not increased, a specific thiamine-dependent defect in pyruvate decarboxylase was considered. Assay of this enzyme in fresh peripheral leucocytes, or after phytohemagglutination stimulation, at low and high concentrations of pyruvate, with and without thiamine added, revealed no abnormality. Negative results were also obtained with cultured skin fibroblasts. A phenotypic variant of the traits described by BLASS and TADA, but limited to liver in this case, is proposed. (Research supported by Grants MRC 1085 and NHW 604-7-643.)

65 *Low Phosphorylase Activity: A Symptom but not a Diagnosis.* GEORGE HUG, WILLIAM K. SCHUBERT and GAIL CHUCK, Children's Hosp. Res. Fndn. Cincinnati, Ohio.

Since our initial description of deficient activity of hepatic dephosphorylase kinase and increased liver glycogen [Science *153:* 1535, 1966], we have studied a total of 7 patients with this condition. Five of these patients have been reported [J. clin. Invest. *48:* 705, 1969]. They and the 2 additional patients, brothers age 4 and 1 ½ years, always had greatly reduced liver phosphorylase activity (about 10% of control) that could be increased to normal by addition of kinase. This rules out phosphorylase deficiency, or type VI glycogenosis. On the basis of 'one enzymatic defect for one type of glycogenosis', we called deficient kinase activity type IX glycogen storage disease. In type IX glycogenosis there is no hyperlipemia, hypoglycemia, acetonuria, or evidence of X-chromosomal inheritance. There is a typical light and electron microscopic appearance of the liver. Biochemically, the skeletal muscle is normal as is the blood sugar response to glucagon. In contrast, in a girl with hepatomegaly and low hepatic phosphorylase activity there is increased glycogen concentration in the skeletal muscle as well as the liver and the glucagon tolerance curve is flat. In her skeletal muscle the amount of *total* phosphorylase is normal but all of it is in the *inactive* form. Phosphorylase kinase is present in this muscle but we found evidence for deficient activity of 3′5′-AMP dependent kinase that normally activates phosphorylase kinase [J. biol. Chem. *243:* 3763, 1968]. Therefore, we have named this new type of glycogen storage disease with a hitherto undescribed enzymatic deficiency type X glycogenosis. (Supported by NIH grants No. AM 13903 and No. RR 00123.)

66 *Human Liver Galactose-1-phosphate Uridyl Transferase: Activity in the Negro Galactosemic.* STANTON SEGAL and SHIRLEY ROGERS, Children's Hosp. of Philadelphia, Pa.

Since the Negro with congenital galactosemia can metabolize substantial amounts of galactose, assays for the deficient enzyme, gal-1-p uridyl transferase, were performed in homogenates of punch biopsies of liver from two Negro patients. Kinetic characteristics of the normal enzyme were determined in homogenates of liver from four non-galactosemic patients undergoing abdominal surgery and in two 50-fold purified preparations of liver obtained within 24 h of death. Kinetic parameters including concentration dependence and Km were similar in the crude homogenates and partially purified normal enzyme. The specific activity of transferase in mμmoles/min/mg protein was 12 and 1.6 in crude homogenates of the normals and galactosemics, respectively. The latter activity was linear with incubation time but showed no concentration dependence on uridine diphosphoglucose. No uridine diphosphogalactose pyrophosphorylase activity, a possible alternate pathway, was detected in the liver of Negro patients and transferase activity with thymidine diphosphoglucose as substrate was negligible. Either these patients possess a non-nucleotide pathway of galactose metabolism or the residual transferase activity accounts for their galactose metabolic capability. Results of *in vivo* metabolic studies with labeled galactose suggests the latter is most likely.

67 *Idiopathic Hypomagnesemia and Osteochondritis.* WILLIAM G. KLINGBERG, Dept. of Ped., West Virginia Univ. Sch. of Med., Morgantown, W.VA.

A previously asymptomatic 5-year-old boy presented with classic carpopedal spasm of 6 h duration. On examination he also had papilledema, decreased DTR's and a negative Chovstek. Serum Mg was 0.8 and K was 2.8 mEq/L. All other serum electrolytes, proteins, enzymes, Ca, PO_4, Glucose, BUN and Creatinine were normal. The EMG and PEG were normal. The EEG showed a few non-specific low waves and mild disorganization. Unexpectedly x-rays of bones showed a mild osteochondritis of shoulders, knees and hips (Legg-Perthes-like). Creatinine clearance was 70 ml/min/1.73 M^2. Gross renal tubular function was normal by concentration and acidification.

A 6-day balance study showed minimal negative Mg balance. When 20 mEq magnesium acetate (MgA) was added *per os* daily, another 6 day balance study showed a positive balance, but barely so (intake 174 mEq, output 132 mEq = +42 mEq). Little is known regarding Mg balance and metabolism in children, but adults with hypomagnesemia given MgAc would excrete very little in the urine. This child continued to excrete 5–9 mEq/day during the balance studies. Potassium was also in negative balance although not enough to be significant. Other ions were in normal balance.

When MgAc orally was increased to 40 mEq/day serum Mg rose to normal. After 6 months therapy the bony lesions have reverted almost to normal. MgAc therapy is still required as hypomagnesemic tetany again occurred when inadequate therapy was given. Is this a specific, partial renal tubular defect in Mg absorption.

68 *Evaluation of Adenine Therapy for Lesch-Nyhan Syndrome.* JOSEPH D. SCHULMAN, MARTIN L. GREENE, WILFRED Y. FUJIMOTO and J. EDWIN SEEGMILLER, NIH, Univ. of Washington, Seattle, and Univ. of California, San Diego (introduced by W. L. Nyhan).

The use of oral adenine to prevent the devastating neurologic consequences of the Lesch-Nyhan syndrome has a sound theoretical basis, and has been proposed for management of this aspect of the disease. We have attempted therapy of two patients with adenine begun in one patient, during the first month of life. Preliminary experiments in rats explored the toxicity of adenine; pretreatment with allopurinol (10 mg/kg) reduced the nephrotoxic effects of the insoluble adenine metabolite, 2,8-dioxyadenine, at adenine doses of 70 mg/kg. Two patients, a 13-year-old male with established neurologic disease and self-mutilation, and a normal-appearing one-month-old boy with documented hypoxanthine-guanine phosphoribosyltransferase (PRT) deficiency, were given adenine in doses up to 65 mg/kg/day. Toxicity was monitored by daily esti-

mations of dioxyadenine content of urinary sediment and close attention to parameters of renal function; in both patients toxicity necessitated reduction in dose or addition of allopurinol. Erythrocyte 5-phosphoribosyl-1-pyrophosphate which was normally elevated (40–70 mμmol/ml) as a consequence of PRT deficiency in these patients, was reduced to 5–15 mμmol/ml during adenine treatment, but not to within the normal range (1–5 mμmol/ml). The high uric acid excretion in both was unchanged. Despite treatment of the younger child for seven months, he developed spasticity, motor retardation and early self-mutilation. The older child also showed no improvement in neurologic dysfunction. We conclude that adenine is of no therapeutic benefit, and is potentially toxic, in treatment of patients with the Lesch-Nyhan Syndrome.

69 *Aspects of Lipid Synthesis Unique to Brown Fat.* Robert E. Greenberg and Charlotte Sumida, Dept. of Ped., Stanford Univ. Sch. of Med., Palo Alto

Chemical thermogenesis, necessary for extra-uterine adaptation, is partly mediated through oxidation of fatty acids in brown fat, requiring a rapid rate of triglyceride turnover. Previous studies from our laboratory indicated that brown fat contains glycerokinase, making it possible for brown fat to reutilize glycerol. Triglyceride synthesis in brown fat, thus, may not be completely dependent on glucose transport. The role of glycerol in triglyceride synthesis in brown fat has been further studied, using both *in vitro* and *in vivo* techniques.

Explants of brown fat were incubated for periods up to 3 days, with either glucose or glycerol as substrate. Insulin was added at varying times, as were tracer quantities of acetate C^{14}, glucose C^{14} or glycerol C^{14}. Glycerol promoted a greater incorporation of acetate C^{14} into mono-, di- and triglycerides of brown fat than did glucose. Incorporation of glycerol C^{14} and glucose C^{14} into lipids of brown fat were both increased by insulin. IP injection of glycerol-2-H^3 into newborn rats was followed by much greater incorporation into lipids of brown as compared to white fat. Net triglyceride synthesis was not demonstrable *in vitro* regardless of substrate or insulin, suggesting marked enhancement of triglyceride turnover by insulin. Increased turnover rate is also indicated by greater conversion of acetate C^{14} to $C^{14}O_2$ in the presence of insulin.

These results suggest the following: (1) Glycerol can be utilized by brown fat as substrate for lipid synthesis; (2) transport of glycerol in brown fat is subject to regulation by insulin; and (3) the anti-lipolytic effect of insulin is not demonstrable in the *in vitro* system used in these studies.

70 *Neonatal Fat Metabolism: Developmental Aspects in Isolated Human Adipose Tissue Cells.* Milan Novak and Ellen F. Monkus, Univ. of Miami Sch. of Med., Dept. of Ped., Miami, FL (introduced by William W. Cleveland).

The *in vitro* metabolism of a suspension of adipose cells, prepared by collagenase disintegration of 5 to 20 mg samples of subcutaneous (white) adipose tissue, was studied in normal newborns 6 h to 6 days of age in comparison with normal adults. In order to relate results to cell number they were calculated in terms of DNA content. Glycerol release was elevated during the first day of life and then decreased below the level found in adults. Glycerol release was less activated by nor-epinephrine in the neonate. In the adult the free fatty acid (FFA) release was consistent with glycerol release (molar ratio of FFA/glycerol of about 3); in the neonate FFA release was much less which suggests that FFA was either being re-esterified or partially oxidized. Oxygen consumption was essentially the same in the adipocytes of neonates and adults as was its activation by nor-epinephrine.

Previous studies using intact adipose tissue fragments indicated an increased glycerol release and also an increased oxygen consumption in young neonates; comparisons were made on a basis of wet weight. The present study not only confirms the previous findings but also shows the increased glycerol release in young neonates to be a property of the individual adipose cell. On the other hand the increased oxygen consumption of neonatal adipose tissue is probably a function of the increased cellularity.

71 *Effect of Folic Acid on Amino Acid Metabolism in Pyridoxine Unresponsive Homocystinurics.* Grant Morrow III, Deborah Meltzer and Lewis A. Barness, Dept. of Ped. Hosp. Univ. of Pa., Univ. of Pa. Sch. of Med., Philadelphia, Pa.

Plasma folate levels were measured in 3 homocystinurics and found to be < 1 ng/ml (normal 5–10 ng/ml). All were on unrestricted, constant protein diets while 2 (A and B) were taking anticonvulsants for seizure control. Patient A had anemia (8.0 G% hemoglobin) and macrocytosis that responded to folic acid. B had macrocytosis but no anemia while C had neither. Plasma folate levels ranged from 14–20 ng/ml after therapy. Within 3 months of discontinuing folic acid in B and C their blood folates had fallen to 2.0 and 2.6 ng/ml. Plasma B_{12} levels and excretion of methylmalonate were normal.

Plasma and urine amino acids responded to folic acid as noted in the table:

	Plasma μM/ml		
	Homocystine	Methionine	Glycine
A	0.067 (0.037)*	0.027 (0.047)	0.235 (0.315)
B	0.206 (0.193)	0.090 (0.439)	0.292 (0.350)
C	0.118 (0.108)	0.176 (0.218)	0.260 (0.246)
	Urine μM/day		
	Homocystine	Methionine	Glycine
A	650 (350)	47 (74)	1298 (2740)
B	471 (658)	111 (314)	2030 (4180)
C	1420 (1410)	123 (144)	1470 (1690)

* Post-folate values in parenthesis.

Methylation of homocystine to methionine requires folic acid. Many homocystinurics may increase methylation to methionine thereby requiring more folate and increasing glycine as a result of demethylation of serine. Some homocystinurics may require long-term folic acid supplementation (A and B) whereas others are unresponsive either biochemically or hematologically (C). (Supported in part by USPHS grants AM-02231 and HD-04837.)

72 *Homocystinuria: Biosynthesis of Cystathionine and Homolanthionine.* Gerald Gaull, Yoshiro Wada, Karmela Schneidman, David Rassin, Harris Tallan and John Sturman, Dept. of Ped. Res., N.Y.S. Inst. Basic Res. Ment. Retard. and Depts. Ped. and Ophthal., Mt. Sinai Hosp. Med. Sch., N.Y.C. (introduced by Horace Hodes).