871 MEASUREMENT OF DNA BREAKAGE AND REPAIR IN ACUTE LYMPHOCYTIC LEUKEMIA (ALL) CELLS. Sharon L. Wenger and Julie Blatt (Spon. by Mark W. Steele). Sch. of Med., Univ. of Pittsburgh, Child. Hosp., Dept. Ped.

Cytogenetic techniques have been used to look at structural and functional chromosome abnormalities in leukemic cells. One method, unscheduled DNA synthesis (UDS), which measures DNA repair in non-dividing cells, has been reported to be elevated in peripheral lymphoblasts and fibroblasts from acute myelogenous leukemia patients when exposed in vitro to DNA damaging agents compared to lymphocytes or fibroblasts from normal individuals. We postulated that karyotype abnormalities may correlate with increases in DNA damage and repair. Bone marrow aspirates from 8 children with ALL and 4 children who had aspirates done for reasons other than malignancy were processed for karyotyping. Samples were evaluated for UDS by treatment with hydroxyurea to stop normal DNA synthesis. Tritiated-thymidine was then added for 22 hours. Cells were harvested and prepared for autoradiography. Grain counts per nucleus averaged 0.86±0.09 for ALL samples with abnormal (structurally or numerically) karyotypes (n=4); 0.51 ±0.13 for ALL samples with normal diploid karyotypes (n=4); and 0.40±0.09 for aspirates from non-leukemic samples with normal karyotypes (n=4). Comparisons of UDS for ALL with abnormal karyotypes to each of the other groups with normal karyotypes was statistically significant ($p<0.05$). These results suggest that leukemic cells with karyotype abnormalities may have a propensity toward increased DNA breakage and repair.

△ 872 CLONING OF cDNA ENCODING HUMAN STEROID 21-HYDROXYLASE Perrin C. White, Bo Dupont & Maria I. New. Cornell University Medical Center, Division of Pediatric Endocrinology & Memorial Sloan-Kettering Cancer Center, Laboratory of Human Immunogenetics, New York.

Deficiency of steroid 21-hydroxylase (21-OH), a cytochrome P-450, is the most common cause of congenital adrenal hyperplasia. It is inherited as a monogenic autosomal recessive trait closely linked to the HLA major histocompatibility complex. We previously used a bovine cDNA clone encoding 21-OH to show that certain patients with 21-OH deficiency carry a deletion involving a 21-OH structural gene. We have now isolated a nearly full length clone encoding 21-OH from a human fetal adrenal cDNA library. This library was constructed by David Russell using the Okayama-Berg system, which tends to yield full-length copies. The library was screened by colony hybridization with the bovine cDNA clone encoding 21-OH, yielding about 0.5% positive colonies. One colony contained an insert of about 2000 base pairs (bp), which was short of a full length copy by about 200 bp corresponding to the 5' end of the mRNA. This clone hybridizes strongly to two Taq I fragments of 3.2 and 3.7 kbp in human genomic DNA, and mapping of cosmid clones shows that these fragments correspond to two 21-OH genes. The 3.7 kbp fragment is deleted on all chromosomes carrying 21-OH deficiency and the HLA haplotype A3;Bw47;DR7, and is also deleted on about 20% of other chromosomes carrying 21-OH deficiency. The 3.2 kbp band is deleted in hormonally normal individuals carrying HLA-A1;B8;DR3, and the corresponding gene may therefore not be active in the adrenal gland. This cDNA clone may be useful in the prenatal diagnosis of 21-OH deficiency.

873 α-L-IDURONIDASE IN LEUKOCTYES AND PLASMA: ISOZYMES OF INTEREST IN BONE MARROW TRANSPLANTATION FOR HURLER SYNDROME. Chester B. Whitley, Caer R. Vitek, William Krivit. U. Minnesota, Dept Pediatrics & Dight Laboratories, Mpls

Three mechanisms are proposed to account for metabolic correction in Hurler syndrome after bone marrow transplantation. Of these, endogenous enzyme replacement is predicated upon the availability and catalytic activity of iduronidase (Idu) in marrow-derived cells and plasma. To characterize enzyme available after transplantation, we studied Idu from normal individuals utilizing the 4-methylumbelliferyl-α-L-iduronide substrate. Idu specific activities were determined for leukocyte subpopulations (mean, n=18): granulocytes 54.7 nmoles/mg-hr, lymphocytes 61.2, and monocytes 47.5. Of further interest were physicochemical characteristics of Idu. In vitro, leukocyte and plasma Idu activities, with optima at pH 3.0-3.4, had less than 1% of maximal activity at pH 7.3. We also quantitated Idu isozymes after separation by DEAE chromatography since enzyme affinity for DEAE has been related to cellular binding and endocytosis. In leukocytes, high-affinity Idu B was the predominant form comprising approximately 90% of total activity. In plasma, Idu B was likewise the major component contributing 64-76% total activity. We conclude that Idu is available in transplantable leukocytes; however, lack of catalytic activity at neutral pH suggests that glycosaminoglycan (GAG) degradation in plasma is insignificant in vivo. Since only lysosomal GAG catabolism is likely to be physiologically important, cellular uptake of enzyme may be crucial. Leukocytes and plasma contain high-affinity Idu B available for endocytosis by deficient cells.

874 NORMAL PYRUVATE OXIDATION IN FRIEDREICH ATAXIA AND CHARCOT-MARIE-TOOTH DISEASE FIBROBLASTS. William G. Wilson (Spon. by Thaddeus E. Kelly). University of Virginia School of Medicine, Department of Pediatrics, Charlottesville.

Methylene blue (MB)-stimulated assays of pyruvate oxidation in cultured skin fibroblasts were used to determine if partial defects in pyruvate metabolism could be demonstrated in cells from patients with Friedreich ataxia (FA) or Charcot-Marie-Tooth disease (CMT). In both a whole cell and a homogenate assay, measuring $^{14}CO_2$ production from labeled pyruvate and lactate, respectively, MB increased pyruvate oxidation 2-to3-fold in all cell lines tested. Pyruvate oxidation in 11 control lines, as measured in the MB-stimulated whole cell assay, was 23.11±1.41 pkat/mg cell protein (mean±S.E.), not significantly different from that found in 4 FA lines (23.8±2.3) or cell lines from 6 related CMT patients (23.9±1.9). In the MB-stimulated homogenate assay, pyruvate oxidation in 4 normal cell lines (9.3±2.4) was not significantly different from that of 4 FA lines (7.0±2.4) or the 6 CMT lines (14.2±2.0). These results provide additional evidence that a primary defect in pyruvate oxidation is not present in FA or CMT patients.

● 875 BIOTINIDASE DEFICIENCY DETECTED BY A STATEWIDE NEONATAL SCREENING PROGRAM. Barry Wolf, Gregory S. Heard, Linda G. Jefferson, Walter E. Nance, Karen A. Weissbecker. Departments of Human Genetics and Pediatrics, Medical College of Virginia, Richmond.

Biotinidase deficiency is an autosomal recessively inherited disorder that usually manifests during infancy or early childhood with seizures, skin rash, alopecia, hearing loss, developmental delay and metabolic compromise, and occasionally results in coma and death. If diagnosed early the disorder can be treated effectively with biotin. To estimate the incidence of biotinidase deficiency and the potential cost-effectiveness of neonatal screening we conducted a pilot program in the Commonwealth of Virginia. After screening 77,145 newborns in ten months we have confirmed the diagnosis in two infants: a female and a male ascertained at 4 mos. and 2 mos. of age, respectively. Each infant had normal EEGs and developmental milestones, and neither had cutaneous or metabolic abnormalities. However, both were slightly hypertonic with brisk reflexes, and the female had abnormal brainstem auditory evoked potentials. Treatment with biotin (10 mg/d) was initiated within 3 days of confirming the diagnosis. Our preliminary estimate of the incidence of the disorder is 1 in 38,500 (95% confidence limits: 1 in 11,700 and 1 in 228,000). Affected infants are not symptomatic at birth, but may develop physical and neurologic abnormalities if untreated. The qualitative screening test for biotinidase activity is simple and inexpensive. Therefore, we recommend that testing for biotinidase deficiency be incorporated into all newborn screening programs.

876 METABOLISM OF PANTETHINE IN NEPHROPATHIC CYSTINOSIS Carl T. Wittwer and Jess G. Thoene, Department of Pediatrics, University of Michigan School of Medicine, Ann Arbor.

Previous in vitro studies with cystinotic fibroblasts have shown D-pantethine to be as effective as cysteamine in decreasing intracellular cystine. We studied the in vivo metabolism and efficacy of oral pantethine (70-1000 mg/kg/day) in three cystinotic children. WBC cystine, plasma cysteamine, plasma and urinary pantothenate, and total urinary sulfur were measured. No pantethine was detected in plasma or urine, but plasma pantothenate was elevated to over 200 times normal. After a single 60 mg /kg dose, an open two compartment model gave rate constants of 0.025/hr for elimination and 1.2/hr for distribution of pantothenate. At optimal pantethine doses, plasma cysteamine levels (ca. 50 nmol/ml) were comparable to those after cysteamine treatment. However, maximal WBC cystine depletion on pantethine was only 60-80%, whereas cysteamine produces over 90% depletion. Pantethine absorption was estimated by pantothenate and sulfur excretion. Only 3-12% of the dose was excreted as pantothenate. At 250 mg/kg/day, ca. 50% of the administered sulfur was excreted. At higher doses, no significant increase in sulfur excretion occurred. An osmotic diarrhea occurred at doses above 350mg /kg/day. Pantethine, pantothenate, and cysteamine were the major osmotic agents. Excess pantethine (10-fold over pantetheine) inhibited pantetheinase from an intestinal biopsy by 30%. Dosing t.i.d. with meals vs q6h increased adsorption and WBC cystine depletion at 250 mg/kg/day. Limited intestinal hydrolysis and absorption of pantethine limit its usefullness in cystinosis.