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 $\mu\text{mol/ml}$) as a consequence of PRT deficiency in these patients, was reduced to 5–15 $\mu\text{mol/ml}$ during adenine treatment, but not to within the normal range (1–5 $\mu\text{mol/ml}$). 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. BARNES, 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 $\mu\text{M/ml}$		
	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 $\mu\text{M/day}$		
	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 Hodges).

Cystathioninuria after administration of homoserine and cysteine has been confirmed in patients with homocystinuria; both precursors are necessary. Homolanthioninuria has been found to occur spontaneously in two of seven patients with homocystinuria and after homoserine loading in two more. A greater capacity for synthesis of cystathionine from homoserine and cysteine (reverse cystathionase) than for its cleavage (forward cystathionase) was demonstrated in rat liver, kidney and pancreas; rat brain showed very low activity in either direction. Homolanthionine synthesis from homoserine and homocysteine was less than 5% of cystathionine synthesis by reverse cystathionase in rat liver, kidney and pancreas; rat brain showed no activity. In liver extracts from two patients with homocystinuria, reverse cystathionase activity was less than cleavage and both were far less than in rat liver; homolanthionine synthesis was relatively greater. Homolanthionine synthesis activity in rat liver was separated from cystathionine synthase by ammonium sulfate fractionation; it was not separated from cystathionase by such fractionation or by chromatography on carboxymethyl-cellulose. *Thus:* (1) Although the administration of homoserine and cysteine results in cystathioninuria it is unlikely to promote formation of any considerable amount of cystathionine in the brain. (2) Whatever the excretion of homolanthionine may have to do with the pathogenesis of homocystinuria, it seems unlikely to have anything to do with the dementia. (3) Cystathionase, acting in the reverse direction is probably responsible for the synthesis of homolanthionine.

- 73 *Tissue Cystathionine in Mice Treated With Cysteine and Homoserine.* PAUL W.K. WONG and RAOUL FRESCO, Chicago Med. Sch. (introduced by David Hsia).

Deficiency of cystathionine may be a factor in the pathogenesis of the abnormalities in homocystinuria. Wong *et al.* [1968] showed that homocystinuric liver was able to synthesize cystathionine from homoserine and cysteine. Hence it may be possible to correct cystathionine deficiency in the brains of these patients.

(1) Homoserine, (2) cysteine, (3) homoserine + cysteine (7.5 $\mu\text{m/g}$) was injected intraperitoneally to mice. It was observed that brain tissue could concentrate these amino acids. At 5 h after injection, brain cystathionine ($\mu\text{m/g}$) was significantly higher in groups (1), (2) and (3) than in controls (4). (1) 0.042 ± 0.013 , (2) 0.069 ± 0.017 , (3) 0.143 ± 0.022 , (4) 0.023 ± 0.011 ($n = 6$).

Ten groups of 25 weaning mice were fed with Purina Chow (PC) or PC + 5%, 2% or 1% of either homoserine or cysteine or both. Mice on diets containing 5% cysteine had initial weight loss and subsequent slow weight gain. These changes were correlated with poor intake. Light microscopy of organs was normal in all groups. Electron microscopy showed 'adaptive' changes in the liver of mice on diets with 2 to 5% cysteine. At 6 months, brain cystathionine ($\mu\text{m/g}$) in mice on PC, PC + 5%, 2% and 1% of both cysteine and homoserine was 0.018 ± 0.005 , 0.112 ± 0.019 , 0.054 ± 0.007 and 0.040 ± 0.003 respectively. The higher brain cystathionine in the experimental groups is significant.

It appears that prolonged feeding of homoserine and cysteine in moderate amounts to homocystinurics is harmless and that cystathionine deficiency may be corrected. Clinical trials to assess the role of homoserine in the treatment of homocystinuria are now justified.

- 74 *Vitamin B₆ Responsive Homocystinuria.* MARGRETTA R. SEASHORE, JOSEPH L. DURANT and LEON E. ROSENBERG, Depts. of Med. and Ped., Yale Univ. Sch. of Med., New Haven, Conn.

In homocystinuria, inborn error due to deficiency of cystathionine synthase activity, methionine and homocystine accumulate in plasma and urine, and cystine disappears. Massive doses of vitamin B₆ (pyridoxine) have corrected these amino acid abnormalities in some homocystinuric patients but not in others. The present studies were undertaken to define the mechanism of this vitamin response. Two homocystinuric males, ages 16 and 18 years, were studied while on diets of known, constant methionine and cystine content. Their plasma and urinary methionine and homocystine concentrations fell to normal within 5 days of B₆ administration (500 mg/day) and, concurrently, cystine appeared. As little as 25 mg of B₆ daily resulted in correction of plasma amino acid abnormalities in one patient, but a larger dose was required in the second. Since B₆ is a cofactor for cystathionine synthase, these observations suggested enhanced synthase activity. Additional studies failed to support this thesis. Urinary sulfate excretion during methionine loading was not increased when the patients were receiving B₆. Furthermore, cystathionine synthase activity in cell-free extractions of cultured skin fibroblasts was absent and was unaffected by addition of pyridoxine or pyridoxal phosphate to the growth medium or the *in vitro* assay system, respectively. These results imply that B₆ responsiveness in homocystinuria involves activation of alternate pathways of sulfur amino acid metabolism rather than correction of the basic metabolic block. This mechanism is unique among the known vitamin-dependent inherited diseases and has important biochemical and therapeutic implications.

- 75 *S-Adenosyl-L-methionine in a Neuroblastoma.* GEORGE M. LYON, Jr. Duke Univ. Med. Center (introduced by F. Stanley Porter).

Soluble extracts of a neuroblastoma did not require additional methyl donor, S-adenosyl-L-methionine (SAM), for maximal catechol O-methyltransferase activity. Prior dialysis of the extract resulted in complete loss of enzymatic activity which could be partially recovered by addition of SAM to the reaction mixture. Tritiated normetanephrine was formed from DL-norepinephrine-7-³H in the presence and absence of added SAM. Using ion exchange and thin layer chromatography a compound was isolated from a deproteinized extract of the tumor which migrated and reacted as authentic SAM in three different chromatographic solvent systems. Examination of tissue from a metastatic tumor in the same patient, obtained following radiation and chemotherapy, revealed similar catechol O-methyltransferase specific activity which was strictly dependent upon added SAM. No SAM was detectable in deproteinized extracts of this tissue. Urine from the patient did not contain detectable quantities of cystathionine, SAM, or S-adenosyl-L-homocysteine at any time. The urine did contain homocysteine as well as a prominent ninhydrin-positive spot which has not yet been identified. Demonstrable SAM in a neuroblastoma from a patient without cystathioninuria suggests that although methionine metabolism may be active in the tumor the metabolites elaborated in the urine are not constant and presumably mirror variable enzymatic activities in the tumor. A difference