5 LINKAGE OF DNA MARKERS ON CHROMOSOME 13 WITH WILSON DISEASE.

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Wilson disease (WD) is an autosomal recessive disorder, characterized by massive copper deposition in the liver, basal ganglia and other organs,due to an impairment of biliary copper excretion. The basic defect in WD is still unknown. Recently a linkage has been reported between the gene for WD and the esterase D locus by determination of esterase D phenotypes in a large inbred Israeli-Arab kindred. This implies that the q14 band of chromosome 13 would be the location of the WD gene, at least in part of the Middle East population.
To examine this assignment we are now studying a number of unrelated WD families of Caucasian origin. Linkage studies are carried out using a cDNA probe of esterase D and random probes from a chromosome 13 library. Preliminary results are from a first five Dutch WD families, with 27 children classified as affected or normal based on serum copper, ceruloplasmin and 24h urinary copper excretion. In these families seven recombinants were found with a probe localised proximal of band q14, and only one with a probe localised in q14. A positive lodscore was obtained for the latter probe (z=0.35 θ=0.15), corroborating the assignment of the WD gene to band q14 of chromosome 13 and suggesting absence of heterogeneity for WD.

6 MECHANISMS OF TRANSPORT OF SODIUM AND CHLORIDE AND THE EFFECTS OF SHORT CHAIN FATTY ACIDS IN THE HUMAN INFANT.

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A major function of the colon is the conservation of salt and water which may be aided by short chain fatty acids. The only previous studies of the mechanisms involved in infants have been in vivo where electrical gradients influence ionic movements. We have carried out a more detailed study of transport in isolated human infant colon using an Ussing Chamber and voltage clamp procedure. Stripped L side colonic mucosa (n=6 pairs) was mounted and bathed in Krebs solution. Under short circuit conditions Na^+ (3.45±1.53 umol/hr/cm^{2}mean 1SD) and Cl^- (0.63±3.61) were absorbed and a residual ion flux consistent with HCO_3^- secretion, approximates Cl^- absorption. Short-circuit current (3.8±0.28) approximates net Na^+ movement. 60 mM acetate increased Na^+ absorption (3.45±1.53 to 7.74±2.25, p<0.05) by a large increase in mucosa to serosa flux (7.24±0.92 to 13.55±1.62, p<0.01). The increased net absorption of Na^+ was markedly reduced by 10^{-4} M amiloride (7.74±2.25 to 1.75±1.72, p<0.01) which was also associated with a marked reduction in tissue conductance. These data clearly show that in the infant Na^+ is absorbed electrogenically and Cl electroneutrally in exchange for HCO_3^- unlike in vivo where Cl^- moves according to the electrical gradient. Short chain fatty acids favourably influence Na^+ salvage and do so via the amiloride Na^+ channel. Thus bacterial metabolism of carbohydrate in the infant colon may be important in the conservation of salt and water.

7 DEVELOPMENT OF EPITHELIAL ELECTROLYTE TRANSPORT PROCESSES IN EARLY CHILDHOOD

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Membrane transport processes are poorly developed in the kidney of the very preterm neonate resulting in reduced ability to acidify urine and increased Na^+ loss. There are close homologies between the transport processes of the distal convoluted tubule and the L colon but little is known of the ontogeny of these processes. We have used non-equilibrium rectal dialysis to investigate rectal electrolyte transport in neonates of different gestational ages: 30-33 wks (A n=15), 34-36 wks (B n=14), 38-44 wks (C n=5), infants (D n=17) and older children (E n=19). Na^+ absorption was highest in A (316±65 nmol/min/cm) and lowest in E (149±44, p<0.005). Plasma aldosterone was markedly elevated in A and B (7570±2500 pmol/l) compared to infant reference values (790±210, p<0.005). Cl^- absorption paralleled Na^+ absorption. HCO_3^- movement provides an indication of anion exchange and in A (59±14), B (26±30) and C (22±20)was absorbed but in D there was little net movement (3±24) and in E (11±16) net secretion.

These data show that colonic, unlike renal, Na^+ transport processes are efficient in the preterm neonate and that aldosterone is an important regulatory hormone. The colon is thus the major organ of Na^+ conservation and the consequences of colonic surgery should be carefully considered. However, anion exchange seems to undergo similar developmental processes in both organs and this might explain the greater susceptibility to acidosis than hyponatraemia in the preterm neonate.

8 LYSINURIC PROTEIN INTOLERANCE (LPI): THE DEFECT IN CATIONIC AMINO ACID TRANSPORT IS EXPRESSED IN PLASMA MEMBRANE OF CULTURED FIBROBLASTS

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Efflux of cationic amino acids lysine, arginine and ornithine at the basolateral membrane (BLM) of intestinal and renal tubular epithelia is defective in patients with LPI. Assuming that BLM of epithelial cells and plasma membrane of parenchymal cells are functionally analogous, we studied Na^+-independent transport of cationic amino acids and homoarginine (a nonmetabolized analogue) in cultured LPI fibroblasts, and measured L-leucine transport as internal control. Net uptake of cationic amino acids was increased in LPI cells; homoarginine/leucine uptake ratio was 0.30 ± 0.07 and 0.26 ± 0.01 (mean ± SEM) ($p<0.05$) in LPI and control cells, respectively, at 10 min. The intracellular pool of cationic amino acids was increased (lys/ala 0.47 ± 0.05 vs. 0.31 ± 0.02; $p<0.01$). The % trans-stimulation of homoarginine efflux was 1.0 ± 0.5 % in LPI homozygotes, 10 ± 0.5 % in heterozygotes and 22 ± 0.5 % in control cells. Our findings imply that the transport mutation in LPI is expressed asymmetrically at the membrane and concordantly at the basolateral membrane of epithelial and plasma membrane of parenchymal cells.

9 POSTNATAL MATURATION OF AMINO ACID TRANSPORT IN THE BRUSH BORDER MEMBRANE OF THE RAT:

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Intestinal amino acid transport in rodents decreases after birth. Simultaneously, changes of other function and structure occur and influence observed uptake rates. Thus, it is equivocally wether the decline is caused by a change of the transport system itself. Rates of uptake of L-phenylalanine into isolated brush border membrane vesicles(bbm) and into an intact mucosal layer(iml) were examined in newborn(n) and adult(a) rats. Uptake decreases significantly($p>.01$) in bbm and iml after birth. Concentration dependent uptake suggests a non-saturable and a saturable component of transport of which constants were determined.

	K_D (mM)		K_t (mM)		V_{max}	
	(iml)	(bbm)	(iml)	(bbm)	(iml	bbm)
(n)	0.25	3.33	2.73	0.26	2.04*	1.52**
(a)	0.15	1.44	1.18	0.12	0.20*	0.41**

* nM/mg weight/min, ** nM/mg protein/40 sec.
Results show a considerable change of the diffusional constants K_D and the V_{max} values during maturation. The change of K_t has only a small effect on the observed differences. This suggests an alteration of the membrane permeability, a decrease of the number of transport sites per unit surface, and an involvement of the brush border transport system in the process of maturating intestinal function.

10 POSTNATAL MATURATION OF SMALL INTESTINAL MICROVILLUS MEMBRANES (MVM).

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To compare different aspects of postnatal MVM maturation, MVM were prepared from rats, starting from newborns to the age of 12 weeks. MVM fluidity was studied by fluorescence polarization using different labels. Cholesterol and phospholipid composition of MVM was analysed. MVM binding studies were performed using radioiodinated probes. Membrane fluidity was increased in newborn MVM, compared to adults. There was a continuous decline of MVM fluidity throughout maturation. Changes in fluidity were accompanied by an increase of cholesterol/phospholipid molar ratio. Continuous maturational changes in membrane glycosylation were indicated by differential specific binding of concanavalin A, soybean agglutinin, and peanut lectin. Contrary to the continuous MVM changes, food protein binding to MVM (cow's milk proteins, wheat gliadin peptides) was decreasing stepwise between 6-8 weeks of age. This was not related to weaning. It remains to be shown how different aspects of postnatal MVM maturation are interrelated, and how they are connected to the development of food protein tolerance or allergy in young individuals.