●1219 PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY EXPRESSED IN LYMPHOCYTES. Douglas S. Kerr and Marilyn M. Lusk, Case Western Reserve University, Rainbow Babies and Children's Hospital, Department of Pediatrics, Cleveland, Ohio.

Pyruvate dehydrogenase complex (PDC) deficiency is usually detected by assaying activity in fibroblasts. Dephosphorylation and phosphorylation of PDC by preincubation with dichloroacetate (DCA) or fluoride (F) increases the difference in activity between normal and abnormal cells. For a rapid alternative assay, we evaluated use of lymphocytes obtained in whole blood and separated by the Ficoll-Paque method. PDC activity in lymphocytes is similar to fibroblasts, linear in relation to time and protein, increased by pre-incubation with DCA, and decreased by pre-incubation with F. Activity remains sufficient for assay in whole blood after 48 hr. DCA stimulated PDC activity (nmol/min/mg protein; mean, range) was compared in lymphocytes from 30 control adults, fibroblasts from 18 control children, and in both from 17 children with lactic acidosis:

Subjects		Lymphocytes	Fibroblasts
Controls		2.03(0.9-3.9)	2.55(1.1-4.1)
PDC Deficient,	Patient A	0.33	0.23
	Patient B	0.90	0.05
	Patient C	0.16	0.83
Other lactic acidosis (n=11)		2.21(1.0-3.8)	2.48(1.0-4.0)

The other 3 patients had reduced PDC in only lymphocytes (1) or fibroblasts (2). PDC deficiency is therefore expressed in lymphocytes as well as in fibroblasts, providing a useful alternative and supplementary assay for diagnosis. Lymphocytes can also be used to detect pyruvate carboxylase deficiency.

1220 ALTERED HEPATIC ENERGY, AMMONIA, AND AMINO ACID METABOLISM IN THE SUBLETHAL ENDOTOXIN RAT MODEL OF REYE SYNDROME. L. Kilpatrick-Smith, M.C. Yoder, S.D. Douglas, M. Erecinska, R.A. Polin. Univ. of Pa. Sch. of Med., Depts. of Pharm. & Peds., Phila., PA.

Administration of a sublethal concentration of E. coli 0111:B4 endotoxin (E) to fasted Sprague-Dawley rats results in Reye syndrome-like metabolic (hyperammonemia, elevated serum lactate, and free fatty acids), hepatic histologic (microvesicular fat deposition) and mitochondrial ultrastructural (pleomorphism and swelling with matrix disruption) changes (Yoder et al, Infect. Immun, in press). In this study, we assessed hepatic alterations in energy production, ammonia metabolism, and determined the amino acid concentration profile in E (0.2 mg/kg) or placebo (5% dextrose in water) treated (C) 250g Sprague-Dawley rats (N=5 each group). At 1, 4 or 12 hrs following E or placebo IP injection, the liver was freeze-clamped. There were no significant differences in metabolic parameters detectable at the 1 hr time point. At 4 and 12 hrs, however, ATP/ADP declined by 25% ($p<0.01$), [lactate] increased by 50% ($p<0.001$), and [urea] increased 67% ($p<0.001$) in E compared to C rats. In addition, mitochondrial [NAD+]/[NADH] increased whereas cytosolic [NAD+]/[NADH] decreased in E compared to C rats. Two-fold increases ($p<0.005$) in [glutamate], [alanine], and [glutamine] and a 55% decrease in [citrulline] ($p<0.05$) were measured in E compared to C animals. These results indicate that a sublethal dose of E disrupts hepatic aerobic energy production, increases glycolysis and proteolysis, and impairs ammonia clearance in the fasted rat. Since the metabolic derangements reported in this study are similar to those observed in patients with Reye syndrome, the current findings lend further support to the relevance of our sublethal endotoxin rat model.

†1221 ELEVATED KETONE OXIDATION IN RAT JEJUNUM EXPOSED TO AMINO-OXYACETATE. R.E. Kimura (Spon. by M. Simmons), Dept. Pediatrics, Univ. of Utah, Salt Lake City, Ut.

Since glutamine is the preferred oxidative substrate in suckling rat jejunum, we determined in developing rat jejunum the activity of alanine aminotransferase, one of three enzymes that converts glutamate to 2-oxoglutarate. The jejunal activity of alanine aminotransferase in cytosolic supernatant is 0.24±0.02 (umol of pyruvate/mg prot/hr) in suckling pups and 0.39±0.04 in postwean pups. Amino-oxyacetate is a known inhibitor of alanine aminotransferase. We determined the effect of amino-oxyacetate on glutamine, glucose and 3-hydroxybutyrate(3-HB) oxidation by adult rat jejunum. Glutamine oxidation to CO_2 and glucose oxidation to lactate, an indicator of glycolysis, is inhibited by 80%. Glucose oxidation to CO_2 is inhibited 40%. 3-HB oxidation is activated by 340%. These data suggest that the major pathway by which glutamine oxidation enters the citric acid cycle is through alanine aminotransferase. Glycolysis is inhibited by amino-oxyacetate. In the absence of glutamine oxidation and glycolysis 3-HB oxidation is activated indicating a high potential for intestinal oxidation of ketones.

SUBSTRATE	GLUTAMINE	GLUCOSE		3-HB
Product (nmol/mg/hr)	CO_2	CO_2	Lactate	CO_2
Control	0.79±0.04	1.46±0.14	59.2±6.7	1.35±0.15
+Amino-oxyacetate 10mM	0.14±0.03	0.87±0.14	11.6±1.5	4.60±0.30

values = means ± S.E.(no.=4) All oxidation rates in which amino-oxyacetate is added are different than control ($p<0.01$).
We conclude that if glucose and glutamine oxidation is suppressed, ketone oxidation can be a major source of energy in rat jejunum.

†1222 ALTERED CEREBRAL SUBSTRATE UTILIZATION BY THE HYPOGLYCEMIC DOG. R Kliegman, S Hulman, S Morton. Case Western Reserve Univ, Rainbow Babies & Childrens Hosp Dept of Pediatrics, Cleve, Oh.

Insulin infusion to 3 hr old term dogs (n=6,control=6) lowered blood glucose (1.31±0.17 vs 8.12±0.29 mM $p<0.001$) and lactate (1.05±0.13 vs 1.72±0.22 $p<0.01$), without altering clinical behavior. Freeze clamped cerebral cortex (<3sec) revealed reduced glycogen content (1.35±0.25 vs 1.98±0.20 umol/g $p<0.05$) and glucose 6-phosphate levels (0.068±0.013 vs 0.115±0.021 $p<0.02$) following hypoglycemia. Cerebral phosphoenolpyruvate and pyruvate levels were augmented during hypoglycemia while cerebral cortical lactate was diminished (0.774±0.054 vs 1.40±0.15 $p<0.01$). Furthermore, citrate (0.210±0.004 vs 0.353±0.009 $p<0.001$) and malate levels (0.103±0.016 vs 0.197±0.022 $p<0.01$) were diminished during hypoglycemia while alpha ketoglutarate was not altered. Cerebral amino acids, aspartate and glutamate were unchanged. However, alanine (0.477±0.041 vs 0.680±0.080 $p<0.05$) and glutamine (6.24 ±0.40 vs 7.68±0.06 $p<0.01$) were reduced during hypoglycemia. In addition, during hypoglycemia cerebral ammonia levels were elevated (1.12±0.24 vs 0.58±0.02 $p<0.02$). Nonetheless, cerebral ATP levels were not affected. These data suggest that neonatal cerebral energy status was not altered by diminished circulating levels of oxidizable cerebral substrates, glucose and lactate. The small pool of cerebral glycogen may be mobilized to contribute to energy production. Nonetheless, the much larger pool of cerebral amino acids via transamination and deamination reactions may become a more important source for cerebral energy production during hypoglycemia than during euglycemia.

●1223 HIGH EXTRACELLULAR PHOSPHATE (Pi) IN VITRO REVERSES THE INHIBITION OF $1,25(OH)_2D_3$ (1,25D) SYNTHESIS IN CHRONIC METABOLIC ACIDOSIS (CMA). C.B. Langman, M.J. Favus, F.L. Coe (Spon. by E.S. Moore). Michael Reese Hosp., Univ. of Chicago., Chicago, Ill.

Low calcium diet (LCD) and low Pi, in vitro, stimulate 1,25D synthesis by proximal tubules (PT). CMA inhibits the rise in serum 1,25D during LCD, but the effect on synthesis is unknown. To investigate in vitro synthesis of 1,25D during CMA, Sherman rats were fed LCD+1.5% NH_4Cl for 10d. to produce CMA (pH=7.34±.03, LCD vs. 7.23±.03, LCD+NH_4Cl, $p<.05$). PT in suspension were incubated for 20 min. in Krebs-Henseleit bicarbonate (KHB) media, pH 7.4, in KHB low in Pi (0.2mM Pi, KHB-Pi), high in Pi (4.6mM Pi, KHB+Pi), low in Ca (0.2mM Ca, KHB-Ca), or low in both (KHB-CaPi), and then for 5 min. at 37°C with 16.6uM $25OHD_3$ to measure 1,25D synthesis. PT from rats fed LCD produced 10±1 pmol 1,25D/mg protein in KHB; 20±2 in KHB-Pi and 21±3 in KHB-CaPi ($p<.005$ vs. KHB for each); 8±1 in KHB+Pi and 11±1 in KHB-Ca (p=NS vs. KHB for each). PT from rats fed LCD+NH_4Cl produced 5±1 pmol 1,25D/mg protein in KHB ($p<.001$ vs. LCD, KHB);8±1 in KHB+Pi($p<.05$ vs. KHB);4±2 in KHB-Pi, 4±2 in KHB-Ca, and 4±1 in KHB-CaPi (p=NS vs.KHB). Pt cAMP from rats fed LCD+1.5% NH_4Cl were similar (p=NS), and were not altered by media Ca or Pi. Conclusions: normalization of pH in vitro did not restore diminished 1,25D synthesis in Pt from rats with CMA. CMA blocks cAMP dependent 1,25D synthesis distal to cAMP generation, and also blocks cAMP independent (low Pi) 1,25D synthesis. High medium Pi, in vitro, reverses the inhibition of cAMP dependent 1,25D synthesis in CMA. CMA may be a state of critical PT Pi depletion.

△1224 VIRAL MEDIATED GENE TRANSFER OF RECOMBINANT HUMAN PHENYLALANINE HYDROXYLASE (PAH) USING RETROVIRUSES. Fred D. Ledley, Hernan E. Grenett, Savio L.C. Woo, Howard Hughes Medical Institute, Department of Cell Biology Baylor College of Medicine, Houston TX (Spons. Arthur L. Beaudet)

Phenylketonuria (PKU) is caused by deficiency of the enzyme PAH. Our laboratory has cloned the full length human PAH cDNA in order to study the molecular basis of PKU and to explore alternative therapies for PKU utilizing recombinant DNA techniques. We have previously reported gene transfer experiments in which the full length human PAH cDNA was recombined with the eukaryotic promotor from human metallathioneine and transfected into mouse NIH3T3 cells which do not normally express PAH. Cells transformed with this recombinant expressed human PAH that was indistinguishable from normal. In the present study the human PAH cDNA has been recombined with a retroviral vector (pzipneo) to produce a defective retrovirus which carries human PAH in place of the viral genome. This recombinant, containing human PAH in a retroviral vector, was transfected into the Ψ2 cell line which constituatively produces the proteins required for viral assembly. Cells transformed with the PAH recombinant produce a defective retrovirus, vZPAH(+), which can be used to infect cells with human PAH but is incapable of sustaining subsequent viral propagation. Tissue culture cells infected with vZPAH(+) exhibit PAH activity and immunoreactive protein characteristic of human PAH. Defective retroviruses containing human PAH provide a highly efficient means for genetic transfer of PAH into a wide range of rodent cells and enables experiments in which human PAH can be transfered into animals as a model for genetic therapy of PKU.