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REVERSAL OF NET SECRETION IN SMALL INTESTINAL SEGMENTS OF ROTAVIRUS-INFECTED MICE BY WHO ORAL REHYDRATION SOLUTION. W G Starkey, J. Collins, S J Haddon, M P Osborne, J Stephen, D C A Candy. Department of Microbiology, Physiology & Paediatrics & Child Health. University of Birmingham, UK.

Glucose-stimulated Na transport is abolished in small intestinal mucosae of rotavirus-infected piglets as measured in Ussing chambers. However in human infants with rotavirus diarrhoea, oral rehydration solutions containing glucose are effective. We have studied by steady state in vitro perfusion water transport in small intestines of 10 day old mice, 3 days post infection with rotavirus.

Net water secretion occurred in rotavirus-infected small intestines perfused with glucose-free Ringer's solution (-5.69(1.11)µl/cm/h; mean (SEM), n=5). Controls absorbed +2.01(0.50)µl/cm/hr.

Water secretion was reversed to net absorption (+5.43(1.11)µl/cm/hr by perfusion with WHO oral rehydration solution.

This points to a greater functional reserve for glucose-stimulated Na and water absorption in rotavirus infected mice as compared with piglets. This is in accordance with the clinical success of WHO oral rehydration solution in humans with rotavirus diarrhoea. It is also in accord with our ultrastructural observations of virus-infected villi in which mucosal damage was less severe compared with piglets.

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THE NATURE & ROLE OF MUCOSAL DAMAGE IN RELATION TO SALMONELLA TYPHIMURIUM-INDUCED FLUID SECRETION IN THE RABBIT ILEUM. T S Wallis, W G Starkey, J Stephen, S J Haddon, M P Osborne, D C A Candy. Departments of Microbiology, Physiology & Paediatrics & Child Health, University of Birmingham, UK.

The time course and nature of mucosal damage induced by two human strains of Salmonella typhimurium (TML and W118)in rabbit ileal loops was assessed by scanning and electron microscopy. Fluid secretion was measured by ml fluid accumulated per cm rabbit ileal loop.

Salmonella-induced fluid secretion (>0.5ml/cm) occurred in the presence or absence of gross mucosal damage. Neither strain induced mucosal ulceration. When damage did occur it involved shortening of villi by loss of tip regions invaded by Salmonellae with concomitant reforming of an intact mucosal surface. Intestinal crypts were not invaded.

Immediately preceeding the onset of fluid secretion, marked infiltration of the mucosa with polymorphonuclear leukocytes was seen. This revives an earlier suggestion that interaction between invading Salmonellae and acute inflammatory cells may be an important factor in the initiation of fluid secretion.

Brush border invasion by Salmonellae, which was maximal in the first 4h after infection, cannot be the immediate cause of fluid secretion, as the latter occurred 2h after initial invasion.

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PATHOGENESIS OF ANTIBIOTIC-INDUCED DIARRHEA NOT DUE TO CLOSTRIDIUM DIFFICILE (CD). A.Fasano,S.Guandalini,M.Migliavacca,A.Ferrara,C.Verga,B.Malamisura,**P.Gianfrilli,M.Alessio,A.Rubino.Inst.Pediatr.2nd Sc.of Medicine, Univ.Naples,**Istituto Superiore di Sanità,Rome, Italy.

We wished to identify,in the in vitro rabbit intestine,the pathogenesis of post-antibiotic non CD-mediated diarrhea.Out of 25rabbits which were given Ampicillin (A) 14 developed diarrhea and were investigated.Results:1)CD and its cytotoxin (absent in all control rabbits)were found in the ileum and/or caecum of 8 rabbits(CD+), while only other enterobacterial species were found in 5(CD-).Clostridium spiroforme was found in one.2) Ileal and caecal transepithelial ion fluxes were measured in Ussing chambers:secretory changes in Na and Cl transport developed in both segments and were more marked(by about 2µEq/hr.cm^2)inCD+. 3)Influx of 5mM Glucose, 1mM Phenylalanine and 2mM Glycyl-Phenylalanine across the brush border of jejunal mucosa was not affected in CD-rabbits,while it was markedly reduced in CD+(respectively,1.5±.06,.27±.05,.55±.07µmoles/hr.cm^2,means ±SE in controls vs.1.12±.08,.32±.01,.59±.08 in CD- and .51±.06,.09±.01,.15±.03 in CD+).In conclusion:1) both CD+ and CD-animals show secretory changes in the small and large intestine,more pronounced in CD-; 2)jejunal influx of nutrients is hampered only in CD+ animals.Thus,the pathogenesis of post-antibiotic CD-mediated diarrhea appears essentially invasive, while that not due to CD appears essentially secretory.

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CHOLERA-LIKE TOXIN AND CYTOTOXIC FACTOR PRODUCTION BY CAMPYLOBACTER JEJUNI/COLI

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Cholera-like enterotoxin (CLT) production by campylobacter jejuni/coli (CJ) was shown in a new medium in which 25 strains of CJ isolated from diarrhoeic stools in children were grown for 24h at 42°. The presence of CLT was demonstrated by means of the Chinese Hamster Ovary Cells (CHO) elongation assay (1). Though the majority of the strains produced CLT, the amount varied significantly from strain to strain. The morphological changes of CHO cells were neutralized by antisera against cholera-toxin (CT) and both porcin and human E.Coli LT.

In a second study, a new cytotoxic factor (CF) killing CHO cells (2), was shown in 24/24 strains of CJ from children with endoscopically and histologically proven invasive colitis but also in 20/26 CJ strains from children with watery diarrhoea. Antiserum against shiga-toxin did not neutralize the CF.

Conclusions:

1) CLT produced by CJ is similar to CT and is produced in small amounts.
2) CF produced by CJ is different from shiga-toxin.
3) Role and importance of CLT and CF in overt intestinal CJ infection is to be further evaluated.

(1) Guerrant et al. Inf. & Imm., 10:326,1974
(2) Goossens et al. Lancet i, 511, 1985

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USE OF CULTURED HUMAN INTESTINAL MUCOSA TO SELECT FOR ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) PRODUCING COLONIZATION FACTORS (CF'S): IDENTIFICATION OF A NEW FIMBRIAL CF IN ETEC SEROGROUP O148. S.Knutton, D.R. Lloyd, and A.S.McNeish. Institute of Child Health, University of Birmingham, Birmingham B16 8ET, U.K.

Three important fimbrial CF's designated CFA/I, CFA/II and E8775 were originally identified in some human ETEC strains because of their haemagglutination (HA) properties. In an attempt to identify new CF's in the many ETEC isolates which lack HA properties we have exploited the ability of human ETEC to adhere to human small intestinal mucosa. ETEC strain B7A(O148:H28) was selected for study because it belongs to a commonly isolated serotype, does not produce CFA/I, CFA/II or E8775 and yet is known to be pathogenic when fed to volunteers. 25ul of a broth culture of B7A was placed onto the mucosal surface of duodenal biopsies maintained in organ culture. The number of mucosally adherent bacteria, assessed by scanning electron microscopy, was observed to increase with time following inoculation and after 10-12 hours virtually the whole of the mucosa was colonized by bacteria. Mucosally adherent bacteria were then subcultured and examined by negative stain electron microscopy. A new fimbrial CF morphologically and antigenically distinct from CFA/I, CFA/II and E8775 fimbriae and consisting of 3-4 nm diameter fibrils was readily identified. This selection and enrichment procedure may provide a general method for the identification of adhesion fimbriae in the many ETEC isolates which lack known CF's.

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ADMINISTRATION OF MULTIPLE DOSES OF ROTAVIRUS (RV) VACCINE RIT-4237 TO NEWBORNS

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The safety and immunogenicity of RIT-4237 live attenuated rotavirus (bovine strain) vaccine were established (1). During the epidemic-free months of July and August 84, maternal and cord blood were obtained from 150 giving-birth mothers and their newborns. After informed consent, 57 newborns were vaccinated orally at birth and at one and two months; 42 infants completed the study. As the vaccine is known labile at low pH (2), gastric acidity was measured at each vaccination. Serum samples for RV-antibodies and faecal samples for RV detection were obtained at each vaccination. RV-antibodies were also assayed in breast-milk. No adverse reaction was observed.

The "take" of the vaccine was estimated by RV-seroneutralizing specific antibodies. Sero-conversion occurred in 28/42 (67%) infants: 8/42 (19%), 9/42 (21%) and 11/42 (27%) after first, second and third vaccinations respectively. No booster reaction was observed. RV-antibodies were low in breast-milk. Whereas there was no difference in gastric pH the geometric mean titer was clearly different in the two groups of responders and non-responders.

Conclusions: 1) Gastric acidity and breast-milk RV-antibodies do not seem to interfere with the "take" of the vaccine. 2) Optimal time for vaccination depends mostly on maternal RV-antibodies interference and therefore on geographical immunity conditions. 3) A multiple dose scheme is advisable.

(1) Vesikari et al. Lancet ii, 807, 1983
(2) Vesikari et al. Lancet ii, 780, 1984