## 59 PATTERNS OF HEp-2 CELL ADHERENCE OF DIARRHOEAGENIC ESCHERICHIA COLI

S Knutton and A S McNeish
Institute of Child Health, Birmingham, U.K.

In 1979 Cravioto et al. showed that most enteropathogenic E. coli (EPEC) strains adhered to cultured HEp-2 cells whereas non-EPEC strains rarely adhered. Two patterns of adherence were described. Most EPEC adhered in localised microcolonies (LA) although a few displayed a diffuse pattern of adherence (DA). A new pattern of adhesion in which bacteria assume a characteristic 'aggregative' pattern (AA) both on the HEp-2 cell surface and between cells has recently been described in diarrhoeagenic E. coli of non-classical EPEC serotypes. To study mechanisms of adhesion we examined by electron microscopy the attachment to HEp-2 cells of strains exhibiting LA, DA and AA. LA was characterised by bacteria intimately attached to cuplike projections of the HEp-2 cell surface with associated disruption of the apical cell cytoskeleton and loss of cell surface microvilli; attachment closely resembling EPEC adhesion to intestinal mucosa. Strains exhibiting DA and AA did not show intimate attachment nor did they cause any alteration of cell surface architecture. A distinct halo surrounding bacteria and separating them from the HEp-2 cell surface was characteristic of AA strains. Specific staining showed DA to be capsule-mediated whereas AA was correlated with the production of specific rod-like fimbriae. These ultrastructural observations show that LA, DA and AA reflect different mechanisms of bacterial adhesion to HEp-2 cells and support preliminary evidence that AA is representative of a new class of diarrhoeagenic E. coli. Based on adherence properties, it would also appear that DA strains of classical EPEC serotypes may not be 'true' EPEC.

## 60 ENTEROPATHOGENIC E. COLI (EPEC) DISRUPT SMALL INTESTINAL BRUSH BORDER MEMBRANE $Na^+/H^+$ EXCHANGE

I.W. Booth, M. Dale, D.R. Lloyd, S. Knutton
Institute of Child Health, University of Birmingham, UK

Despite a highly specific morphological lesion of the small intestinal brush border membrane, the mechanisms whereby EPEC produce diarrhoea are unknown; mucosal invasion and toxin production are not thought to be important factors. We have therefore studied the effects of the rabbit EPEC strain, RDEC-1, upon $Na^+/H^+$ exchange at 60 sec in rabbit small intestinal brush border membrane vesicles (BBMV), following incubation at 37°C with RDEC-1. Before incubation, BBMV showed a 3.4-fold enhancement of $Na^+$ uptake in the presence of an outwardly directed $H^+$ gradient ($p<0.0001$), indicating intact $Na^+/H^+$ exchange. However, after 60 min incubation with RDEC-1; $H^+$-stimulated $Na^+$ uptake was markedly reduced to 43% of original enhancement ($p<0.01$) but reduced to only 90% in control BBMV (NS).

The possibility of non-specific BBMV damage by spontaneous acidification of the incubation media by RDEC-1 (to pH 4.9 from pH6.0) was investigated in control experiments, by manipulation of intra- and extra-vesicular pH to 4.9, in the absence of RDEC-1; $Na^+/H^+$ exchange remained intact. Depression of $Na^+/H^+$ exchange after 60 min incubation with a non-virulent strain of E. coli was not significantly different from controls.

These data indicate that EPEC disrupt brush border $Na^+/H^+$ exchange, which may play an important role in the pathogenesis of EPEC-diarrhoea.

## 61 INTERACTION OF STRAINS OF Salmonella typhimurium OF DIFFERENT VIRULENCE WITH ILEAL MUCOSAE in vitro.

DCA Candy, KJ Worton, TS Wallis, WG Starkey, AJ Spencer SJ Haddon, MP Osborne, J Stephen
Institute of Child Health, and Departments of Physiology and Microbiology, University of Birmingham, UK.

S. typhimurium strains TML and W118 isolated from humans with cholera-like diarrhoea invaded rabbit ileum in vivo and induced fluid secretion. Avirulent strains, LT7, M206 and SL1027, invaded without inducing fluid secretion; Thaxl was non-invasive and non-fluid secreting. We have tested strains of S. typhimurium of varying virulence for ability to interact with rabbit ileal mucosa in an asymmetric organ culture system to determine if differences in the degree of mucosal association could explain differences in virulence. Strains were also tested for motility and presence of flagellae. Mean(SEM) numbers of organisms (log cfu/g wet wght ileal mucosa) associated with the mucosa after 30min incubation and washing were: TML 6.17(0.17;n=15) W118 6.14(0.27;n=7) LT7 6.31(0.11;n=5) M206 6.14(0.11;n=5) SL1027 5.49(0.21;n=6 P vs TML<0.025) Thaxl 4.88 (0.15;n=7 P vs TML<0.0005). All strains except Thaxl had flagellae, and all except Thaxl and SL1027 were motile. Thus flagellae may enhance association of strains of S. typhimurium with ileal mucosa. When strains are motile association is further enhanced. Decreased association with ileal mucosa may explain in part the decreased virulence of strains SL1027 and Thaxl, but not LT7 and M206. Thus factors other than functional adhesins are involved in determining virulence of S. typhimurium.

## 62 NUTRITIONAL MANAGEMENT MODIFIES THE INTESTINAL PERMEABILITY CHANGES IN ACUTE GASTROENTERITIS.

E. Isolauri, M. Juntunen, S. Wiren, P. Vuorinen, T. Koivula. University of Tampere, Finland.

To study the effects of early home management of acute diarrhoea on intestinal permeability, 55 children aged 3-25 (mean 13) months with acute gastroenteritis (65 % rotavirus) were given 4 g lactulose (lacl) and 0.8 g mannitol (man) after oral rehydration. An aliquot of urine passed in the subsequent 5 h was preserved for sugar analysis by gas-liquid chromatography. The patients showed a significant increase in lacl/man excretion compared to 28 controls. This was due to decreased excretion of man whereas the differences in lacl were insignificant. The patients given uninterrupted feedings in addition to adequate fluid replacement prior to hospitalization (n = 21) had a normal lacl/man excretion, mean 0.03 with 95 % confidence interval (CI) 0.01-0.05 compared to those fasted with inadequate (n = 13) or adequate (n = 21) fluid replacement (mean 0.25, 95 % CI 0.15-0.44 and mean 0.12, 95 % CI 0.09-0.18, respectively) ; F = 19.58, $p < 0.001$. This was caused by increased excretion of lacl while the excretion of man remained unchanged. In 14/55 patients retested after 2 days realimentation in hospital, the lacl/man urinary recovery did not differ from the admission level, mean difference in excretion ratio was 0.01 with 95 % CI from -0.08 to 0.1. These results indicate that fasting maintains the increased intestinal permeability caused by the infection whereas early feeding may hasten the recovery from acute gastroenteritis. Therefore, the results suggest that early feeding at home is beneficial.

## 63 MODULATION OF THE SECRETORY RESPONSE TO CHOLERA TOXIN BY DIETARY FAT

F A Sagher, J A Dodge
Department of Child Health, Queen's University, Belfast.

The effects of dietary fat on jejunal water, solute and glucose absorption, and on cholera toxin-induced secretion were studied in 4 groups of rats, using an in vivo steady state perfusion technique.

Weanling rats were fed isocaloric diets with 40% of their total calories as fat, (varied in the degree of saturation and w6/w3 ratio) for 7-9 weeks. The absorption of water, electrolytes and glucose in steady state perfusion was altered dramatically, with highest rates of water and electrolyte absorption in the high polyunsaturated group.

Feeding either high polyunsaturated or high monounsaturated diets decreased the water and electrolyte secretion in response to cholera toxin compared to control chow diet (4% fat) or feeding high saturated fat diet.

We conclude that variation in the fatty acid profile of the diet alters the transport properties of the fat jejunum. This may explain the beneficial effects of added dietary fat in some diarrhoeal disorders.

## 64 ORAL REDHYRATION SOLUTIONS - EXPERIMENTAL STUDIES OF NET WATER AND ELECTROLYTE ABSORPTION.

B.K Sandhu, G.Pillai, D. Burston, M.J Brueton

WESTMINSTER CHILDREN'S HOSPITAL, LONDON, SW1P 2NS.

Net water absorption, unidirectional fluxes and sodium and potassium absorption from oral rehydration solutions (ORS) of varying solute compositions were studied in normal and secreting rat jejunum using an invivo steady state perfusion technique. Tritiated water and phenol red were used as markers with at least five animals in each group.

The addition of glycine to ORS was found to be ineffective in animal and clinical studies. The effects of other amino acids including leucine which has a sodium independent transport pathway have now been investigated. The standard ORS perfused (A) was the World Health Organization formula containing in mmol/l Na 90, and glucose 110. The other solutions perfused contained in mmol/l Na 90 and glucose 60 plus in B leucine 50, C alanine 50, and D phenylalanine 50. In a second series of experiments cholera toxin 75mcg was instilled into the gut prior to perfusion. The net water absorption in normal gut in µl/min/g ± 1SD was for A 17.4 ± 3.7, which was significantly less than for B 43.9 ± 7.2 ($P<0.001$), C 24.0 ± 4.3 ($P<0.05$) and D 32.5 ± 5.3 ($P<0.001$). In the cholera toxin treated gut perfusion with B resulted in net absorption (9.4 ± 9.8 µl/min/g) whereas net secretion continued with solutions A, C and D, the rates being respectively -2.4 ± 3.0, -4.8 ± 8.2 and -7.7 ± 5.0 µl/min/g. The associated changes in unidirectional fluxes and electrolyte absorption will also be discussed. This animal study suggests that clinical studies in children using ORS containing leucine should be considered.