698 PLACENTAL COPPER TRANSPORT IN THE BRINDLED MOUSE. Adolfo D. Garnica; James Bates; Owen M. Rennert. Univ. Okla., Dept. Pediatr., Okla. City.

The X-linked mouse mutant brindled is a model for Menkes syndrome. In young hemizygotes, reduced liver and brain copper concentrations are associated with neurologic dysfunction. In fetuses copper concentrations in placenta and kidney are higher in brindled than controls while those in liver and carcass are lower. To treat the copper deficiency in brindled young, heterozygotes were injected at 16 or 18 days gestation with copper, 6 mcg/g/dose, as cupric chloride, 18 and 6 hours before sacrifice. Placental, carcass, and hepatic copper concentrations in brindled fetuses increased ($p>0.006$). Injection of methylprednisolone, 5 mcg/g, 20 hours before the copper, to increase fetal hepatic copper storage through metallothionein induction, resulted only in further increase in the carcass copper concentrations. These data suggest: placental copper transport in brindled fetuses is impaired; hepatic copper binding capacity of control and brindled fetuses is limited and cannot be augmented by pretreatment with methylprednisolone; extra-hepatic copper binding may be increased with methylprednisolone, which implicates induction of extra-hepatic metallothionein in both groups of animals.

699 DEFECTIVE HEPATIC COPPER STORAGE IN THE BRINDLED MOUSE. Adolfo D. Garnica; James Bates; Owen M. Rennert. Univ. Okla., Dept. Pediatr., Okla City.

The X-linked mouse mutant brindled is a model for Menkes syndrome. As such, young hemizygotes and heterozygotes demonstrate low hepatic copper contents and serum ceruloplasmin concentrations, which cannot be corrected with parenteral copper. In adult brindled animals ceruloplasmin and hepatic copper are not significantly different from those in normal animals. Measurement of copper concentrations in hepatic subcellular fractions of young brindled animals demonstrates reduced copper concentrations in all fractions but especially in mitochondrial and nuclear fractions. Parenteral copper, 10 mcg/g/day x 5 days, as cupric chloride solution, in young control animals results in increased copper concentrations predominantly in nuclear, mitochondrial, and supernatant fractions. In contrast, identical treatment of young brindled animals demonstrates increases in copper concentration primarily in the supernatant fraction. No difference was observed in copper concentrations of subcellular fractions from similarly treated adult brindled heterozygotes when compared with treated controls. These data implicate a defect in hepatic copper retention in brindled young, predominantly involving the nuclear and mitochondrial fractions, associated with impaired copper incorporation into ceruloplasmin.

● 700 CYSTINE EXODUS FROM LEUKOCYTE GRANULAR FRACTIONS IS STIMULATED BY ATP AT SUB-SATURATING CYSTINE LEVELS. Alice A. Greene, Karen F. Clark, Margaret L. Smith, and Jerry A. Schneider, University of California, San Diego, Department of Pediatrics, La Jolla, California.

Cystine exodus from the granular fractions of fibroblasts, lymphoblasts, and rat liver is stimulated by ATP. We observe a similar ATP effect in leukocytes. N-ethyl maleimide (NEM), an inhibitor of the lysosomal $H^+$-translocating ATPase ($H^+$ATPase), eliminated the stimulation by ATP, but had no effect on basal exodus. In a series of three experiments on different days the % cystine loss in 30 min. at 37° was 54.5±1.9 (mean±SD) in pH 7.0 Hepes/sucrose buffer, 51.6±3.7 in buffer with 1 mM NEM, 76.6±4.8 in buffer with 2 mM MgATP, and 57.3±8.1 in buffer with NEM and MgATP. The initial concentration of cystine in these experiments ranged from 19 to 52 nmol cystine/unit β-hexosaminidase (cys/hex). The ATP effect disappeared when the load was increased to 1200 nmol cys/hex. The cystine loss for the same four conditions as above was 25.2, 29.8, 23.6, and 20.1%, respectively. At initial loads from 22-117 cys/hex, ATP stimulated exodus 2.2±0.6 (mean±SD, n=5) fold, whereas at loads from 800-1300 cys/hex, ATP stimulated exodus 1.1±0.2 (n=3) fold. The increased rate in the presence of ATP may represent improved binding of the cystine to the partially saturated inner transporter resulting from conformational or charge optimization brought about by the $H^+$ATPase. The effect disappears either in the presence of the $H^+$ATPase inhibitor NEM or when the transporter becomes saturated by high load levels.

● 701 CYTOGENETIC AND MOLECULAR STUDIES OF NON-DISJUNCTION IN TRISOMY 13. Terry J. Hassold, Patricia A. Jacobs and Michael Sheldon (Spon. by Maria I. New) Cornell University Medical College, New York Hospital, Department of Pediatrics, New York, N.Y.

We have been using chromosome heteromorphisms and restriction fragment length polymorphisms (RFLPs) to investigate the mechanism of origin of trisomy 13, and the possible association between errors of recombination and the non-disjunctional event leading to trisomy. To date, we have studied 33 liveborn or spontaneously aborted trisomy 13 conceptions. By combining analysis of chromosome heteromorphisms with analysis of seven probes detecting chromosome 13 RFLPs, we have been able to determine the parental origin of 23 cases, with 20 being maternal and 3 paternal in origin.

In eight cases in which we have both cytogenetic and molecular information, we can make inferences regarding recombination in the two non-disjoined chromosomes. We have evidence for recombination in two of three cases in which the extra 13 originated in maternal meiosis I and in both instances this observation is based on results from several probes. This suggests that absence of recombination due to pairing failure is unlikely to be an important mechanism in the genesis of human trisomy. Furthermore, analysis of recombination in all eight cases provides no evidence for an association between reduced recombination and non-disjunction leading to trisomy 13, as has recently been suggested to be the case for trisomy 21.

● 702 **cDNA AND GENOMIC CLONES FOR HUMAN PLATELET GLYCOPROTEIN IIb (GPIIb).** Randy A. Heidenreich, Elias Schwartz, Saul Surrey, Joel Bennett, Paul LaRocco, Robin Eisman, and Mortimer Poncz. University of Pennsylvania School of Medicine and The Children's Hospital of Philadelphia.

The platelet GPIIb-IIIa heterodimer complex is essential for platelet aggregation and is a member of a family of adhesive protein receptors. GPIIb, the larger component of this complex, is composed of two disulfide-linked subunits: GPIIbα and GPIIbβ. A cDNA expression library was constructed using mRNA from a human erythroleukemia (HEL) cell line expressing GPIIb-IIIa in λgt11, and the library was screened using a polyclonal antibody against GPIIb. Positive clones were isolated and a 3.3 kb cDNA clone was shown to contain DNA sequence information for the amino acids at the N-termini of both GPIIbα and GPIIbβ. The IIbα sequence begins 30 bp downstream from the 5'-end of the clone, while the IIbβ starts 700 bp upstream from the poly-A tail at the 3'-end of the clone. Northern blot analysis indicates a GPIIb mRNA of 4.1 kb. Analysis of the DNA sequence provides the first complete amino acid sequence for GPIIbα and GPIIbβ, including hydrophobic domains, glycosylation sites, and proline-rich areas. A 16.3 kb human genomic clone has been isolated; exons span at least 12 kb of this clone. Current studies are defining the intron-exon organization of the gene. These findings will allow a direct approach to analysis of the genetic defect(s) causing thrombasthenia, and may lead to means for prenatal diagnosis of this platelet defect.

703 ON THE MECHANISM OF BRAIN DYSFUNCTION IN HYPERPHENYLALANINEMIA. Frits A. Hommes, Medical College of Georgia, Department of Cell and Molecular Biology, Augusta, Ga.

A high phenylalanine (Phe) level, such as observed in untreated or poorly controlled PKU, results in inhibition of the oligodendroglial specific ATP sulfurylase, the first enzyme of sulfate activation. A decreased rate of synthesis of sulfatides results from this inhibition and consequently there are less acidic glycolipids available to protect, particularly myelin basic protein, from proteolytic degradation. This leads to an increased turnover of myelin which is not compensated by an increased rate of synthesis. The myelin sheath disintegrates and the initially formed labile synaptic contact regress. As a result there will be fewer synaptic contacts in the mature brain. As the proper formation of synapses is recognized as the basis of the dynamic integrative capacities of the central nervous system, the scenario outlined above provides a mechanism for the brain dysfunction. Experimental evidence for several steps of this scenario is available: inhibition of ATP-sulfurylase, localization of the Phe-sensitive ATP-sulfurylase in those areas of the brain most affected by the high Phe condition, the developmental profile of the Phe sensitive ATP-sulfurylase, precisely coinciding with myelination, i.e. active during the vulnerable period of brain development, increased vulnerability to proteolytic degradation and turnover of myelin in the absence of acidic glycolipids. Since inhibition of ATP-sulfurylase is not limited to Phe, but can be observed with other amino acids as well, the scenario may be of a quite general nature.