11

OTNÆSS, A.-B.* and HALVORSEN, S. and ØRSTAVIK, I.*
Institute for Experimental Medical Research, Pediatric Dept.
and Microbiological Dept., Ullevål Hospital, Oslo 1, Norway.

THE EFFECT OF HUMAN MILK FRACTION ON ROTAVIRUS AND E. COLI HEAT LABILE ENTEROTOXIN.

Breast fed infants suffer less from gastrointestinal infections compared to artificially fed infants. We have studied the protective effect of fractions of milk from Ethiopian and Norwegian women against rotavirus and *E. coli* heat labile enterotoxin *in vitro*. Human milk was fractionated by ammonium sulphate precipitation (50%) and column chromatography (Ultrogel AcA 44). Rotavirus specific antibodies were detected by immunofluorescence in 15 out of 24 samples before fractionation and in 11 out of 13 of the concentrated immunoglobulin-rich fractions. Rotavirus infection of LLC-MK2 cells was inhibited by the concentrated immunoglobulin-enriched milk fractions, as well as by some immunoglobulin-depleted fractions, indicating that the milk may contain rotavirus neutralizing activity of non-immunoglobulin nature. No difference in the anti-rotavirus activity between Ethiopian and Norwegian milk samples was observed. The Norwegian and Ethiopian milk samples inhibited the *E. coli* heat labile enterotoxin when the toxin was tested by enzyme-linked immunosorbent assay. Upon fractionation this inhibitory activity was not associated with the immunoglobulin-rich fractions, and gel filtration experiments indicated a molecular weight of more than 400 000.

12

W. BAUMANN (Intr. by J. SPRANGER), Department of Pediatrics, Johannes Gutenberg-University of Mainz, FRG
Liver diseases and hepatitis B virus antigens and antibodies in chronic HBsAg carriers in childhood

Liver biopsies were obtained from 109 children who had been chronic carriers of HBsAg for more than 6 months. The specimens were examined for the presence of intracellular HBsAg, HBeAg and HBeAg by direct immunofluorescence. Sera were tested for HBeAg, virus B specific DNA polymerase, anti-HBs, anti-HBe and anti-HBc. On the basis of accepted histological criteria we found chronic active hepatitis (CAH) in 56 and chronic persistent hepatitis (CPH) in 19 children. 15 cases had minimal changes (minimal hepatitis, MH) and 19 normal liver tissue (healthy HBsAg carriers, HC). Children with CAH and CPH had HBeAg, DNA polymerase and anti-HBc in their serum. HBcAg and HBeAg were found in 5-50% of the nuclei and HBsAg mostly in a lower percentage in the cytoplasm of hepatocytes. All cases with MH and 10 of 19 HC had also HBeAg positive sera. DNA polymerase showed higher activities and anti-HBc lower titers compared with CAH and CPH. HBcAg and HBeAg were detected in 50-90% of liver cell nuclei. 9 HC had only HBsAg in their hepatocytes and showed anti-HBe in serum. MH and HC have been first described in childhood and differentiated from other types of chronic HBsAg positive hepatitis. HC occurred both with and without persistent virus B infection. Furthermore, the study demonstrates that the presence of HBeAg and DNA polymerase in serum are closely associated with HBcAg and HBeAg expression in liver cell nuclei. It is suggested that immune responsiveness determines the variable reaction patterns of virus B antigens and antibodies as well as the form of hepatitis.

13

Comparison of the Epstein-Barr virus (EBV) antibodies against viral capsid antigen (VCA) and early antigen (EA), in two populations of hospital children from 0 to 15 years old differing with regard to socio-economic status. J. LEVY*, M.E. LAMY*, A.M. FAVART*, G. ZISSIS*, M. VAINSEL, 1 Dept. Pediatrics (Prof. Daëhy) 3 Lab. Virology, University of Brussels-2 Lab. Virology, Catholic University Louvain, Belgium.

We have studied the presence of antibody against EBV in two populations: the first included 561 children mostly immigrants of low socio-economic status and the second included 2189 children representing a large sample of the Belgian population. From 1 to 5 years of age, the percentage of positive sera against VCA rose rapidly within the first population (69% by the age of 2, 79% by the age of 3 and 78% by the age of 4). In the second group, the percentage of VCA positive sera rose more slowly (49% by the age of 2, 53% by the age of 3 and 49% by the age of 4). The differences between the two groups are statistically significant ($\alpha < 0.05$). From the age of 5, no more difference was found. The percentage of EA positive sera in the 2 groups were respectively 31% and 21%. In spite of the fact that the difference between the 2 groups was not statistically significant, these high percentages of EA positive sera reflected a status of active immunization in the age groups of 1 to 5 years in the 2 populations. By contrast, in a group of school children of 10 to 15 years, we have found only 12% of EA positive sera. This difference is statistically significant ($\alpha < 0.05$). In the first population, 55 patients (9.75%) had anti EA titer $> 1/16$, suggestion of a recent infection. Eleven of them (20%) had a clinical picture suggesting of actual EBV infection.

14

THE INTERFERON SYSTEM: A NEW ASSAY AND ITS CLINICAL IMPLICATIONS. STANLEY LEVIN and THALIA HAHN*, Pediatric Research Dept., Kaplan Hospital, Rehovot, Israel.

Prevention of viral replication is a major function of the Interferon (IF) system in man, and can be divided into 2 phases. Firstly, the production of IF by cells following stimulation by any of a number of inducers, including viruses; and secondly, the action of this IF on other cells, leading to the production of anti-viral proteins which become activated when a virus enters the cell, thereby preventing replication of the virus. We have developed an assay system which simultaneously examines the blood IF, the ability of mononuclear cells to produce both type I and type II IF, and whether the cells have been primed into an anti-viral state. Further this assay will show whether in the absence of *in vivo* cell protection from viral replication, extrinsic IF is able to prime the anti-viral state and at what dosage level. Our studies show that healthy children and adults have virtually no IF in the blood, that their mononuclear cells produce both types I and II IF in high titre when stimulated, and that their cells will promote good viral replication which can be inhibited with extrinsic IF. On the other hand children with suspected viral disease have good blood IF levels, their IF production by mononuclear cells is somewhat suppressed, and their cells will not promote viral replication indicating a good anti-viral state. Pharmacodynamic IF studies on patients with severe viral infections receiving IF therapy indicate the importance of this combined assay, and in particular the value of determining the anti-viral state of the patient's cells.

15

K.-G. SABEL*, B.O. ERIKSSON* and L.-G. FRIBERG* (Intr. by P. Karlberg), Dept of Pediatrics, Univ of Göteborg, Göteborg, Sweden. Metabolic effects of symptomatic ventricular septal defect (VSD) in infancy.

Feeding difficulties play a major role in the clinical picture of severe congestive heart failure (CHF) in infancy resulting in poor weight-gain and reduced fat stores and muscle mass. Muscle enzymes and substrates were studied during diagnostic heart catheterization in 20 infants, 1-13 months, with symptomatic VSD. Biopsies from the gracilis muscle were immediately frozen in liquid nitrogen. In a subsample of patients *i.v.* glucose tolerance tests (0.5 g/kg) and insulin determinations were performed with simultaneous sampling from femoral artery (FA) and main pulmonary artery (MPA). In muscle the conc of ATP, creatine-phosphate and glycogen were low compared to healthy controls. Succinate dehydrogenase activity (as indicator of aerobic energy capacity) was decreased, while lactate dehydrogenase (anaerobic glycolysis) was not. Infants with manifest CHF and poor weight-gain had slower disappearance rate of glucose (k_d) and lower insulin response than infants who had overcome the failure and were gaining weight. Insulin levels in MPA and FA did not differ significantly. Max insulin response (FA) did not correlate to the degree of left to right shunt. Reduced muscle blood supply, nutritional deficiency and inactivity may be responsible for the muscle enzyme and substrate reductions. The differences in k_d and insulin response may be due to calorie deficiency and/or inhibition of insulin release by high catecholamines present in CHF. Increased insulin clearance by the lung in large left to right shunts is not supported by our results.

16

J. KNUDTZON* (Intr. by O. Trygstad) Pediatric Research Institute, Rikshospitalet, Oslo, Norway. The *in vivo* effect of thyrotropin-releasing hormone (TRH) on plasma levels of pancreatic specific glucagon (G), insulin (I), blood sugar (BS) and free fatty acids (FFA) in rabbits.

TRH immunoreactivity and bioactivity have been demonstrated in pancreatic islets, but studies with isolated rat pancreas have failed to show any direct stimulatory effect of TRH on G and I. In order to study the *in vivo* effect, TRH "Roche" was injected intravenously into fasted rabbits, and blood samples were obtained from the ear vein. Compared to saline controls ($n=5$), the peak increases (mean $\pm$ SEM) for G (256 ± 30 pg/ml) and FFA (2.4 ± 0.2 mmol/l), and for I (23 ± 4 μ U/ml) and BS (1.8 ± 0.3 mmol/l), were found within 15 and 60 min. after injection of 11 nmol ($n=5$, $p=0.005$) and 22 nmol ($n=5$, $p=0.05$) of TRH respectively. The analogous tripeptide pyroGluHisGlyOH did not influence G and I. A significant increase of G was observed with 1,1 nmol TRH. The increase of G was augmented by simultaneous injection of TRH and insulin, and was nearly abolished in fed rabbits. It is suggested that TRH may have physiologic significance in modulating G, I, BS and FFA. In contrast to the effects of an alanine load, the increases of G and I are not significant within the first 5 min. This may suggest an indirect effect by humoral or neural mechanisms, possibly via the brain.