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Antibacterial Action of Bismuth in Relation to *Campylobacter pyloridis* Colonization and Gastritis

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Abstract. Colloidal bismuth subcitrate (CBS, De-Nol®) heals duodenal ulcers but with a lower relapse rate than cimetidine, perhaps due to inhibition of *Campylobacter pyloridis* (CP) organisms. To test this hypothesis we studied gastric mucosal histology in three groups of ulcer patients treated with either cimetidine, CBS, or CBS in combination with an antibiotic.

Cimetidine had no effect on CP or gastric mucosal histology but with CBS therapy there was a significant reduction in the number of bacteria ($p < 0.0001$). However, relapse of both CP infection and gastritis usually occurred once CBS was withdrawn. When CBS was combined with amoxycillin or tinidazole, long-term disappearance of both CP bacteria and gastritis was achieved ($p < 0.0001$). In ultrastructural studies 30–90 min after single oral doses of CBS or bismuth subsalicylate, CP had detached from the gastric epithelial cells and exhibited structural degradation associated with the selective deposition of a particulate bismuth complex within and upon the surface of the organisms. In vitro, CP and other campylobacters were inhibited by bismuth compounds at 25 mg/l but they were resistant to cimetidine and ranitidine. CBS has a powerful antibacterial effect against CP but relapse of infection is common after CBS alone. In combination with antibiotics however, eradication of CP and long-term healing of gastritis occurs. In such cases the gastroduodenal mucosa is intact, and less likely to ulcerate.

In an attempt to explain the healing action of colloidal bismuth subcitrate (CBS, De-Nol®) in peptic ulceration, it has been suggested from animal ulcer model studies that CBS binds to the base of peptic ulcers [1] and impairs the progress of hydrogen ions through the mucus layer [2]. While this mechanism might lead to ulcer healing, it does not account for the lower relapse rates reported for duodenal ulcers (DUs) healed

with CBS as compared with H_2-receptor antagonist therapy [3, 4].

Gregory et al. [5] studied the ultrastructure of DU borders before and after treatment with CBS or cimetidine, and observed that healing was 'more complete' with CBS, perhaps explaining why relapse occurred less often. He was apparently unaware, however, that inhibition of so-called 'gastric spirochetes' by bismuth salts had previously been proposed to explain the healing of gastric ulcers by Freedberg and Barron [6] in 1940; indeed, Gregory's pretreatment micrographs featured adherent bacterial cells similar to the curved or spiral organisms now known as *Campylobacter pyloridis* (CP).

There is now substantial evidence that CP colonization of gastric mucosa is present in most DU patients [7–9], and that the organism is also to be found in the duodenal cap [10], when gastric type epithelium extends beyond the pylorus [11]. If CP colonization is responsible for active chronic gastritis and is also etiologically related to peptic ulceration as has been suggested [12], then appropriate antibacterial therapy should emerge as the rational approach to treatment of both conditions.

During 1983 and 1984, preparatory to a larger clinical trial now in progress, we performed a pilot study based on analysis of sequential endoscopic biopsies. Initially, the H_2-receptor antagonist cimetidine and CBS were compared for their effects on CP and gastric histopathology. A further study followed, of patients receiving different forms of combination therapy aimed at achieving long-term, or permanent, eradication of the gastric organism. In parallel with the clinical studies, we observed the in vitro effect of bismuth compounds on cultures of CP and other bacteria. Finally, evidence for direct effects of bismuth on CP organisms in vivo and on affected epithelial cells was sought by electron microscopy of biopsy specimens obtained shortly after the administration of oral bismuth medications.

Because of the small number of patients there has been no attempt to analyze clinical data, ulcer healing rates or ulcer relapse rates in this paper.

Patients and Methods

The effect of therapy on active chronic gastritis was studied in consecutive patients presenting with gastric or duodenal ulceration and coexistent gastric CP colonization. Initially, 15 patients were randomized to therapy with either cimetidine or De-Nol® liquid. In the light of data obtained in that study a separate group of 11 patients received combination therapy with De-Nol® tablets and an antibiotic, continued until CP were no longer demonstrable. For ultrastructural studies, 9 more patients scheduled to receive bismuth therapy for CP-associated gastritis were biopsied at time points between 30 and 95 min and at 24 h after taking oral bismuth medication. Three patients received De-Nol® liquid, 4 received De-Nol® tablets, 1 received bismuth subsalicylate tablets (Pepto-Bismol®) and 1 bismuth subsalicylate liquid. The trial was approved by the Fremantle Hospital Human Rights Committee, and no patient refused consent.

Endoscopy and Biopsy

After an overnight fast the patients were given premedication with valium, atropine and phenoperidine. The instrument used was a Fujinon GIFP fiberoptic gastroduodenoscope. When the examination was complete and the usual biopsies thought necessary for diagnostic purposes had been taken, three extra biopsy specimens were taken from areas of the antrum apparently uninvolved by any local lesion such as an ulcer. Two of these were fixed in 10% buffered formalin for paraffin sectioning and histological examination, the third was placed in a drop of 10% glucose and transferred to the microbiology laboratory immediately. For ultrastructural studies biopsy

specimens from the antrum and duodenal cap were fixed by immersion for at least 2 h in 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.2.

Therapeutic Studies

Cimetidine vs. CBS: 15 patients were allocated alternatively to therapy with either cimetidine 200 mg t.d.s. and 400 mg nocte, or liquid De-Nol® 5 ml q.i.d. Endoscopic biopsy was repeated after 6 weeks, 1 day after finishing therapy. Patients then remained off ulcer medication and were re-examined by endoscopy and biopsy at 12 weeks. For analysis we graded biopsy specimens taken at baseline, 6 weeks and 12 weeks.

CBS and antibiotic: When the outcome of the above study was known, 11 other patients were treated with combined therapy consisting of De-Nol® tablets 1 q.i.d. for 4 weeks, with the addition of an antibiotic during the 3rd and 4th week of the course. Preferably, tinidazole was given in a dose of 500 mg twice daily from day 18 to 28. If the patient's CP isolate was found to be resistant to tinidazole, amoxicillin 500 mg q.i.d. was prescribed from day 14 to day 28. All treatment ceased after 4 weeks and endoscopic antral biopsy was repeated 2 weeks later, i.e. at 6 weeks. If CP was not detected, then no further therapy was given. Once a CP-negative biopsy had been obtained no further therapy was prescribed, and the patient was rebiopsied 6 weeks later. For analysis in this group, we graded biopsies taken at baseline, 2 weeks after clearance of CP ('6 weeks'), and 6 weeks later ('12 weeks'). (Only 9 patients could be assessed; in 2 cases CP were not cleared.)

Histology

The two antral biopsy specimens were processed in the same paraffin block and mounted on the same slide. Sections were stained with hematoxylin and eosin to evaluate for gastritis, with the periodic acid–Schiff stain to demonstrate mucinogenesis of the epithelium, and with the Warthin-Starry silver stain to demonstrate CP organisms.

Grading of Gastritis and CP Bacteria (table I)

The sections were graded blind and in random order. The grading used was that described by Whitehead et al. [13] as modified by Warren and Marshall [14], with the addition of an assessment of mucinogenesis of the epithelium [J.R. Glancy, pers. commun.]. Three components of the epithelium were graded to indicate the severity of the gastritis. These were polymorphonuclear leukocytes (PMNs), lymphoctes and plasma cells (MONOs) and mucus depletion (Mucus Depl). Each component was given a score of between 0 and 3. Intestinal metaplasia and CP organisms were similarly graded but were not included as indicators of gastritis. For each parameter

Table I. Criteria used for grading of gastritis and CP infection

Score	Histology
MONOs	
0	Plasma cells and lymphocytes present in normal numbers
1	Minor, equivocal increase of mononuclear cells
2	Moderate increase
3	Maximal or submaximal numbers of cells present in most of the section
Mucus depletion	
0	No mucus depletion, 50% of the epithelial cell depth is intracellular mucus
1	Mild mucus depletion, most evident in the superficial glands
2	Obvious decrease in mucin content of epithelial cells
3	Severe mucus depletion
PMNs	
0	Polymorphs difficult to find
1	Minor increase but cells not in groups or invading glands necks
2	Cells definitely increased, in groups, and/or invading gland necks
3	Groups of cells visible in nearly every field
Score Culture/Histology	
CP infection	
0	No curved bacilli seen in histology sections or gram-stained smears; cultures also negative for CP
1	Occasional curved bacilli seen after searching, or negative histology but positive microbiology
2	Many curved bacilli seen in most fields
3	Large numbers of curved bacilli in every field

increasing abnormality was indicated by a higher score. Thus, by summing the values obtained for PMNs, MONOs and Mucus Depl, a total score of between 0 and 9 could be given to each biopsy. In this scheme, severe gastritis was reported by a score of from 5 to 9, whereas scores less than 3 were of doubtful significance.

Statistical Analysis

Analysis of gastritis grades was performed using the SPSS-X statistical package. Patients were studied in three groups (cimetidine vs. CBS vs. CBS + antibiotic), at three times (baseline vs. 6 weeks vs. 12 weeks).

Microbiology

Gram staining of smears and culture of bacteria were performed routinely, as previously described [12]. Sensitivity testing was performed by agar plate dilution in brain-heart infusion blood agar. Initially, bismuth citrate solution, prepared as described by Wilson and Blair [15], was tested against 13 routine isolates of CP and a range of other enteric organisms (table II). Another 12 isolates of CP were then tested against De-Nol® liquid, bismuth subsalicylate liquid, cimetidine tablets, cimetidine intravenous solution and ranitidine tablets.

Plates containing the test drug at concentrations of between 6.25 and 400 mg/l were prepared. A heavy inoculum of each of the 12 CP isolates was streaked onto each plate and incubated for 72 h. The minimal inhibitory concentration (MIC) was taken as that of the first plate on which no growth occurred. On each occasion, control plates of the test isolates were incubated with the test plates.

Electron Microscopy

For ultrastructural studies, and localization of bismuth deposition sites, the glutaraldehyde-fixed biopsy specimens were embedded in epoxy resin, sectioned and stained with uranyl acetate and lead citrate essentially as described previously [16]. Additionally, lead and uranyl acetate staining was omitted in some instances to facilitate the localization of trace amounts of bismuth complex deposited within or adjacent to the bacterial cells.

Table II. In vitro sensitivity of various microorganisms to bismuth citrate

Organism	Isolates tested, n	MIC mg/l
Campylobacter pyloridis	3	12
Campylobacter pyloridis	10	25
Campylobacter jejuni	2	25
Campylobacter coli	1	25
Bacteroides fragilis	4	128
Clostridium difficile	2	256
Peptococcus magnus	2	256
Vibrio parahaemolyticus	1	1,000
Pseudomonas	1	1,000
Vibrio cholera	1	2,000
Clostridium welchii	1	2,000
Clostridium septicum	1	2,000
Aeromonas hydrophila	2	4,000
'Oxford' *Staphylococcus aureus*	1	>4,000
Shigella flexneri	1	>4,000
Salmonella adelaide	1	>4,000

Results

Effects on Gastric Histology: Cimetidine vs. CBS vs. CBS + Antibiotic

Baseline comparison of the three groups, histological grades for PMNs, MONOs, Mucus Depl and CP, were the same for the three treatment groups at the baseline biopsy. As shown in table III and figures 1–3, all groups had moderate to severe active chronic gastritis prior to therapy (i.e. grade ≥ 5). The group treated with CBS + antibiotic was significantly younger than the other two groups ($p = 0.03$).

Effect of Therapy

Cimetidine (7 patients): At 6 weeks there was no discernible change in the number of demonstrable CP or in any of the other parameters tested (fig. 1). The overall gastri-

Table III. Baseline comparison of the three treatment groups

Group	n	Age	Males	DU	GU	Baseline grade of gastritis (SD)
Cimetidine	7	50	3	5	2	6.0 (1.53)
CBS	8	57	6	6	2	5.8 (1.39)
CBS + antibiotic	9	37	7	8	1	6.6 (1.24)

tis score in this group was 6.0 (SD 1.5) at baseline, 6.8 (1.8) at 6 weeks , and 7.3 (1.4) at 12 weeks. These differences were not statistically significant. It was concluded that cimetidine had no demonstrable effect on CP organisms, and did not heal gastritis.

CBS alone (8 patients): At 6 weeks (i.e. 24 h after ceasing CBS therapy) numbers of demonstrable CP organisms had decreased in 7 patients (fig. 2). Three of these patients were negative for CP on histology and on culture, 2 were CP negative on histology but had remained positive on culture, and 2 patients had low numbers of bacteria detectable by both tests (the remaining patient, in whom CP were not reduced, is discussed below). The highly significant reduction in CP ($p < 0.0001$) was accompanied by significant reductions in PMNs ($p < 0.0001$) and in Mucus Depl ($p < 0.05$). MONOs were unchanged. The gastritis score for the group declined from 5.75 (SD 1.4) at baseline to 2.9 (1.2) at 6 weeks ($p < 0.001$). The histological improvement seen at 6 weeks disappeared after CBS therapy was discontinued. At 12 weeks, 7 of the 8 patients were rebiopsied and scores for PMNs and Mucus Depl had risen in 6 of these, giving a mean gastritis score for the group of 5.6 (SD 1.8), which was not significantly different from the baseline score. In these 6 patients, CP were once more present in large numbers.

There were 2 patients treated with CBS alone in whom the gastritis did not follow the trend for the rest of the group. One patient, a 44-year-old woman, ran out of CBS after 5 weeks so had been off the drug for 1 week when the second biopsy was taken. She was the only patient in whom moderate numbers of CP organisms could be found at the 6 weeks' biopsy. She was also the only patient in whom gastritis had not improved at 6 weeks. Her scores were 4.0, 4.0 and 5.0 (fig. 4, noncompliant). The other case of interest was an 84-year-old man in whom CBS therapy alone cleared the bacteria. He had improved histologically by the 6th week and was the only case in this group to maintain the improvement after CBS treatment was ceased. His scores were 8.0, 4.0 and 2.0 (fig. 4, CP negative).

CBS plus antibiotic (9 patients): In these patients eradication of CP was accompanied by a striking histological improvement in the gastritis in the biopsy at 6 weeks (fig. 3). Significant reductions in PMNs ($p < 0.0001$), MONOs ($p < 0.01$) and Mucus Depl ($p < 0.001$) had occurred as compared with the baseline biopsy. In the 7 patients who were rebiopsied at 12 weeks the histological improvement was maintained. Reduction in

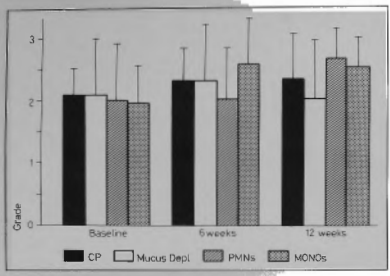

Fig. 1. Gastritis score (mean ± SD) for cimetidine-treated group (n = 7). No significant change in any of the parameters tested.

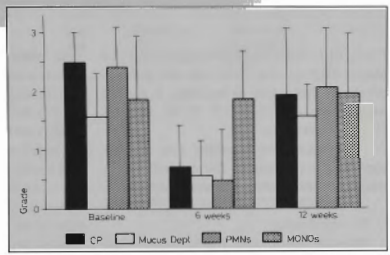

Fig. 2. Gastritis score (mean ± SD) for CBS-treated group (n = 8). Significant change in CP ($p < 0.0001$), Mucus Depl ($p < 0.05$) and PMNs ($p < 0.0001$) at 6 weeks.

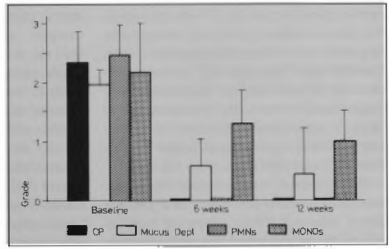

Fig. 3. Gastritis score (mean ± SD) for CBS + antibiotic-treated group (n = 9). Significant changes both at 6 and 12 weeks in CP ($p < 0.0001$ resp. $p < 0.01$), Mucus Depl ($p = 0.01$ resp. $p < 0.01$), PMNs ($p < 0.0001$ both) and MONOs ($p < 0.01$ resp. $p = 0.01$).

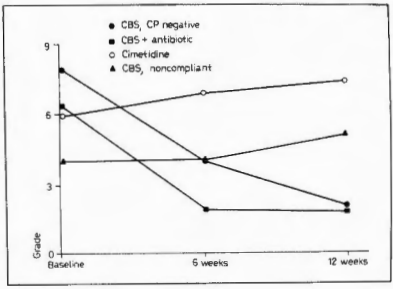

Fig. 4. Gastritis score of special cases.

PMNs ($p < 0.0001$), MONOs ($p = 0.01$) and Mucus Depl ($p < 0.0001$) was still present. Scores in this group at baseline, 6 and 12 weeks were 6.6 (1.2), 1.9 (0.78) and 1.6 (1.34), respectively.

Histological improvement had not occurred in 2 patients of the original 11, in whom CP eradication was not achieved.

In vitro Studies

Bismuth sensitivities (table II, IV): The species of bacteria tested and the MIC for each is given in table II. CP was the organism most sensitive to bismuth compounds, its growth was inhibited by bismuth citrate, De-Nol® and Pepto-Bismol® at concentrations of 25 mg/l or less (table IV). Other campylobacters were also very sensitive to bismuth compounds. Moderate sensitivity was exhibited by bacteroides and *Clostridium difficile* (table II).

Fecal bismuth was measured by atomic absorption spectrophotometry in 2 patients taking standard CBS therapy of 1 tablet q.i.d. (108 mg elemental bismuth/tablet). In both cases the concentration of elemental bismuth was greater than 4 mg/g of feces.

Cimetidine and ranitidine sensitivities (table IV): All CP isolates tested were comparatively resistant to cimetidine, inhibition being noted only at concentrations of 400 mg/l and above. There was no difference between the tablet and the intravenous preparation of cimetidine. Ranitidine showed no inhibitory activity for any of the isolates at the concentrations tested.

'Bismuth mirrors': This interesting incidental phenomenon was noted during preliminary studies when discs containing 0.25 mg of bismuth citrate were placed on plates inoculated with CP or other organisms. In most cases, reflective mirrors, presumably of metallic bismuth, developed in a 10-mm radius circle around the disc. Direct contact with the bacteria was not necessary for this phenomenon to occur. If a bismuth citrate disc was placed on a sterile plate of bacteriological agar and this plate was incubated upside down over a bacterial culture, the mirror still developed on the sterile plate.

Table IV. Sensitivity of 12 CP isolates to CBS, bismuth subsalicylate, cimetidine and ranitidine

Number of isolates inhibited by	Drug concentration, mg/l					
	6.5	12	25	200	400	>400
CBS	4	8				0
Bismuth subsalicylate	1	9	2			0
Cimetidine tablets	0	0	0	0	4	8
Cimetidine liquid	0	0	0	0	4	8
Ranitidine liquid	0	0	0	0	0	12

Table V. Ultrastructural localization of bismuth

Drug	Time min	Detachment from epithelial cells	Sub. memb. Bi deposits in CP	CP lysis and large Bi granules
CBS liquid 5 ml	30	−	+	−
CBS liquid 5 ml	60	−	++	+−
CBS liquid 10 ml	70	++	++	+++
CBS liquid 10 ml	95	+++	++	+++
CBS tablet	40	−	+	−
CBS tablet	70	+	++	++
CBS tablets 1 q.i.d.	24 h, no intact bacteria, subcellular fragments only			
CBS tablets 1 q.i.d.	24 h, no intact bacteria, subcellular fragments only			
Bismuth subsalicylate 30 ml	80	+	++	++
Bismuth subsalicylate tablets ×4	150	+	+	+

The best mirrors were produced by *Aeromonas hydrophila*. Mirrors did not develop on plates which were not in connection with a bacterial culture. We invite comment on the origin and significance of this phenomenon.

Ultrastructural Localization of Bismuth (table V)

In all biopsy specimens for electron microscopy taken prior to administration of bismuth compounds, moderate to large numbers of flagellate CP organisms were present (fig. 5a). They were located deep to the mucus layer, in intimate relation to the luminal surfaces of the antral epithelium. Where bacterial colonization was heavy the related epithelium invariably showed irregular flap-like or smooth bulging protrusions, and partial to complete loss of the surface microvilli.

The sectioned bacteria (fig. 5b, c) appeared as curved rod-like or spiral profiles

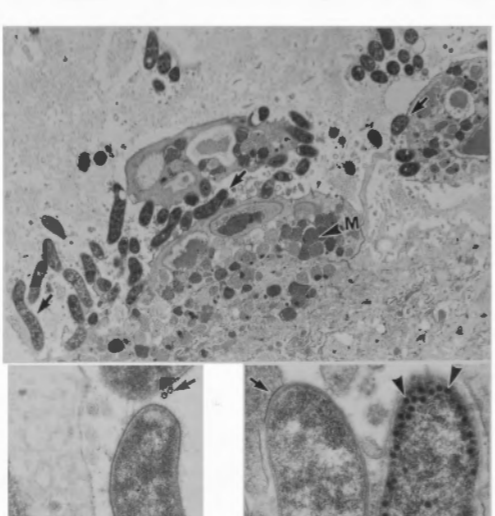

Fig. 5. Baseline pretreatment appearances of CP bacteria on the gastric antral mucosa. **a** Numerous curved and rod-like bacterial profiles (arrows) in close relation to bulging epithelial cell surfaces depleted of microvilli. × 5,400. **b** Detail view of a typical intact CP organism; note the close-fitting cell wall, rounded ends, and two sectioned sheathed flagella (arrow). × 30,000. **c.** Bacteriophage-like particles (arrowheads) within a CP organism, as found in antral tissue from 2 cases. Also note the point of intimate bacterial adherence to epithelium at upper left (arrow). × 40,000.

with bluntly rounded ends; some dividing forms were often noted. With rare exceptions, the individual organisms were structurally intact, with smooth cell walls closely adhering to the cytoplasmic membrane, and enclosing a relatively homogeneous cytoplasm comprising dispersed bacterial ribosomes together with focal pale areas containing sheaves of nuclear fibrils.

An incidental but interesting finding, in 2 cases only, was the presence in some intact bacteria of intracellular bacterophage-like particles, about 40 nm in diameter (fig. 5c). Remnants of very occasional lysed organisms adjacent to apparently released phage particles were also found.

In the two biopsy specimens obtained at 30 and 40 min after oral CBS was taken, no obvious changes in the appearance, location, or numbers of bacteria were detected

In the 4 patients biopsied between 60 and 95 min after ingestion of liquid or tablet CBS, marked changes were evident in the organisms and their relationships to the epithelium. Firstly, they were now mostly within the gastric mucus layer, rather than beneath it, having evidently lost ahderence to the antral epithelium. Secondly, a majority of the bacterial profiles were irregular or fragmenting; showing degrees of structural degradation ranging from focal vacuolation beneath the cell wall, to gross vacuolation with retraction and condensation of the bacterial contents (fig. 6a, b). Smaller lysed remnants were also present as coiled cell-wall and flagellar fragments. Thirdly, deposits of extraneous electron-dense material, ranging from minute particles less than 6 nm, in diameter to large irregular aggregates of more than 250 nm, were present on the external surfaces and to a lesser extent within the damaged bacteria (fig. 6a, b). No such deposits were visible on nearby gastric epithelial cell membranes or their microvilli. Identical dense particles, and aggregates, have previously been reported ultrastructurally and characterized as 'bismuth complexes', demonstrable in the gastrointestinal tract (but, interestingly, not in the stomach) of rodents and human subjects after oral dosage with CBS preparations [17].

In our 2 patients receiving bismuth subsalicylate, biopsied 80 and 150 min later, similar changes in bacterial location and morphology were present, accompanied by bismuth complex deposition; but the proportion of degraded organisms appeared rather less than in the CBS-treated cases.

Later inspection of unstained thin sections (omitting lead and uranyl acetate enhancement) disclosed traces of small particulate bismuth complex inside many of the still intact bacterial cells (fig. 6c): it was revealed as rows or clusters, in the intermembranous plane between the cell wall and the cytoplasmic membrane. The same feature was demonstrable in unstained sections of biopsy specimens taken at the earliest time point (30 min after CBS dosage) and in which the routinely stained sections had revealed no gross signs of bacterial injury or change. No similar particulate deposits, or aggregates, were demonstrable in biopsy specimens taken prior to medication with the bismuth compounds.

In the specimens obtained 24 h after commencing CBS therapy, CP and deposits of bismuth complexes were no longer found, apart from possible fragments of bacterial debris in the mucus layer. The antral epithelium was still irregular, and depleted of mucin granules and microvilli. On further biopsy of 1 of these cases, obtained 4 weeks later, there was evident restoration of nor-

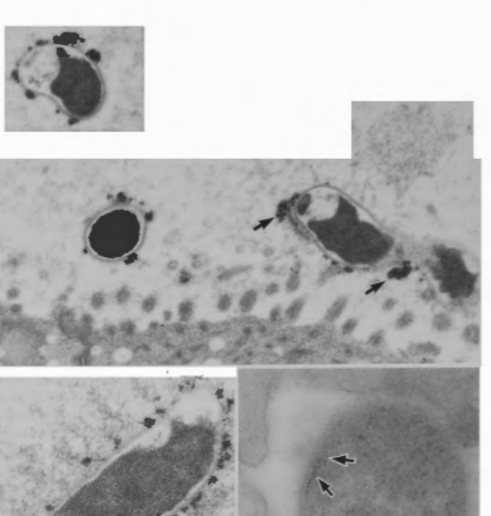

Fig. 6. Appearance of CP bacteria as found in biopsy specimens between 30 and 150 min after oral De-Nol®. **a** Deformed and vacuolated CP with opaque aggregates of bismuth complex deposited on the cell walls (arrows): biopsy 70 min after a single dose of liquid De-Nol®. × 24,000. **b** Typical longitudinal profile of structurally degraded organism, free in the gastric mucus 95 min after commencing De-Nol® treatment. Note irregular retraction of bacterial contents, surrounding cavitation, and external bismuth deposits. Compare with figure 5b. × 40,000. **c** Focal penetration of particulate bismuth complex (arrows) under the cell wall of a residual (still intact) organism 60 min after De-Nol® liquid ingestion. Section is unstained. × 75,000.

mally-aligned epithelium with well-developed microvilli.

In each patient a duodenal cap biopsy specimen was examined for presence of CP and bismuth complex deposition. The bacteria were not seen unless gastric rather than duodenal epithelium was present. In such cases the appearances paralleled those in the accompanying antral specimen. On true duodenal epithelium no CP were seen. In some cases small particulate deposits of bismuth complex were detected within the glycocalyx or between the long intestinal-type microvilli, as previously reported by Coghill et al. [17]; but there were no large deposits as noted in apparently selective association with the CP in the stomach.

Discussion

Our in vitro studies demonstrate that bismuth subcitrate is highly active in inhibiting growth of Campylobacters, particularly CP. As shown in table II, bismuth subcitrate could also perhaps be used in the treatment of *C. difficile* infection and cholera. The fecal bismuth concentration found in patients taking regular doses of CBS indicates that any gut organism with an MIC less than 4,000 mg/l could be vulnerable to the drug.

Although we used different CP isolates for the bismuth citrate MIC studies, very similar MICs were obtained for CBS liquid and bismuth subsalicylate liquid, all being inhibitory to CP organisms at a concentration of 25 mg/l or less. Disc tests [12] have shown that bismuth subnitrate (Roter®) tablets are similarly active. It is possible therefore that all soluble bismuth compounds are effective in inhibiting Campylobacter growth.

The 'bismuth mirrors' observed during our in vitro studies led us to suspect that bismuth might similarly precipitate in the human stomach colonized with CP organisms. In rat studies, bismuth concentrations are higher where epithelium is colonized by other types of spiral bacteria, e.g. the cecum [18, 19]. In our ultrastructural studies, both CBS and bismuth subsalicylate appeared to exert a rapid antimicrobial effect against CP. In the first hour after ingestion, small amounts of bismuth complex had evidently entered the bacteria and were demonstrable beneath the bacterial cell wall. At 70 min many organisms had lysed and the remaining organisms mostly showed degenerative changes with ballooning of the cell wall. At this time also, larger deposits of a bismuth complex were present adhering selectively to the bacterial cell walls and to subcellular bacterial debris in the gastric mucus. Such deposits were not seen in duodenal mucus, or in gastric mucus in the absence of CP organisms.

In vivo as well as in vitro therefore, CP bacteria seem to be associated with precipitation of a soluble bismuth complex from the surrounding mucinous environment. The apparent concentration of bismuth complexes in the vicinity of the organisms might be explained by an altered pH immediately adjacent to the bacteria. CP are powerful urease producers and in the presence of extracellular urea can be expected to generate ammonium ions [20]. CBS is more soluble in an alkaline medium [21] and precipitates in acid. Sudden reversion to a lower pH after CP organisms become metabolically inactive could explain the selective precipitation of bismuth complex we have described.

The mechanisms underlying the observed antibacterial effects of CBS and other bismuth compounds, in vitro and in vivo, have

yet to be clarified. After oral administration of CBS the rapid onset of lytic changes amongst bacteria on the gastric mucosa and their subsequent disappearance within 24 h, suggests a direct bactericidal action. However, bismuth is in the same chemical group as arsenic in the table of elements and, like the trivalent arsenicals [22], CBS could perhaps inactivate bacterial enzymes. Thus, the blocking of some vital metabolic functions (even temporary impairment of which might render the organisms more suceptible to other more lethal influences in the gastric environment), might equally account for the observed clearance of CP from the stomach. Indirect support for such a concept comes from in vitro studies in progress showing that CP organisms inactivated by exposure to growth-inhibiting concentrations of CBS can remain viable for several hours and are capable of resuming normal growth on transfer to a CBS-free medium [Armstrong et al., unpubl.].

Our in vitro findings are paralleled by clinical experience. In patients treated with CBS alone, less CP bacteria were demonstrable on histology or by culture. In these patients the gastritis improved suggesting that CP organisms were responsible for the epithelial cell changes and inflammatory infiltrate, as proposed in recent reviews [23, 24]. An alternative hypothesis that bismuth compounds are anti-inflammatory has little evidence to support it at this stage. When therapy ceased the bacteria usually returned and the gastritis worsened. An exception was the 80-year-old man in whom CP was effectively eradicated by CBS therapy alone (fig. 4). In this patient the mucosal histology continued to improve even after CBS therapy ceased. In contrast, patients treated with cimetidine showed no detectable change in the numbers of CP present, or in the severity of the histological lesions.

On CBS therapy, mucus production increases and the gastritis resolves during treatment. After a peptic ulcer has been healed with CBS therefore the gastric or duodenal epithelium in its vicinity can be expected to revert to normal levels of mucinogenesis, with reduced inflammation. In most cases, however, the bacteria will soon return when the CBS is discontinued. The histological appearance will again deteriorate, mucinogenesis will be reduced, and the mucosa become once more suceptible to the erosive effects of acid. By the same token, if CBS therapy has completely eradicated CP and the epithelium remains histologically within normal limits, ulcer relapse would not of course be expected.

This proposed sequence may explain the difference in relapse rates observed between CBS and the H_2 receptor antagonists [3, 4]. Duodenal ulcer disease has a relapse rate of 80% per annum as seen in, for example, a cimetidine-treated group [25]. In most patients treated with CBS alone the bacteria are suppressed, but not eradicated completely, so relapse still occurs. However, relapse is slightly delayed due to the temporary improvement in the histology. In addition, a few patients taking CBS are indeed cleared of the bacteria and do not relapse. In this model, if 20% of the patients treated with CBS alone are permanently cleared of CP, the 12-month relapse rate for DUs so treated would be 50–60% [3, 4].

We naturally were disappointed to find that CBS alone usually did not eradicate CP. Other investigators have also reported difficulty in eliminating the organism. In a study of a similar group of patients, Langenberg et al. [26] observed that long-term clearance of

CP was uncommon with either CBS alone or amoxycillin 500 mg t.d.s. for 21 days. As our study involved patients with ulcer disease we chose to use CBS as the ulcer-healing agent, in combination with the antibiotic. In this way, patients received effective ulcer therapy and were taking two drugs which were both inhibitory to CP. By using tinidazole and CBS as the first-line therapy, and treating failures or resistant organisms with CBS plus amoxycillin, we were able, seemingly, to eradicate the organisms from the stomach in 9 of 11 patients (two treatment failures were excluded from analysis, their histology did not change). As expected, healing of gastritis was long-lasting once CP had been eradicated. Five of the patients have been rebiopsied 12 months after treatment and no relapse of CP infection or of histological gastritis has yet occurred.

We have demonstrated that it is possible with combination therapy to eradicate CP infection and heal gastritis. It will now be possible to test the hypothesis we have advanced, that CP-associated gastroduodenitis is the mucosal defect underlying peptic ulcer disease. Whether CBS alone, antibiotics alone, or combinations of the two are used is largely irrelevant to this hypothesis. We predict that any ulcer drug which fails to eliminate CP colonization of the gastric mucosa will be ineffective in preventing ulcer relapse when the medication is discontinued.

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