

**765** AMINO ACID EFFLUX FROM NORMAL AND CYSTINOTIC LEUKOCYTE LYSOSOMES. R. Steinerz, F. Tietze, D. Raiford, and J.D. Schulman, NICHD and NIAMDD, NIH, Bethesda, MD

Decreased cystine (C) efflux from lysosomes could explain C storage in cystinosis. We studied efflux of C and leucine, methionine, phenylalanine, tryptophan, and cysteine from isolated normal (NL) and cystinotic (CY) lysosome-rich leukocyte fractions loaded by incubation with amino acid-methyl esters. The T-1/2 (min) for efflux of the radioactive amino acid produced by esterase activity was determined as described by Reeves (J. Biol. Chem. 254: 8914, 1979). For leu and trypt, efflux was compatible with a diffusion model: strongly temperature dependent and unaltered by exogenous cations, ATP, or amino acid. T-1/2 was comparable for leucine in NL and CY ( $9.33 \pm .60$ [SEM] vs  $9.18 \pm .92$ ). NL T-1/2 was  $13.7 \pm 1.03$  for meth,  $9.35 \pm 1.19$  for phe,  $22.6 \pm 3.97$  for trypt. In contrast, T-1/2 for C was much longer (NL  $92.0 \pm 15.9$ , CY  $101 \pm 27.1$ ). Apparent similarities between NL and CY efflux of C at these low rates require cautious interpretation. C efflux from NL and CY increased upon exposure of lysosomes to 1mM cysteamine during efflux ( $20.6 \pm 1.58$  vs  $26.1 \pm 2.74$ ); in contrast, 1mM reduced glutathione (GSH), a presumably less penetrant thiol, did not cause a change in C efflux. Efflux of other AA was unaltered by 1mM cysteamine or GSH. We conclude: there is normal leu efflux and thus no general defect in lysosomal amino acid efflux in CY; the efflux of C is exceptionally slow in both NL and CY; cysteamine enhances C efflux selectively in isolated lysosomes, probably by penetration and subsequent reduction of C to cysteine.

**766** CYSTINOTIC FIBROBLASTS ACCUMULATE CYSTINE FROM CYSTINE BUT NOT CYSTEINE RESIDUES OF EXTRACELLULAR PROTEINS Jess G. Thoene, Rosemary M. Lemons, Katherine

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Cystine depleted cystinotic fibroblasts reaccumulate cystine when incubated in cystine free medium supplemented with a variety of disulfide (cystine)-containing proteins. The cystine accumulation is linear with the concentration of added protein. Correlation of the amount of degradation of  $^{125}I$  labelled protein with cystine accumulation yields a significant correlation ( $r=0.97$  for bovine serum albumin [BSA], and  $r=0.81$  for bovine insulin). Similar results have been found for human and rat albumin, and bovine  $\gamma$  globulin & thyroglobulin. This cystine accumulation is inhibited by inhibitors of proteolysis, and inhibitors of selective endocytosis. Reduced BSA (RBSA) produced by pre-treatment with mercaptoethanol in 6 M urea yields diminished cystine accumulation even though more of the reduced protein is degraded:  $0.18$  nmol/ $10^6$  cells cystine accumulated and  $0.50$  mg/ $10^6$  cells RBSA degraded in 24 hrs;  $0.70$  nmol/ $10^6$  cells cystine accumulated and  $0.20$  mg BSA degraded in 24 hrs. Ovalbumin (OVA), which contains 4 mol of cysteine/mol of protein and 1 mol cystine yields no cystine accumulation after 24 hrs incubation in which  $0.86$  mg/ $10^6$  cells OVA was degraded. Addition of OVA or RBSA to non-depleted cystinotic fibroblasts produces cystine depletion (30% in 4 hrs, 55% in 24 hrs). We conclude that cystinotic cells accumulate cystine through the lysosomal retention of cystine, but not cysteine residues following their release from peptide chains during proteolysis. Supported by USPHS AM 25548.

**767** GENETICS OF OSTEOGENESIS IMPERFECTA TYPE II. Petros Tsipouras, Edward P. Shields, Deborah Silberberg and F. Clarke Fraser. Department of

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Osteogenesis Imperfecta Type II (OI, lethal perinatal) has been presumed to be inherited as an autosomal recessive trait. In an attempt to test this hypothesis questionnaires were sent to all University Departments of Medical Genetics in Canada, requesting information about their ascertained cases of OI Type II. Twenty-three kindreds were ascertained with a total of 23 sibs of which 4 (17.4%) were affected. By the "singles" method of Davie (Ann. Hum. Gen. 42, 507, 1979) the estimated segregation frequency was  $0.17 \pm 0.024$ . The sex ratios of affected and normal individuals did not differ significantly from expectation. There was no recognized parental consanguinity. The segregation ratio is significantly lower than  $0.25$  ( $P < 0.01$ ), suggesting the presence of either selective prenatal mortality or of genetic heterogeneity. These findings support the contention of Young and Harper (Lancet I, 432, 1980) that the empiric recurrence risk for OI Type II is less than 25%.

**768** ENZYME MANIPULATION: ENHANCEMENT OF THE RESIDUAL ARYL SULFATASE B (rASB) ACTIVITY IN FELINE (f) MUCO-

POLYSACCHARIDOSIS (MPS) VI BY DTT. Deborah T. Vine, Margaret M. McGovern, Edward Schuchman, Mark E. Haskins, Robert J. Desnick. Mt. Sinai Sch. of Med., Div. of Med. Genet., NYC.

Both human and f MPS VI result from deficient ASB activity ( $\sim 6\%$  of normal) and accumulation of dermatan sulfate (DS). Normal human, normal f and f MPS VI hepatic ASB were purified (spec. act. 55, 106, 2.7  $\mu\text{mol/h/mg prot.}$ , resp.) and molecular weights (MW) were determined by PAGE ( $\sim 80,000$ ,  $160,000$ ,  $78,000$ , resp.) and Sephadex G-200 chromatography ( $\sim 45,000$ ,  $110,000$ ,  $53,000$ , resp.). These data were consistent with human ASB and rASB being monomers and the normal f ASB a dimer. When the rASB was incubated with 1 mM DTT, the MW was  $160,000$  (PAGE) and  $100,000$  (Sephadex), suggesting that DTT caused dimerization of the rASB. The DTT-treated purified rASB was increased 5-fold and had markedly increased cryostability. Normal f and f MPS VI buffered whole bloods were incubated  $\pm 10$  mM DTT, leukocytes isolated, ASA and ASB separated by DEAE-cellulose chromatography, and DS determined by cellogelelectrophoresis. The WBC rASB activity was increased 6-fold with DTT (spec. act.  $0.025 \rightarrow 0.164$ ) and the DS was markedly decreased ( $0.37 \rightarrow 0.02$   $\mu\text{g/ug prot.}$ ); DTT had no effect on ASA, normal ASB, or normal DS levels. These findings suggest that the mutation in f MPS VI may involve a cysteine residue which hinders dimerization and that -SH reduction results in dimerization with enhanced rASB activity. Thiol-induced subunit association provides a novel approach for enzyme manipulation therapy which can be tested *in vivo* using the animal model.

**769** ASSIGNMENT OF THE HUMAN GENE FOR LIVER TYPE PHOSPHOFRUCTOKINASE ISOZYME TO CHROMOSOME 21 USING SOMATIC CELL HYBRIDS. Shobhana Vora and Uta Francke. Colum-

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Human phosphofructokinase (PFK, E.C.2.7.1.11) is under the control of three structural loci which code for M (muscle), L (liver), and P (platelet) type subunits. Human diploid fibroblasts express all 3 genes; random tetramerization of resulting subunits produces various isozymes. We have analyzed 17 human x Chinese hamster hybrids segregating human chromosomes for the expression of human L subunits. Since electrophoresis does not distinguish between the rodent or human PFKs, we employed an anti-human L monoclonal antibody which does not react with rodent PFK. The expression of human L subunits in the hybrids was detected by the enzyme-immunoprecipitation technique using staphylococci bearing protein A as an immunoadsorbent. 12 out of 17 hybrids expressed human L subunits and retained chromosome 21 whereas 5 did not express human PFKL and lacked chromosome 21. There was discordant segregation of all other human chromosomes and PFKL expression in these hybrids. The mean RBC PFK of 6 individuals with trisomy 21 was found to be elevated (147% of normal). A specific increase in L subunits in trisomic RBC was evident chromatographically by an increase in L-4 species and immunologically by decreased precipitation with anti-M monoclonal antibody. We conclude that the PFKL gene is located on chromosome 21 and that the previously noted elevation of RBC PFK in individuals with trisomy 21 is due to a gene dosage effect.

**770** REGIONAL ASSIGNMENT OF HUMAN PBGD-DEAMINASE (PBGD) AND ESTERASE A (ESA4) TO 11q23  $\rightarrow$  11qter. Ai-Lan Wang, Francisco X. Arredondo-Vega, Philip F. Giampietro, Moyra Smith, W. French Anderson, R.J. Desnick. Mt. Sinai Sch. of

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Human porphobilinogen (PBG) deaminase, the heme biosynthetic enzyme deficient in acute intermittent porphyria, and ESA4 have been mapped to the long arm of chromosome 11 by somatic cell hybridization techniques. Man-mouse hybrids derived from the fusion of 2S mouse erythroleukemia (MEL) TK<sup>-</sup> cells and human HGPR<sup>-</sup> fibroblasts have been used for studies on the induction and expression of human PBGD. PBGD was examined by isoelectric focusing, immunoprecipitation and rocket immunoelectrophoresis. Among 120 sub-clones generated from one primary clone (XX-8), a clone was found which contained a single human chromosome consisting of a 4/11 translocation (4q12::11q11). This clone was human PBGD and ESA4 positive and LDHA negative. From examination of other primary and secondary clones, it was possible to rule out assignment of PBGD to chromosome 4. Further regional localization was achieved by characterization of 2S MEL (HGPR<sup>-</sup>) x human fibroblast (GM 3552) hybrids containing an X/11 translocation (X;11)(q25-26;q23). One clone (MX/11-2) contained only the intact X/11 translocated chromosome and was LDHA positive. In a second clone (MX/11-3)(LDHA negative), the 11p portion of the translocated chromosome was deleted and the remainder was translocated to a mouse chromosome. Both clones were negative for human PBGD and ESA4. These results demonstrate that human PBGD and ESA4 are encoded on human chromosome 11 in the region 11q23  $\rightarrow$  11qter.