Increased adhesion of *Escherichia coli* to mucosal cells from infants with protracted diarrhoea: a possible factor in the pathogenesis of bacterial overgrowth and diarrhoea

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SUMMARY Mucosal adhesion of bacteria has been studied in eight infants with protracted diarrhoea and malnutrition, using a buccal epithelial cell technique. A known non-adhesive strain of *Escherichia coli* (O1:K1:H7) adhered to a significantly greater ($p < 0.001$) proportion of buccal epithelial cells from patients with protracted diarrhoea, compared with children with acute diarrhoea, healthy infants, and healthy adults. Also, Enterobacteria isolated from the jejunum or stools of patients with protracted diarrhoea adhered to far greater numbers of their own buccal epithelial cells compared with healthy adults. These results suggest that bacterial adhesion may play an important role in the pathogenesis of protracted diarrhoea.

Diarrhoea is one of the leading causes of mortality and morbidity in young children throughout the world, particularly in the developing countries where the diarrhoea/malnutrition interaction is so common.¹⁻⁶

Although the pathophysiological mechanisms operating in the genesis of the diarrhoea are poorly understood, bacterial overgrowth of the small intestine in such patients is common.²⁻⁷ Adhesion of bacteria to the mucosal surface is probably an essential prerequisite to their proliferation within the small intestine and therefore to their capacity to induce disease.⁸

In this paper, we report our findings of studies of adhesion of a previously studied strain of *E coli*⁹ to buccal epithelial cells from infants with protracted diarrhoea and malnutrition. The preliminary results have been included in a previous communication.¹⁰

Methods

SUBJECTS

Protracted diarrhoea is defined as the passage of four or more watery stools per day for a period of more than two weeks during which time there is no

weight gain or loss of weight.¹¹

Eight patients with protracted diarrhoea aged between three and 44 weeks (mean age on admission 21 weeks) were studied in whom investigations failed to reveal an established cause for the diarrhoea. All were below the third centile for weight and two of the patients subsequently died (Table 1). Jejunal biopsies were normal in four, the fifth had partial villous atrophy; biopsies were not performed in the remaining three patients. Anaerobic and aerobic cultures of jejunal fluid were performed in four patients and all had viable counts of 10^5 /ml or more of Enterobacteriaceae.

In order to control the study three other groups of subjects were studied: (1) Ten children aged one to 22 months (mean 8.4 months) who developed acute diarrhoea. Serotypable strains of *E coli*¹² were isolated from the stools of each of the 10 children; viruses were not visible on electronmicroscopy in any of the patients. (2) Thirteen healthy infants aged four to 24 months (mean 9.2 months) who were attending an infant welfare clinic. (3) Ten healthy adults.

ADHESION ASSAY

Bacterial adhesion to buccal epithelial cells was studied by a modification of the method of Ofek *et al*¹³ as described by Candy *et al*¹⁴ and the percentage of buccal epithelial cells with adhering gram-

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* Since this paper was submitted Professor Harries has unfortunately died.

Table 1 Clinical details, jejunal mucosal morphology, and jejunal fluid culture results in the infants with protracted diarrhoea

Patient	Age (weeks)		Weight on admission (kg)	Jejunal biopsy	Jejunal fluid culture		Age (months) at discharge or death*
	Onset	Admission			Coliform species	Viable count (organisms/ml)	
1	2	3	2.3	N	<i>E coli</i>	4.3×10^6	25 ⁺
2	2	20	3.3	N	<i>P mirabilis</i>	1.4×10^5	29
3	3	44	7.5	N	<i>E cloacae</i>	1.4×10^5	13
4	17	40	7.4	N	—	—	11
5	24	25	4.2	PVA	<i>E coli</i>	1.0×10^5	8
6	1	1	2.8	—	—	—	5 ⁺
7	20	33	3.5	—	—	—	9
8	4	4	2.9	—	—	—	2

N = normal. PVA = partial villous atrophy.

negative bacilli was recorded by examining 100 cells by light microscopy. In all experiments a 'cell control' was included in which the buccal epithelial cells were incubated in the absence of bacteria.

BACTERIA

E coli O1:K1:H7 which does not adhere to human fetal intestine,⁹ *E coli* (untypable), cultured from the upper small intestinal juice of case 1, *Proteus mirabilis* and *E cloacae* from cases 2 and 3 respectively, and *E coli* serogroups O111 and O119 cultured from the stools of cases 4, 6, 7, and 8 were studied (Table 2).

E coli O1:K1:H7 was studied with buccal epithelial cells from both the patients and the other three groups. The bacteria isolated from the patients with protracted diarrhoea were studied with buccal epithelial cells from the patients and from a healthy adult volunteer.

Table 2 Adhesion of Enterobacteria isolated from infants with protracted diarrhoea

Patient	Enterobacteria	% Adhesion	
		Patient†	Adult‡
1	<i>E coli</i> (non-agglutinable)*	86	52
2	<i>P mirabilis</i> *	98	59
3	<i>E cloacae</i> *	90	4
4	<i>E coli</i> O111	100	9
5	<i>E coli</i> O119	60	9
6	<i>E coli</i> O111	83	NT
7	<i>E coli</i> O1, O111	9	0

* Jejenum isolates.

† Tested with buccal epithelial cells from patient from whom the Enterobacteria were isolated.

‡ Tested with buccal epithelial cells from a healthy adult (different adult donor used in each test).

NT = not tested.

HAEMAGGLUTINATION TESTS

Enterobacteria were screened for colonisation factors I and II (CFA/I and CFA/II) by mannose-resistant haemagglutination of species-appropriate erythrocytes¹⁵ and for Type I fimbriae by mannose-sensitive agglutination of guinea pig erythrocytes.¹⁵

STATISTICAL METHODS

The significance of differences between results in the various groups studied was assessed using the χ^2 test.

Results

ADHESION OF *E COLI* O1:K1:H7 (Figure)

E coli O1:K1:H7 adhered to 18–96% of buccal epithelial cells from the infants with protracted diarrhoea, 0–24% from those with acute diarrhoea, 0–41% of healthy infants, and 0–8% of healthy adults. The proportion of subjects with *E coli* adhering to 25% or more buccal epithelial cells was seven of eight with protracted diarrhoea, none of 10 with acute diarrhoea, two of 13 healthy infants, and none of the adults. Thus this known non-adhesive strain of *E coli* adhered to 25% or more of buccal epithelial cells from a significantly greater proportion of infants with protracted diarrhoea compared with each of the other groups ($p < 0.001$).

ADHESION OF ENTEROBACTERIA ISOLATED FROM THE JEJUNUM OR STOOLS OF INFANTS WITH PROTRACTED DIARRHOEA (Table 2)

Six of the seven strains of Enterobacteria adhered to 50% or more of buccal epithelial cells from the patients from whom they were tested. Bacteria from six of the patients were then tested with buccal epithelial cells from healthy adult donors, and only two of the strains adhered to more than 50% buccal epithelial cells.

It is tempting to speculate that malnutrition and/or genetic predisposition may influence the adhesive properties of mucosal cells, and explain why some children develop protracted diarrhoea after an acute enteric infection.

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