been interpreted as indicating that unknown intraluminal or mucus layer factor(s) might interfere with antimicrobial action. A recent report raises the possibility of antimicrobial resistance consequent on previous suboptimal exposure,[9] which emphasises the need for an effective treatment regimen with a lower relapse rate.

Administration of bismuth subsalicylate (BSS) results in a high rate of *H pylori* eradication (75% eradication after four weeks), comparable to that of bismuth subcitrate (CBS).[10] As the subsalicylate salt is insoluble in the stomach and peak plasma concentration does not occur until 1–2 hours after ingestion, absorption must occur in the duodenum or jejunum. In contrast, CBS is a very soluble salt and is rapidly absorbed from the stomach, and the peak plasma bismuth concentrations occur 0·25 to 0·5 hour after the dose.[11] The significant eradication of *H pylori* by BSS tends to suggest that the anti-*H pylori* effects are due to absorption of bismuth from the duodenum and subsequent exposure by way of systemic delivery. This is logically consistent with histological evidence which indicates that *H pylori* inhabit the deeper part of the gastric mucus film and often insinuate themselves between the epithelial cells[7 12] sequestering themselves from gastric juice by sitting deep to the mucus layer.[1] This intimate adherence of the bacteria to the gastric epithelial cells and its histopathological implications for disease production has been shown in a recent study.[13] Systemic delivery only requires good contact with gastric secretion or interstitial fluid or diffusion into the gastroduodenal lumen.

A practical extension of this hypothesis is that antimicrobial treatment should be given systemically to yield improved results (Figure). Currently effective treatments (amoxicillin, erythromycin, tetracycline, or metronidazole) might be given intravenously initially for several days before continuing with oral treatment. CBS can be given orally since a high initial postabsorptive plasma peak occurs particularly with the tablets, indicating rapid absorption at the gastric site[11 14] hence, of necessity, contact with *H pylori*. Enterohepatic circulation of bismuth[15 16] could be of benefit as it would effectively prolong the exposure time of the bacteria to agents in the circulation.

We believe that this issue of luminal gastric drug delivery *v* systemic delivery is of critical importance for drug formulation and overall treatment strategy.

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Diabetes and cholelithiasis

SIR,—We read with interest the study by Laakso *et al* on serum lipids and lipoprotein in patients with non-insulin diabetes and with gall stone disease.[1] The association between diabetes and cholelithiasis has been defined also in the Italian population,[2 3] but the reason for this association has not yet been clarified. It is generally held that patients with diabetes secrete a more lithogenic bile than non-diabetics.[4] In the few studies that have compared diabetics with age, sex, and weight matched controls, however, neither the insulin dependent diabetics nor the insulin independent diabetics had a bile cholesterol saturation index higher than that of control subjects.[5] Therefore, the secretion of a lithogenic bile by diabetics does not satisfactorily explain the observed frequency of gall stones in the diabetic population.

In one of our previous reports[6] on 120 patients affected by type II diabetes we too observed that in diabetics with gall bladder disease fasting plasma insulin concentrations and daily average plasma insulin concentrations were appreciably higher than in diabetics without gall bladder disease. But we failed to show any differences in serum total and high density lipoprotein cholesterol, triglycerides, glycated haemoglobin HbA_1, body mass index, and duration of diabetes between patients with gall stones and those without gall stones. In our study, in non-insulin dependent diabetes increased plasma insulin concentrations seemed to be associated with an increased risk of gall stones regardless of plasma triglycerides, plasma cholesterol, and obesity.

We believe, however, that it is necessary to take into account the effects of diet and, above all, prolonged use of oral hypoglycaemic agents which may bias the interpretation of plasma lipid pattern and the level of insulinaemia. Furthermore, it is likely that the patient's awareness of his gall stone condition (particularly if it is symptomatic) may cause pronounced changes in eating habits. In our study, in fact, all the patients were being treated with oral hypoglycaemic agents in addition to a restricted diet.

Because cholesterol gall stones are generally thought to be the result of an altered lipoprotein metabolism information about the role of blood lipids in gall stone disease may provide indirect clues about the changes in lipid metabolism that are associated with their formation. But considerable controversy exists about the relation between plasma and biliary lipid compositions. Moreover, it is now generally recognised that a simple concept of lithogenic bile is inadequate to account for cholesterol gall stone development.

We agree with Laakso *et al* that changes in plasma insulin and serum lipid concentrations do not account for increased prevalence of gall stone disease in non-insulin diabetes. Abnormal gall bladder motility may play an important part in the pathogenesis of gall stone disease in these patients. Recently, impaired emptying of the gall bladder in diabetics was shown and the presence of an autonomic neuropathy seems to be a risk factor for such an impairment.[6]

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Reply

SIR,—Pazzi *et al* have shown an association between high insulin concentration and cholelithiasis in non-insulin dependent diabetes similar to what we have reported.[1] In our study we also found a significant positive correlation between insulin concentration and very low density lipoprotein triglyceride concentration and a negative correlation between insulin concentration and high density lipoprotein cholesterol concentration. In contrast, Pazzi *et al* did not find any association between lipid and lipoprotein concentrations and gall stone disease. They proposed that the effects of diet and prolonged use of oral hypoglycaemic agents could explain the association of a high insulin concentration and gall stone disease. While we have presented similar explanations for this association, we have also proposed that a high insulin concentration in patients with gall stone disease is related to insulin resistance.[2] Indeed, our preliminary unpublished data show that

whole body insulin-mediated glucose uptake, evaluated by the euglycaemic clamp technique, tends to be lower in non-insulin dependent diabetic patients with gall stone disease than in diabetic patients without gall stone disease. Therefore, the risk of cholelithiasis could be in part due to defects in glucose metabolism. A high insulin concentration has been shown to be a good marker of insulin resistance,[2] and we have shown the association between insulin resistance and lipid and lipoprotein abnormalities in patients with non-insulin dependent diabetes.[3] Therefore, the correlation of a high insulin concentration with lipid and lipoprotein abnormalities which we found in patients with gall stone disease[1] is expected.

In any case, the possibility that a high insulin concentration is only a marker of impaired insulin-mediated glucose uptake in diabetic patients with gall stone disease is a novel finding which deserves further study. The relation of insulin resistance and gall stone disease is, however, of biological importance only if this association can be shown in non-diabetic subjects.

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Gall bladder epithelium and cholesterol gall stones

SIR,—The ultrastructural findings by Sahlin and colleagues[1] do not show any statistically significant increase in mucous secretory granules of the gall bladder epithelial cells in patients with cholesterol gall stones compared with control patients without gall stones but with polyps. Polyps of the gall bladder may be involved in the natural history of the gall stone disease,[2] and fragments of gall bladder mucosa taken from such patients do not seem to us appropriate control tissues. Sahlin *et al*, furthermore, do not say where 'a strip of the gall bladder wall was excised' and how many specimens were examined in each patient.

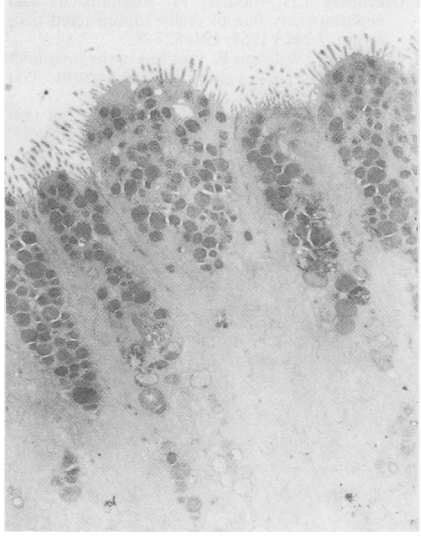

Figure 1: Ultrathin section uranyl acetate-lead citrate stained. An area of 'mucous metaplasia.' Many mucous secretory granules are recognisable in the apical part of the cells. Original magnification × 7000.

Figure 2: Ultrathin section stained with Thiéry's method. A mucus layer is suspended above metaplastic cells. Original magnification × 45 000.

We investigated ultrastructural morphology of gall bladder mucosa in 15 patients with cholesterol gall stones (data submitted for publication). Fragments of tissue were taken from the fundus, body, and neck. Ten minute specimens of about 1 mm^3 in diameter were obtained from each fragment. Transmission electron microscopy, using a specific method to stain glycoprotein and mucoproteins (Thiéry's method), showed at least one area of 'mucous metaplasia' (Fig 1) (similar to that which other workers have called gastric metaplasia) in at least one specimen from each patient. A layer of visible mucus was observed suspended only above the metaplastic areas (Fig 2), and intercellular spaces appeared much tighter in fully metaplastic epithelium than in the ordinary one, further indicating a progressive tendency to assume a secretory function. We suggested that the increase of mucous glycoproteins may be a zonal event: fully metaplastic zones may functionally represent 'nucleating areas' and the areas of gall bladder lumen near 'mucous metaplasia' might be the ones in which the onset of nucleation occurs.

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Immunological response to *Cryptosporidium* species

SIR,—The case report by Jacyna *et al* describing a patient with colonic *Cryptosporidium* infection and IgA deficiency, is informative.[1] Specifically, this report provides evidence that intestinal IgA antibody is important for protection against *Cryptosporidium* infection.

One comment by the authors – namely, that cell mediated immunity is the primary mechanism of host defence against cryptosporidiosis, is not well supported by currently available evidence. Clearance of an infection by cell mediated immunity means that effector cells such as macrophages or cytotoxic lymphocytes kill the responsible micro-organism. Recent studies with *Cryptosporidium*-infected mice indicate that clearance of the parasite is not inhibited by selective depletion of cytotoxic ($CD8^+$) T lymphocytes.[2] This observation argues strongly against a role for T cell mediated cytotoxicity in protection against *Cryptosporidium* infection, at least in mice. By contrast, selective depletion of helper ($CD4^+$) T lymphocytes prolongs murine cryptosporidiosis.[2] It can, therefore, be concluded that protective immunity against *Cryptosporidium* infection in mice is dependent on helper T lymphocytes, but this conclusion does not necessarily imply that the immunity is cell mediated.

Although it is unclear how $CD4^+$ T lymphocytes confer protection against *Cryptosporidium* infection, a testable possibility is that they do so by triggering a *Cryptosporidium*-specific antibody response in the intestine.[3] What is the evidence that antibody protects against *Cryptosporidium* infection? Besides the report by Jacyna *et al*,[1] several recent studies support the idea that protection against *Cryptosporidium* requires the presence of antibody. For example, enteral administration of bovine colostrum containing *Cryptosporidium*-specific antibodies can diminish the intensity of *Cryptosporidium* infection in human subjects and in calves.[4-6] In addition, the infectivity of *Cryptosporidium* sporozoites for mice can be reduced or eliminated by incubating the sporozoites with antibodies that bind to their surfaces.[7] This particular finding raises the possibility that protective *Cryptosporidium*-specific antibody acts mainly, or exclusively, against *Cryptosporidium* lifecycle stages that occur extracellularly, in the intestinal lumen (sporozoites and merozoites).[8] Unlike these extracellular stages, *Cryptosporidium* trophozoites are attached to intestinal epithelial cells, and are covered on their luminal aspect by an envelope of host cell origin.[9] Conceivably, this envelope may prevent luminal antibody from binding to *Cryptosporidium* trophozoites in vivo.

The lifecycles of *Cryptosporidium* and *Giardia* species have little in common with each other. None the less, evidence accumulated over recent years suggests that antibody plays a major part in the development of protective immunity against both these genera of intestinal parasite.[1 10]

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