35 EVALUATION OF A SPECIFIC TEST FOR 'ATTACHING AND EFFACING' ENTEROPATHOGENIC ESCHERICHIA COLI

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Serotyping remains the main diagnostic test for enteropathogenic Escherichia coli (EPEC). We have recently developed a fluorescent actin staining test (FAS test) which uniquely identifies the 'attaching and effacing' ('A & E') membrane lesion produced when EPEC adhere in vivo to human small intestinal mucosa or in vitro to tissue culture cells. The object of this study was to use the FAS Test to examine A) serotypable EPEC isolates and B) E. coli isolates from children with diarrhoea for their ability to adhere to cultured human embryonic lung cells and produce an 'A & E' lesion. 15 of 44 serotypable EPEC strains and 6 of 297 E. coli isolates were positive in the FAS test. All 6 FAS Test-positive E.coli were subsequently shown to adhere also to cultured human small intestinal mucosa and to produce an 'A & E' leion. Only 1 of the 6 was subsequently found to belong to a classical EPEC serogroup (0127). These results show: 1. The FAS test identifies 'A & E' E. coli which would not have been detected by serotyping. These 'A & E' E.coli which belong to non-classical EPEC serogroups are most probably human E.coli pathogens which have previously gone unrecognised. 2. There is no correlation between EPEC serotyping and the ability to cause the 'A & E' lesion. 3. The FAS Test is a useful addition to the current range of diagnostic tests for human E. coli enteric pathogens.

36 THE EFFECT OF MATERNAL PROTEIN MALNUTRITION AND ETHANOL EXPOSURE ON EGF BINDING TO NEONATAL RAT HEPATOCYTES

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Epidermal Growth Factor (EGF) plays an important role in hepatocellular maturation and regeneration. We have shown that foetal and neonatal rat hepatocytes have fewer EGF receptors with reduced affinity for ligand compared to adult cells suggesting down-regulation of this receptor in developing cells.
In order to evaluate the effect of maternal malnutrition and chronic ethanol exposure on EGF binding to neonatal hepatocytes female Sprague-Dawley rats were fed 1 of 3 diets two weeks prior to breeding until after parturition: 1) Rat chow (23% protein) with water ad lib (C), 2) liquid Lieber-Decarli diet with 10% protein (LP), and 3) the LP diet with 36% of maltose-dextrin calories replaced by ethanol (ELP). Isolated hepatocytes were prepared from 6 day old neonatal livers by collagenase digestion of minced livers. Binding was assessed by incubating cells at 37 C with varying concentrations of 125I-EGF (0.16 nM-7.8 nM) for 60 minutes. There was an increase in both the number of surface receptors (B_{max} (x 10^4/cell, mean +/- SEM n=3:) - C = 1.78 +/- 0.35; LP = 2.35 +/- 0.56 p=NS; ELP = 3.53 +/- 0.55; P<0.02.) and the binding affinity for EGF (K_d x 10^{-10}M C = 6.54 +/- 0.34; LP = 5.17 +/- 1.12 p = NS; ELP = 4.69 +/- 0.7 p<0.02) in hepatocytes from both treated groups compared to controls although this was only significant in group ELP.
It is concluded that the combination of maternal protein malnutrition and ethanol exposure in utero alters the regulation of EGF processing in developing hepatocytes which may retard hepatocellular maturation and development.

37 HLA-DQ SPECIFIC ALLELES ASSOCIATED WITH CELIAC DISEASE(CD) ARE EXPRESSED IN THE SMALL INTESTINAL MUCOSA (SIM)

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Previous studies at DNA and product level of B cell lines on CD patients have shown a strong association between CD and the HLA-DQα2.3 and HLA-DQ β2.7 alleles[1]. The rat monoclonal SFR-2 DQ5 which specifically recognizes the HLA-DQα2.3 specificity[2] and the monoclonal directed against the HLA-DQβ2.7 specificity (MC Mazzili, Italy) have been used to detect the expression of these specificities at the SIM in 7 CD patients and 6 non-CD patients. The mouse monoclonal SPV-L3 against HLA-DQ backbone was used as positive immunoperoxidase control. An immunoperoxidase technique on frozen tissue sections of jejunal biopsy specimen was used.
Results: Positive specimens showed an infiltration of positive lymfocytes and histiocytes in the lamina propria. Positive results at the intestinal level correlated with HLA typing of the patients and controls. The epithelial cells were negative.

	Peripheral blood					Small intestine		
	DR3	DR7	DR3/7	DR-/-	DQw2	DQα2.3	DQβ2.7	DQ
CD	5	5	3	0	7	6	7	7
Control	1	0	0	5	1	3	1	6

Conclusion: The results show that at intestinal level the HLA-DQ specific alleles associated with CD are not expressed in the epithelial cells but abundantly present in lamina propria cells of celiac patients. This distribution may support the hypothesis that these DQ molecules are involved in the regulation of the intestinal immune response to gluten.

1. Roep etal. Hum Immunol 1988;23:271-9
2. Amar etal. J Immunol 1987; 138:3986-90

38 METABOLISM OF FETAL BILE ACIDS IN HEALTHY NEONATES

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In order to clarify the bile acid metabolism during the neonatal period, we measured fetal bile acids in the urine of full term neonates using specific quantitative assay by capillary GC with negative ion chemical ionization MS. Spot urine samples of 15 full term neonates were taken 1-5 days after birth. As controls, spot urine samples of 10 health children aged 4-8 years were analysed. Bile acids were extracted from urine using Bond-Elut C_{18} cartridge. After solvolysis and alkaline hydrolysis, the free bile acids were derivatized to the pentafluorobenzyl ester and trimethylsilyl ether.

In the neonates, the percentage of total 1β- hydroxylated bile acids was significantly higher than that in the older children. The percentages of 3β, 12α-5-cholenic acid and hyocholic acid in the neonates were high compared to those in the older children. The ratios of 1β -CA/CA and 1β -CDCA/CDCA in the neonates were significantly higher than those in the older children. The ratios of CA/CDCA and 3β , 12α -5-cholenoic acid / 3β -5-cholenoic acid were also significantly higher in the neonates than those in the older children. It suggests that 1β and 12α hydroxylation of bile acids are important metabolic pathways of bile acids in neonates.

39 IMPROVEMENT OF CHOLESCINTIGRAPHY IN THE DIFFERENTIAL DIAGNOSIS OF EXTRAHEPATIC BILIARY ATRESIA

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The introduction of halogenated analogs of iminodiacetic acid (IDA) has increased the effectivity of cholescintigraphy in the differential diagnosis of extrahepatic biliary atresia (EHBA). We attempted to evaluate cholescintigraphy with regard to its potential in increasing the effectivity and predictive value in the diagnosis of EHBA.
We studied 16 patients (3 with EHBA, weight = 2,45-6 kg, direct bilirubin 1,6-17,9 mg/dl) by 18 tests. 99mTc mebrofenin (n=5) and 99mTc Iodida (n=13) were used as tracers in intermittant sequential scintigraphy to 24 hrs. The data were evaluated by a computer program to obtain 13 kinetic parameters, and were compared with the results of the conventional scintigraphic interpretation.
The positive predictive value of the test increased to 80 % by measurement of the maximum intestinal activity, and to 100 % by evaluation of the hepatic tracer clearance. Combining the maximum intestinal activity with the hepatic tracer retention or clearance led to an effectivity and positive predictive value of 100 %.
The examination of liver indices (± adjustment for background activity) had no value.
Conclusions: An automated evaluation of data characterizing the excretion of halogenated IDA derivatives into the intestine has the potential of allowing the differentiation of EHBA from intrahepatic diseases with high probability. We plan to perform prospective studies on larger numbers of infants.

40 THE PRVENTION OF PERINATAL TRANSMISSION OF HEPATITIS B VIRUS (HBV) INFECTION: A COMPARISON OF TWO PROPHYLACTIC SCHEDULES

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During the two year period 1986/87, 271 babies were born to hepatitis B surface atigen carrier mothers in the English West Midlands. Babies were allocated sequentially into either treatment Group A (4 doses of HBVax, Merck Sharp & Dohme, 10 mcg. i.m. at birth, 1, 2 and 6 months) or treatment Group B (250 I.U. hepatitis B immunoglobulin (HBIG) at birth, combined with the same vaccine schedule as Group A). 172 babies were enrolled and data was available for analysis on 150 (87%).
REsults:

Mothers HBe status at delivery	HBe Ag+		HBe Ag-/Ab-		Anti Hbe+	
Treatment Group	A	B	A	B	A	B
No of babies enrolled	12	15	21	14	48	40
Babies immune at last test	4	11	20	14	45	37
Babies becoming HBsAg+	5	1	0	0	0	0
Outcome unknown	3	3	1	0	3	3

Conclusions
In circumstances in which HBIG is not available a 4 dose vaccine schedule can protect at risk infants from perinatal transmission of HBV. In babies born to the less infectious carrier mothers in our study (i.e. those not HBe Ag+) the addition of HBIG to the schedule conferred no added benefit. In infants born to HBe Ag+ mothers protection was enhanced by addition of HBIG although transmission was still not prevented in every case. In utero infection and slow response to vaccine may be implicated failure mechanisms.