†1225 PKU AND MILD HYPERPHENYLALANINEMIA (MHP) IN SIBLINGS: BIOCHEMICAL CHARACTERIZATION AND MOLECULAR RFLP ANALYSIS OF THE PHENYLALANINE HYDROXYLASE (PAH) GENE. Fred D. Ledley, Harvey L. Levy, Alan S. Lidsky, Savio L.C. Woo. Howard Hughes Medical Institute, Baylor College of Medicine, Dept. of Cell Biology, Houston; Harvard Medical School, Depts. of Neurology and Pediatrics, Children's Hospital, Boston.

Mutations in the PAH gene can cause either classical phenylketonuria (PKU) or MHP. The genetic relationship between these different forms of PAH deficiency is complex. We report biochemical characterization and restriction fragment length polymorphism (RFLP) analysis of the PAH gene using cloned PAH in two families in which some siblings have PKU and others have MHP. In both families the mother has MHP and the father is biochemically normal. The first family has one child with PKU, one child with MHP, and two normal children. The second family has one child with PKU, three children with MHP, and one normal child. Haplotype analysis of the PAH gene using RFLPs demonstrates that in each family one allele from the father segregates with all the affected children (both PKU and MHP) while one of the maternal alleles segregates with PKU and the other with MHP. This analysis identifies several mutant PAH alleles which can cause either PKU or MHP. These findings demonstrate that, at least in these families, there is extensive overlap between those mutations causing PKU and those causing MHP. The two distinctly different clinical phenotypes reflect the effects of pairs of mutant alleles, presumably representing a variety of different mutations, which together have distinctive biochemical and pathophysiological consequences.

1226 THE EFFECT OF FASTING ON OVINE FETAL AND MATERNAL GLUCONEOGENIC ENZYMES, James A. Lemons, Helen C. Moorehead, Debra Reyman, George P. Hage, Indiana University School of Medicine, Indiana University Hospitals, Department of Pediatrics, Indianapolis.

Fetal and maternal sheep were studied to determine whether changes in gluconeogenic enzyme activities could be detected in the liver and/or kidney associated with nutritional deprivation. Thirteen ewes and 16 fetuses were sacrificed in the fed state while 13 ewes with 17 fetuses were sacrificed after five days of fasting, all at 125 days gestation (term = 147 days). Fetal weight was decreased in the fasted versus fed group (2.86 ± .56 versus 3.61 ± .58 kg, p <.001). Weights of fetal liver (66 ± 16 versus 105 ± 27 gm), fetal kidney (9.3 ± 1.9 versus 12.4 ± 1.9 gm) and maternal kidney (74.9 ± 8.3 versus 88.0 ± 9.3 gm) were all significantly decreased with fasting (p <.001). Tissues were analyzed for glucose-6-phosphatase (G-6-P), fructose-1,6-diphosphatase (FDP), glutamate-oxaloacetate aminotransferase (GOT) and glutamate-pyruvate aminotransferase (GPT). In both fetus and mother, G-6-P and FDP were increased during fasting when measured per gm liver tissue and per mg protein (p <.001). GOT and GPT were also increased in fetal liver but not in kidney with fasting. However, activities of the enzymes per whole fetal liver or kidney were not different between fed and fasted states. Therefore, although gluconeogenic enzyme activities increased in relative activity per unit weight in fetal tissues during fasting, total enzyme activity is unchanged due to a decrease in liver and kidney mass.

†1227 UREA CYCLE ENZYMES DURING FASTING IN THE OVINE FETUS, James A. Lemons, Philip J. Snodgrass, Indiana University School of Medicine, Indiana University Hospitals, Veterans Administration Hospital, Departments of Pediatrics and Medicine, Indianapolis.

Activities of five urea cycle enzymes were measured in maternal and fetal sheep liver during the normal fed state and following five days of fasting. Six ewes and 10 fetuses were studied in both the fed and fasted periods at 132 days gestation (term = 147 days) for liver protein, DNA and enzyme levels. Results indicated that protein and DNA contents remained unchanged during fasting in both the maternal and fetal liver. Fetal liver weight was decreased during fasting from 108 ± 23 to 71 ± 21 gm (mean ± SD). Fed state fetal enzyme levels were at 25-50% of maternal values per mg DNA and 50-125% of maternal levels per gm liver. After fasting, four of the five enzymes increased by 2.3 to sixfold (per mg DNA) in fetal liver; only one enzyme (AS) increased significantly in maternal liver. Activities of each enzyme per whole fetal liver gave similar results.

	Units per mg DNA				
	CPS	OTC	AS	AL	ARG
Fetal-fed	25	1334	12	29	46281
Fetal-fasted	149	1885	53	67	137156

These data indicate that the in vivo studies which demonstrate a doubling in fetal urea production in the fasted sheep in latter gestation are associated with parallel increases in the fetal hepatic activities of several enzymes responsible for urea synthesis.

●1228 LACTATE IS A GLYCOGENIC PRECURSOR IN THE OVINE FETUS. Lynne L. Levitsky, John B. Paton, David E. Fisher, Pritzker Sch. of Med., Univ. of Chicago, Michael Reese Hosp., Department of Pediatrics, Chicago, Ill.

Glucose (Glu) levels are exceedingly low in the ovine fetus and the ungulate liver has little measurable glucokinase. Nonetheless, at term, the fetal (Fet) sheep liver contains significant glycogen stores. Recent suggestions that glycogenesis in the adult may proceed through the gluconeogenic pathway led us to examine the capacity of lactate (Lac) to serve as a glycogenic precursor in the chronically catheterized Fet sheep. Six ovine fetuses were prepared with aortic (Ao) and inferior vena cava (IVC) catheters. On day 5 post surgery, after a maternal (Mat) 48 hour fast, a steady state Mat venous infusion of Glu was begun sufficient to raise Mat blood Glu from 19.4±1.6 mg/dl to 48.6±6.2 mg/dl. A simultaneous infusion of 300 uC ($U\text{-}^{14}C$) Lac was administered to the Fet IVC over a 5 hour period to steady state. Six Fet Ao and Mat art. samples were obtained q 10 min during the last 60 min of the infusion. The fetus and ewe were then sacrificed and liver removed for determination of glycogen and ^{14}C glycogen enrichment. Minimal radiolabel was found in Mat blood or liver glycogen. Fet blood levels were Lac 13.6±1.3 mg/dl (SA 42±9 $uc/mgx10^3$), fructose 70.2±10.4 mg/dl (SA 1.0±.2 $uc/mgx10^3$), Glu 23.2±4.3 mg/dl. No Lac-derived labelled Fet Glu could be reliably detected. Fet hepatic glycogen was 40.0±6.5 mg/g liver (SA 1.4±.5 $uc/mgx10^3$). The SA of glycogen and absent Glu labelling suggests that Lac is a precursor of Fet glycogen through the gluconeogenic path. This is teleologically important, since placental conversion of Mat Glu to Lac maintains higher levels of Lac in the Fet circulation and seems to be a means for Fet conservation of energy stores.

1229 GEL ELECTROPHORETIC(GE) ANALYSIS OF PROTEINURIA IN PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM). Kenneth V. Lieberman, Mary E. Witt, Fredda Ginsberg-Fellner. Mt. Sinai School of Med, Dept of Ped, NY, NY.

Clinically significant proteinuria indicates a poor renal prognosis in IDDM patients. Microalbuminuria presages worsening proteinuria. Other techniques to evaluate proteinuria may allow an earlier prediction of nephropathy in time to use aggressive therapy to alter morbidity. We analyzed the patterns of proteinuria in 14 IDDM patients (compared with 8 controls) by a novel protocol utilizing spot (random, resting, single void) urines. All patients had < 1 gm/day proteinuria. Diabetic patient characteristics(diab indices): ages 3-24, duration diabetes 1.5-16 yrs, mean $HgbA_{1c}$(mHA) 4.6-8.6%(n1<4.8%), current $HgbA_{1c}$ 3.4-10.9%. Spot urines were: obtained sterilely, micropore filtered, dialyzed, lyophylized, subjected to sodium dodecyl sulphate polyacrylamide GE, stained with Coomassie Blue and then analyzed by an automatic gel scanner/integrator. Peaks were grouped into the following fractions: %albumin(alb), %high molecular weight(hmw,>alb), %low molecular weight(lmw,<alb). The protein to creatinine ratio (p/c) was also determined for each specimen. Fractional ratios were obtained by multiplying each fraction(%) by p/c yielding: hmw/c, alb/c, lmw/c.

Means+SEM	p/c	hmw/c	alb/c	lmw/c
diabetic	.21±.04	.02±.004	.11±.03	.08±.01
normals	.12±.02	.01±.003	.06±.01	.04±.01
p	.03	.25	.07	.05

Total p/c and lmw/c are significantly increased in diabetics with normal creatinine clearances. Multiple regression analysis was performed on the diabetic data; fractional ratios were regressed against all the diab indices. Although none of the correlations reached statistical significance, there was a trend toward higher fractional ratios with increasing age and mHA. The ease of acquisition of spot urine samples, the significant elevation of p/c in diabetics and the trends relating to glucose control makes further exploration with a larger, more heterogeneous sample appear promising.

1230 OCULAR INVOLVEMENT IN NIEMANN-PICK DISEASE, TYPE B. M. Lipson[1], J. O'Donnell[2], J.W. Callahan[3], D. Wenger[4], S. Packman[5], [1]Dept. Pediatrics, Kaiser Permanente Medical Center, Sacramento; Dept. of [2]Pediatrics and [3]Ophthalmology, University of California San Francisco; [4]Research Institute, Hospital for Sick Children, Toronto; [5]Dept. of Pediatrics, University of Colorado Health Sciences Center, Denver.

Niemann-Pick disease (NPD) is a heterogeneous group of disorders usually designated types A-E. Each type is characterized by the accumulation of excessive sphingomyelin. This classification scheme fails for an ever-growing number of NPD patients. We report a 7½ year old male with type B Niemann-Pick disease with normal neurological and intellectual function, but who does have cherry red spots. This patient represents the fourth case of a child with the clinical and biochemical findings of type B NPD, but with evidence of involvement of neural tissue. These patients may represent a distinct clinical variant of NPD, in whom the natural history has not yet been elucidated. The existence of this subset of type B NPD has implications for genetic and biochemical studies in NPD, as well as for genetic and prognostic counseling of families.