 9 months after ulcer production. Teir and Räsänen (1961) have compared mitotic activity in non-diseased portions of gastric mucosa of patients with gastric ulcer, duodenal ulcer and gastric carcinoma and have found greater activity in stomachs containing ulcers and carcinomata. Liavåg (1968) has studied 5 patients with gastric ulcers after giving Colcemid before gastrectomy, and the ulcer margins were also inspected. In 2 patients greater activity was found there than elsewhere, while in the remainder activity was lower. Persisting mitotic activity has been found at the edges of chronic venous ulcers (Adair, 1977) with a significant increase in numbers compared with normal skin. The results of the present study are compatible with these observations. Mitotic activity is still present at the edges of chronic gastric ulcers even in the gland immediately adjacent to the margin, and the median value of counts from ulcer biopsy samples was significantly greater than that from

control specimens. The increase in gland cell population and the appearance of mitotic figures in surface cells suggest that the increase in mitotic activity reflects a true increase in mitotic rate.

Cell migration and other features

Flattened migrating cells are a characteristic feature of the ulcer margin in experimental acute lesions (McMinn, 1969) and similar cells have been examined by electron microscope in the monkey (Johnson and McMinn, 1965). The ultrastructural features of migrating cells seen in this investigation are similar to those previously described. Loss of basal lamina presumably allows change in cell orientation and freer movement of the plasma membrane. Desmosomes maintain cell-to-cell continuity and no morphological abnormality was detected in these structures. Tight junctions persist at the cell surface and probably effect a seal against the external milieu, thus protecting the deeper parts of the cell. Dedifferentiation with loss of mucin granules is a regular feature of migrating cells, allows cell metabolism to be wholly directed towards movement, but of course may make the cell more vulnerable to acid/pepsin attack. The presence of microfilaments, shown to be actin-like in other cell types (Ishikawa, Bischoff and Holtzer, 1969), provides a mechanism for cell movement. Cell-substrate contacts, although identified in fibroblasts *in vitro* (Abercrombie, Heaysman and Pegrum, 1971), were not seen here and probably cell-substrate adhesion is tenuous even under favourable circumstances. Cells of migratory type are thus certainly present in the chronic situation and are capable of locomotion and cell-to-cell adhesion as judged by morphological criteria.

The neutral mucosubstances found in ulcer regions are similar to those seen and described in normal pyloric mucosa (Gad, 1969). The invariable association of gastitis with gastric ulcers is well described (Dawson and Morson, 1972) and evidence from both man (Gear, Truelove and Whitehead, 1971) and animals (Lawson, 1966) favours this as being the primary change predisposing to ulceration. The changes in mucin content and the presence of mitotic figures in surface cells at ulcer margins are evidence for incomplete cell differentiation. The finding of cell types with ultrastructural features of gastric surface cells and intestinal absorptive cells is in agreement with that of Tarpila, Telkka and Siurala (1969) and supports the idea that intestinal cells found in the stomach are truly metaplastic (Magnus, 1937). Infiltration with polymorphs, particularly if intracellular, implies inflammatory destruction of the epithelium and this may of course also apply to migrating epithelium within which polymorphs were sometimes seen. Epithelium is dependant upon mesenchyme for normal establishment and development (Grobstein, 1969) and the mesenchymal abnormalities seen at ulcer margins may be important in this respect. The epithelium at the edges of chronic ulcers is certainly unstable and this may influence re-establishment of migrating cells.

This work was carried out while I was in receipt of a Bernard Sunley Research Fellowship.

I should like to thank Professor R. M. H. McMinn for his advice and help and Mr. S. Gschmeissner, Mr R. Hutchings and technical staff of the Department of Anatomy for their assistance.

I am grateful to Professor H. Ellis of the Westiminster Hospital and Mr J. Bradbeer, Mr J. Pendower and Dr J. A. Parrish of Mayday Hospital, Thornton Heath, for allowing patients under their care to be studied.

REFERENCES

ABERCROMBIE, M., HEAYSMAN, J. E. M. & PEGRUM, S. M. (1971) The Locomotion of Fibroblasts in Culture. IV. Electron Microscopy of the Leading Lamella. *Expl. Cell Res.*, **67**, 359.

ADAIR, H. M. (1977) Epidermal Repair in Chronic Venous Ulcers. *Br. J. Surg.*, **64**, 800.

ADAIR, H. M. (1978) Epithelial Mitotic Activity in Experimental Gastric Ulcers. *J. Anat.* In Press.

BERTALANFFY, F. D. & LAU, C. (1962) Cell renewal. *Int. Rev. Cytol.*, **13**, 357.

CAPPER, W. M. (1967) Factors in the Pathogenesis of Gastric Ulcer. *Ann. R. Coll. Surg.*, **40**, 21.

COOK, D. (1946) *Ulcer*. Chicago: Medical Centre and Foundation Fund, p. 1.

COX, H. T., POLLER, L. & THOMSON, J. M. (1967) Gastric Fibrinolysis. A Possible Aetiological Link with Peptic Ulcer. *Lancet*, **1**, 1300.

DAVENPORT, H. W. (1968) Destruction of the Gastric Mucosal Barrier by Detergents and Urea. *Gastroenterology*, **54**, 175.

DAWSON, I. M. P. & MORSON, B. C. (1972) *Gastrointestinal Pathology*. Oxford: Blackwell Scientific Publications, p. 110.

DRAGSTEDT, L. R. (1969) Peptic Ulcer. An Abnormality in Gastric Secretion. *Am. J. Surg.*, **177**, 143.

DU PLESSIS, D. J. (1975) The Aetiology of Gastric and Duodenal Ulcer. In *Surgical Forum—Gastric Surgery*. Ed. Sir Rodney Smith. London: Butterworth Group, p. 1.

GAD, A. (1969) A Histochemical Study of Human Alimentary Tract Mucosubstances in Health and Disease. I. Normal and Tumours. II. Inflammatory Conditions. *Br. J. Cancer*, **23**, 52, 64.

GEAR, M. W. L., TRUELOVE, S. C. & WHITEHEAD, R. (1971) Gastric Ulcer and Gastritis. *Gut*, **12**, 639.

GROBSTEIN, C. (1969) Epithelio—Mesenchymal Interactions in Relation to Reparative Processes. In *Repair and Regeneration*, Eds J. E. Dunphy and W. Van Winkle Jr. New York: McGraw-Hill, p. 57.

IRVINE, W. J. (1968) Autoimmune and Hypersensitivity Phenomena in Alimentary Diseases. In *Recent Advances in Clinical Pathology*, Series V, Ed. S. C. Dyk, London: J. & A. Churchill, p. 497.

ISHIKAWA, H., BISCHOFF, R. & HOLTZER, H. (1969) Formation of Arrowhead Complexes with Heavy Meromyosin in a Variety of Cell Types. *J. Cell Biol.*, **43**, 312.

JOHNSON, F. R. & MCMINN, R. M. H. (1965) Wound Healing in Gastric Mucosa. In *Microscopie Electronique et Physiopathologie Digestif*, Ed. A. Claude, Brussels, p. 39.

JOHNSON, H. D. (1957) Etiology and Classification of Gastric Ulcers. *Gastroenterology*, **33**, 121.

KUKRAL, J. C. (1968) Gastric Ulcer: an Appraisal. *Surgery*, **63**, 1024.

LAWSON, H. H. (1966) Gastritis and Gastric Ulceration. *Br. J. Surg.*, **53**, 493.

LIAVÅG, I. (1968) Mitotic Activity of Gastric Mucosa. A Study by Means of Colcemid. *Acta path. microbiol. scand.*, **72**, 43.

LIPKIN, M. & BELL, B. (1968) Cell populations. In *Handbook of Physiology*, Vol. 5, Section 6, Section Ed. C. F. Code, Washington D.C.: American Physiological Society, p. 2861.

MAGNUS, H. A. (1937) Observations on the Presence of Intestinal Epithelium in the Gastric Mucosa. *J. Path.*, **44**, 389.

MCMINN, R. M. H. (1969) *Tissue Repair*, New York: Academic Press, p. 231.

OI, M., ITO, Y., KUMAGAI, F., YOSHIDA, K., TANAKA, T., YOSHIKAWA, K., MIHO, O. & KIJIMA, M. (1969) A Possible Dual Control Mechanism in the Origin of Peptic Ulcer. *Gastroenterology*, **57**, 280.

OLCH, P. D. & HARKINS, H. N. (1969) A History of Gastric Ulcer. In *Surgery of the Stomach and Duodenum*, 2nd ed., Ed. H. N. Harkins and L. M. Nyhus, London: J. & A. Churchill, p. 1.

REEVE, D. R. E. (1974) The Healing of Chronic Colonic Ulcers in the Rat. *Br. J. exp. Path.*, **55**, 533.

REGISTRAR GENERAL'S STATISTICAL REVIEW OF ENGLAND AND WALES FOR THE YEAR 1973. (1975) H.M.S.O. London, p. 94.

SUZAKI, K. (1966) Mitotic Activity and Renewal Rate of Epithelial Cells of Digestive Tract in Cancer Patients. I. Observations on the Stomach and Duodenum in the Patients with Gastric Cancer or Peptic Ulcer. *Gann.*, **57**, 453.

TARPILA, S., TELKKA, A. & SIURALA, M. (1969) Ultrastructure of Various Metaplasias of the Stomach. *Acta path. microbiol. scand.*, **77**, 187.

TEIR, H. & RÄSÄNEN, T. (1961) A Study of Mitotic Rate in Renewal Zones of Non-diseased Portions of Gastric Mucosa in Cases of Peptic Ulcer and Gastric Cancer, with Observations on Differentiation and So-called "Intestinalization" of Gastric Mucosa. *J. natn. Cancer Inst.*, **27**, 949.

TEIR, H., SCHAUMANN, A. & SUNDELL, B. (1952) Mitotic Ratio and Colchicine Sensitivity of the Stomach Epithelium of the White Rat. *Acta anat.*, **16**, 233.

WHITEHEAD, R. (1973) *Mucosal Biopsy of the Gastrointestinal Tract*, Philadelphia: W. B. Saunders Co. Ltd.

WILLEMS, G. (1972) Cell Renewal in the Gastric Mucosa. *Digestion*, **6**, 46.