## 77 ISOLATION OF APICAL MEMBRANES FROM HORMONE-SENSITIVE RAT COLONIC CRYPT CELLS.

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In order to study hormone-dependent transport properties of VIP-sensitive colonic crypt cells were prepared using chelators and mechanical forces. Thymidine kinase activities in crypt cells and absorptive cells were 77±8 and 9±2 nmol/min/mg protein respectively. Hormone sensitivity was shown by a dose-dependent increase of cAMP levels up to 18-fold over basal with $10^{-6}$M VIP. From this preparation apical membranes were isolated by standard procedures. Membrane purity was assessed by electron microscopy and marker enzyme assays: In the final membrane preparation increase of relative specific activity (nmol/min/mg protein) - as compared to the intact cells - was 10-fold for alkaline phosphatase, 2-fold for Na-K-ATPase. Succinate dehydrogenase was decreased by 50%.

Conclusions: 1.Thymidine kinase concentration in isolated colonic crypt cells is 8-fold higher than in absorptive cells. 2.This preparation is VIP-sensitive. 3. Apical crypt cell membranes were partially purified (ALP: 10-fold) with only minor contamination with basolateral membranes and mitochondria.

## 78 LONG TERM FOLLOW-UP (over 10 years) IN CONFIRMED COELIAC DISEASE

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Since 1973 we performed a total of 2040 jejunal biopsies in children. 192 showed total villous atrophy : 64 coeliac disease (C.D) were confirmed according to ESPGAN criteria. The progress of C.D in this teenage group was assessed in 26 patients (16 girls, 10 boys) over a 10 years period. Follow-up of theses patients consisted in clinical examination, blood tests and formal dietary check every 6-12 months, jejunal biopsy control every 3-4 years. A strict diet without gluten was proposed until puberty. If it was refused or considered imperfect, a normal diet with gluten was given. RESULTS : GROUP 1 : 11 patients considered their diet to be strict : 3 had normal mucosae and 8 had persistent partial villous atrophy proving a faulty regimen. The staturo-ponderal growth was normal between + 1 and - 2 SD and all of them reached puberty without delay. GROUP 2 : 15 patients considered their diet to be normal with substantial amounts of gluten. 11 had flat mucosae without digestive symptoms. The staturo-ponderal growth was between - 1 and - 2.5 SD with normal puberty. 4 patients (3 girls and 1 boy) had normal mucosae (intra epithelial lymphocyte count <15%) after 7-8 years of normal diet (eating 5 g of gluten per day at least). The height and weight growth and puberty were without delay. Blood tests were normal without nutritional deficiencies.

Our experience show that compliance with a strict gluten free diet is variable ; most commonly refused in asymptomatic patients. The possibility of total mucosal improvement on normal diet is asserted on repeated control biopsies in confirmed C.D.

## 79 INTESTINAL WATER AND SOLUTE TRANSPORT IN A NEONATAL RAT MODEL OF ROTAVIRUS DIARRHOEA

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Entire small intestine has not been used for transport studies in animal models of rotavirus (RV) diarrhoea. We have studied water and electrolyte transport in a neonatal rat model of RV diarrhoea using in situ steady state perfusion of the whole small intestine. Perfusion with an isotonic plasma electrolyte solution without glucose at 48 hours post oral challenge with rate RV showed net water secretion (-21.5 ±7.3 ul; Cont: +67.9 ± 8.5 ul/min/g dry weight of gut). To determine whether net water secretion can be reversed by solutions containing glucose (G) or glucose polymer (GP), the rats were perfused with six different solutions. Two were hypertonic: A) Na 35, G 200 mM/l; B) Na 90, G 111; and four were hypotonic: C) Na 60, G 90; D) Na 60, G 111; E) Na 60, GP 9 ; and F) Na 60, GP 18 mM/l. Hypertonic solution A, containing low sodium and high glucose, caused further secretion (-38.2 ± 8.0 ul/min/g). The other hypertonic and all hypotonic solutions reversed secretiont o absorption, although net water absorption was always significantly greater from hypotonic solutions, being maximal from the solution containing 18 mM GP. Solutions containing 60 mM sodium showed net sodium secretion, 90 mM induced absorption. This model shows that RV causes a secretary state in the small intestine, which can be reversed by glucose electrolyte solutions. Maximal water absorption occurs from a hypotonic solution containing GP. High concentration of glucose leading to hyperosmolality causes secretion in rotavirus diarrhoea.

## 80 T CELL-MEDIATED DAMAGE IN THE HUMAN COLON IN ORGAN CULTURE A NEW IN-VITRO MODEL OF COLITIS

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Immune mechanisms may be important in the pathogenesis of colonic inflammation To test this hypothesis, an organ-culture model was used to study the effects of T cell activation in human colonic mucosa.
Lamina propria T cells in expant cultures of human fetal colon (16-20 weeks gestation, n=10) were activated in-situ using poke-weed mitogen or anti-$CD^3$ atibody, and compared with controls.
After 3 days of culture, immunohistochemistry demonstrated a 2-4 fold increase in crypt epithelial cell proliferation in T cell stimulated explants compared with controls; this was associated with a decrease in crypt goblet cell numbers of up to 20 fold. After 5 days, the surface epithelium in stimulated explants appeared flttened with severe loss of goblet cells, and by day 7, severe mucosal damage was observed under both light and electron microscopy. A fall in crypt goblet cells without hyperplasia had occurred in control cultures by day 7; however, the epithelium remained intact, and the degree of mucous depletion, and the rate at which it occurred, were less than in stimulated explants.
Goblet cell depletion, crypt hyperplasia, and epithelial damage are common histological findings in colonic inflammation (particularly ulcerative colitis) These experiments suggest that T cells may have an important role in the pathogenesis of colitis. The model provides a new system whereby the mechanisms of T cell-mediated damage in the human colon can be studied.

## 81 THE ROLE OF SURGERY IN CROHN'S DISEASE OF CHILDHOOD

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There are few reprots of the outcome of surgery for Crohn's disease in childhood. A retrospective study was thus performed of 67 children who have required surgery out of 167 histoogically proven cases of Crohn's disease presenting between 1979-88. Cases were divided into four grops on the basis of disease location.
In group 1 (pan-enteric disease, n=3), indications for surgery were failed medical treatment and local sepsis. Results were disappointing; little was achieved to help symptoms or aid growth.
In group 2 (diffuse small bowel disease, n=7), surgery was performed for pain or sub-acute bowel obstruction. Results were good, with low morbidity and a lengthy remission in 6/7 cases.
In group 3 (ileo-caecal disease, n=30), the results were also impressive. All children had right hemicolectomies, most for poor growth. Long-lasting remission was achieved in 24/30 (80%), with significant acceleration of growth and puberty in most cases.
In group 4 (colitis, n=27), the results of surgery were mixed. All of the 7 who had staged resections did badly, as did 3/4 cases given a loop ileostomy to divert faecal flow. Best results were achieved in the 16 children who had a sub-total colectomy, ileostomy and mucous fistula as the primary procedure; 15 have remained well, with only minor problems subsequently.

In conclusion, outcome following surgery depended on disease location, but was also influenced by the type of operation. In addition to symptomatic relief, the principal benefits of surgery were acceleration of growth and pubertal advancement.

## 82 EXPERIMENTAL ROTAVIRUS INFECTION IN NEONATAL RATS.

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Although rotavirus (RV) is the commonest cause of acute diarrhoea in children, its pathogenesis remains uncertain. We, therefore, have studied the clinical course and the small intestinal morphometry and pathophysiology of neonatal rats with experimental RV infection. Eight day old rats (n=6 minimum per group) inoculated orally with rat RV, were studied at 12-96 hours post inoculation (HPI) for perfusion, or 12-168 HPI for morphometry at 6 hour intervals up to 24 HPI, and then at 24 hour intervals. Clinical diarrhoea occurred at 24 HPI, which continued for 4-5 days. RV was excreted in stool from 24-72 HPI. Body weight was significantly less than controls ($p<0.01$) from 24-96 HPI, but no difference was seen at 168 HPI. Small intestinal morphometry showed significant villous destruction, i.e. a reduction in villous height, by 12 HPI ($p<0.04$) and increased crypt depth (CD) by 48 HPI ($p<0.01$) in ileum, which continued up to the end of study period (168 HPI). In jejunum, significant villous atrophy was first noted at 18 HPI ($p<0.01$) and increased CD at 72 HPI ($p<0.01$), which continued until 72 and 168 HPI respectively. Steady state perfusion in situ of the whole small intestine with plasma electrolyte solution with no glucose showed reduced absorption of water than controls at 12, 72 and 96 HPI ($p<0.01$), and net secretion at 18, 24 and 48 HPI. This rat model shows that villous destruction by RV is associated with transport defects and both occur before clinical diarrhoea. Crypt hyperplasia occurs afterwards and persists.