Gut, 1989, **30**, 917–921

Effect of bismuth subcitrate on amphibian gastroduodenal bicarbonate secretion

C J SHORROCK, J R CRAMPTON, L C GIBBONS, AND W D W REES

From the Department of Gastroenterology, Hope Hospital, University of Manchester School of Medicine, Salford

SUMMARY **The ulcer healing and cytoprotective properties of colloidal bismuth (De-Nol) are well established although its mode of action is unclear. We have examined the action of bismuth subcitrate, the active ingredient of De-Nol, on gastroduodenal bicarbonate secretion by isolated amphibian mucosa. Addition of bismuth subcitrate (10^{-6} to 10^{-4} M) to the luminal solution produced a dose dependent increase in bicarbonate secretion from both gastric and duodenal mucosae without a change in transmucosal potential difference. The magnitude of this stimulation was greater for gastric than duodenal mucosae at all dose ranges. A second bismuth salt, bismuth oxynitrate, produced similar increases in bicarbonate secretion from gastric mucosae. Pretreatment of gastric mucosa with the cyclooxygenase inhibitor, indomethacin (10^{-5} and 10^{-4} M), did not abolish the secretory response to bismuth subcitrate. Similar treatment with the chloride transport inhibitor, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) (10^{-3} M) prevented the secretory response to bismuth subcitrate.**

Bismuth compounds have been used for over 200 years in the treatment of various gastrointestinal disorders. Colloidal bismuth subcitrate (CBS) has been shown to protect the gastric mucosa against a wide variety of damaging agents in animals[1–3] and to heal gastric[4] and duodenal[5] ulcers in man. Moreover, compared with H_2 receptor antagonists, most studies have shown De-Nol to reduce the duodenal ulcer relapse rate after healing.[6–8] The reasons for this lower relapse rate are unclear but may, in part, be because of De-Nol's bactericidal action against *Campylobacter pylori*.[9]

De-Nol is a colloidal suspension at neutral pH but precipitates in an acid environment, binding to an ulcer base or mucosal defect. This local effect, by forming a physical barrier to acid and pepsin, was thought to represent its primary mechanism of action.[10] Recent evidence, however, suggests a more complex action possibly involving the formation and release of endogenous prostaglandins.[3 11]

As the mucus-bicarbonate barrier may act as an important defence mechanism against acid and pepsin,[12 13] we have examined the effect of bismuth on bicarbonate secretion by gastroduodenal mucosa.

Address for correspondence: Dr W D W Rees, Hope Hospital, Eccles Old Road, Salford M6 8HD.

Accepted for publication 29 November 1988.

Methods

ANIMALS

Studies on bicarbonate secretion were performed on gastric mucosa obtained from *Rana temporaria* (Xenopus, UK) and, in order to obtain mucosa of suitable area, on duodenal mucosa from *Rana catesbeiana* (St Croix Biologicals, Minneapolis, Minnesota, USA). All animals were kept at 21°C and used within three weeks of purchase.

GASTRIC MUCOSA

Secretory and electrical activities of gastric mucosa were examined using methods previously described in detail.[14] Briefly, the gastric mucosa was dissected free of muscularis externa and mounted as a membrane between two halves of perspex chambers (1·8 cm^2 surface area). Each surface was bathed with 20 ml of solution circulated by gas lifts. The unbuffered luminal side solution was gassed with 100% oxygen and the buffered serosal side solution with a mixture of 95% oxygen and 5% carbon dioxide. The pH of the luminal solution was maintained at pH 7·4 by infusion of 5 mM hydrochloric acid using a pH stat system (ABU13 and TT2, Radiometer, Copenhagen, Denmark).

Spontaneous acid secretion by gastric mucosa was

inhibited by 10^{-3} M cimetidine added to the serosal side solution to allow measurement of the alkali output. Transmucosal potential difference was recorded by means of matched calomel electrodes. The mucosal and serosal side solutions were maintained at 20°C by water jackets circulated through by a Haake G circulator (Haakemess-Technik, W Germany).

DUODENAL MUCOSA

The duodenal mucosa was stripped of muscularis externa and mounted as an intact tube on glass cannulae as previously described.[15] The luminal surface was bathed with 10 ml of unbuffered solution, circulated by a 100% oxygen gas lift and maintained at pH 7·40 using pH stat titration as described above. The serosal surface was bathed with 120 ml of buffered solution gassed with a mixture of 95% oxygen and 5% carbon dioxide. Transmucosal potential difference was measured and recorded as described above and the bathing solution maintained at 20°C.

BATHING SOLUTIONS

The serosal side of the mucosa was bathed by a solution containing Na^+ 102·4 mM; K^+ 4·0 mM; Ca^{++} 1·8 mM; Mg^{++} 0·8 mM; Cl^- 91·4 mM; HCO_3^- 17·8 mM; $H_2PO_4^-$ 0·8 mM; SO_4^{2-} 0·8 mM; and glucose 2 mM (osmolarity=220 mOsm and a pH=7·20). The luminal solution was unbuffered with HCO_3^- and $H_2PO_4^-$ being replaced by mannitol (11·3 mM) to produce the same osmolarity. Titrant acid had the same composition as the luminal solution but in addition contained 5 mM hydrochloric acid.

DRUGS AND CHEMICALS

Bismuth subcitrate was obtained as an amorphous powder (Gist-Brocades, Delft, The Netherlands) and prepared as a suspension in water. One hundred microlitre volumes of this constantly stirred suspension were added to the luminal or serosal side solution so that the final concentration ranged between 10^{-6} and 10^{-4} M. Bismuth subcitrate itself possesses very little buffering capacity, even at much higher concentrations, but solutions were titrated to pH 7·4 before addition to the chambers. The resultant solutions were stable and there was no change in pH when titrated bismuth subcitrate was added to gassed bathing solution in the presence of an inert membrane instead of mucosa. Bismuth oxynitrate (Sigma Chemicals, England) was also prepared in stock solution (pH 7·40) and added to the luminal solution to produce final concentrations between 10^{-6} and 10^{-4} M. There was no disturbance of the pH equilibrium of luminal solution on adding this compound.

Indomethacin (Sigma Chemicals, England) was prepared as a stock solution and added to the serosal side solution to produce a final concentration of either 10^{-5} or 10^{-4} M.

STATISTICAL ANALYSIS

Secretory rate and potential difference were recorded at five minute intervals and mean values for consecutive 15 minute periods calculated for each experiment. Results are expressed as means standard errors (SE) and the statistical significance calculated by comparing 15 minute values obtained before and after addition of a compound using Student's paired *t* test.

Results

Addition of 10^{-6} to 10^{-4} M bismuth subcitrate at pH 7·40 to the luminal solution produced a linear dose dependent stimulation of alkali secretion from gastric mucosa (Fig. 1) which persisted during the period of observation (10^{-6} M=21% increase from 0·19 (0·04) to 0·23 (0·05) μmol/cm²/h, n=10, p<0·02; 10^{-5} M=41% increase from 0·17 (0·04) to 0·24 (0·04) μmol/cm²/h, n=10, p<0·001; 10^{-4} M=68% increase from 0·27 (0·05) to 0·47 (0·06) μmol/cm²/h, n=10, p<0·001) (Fig. 2). Transmucosal potential difference across the tissues was not altered.

In duodenal mucosa, bismuth subcitrate again produced significant stimulation of bicarbonate

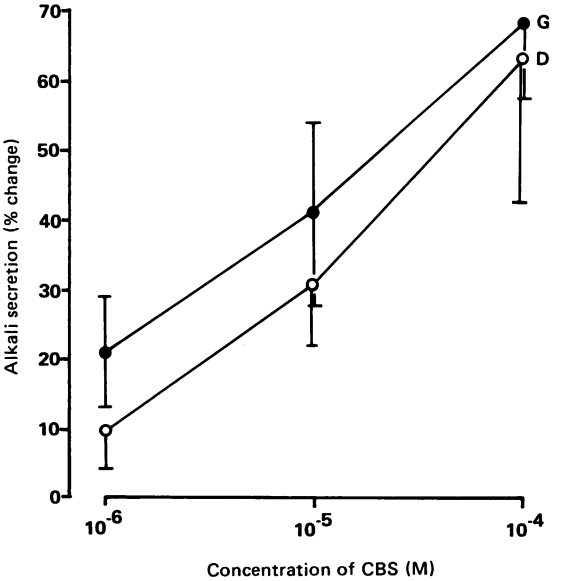

Fig. 1 *Dose response curves to bismuth subcitrate for gastric (G) and duodenal (D) mucosa. Each point represents mean (SE) change in secretion (n=10 for G, n=6 for D).*

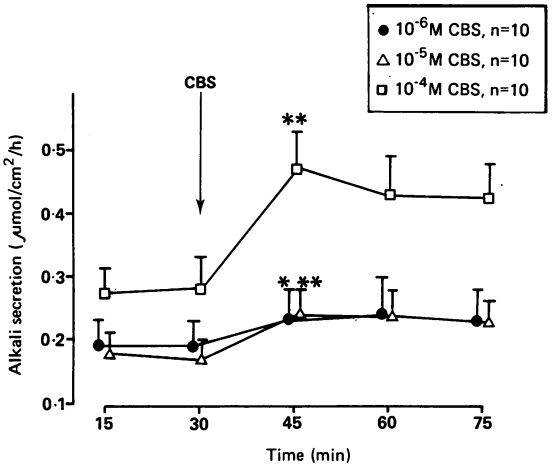

Fig. 2 *Effect of luminal bismuth subcitrate on alkalinisation of gastric mucosa (means (SE), n=10, *p<0·02 compared with basal secretion; **p<0·001 compared with basal secretion).*

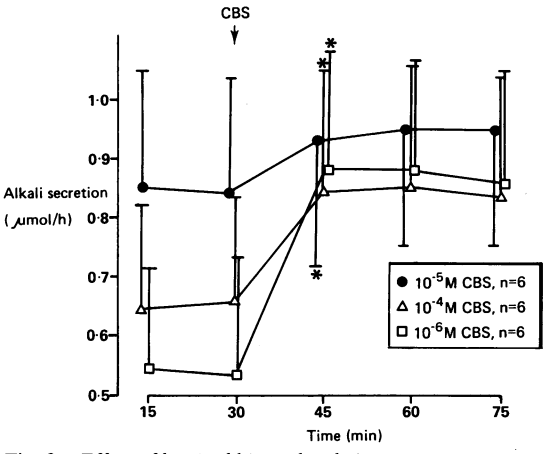

Fig. 3 *Effect of luminal bismuth subcitrate on alkalinisation of duodenal mucosa (means (SE), n=6, *p<0·05 compared with basal secretion).*

secretion without change in transmucosal potential difference (10^{-6} M=10% increase from 0·84+0·20 to 0·93+0·20 μmol/h, n=6, p<0·05; 10^{-5} M=31% increase from 0·64+0·18 to 0·84+0·20 μmol/h, n=6, p<0·05; 10^{-4} M=63% increase from 0·54 (0·17) to 0·88 (0·22) μmol/h, n=6, p<0·05) (Fig. 3). The variability in basal alkali secretion and large standard errors are the result of the variation in resting alkali secretion between animals and to the fact that the duodenum is mounted as a tube, not as a fixed surface area.

At each concentration studied, the magnitude of stimulation of bicarbonate secretion was greater in gastric than duodenal mucosa (Fig. 1). Serosal side addition of 10^{-6} to 10^{-4} M bismuth subcitrate did not alter the rate of alkali secretion from either gastric or duodenal mucosa. Addition of a different bismuth salt, bismuth oxynitrate, at pH 7·40 to the luminal solution had no effect on bicarbonate secretion at a concentration of 10^{-6} M but 10^{-5} and 10^{-4} M concentrations significantly stimulated gastric bicarbonate secretion without change in transmucosal potential difference (10^{-5} M=23% increase from 0·94 (0·3) to 1·16 (0·3) μmol/cm²/h, n=7, p<0·02; 10^{-4} M=40% increase from 0·37 (0·07) to 0·52 (0·08) μmol/cm²/h, n=7, p<0·002). Serosally applied bismuth oxynitrate had no effect on alkali secretion.

MECHANISMS OF ALKALI SECRETION INDUCED BY BISMUTH

Because the increase in gastroduodenal alkali secretion was not accompanied by changes in transmucosal potential difference, the secretory response

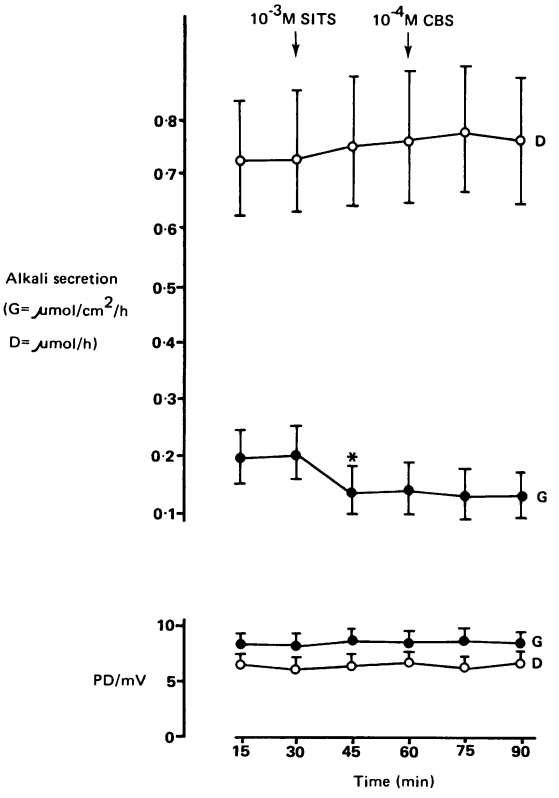

Fig. 4 *Effect of serosal SITS (10^{-3} M) pretreatment on alkalinisation of gastric (G) and duodenal (D) mucosa produced by luminal bismuth subcitrate (10^{-4} M) (n=4 for both G and D, means (SE), *p<0·05 compared with basal secretion).*

is unlikely to result from mucosal damage with increased passive diffusion of bicarbonate or electrogenic transport of this ion across the epithelial cell membrane. It thus seems likely that stimulation of bicarbonate secretion by bismuth occurs by an electroneutral ion exchange mechanism, such as Cl^-/HCO_3^- exchange. To confirm this, the effect of SITS, a chloride transport inhibitor, on the bismuth subcitrate effect was studied. Addition of 10^{-3} M SITS to the serosal side solution of gastric mucosa from *Rana temporaria* produced a significant fall in basal bicarbonate secretion (from 0·19 (0·02) to 0·14 (0·01) μmol/cm²/h, n=4, p<0·05) and completely prevented the stimulation of bicarbonate secretion produced by 10^{-4} M bismuth subcitrate (Fig. 4).

In duodenal mucosa, serosally applied 10^{-3} M SITS abolished the stimulation of bicarbonate secretion produced by 10^{-4} M bismuth subcitrate but did not alter basal bicarbonate secretion (basal secretion= 0·73 (0·10) μmol/h, after 10^{-3} M SITS=0·76 (0·12) μmol/h and after 10^{-4} M CBS=0·79 (0·11) μmol/h, n=4, p=NS) (Fig. 4). In both gastric and duodenal mucosae, luminal application of 10^{-3} M SITS produced the same effect as that applied serosally.

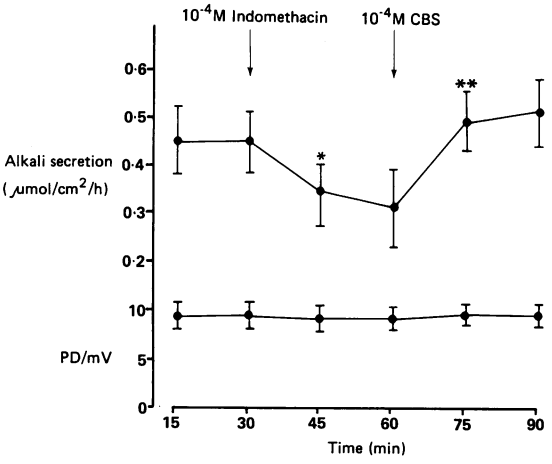

Fig. 5 *Effect of serosal indomethacin (10^{-4} M) pretreatment on the increased gastric alkali secretion produced by luminal bismuth subcitrate (10^{-4} M) (n=6, means (SE)). There is an initial fall in alkali secretion from basal values with indomethacin (*p<0·05) followed by a stimulation of secretion by CBS (**p<0·001 compared with the secretory rate immediately before the addition of CBS).*

ROLE OF ENDOGENOUS PROSTAGLANDINS IN THE BISMUTH RESPONSE

Serosal application of 10^{-5} M and 10^{-4} M indomethacin, doses known to inhibit mucosal cyclooxygenase,[16] produced a significant fall in alkali secretion from basal values (Fig. 5 – shown for 10^{-4} M indomethacin only) but did not prevent the increase in gastric alkali secretion produced by 10^{-4} M bismuth subcitrate (61% increase from 0·31 (0·06) to 0·50 (0·08) μmol/cm²/h, n=6, p<0·001, with 10^{-4} M indomethacin (Fig. 5); 50% increase from 0·18 (0·02) to 0·27 (0·02) μmol/cm²/h, n=4, p<0·05, with 10^{-5} M indomethacin).

Discussion

Our results show that bismuth subcitrate increases alkali secretion from gastroduodenal mucosa by a prostaglandin independent mechanism. As transmucosal potential difference was not altered by bismuth subcitrate and oxynitrate, in either gastric or duodenal mucosa, the increased transport of bicarbonate is unlikely to be either electrogenic or as a result of increased passive diffusion across damaged epithelium. In support of a transcellular, electroneutral Cl^-/HCO_3^- exchange mechanism is the observation that bismuth stimulated alkali secretion was prevented by pretreatment of mucosa with the chloride transport inhibitor, SITS. Such an exchange mechanism accounts for the bulk of basal gastric bicarbonate secretion and may be activated in duodenal mucosa, which normally secretes bicarbonate by an electrogenic process, by encephalins, GIP and glucagon.[14 15 17] The finding that a different bismuth salt, bismuth oxynitrate, produced similar stimulation of gastric bicarbonate secretion suggests that the metallic cation is the ingredient responsible for the secretory stimulation. This is of particular interest as we have recently described stimulation of gastroduodenal alkali secretion by another metallic ion, aluminium, which is an ingredient of certain antacids and the ulcer healing drug, sucralfate.[18]

It has recently been suggested that the protective action of colloidal bismuth may be mediated by mucosal generation and luminal release of protective prostaglandins.[3 11] Because exogenous prostaglandins of the E and F series stimulate gastroduodenal bicarbonate secretion[19] and suppression of endogenous prostanoid synthesis by non-steroidal anti-inflammatory drugs leads to its inhibition,[20] it seems possible that the mode of action of colloidal bismuth subcitrate could be mediated by enhanced prostaglandin formation. There are, however, a number of important flaws in this hypothesis. First, stimulation of duodenal bicarbonate secretion by exogenous prostaglandins occurs *via* an electrogenic mechanism and is therefore associated with an increase in transmucosal potential difference.[15] In our experiments the duodenal response to bismuth subcitrate was non-electrogenic. Second, indomethacin, in concentrations which abolish cyclooxygenase activity,[16] failed to abolish the gastric

alkali stimulation produced by bismuth subcitrate. Therefore, whilst our results confirm the stimulatory action of bismuth on gastric alkali secretion reported by Konturek,[21] our observations imply a prostaglandin independent mechanism. The reasons for this discrepancy are not immediately clear, although different experimental models have been used.

Stimulation of bicarbonate secretion by bismuth subcitrate would be expected to enhance the protection offered by the mucus-bicarbonate barrier overlying healthy epithelium and provide protection from mucosal damaging agents. This enhancement of the barrier would theoretically prevent recurrence of peptic ulcer after initial healing and prevent NSAID induced gastric damage or stress ulceration in severely ill patients. It is difficult, however, to envisage what importance this effect would have in accelerating chronic peptic ulcer healing. Once such an ulcer has formed, the normal mucus-bicarbonate barrier disappears from ulcerated tissue and is replaced with a layer of sloughed epithelial cells, fibrin and mucus covering the damaged mucosa, into which diffuses alkali from interstitial tissue.[22] Although the effect of bismuth subcitrate on gastroduodenal bicarbonate secretion by intact epithelium is interesting and relevant to mucosal protection, we suspect it may be of little relevance to chronic ulcer healing.

The authors are grateful to the Department of Medical Illustration, Hope Hospital for providing the figures. Dr Shorrock is a British Society of Gastroenterology Research Fellow. This work was supported in part by a grant from Gist-Brocades.

References

1 Konturek SJ, Radecki T, Piastucki I, Drozdowicz D. Advances in the understanding of the mechanism of cytoprotective action by colloidal bismuth subcitrate. *Scand J Gastroenterol* 1986; **21** (suppl 122): 6–10.
2 Wieriks J, Hespe W, Jaitly KD, Koekkoek PH, Lavy UI. Pharmacological properties of colloidal bismuth subcitrate CBS, DE-NOL. *Scand J Gastroenterol* 1982; **17** (suppl 80): 11–6.
3 Hall DWR, Van Den Hoven WE. Gastric mucosa protection and prostaglandin E_2 generation in rats by colloidal bismuth subcitrate. *Arch Intern Pharmacodyn Ther* 1987; **286:** 308–19.
4 Tytgat GNJ, Rauws E, Langenberg W. The role of colloidal bismuth subcitrate in gastric ulcer and gastritis. *Scand J Gastroenterol* 1986; **21** (suppl 122): 22–9.
5 Kang JY, Piper DW. Cimetidine and colloidal bismuth in treatment of chronic duodenal ulcer. Comparison of initial healing and recurrence after healing. *Digestion* 1982; **23:** 73–8.
6 Martin DF, Hollanders D, May SJ, Ravenscroft MM, Tweedle DEF, Miller JP. Difference in relapse rates of duodenal ulcer after healing with cimetidine or tripotassium dicitrato bismuthate. *Lancet* 1981; i: 7–10.
7 Hamilton I, O'Connors HJ, Wood HC, Bradbury I, Axon ATR. Healing and recurrence of duodenal ulcer after treatment with tripotassium dicitrato bismuthate (TDB) tablets or cimetidine. *Gut* 1986; **27:** 106–10.
8 Dobrilla G, Vallaperta P, Amplatz S. Influence of ulcer healing agents on ulcer relapse after discontinuation of acute treatment: a pooled estimate of controlled clinical trials. *Gut* 1988; **29:** 181–7.
9 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; i: 1311–4.
10 Lee SP. A potential mechanism of action of colloidal bismuth subcitrate: diffusion barrier to hydrochloric acid. *Scand J Gastroenterol* 1982; **17** (suppl 80): 17–21.
11 Konturek SJ, Radecki T, Piastucki I, Brzozowski T, Drozdowicz D. Gastrocytoprotection by colloidal bismuth subcitrate (De-Nol) and sucralfate. Role of endogenous prostaglandins. *Gut* 1987; **28:** 201–5.
12 Rees WDW, Turnberg LA. Mechanisms of mucosal protection: A role for the mucus bicarbonate barrier. *Clin Sci* 1982; **62:** 342–8.
13 Flemstrom G. Gastric and duodenal mucosal bicarbonate secretion. In: Johnson LR, ed. *Physiology of the gastrointestinal tract.* New York: Raven Press, 1986: 842–7.
14 Flemstrom G. Active alkalinisation by amphibian gastric fundic mucosa in vitro. *Am J Physiol* 1977; **233:** 1–12.
15 Flemstrom G. Stimulation of HCO_3^- transport in isolated proximal bullfrog duodenum by prostaglandin. *Am J Physiol* 1980; **239:** 198–202.
16 Crampton JR, Gibbons LC, Rees WDW. Effects of sucralfate on gastroduodenal bicarbonate secretion and prostaglandin E_2 metabolism. *Am J Med* 1987; **83** (suppl 3B): 14–8.
17 Flemstrom G, Garner A. Gastroduodenal HCO_3^- transport: characteristics and proposed role in acidity regulation and mucosal protection. *Am J Physiol* 1982; **242:** 183–93.
18 Crampton JR, Gibbons LC, Rees WDW. Stimulation of amphibian gastroduodenal bicarbonate secretion by sucralfate and aluminium: role of local prostaglandin metabolism. *Gut* 1988; **29:** 903–8.
19 Konturek SJ, Tasler J, Bilski J, Kania J. Prostaglandins and alkaline secretion from oxyntic antral and duodenal mucosa. *Am J Physiol* 1983; **245:** G539–46.
20 Garner A, Flemstrom G, Heylings JR. Effects of anti-inflammatory agents and prostaglandins on acid and bicarbonate secretion in the amphibian isolated mucosa. *Gastroenterology* 1979; **77:** 451–7.
21 Konturek SJ, Bilski J, Kwiecien N, Obtulowicz W, Kopp B, Oleksy J. De-Nol stimulates gastric and duodenal alkaline secretion through prostaglandin dependent mechanism. *Gut* 1987; **28:** 1557–63.
22 Wallace JL, Whittle BJR. Role of mucus in the repair of gastric epithelial damage in the rat. *Gastroenterology* 1986; **91:** 603–11.