559 ENZYME THERAPY: IMMUNE RESPONSE TO ERYTHROCYTE- AND LIPOSOME-ENTRAPPED ENZYME IN β-GLUCURONIDASE DEFICIENT MICE. Lynn D. Steger, Morris B. Fiddler, Steven D. Douglas and Robert J. Desnick. University of Minnesota, Dept. of Pediatrics and Genetics, Minneapolis, MN 55455.

Entrapment of enzyme (E) in autologous erythrocytes (RBC) or liposomes (LIPO) has been studied as a strategy for replacement therapy. Using a murine system, the immunogenicity of entrapped (entp) bovine β-glucuronidase in RBC and LIPO was assessed after sensitization (sens) by repeated injection of RBC and LIPO ± entp E. RBC-entp E elicited no antibody (ABY) response assessed by C'-dependent lysis, double diffusion and passive hemagglutination (HA); little, if any, response was detected by HA against intact murine RBC. The fate of entp E after repeated prior injections was similar to that observed in unsens mice. Also, RBC-entp E was protected from ABY in mice previously sens with unentp E. Repeated injection of LIPO ± entp E induced an altered response to subsequent injection of LIPO entp E. Although no humoral ABY was detected, the in vivo fate of the E was markedly altered; the hepatic uptake was more rapid, max levels were reduced (~35 v 75% dose), and the E was cleared rapidly (~1 d v 8 d). An altered tissue fate was also observed after one injection of buffer-entp LIPO 2 d prior to a time course experiment; similar results were found when E-entp LIPO were injected 10 d after a single sens. These results suggest that RBC entrapment may protect exogenous E from immunologic surveillance for E replacement. However, both buffer- and E-entp LIPO induce immunologic and/or physiologic responses which mandates further study prior to clinical trials of LIPO-entp agents.

560 CYSTINOTIC FIBROBLASTS ACCUMULATE CYSTINE FROM INTRACELLULAR PROTEIN CATABOLISM. Jess G. Thoene, David G. Ritchie, Robert G. Oshima, and Jerry A. Schneider. Department of Pediatrics, University of California, San Diego, La Jolla, California.

Nephropathic cystinosis is an autosomal recessive disease characterized clinically by renal tubular and glomerular dysfunction and biochemically by intralysosomal cystine accumulation. We have recently reported that aminothiol treatment of cultured fibroblasts from patients with cystinosis produces rapid and complete removal of the stored cystine ([1]). Subsequent studies ([2]) showed that such cystine-depleted cells can reaccumulate cystine during incubation in culture medium lacking cystine. Thus, cystine appears to accumulate in these cells from an intracellular source. Potential intracellular sources include (1) GSH, (2)protein, and (3) cystathionine. No activity of the cystathionase pathway has been found by previous investigations in these cells, and none was found under the conditions of cystine depletion in the current investigations. Cells depleted of >80% of their GSH content reaccumulate cystine to the same extent as non-GSH depleted controls. Cells selectively labelled with ^{35}S-cystine in either the GSH + protein pools or GSH pool alone only reaccumulate labelled cystine when the protein pool is labelled. Known inhibitors of protein degredation (NH_4Cl and chloroquin) reversibly inhibit cystine reaccumulation in cystine-depleted cells. We conclude that the intralysosomal cystine accumulation observed in cells cultured from patients with cystinosis is derived from protein catabolism. ([1]) Thoene, J.G. et al. J.Clin.Invest.58:180, 1976. ([2]) Oshima, R.G. et al. J.Biol.Chem. 251:4287, 1976.

561 CHROMOSOME POLYMORPHISMS IN THE IDENTIFICATION OF THE ORIGIN OF SUPERNUMERARY MARKER CHROMOSOMES. Kathleen E. Toomey, T. Mohandas and Michael M. Kaback, UCLA-Harbor General Hospital, Division of Medical Genetics, Torrance, Ca.

Q banded chromosome polymorphisms have been shown to segregate in a Mendelian manner in humans and may be used to determine the origin of marker chromosomes. Recent studies of Q polymorphisms on 2 patients, both mosaic for a supernumerary marker chromosome, and their families, yielded information regarding the origin of the marker. Patient J.C., presented with classic features of Cat-Eye Syndrome: anal stenosis, fundal coloboma and preauricular tags and pits. The marker chromosome found was dicentric, as revealed by C banding, and the size of a G group chromosome. Comparison of Q banded karyotypes of the patient and her parents showed her to be 46XX/47XX, t(13;22)(13pter-->13q11::22pter-->22q11). E.S., a male evaluated for severe psychomotor retardation, cleft palate and congenital heart disease was found to be mosaic for a small bisatellited, metacentric chromosome. Comparison of polymorphisms of parents and child eliminated chromosomes 14, 15 & 22 from contributing to the origin of the marker. It is interpreted as arising post-zygotically from breaks in both number 13 chromosomes with subsequent reunion of the 2 short arms and satellite fragments such that the karyotype is 46XY/47XY, t(13;?13)(13pter-->13q11::?13pter-->?13q11). The data obtained from such studies is of importance in 1) determination of the frequencies of involvement of acrocentric chromosomes in such rearrangements, 2) defining phenotypic patterns associated with supernumerary markers and 3) facilitating accurate prenatal counseling when such a marker is detected in amniotic fluid cell cultures.

562 I-CELL DISEASE: IMPAIRED FIBROBLAST UPTAKE OF β-HEXOSAMINIDASE IS NOT DUE TO EXCESS SIALIC ACID. G.D. Vladutiu & M.C. Rattazzi, Children's Hospital of Buffalo, Dept. Pediatrics, SUNYAB, Buffalo, N.Y.

Cultured skin fibroblasts from patients with I-cell disease (ICD; mucolipidosis II) are characterized by deficiency of lysosomal enzymes, including β-hexosaminidase (β-hex), which are greatly increased in culture fluid. Whereas normal fibroblast-excreted lysosomal enzymes are pinocytosed by ICD cells, ICD-excreted enzymes are not pinocytosed either by non-ICD cells or, presumably, by ICD cells. Our previous finding of excess sialic acid on ICD-excreted β-hex suggested that its presence may result in impaired enzyme uptake, perhaps by masking galactose residues necessary for recognition by a receptor analogous to that on hepatocyte plasma membrane. Desialylation of ICD-excreted β-hex with Cl. perfringens neuraminidase, however, did not enhance enzyme uptake by β-hex deficient, non-ICD cells. Similarly, β-hex from normal plasma was not taken up by these cells whether or not sialic acid had been removed. In contrast, β-hex from normal seminal fluid, also similar to ICD-excreted β-hex in net charge and neuraminidase sensitivity, was pinocytosed irrespective of sialic acid removal. Thus the presence of neuraminidase-susceptible sialic acid is not responsible for impaired β-hex uptake, and the recently reported neuraminidase deficiency in ICD fibroblasts (Thomas et.al., Biochem. Biophys. Res. Comm. 71: 188, 1976) may not be directly related to this phenomenon. Carbohydrate residues other than galactose may be involved in recognition and uptake of lysosomal enzymes by fibroblasts, which may be masked or absent in ICD-excreted β-hex.

563 ETHNIC ORIGIN OF CYSTIC FIBROSIS (CF) FAMILIES Chun-I Wang (Sponsored by Maurice A. Kogut), Univ. So. Cal. Sch. of Med and Children's Hospital, L.A., Dept. of Pediatrics, Los Angeles, CA

Beginning with the very first interview of parents with CF children seen at this center, an on-going study of the genealogy of each family was conducted. The pedigrees of 220 families with 293 CF children provided satisfactory data for an analysis of their ethnic origins. The majority (95.5%) were from 3 ethnic groups, namely, Germans (19.4%), Irish (18.7%), and English (17.4%). The next frequent groups were French (8.8%), Scotch (7.0%), and Dutch (6.7%), totaling 23.4%. The rest were Swedes (3.3%), Italians (3.1%), Polish (3%), Norwegians (2.4%), Spanish (1.6%), and Russians (1.7%), adding to 15.3%. Danes, Hungarians, Austrians, Yugoslavians, Czechoslovakians, Portuguese, Greeks, Romanians, and Finnish, each less than 1%, made up the remaining 5.8%. There were 13 (6%) Jewish families whose ancestors were from Germany, Austria, Poland, Hungary, and Russia. Three other families had Jewish intermarriages in their pedigrees. There were also two Gypsy families of Romanian and Greek origin. Among the non-Caucasians, only 10 families were American Negroes and one family was Asian. The latter was from Korean father of Chinese origin and an Okinawan mother who had no known intermarriages with the Caucasian race. Two other Caucasian families had Phillipine ancestors and one other family had an ancestor of Hawaiian Chinese. Intermarriages with the American Indians were present in at least 10% of the Caucasian population studied

564 TRANSAMINATION OF L-HOMOCITRULLINE TO A NEWLY-DESCRIBED CYCLIC METABOLITE. Ronald W. Wilson, Jacquelyn K. Huff, and Arthur F. Kohrman. Michigan State University, College of Human Medicine, Department of Human Development, East Lansing 48824

Elevated levels of serum and urinary L-homocitrulline (HC) are seen in citrullinemia and in several other genetic disorders of the urea cycle and lysine catabolism. In recent studies on the metabolism of HC in rats and mice we have found that HC is converted to a previously undescribed compound (Fed. Proc. 35:1478, 1976). We now report evidence indicating that the initial step in the formation of the new compound is a transamination. The conversion of HC to the cyclic derivative, in vitro, proceeds in mouse liver homogenates, but not in preparations of kidney, brain, spleen, lung, muscle or intestine. The most effective amino-group acceptors for the reaction are pyruvate and glyoxylate; α-ketoglutarate, oxaloacetate, α-ketomalonate, α-ketobutyrate, and α-ketoisocaproate are active but less effective. This transamination may represent a significant route for the disposal of HC and, possibly, other alpha-amino acid intermediates which accumulate in disorders of urea, lysine, and ammonia metabolism. This reaction may also contribute to the depletion of available α-ketoacids in clinical situations with elevated levels of HC, homoarginine, citrulline, and similar compounds.