The Society for Pediatric Research announces the results of the 5th Annual **Young Investigator Award** for pediatric research.

The winner is **Arthur L. Horwich, M.D.**, Assistant Professor, Dept. of Human Genetics, Yale University School of Medicine, New Haven, CT.

This presentation will be made during the SPR Plenary Session on Thursday morning (April 30, 1987) in the **Grand Ballroom.**

MOLECULAR STUDY OF ORNITHINE TRANSCARBAMYLASE: OTC DEFICIENCY AND ANALYSIS OF MITOCHONDRIAL TARGETING OF THE OTC PRECURSOR. Arthur L. Horwich, M.D., Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

Ornithine transcarbamylase (OTC) is a mitochondrial matrix enzyme that catalyzes the second step of the urea cycle. X-linked deficiency of OTC often results in lethal neonatal hyperammonemia in affected hemizygous males. As many as 20% of females heterozygous for OTC deficiency may exhibit clinical symptoms including hyperammonemic episodes and mental retardation, usually associated with dietary protein intolerance.

The isolation of both cloned cDNA and genomic sequences encoding human OTC has permitted a molecular analysis of families with OTC deficiency. Southern blot analysis revealed a gene deletion in four probands out of 50 examined. The majority of OTC-deficient males do not exhibit detectable deletions and most likely harbor either single-base substitutions or small deletions. For these families, we have utilized linkage with restriction fragment length polymorphisms (RFLPs) localized within the OTC gene to identify the presence of a mutant allele. Four such RFLPs have been identified and ~80% of females are heterozygous for one or more of the polymorphisms.

Where an OTC-deficient proband is identified, there is an approximately 1:3 chance, according to Haldane, that a spontaneous mutation is involved, and, thur, that the recurrence risk for subsequent pregnancies is nearly zero. Two types of information can identify the situation in which the mother is an obligate heterozygous carrier, where there is a 50% risk that a male pregnancy will be affected. One involves pedigree analysis, to identify other affected males or heterozygous females. The other involves an allopurinol loading test which has recently replaced protein challenge as a safe means of identifying partial OTC deficiency. Pedigree analyses and heterozygote detection have permitted prenatal testing of seven at-risk male pregnancies using RFLP analysis of DNA derived either from chorionic villi or amniocytes. Four pregnancies were identified as being affected and enzyme analyses performed on fetal liver confirmed deficiency.

We have also utilized human OTC as a model for study of the biogenesis of mitochondrial proteins, most of which are synthesized in the cytosol as precursors and post-translationally directed to the organelles by cleavable NH_2-terminal amino acid sequences known as leader peptides. In previous studies, we showed that the OTC leader peptide contains information not only necessary but sufficient to direct the subunit precursor to mitochondria. When the peptide was fused to a protein normally localized to the cytosol, the fused protein was directed to the mitochondria. We have now carried out a detailed mutational analysis of the human OTC leader to define the structural elements required for import of the precursor and processing of its leader peptide. Fusion constructs revealed that neither the NH_2-terminal 12 or 16 residues could direct import whereas a leader peptide comprising residues 8-25 could do so. Remarkably, deletion of residues 30-37 containing the normal site of cleavage to mature OTC had no deleterious effect on import or processing indicating a lack of requirement for specific primary structure at this site. Nearly 30 site-directed and random substitutions--single, double, and even triple--have been produced and found to impair import and/or cleavage only when either positively charged arg residues were replaced by charge-neutral ones or where an acidic residue was introduced in place of a charge-neutral one. Of the 4 arg residues in the leader (positions 6,15,23,26), arg 23 was most critical and appeared to be part of an α-helical region. We conclude that the OTC leader directs import via at least two elements: net positive charge and a crucial midportion segment centered over arg 23.

Honorable mention is given to the following individual whose outstanding accomplishments qualified him for consideration for this award.

David Williams, M.D.
Children's Hospital, Boston