

1003 DOES HEME OXYGENASE ACTIVITY DEPEND ON THE VINYL SUBSTITUENTS OF HEME? Rolf R. Engel, James S. Levine, Cheryl Ellis, Michael D. Levitt, U of M Medical School, Hennepin County Medical Center and Minneapolis VA Hospital, Departments of Pediatrics and Medicine, Minneapolis, MN

Since cytochrome c and myoglobin are heme proteins, we tested the assumption that their catabolism contributes to endogenous CO production (Vco). Adult mice were injected with NaHCO₃ solution or this solution containing equimolar amounts of myoglobin, cytochrome c, freshly dissolved hematin or hematin stored for 24 and 48 hrs. After the injection, each mouse was placed in a glass chamber from which gas was sampled at 4 and 23 hrs. Within 4 hrs of injecting myoglobin or freshly prepared hematin, the Vco was 2 to 4 fold greater than for control mice. Tin protoporphyrin prevented most of this rise in Vco suggesting that heme oxygenase mediated the reaction. In contrast, Vco 4 hrs after injecting either cytochrome c or stored hematin was indistinguishable from control mice. However between 4 to 23 hrs Vco increased and was comparable to myoglobin or fresh hematin treated animals. The cytochrome c heme differs from myoglobin or hemoglobin only in that the 2 vinyl groups are covalently linked to cysteine. Storage of hematin also alters these vinyl groups (NEJM 315:235, 1986). While these in vivo studies suggest that heme cleavage at the α -methene bridge requires 2 vinyl groups on the adjacent pyrrole rings, in vitro studies (J Biol Chem 257:9944, 1982) suggest alternative explanations. The delayed increase in Vco from cytochrome c and aged hematin suggests either slow conversion to compounds that are cleaved by heme oxygenase or excretion and CO formation by enteric bacteria,

1004 REDUCING LITTER SIZE BY ABLATION ENHANCES GROWTH AND INSULIN SECRETION IN THE FETAL RAT. Sandra L. Finley, Edward S. Ogata, Northwestern Univ. Med. School, Depts. of Peds., Ob/Gyn, Chicago, IL.

Fetal growth is inversely related to litter size. Little is known about the mechanisms responsible for this or its potential consequences in the newborn. To address this, we developed a fetal ablation model in the rat. After anesthetizing the mother on day 15 (term 21.5 days), we ligated selected arteries in the secondary cascade of uterine vessels. This ablates individual fetuses. Ablation (A) reduced fetal number compared to controls (C) (5+2 v 8+3) and accelerated fetal growth (Birthweight 5.34±.05 v 5.01±.09 g, p<.01). A pups had increased liver mass .289±.005 v .254±.005 g, p<.001) but similar liver/body ratios compared to C suggesting symmetrical growth enhancement. To some extent accelerated growth was mediated by insulin, a critical growth stimulating hormone, since plasma insulin conc were significantly elevated in A v C newborns (112.2±14.2 v 67.0±10.1 uU/ml, p<.001). A pups developed hypoglycemia at 0 and 20 minutes of life (20 min 78.2±2.6 v 91.0±4.8 mg/dl, p<.01). This resulted from a carryover of fetal hyperinsulinism (20 min 211.2±16.0 v 52.5±19.0 uU/ml, p<.01) since plasma glucagon conc did not differ (20 min 736.7±80.0 v 655.0±99.0 pg/ml) and hepatic glycogen conc remained elevated in A pups (20 min 71.4±3.2 v 52.08±6.4 mg/g liver, p<.01). Restricting litter size may enhance blood flow to the remaining fetuses thereby enhancing metabolic fuel supply, fetal growth, and hepatic glycogen deposition. Increased insulin availability may mediate these changes and also cause neonatal hypoglycemia.

1005 EXOGENOUS INSULIN ACCELERATES FETAL GROWTH AND CAUSES NEONATAL HYPOLYCEMIA IN THE RAT. Sandra L. Finley, Edward S. Ogata, Northwestern Univ. Med. School, Depts. of Peds., Ob/Gyn, Chicago.

With maternal diabetes in pregnancy, the fetus receives a surfeit of metabolic fuels and as a consequence increases insulin secretion. Insulin stimulates the growth of specific tissues and the combination of increased fuels and insulin causes macrosomia. To assess the effect of hyperinsulinism alone upon the fetus, we injected fetal rats *in situ* with insulin (I) or saline (S) on day 18 (term 21.5 days). One day following injection, I and S fetuses were of similar weight. Fetal/maternal glucose ratios did not differ between I and S fetuses (.63-.75); however I fetuses had elevated plasma insulin (543.3±74.9 v 139.0±27.0 uU/ml, p .01) and diminished glucagon conc (151.8±24.9 v 267.7±35.0 pg/ml). By day 20, I>S fetuses (4.58±.09 v 3.16±.07g, p<.001). Insulin (165.8±19.6 v 181.2±24 uU/ml) and glucagon were equivalent between I and S fetuses. Newborn I pups weighed 5.21±.05 and S, 4.93±.09g (p<.001). While initially normoglycemic (0-60 min), I pups developed hypoglycemia at 120 and 240 min of life. This resulted from a sustained insulin effect since while S newborns decreased plasma insulin, I pups had relatively elevated values at 60, 120, and 240 min (60 min I 46.8±3.8 v 22.7±3.7 uU/ml, p<.01) and plasma glucagon did not differ (60 min 429.6±50.2 v 359.3±32 pg/ml). For these reasons, hepatic glycogen conc in I pups remained elevated (20 min 54.9±6.0 v 36.4±6.25 mg/g liver, p<.01). Insulin can stimulate fetal growth without the provision of extra fuels. Fetal hyperinsulinism is associated with delay in the normal decrease in insulin during the neonatal period and causes hypoglycemia.

1006 EFFECT OF CYSTINE DIMETHYLESTER (CDM) ON RENAL TUBULAR FUNCTION, A MODEL OF FANCONI SYNDROME. John W. Foreman, Judithann Lee, Margaret Ann Bowring, Stanton Segal Univ. of Penn. School of Medicine, The Children's Hospital of Philadelphia, Dept. of Ped., Phil.

Fanconi syndrome is an enigmatic disorder globally affecting proximal tubule solute handling. Cystinosis is a common cause of this syndrome and is characterized biochemically by increased intracellular cystine. Taking advantage of the ability of CDM to load normal lysosomes with cystine, we examined the effect of this compound on rat renal tubular transport in vivo and in vitro. Normal adult rats were injected with 400 μ moles BID of CDM for 4 days and then urine was collected overnight. There was no difference between controls injected with saline and the treated rats in plasma creatinine, glucose, phosphate, and amino acids except for cystine. Urine volume increased by 133%, as did the excretion of phosphate (42%), glucose (35%) although the actual amount lost was quite low, and α -amino nitrogen (17%) with a generalized aminoaciduria. Renal tissue levels of cystine were not increased but cysteine levels were.

The effect of CDM on isolated rat renal tubule transport was studied by incubating tubules with 2mM CDM for 10 min. removing them from the CDM buffer, and resuspending them in buffer with labeled substrates. CDM markedly inhibited the initial and subsequent uptake of 2mM α -methyl glucoside, 0.1 mM glycine, 0.1 mM taurine, and 0.025 mM lysine when compared to control tubules handled in a similar fashion without CDM. Both treated and control tubules appeared viable as measured by trypan blue exclusion. Tissue cystine was markedly increased with CDM. These studies indicate that CDM can induce a new model of Fanconi syndrome both in vivo and in vitro.

1007 SULFUR AND METHYL BALANCES IN A MAN WITH HEPATIC METHIONINE ADENOSYLTRANSFERASE (MAT) DEFICIENCY W.A. Gahl, I. Bernardini, J.D. Finkelstein, A. Tangerman, J.J. Martin, H.J. Blom, K.D. Mullen, H.S. Mudd, NICHD, NIDDKD, NIMH, Bethesda, MD; VA Med. Center, Wash., DC; St. Radboud U. Hosp., Netherlands.

MAT converts ATP and methionine (met) to S-adenosylmethionine (SAM), a methyl donor and intermediate in transsulfuration to homocysteine (HS). HS is catabolized to inorganic sulfate for excretion, or is remethylated using betaine or N⁵-methyltetrahydrofolate (formed by methylenetetrahydrofolate reductase, MTHFR). We studied sulfur and methyl balances in a man with severe but incomplete hepatic MAT def. This clinically normal individual lives with 20-30X normal plasma met (0.72 mM) and excretes 2.7 mmol/day of met and L-met-d-sulfoxide. His hepatic SAM is low, 18 nmol/gm wet wt. Steady state balances revealed that he converts only 65% of ingested sulfur to inorganic sulfate (nl, 80.1±5.3%). Even with a large met load, he converted only 6-7 mmol/day of met to inorganic sulfate, yet he produced at least 18 mmol/day of SAM and HS. Of the 18 mmol, 9.5 was remethylated, and 6.6 mmol of the methyl groups were contributed by N⁵-methyltetrahydrofolate. This methylenogenesis appeared very high considering his enormous met load. These findings support the view that SAM regulates the distribution of HS between remethylation and transsulfuration. In the patient, a low steady state SAM may fail to inhibit MTHFR or activate cystathionine synthase; the consequence would be enhanced HS methylation, as observed. Measurement of α -keto- γ -methylthiobutyrate production in this patient also placed an upper limit on the capacity of met transamination to catabolize met in the presence of an elevated body load of met.

1008 SERUM GLYCOSYLATED PROTEINS (GP): PREDICTORS OF MATERNAL-FETAL HYPERGLYCEMIA AND FETAL B.W.T.? Geeti Chosh, R.S. Pildes, Samuel Richton, Cook County Hosp., Div. of Neonatology, Univ. of Illinois, Chicago.

The usefulness of serum GP in detection of subtle hyperglycemia was studied in 3 groups of mother/infant pairs: 20 normal/20 AGA(GrI): 20 normal/20 MACRO, > 4.0kg (GrII) and 9 diabetic mothers/10 neonates (GrIII). Two diabetics were class A, 4 class B and 3 class C. Maternal serum HbA_{1c} and GP and cord GP were measured at delivery. Maternal age, gravidity, parity and gest. age were similar. GrII mothers gained more wt (p<.01) than GrI (mean±SD, 41±13 vs 28±7 lbs resp.) but not sig. diff. than GrIII mothers (33±12 lbs). B.Wt. and B.Wt.ratios were greater (p<.01) in GrII (4.4±.3kg; 1.39±.1 resp.) than in GrI (2.9±.3kg; 0.9±.08) or GrIII(3.3±.9kg; 1.08±.25) neonates. HbA_{1c} and GP were:

	I	II	III
Maternal HbA _{1c}	6.74±0.78	6.88±1.13	7.46±1.58
Maternal GP	9.75±2.49	10.05±1.99	13.77±2.31*
Cord GP	8.60±1.69	9.10±1.29	12.30±1.86*

III vs II or I GP values were above the 95th Zile in 44% of moms and 80% of newborns in GrIII as compared to 5% in GrII or GrI patients (p<.01). Maternal GP correlated sig. (p<.001) with cord GP in GrI (r=.87) and GrII (r=.77) but not in GrIII pairs. B.Wt., B.Wt.ratio and plasma glucose at 1hr correlated poorly with HbA_{1c} and maternal or cord GP; however, B.Wt. and B.Wt.ratio correlated sig. (p<.01) with maternal wt. gain. In summary, 1) GP are more useful than HbA_{1c} in detecting short term hyperglycemia in diabetic mothers and infants at delivery; 2) glucose intolerance in macrosomic newborns and their mothers may be too subtle for detection by serum GP; 3) B.Wt. can be sig. correlated with maternal wt. gain but not with serum GP. **p<.001