7 C. KATTAMIS, ANNA METAXOTOU-MAVROMATI*, W.G.WOOD* and D. WEATHERALL*. 1st Department of Pediatrics Athens University, Athens, Greece and Nuffield Department of Clinical Medicine, Oxford University, Oxford, England.
Globin chain synthesis and clinical and hematologic findings in double heterozygotes for the silent and classical β-thalassemia genes, and their parents.

Detailed hematologic and globin chain synthesis studies were performed in a special group of six patients with thalassemia syndrome who were offsprings of segregation of classical β-thalassemia trait (increased HbA_2 and normal or slightly elevated HbF), to "silent" β-thalassemia trait (normal HbA_2 and HbF, but impaired β-chain synthesis). Clinically these patients varied widely, from those severely affected which necessitated regular transfusions since the first year of life, to others with an intermediate clinical course without transfusion requirements. The levels of both HbA (28-80%), and HbF (18-70%), varied widely. Of interest were the results of globin chain synthesis in the patients with the "silent" β-thalassemia gene and their siblings; the ratio of a to non-a chains ranged from 1.43 to 3.02 indicating a considerable variation in the degree of impairement of β-chain synthesis. This favors the assumption that a heterogeneity in "silent" β-thalassemia gene may exists, and that the extreme variation in the clinical severity observed in double heterozygotes patients, could be associated with heterogeneity of the "silent" thalassemia gene.

8 G. SCHOECH*, G. HELLER-SCHOECH* and H. BAISCH* (Intr. by K.H. Schäfer). Universitäts-Kinderklinik, Hamburg, West Germany.
Analysis of urinary nucleobases and nucleosides: a new diagnostic means for following normal and malignant growth processes.

Growth and differentiation processes are accompanied by an altered turnover of nucleic acids. In contrast to their major constituents, the modified nucleosides and nucleobases as minor nucleic acids components cannot be recycled. Only part of them can be catabolized. So the quantity and pattern of their excretion in urine represent new indicators of growth and differentiation. Normal and modified nucleobases and nucleosides are measured at the picomole level by cation exchange HPLC. Plotted against age, the excretion curves of the modified nucleobases and nucleosides are in accordance with the shape of the known growth velocity curve. As a consequence of the organ specific nucleic acid equipment and the varying relative growth velocity of specific organs, the nucleobase excretion patterns of young children differ significantly from those of older ones. When adequately age-correlated, many children and adults with different malignancies show significant quantitative and qualitative aberrations in their urinary nucleobase pattern. These are normalized by a successful cytostatic therapy. So, the outlined principle and method should be well suited to follow normal and malignant growth and differentiation processes.

9 MOE, P.J., BRATLID, D. Department of Pediatrics, University Hospital, University of Tromsø, Norway.
HIGH DOSE METHOTREXATE TREATMENT OF CHILDREN WITH ALL.
A clinical trial with HDM as consolidation therapy in ALL was started in June 1975. Three courses of HDM, 500 mg/sq.m. at 3-weekly intervals have been used in 74 children with ALL and one with AML. Fourty-six of 53 patients in primary complete remission have been in sustained remission for 6-39 months.
The pharmacokinetics of HDM treatment were studied in 11 of these patients and in six other patients with far advanced lymphatic leukemia and other malignancies given a much higher dose of methotrexate (2790 ± 1010 mg/sq.m.). The decay of serum concentration of methotrexate was found to follow a diphasic curve, with an initial serum half-life of 4.8 hours followed by a second half life of 34.4 hours. Apparent volume of distribution was 56.8 litres/sq.m. and distribution equilibrium was not reach before approx. forty hours after start of infusion. Significant levels of methotrexate were found in the cerebrospinal fluid, but the penetration to the cerebrospinal fluid was slow. The concentrations are, however, comparable to those obtained in ventricular fluid after lumbar administration of from 6.25 - 12.5 mg methotrexate/sq.m. Urinary excretion of methotrexate was considerable, and might exceed the limit for methotrexate solubility if the urine is not alkalinized.

10 F.Tzortzatou* (Intr.by A.Constantopoulos). First Department of Pediatrics of Athens University "St.Sophie's" Children's Hospital, Athens, 608, Greece

Reversibility of the toxicity of methotrexate by dihydrofolate in mice

Methotrexate (MTX) is generally believed to be a potent inhibitor of dihydrofolate reductase (DHFR), inhibition of this enzyme leading to cell death. However, the intracellular binding of MTX to DHFR was found by a cytochemical method to be weak and reversible. Thus MTX was readily displaced by the physiological substrate, dihydrofolate (FH_2). The displacement of MTX by FH_2 has been confirmed by experiments in mice. A group of 68 AKR mice was studied, 8-12 weeks old (12-26 g). MTX was injected i.p. in the lethal dose of 200 mg or 300 mg/kg FH_2 was injected in the same doses as MTX prior or simultaneously or up to 72 hrs after MTX. The younger and lighter mice survived when FH_2 was given 1 hr prior to the lethal dose of MTX, and the older and heavier mice survived even when FH_2 was given up to 17 hrs after MTX. These results suggest that FH_2 reversed the toxicity of a lethal dose of MTX and protected the animals when equal amounts of FH_2 were given 1 hr before or simultaneously or up to 17 hrs after MTX.

11 H. PRZYREMBEL, B. BRAUSER*, and H.J. BREMER - University Children's Hospital Düsseldorf, Inst. Physiol. Chem. Phys. Biochem. Cell Biol. Munich:
Myopathy with lactic acidosis and altered redox state of the respiratory chain cytochromes (cyt).

A 16-years old girl has progressive loss of muscular strength, on exertion muscle pains, lactic acidosis and ketosis. Load tests with glucose, fructose, alanine, and lactate show normal metabolism at rest. Lactate accumulation is only dependent on muscular activity. Investigation of an open muscle biopsy showed normal glycogen content and normal activities of glycogenolytic enzymes (Dr. Schaub, Munich) and mild mitochondrial abnormalities on electron microscopy (Prof. Peiffer, Prof. Schlote, Tübingen). Muscle mitochondria were studied by O_2polarography and spectroscopy according to Brauser, 1968: respiration rate with succinate was normal, it was decreased with NADH linked substrates (malate/glutamate and malate/pyruvate 10 % of normal). Contents of cyt were normal. The percentage of cyt b reduced was minimal, while that of cyt c was increased. Reduction of cyt b occurred only after antimycin. Therapeutic trials with riboflavin, succinate, and long-chain fatty acids have been without effect. Possible explanations for these findings are: 1) increased negativity of the cyt b potential with normal flow of electrons and 2) direct flow of electrons from succinate dehydrogenase to cyt c.

12 I.SIPILÄ[X], O.SIMELL[X], J.RAPOLA[X] & K.SAINIO[X] (Intr. by J.Perheentupa). Children's Hospital, University of Helsinki, Helsinki, Finland.
Muscle pathology in hyperornithinemia with gyrate atrophy of the choroid and retina (HOGA).

HOGA is an autosomal recessive disease, which is clearly enriched in Finland. Our 30 patients have a 10-20-fold elevation of ornithine levels in plasma and urine. The clinical disease appears at the age of 5-9 years with an impairment of vision, myopia and dark blindness, and proceeds to practically total blindness by age 30 years. A defect in ornithine transaminase has been demonstrated in the patients' fibroblasts and lymphocytes.

We have studied the muscles of 20 patients. Clinically they had a varying degree of muscle atrophy but normal strength. Most of them reported a moderately decreased capacity to fast muscular performance. Needle biopsies revealed a decreased proportion of type II fibres; in some specimens they were almost absent. In electron microscopy, subsarcolemmal sites of type II fibres showed tubular aggregates. Light microscopy revealed corresponding histochemical alterations. In electromyography, myopathic patterns were constantly obtained from a part of the proximal muscle fibres.

The muscular pathology showed no clear correlation with the severity of visual impairment. Presumably it arises by the same mechanism as the retinal and choroidal atrophies. Muscle is easy to sample and presents a model for studies on the pathogenesis of the progressive atrophy which appears to be a process characteristic of the disease. Our findings also emphasize the generalized nature of the disease in HOGA.