B. KREMPEN* and J. LUCKEY* Department of Pathology, University of Heidelberg, F.R. Germany. Functional structure and strenght of the growth apparatus in normal and in uremic rats.

Epiphyseolysis is a rare condition in healthy children, but it is a common finding in children with chronic uremia.

We therefore tried to analyse the tensile strenght and the functional structure of the proximal epiphyseal cartilage in normal and uremic rats.

Material and methods

Tibiae of Wistar rats (250g, uremia of four weeks duration, sham operated controls) were excised and freed from the surrounding soft tissue. In order to measure the tensile strenght of the growth apparatus, we prepared two bore wholes within the epiphyseal and diaphyseal bone, which served as a suspension in a ZWICK material testing machine.

Non mineralized surfaces of cartilage, which were exposed by the final rupture, were prepared by the critical point drying method. Mineralized structures of cartilage and bone were exposed by digestion of unmineralized organic material by sodium hypochlorite. The specimens were analysed by scanning electron microscopy. Collagen fiber systems were either studied in serial sections using transmission electron microscopy, or by scanning electron microscopy after a dissolution procedure of the cartilage matrix by a papain treatment.

Results

Tensile strenght of normal rats was 56,73 +/- 6,03 N. In uremic rats we found a significant reduction of tensile strenght of 39,90 +/- 4,96 N.

Electron microscopical studies: Epiphyseal cartilage and epiphyseal bone are connected by numerous plugs of cartilage, which insert in holes of the epiphyseal bone plate. This structure prevents a sliding of the epiphysis.

In columnar cartilage several columns are bound together to bundle pillars by collagen fibers. Longitudinally arranged fiber systems and a lattice system of shearing fibers can be identified in between these columns. Each pillar is connected with the epiphyseal bone plate by a radially orientated fiber system which resembles a system of shrouds. Neighbouring cells within the columns are bound together by a densely packed wickerwork of crossing fibers.

In the zone of mineralization longitudinal septa of cartilage show globiform mineral deposits. These globiform surface structures enlarge the interface between the chondrocytes and the mineralized matrix, increasing the adhesive strenght in this region. Longitudinal septa are gradually transformed into metaphyseal bone by enchondral ossification. Thus a continuous functional system of epiphyseal bone, growth cartilage and metaphyseal bone is formed.

O. SIMELL, Children's Hospital, University of Helsinki, Helsinki, Finland. Serum and tissue alkaline phosphatases and electron microscopy of the bone lesions in the juvenile hypophosphatasia syndrome

Abstract not received

ELEANOR DAFFORN-IERODIAKONOU*, A. MORAKIS*, C. VRETOS* and P. LAPATSANIS* Paediatric and Orthopaedic Unit "Aghia Sophia" Children's Hospital, Athens, Greece. Hyperphosphaturia producing collapse of vertebral body.

A child age 9 years complained of pains in both legs from November 1975 and was unable to walk 1 month later. On clinical examination he had pain on pressure over L3 and L4 lumbar vertebrae, but no other abnormal clinical findings. Body height 142 cm (90th percentile). The following tests were abnormal: serum phosphorus (P) 3.6-4.0mg/dl, UP 0.88-1.1. X-ray showed a collapse of L4. The patient was put in a plaster jacket and started on 2g of phosphorus daily by mouth. 3 months later he was able to walk again and in another 7 months painfree and the plaster was removed. His height was 145 cm (90th percentile), serum Ca 11 mg/dl, serum P 3.7 mg/dl UCa_{0.07}, UP 1.15. X-ray of spine showed a marked improvement of L4. The treatment with oral administration of phosphate was continued and the patient reviewed 12 months after removal of his plaster in September 1977. X-ray showed a continuing improvement of L4, serum Ca 9.9 mg/dl, serum P 4.3 mg/dl, urinary Ca and P excretion in 24 hours 73 and 1385 mg respectively. One year later in September 1978 whilst still on phosphate therapy his results were serum Ca 9.7 mg/dl, serum P 4.3 mg/dl and x-ray improvement in shape of L4. This condition seems to be due to a renal tubular handicap for handling P resulting in phosphaturia but producing only an isolated bone lesion without rickets. Since there is healing with phosphate alone and his father also has abnormal phosphaturia but no bone lesions it could be an atypical form of vitamin D dependent rickets.

R. STEENDIJK* Department of Paediatrics, University Hospital, Binnengasthuis, Amsterdam, The Netherlands. Statural growth and serum-P in hypophosphataemic rickets.

In cross-sectional studies the average height of 37 untreated boys and girls with X-linked hypophosphataemic rickets (X-LH) between 1 and 13 years of age was 2 standard deviations below the normal mean, but there was no relation between height and serum-P in single determinations (Steendijk and Latham, *Helv Paed Acta* 26: 179, 1971). Sustained increased growth rates have only rarely been observed during therapy with high doses of vitamin D or dihydrotachysterol,

and it is not known whether those occurred following a sustained rise in serum-P. Catch-up growth during treatment with oral phosphate supplements, causing a rise in serum-P, has more often been seen, but only in short-term studies. To establish a relation between the level of serum-P during childhood and adolescence, and adult height in a long-term longitudinal study 8 patients with X-LH were followed for periods of 12 to 17 years until adult height had been reached. The patients were treated with dihydrotachysterol in high doses but did not receive phosphate supplements. Since serum-P was not constant during the years of treatment it was necessary in every patient to derive an average value from the serum-P determinations. This was done in the following manner: serum-P was determined at least 4 times per year in each patient, and the average serum-P value per year of age was calculated. From these mean values, the average serum-P (av. serum-P) for the whole period of observation was determined. This final value was correlated with adult height, expressed as standard deviation score (SDS). The coefficient of correlation (r) was 0.789 (p < 0.05) and the regression equation was:

$$\text{Height (SDS)} = 7.64 \times \text{av. serum-P} - 24.51$$

Av. serum-P ranged from 2.77 to 3.17 mg/100ml and adult height ranged from 0.04 to -3.34 SDS. Within this range of values a reduction of av. serum-P by as little as 0.1 mg/100ml would entail a reduction of adult height by 0.77 SDS (1 SDS equals 6.8 cm in men and 6.2 cm in women). This relatively large effect of a small change in serum-P may help to explain why a relation between serum-P and height has not been found before.

I. MCKINLAY*, J.D. CROMBIE*, N.R. BELTON* and D.G.D. BARR* Royal Hospital for Sick Children, Department of Child Life & Health, University of Edinburgh, Edinburgh, United Kingdom. Effects of calcitonin in a case of congenital hypophosphatasia.

A female infant with congenital limb deformities developed cranio-tabes and x-ray revealed rachitic metaphyses. Initial biochemistry showed serum calcium 11.6 mg%, phosphate 7.8 mg%, alkaline phosphatase 6 King Armstrong units. Subsequent alkaline phosphatase values were persistently low (range 0.9-2.7 K.A. Units) and a diagnosis of the severe form of congenital hypophosphatasia was made supported by levels of alkaline phosphatase in cultured cells and tissues. Family studies showed a pattern consistent with autosomal recessive inheritance.

At the age of three months she was irritable, feeding poorly and failing to thrive. Porcine calcitonin 50 M.R.C. Units/day was given intramuscularly for three weeks. Within two weeks hardening of the skull bones was noted with disappearance of cranio-tabes which recurred, however, six weeks later. X-rays over the following months showed increased mineralisation and periosteal new bone. Metabolic balance studies for calcium, phosphorus and magnesium before, during and after calcitonin therapy showed an alteration from an average positive calcium balance of 15 mg/day to 103 mg/day with treatment. No consistent change in phosphate or magnesium balance was evident. At the time of calcitonin treatment evidence of craniosynostosis developed. The child died of chest infection at 14 months of age.

In this case of severe congenital hypophosphatasia there is evidence of clinical, radiological and biochemical response to calcitonin. Treatment may have precipitated craniosynostosis and failed to alter the grave prognosis. It is possible that smaller doses of calcitonin over a longer period of time would be safer and more effective.

H.W. KRETH* (Intr. by V. ter Meulen). Institute of Virology and Immunobiology, University of Würzburg, W-Germany. Cell-mediated immunity in acute and chronic measles virus infections.

While the etiology of subacute sclerosing panencephalitis (SSPE) by a measles-like virus is now firmly established, the pathogenesis of SSPE is not understood. It has been suggested that the disease might be due to a specific unresponsiveness at the level of T cells (Burnet, *Lancet* ii, 610, 1968). To test this hypothesis specific T cell functions were measured by direct lymphocyte-mediated cytotoxicity in vitro using measles virus-infected lymphoblasts as target cells in 4- hr Cr-51 release assays. Results: Cells with the properties of cytotoxic T lymphocytes (CTL) were only present in children with acute measles and could be found among peripheral blood lymphocytes for up to 7 days after onset of measles rash. These cells were functionally restricted by the HLA system and belonged to the FcR_γ negative pool of lymphocytes. Such cells could neither be detected in measles convalescents nor in patients with SSPE. It is proposed that in order to assess specific T cell immunity in donors beyond the acute stage of measles, memory T cells must first be restimulated in vitro into secondary populations of CTL before testing direct lymphocyte-mediated cytotoxicity.

H. SIEMES*, M. SIEGERT*, and F. HANFELD. Kinderklinik der Freien Universität Berlin, F.R.G. Determination of Oligoclonal Gammaglobulin in the CSF of Children with Subacute and Chronic CNS-Infections by Quantitative Agarosegel Electrophoresis.

CSF-proteins of 1300 children and adolescents with different neurologic diseases and of 75 normal controls were examined by zone electrophoresis in agarosegel and by immunologic techniques. The quantitative evaluation of the phoretograms by an analog computer revealed in 68 patients who suffered from subacute or chronic CNS-infections marked changes of the γ -globulin profile. 32 out of

37 children with congenital infections showed a marked increase of γ -globulin during the first two years. One half of these patients revealed 1-5 oligoclonal IgG-fractions (comprising 0.6 - 15.5 % of total protein), that persisted for at least several months. Though γ -globulin was increased only in 3 out of 6 patients with multiple sclerosis, 2-5 oligoclonal IgG-fractions (5.5 - 20.4 % of total protein) appeared in all cases. In 16 children suffering from subacute sclerosing panencephalitis oligoclonal IgG (5-7 subfractions) amounted to 20.1 - 56.1 % of total protein. Furthermore, 1-5 oligoclonal IgG bands (8.2 - 35.5 % of total protein) were found in the CSF of 5 children during prolonged non-bacterial meningitis, in 2 patients with chronic aseptic meningoencephalitis, and in 2 cases of subacute bacterial meningitis. Since oligoclonal IgG occurred only in the CSF of cases of subacute or chronic neurologic infections (with the exception of leukemia and medulloblastoma) this finding could mean persistence of antigen in the CNS and pathologic invasion of lymphocytes into the CNS with selective proliferation of antigen-stimulated clones.

45 G. RABENDING*, K. JAHRIK, F. HEYDENREICH*, R. PERLWITZ* and M. KOCH* University of Greifswald, GDR. Interval analysis of repetitive complexes in SSPE.

The time intervals between biologic phenomena (f.e. between R-waves of ECG or respiration excursions) are not always very constant. Nevertheless such events are called "periodic". In fact the train of intervals depends on periodic and superimposed random factors (=noise). A significant task of an analysis of biologic rhythms is to separate the really periodic signals from the basic noise, and to visualize them clearly. The knowledge of the periodicity of repeated phenomena is interesting for identifying a possible functional pace-maker. It is a presupposition to start a cross correlation for statistical investigations of the kind of interrelations of different periodic events. A suitable method to detect such a periodicity is the spectral analysis.

We used it to answer the question, if the repetitive complexes (RA-DERMECKER complexes) in the EEG of SSPE are indeed "periodic" rhythms. After discussion of some methodological problems of autocorrelation and the variance spectra, the results of a statistic analysis of 18 tracings from seven children with SSPE are demonstrated. It could be shown, that the intervals between the typical high voltage complexes (wave bursts) are intraindividually highly constant. Over a longer time during the course of disease the length of intervals has a tendency to decrease. The diminution of the time span between the complexes follows a potency equation with negative exponent. The demonstrated way to analyse autocorrelations is generally useful for examinations of repeated, probably periodic biologic phenomena, which can mathematically be described as "point processes".

46 M.J. DILLON, V. SHAH* and M.D. MITCHELL* Renal Unit, Hospital for Sick Children and Queen Elizabeth Hospital for Sick Children, London, and Muffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford.

Bartter's Syndrome: 10 cases in childhood. Results of long term Indomethacin therapy including effects on Prostaglandins in urine.

Ten children with Bartter's Syndrome were studied. Their ages at diagnosis ranged from 3 months to 15 years. There was an equal sex distribution. A wide spectrum of severity in terms of clinical and biochemical features was found. In addition to those findings considered diagnostic of the condition, some patients were shown to have hypercalcaemia, hypophosphataemia, hypercalciuria, nephrocalcinosis, rickets and urine acidification defects. Two affected children were siblings. Six children were treated over prolonged periods (6 - 24 months) with Indomethacin. The effects of treatment were monitored in several ways, including regular measurement of PRA, plasma aldosterone, G.F.R. and PGE₂, PGF₂ and PGFM in urine. Remarkable clinical and biochemical improvement was documented including catch-up growth in all cases. In spite of this, PG findings in urine were inconsistent and tolerance to Indomethacin appeared to develop in some children. Although PG synthetase inhibitors have revolutionized treatment of Bartter's Syndrome it is questioned whether excess renal PG production is the primary cause of the disorder or just another epi-phenomenon.

47 K. KRUSE*, H. BARTELS and R. ZIEGLER*. Dept. of Pediatrics, Univ. of Kiel, and Dept. of Internal Medicine, Univ. of Ulm, GFR.

Parathyroid function and serum calcitonin in children receiving phenytoin drugs. In various bone diseases (b.d.) associated with secondary hyperparathyroidism, serum calcitonin (ct) levels are high, which seems to inhibit demineralisation. In pigs, phenytoin inhibits ct secretion. To further elucidate the pathogenesis of anticonvulsant b.d., serum ct levels and several parameters of parathyroid function were studied in 29 anticonvulsive treated children and 35 controls. In patients, mean values of serum calcium and phosphate, urinary calcium/creatinine (Uca/cr), and phosphate reabsorption (TmPO₄/GFR) were significantly decreased, serum alkaline phosphatase (AP), parathyroid hormone (iPTH) and urinary cyclic AMP (UcAMP) were significantly increased. AP and iPTH were correlated to UcAMP (p<.001), whereas iPTH and UcAMP were inversely correlated to Uca/cr (p<.001 and p<.05). Serum ct levels were significantly decreased (median 100 pg/ml, range <100-520 pg/ml; controls:

median 150 pg/ml, range <100-740 pg/ml, p<.05). ct levels were low ($\leq$ 200 pg/ml) even in 6 patients with obvious hyperparathyroidism (iPTH 6.2-12.1 pmol/l, controls: <5.3 pmol/l). Thus, drug induced reduction of ct mediated inhibition of skeletal resorption is probably an additional pathogenetic factor in anti-convulsant bone disease.

48 P. WIELAND*, U. TRECHSEL*, K. VETTER*, H. SCHNEIDER*, A. HUCH*, H. FLEISCH* and J.A. FISCHER* (Intr. by: A. Prader) Univ. of Zurich and Berne, Switzerland. Perinatal calcium homeostasis: Maternal and cord serum concentrations of Ca, Mg, vit. D-metabolites and parathyroid hormone (PTH).

Ca, Mg, 25(OH)D, 24,25- and 1,25(OH)₂D and PTH (estimated with antibodies which mainly recognize intact PTH-(1-84)) were measured in the serum of normal non-pregnant adults (C) as well as at delivery. Maternal venous (M) and umbilical venous (UV) and arterial (UA) blood was obtained from normal mothers and neonates at term (N=12). Mean levels ($\pm$ SE) amounted to:

	Ca	Mg	25(OH)	24,25-	1,25(OH) ₂ D	PTH
	mg/dl		nmol/l			ng/ml
C	9.0 $\pm$ 0.1	2.06 $\pm$ 0.06	54.0 $\pm$ 4.3	4.48 $\pm$ 0.68	0.10 $\pm$ 0.01	0.20 $\pm$ 0.02
M	9.1 $\pm$ 0.1	1.62 $\pm$ 0.05	45.4 $\pm$ 9.8	1.46 $\pm$ 0.22*	0.31 $\pm$ 0.04*	0.26 $\pm$ 0.03
JV	10.4 $\pm$ 0.1*	1.68 $\pm$ 0.03	26.7 $\pm$ 5.8*	0.55 $\pm$ 0.16*	0.12 $\pm$ 0.01*	0.07 $\pm$ 0.01*
UA	10.5 $\pm$ 0.1	1.72 $\pm$ 0.04	26.6 $\pm$ 5.3	0.61 $\pm$ 0.19	0.16 $\pm$ 0.02*	0.07 $\pm$ 0.01

* p<0.02 to <0.001 vs. preceding line. 24,25(OH)₂D was lower and 1,25(OH)₂D higher at the end of pregnancy than in non-pregnant controls, presumably in response to raised fetal demands of calcium. Vitamin D-metabolites were lower in cord than in maternal blood possibly because of a diffusion barrier across the placenta and/or different affinities of binding proteins. However, higher levels of 1,25(OH)₂D in UA than in UV suggest that the fetus also participates in the synthesis of 1,25(OH)₂D. Finally serum levels of calcium were higher in UV than in M and fetal PTH was undetectable or low.

49 E. SULYOK*, F. VARGA and I.F. CSABA* Department of Obstetrics and Gynecology University of Pécs, Hungary. Postnatal development of renal sodium handling in premature infants.

In an attempt to estimate the contribution of the specific defect in proximal and distal tubular reabsorption of Na to renal salt wasting, fractional Na excretion, distal tubular Na delivery and distal tubular Na reabsorption were determined using clearance method in 11 healthy premature infants. The mean birth weight and mean gestational age was 1670 g /range: 1140-2120g/ and 31.7 weeks /range: 27-35 weeks/, respectively. The study was performed on the 7-th day and in weekly intervals thereafter up to the 6-th week of life. It was demonstrated that Na clearance and fractional Na excretion decreased significantly with increasing postnatal age /p<.001/. There was no significant alteration in either osmolar or free water clearances. Distal tubular Na delivery steadily decreased from 4.96 $\pm$ 0.66 /mean $\pm$ SE/ in the first to 3.30 $\pm$ 0.41 ml/min/100 ml GFR in the 6-th week of life /p<.05/. Distal tubular Na reabsorption was 69.5 $\pm$ 2.36 % in the first week then rose significantly to reach a value of 83.7 $\pm$ 1.85 % in the second week /p<.001/ and remained practically unchanged thereafter. It is suggested that the more rapid improvement of distal tubular Na reabsorption in premature infants might be resulted from the forced stimulation by the excessively activated renin-angiotensin-aldosterone system.

50 G. SCHOECH* and J. THOMALE* (Intr. by K.H. Schäfer). Universitäts-Kinderklinik, Hamburg, West Germany. A new method for the rapid

analysis of normal and modified nucleosides and nucleobases in various body fluids.

Recently, normal and modified urinary nucleosides and nucleobases have been shown to reflect processes of proliferation, differentiation, and malignant transformation. To realize the theoretical implications of those findings and ideas for clinical applications it was necessary to develop a highly sensitive, rapid, and reliable analytical procedure. Several prefractionation steps were combined to isolate bulk nucleosides and nucleobases simultaneously. Finally, both were analysed by reversed phase HPLC (detection limit: 10⁻¹³ mole per substance; about 1ml of urine, serum or spinal fluid is sufficient for a complete analysis). The registration and comparison of the analytical data has been automated on the basis of creatinine-, age- and sex-correlated regression equations of normal values. Actually, 17 normal and modified nucleosides and nucleobases are routinely measured. We use this method presently to control effects of cytostatic therapy as well as normal growth velocity.

Schoech, G. et al. Helv. Paediat. Acta, Suppl. 38, 1-171, 1977.