●1021 DIHYDROPTERIDINE REDUCTASE DEFICIENCY: CLONING OF THE DIHYDROPTERIDINE REDUCTASE GENE, ANALYSIS OF MUTANT CELLS, AND PROSPECTS FOR GENETIC THERAPY. Fred D. Ledley, Jean Lockyer, Seymour Kaufman, Sheldon Milstein, Savio L.C. Woo. (Spon. by Arthur Beaudet) Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, TX, and Laboratory of Neurochemistry, National Institutes of Health, Bethesda MD.

Deficiency of the enzyme dihydropteridine reductase (DHPR) causes a syndrome of hyperphenylalaninemia and mental retardation but is refactory to conventional dietary therapy. An antibody against sheep DHPR was used to identify clones for human DHPR from a human liver cDNA expression library. The full length cDNA clone comprises 1560 bases, codes for a protein of 244 amino acids and 25,774 daltons, and the predicted amino acid sequence matches an incomplete amino acid sequence of sheep DHPR. The cDNA was recombined in an eukaryotic expression vector and introduced into cultured cells by DNA mediated gene transfer. Cells transformed with the recombinant gene expressed DHPR protein and enzymatic activity at levels 50% of human liver. Analysis of fibroblasts from five unrelated individuals genetically deficient in DHPR by southern and northern blotting indicates that the DHPR gene is grossly in tact and that DHPR mRNA is transcribed. These results indicated that the mutations causing DHPR deficiency are not large deletions of the DHPR locus. These experiments also demonstrate the feasibility of reconstituting DHPR activity by gene transfer of the recombinant clone and introduces the possibility of exploring somatic gene replacement therapy of DHPR deficiency.

1022 FOLATE DEFICIENCY INDUCED HOMOCYSTEINEMIA. J.Y. LIN, S.S. Kang, J. Zhou and P.W.K Wong. Rush Medical College, Presbyterian-St. Luke's Medical Center. Department of Pediatrics, Section of Genetics, Chicago, Illinois.

In humans on a normal diet, about 50% of homocysteine is remethylated to methionine with methyltetrahydrofolate as the methyl donor. Hence, folate deficiency may result in inadequate synthesis of the methyl donor, leading to homocysteine accumulation. Moderate homocysteinemia has been associated with atherosclerotic vascular disorders in humans but other contributory factors have not been excluded.

To study the effect of isolated folate deficiency, 2 groups of 45 rats were given (1) adequate folate or (2) folate deficient chemically defined diets. Five rats in each group were killed biweekly for serum chemical analysis. B12 and pyridoxine remained normal in both groups throughout the experiments.

	Gr(1) Folate	Homocyst.	Gr(2) Folate	Homocyst.
Weeks	ng/ml	nmol/ml	ng/ml	nmol/ml
4	> 20	4.2±0.7	2.9±1.5	6.4±1.2
10	> 20	3.9±0.9	2.5±0.7	7.9±1.5
12	> 20	4.4±0.8	1.4±0.1	7.4±1.3
20	> 20	3.4±0.7	1.3±0.1	8.3±1.2

The differences in folate and total homocysteine between the 2 groups were statistically significant ($P < 0.001$). Serum folate in rats from 4-10 weeks and from 12-20 weeks was similar respectively to low normal and subnormal level in humans observed to have moderate homocysteinemia. This raises questions regarding normal serum folate values and folate requirement in humans.

1023 MIGRAINE AS A CONSEQUENCE OF EUGLYCEMIA DIABETIC CONTROL: C. Charlton Mabry, Univ. KY. Col. Medicine, Dept. of Pediatrics, Lexington, KY.

Euglycemia in insulin dependent diabetes mellitus (IDDM) is possible with intensive insulin treatment. Though control may be improved, the risks of hypoglycemia are increased. We have encountered a patient with IDDM who attempted intensive insulin treatment only to experience debilitating migraine attacks in association with insulin induced hypoglycemia.

Seven year-old boy developed IDDM and managed by conventional insulin therapy (2 injections mixed insulin/day, urine glucose monitoring) for two years. On occasion he experienced early AM headaches with vomiting in association with hypoglycemia. Neurologic evaluations indicated that he was experiencing migraine attacks. At the same time he was enrolled in an adult diabetic clinic which had the goal of maintaining his blood glucose between 100-160 mg/dl. Frequent severe usually early AM headaches with prolonged vomiting triggered by hypoglycemia led to metabolic derangement and many hospitalizations. over the next 18 months. Maintenance propranolol started for migraine prevention; intensity and frequency of hypoglycemia and migraine attacks increased. Conventional diabetic management resumed with lessening of frequency and severity of hypoglycemia and migraine episodes. Then propranolol discontinued, and no symptomatic hypoglycemia, migraine or hospitalization for past year.

Counter regulatory mechanisms to prevent insulin induced hypoglycemia are usually intact in the young diabetic, and migraine is an unexpected complication of hypoglycemia, particularly in a child. This boy showed symptoms of hypoglycemia. that had a unique and repetitive expression. He also showed that children may not tolerate an adult oriented intensive insulin management plan.

1024 DISAPPEARANCE OF ISLET CELL ANTIBODIES (ICA) MAY BE ASSOCIATED WITH EITHER DETERIORATION OR AMELIORATION OF ISLET CELL FUNCTION. Duncan R. MacMillan, Michael B. Foster and Mary P. Key (Spon. by B.F. Andrews). University of Louisville School of Medicine, University of Louisville Affiliated Hospitals, Department of Pediatrics, Louisville, Kentucky.

Disappearance of serum ICA following diagnosis of diabetes is well documented and may be related to a decline in available antigen. Reversion of ICA positive to negative is otherwise rare. We report two exceptional cases: Case 1 whose reversion from ICA positive to negative was followed in 29 months by type 1 diabetes and Case 2 who presented with insulinopenic ICA positive diabetes mellitus with progressive decline in insulin requirements, loss of ICA and return to normal glucose tolerance over 33 months. Case 1 suggests that ICA may be a transient marker of a persisting underlying immune process leading to complete islet destruction.

Case #	Age (yr)	Sex	Race	Wt %ile	INITIAL STUDIES Glucose (mg/dl)	Insulin (uu/ml)	A_1 Hgb %	ICA	SUBSEQUENT STUDIES (2-3 yr) Glucose (mg/dl) GTT Max	Insulin (uu/ml)	A_1 Hgb %	ICA
1	3	F	W	50	--	--	6.7	+	500	10.9	10.7	Neg
2	12	M	B	95	474	32	10.1	+	137	109	6.5	Neg

Case 2 suggests that ICA does not necessarily indicate inevitable progression to total islet cell destruction and that cessation of immune islet cell destruction may occur in association with disappearance of ICA, allowing for islet cell regeneration to occur.

▲1025 HYPERINSULINEMIA IN TRANSGENIC MICE CARRYING MULTIPLE COPIES OF THE HUMAN INSULIN GENE: EVIDENCE FOR A GENE DOSAGE EFFECT. S. Lee Marban and John D. Gearhart. (Spon. by M. Douglas Jones, Jr.) The Johns Hopkins Hospital, Depts. of Pediatrics and Physiology. Baltimore, MD 21205

We are investigating insulin gene expression in transgenic mice carrying multiple copies of the human preproinsulin gene. An 8.8 kb EcoRI-Hind III human genomic DNA fragment containing the insulin gene was introduced into 1-cell mouse embryos by pronuclear microinjection. This DNA fragment includes 2kb of 5' and 5.5kb of 3' human flanking sequences. Two lines of insulin transgenic mice have been established that differ in copy number of the human gene: one line has 4 copies integrated per cell, the other 16 copies per cell. Total serum insulin levels were determined by RIA (Cambridge Medical). Fasted insulin levels in transgenic mice (mean ± SEM = 0.48 ± 0.08 ng/ml, n=37) were significantly higher than in controls (0.10 ± 0.01 ng/ml, n=10; $p < 0.001$). Comparing the 2 transgenic lines, there was considerable variation in insulin levels but those animals with more copies of the human gene have higher insulin levels (0.74 ± 0.20 ng/ml, n=11) than those with fewer copies (0.37 ± 0.06 ng/ml, n=26). Both lines have significantly higher insulin levels than control ($p < 0.01$). This is in contrast with previously reported insulin transgenics (Bucchini et al, PNAS 83:2511) in which insulin was not overexpressed. In this model system, insulin gene expression correlates with gene copy number, suggesting a direct gene dosage effect.

●1026 ASPARTAME INTAKE AND ITS EFFECT ON PHENYLALANINE (PHE) AND PHE METABOLITES. Reuben Matalon, Kimberlee Michals, Debra Sullivan and Paul Levy. University of Illinois at Chicago, Departments of Pediatrics, Nutrition and Medical Dietetics and Epidemiology and Biometry, Chicago, Illinois.

Aspartame a methyl ester L-aspartylphenylalanine, is a widely used sweetener. The intake of aspartame was studied in 51 adults 23 of whom were carriers for phenylketonuria (PKU). The study was divided into a loading test followed by chronic intake for a period of 12 weeks. Two doses of aspartame were used in the loading test 50mg/kg and 100mg/kg. Blood levels of phe were measured hourly and the aromatic acid metabolites of phe and phenylethylamine (PEA) were estimated in the urine. During the chronic intake blood phe and urine metabolites were determined every two weeks. The levels of blood phe rose from 5-10 fold in the carriers for PKU on 100mg/kg load as opposed to 2.5 to 6 fold in the normal controls. On 50mg/kg the carriers response was increased from 2 to 7 times the normal levels and from 2 to 3.5 in the control group ($P < .02$). During the chronic intake 19 carriers had blood phe ranges of 0.84 to 18.8mg/dl; five of these individuals had levels above 10mg/dl. Among the controls(16) the range of blood phe was 0.8 to 11.1mg/dl. The aromatic acid metabolites 3 and 4 OH phenylacetate and 4 OH phenyllactate were increased more than 10 fold in the carrier group, and were less elevated in the controls but still significantly higher than baseline levels. The urinary levels of PEA were increased in both groups ranging from 5-40 times the normal levels. These data are of concern since blood phe levels are much higher than anticipated from previous published reports.