WGMM—Abstracts for Poster Presentations

235 INTRACELLULAR PHOSPHATE METABOLISM IN METABOLIC DISORDERS

Vitamin D dependent rickets (VDDR) and renal tubular acidosis (RTA). Case reports. P. Lapatsanis, G. Vrionis, H. Salvanos, A. Challa. Dept. of Paediatrics, University of Ioannina, Greece.

Red cell inorganic phosphate (Pi) was studied in two siblings (a 3y-old girl and a 2m-old boy) with VDDR and another infant (2m-old boy) with RTA before and during therapy. The girl was already on 2μg $1\alpha OHD_3$/d which was discontinued and the boy was followed from the age of two months. In both cases the parameters studied were normal at start and were followed regularly until SeCa had fallen respectively to 6.52 and 6.38mg/dl, when $1\alpha OHD_3$ administration commenced. At that time plasma Pi (PlPi) also fell by 1-1.5mg/dl while red cell Pi (RBCPi) hardly changed. Consequently the distribution of phosphate ions across the erythrocyte membrane changed markedly. All parameters studied started normalising from the first weeks of treatment. In the case of RTA at the time of diagnosis when blood pH was 7.27, HCO_3^- 6mEq/l and Cl^- 118mEq/l SeCa was 8.5mg/dl, PlPi 4.6mg/dl and RBCPi abnormally low (0.7mg/dl). After the commencement of HCO_3^-, SeCa started rising while PlPi showed a transient fall and began rising after a week when $1\alpha OHD_3$ (0.3μg/d) and citrate were given. RBCPi showed a steady rise and within 2 months it had reached physiological levels (2.8mg/dl). At that time SeCa was 9.8mg/dl and PlPi 5.7mg/dl. Metabolic acidosis seems to be responsible for the depletion of erythrocyte Pi and probably in other cells too, while the correction of pH leads to repletion even before HCO_3^- and Cl^- ion concentrations normalise.

236 VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEIN (CaBP 9k-Da) IN EPIPHYSEAL CARTILAGE IN GROWING RAT : EXTRACELLULAR AND CYTOPLASMIC LOCALIZATION.

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Calcium-binding protein is a molecular expression of the hormonal action of $1,25(OH)_2D_3$. To further evaluate the direct action of this hormone on growth cartilage, CaBP 9k-Da was localized by immunohistochemistry.

Tibial growth cartilage from 21 d old rats was fixed in formaldehyde or Carnoy's solution and frozen. Cryosections were incubated with diluted antiserum followed by peroxidase-conjugated protein A. Control sections were reacted with normal rabbit serum or antiserum treated with excess CaBP.

CaBP, found in the cytoplasm, was first encountered in maturing chondrocytes and was not detected in either resting or proliferative cells. Present in large quantities in the region of the rough endoplasmic reticulum of the upper hypertrophic cells, CaBP was then found in the cytoplasmic processes. CaBP became exteriorized and accumulated in those extracellular lateral regions where matrix vesicles are known to occur. CaBP concentration decreased progressively in the lower hypertrophic cells as the lateral edges of the longitudinal septa underwent calcification. Then CaBP was present in the extracellular matrix where it was exclusively concentrated in the lateral edges of the longitudinal septa.

These findings raise the possibility that CaBP 9k-Da may be involved in the matrix vesicle associated process of cartilage calcification.

237 NUCLEAR LOCALIZATION OF VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEIN (CaBP 28k-Da) IN THE EPIPHYSEAL CARTILAGE IN GROWING RAT.

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The presence of CaBP (28k-Da) was localized in the rat epiphyseal plate by indirect immunohistochemistry using a specific antiserum in the rabbit against rat kidney CaBP.

Tibial epiphyseal cartilage from 21 d old rats was fixed in Carnoy's solution and frozen. Cryosections were incubated with diluted antiserum followed by peroxidase conjugated protein A. A series of controls were performed including the non immune rabbit serum instead of the specific anti-CaBP antibody.

CaBP was found exclusively in the nuclei of the chondrocytes. It was not uniformly distributed throughout the epiphyseal cartilage : it was selectively located in the proliferative and maturing chondrocytes ; specific staining was first observed in the nuclei of the resting chondrocytes. CaBP was not detected in the hypertrophic chondrocytes.

The data show the exclusive intranuclear localization of CaBP in chondrocytes implicated in proliferative activity. These findings raise the possibility that CaBP 28k-Da may be involved in the mitotic activity of the chondrocytes acting as a regulator of the intranuclear calcium. Furthermore, the localization of 28k-Da CaBP, which is different from that of 9k-Da CaBP (reference to preceeding summary) suggests a specific role for each protein in the epiphyseal cartilage.

238 ENAMEL DEFECTS IN DECIDIOUS DENTITION OF LOW BIRTH WEIGHT INFANTS.

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Enamel defects have been observed in the deciduous dentition of preterm infants, but few systematic studies have been carried out. A dental examination is part of the study of a cohort of infants weighing 2000g or less at birth for which comprehensive neonatal and maternal medical histories are available. The Fédération Dentaire Internationale index for developmental defects of enamel was used to record opacities (defective calcification), hypoplasis (deficient amount of enamel) or discolouration. Data from 86 children aged 3-5 years showed a much higher prevalence of defects than that found in a normal population (Murray J Shaw L Arch Oral Biol 1979; 24: 7-13. 71 (81%) were affected with 49 (56%) having hypoplasia of one or more incisor. In our study, enamel was frequently missing from the incisal edge of incisors. This enamel begins to calcify at 4 months of intrauterine life and is maturing at birth. 16 children who had major neonatal illness all had hypoplasia of the incisal edge of one or more incisor. This may indicate developmental disturbances in the second half of pregnancy or reflect post-natal influences on maturation. Trauma from endotracheal intubation has been postulated as an aetiological factor but due to the symmetry of the lesion the authors favour sytemic disturbances.

239 THE USE OF PHOSPHATE SUPPLEMENTS IN EXTREMELY LOW BIRTHWEIGHT INFANTS TO PREVENT RICKETS OF PREMATURITY

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During the years 1981-1983 inclusive, 26 of 48 neonatal survivors (54%) of birthweight less than 1000g developed radiological rickets despite an intake of 2000 units of Vitamin D daily from 7 days of age. The rickets was quantified using the radiological grading of Koo et al, grades 2 and 3 being accepted as diagnostic. From this early survey, we concluded that rickets of prematurity was due to factors other than Vitamin D deficiency in our unit. From January 1984 until January 1985, infants of birthweight less than 1000g were given 1000U of Vitamin D per day and in addition 1ml of buffered sodium phosphate BP (26.6mg phosphate) per day from Day 1 of life. The babies were managed on expressed breast milk - either fresh mother's own or donor pasteurized, usually introduced within 3 days of birth. The incidence of rickets was reduced in the 31 infants surviving for 28 days or more (54% to 43%). In addition, the severity of the condition was reduced. In a control group of infants 1001-1250g in birthweight who were given Vitamin D supplements but no additional phosphate, the incidence of radiological rickets did not alter from previous years.

We conclude that phosphate supplementation to extremely low birthweight babies on adequate Vitamin D supplementation reduces both the incidence and severity of rickets of prematurity but does not abolish it. It is likely that other nutritional supplements (possibly calcium) are also required.