553 EFFECT OF POKEWEED MITOGEN AND RICIN ON PROTEIN SYNTHESIS IN CULTURED HUMAN LYMPHOID LINES. Richard A. Polin, Roger Kennett. (Spon. by William Mellman). Univ. of Pa. Sch. of Med. and The Children's Hospital of Phila., Department of Human Genetics. Philadelphia, PA.

Pokeweed mitogen (PWM) and ricin are both lectins derived from plant seeds. They are glycoproteins and share the ability to agglutinate a variety of animal cells particularly erythrocytes. Preliminary to development of a system to screen for the effect of environmental mutagens on the frequency of lectin resistant mutants we studied the effect of these 2 lectins on protein synthesis in long-term lymphoid lines. Ricin, a highly toxic compound is a known inhibitor of protein synthesis in other cell systems. PWM has primarily been evaluated for its mitogenic effect. Four lymphoid lines were studied: 8866 and GM1531 which are B cell lines, and CCRC/CEM and MOLT 4 which are considered to be T cell lines. Ricin (50 ug/ml) completely inhibited protein synthesis by 2 hours in all 4 lines as measured by the uptake of ^{3}H leucine. PWM appeared more specific and at a concentration of 500 ug/ml inhibited protein synthesis only in the B lines (8866 and GM1531). This effect was maximal at 5 hours. To investigate the reason for the differential effect of PWM on T & B cells, ^{125}I labeled PWM was incubated with 8866 and MOLT 4 to see if an increased binding to B cells could be demonstrated. A significantly greater number of counts bound to 8866 compared to MOLT 4. As lectins bind to cell surface carbohydrates, the possibility that this may represent a difference in the composition or arrangement of the surface glycoproteins is being investigated.

554 EVIDENCE FOR THE ROLE OF RIBONUCLEOTIDE REDUCTASE INHIBITION IN ADENOSINE DEAMINASE DEFICIENCY. SH Polmar, EM Wetzler, RC Stern, DW Martin, Jr. Case Western Reserve Univ., Cleveland and U. of Calif., San Francisco.

Adenosine deaminase (ADA) deficiency is associated with a form of severe combined immunodeficiency disease (SCID). Enzyme replacement therapy has restored immunologic competence in some ADA-SCID patients (Polmar et al. N Engl J Med 295:1337, 1976). Their lymphocytes, however, remain ADA deficient. Enzyme therapy also reduces the high levels of deoxyATP (dATP) present in these patients prior to therapy. The effects of adenosine and deoxyadenosine upon proliferation of ADA deficient lymphocytes were studied using ^{3}H-leucine incorporation as a measure of cell proliferation. Studies were carried out upon lymphocytes from one ADA-SCID patient and three normal individuals whose lymphocytes were made ADA deficient with the ADA inhibitor EHNA.

ADA deficient lymphocytes were 100-1000 times more sensitive to inhibition by deoxyadenosine than by adenosine. Inhibition could be partially reversed (up to 78%) by low concentrations (1-10 uM) of deoxycytidine and to a lesser extent by thymidine, but not by other ribo- or deoxyribonucleosides. Addition of both deoxycytidine and thymidine completely reversed inhibition.

Deoxyadenosine's inhibitory effects appear to be mediated through dATP, a potent inhibitor of ribonucleotide reductase. Deoxycytidine and thymidine can bypass this block and supply these cells with deoxypyrimidine nucleosides for DNA synthesis. These data suggest that deoxycytidine and thymidine supplementation may be clinically useful in the therapy of ADA-SCID patients.

555 INTRAHEPATIC ALPHA 1-ANTITRYPSIN ACCUMULATION AND Pi TYPE; A CORRELATION STUDY. Hope H. Punnett, Dale S. Huff, Harold W. Lischner, Mildred L. Kistenmacher, and Frank Greenberg, Temple University School of Medicine, St. Christopher's Hospital for Children, Departments of Pediatrics, Pathology and Immunology, Philadelphia, Pa.

A retrospective search for propositi to study familial alpha 1-antitrypsin (AAT) deficiency was initiated through an immunohistochemical study of pathological specimens from children with liver disease. A modification of the immunoperoxidase method of Sternberger et al (J. Histochem. Cytochem. 18:315, 1970) was used to identify intrahepatic accumulation of AAT, followed by Pi typing of the children and/or their parents, using isoelectric focusing, acid starch gel and crossed immunoelectrophoresis.

Ten patients with significant AAT accumulation have been Pi typed thus far. Four children were PiZZ; four were PiMM. Both groups included children with portal cirrhosis, neonatal hepatitis and/or biliary atresia. Two children with liver lesions resembling tyrosinosis were typed as MZ and M (tentative). Malton

An additional child (deceased) is presumed to have been AAT deficient on the basis of serum electrophoresis; his parents are unavailable for typing. The immunohistochemical identification of AAT in MM and non-M individuals suggests that AAT accumulates in damaged livers regardless of the Pi type. The identification of AAT in the liver cannot be considered proof of PiZZ genotype, but must be followed by typing. Supported in part by NIH grants CA 19834 and RR5624.

556 ENZYME THERAPY IN GM2 GANGLIOSIDOSIS: CARBOHYDRATE-SPECIFIC HUMAN β-HEXOSAMINIDASE UPTAKE BY FELINE LIVER. Mario C. Rattazzi, Children's Hospital of Buffalo, Dept. of Pediatrics, SUNY at Buffalo, N.Y.

In our studies on cats with genetic Gm2 gangliosidosis as models for enzyme therapy, we characterized a mechanism responsible for rapid plasma clearance ($t_{½} \sim 3$ min) of human β-hexosaminidase (β-hex)injected into normal cats. Preferential hepatic uptake was shown by recovery studies; circulatory bypass of liver markedly impaired enzyme clearance ($t_{½} \sim 60$ min). Carbohydrate-specific uptake, suggested by slow clearance of periodate-treated β-hex ($t_{½} \sim 60$ min) was confirmed by impaired clearance of normal β-hex obtained by injection of terminal N-Acetyl glucosamine- and mannose-rich glycoproteins. Ovomucoid, ovalbumin and ribonuclease-B (final plasma concentration, fpc 0.1, 0.4 and 0.3 mM, resp.) markedly inhibited clearance ($t_{½} \sim 60$ min). Terminal galactose-rich,desialylated orosomucoid and fetuin had no significant effect. Marked inhibition of β-hex clearance ($t_{½} \sim 60$ min) was also obtained by injection of mannose, N-Acetyl glucosamine and L-fucose (fpc 0.15, 0.7, and 0.7M, resp.), but not of glucose or galactose (fpc 0.7M). Thus the feline liver receptor involved in β-hex clearance recognizes terminal mannosyl- and N-Acetyl glucosaminyl-, but not galactosyl residues on glycoproteins. A hepatic receptor with similar specificity, also clearing exogenous lysosomal enzymes, has recently been described in the rat. This suggests that the same mechanism may be present in other mammalian species including man, and may be of critical importance in lysosomal enzyme replacement in humans.

557 PRENATAL DIAGNOSIS IN THE FIRST TRIMESTER VIA ENDOCERVICAL SAMPLING. Samuel A. Rhine and Aubrey Milunsky. Eunice Kennedy Shriver Ctr. and Harvard Medical Sch.

Successful prenatal sex detection in the first trimester utilizing exfoliated trophoblast in endocervical smears led to the development of procedures for sampling and culturing this tissue for chromosomal analysis. Initial studies yielded fetal chromosomes in 26% of samples obtained. We have continued this study testing single and double sampling procedures in the 1st and 2nd trimesters from samples obtained at the time of abortion. From a total of 53 1st trimester samples, growth was observed in 37 (70%) and chromosomes were demonstrated in 26 (49%). However after double sampling for the last 20 cases, karyotyping was successful in 16 (80%). Only 9 of 24 specimens from the 2nd trimester grew but chromosomal analysis was unsuccessful. A number of unusual cell types have been found in the endocervical samples immediately after sampling. These include mosaic sheets of epithelioid cells, ameboid cells, and small round amebocytes. The most striking novel structure found in all samples is a huge (>2100 μm), enucleate, multivacuolated cell rich in cytoplasmic RNA. We are investigating the potential utilization of these various cells for early prenatal diagnosis of inborn errors. While the exact fetal origin of this exfoliated tissue is uncertain, photomicrography and stereomicroscopy have enabled us to propose a novel model of membrane and trophoblast anatomy in early pregnancy. Meanwhile we are optimistic that endocervical sampling in the first trimester will become a useful adjunctive method for prenatal diagnosis.

558 BINDING OF ^{125}I-LABELED PROTEASES TO PLASMA PROTEINS IN CYSTIC FIBROSIS. Giovanni Romeo, Joann C. Blessing-Moore, Marilyn Parsons, Amy N. Bossen and Luigi L. Cavalli-Sforza (Sponsored by Howard Cann) Stanford University School of Medicine, Depts. of Genetics and Pediatrics, Stanford, CA.

Trypsin binding immunoglobulins (TbIg) especially of IgG class (TbIgG), have been identified in cystic fibrosis (CF) patients. Samples of plasma or serum from 53 CF patients, 90 relatives of CF patients, and 159 controls were incubated with porcine or bovine ^{125}I-trypsin, electrophoresed on poly-acrylamide gel and auto-radiographed. The main binding protein for ^{125}I-trypsin was α_2-macroglobulin (α_2M). No difference in electrophoretic migration of ^{125}I-trypsin α_2M complexes was observed between patients and controls. However, trypsin binding to IgG was observed in 80% of CF patients, 30% of their mothers, 3% of controls and in 2 patients with chronic pancreatitis. Binding of trypsin to IgG appears to occur through the Fab portion of the molecule. This strongly supports the antibody nature of TbIgG. The correlation of the presence of TbIgG in CF patients and history of intake of pancreatic extracts supports a possible immunization process. It remains to be established whether TbIgG is active only against exogenous trypsin from pancreatic extracts or also against endogenous human trypsin. Our immunoprecipitation assay of the binding of ^{125}I-trypsin to α_2M and TbIgG did not confirm previous reports of a decreased trypsin binding to α_2M in whole plasma from CF patients.