841 L-PIPECOLIC ACID (PIP) CATABOLISM IN THE RABBIT: A MODEL FOR PIP METABOLISM IN MAN. Stephanie J. Mihalik and William J. Rhead (Spon. by Jean Robillard), University of Iowa, College of Medicine, Department of Pediatrics, Iowa City, Iowa.

Elevated circulating levels of PIP, a putative neurotransmitter, are found in Zellweger syndrome (ZS) and hyperpipecolic acidemia (HPA). As these elevations and the mammalian PIP degradation pathway are poorly understood, we studied L-PIP degradation to α-aminoadipic acid (AAA) in the rabbit. Tissue homogenates converted pure L-[2,3,4,5,6-^{3}H]PIP to ^{3}H-AAA in both rabbit liver and kidney (13.9±9.8 and 34.1±15 pmols/mg prot/hr). Addition of FAD, NAD, and NADP did not raise AAA production and no ^{3}H-AAA was formed in brain homogenates. ^{3}H-AAA identity was confirmed by coelution and re-elution with cold AAA on two paper and one thin layer chromatographic systems. Kidney cortex mitochondria (KCM) formed ^{3}H-AAA from ^{3}H-PIP 14-fold better than tissue homogenates (485±354 pmol/mg prot/hr) and converted 1-2% of the ^{3}H-PIP to ^{3}H-AAA. Addition of 50mM glutamate, a transaminase inhibitor, and 1.4mM cold AAA increased AAA recovery 2.7 and 2.8 fold, respectively. Glutamate and cold AAA lowered ^{3}H release from ^{3}H-PIP 45 to 50%, confirming the blockade of ^{3}H-AAA degradation by these compounds. Thus, KCM degrade L-PIP to AAA; the apparent mitochondrial location of this pathway is consistent with its location on the electron transport particle of P. putida, although we cannot totally exclude peroxisomal involvement in mammalian PIP degradation. Further studies on PIP metabolism in rabbit KCM and peroxisomes and in ZS and HPA tissues and fibroblasts are in progress.

842 A PREVIOUSLY UNREPORTED DE NOVO FRAGILE SITE [fra(2)(q37)] IN A BOY WITH DEVELOPMENTAL DELAY, HYPOTONIA AND DYSMORPHISM. Navnit S. Mitter, Lytt I. Gardner and Jannell Welsh-Sloan, SUNY Upstate Medical Center, Depts. of Pathology and Pediatrics, Syracuse.

The patient was born at term with a birth weight of 2.84 Kg. At age 12 months he was not sitting up or responding. At 15 months he could not stand, with hypotonia of legs and a ? history of myoclonic seizures. At 21 months he presented as a thin boy with hypertelorism, epicanthal folds, pectus excavatum, hammer-toe deformities of the great toes and a head that appeared small. Analysis of GTG-banded, cultured peripheral lymphocytes revealed a karyotype of 46,XY,fra(2)(q37). The fragility on chromosome 2 was present in 100% of the cells examined. The appearance of the fragile site varied from that of a secondary constriction to an isochromatid break. It was seen in lymphocytes cultured in Ham's F-10 synthetic medium, plus 20% calf serum. No inducing agents like FUdR, BUdR, anti-folates or distamycin were present. The parents had normal karyotypes. It is of interest that the father was exposed to Agent Orange spray in Vietnam in 1968 and 1969. There are two areas of possible causality which are unresolved in this case: the relation of the dysmorphism and developmental delay to the fragile site, and in turn its relationship (if any) to Agent Orange.

● **843** AMNIOTIC MEMBRANE IMPLANTATION IN MUCOPOLYSACCHARIDOSIS. Joseph Muenzer, Elizabeth F. Neufeld, George Constantopoulos, Rafael C. Caruso, Muriel I. Kaiser-Kupfer, Anita Pikus, Harold D. McDonald and Michael A. Zasloff. (Spon. by James B. Sidbury). NICHD, NINCDS, NEI, NCI, CC, NIH, Bethesda, & UCLA Sch. of Med., Dept. of Biol. Chem., Los Angeles.

Amniotic membrane implantation has been performed as an approach to enzyme replacement in mucopolysaccharidosis (MPS). The rationale for this study is based on the findings that cultured amniocytes secrete lysosomal enzymes and that amnion membranes are non-immunogenic. 19 patients (MPS I,II,III), ages 3 to 16 yr., have had implantation of human amniotic membranes obtained from elective repeat C-sections. The subcutaneous implants in the abdominal wall have been well tolerated with follow-up between 4 to 12 mo. The effects of amniotic membrane implantation on the clinical status of the patients was evaluated by comparing the following studies pre- and 6 mo. post-implantation: EKG, echocardiography, head and liver CT, liver/spleen scan, skeletal survey, fundus exam, electroretinography, audiological assessment, auditory brain stem response and joint range of motion. Objective clinical improvement was noted as an increased range of motion in 3 of 18 patients. Biochemical analysis demonstrated no change in serum or WBC enzyme activity. Urinary GAG transiently decreased in 2 of 10 patients with levels returning to pre-treatment range at 6 mo. post implantation. Biopsy of the implantation site at 6 mo. post implantation demonstrated a foreign body reaction with no evidence of amnion tissue. Amniotic membrane implantation appears to have limited success as a means of enzyme replacement.

844 A NEW INFANTILE ACUTE LEUKEMIA WITH A UNIQUE CHROMOSOMAL t(4;11) REARRANGEMENT. Daniel P. O'Malley, Roshni Kulkarni, James V. Higgins (Spon. by Marshall Klaus), Michigan State Univ., Dept. of Pediatrics, E. Lansing, MI

An unreported interstitial variation of the t(4;11) that is found in infantile acute lymphocytic leukemia (ALL) is presented that sheds new information on the genetic basis for neoplasia. The chromosome 4 breakpoints are at the q21q31 bands with the interstitial genetic material inserted within chromosome 11 at band q23. The typical t(4;11) shows a non-interstitial translocation with the breakpoints at bands 4q21 and 11q23.

A 3 month old female was diagnosed as having non-T/non-B ALL. ALL therapy induced a 14 month remission which was followed by an M3 relapse. The relapse immunological studies suggested poorly differentiated AMMoL. Relapse cytogenetic studies revealed 7 consistent cell lines: normal 46,XX; 46,XX,t(4;11)(q21q31;q23) and 5 derivative clones, with additional chromosome rearrangements, of the t(4;11) cell line. Subsequent cytogenetic studies showed relative frequency changes of the 7 cell lines which appeared to correspond to therapy adjustments.

Fewer than 30 cases of t(4;11) infantile ALL have been reported and they show a consistent disease progression: initial diagnosis of non-T/non-B ALL which then evolves into poorly differentiated acute myelomonocytic leukemia (AMMoL). The consistent clinical course of t(4;11) infantile ALL may be defined by the t(4;11) occurring in an undifferentiated-pluripotent stem cell. This case shows not only the classical disease progression of t(4;11) infantile ALL, but the unique t(4;11) rearrangement appears to show a significance of the 4q21 band interfacing with the 11q23 band regarding neoplastic processes.

△ **845** MOLECULAR BASIS OF GROWTH HORMONE (GH) GENE DELETIONS. John A. Phillips, III, Ellson Y. Chen, and Peter H. Seeburg. Vanderbilt University, Department of Pediatrics, Nashville and Genentech, Department of Molecular Biology, South San Francisco.

A rare type of familial isolated GH deficiency (type 1A) is caused by homozygosity for deletion of the structural gene for GH (GH-N). This gene is normally located on the 5' end of the 40-kb GH gene cluster (5'-GH(N)-CS(L)-CS(A)-GH(V)-CS(B)-3'). Six of eight such deletions we have studied differed in either their size (6.7 versus 7.6-kb) or in their pattern of restriction fragments which contain the non-deleted components of the cluster. Since these differences suggested GH-N deletions had occurred multiple different times we analyzed the DNA sequence of a novel fragment produced by a deletion to determine the mechanism of the deletion. The DNA studied was from a subject with a 7.6-kb deletion in which the BAM HI derived 8.3-kb genomic fragment normally containing the CS-L gene was replaced by a unique 9.3-kb fragment. We inferred this unique fragment resulted from fusion of sequences which normally flank the GH-N gene. The complete DNA sequence of the 9.3-kb fragment was determined and the point of transition from sequences normally 5' to GH-N to those normally 3' was detected. The junction of these flanking sequences occurred within the 29-bp sequence TCAGCAGAAGTGGAATTCAGGACTGAATC. Perfect repeats of these 29 bases lie 497-bp 5' and 7082-bp 3' to the GH-N cap site. Since the 7.6-kb genomic deletions would result from unequal crossing-over between these two homologous copies these repeats may comprise hotspots for recurrence of such deletions.

† **846** LYSOSOMAL AMINO ACID TRANSPORT:DESCRIPTION OF A NEW SYSTEM. Ronald Pisoni, Jess Thoene and Halvor Christensen, Depts. of Biological Chemistry and Pediatrics, University of Michigan, Ann Arbor.

Lysosomes export amino acids derived from proteolysis into the cytoplasm against a concentration gradient. A system specific for cystine transport has recently been described which is defective in cystinotic tissues, accounting for the lysosomal cystine accumulation found in that condition. We here describe a second lysosomal amino acid transport system. Crude granular fractions were prepared from normal and cystinotic fibroblasts and loaded by exposure to 60 μM ^{14}C-L-lysine methylester. The loaded fractions were then placed in transport buffer and the half-life for exodus measured as radioactivity retained on glass-fiber filters after filtration of aliquots of the incubation mixture. We found trans-stimulation of ^{14}C-lysine efflux by arginine, lysine, ornithine, diaminobutyrate, histidine, 2-aminoethyl-L-cysteine and the mixed disulfide of cysteine and cysteamine. The $T_{1/2}$ for lysine at 25°C and pH 6.5 was 24 ± 2 min, but fell to 2.5 ±1.5 min in the presence of 2 X 10^{-3}M of the above-listed compounds in the incubation mixture. Lysine efflux was not Na^+ dependent but was stereospecific for L-isomers and was inhibited by chloroquin. This system is intact in cystinotic fibroblasts. Since it recognizes the mixed disulfide of cysteine and cysteamine, we propose that cystine-depletion of cystinotic fibroblasts occurs via this intact transport system after conversion of cystine to the mixed disulfide by reaction with cysteamine.