COMPARISON OF CAPILLARY AND VENOUS BLOOD SAMPLING FOR QUANTITATION OF PLASMA CHOLESTEROL. T. Ishikawa, J. Morrison, R. Fallat, D. Parsons, R. Tsang and C. J. Glueck (intr. by J. Sutherland). Univ. Cincinnati; Col. Med., Cin. Gen. Hosp., Dept. Med., Ped., Cincinnati.

Plasma (P) cholesterol (C) levels in 181 subjects measured using a capillary blood gas-liquid chromatography(GLC) micromethod (20 µl of plasma), were compared to C levels in venous blood using ferric-chloride automated colorimetric methods (AA-1). For the GLC, 20µl of plasma was saponified with tetramethylammonium hydroxide-isopropanol (with 5 α cholestane as internal standard) and was extracted with tetrachlorethylene:methyl butyrate 1:3 v/v. After addition of water, the tetrachlorethylene lower phase was injected into the GLC and C concentration determined by peak-height ratio comparison with reference P. In 181 subjects C (mean ±SD) for the AA-1 method (which required 5 ml plasma and venesection) was 235± 54 essentially identical to C measured by the GLC method, 235±57 mg/100ml. The correlation of the C levels by AA-1 and GLC was very close, r=0.96, p<.001. Two-way analysis of variance revealed no differences between C by AA-1 or GLC. There were no differences between C by AA-1 or GLC for subjects with plasma triglycerides >200 mg/100 ml and those with TG<200. The close concordance of the GLC capillary method with the venous blood AA-1 method allows reduction in sample size to 20 µl plasma, negates the necessity for venesection, and facilitates lipid studies in infants and children.

TISSUE DISTRIBUTION AND TOXICITY OF LEAD FOLLOWING IRON SUPPLEMENTATION. Joseph Kochen* and Yigal Greener*, Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. of Pediatrics, The Bronx, New York (Intr. by Laurence Finberg)

It has been postulated that iron(Fe) deficiency enhances the toxic effects of lead(Pb) in younger children. The present study shows that Fe supplementation has pronounced effects on the distribution and toxicity of Pb. The addition of $FeCl_3$ (300µg Fe per 100ml plasma)to human blood resulted in a marked decrease in the uptake of added Pb210 by red blood cells. At equilibrium, 86.5±6.1%[14] of the added Pb remained in the plasma as compared to 15.6±4.0%[18] in the absence of additional Fe. The retained plasma Pb in the presence of added Fe is in the form of a stable macromolecular Pb complex. In the rat, intravenous administration of Fe followed by Pb, resulted in a similar failure of red cell Pb uptake. This was accompanied by an 80.3±38.6%[11] increase in Pb uptake by the liver and a 38.4±10.0%[11] decreased Pb uptake by the kidneys. Toxicity studies performed by injecting human plasma containing 55µgPb into the yolk sacs of 5 day chick embryos resulted in 88% mortality by day 12. Among these embryos,94% showed gross brain hemorrhage and hydrocephalus. Similar embryos injected with Pb in plasma pretreated with Fe, showed a decrease in mortality(41%) and a markedly reduced incidence of gross brain damage(26%). These findings demonstrate an interaction between plasma constituents and Pb, which under conditions of Fe excess changes the tissue distribution of Pb and protects against its neurotoxic effects.

NORMAL PRODUCTION AND INCREASED OXIDATION OF GLUCOSE ASSOCIATED WITH EPINEPHRINE DEFICIENCY IN "KETOTIC" HYPOGLYCEMIA. Douglas S. Kerr, Hazel Macdonald, Hazel M. Robinson, and David Picou, Univ. West Indies, Tropical Metabolism Research Unit, Kingston, Jamaica (Intro. by LeRoy W. Matthews)

Neither the current hypothesis that "ketotic" hypoglycemia results from deficient gluconeogenesis nor the alternative of increased glucose oxidation has been adequately tested. Three affected children (H) were compared with their unaffected identical twins (C) while fasting until hypoglycemic or nearly glycogen depleted. Plasma glucose production (flux, measured by constant infusion of ^{13}C-glucose) was the same earlier in the fast during glycogenolysis (H = 19, C = 19 µmoles/min/kg), as well as later when it was derived almost entirely from gluconeogenesis (H = 15, C = 16 µmoles/min/kg). Potentially available glycerol or amino acids for gluconeogenesis (determined from respiratory calorimetry and N excretion) were not less in H (alanine was lower). However, glucose oxidation (by calorimetry) was greater in H while glucose fell rapidly preceding symptoms (H = 17, C = 8 µmoles/min/kg). Urine epinephrine rose in C as glucose fell, but not in H until after becoming hypoglycemic and the maximum was much less (H = 0.6, C = 1.6 ng/min/kg). There was also a delayed rise in plasma lipolysis products and failure to suppress insulin in H. Inhibition of glycolysis by infusion of 2-deoxyglucose raised epinephrine, glucose, and FFA in C, but not in H. In conclusion, acquired epinephrine deficiency can account for this impaired transition from glucose to fat oxidation resulting in hypoglycemia.

GLUCOSE DISPOSAL, HORMONAL CHANGES, AND PLASMA ALANINE LEVEL IN NEONATAL SEPSIS. R.D. Leake, R.H. Fiser, Jr., & W. Oh. Dept. of Ped., UCLA Sch. of Med., Harbor Gen. Hosp., Torrance, Ca.

Glucose (G) disposal rate, plasma insulin (I) and plasma alanine (A) levels were studied in 8 full term infants (mean age=25 days) during acute infection and convalescence, 2 days and 8 days following antibiotic therapy for septicemia in 6 infants and/or meningitis in 5. Five infants had gram positive; 2, gram negative; and 1, viral infection. Following a 1-2 hour constant G infusion (6 mg/kg/min), baseline G, I, and A samples were obtained. An intravenous glucose tolerance test (GTT) was performed giving 1 gm/kg G (in 50% solution). Peripheral blood G was obtained for calculation of G disposal rate (K_+) and at 30 to 45 min. for plasma I levels. Four nonseptic infants underwent similar studies.

The results show that (1) glucose utilization is enhanced during the acute phase of infection, and although decreased, remains high during convalescence (K_+ acute=3.7 + 0.3 vs. control=1.8 + 0.1, p<.01, and convalescence=2.5 + .23), (2) insulin levels were similar during the acute phase and convalescence and were not significantly different from controls, (3) basal A levels in septic infants were similar to control values indicating no deficiency of A as substrate for gluconeogenesis, and (4) no difference was observed in any parameter between gram positive and gram negative infection. Thus, enhanced glucose utilization during infection occurs in the absence of elevated insulin levels, suggesting that another "insulin-like" factor is present during infection.

DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA BY MEASUREMENT OF CHOLESTEROL SYNTHESIS IN SKIN FIBROBLASTS Avedis K. Khachadurian,* Mark Lipson* and F. S. Kawahara* (Intr. by H. L. Nadler) Clinical Research Center, Children's Mem. Hosp. Northwestern Univ. Med. Sch., Chicago and Col. of Med. and Dentistry of New Jersey, Rutgers Med. Sch., Piscataway, New Jersey

Incorporation of acetate into digitonin precipitable fraction (DPF), fatty acid fraction (FAF) and CO_2 were measured in monolayers of cultured skin fibroblasts from 4 homozygotes (HH) for familial hypercholesterolemia(FH), 6 heterozygotes (Hh), 10 controls (hh) and 4 subjects with type 4 and 5 hyperlipidemia. In cells preincubated for 20 hours in lipid free medium, counts per min per mg of protein (CpMp) in DPF were: HH, 19,600; hh, 20,700. In cells preincubated in standard medium with cholesterol (C) concentration of 3.5 mg%, CpMp were (mean ± SD): HH 3868 ± 2356; Hh, 725 ± 160; hh, 177 ± 79; type 4-5 hyperlipidemia, 168 ± 91. Counts in FAF and CO_2 were similar in the 4 groups. Variation in time of incubation (30 to 240 min) and acetate concentration (0.03 to 1 µmole per flask) had no effect on direction or magnitude of differences. Increasing C in preincubation medium to 50 mg% did not suppress further the counts in HH. Results are in agreement with findings in liver slices (Lancet II, 778, 1969) and add further support to the hypothesis that the metabolic defect in FH is a derangement of the feedback inhibition of C synthesis. Fibroblast assay could be a useful tool for the etiological diagnosis of hyperlipidemia.

BRAIN FREE AMINO ACID CONCENTRATIONS AND L-GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY IN A PATIENT WITH VITAMIN B6 DEPENDENCY SEIZURES. Harvey L. Levy, J. Thomas Coulombe, and Ira T. Lott. Harvard Med. Sch. and Mass. Gen. Hosp., Dept. of Neuro., Boston.

The basic biochemical defect in B6 dependency seizures has not been elucidated, though it has been postulated that brain GAD or perhaps another system may be B6 dependent in such patients. We have examined free amino acids (umoles/gm fresh tissue) and GAD activity in brain from one of the original patients, who died in status epilepticus after not having been given B6 for 3 days. Glutamic acid was increased in frontal (71.8 vs. 47.8 ± 11.9) and occipital lobes (68.0 vs. 48.8 ± 10.1) whereas GABA was decreased (frontal 1.3 vs. 7.4 ± 2.8; occipital 1.1 vs. 10.1 ± 4.0). Cystathionine (cysta) was increased in both frontal (4.4 vs. 2.0 ± 1.6) and occipital (11.3 vs. 3.3 ± 1.4) lobes but cystine was virtually undetectable in both patient and control brains. Frontal lobe GAD activity, as measured by $^{14}CO_2$ formation from DL-glu (1-^{14}C), was undetectable in the patient without added pyridoxal -5'-PO_4 (PLP) as was true in 3 of 4 controls. However, activity was detectable by the addition of as little as .1 uM PLP (30 cpm/mg protein vs. 0-160 in controls) and was enhanced comparable to controls upon further PLP additions (.2-100 uM). Thus GLU and GABA concentrations suggested reduced GAD activity in vivo but no specific B_6 dependency could be shown. Cysta increase suggested the possibility of B_6 dependent brain cystathionase deficiency.