*Bias in resulting estimate of distribution*

It is perhaps more surprising that any method of estimating the time of infection by a single time point will result in the estimation of a biased distribution of incubation time. The exact resulting perturbation of the distribution is difficult to predict but plainly, for example, the variance will be overestimated, as the times subject to analysis will have one variance component in themselves (the natural variability of incubation times) and one additional variance component from the estimation of the time of infection. This second source of variance must of necessity be greater than zero, as the true times of infection are unknown. Taylor *et al* tried to correct for this effect,[17] but we believe that the resulting distribution remains biased. We do not, however, know of any other method of correction.

*Fitting of distributions*

It is tempting to try to fit known distributional forms to data in order to stabilise estimates and be able to make predictions about the future course of infection. We are not convinced about the soundness of this approach. There is at present no way of choosing the correct distribution, and knowing or being able to estimate the left tail of the incubation times (in this study we barely reached the 10% level in the cumulative incidence of AIDS) does not carry much information about the central parts of this distribution.

We have checked the applicability of the often used Weibull distribution[4,5] as a description of the present data by graphical methods—plotting estimated log cumulative hazard against log time, a plot that should yield approximately straight lines if the Weibull distribution were appropriate—and found that time to the onset of AIDS could be thus described but not time to the occurrence of first symptom. The parametric fitting of Weibull distributions to the haemophiliac and transfusion recipient groups separately, for example, resulted in estimates of the corresponding median incubation times before the onset of AIDS, which were 13·4 (SE 2) and 5·7 (1) years, respectively.

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# Adhesive Escherichia coli in inflammatory bowel disease and infective diarrhoea

D A Burke, A T R Axon

## Abstract

**The clinical features of ulcerative colitis and Crohn's disease are similar to those of infections of the bowel, although their cause is uncertain. Many bacteria that cause intestinal diseases adhere to the gut mucosa, and adhesion of pathogenic Escherichia coli is resistant to D-mannose. The adhesive properties of isolates of E coli were assessed by assay of adhesion to buccal epithelial cells with mannose added. The isolates were obtained from patients with inflammatory bowel diseases (50 with a relapse of ulcerative colitis, nine with ulcerative colitis in remission, 13 with Crohn's disease, and 11 with infectious diarrhoea not due to E coli) and 22 controls.**

**The median index of adhesion to buccal epithelial cells (the proportion of cells with more than 50 adherent bacteria) for E coli from patients with ulcerative colitis in relapse was significantly higher (43%) than that for controls (5%) and patients with infectious diarrhoea (14%). The index was not significantly different among isolates from patients with ulcerative colitis in relapse, Crohn's disease (53%), and ulcerative colitis in remission (30%). If an index of adhesion of >25% is taken as indicating an adhesive strain 86% of isolates of E coli from patients with inflammatory bowel disease were adhesive compared with 27% from patients with infective diarrhoea and none from controls.**

**The adhesive properties of the isolates from patients with inflammatory bowel disease were similar to those of pathogenic intestinal E coli, raising the possibility that they may have a role in the pathogenesis of the condition; the smaller proportion of adhesive isolates in patients with infective diarrhoea due to other bacteria suggests that the organism may be of primary importance rather than arising secondarily.**

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## Introduction

Mucosal adhesion is a virulence factor that is expressed by pathogenic *Escherichia coli* in the intestine. Enterotoxigenic strains of *E coli* are recognised

pathogens, but in both animals and humans the production of enterotoxin alone is not sufficient to produce disease in all cases.[1,2] Intimate mucosal association is necessary for the pathogenicity of enterotoxigenic *E coli* to be fully expressed and such strains adhere to intestinal mucosa with the fimbrial antigens colonisation factor antigens I and II, which are plasmid mediated and whose adhesion is resistant to mannose.[3-6] The mechanisms whereby enteropathogenic *E coli* that does not produce toxin causes diarrhoea are not fully understood, but again adhesion that is resistant to mannose seems to be essential for pathogenicity.[7-12] Mucosal adhesion of *E coli* is also important in non-gastrointestinal disease, being found in strains causing infections of the urinary tract.[13]

Dickinson *et al*, using a HeLa cell technique, showed an adhesive property of *E coli* isolated from patients with ulcerative colitis,[14] and this observation has been confirmed by an assay of adhesion to buccal epithelial cells.[15] It is unclear, however, whether these adhesive organisms are primary pathogens or are secondary to the disease, perhaps arising as a result of altered transit through the bowel or inflamed host tissue.

We assessed the prevalence of *E coli* showing mannose resistant adhesion in four groups of patients: one group with ulcerative colitis, one with Crohn's disease, one with infective diarrhoea, and a control group.

## Patients and methods

*E coli* was isolated from the stools of 50 patients who presented with a relapse of ulcerative colitis before treatment of the episode, nine with ulcerative colitis in remission, 13 with Crohn's disease, 11 with infectious diarrhoea (one with salmonellosis and 10 with campylobacter enterocolitis), and 22 controls. The controls comprised staff and patients who were not receiving any treatment and did not have evidence of inflammatory bowel disease. One colony of *E coli* was chosen at random from each patient to be tested for adhesion. Ten colonies in all were kept from each patient for future analysis. All isolates were stored on slopes of Dorset egg medium and protected from light. *E coli* E851/71, a non-toxigenic enteropathogenic strain that adheres to Hep 2 cells (originally isolated from a patient with infantile diarrhoea),[16] and *E coli* SC13, a recognised non-adhesive strain, were included as standard strains.

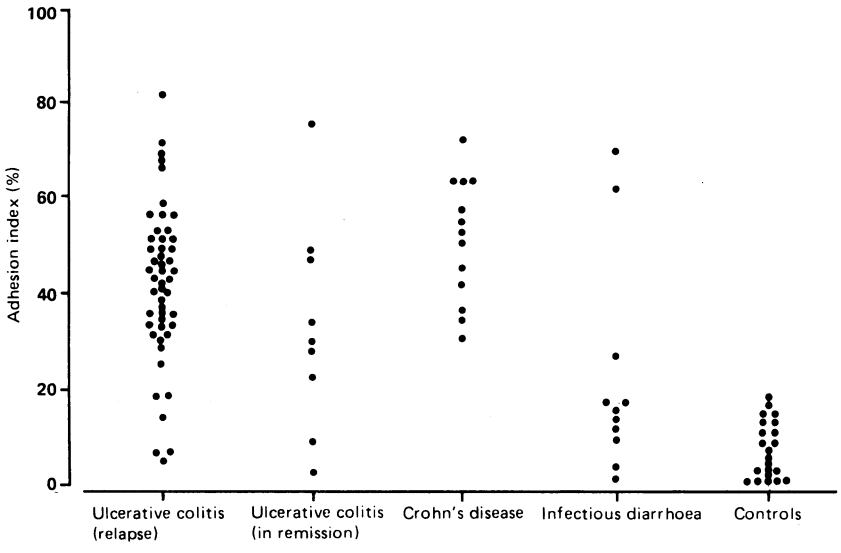
Adhesion was determined by assaying adherence of the isolates to buccal epithelial cells. Isolates were independently coded and grown for 18 hours at 37°C on slopes of Oxoid blood agar base No 2. Buccal epithelial cells were obtained from a single donor by gently scraping the buccal mucosa with a sterile spatula. The cells and bacterial cell suspensions of bacteria were incubated together as previously described at room temperature in the presence of D-mannose.[15] After incubation the cells were harvested by filtration and washing over a 5 μm millipore filter (Nucleopore). An impression smear was made on to a clean glass slide, dried in air, fixed in methanol, and stained by Gram's method. A control comprising epithelial cells without added bacteria was included in each assay.

An index of adhesion was derived by inspecting 100 non-overlapping epithelial cells in at least 10 high power fields and recording the number with more than 50 adherent Gram negative rods minus the number of such cells present in the control. Results are expressed as the proportion of buccal cells with more than 50 adherent bacteria. Statistical analysis was by the Mann-Whitney U test.

## Results

The figure shows the index of adhesion of the *E coli* isolates. The median index of *E coli* isolated from patients with a relapse of ulcerative colitis was significantly higher (43%, range 5-81%) than that for controls (5%, 0-16%; $p<0.001$) and infectious diarrhoea (14%, 0-68%; $p<0.005$) but not significantly different from that of isolates from patients with Crohn's disease (53%, 30-71%) and ulcerative colitis in remission (30%, 2-75%). There was no significant difference between the index of adhesion of *E coli* isolated from patients with a relapse of ulcerative colitis who were receiving sulphasalazine (n=22) and those who were not (n=28). The mean index of adhesion of the standard control strains, E851/71 and SC13, was 47% and 6%, respectively.

Previous workers have taken an index of adhesion to buccal epithelial cells of >25% to differentiate between adhesive and non-adhesive strains.[17] By this criterion 86% of our isolates from patients with inflammatory bowel disease were adhesive strains compared with 27% from patients with infectious diarrhoea and none from the controls.




*Adhesion index (proportion of buccal epithelial cells showing >50 adherent Gram negative bacteria in presence of D-mannose) of isolates of E coli from patients with inflammatory bowel disease and controls*

## Discussion

The clinical features of ulcerative colitis and Crohn's disease in relapse are similar to those of dysentery and other infectious colitides.[18-21] The intimate association of micro-organisms with the colonic mucosa and the difficulties in inducing experimental colitis in germ free animals suggest that bacteria may have a role in the pathogenesis of inflammatory bowel disease.[22] Several studies in which the proportion of *E coli* to other viable bacteria was compared in inflammatory bowel disease and controls have reported conflicting results.[23-26] Indeed, diarrhoea from any cause may alter the relative proportion of organisms in the faeces.[27] A more relevant approach is to look for qualitative differences between organisms isolated from patients and controls.

Our finding that a significant proportion of patients with inflammatory bowel disease harboured in their stools *E coli* with an adhesive property resistant to mannose that was reminiscent of that expressed by recognised pathogenic *E coli* suggests that this organism may have a role in the pathogenesis of inflammatory bowel disease. An alternative explanation is that it was acquired secondarily. The difference in the proportion of adhesive *E coli* present between the group with inflammatory bowel disease and the

group with campylobacter enteritis, which also gives rise to intestinal inflammation, does not support this view. Our observation cannot necessarily be extended, however, to other infectious diarrhoeal diseases. In a previous study the proportion of patients carrying adhesive *E coli* was higher during a first attack of idiopathic colitis than during relapse.[28] If the disease itself was the underlying cause for the presence of adhesive *E coli* the reverse might have been expected.

All bacteria were grown and subcultured in vitro, so the adhesive property is not likely to be a transient effect induced by a factor within the gut lumen. The adhesive mechanisms, both fimbrial and afimbrial, described for recognised pathogenic intestinal *E coli* are genetically controlled: colonisation factor antigens I and II of enterotoxigenic *E coli* and the enteroadhesive factor of enteropathogenic *E coli* are mediated by plasmids.[3 29 12] This raises the possibility that the adhesive *E coli* in patients with inflammatory bowel disease acquire the property from some other source. There are anecdotal reports of inflammatory bowel disease occurring after infection with recognised intestinal pathogens.[30-32] Plasmids coding for virulence factors have been transferred among different strains of *E coli* and from other pathogenic intestinal bacteria to *E coli*.[3 33-35] Such transfer to commensal *E coli* in vivo might result in the expression of virulence antigens.

We excluded bacterial adhesion by type 1 fimbrias in this study by including mannose in the adhesion assay and by growing the *E coli* on a solid medium.[36] There is no evidence to suggest that fimbrial adhesions sensitive to mannose are associated with the pathogenicity of intestinal *E coli* in humans.

Controversy exists as to whether Crohn's disease and ulcerative colitis are separate diseases. Interestingly, we found adhesive *E coli* in similar proportions of patients with both conditions.

There is a long held view that infectious agents may have a role in the pathogenesis of inflammatory bowel disease. Several workers have shown that isolates of *E coli* obtained from patients with ulcerative colitis can degrade mucins and produce necrotoxins and haemolysins to a greater extent than those from control subjects.[23 37] The qualitative differences between *E coli* from patients with inflammatory bowel disease and the normal faecal flora observed in this and previous studies suggest that these strains may have a role in the pathogenesis of the disease.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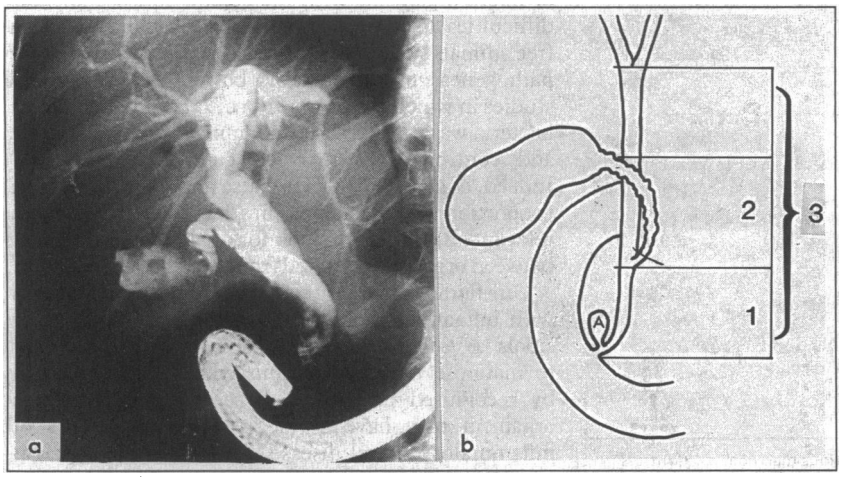
## Correction

**Does low entry of cystic duct predispose to stones in the common bile duct?**

We regret that the radiograph in this article by Professor P C Bornman and others (2 July, p 31) was left out of some copies of the *BMJ*. The illustration was correct when passed for press but fell off during the prepress production stage.




(a) *Left sided entry of cystic duct at level of ampulla of Vater.* (b) *Measurements of segments of bile duct: 1 ampulla of Vater to entry of cystic duct, 2 contiguous segment of cystic duct and common hepatic duct, and 3 total length of bile duct. A=Ampullary diverticulum*