143 THE INFLUENCE OF ACUTE ACID-BASE CHANGES ON BICARBONATE HANDLING IN TJE NEWBORN RABBIT.

A.J. v.d. Heijden, J.P. Guignard and E. Gautier.

The ability of the newborn kidney to reabsorp filtered bicarbonate seems to be limited when compared to that of the adult kidney. This may be due to renal or extrarenal factors which are still ill-defined. Bicarbonate handling during acute acid-base changes was studied in newborn rabbits before the end of nephrogenesis. Fourty anesthetized mechanically-ventilated rabbits aged 5-12 days were studied during hypercapnic acidosis, metabolic acidosis or metabolic alkalosis. Inulin was used as a marker of glomerular filtration rate. Control newborn rabbits were in a state of hypochloremic metabolic alkalosis (blood pH 7.49, HCO_3 31 mmol/l, Cl 83 mmol/l) which was not present in adult rabbits (pH 7.41, HCO_3 19.5, Cl 100). This was ascribed to elevated urinary chloride losses in the first weeks of life. Hypercapnia with a $PaCO_2$ of 80-100 mm Hg induced a significant rise in bicarbonate reabsorption, resulting in a possitive correlation between bicarbonate reabsorption and $PaCO_2$ ($y= 20.4 + 0.15 x$, $r= 0.86$, $p<0.01$). During metabolic acidosis induced by infusing NH_4Cl and metabolic alkalosis induced by infusing $NaHCO_3$, bicarbonate reabsorption was mainly dependent on the filtered bicarbonate load in a wide range of serum bicarbonate levels (from 19 to 39 mmol/L). We conclude that in the chloride losing alkalemic newborn rabbit, bicarbonate renal handling responds to the same physiologic variables as present in adult animals.

144 CAFFEINE DOES NOT PREVENT HYPOXAEMIAS IN PREMATURE INFANTS: A RANDOMISED CONTROLLED TRIAL

Bucher HU, Duc G. Department of Neonatology, University Hospital, Zurich, Switzerland

Spontaneously breathing preterm infants 48 h old, of 32 weeks gestation or less, were assigned randomly to receive caffeine citrate (loading dose 20 mg/kg, maintenance dose 10 mg/kg/day) or placebo (NaCl 0.9%). To demonstrate a reduction from 50% to 25% in the proportion of infants with >6 hypoxaemias (decrease in $tcPo_2$ of 20% within 20 sec)/12 h required a sample size of 25 per group (50% power, 5% type I error). $tcPo_2$ was recorded continuously for 50 h and analised by computer. The two groups did not differ significantly in gestational age, birth weight, delivery mode, sex distribution, Apgar scores. Mean serum concentration (SD) of caffeine 2 h after the second maintenance dose was 96.0 (34.5) umol/l in the caffeine group and 9.3 (12.8) umol/l in the placebo group. The proportion of infants with hypoxaemias in the caffeine group did not drop below the values of the control group.

Group	N	gestat.age M (SD) wks	infants with hypoaemias before	12	24	36	48 h
Caffeine	25	30.3 (1.8)	52%	60%	44%	68%	56%
Placebo	25	30.4 (2.0)	60%	56%	44%	56%	48%

In conclusion our data do not confirm the efficacy of caffeine in preventing hypoxaemias in premature infants.

145 CHORIONIC VILLI PHOSPHOHYDROLASE ACTIVITY - USE FOR PRENATAL DIAGNOSIS OF GLUCOSE-6-PHOSPHATASE DEFICIENCY?

Barash, V. Khassis, S., Riskin, A. Dept. Biochemistry, Hadassah University Hospital, Jerusalem, Israel.

Prenatal diagnosis of Type I glycogenosis has not, so far, been possible since activity of glucose-6-phosphatase (G-6-p'ase) cannot be demonstrated in amniotic fluid fibroblasts. The introduction of chorionic villi sampling (CVS) raised the possibility of using this tissue for prenatal diagnosis of glycogenosis I. In view of conflicting reports on activity of G-6-p'ase in the placenta, phosphohydrolase activity with G-6-P and β-glycerophosphate (βGP) was assayed on villi homogenates and on the microsomal fraction (week 10-12). The pH curve obtained with both substrates showed maximal activity at pH 4 and no peak at pH 6.5. Km was 20 and 8 mM for G-6-P and βGP, respectively. Vmax and enhancement of activity by treatment of the microsomal fraction with detergents (Triton-X100 and desoxycholate) were similar for both substrates. Activity was not affected by preincubation of the preparation at pH 5 and 37°C. Activity of PPi-glucose phosphotransferase, known to be characteristic of glucose-6-phosphatase, was only 5% of that of phosphohydrolase. These results do not support the existence of specific G-6-p'ase activity in CV and the possibility to use CVS for prenatal diagnosis of glycogenosis I.

146 GLYCOGEN METABOLISM IN CHORIONIC VILLOUS SAMPLINGS: POSSIBILITIES FOR PRENATAL DIAGNOSIS OF GLYCOGEN STORAGE DISEASES.

Shin, Y., Friedel, J., Besser, A., Rieth, M., Gerg, J. and Endres, W. University of Munich, Children's Hospital, Munich, F.R.Germany.

Various enzymes of which the deficiency causes a type of glycogenoses have been investigated in 20 control samplings of chorionic villi (CVS) between 8 and 12 weeks of gestation. Except glucose 6-phosphatase which is either not expressed or not yet developed, the activity of seven enzymes were well measurable in CVS. The glycogen content was 0.2-0.8 g per 100 g CVS; the activity of 1,4-α-glucosidase (4G), 3.0-16.0 nmol/min/mg protein (U); amyloglucosidase (6G), 0.12-0.56 U; brancher, 0.40-0.95 U; phosphorylase (PL), 0.2-1.5 U (a form) or 3.0-15.0 U (total); phosphorylase kinase (PK), 1.3-7.0 µmol/min/mg protein; phosphofructokinase (PF), 1.8-8.5 U; glycogen synthase, 0.1-0.3 U (I-form) or 0.4-3.0 U (D-form). Prenatal diagnosis of M.Pompe was performed in a pregnancy at risk by chorionic biopsy at the 8th week as well as by amnioscentesis at the 17th week. The 4G activity in CVS and in amniotic cells was in the range of heterozygotes, as was in leucocytes of cord blood at birth. Glyocgenosis type III can also be diagnosed prenatally by direct assay of 6G in CVS. Investigation of PL, PK and PF was done by isoelectrofocusing and kinetic studies in order to explore diagnostic ways of severe forms of respective deficiencies.

147 NEW VARIANTS OF PHOSPHORYLASE KINASE DEFICIENCY.

Bashan N, Potashnik R, Ehrlich T, Abarbanel J and Moses SW. Faculty of Health Sciences, Ben-Gurion University of the Negev and Pediatric Laboratory, Soroka Medical Center, Beer Sheva, Israel.

Three variants of phosphorylase kinase (PK) deficiency have been described, differing from one another by their genetic mode of inheritance and their organ involvement. The present report describes two additional variants for GSD IX: The first, in which the PK deficiency was reduced in muscle only (activity was 0.3 as compared to 2.6±1.5 U phosphorylase a/mg prot/min), with no indication of liver, erythrocyte or leukocyte involvement. The second variant presented in a 2-year-old boy with hepatomegaly and a slight tendency to fasting hypoglycemia. Blood cell PK activities were measured and are summarised in the table.

	Red Blood Cells (RBC) exogenous substrate U phos. a/g Hb/h	Red Blood Cells (RBC) endogenous substrate U phos. a/g Hb/h	White Blood Cells (WBC) exogenous substrate U phos. a/g prot/h	White Blood Cells (WBC) endogenous substrate U phos. a/g prot/h
patient	0	0	3800	0
control	20	0.25	5000	240

The K_m of leukocyte PK to phosphorylase b was normal. The difference in the patients' enzyme activity found in WBC as compared to RBC may be explained by the production of an unstable PK. The detection of additional variants of PK is not surprising in view of the fact that PK consists of 4 subunits which are coded on different chromosomes; thus various mutations are expected to be expressed in different tissues and to follow diverse modes of inheritance.

148 LYSOSOMAL GLYCOGEN STORAGE DISEASE WITHOUT DEFICIENCY OF ACID α-GLUCOSIDASE.

K.Ullrich, D.von Bassewitz, J.Shin*, R.Korinthenberg, K.von Figura, University of Münster and München*, FRG.

Two siblings (50 and 64 y old)presented with weakness and pain of trunk and lower limb muscles. Serum,CPK,ECG,echocardiography and NCV were normal.The EMG demonstrated myopathic and pseudomyotonic discharges. Ultrastructural examination revealed lysosomal glycogen storage in sceletal muscle,fibroblasts and blood lymphocytes. By immunoprecipitation no deficiency of acid α-glucosidase was found in fibroblasts, leucocytes,lymphocytes and urine(substrates: p-N-phenyl-α-D-glucopyranoside,maltose,isomaltose, glycogen).Heat stability,pH-profile and Km(p-N-phenyl-substrate,1.5 mM)of the enzyme were normal.Previous studies presented a defective post-translational modification of acid α-glucosidase in late onset forms of GSD II (FEBS Lett.150,69,1982). We therefore studied the incorporation of radioactive leucine into the enzyme by immunoprecipitation,gel-electrophoresis and fluorography (J.Biol.Chem.255,4937,1980).Synthesis of precursor α-glucosidase such as processing of the enzyme to it's mature forms was found to be normal in the cultivated fibroblasts of the patients.