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**SYMBIOTIC INTERACTIONS BETWEEN THE COLONIC MICROFLORA AND THE PROTEIN TURNOVER IN INFANTS**

Heine, W., Mohr, Christa, Wutzke, K.D.

Children's Hospital, WPU Rostock, GDR

The utilization of  $^{15}\text{N}$ -nitrogen from  $^{15}\text{N}$  labeled *Saccharomyces cerevisiae* cells and  $^{15}\text{N}$  labeled Bifido-bacteria for whole body protein synthesis was studied in a total of 9 infants by means of oral or colonic pulse labelings. The microbes were harvested from synthetic culture mediums containing  $^{15}\text{N}$  ammonium chlorid as the only source of nitrogen.  $^{15}\text{N}$  enrichment of the cells amounted to 95%. A tracer dose of 3 mg  $^{15}\text{N}$ /kg was chosen. The pulse labeling was followed by a 48 hours collection of urine and feces. Following oral single pulse labeling of the native and heat-treated yeast cells 77% on an average of the heavy nitrogen was retained in the protein pool. Comparatively high utilization rates of microbial  $^{15}\text{N}$  were found after instillation of  $^{15}\text{N}$  labeled Bifido-bacteria into colonic segments in 3 infants with colostomies. The retention was confirmed by elevated  $^{15}\text{N}$  concentrations of the plasma proteins. The results show that microbial nitrogen is intensively used for whole body protein synthesis in infants. The colonic microflora contributes to improvement of non essential endogenous and exogenous nitrogen.

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**THE IMMUNOLOGICAL RESPONSE TO GLIADIN IN COELIAC DISEASE SEEMS NOT TO BE OF PRIMARY PATHOGENETIC IMPORTANCE.**

Steffen Friis, Hans Sjöström and Ove Norén.

Department of Biochemistry C, The Panum Institute, The University of Copenhagen, 3-Blegdamsvej, DK-2100 Copenhagen Ø, DENMARK.

Patients with untreated coeliac disease have circulating antibodies against gliadin. We have previously demonstrated that the gliadin-antibody pattern is dominated by reactivity against a few polypeptides, tentatively identified as gamma-gliadins by their migration in electrophoresis. A varying degree of reactivity to alfa- and beta-gliadin was detected, but the sera did not react with glutenins. It was accordingly hypothesized that the reaction pattern was due to a specific primary reactivity against some gliadin polypeptides, with a secondary immunization with other polypeptides, initiated by epithelial damage and increased intestinal permeability.

Gliadin, digested with pepsin and trypsin (PT-gliadin), has been shown to be toxic. In order to further elucidate the connection between gliadin antigenicity and toxicity, the antibody reactivity in coeliac sera against PT-gliadin was investigated by immunoblotting. The enzymatic digestion of gliadin accomplished low molecular weight polypeptides, which notably did not display antibody reactivity.

It is therefore suggested that characteristic gliadin antibody pattern in patients with coeliac disease is not of primary pathogenetic importance, but instead correlated to common HLA-antigens of most of the patients.

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**CHANGES IN THE LIVER HBV-DNA PATTERN EXPRESSION IN CHILDREN WITH HBV CHRONIC INFECTION**

G.Moralada, J. Bartolome, M. Ruiz-Moreno, J.C.Porres, V.Carreno Pediatric and Gastroenterology Depts. Fundación Jimenez Diaz. UA Madrid, SPAIN.

The antiviral effect of r-IFN $\alpha$  on HBV replication has been proven "in vivo". However there is no information about the changes induced by the r-IFN $\alpha$  in the patterns of HBV-DNA, specially in children. The aim of this work was to study the effect of the r-IFN $\alpha$  over the liver HBV-DNA patterns in children with chronic hepatitis B.

30 children with CAH histologically proved were included. All of them had HBeAg and serum HBV-DNA for at least 6 months prior to the beginning of the study. Two liver biopsies were obtained from all the patients, one just before the treatment and the second at the 15th month.

HBV-DNA was tested in serum by dot blot and in liver by southern blot hybridization.

In the first liver sample all children had replicative intermediates of the viral DNA and in one of them the HBV-DNA was also integrated in the host genome. In the second liver biopsy, in the children who did not respond to the therapy (HBV-DNA+ in serum) the replicative forms of the HBV-DNA remained in the liver and in 3 children integrated HBV-DNA was detected simultaneously. Among the 9 patients who lost serum HBV-DNA at the end of the therapy, in 8 of them the viral DNA was undetectable in their liver, in the other child integrated and episomal forms non replicatives of the HBV-DNA were detected. None of the responder children lost the HBeAg in serum. To investigate this fact we looked for the presence of HBV-DNA in peripheral blood cells by dot-blot. None of these children showed viral DNA in these cells.

In conclusion, HBV-DNA can be integrated in the host genome early in the natural history of the HBV-chronic infection. Our results suggest the antiviral effect of the r-IFN $\alpha$  of hepatic level. The persistence of HBeAg in the responder children is not maintained at expenses of HBV-DNA expression in mononuclear blood cells.

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**PREDICTIVE FACTORS OF THE RESPONSE TO RECOMBINANT INTERFERON IN CHRONIC HEPATITIS B IN CHILDHOOD**

M.Ruiz-Moreno, V.Carreno, J.Jimenez, J.Bartolome, A.Moreno and J.C.Porres Pediatric and Gastroenterology Depts.Fundacion Jimenez Diaz UA Madrid SPAIN

The antiviral effect of recombinant interferon (rIFN) therapy of chronic hepatitis type B in children has been demonstrated. In order to determine the predictive factors of the response to rIFN, the basal features of responder and non-responder patients (age, sex, ALT levels, HBV-DNAp activity, time of HBeAg carriers, Knodell's index and percentage of infected cells of HBeAg in the liver biopsy) have been evaluated. Twenty eight children (mean age 2-14 years) with viral replication markers (HBV-DNA positive) have been included. Eight children had received 10 MU of rIFN-a2C (Boehringer Ingelheim)/m<sup>2</sup> body surface, I.M., twice a week during six months; 8 children were treated with 7,5 MU under the same conditions and 12 received 10 MU of rIFN-a2A (Roferon)/m<sup>2</sup> body surface I.M., thrice weekly during 3 months. All of them had a histologically proven CAH. At the end of the follow-up period (15 months) 8 children became HBV-DNA and HBeAg negative and were considered as rIFN-responders and 20 remained positive for these markers (non-responders).

The responders patients had a significantly lower activity of HBV-DNAp (265.7±323.6 vs 719.3±480.3, p(0.05) and less percentage of HBeAg infected liver cells than the non-responders (14.17 ± 6.83 vs 58.28 ± 32.73 p(0.05). In addition, the ALT (210.5±75.2 vs 128.8±76.1, p(0.05) and the liver Knodell's index of histological activity in the liver biopsies (12.25±2.41 vs 7.64 ± 3.23 p(0.05) were higher in the responders than in the non-responders. In conclusion, the children who responded to rIFN therapy had a more active liver disease (ALT, Knodell's index). Furthermore these patients had a relatively low level of viral replication (HBV-DNAp, HBeAg in liver cells).

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**PANCREATIC FUNCTION IN ESSENTIAL FATTY ACID DEFICIENT (EFAD) RATS**

Hjelte L, Ahren B; Andrén-Sandberg Å; Böttcher G\*\* Strandvik B.

Dept of Paediatrics, Karolinska Institute, Huddinge Univ Hospital, Stockholm, and Depts of Surgery\* and Histology\*\*, Univ of Lund, Lund, Sweden.

Patients with cystic fibrosis (CF) have EFAD which might contribute to their symptoms (Scand J Gastroenterol 1988; 23 (Suppl 143):1-4). About 90% of the patients have pancreatic insufficiency and 5-10% develop diabetes mellitus. Pancrease has been shown to be highly sensitive to EFAD as revealed by the triene-tetraene ratio. To study the influence of EFAD on pancreatic endocrine function the insulin response was studied after administration of glucose intravenously in infusion or bolus and in addition the glucagon response after arginine infusion in EFAD female rats (120 days old) and age-matched controls. The exocrine function was studied in isolated pancreatic acinar cells after stimulation with alcohol and carbacholine chloride. The amount of secreted amylase was assessed colorimetrically in computerized spectrophotometer. Gastrointestinal peptides were studied by immunohistochemical methods. The EFAD rats showed a higher insulin secretion than control rats with all stimulations whereas no differences in glucose and glucagon levels were seen. The isolated pancreatic acinar cells showed a normal amylase secretion in EFAD rats. Morphologically no changes were seen in the pancreas and the immunohistochemistry pattern of insulin-, glucagon-, somatostatin- and pancreatic polypeptide cells was not different from the controls. The results of this study indicate that the endocrine pancreas seems more sensitive to EFAD than the exocrine part.

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**UNCOUPLING OF BILIARY LIPID SECRETION FROM BILE ACID SECRETION BY ORGANIC ANIONS, DUE TO INTRACANALICULAR INTERACTION WITH BILE ACIDS.**

H.J.Verke, M.J.Wolbers, R.Havinga, R.J.Vonk, F.Kuipers Dept.of Pediatrics,University of Groningen,The Netherlands.

Biliary secretion of phospholipids (PL) and cholesterol (CH) is regulated by bile acids (BA). However, a number of organic anions (OA) has been shown to inhibit PL and CH secretion without affecting that of BA. The mechanism of this OA-effect is unclear. We studied this uncoupling phenomenon with 3 different OA in normal Wistar (NW) rats and Groningen Yellow (GY) Wistar rats. The GY strain has a genetic defect in biliary secretions of various OA (JCI 81: 1593-9, 1988). NW and GY rats with 8-day bile diversion were injected intravenously with ampicillin (18 mol.100 g BW), sulfated taurothiocholic acid (STLC, 1.0 umol/100gBW) or idocyanine green (ICG, 0.6 umol/100g BW). At 1 hr after injection recoveries in bile were: ampicillin, 4.1% and 0.5%; STLC 98% and 32%; ICG, 39% and 9%, in NW and GY rats, respectively. Ampicillin and STLC caused a strong uncoupling in NW rats (maximal BA.(PL+CH) ratio: +70% (ampicillin) and +147%(STLC), but no (ampicillin) or a much smaller (STLC +65%) uncoupling in GY rats. ICG injections did not induce an uncoupling, either in NW rats or in GY rats. The hepatic uptake of the used OA appeared to be unaffected in GY rats. Gel filtration chromatography (Sephacrose CL-4B) showed that ampicillin and STLC coeluted with BA, while ICG coeluted with the PL and CH fraction. We conclude that the uncoupling of biliary PL and CH from BA secretion by ampicillin and STLC is not due to disturbance of processes at intracellular or bile canalicular membrane level, but to interactions with BA inside the canalicular lumen which disturb PL and CH solubilization.