

# Effect of bismuth subcitrate on amphibian gastroduodenal bicarbonate secretion

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**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<sup>1-3</sup> and to heal gastric<sup>4</sup> and duodenal<sup>5</sup> ulcers in man. Moreover, compared with H<sub>2</sub> receptor antagonists, most studies have shown De-Nol to reduce the duodenal ulcer relapse rate after healing.<sup>6-8</sup> The reasons for this lower relapse rate are unclear but may, in part, be because of De-Nol's bactericidal action against *Campylobacter pylori*.<sup>9</sup>

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.<sup>10</sup> Recent evidence, however, suggests a more complex action possibly involving the formation and release of endogenous prostaglandins.<sup>3,11</sup>

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

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## 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.<sup>14</sup> Briefly, the gastric mucosa was dissected free of muscularis externa and mounted as a membrane between two halves of perspex chambers (1.8 cm<sup>2</sup> 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  $20^{\circ}\text{C}$  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.<sup>15</sup> 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  $20^{\circ}\text{C}$ .

#### BATHING SOLUTIONS

The serosal side of the mucosa was bathed by a solution containing  $\text{Na}^+$  102.4 mM;  $\text{K}^+$  4.0 mM;  $\text{Ca}^{++}$  1.8 mM;  $\text{Mg}^{++}$  0.8 mM;  $\text{Cl}^-$  91.4 mM;  $\text{HCO}_3^-$  17.8 mM;  $\text{H}_2\text{PO}_4^-$  0.8 mM;  $\text{SO}_4^{2-}$  0.8 mM; and glucose 2 mM (osmolarity=220 mOsm and a pH=7.20). The luminal solution was unbuffered with  $\text{HCO}_3^-$  and  $\text{H}_2\text{PO}_4^-$  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)  $\mu\text{mol}/\text{cm}^2/\text{h}$ ,  $n=10$ ,  $p<0.02$ ;  $10^{-5}$  M=41% increase from 0.17 (0.04) to 0.24 (0.04)  $\mu\text{mol}/\text{cm}^2/\text{h}$ ,  $n=10$ ,  $p<0.001$ ;  $10^{-4}$  M=68% increase from 0.27 (0.05) to 0.47 (0.06)  $\mu\text{mol}/\text{cm}^2/\text{h}$ ,  $n=10$ ,  $p<0.001$ ) (Fig. 2). Transmucosal potential difference across the tissues was not altered.

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

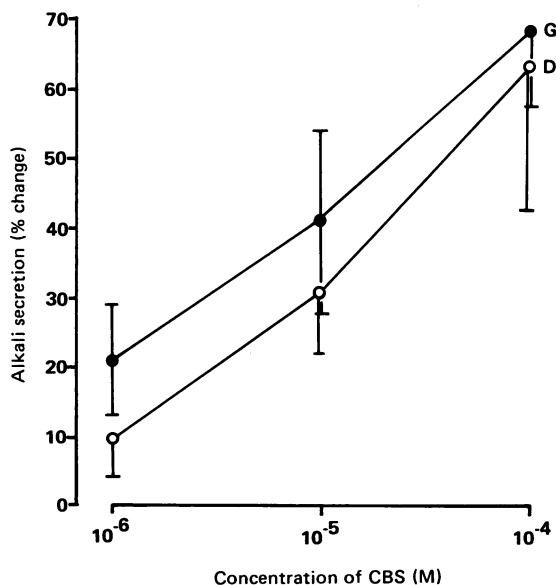


Fig. 1 Dose response curves to bismuth subcitrate for gastric (G) and duodenal (D) mucosa. Each point represents mean (SE) change in secretion ( $n=10$  for G,  $n=6$  for D).

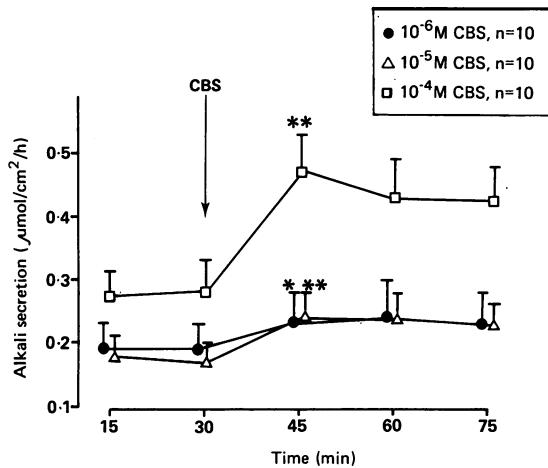


Fig. 2 Effect of luminal bismuth subcitrate on alkalinisation of gastric mucosa (means (SE), n=10, \*p<0.02 compared with basal secretion; \*\*p<0.001 compared with basal secretion).

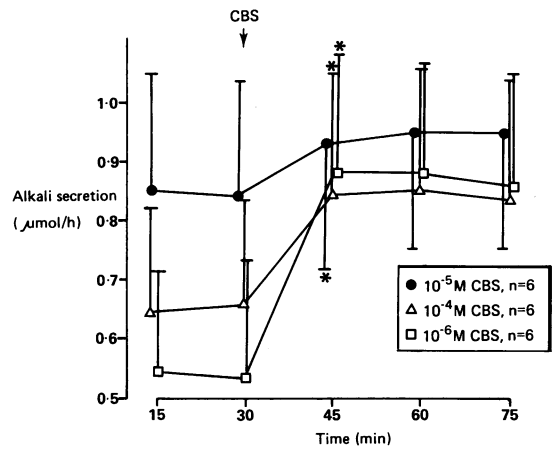


Fig. 3 Effect of luminal bismuth subcitrate on alkalinisation of duodenal mucosa (means (SE), n=6, \*p<0.05 compared with basal secretion).

secretion without change in transmucosal potential difference ( $10^{-6}$  M=10% increase from  $0.84 \pm 0.20$  to  $0.93 \pm 0.20$   $\mu\text{mol/h}$ , n=6,  $p < 0.05$ ;  $10^{-5}$  M=31% increase from  $0.64 \pm 0.18$  to  $0.84 \pm 0.20$   $\mu\text{mol/h}$ , n=6,  $p < 0.05$ ;  $10^{-4}$  M=63% increase from  $0.54$  (0.17) to  $0.88$  (0.22)  $\mu\text{mol/h}$ , 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)  $\mu\text{mol/cm}^2/\text{h}$ , n=7,  $p < 0.02$ ;  $10^{-4}$  M=40% increase from  $0.37$  (0.07) to  $0.52$  (0.08)  $\mu\text{mol/cm}^2/\text{h}$ , 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

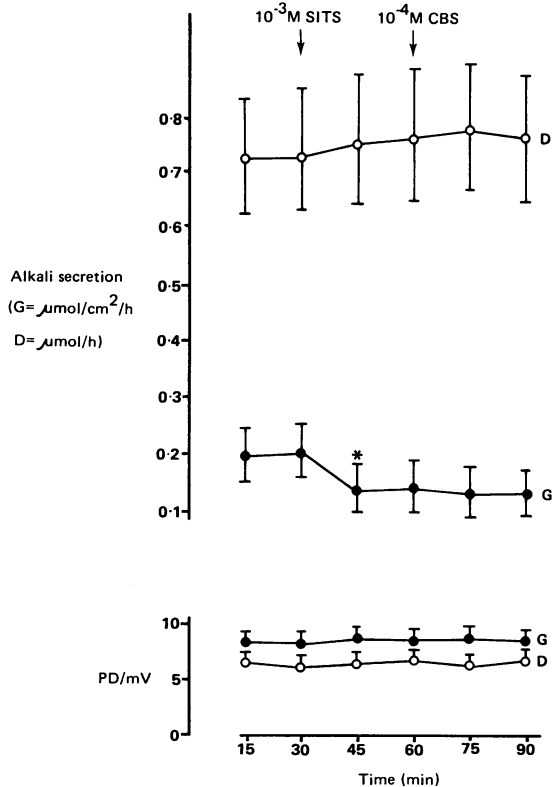


Fig. 4 Effect of serosal SITS ( $10^{-3}$  M) pretreatment on alkalinisation of gastric (G) and duodenal (D) mucosa produced by luminal bismuth subcitrate ( $10^{-4}$  M) (n=4 for both G and D, means (SE), \*p<0.05 compared with basal secretion).

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  $\text{Cl}^-/\text{HCO}_3^-$  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)  $\mu\text{mol}/\text{cm}^2/\text{h}$ ,  $n=4$ ,  $p<0.05$ ) 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)  $\mu\text{mol}/\text{h}$ , after  $10^{-3}$  M SITS = 0.76 (0.12)  $\mu\text{mol}/\text{h}$  and after  $10^{-4}$  M CBS = 0.79 (0.11)  $\mu\text{mol}/\text{h}$ ,  $n=4$ ,  $p=\text{NS}$ ) (Fig. 4). In both gastric and duodenal mucosae, luminal application of  $10^{-3}$  M SITS produced the same effect as that applied serosally.

#### 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,<sup>16</sup> 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)  $\mu\text{mol}/\text{cm}^2/\text{h}$ ,  $n=6$ ,  $p<0.001$ , with  $10^{-4}$  M indomethacin (Fig. 5); 50% increase from 0.18 (0.02) to 0.27 (0.02)  $\mu\text{mol}/\text{cm}^2/\text{h}$ ,  $n=4$ ,  $p<0.05$ , 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  $\text{Cl}^-/\text{HCO}_3^-$  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 duo-

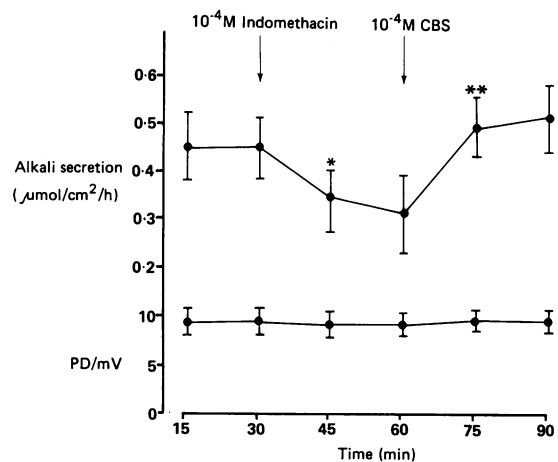


Fig. 5 Effect of serosal indomethacin ( $10^{-4}$  M) pretreatment on the increased gastric alkali secretion produced by luminal bismuth subcitrate ( $10^{-4}$  M) ( $n=6$ , means (SE)). There is an initial fall in alkali secretion from basal values with indomethacin (\* $p<0.05$ ) followed by a stimulation of secretion by CBS (\*\* $p<0.001$  compared with the secretory rate immediately before the addition of CBS).

denal mucosa, which normally secretes bicarbonate by an electrogenic process, by encephalins, GIP and glucagon.<sup>14,15,17</sup> 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.<sup>18</sup>

It has recently been suggested that the protective action of colloidal bismuth may be mediated by mucosal generation and luminal release of protective prostaglandins.<sup>3,11</sup> Because exogenous prostaglandins of the E and F series stimulate gastroduodenal bicarbonate secretion<sup>19</sup> and suppression of endogenous prostanoid synthesis by non-steroidal anti-inflammatory drugs leads to its inhibition,<sup>20</sup> 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.<sup>15</sup> In our experiments the duodenal response to bismuth subcitrate was non-electrogenic. Second, indomethacin, in concentrations which abolish cyclooxygenase activity,<sup>16</sup> 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,<sup>21</sup> 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.<sup>22</sup> 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.

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