HL-A PHENOTYPE OF THE FETUS DETECTED ON CULTURED AMNIOTIC FLUID CELLS. Howard M. Cann, Ruta Radvany and Rose Payne. Stanford Univ. Sch. of Med., Depts. of Ped. and Med., Stanford, CA.

Cells cultured from amniotic fluid (AF) specimens obtained at 15-18 weeks of gestation for antenatal detection of chromosomal abnormalities have been typed for HL-A antigens by the fluorochromasia microcytotoxicity test. Fibroblasts growing in four AF cultures were tested for 31 antigens with 100 antisera. In each culture, one maternal and one paternal haplotype was detected on AF fibroblasts, indicating that these are of fetal origin. The HL-A phenotype of the cells dividing in AF culture is stable as shown by concordance of antigenic specificities of the same culture tested at different tissue culture generations. In one of the four cultures, specificities determined by both maternal haplotypes were detected. This finding might indicate contamination of the AF culture with maternal cells, but relatively weak serological reactions and the extra specificity involved suggest that cross reaction is the more likely explanation.

HL-A typing can clearly demonstrate the fetal origin of cells dividing in AF culture, a useful observation when there is concern about contamination by maternal cells. Determination of the fetal HL-A phenotype provides a potential method for antenatal detection of an autosomal dominant disorder by syntenic relationships. This method will be realized when loci of genes determining such disorders are found to be closely linked to the HL-A region.

MUCOPOLYSACCHARIDOSIS VI: STUDY OF A MILD CASE. N. Di Ferrante, B.H. Hyman, W. Klish, P.V. Donnelly, B.L. Nichols and J. Gniot-Szulzycka. Baylor Col. of Med., Hou., Tx. 77025.

A 17 year old male with decreased vision, photophobia and ocular pain had ocular, cutaneous, facial features, skeletal changes and cardiac involvement suggesting a mild mucopolysaccharidosis. Particular findings were corneal cloudiness, hyperpigmentation and hypopigmentation of both fundi, low amplitude ERG, delayed VER, left ventricular hypertrophy, carpal tunnel syndrome and an I.Q. of 115. All peripheral neutrophils and 39% of the lymphocytes had metachromatic granulations. Urinary GAG were 36.2 mg/day, 96% being dermatan sulfate, 3% heparan sulfate; the former poorly degraded. Urinary arylsulfatase A was low and B absent. Cultured skin fibroblasts had increased accumulation of $^{35}SO_4$ and decreased degradation of intracellular GAG. Concentrates of their media corrected the increased accumulation of $^{35}SO_4$ in all types of mutant fibroblasts except those from type VI (severe variant). However, concentrates from media of the latter ones corrected in part the patient's fibroblasts, which were also corrected by subfractions $\beta+\gamma$ of arylsulfatase B, but not appreciably by α. The latter corrected the abnormality of fibroblasts from the severe variant while $\beta+\gamma$ did not. The possibility that the variants might represent defects of specific subunits of arylsulfatase B is being investigated. Supported by USPH AM-10811, RR-00188, HL-05435, GM-19513, the National Foundation-March of Dimes and the Robert A. Welch Foundation.

A NEW VARIANT OF GALACTOSEMIA. Claramma M. Chacko*, Ira K. Brandt, Rebecca S. Wappner* and Henry L. Nadler. Northwestern and Indiana Univs., Dept. Peds., Chicago and Indianapolis.

Galactosemia is an autosomal recessive defect in which the enzyme gal-1-P uridyl transferase (transferase) is decreased or absent in several tissues. Several variants of this enzyme have previously been described. We report here a family in which the proband CB has a new variant of transferase. CB developed anorexia and jaundice shortly after being placed on Similac. The diagnosis of galactosemia was considered when the Beutler fluorescence screening test revealed no detectable transferase activity. Studies of transferase properties; i.e. activity, heat stability at 50° C, and starch gel electrophoretic mobility of family members revealed the following.

	Activity	Stability	Mobility
CB	9.3	+	Fast
Mother	9.3	+	Normal
Father	18.0	-	Fast
Brother	24.0		Normal
Paternal grandfather	19.1	+	Fast
Paternal grandmother	28.0	-	Normal
Normal control	18.0-32.0	+	Normal

These data suggest that at least three alleles are present at the transferase loci in this family. The mother is a classic heterozygote; the father is a double heterozygote for the fast-moving and unstable transferase; and the patient is a double heterozygote for the fast-moving and classic galactosemia transferase.

BIMODALITY OF THE PROPORTION OF Hb Gα-PHILADELPHIA SUGGESTING HETEROGENEITY IN THE NUMBER OF HEMOGLOBIN ALPHA CHAIN LOCI Dublin, P.A., Jr., Cates, M., and Rucknagel, D.L. Howard University College of Medicine, Sickle Cell Center, Washington D.C. Heterozygotes for beta chain variants have 30 to 40% of the abnormal molecule; whereas alpha chain variants have 15-20%. This and other evidence suggests that there are two Hbα loci in man. Homozygotes for the Hb J-Tongariki gene have no Hb A, suggesting that Melanesians have only one locus. We have recently quantified the hemoglobin components of members of two Negro families in which Hb Gα-Philadelphia is present. Four heterozygotes for Hbα^G only possessed 28.4%, 35.5%, 37.7% and 40.9% of Hb G. When these data are combined with that obtained from eleven other previously studied heterozygotes the values appear to be bimodally distributed around means of 30% and 40% Hb G. These are consistent with the hypothesis that two types of chromosomes bearing alpha chain structural loci exist, one having only a single Hbα locus and another having two. Hb G is a mutant on the chromosome having one locus. Those persons having 40% Hb G presumably have only one Hbα locus on the homologous chromosome. Those having 30% Hb G presumably have two loci and thus two Hbα^A genes on the chromosome homologous to that having the gene for Hb G. In one family three persons heterozygous for the genes for Hb G and Hb C were found. Their electrophoretic patterns contained four components, Hb A, Hb G, Hb C, and the Hb G/C hybrid.

EVALUATION OF THE FETO-MATERNAL AMINO ACID METABOLISM AS AN AID TO PRENATAL DIAGNOSIS OF GENETIC DISORDERS AND FETAL DEVELOPMENTAL STUDIES. Louis Dallaire, Michel Potier and Serge B. Melançon. Dept. of Ped., Univ. of Montreal and Ped. Research Ctr., Ste-Justine Hosp. Montreal, Quebec, Canada.

Amino Acid (AA) concentrations in 111 normal amniotic fluids (AF) obtained by transabdominal amniocentesis between 10 and 40 weeks of pregnancy and 89 corresponding maternal plasmas (MP) were measured using a Beckman 121 automatic AA analyser. Scatter diagrams of concentration versus gestational age were plotted for each amino acid. All calculations were made using a control data Serial 6600 computer system. Thirty six AA's and related compounds were identified in AF and MP samples. The concentration of eight AA's: Phe, Tyr, Val, Leu, Ile, Lys, Ala and His decreased towards the end of pregnancy in AF but the Phe/Tyr ratio remained constant. Our results show that the great variation in Lys concentration between the 10th to the 20th week does not permit fetal age correlation studies. In the study of high risk pregnancies there is a marked elevation of AA concentrations in sacs containing two fetuses. In addition, the presence of β-aminoisobutyric acid in AF may be an indication of fetal distress.

Near term (38 to 41 weeks) all AA values were lower in AF as compared with the first voided urine samples. In cord blood all AA's except Met, Pro and Ans were more elevated than in AF samples. These findings suggest that certain AF amino acid concentrations are related to fetal development. Supported by the Medical Research Council of Canada grant MA-4741.

HOMOCYSTINURIA DUE TO CYSTATHIONINE SYNTHASE (CS) DEFICIENCY: INVESTIGATIONS IN CULTURED LONG-TERM LYMPHOCYTES, FETAL SKIN FIBROBLASTS AND AMNIOTIC FLUID CELLS. L.D. Fleisher, N.G. Beratis, H.H. Tallan, K. Hirschhorn, and G.E. Gaull. Dept. Ped. & Clin. Gen. Ctr., Mt. Sinai Sch. Med., N.Y., N.Y.; Dept. Ped. Res., N.Y. State Inst. Res. Ment. Retard., Staten Island, N.Y.

Systematic delineation of optimal assay conditions for CS in adult skin fibroblasts (fib.) enabled us to differentiate obligate heterozygotes (het.) from affected individuals and controls (BBRC 55:38, 1973). We have now extended our studies to long-term lymphoid lines (LTL) and cultured fib. of fetal origin. We have detected CS activity in LTL from 12 normal donors (mean ± SEM=9.49 ± 0.98 nmoles/mg prot./h), its deficiency in a line from an affected individual (0.88), and intermediate activity in 3 obligate het. lines (3.21 ± 0.37), with no overlap. Activity in skin fib. from 5 control fetuses was 32.9 ± 5.06 and not different from control amniotic fluid (AF) cell cultures (40.7 ± 4.58) ($.25 > p > .01$); both differed from adult skin fib. (21.0 ± 1.71) ($p < .001$). Activity in AF cells from an obligate het. mother was 73.8; prenatal diagnosis of a normal fetus was confirmed after birth by assay of skin fib. Thus: (1) LTL, which proliferate vigorously in apparently permanent cultures, are ideal for detection and study of the enzymatic defect in homozygotes and het. for CS deficiency. (2) CS values from adult skin fib. cannot be used as controls for AF cells. (3) Requirements for prenatal diagnosis of CS deficiency have now been met. (4) CS activity in het. LTL, as in liver and skin fib., was less than 50% of normal.