●1188 DEFICIENCY OF ELECTRON TRANSFER FLAVOPROTEIN (ETF) OR ETF DEHYDROGENASE (DH) IN GLUTARIC ACIDEMIA TYPE II (GA2) FIBROBLASTS. FE Frerman and SI Goodman Medical College of Wisconsin, Dept of Microbiology, Milwaukee, and Univ of Colo Sch of Medicine, Dept of Pediatrics, Denver.

GA2 is an inborn error of metabolism characterized by nonketotic hypoglycemia, metabolic acidosis, and excretion of metabolites of flavoprotein dehydrogenases that transfer electrons to the respiratory chain via ETF and ETF DH. Defects in these proteins have been proposed to explain the discrepancy between the apparent dehydrogenase deficiencies in vivo and in whole mutant fibroblasts, and their normal catalytic activities when asayed in vitro.

Antisera to pork liver ETF and ETF DH were raised in rabbits; both cross-reacted with human mitochondrial proteins of the same molecular weights. Normal human fibroblasts produce CRM+ polypeptides, as shown by Western blotting and localization with peroxidase-conjugated antirabbit IgG. Of four GA2 lines examined, two (unrelated patients with neonatal onset and congenital anomalies) were CRM- for ETF DH and CRM+ for both ETF subunits. The two remaining lines (neonatal onset w/o anomalies) were CRM+ for ETF DH; one was deficient in both ETF subunits and the other was deficient only in the α polypeptide. Study of liver mitochondria confirmed CRM- for ETF DH in one patient, also showing a decrease in the characteristic EPR signal of ETF DH, and confirmed CRM- for the α polypeptide of ETF in another.

These results show that there is a defect in this branch of the respiratory chain in GA2, and that there is biochemical and (probably) genetic heterogeneity in the condition.

1189 IDIOPATHIC INFANTILE HYPERCALCEMIA. Paul R. Goodver, Agnes Frank, and Bernard S. Kaplan. McGill University, The Montreal Children's Hospital, Department of Pediatric Nephrology, Montreal, Quebec.

Idiopathic infantile hypercalcemia is a rare inborn form of severe hypersensitivity to vitamin D which tends to abate by 1 year of age. We report clinical studies of an infant with this syndrome which bear on the pathogenetic mechanism. Our patient was born at term to a diabetic mother and was transiently hypocalcemic in the first week of life. From 1-3 months, he was irritable and failed to thrive on breast milk (Ca = 31.5 mg/dl) and supplementary vitamin D_2 (400 U/day). At 3 months, hypercalcemia (17.7 mg/dl) was recognized in association with depressed serum levels of PTH (undetectable), normal TPR (90%), 1,25 dehydroxy-D3 (11 mg/ml) and 25-hydroxy-D3 (< 17 ug/ml) and hypercalciuria (U Ca/creat = 1.3). Parents were unaffected and there was no evidence of malignancy, subcutaneous fat necrosis or stigmata of Williams syndrome. Radiologic bone density was increased and GFR was reduced secondary to nephrocalcinosis demonstrable by ultrasound. Hypercalcemia resolved with prednisone therapy (1 mg/kg/d) and GFR returned to normal, but growth was poor. On an oral calcium binding agent (cellulose phosphate, 0.5 g x 5/d) serum calcium has been well controlled, U Ca/creat has decreased to 0.4 and growth has accelerated. We suggest that hypercalcemia in this syndrome results from the post-natal induction of unregulated intestinal vitamin D receptors allowing massive hyperabsorption of dietary calcium. Studies of vitamin D receptors in the patient's fibroblasts are in progress.

1190 EFFECTS OF ANESTHESIA ON BILE PIGMENT EXCRETION. Glenn R. Gourley, William Mogilevsky, Frank L. Siegel, Gerard B. Odell. U. of Wisconsin Hospitals, Depts. of Pediatrics and Physiological Chemistry, Madison WI.

Anesthetic agents can alter hepatic glucuronidation. Under diethyl ether (E), pentobarbital (P) or ketamine (K) (n=5 each), rats received bile duct and venous catheters. Basal bile was collected for 300 min without further anesthesia. Bilirubin diglucuronide (DG) was quantified by HPLC, total bilirubin excretion (μg/100 gm body wt/min) by Van den Bergh analysis and bile flow (μl/100 gm body wt/min) by weight.

			0-20 min	60-90 min	270-300 min	Sig
Data are expressed as mean±SE. Significance is based on ANOVA.	E	%DG	36.4±3.8	53.0±3.8	58.0±2.8	p < .01
		Flow	4.0±0.4	4.5±0.6	3.8±0.5	NS
		Excr	0.24±0.03	0.25±0.02	0.37±0.02	p < .01
	P	%DG	59.0±2.2	65.0±2.6	63.0±3.2	NS
		Flow	4.3±0.8	3.8±0.7	3.0±0.4	NS
		Excr	0.31±0.02	0.27±0.02	0.34±0.03	NS
	K	%DG	54.6±1.1	56.0±4.2	52.9±3.9	NS
		Flow	8.0±1.1	5.2±1.1	3.6±0.5	p < .05
		Excr	0.39±0.02	0.31±0.02	0.74±0.08	p < .01
	Sig	%DG	p < .01	NS	NS	
		Flow	p < .01	NS	NS	
		Excr	p < .01	NS	p < .01	

E anesthesia is associated with a reversible suppression of DG formation and total bilirubin excretion. Flow and excretion are variable with K. P provided the most uniform excretion data.

●1191 EFFECTS OF VALPROIC ACID (DEPAKENE) ON KETOGENESIS (K), GLUCONEOGENESIS (G), AND UREAGENESIS (U) BY RAT HEPATOCYTES. Daniel E. Hale, Barbara E. Corkey, and Charles A. Stanley. Depts of Pediatrics and Biochemistry, University of Pennsylvania School of Medicine, Children's Hospital of Philadelphia, Philadelphia, PA.

Recently there have been reports of a Reye's-like hepatoencephalopathy with hyperammonemia, fatty infiltration of the liver, hypoglycemia, and progressive obtundation, associated with the use of the anticonvulsant, valproic acid (VPA). We evaluated the effects of VPA on liver metabolism with the consideration of developing a model for studying Reye's syndrome. Rates of K (umol/hr/gm dry weight) by rat hepatocytes incubated with 0.5 mM VPA (optimal serum levels 0.5 mM - 1.0 mM) and ketogenic substrates are presented below:

	Oleate	Octanoate	Butyrate
Control	334	346	245
VPA (% Inhibition)	80	29	23

VPA inhibited U by 75% and G by 60%. With isolated mitochondria, VPA (20uM) inhibited oxidation of palmityl carnitine, but had no effect on pyruvate oxidation, suggesting that VPA inhibits an enzyme(s) of fatty acid oxidation. The inhibition of U and G by VPA may reflect decreased fatty acid oxidation with consequent decreased acetyl CoA and NADH or specific inhibition of other enzymes of these pathways. Inhibition of U and G is also consistent with the clinical picture seen in VPA toxicity. VPA is more likely to be toxic under fasting conditions. This model may be useful for the study of metabolic abnormalities associated with Reyes Syndrome.

●1192 THE EFFECT OF INSULIN ON FETAL GLUCOSE UTILIZATION AND OXIDATION. William W. Hay,Jr., Huei K. Meznarich, John W. Sparks, Giacomo Meschia, and Frederick C. Battaglia. Division of Perinatal Medicine, University of Colorado School of Medicine, Denver, Colorado.

We have shown previously that fetal glucose uptake, utilization, and oxidation rates are directly related to glucose concentration [G]. However, [G] and insulin concentration [I], are also directly related. In order to measure the specific effect of [I] independent of [G] on fetal glucose utilization (GUR) and oxidation (GOxR) rates we performed glucose clamp experiments in 14 chronically catheterized unstressed fetal lambs. During a 1/2 hr. control period mean arterial [G] was determined and then maintained ± < 0.5 mg/dl by a variable glucose infusion (GIR) during a 2 hr. period of hyperinsulinemia. Net umbilical oxygen (UO_2U) and glucose (UGU) uptakes (Fick Principle), and the fraction of glucose oxidized (GOxFx), GUR and GOxR (^{14}C-U-glucose tracer) were measured simultaneously during control and hyperinsulinemia periods.

	[G]a	[I]	UO_2U	GIR	UGU	GUR	GOxR	GOxFx.
	mg/dl	μU/ml	mmol/min/kg	mg/min/kg				
Control: X̄	17.23	22	0.308	-	4.51	4.74	2.63	0.568
SEM	1.56	2	0.011	-	0.47	0.52	0.21	0.109
High X̄	16.55	323	0.338	4.06	5.19	9.11	4.57	0.538
Insulin SEM	1.58	47	0.011	0.48	0.33	0.96	0.39	0.049

Conclusions: 2 hrs. of hyperinsulinemia at constant [G] increased UO_2U by 10% (p<0.02), and doubled glucose utilization and oxidation rates, although the fraction of glucose oxidized and thus the partition of glucose metabolism was not changed.

●1193 THIAMINE UPTAKE BY HUMAN FIBROBLASTS. Richard E. Hillman, Barbara Witte, JoAnne C. Kelly, Washington University School of Medicine, St. Louis Children's Hospital, St. Louis.

Thiamine requiring enzymes are commonly studied in cultured fibroblasts but the mechanism of thiamine accumulation had not been defined in these cells. Thiamine is concentrated greater than in the medium by 30-60 minutes and reaches apparent concentrations 6 times the medium by 2-3 hours. Kinetic studies demonstrate that uptake is saturable and the apparent Km(.15-3 μM) and Vmax (.6-.8 p moles/mg/min) are similar to those in gut and brain. However, the uptake process is quite different. Accumulation is highly specific but not energy dependent. Before thiamine is phosphorylated it is bound to at least two <40,000 MW cytoplasmic proteins. Uptake and binding are competitively inhibited by pyrithiamin but only slightly by TP or TPP. Thiamine efflux is biphasic with rapid efflux occuring over 30-60 minutes followed by a slow phase lasting at least several hours, probably reflecting phosphorylation.

We conclude that thiamine uptake in these cells is more comparable to steroid hormone uptake than to thiamine uptake by specialized transport tissue.