Cystathioninuria after administration of homoserine and cystein 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 μm/g) was injected intraperitoneally to mice. It was observed that brain tissue could concentrate these amino acids. At 5 h after injection, brain cystathionine (μm/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. Electronmicroscopy showed 'adaptive' changes in the liver of mice on diets with 2 to 5% cysteine. At 6 months, brain cystathionine (μm/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 B_6 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, in inborn error due to deficiency of cystathionine synthase activity, methionine and homocystine accumulate in plasma and urine, and cystine disappears. Massive doses of vitamin B_6 (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 B_6 administration (500 mg/day) and, concurrently, cystine appeared. As little as 25 mg of B_6 daily resulted in correction of plasma amino acid abnormalities in one patient, but a larger dose was required in the second. Since B_6 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 B_6. 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 B_6 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-^{3}H 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

can also be found between primary and metastatic tumor; an alteration possibly due to treatment.

76 *Apparent Saturation of Diphenylhydantoin Metabolism in Children.* LORNE K. GARRETTSON and OK KYUNG KIM, Dept. of Ped., State Univ. of N.Y. at Buffalo, Buffalo, NY (introduced by W.J. Rahill).

Many patients show an unexpectedly great increase in serum concentration of diphenylhydantoin (DPH) following moderate increases of dose. Published studies of DPH serum levels [LOESER, Neurol. *11:* 424, 1961 and REMMER *et al.*, D.M.W. *94:* 1265, 1969] show patterns of increase and decline which are not compatible with a metabolic rate that is a sum of first order processes.

In two girls, ages 10 and 11, who showed signs of DPH toxicity on oral dose of 10.3 and 5.5 mg/kg/d, serum and urine DPH concentration and urinary concentration of total hydroxylated metabolite of DPH (HPPH) were measured using published methods. In the first case, total daily HPPH excretion was 119.5, 195.6, 133.4 and 198.4 mg on days 1 through 4 after cessation of therapy. On the first 3 days there were small losses due to incontinence. During the same period, serum DPH declined from 43.8 to 11.8 mg/l forming an apparent straight line on linear coordinates. Total daily urine DPH fell from 7.4 to 4.9 mg. In the second case, total HPPH values on days one through 5 after therapy ceased were 115.2, 131.3, 143, 111.9 and 73 mg with significant losses only on day 1. Serum declined from 47.6 to 25.2 mg/l. Total daily excretion of DPH varied with urinary output. U/P ratio remained constant. Nearly constant metabolite production at the time of declining high serum levels of DPH in both, and an apparent zero order serum decay in one case, suggests saturation of DPH metabolism. Above the saturating dose, serum levels would rise excessively following any increment in dose. (NIH 5-M01-RR-77 and UHF of WNY CL-6-CH-69.)

77 *Experimental Congenital Lipoid Adrenal Hyperplasia: Prevention of Defects Produced by Aminoglutethimide.* ALLEN S. GOLDMAN, Children's Hosp. of Philadelphia, Philadelphia, Pa.

Aminoglutethimide, a selective inhibitor of cholesterol desmolase, produces in rats the fetal adrenal and testicular defects and hypospadias in male fetuses characteristic of the human disease postulated to be due to a genetic defect in this enzyme, but unlike the disease, virilizes female fetuses. The effects of testosterone (1 mg/kg) and corticosterone (10 mg/kg) administered to pregnant rats treated with aminoglutethimide (100 mg/kg) from days 13 to 20 were studied. In the mothers, testosterone did not significantly affect the marked adrenal enlargement or increase of adrenal size and cholesterol content, but corticosterone reduced the increase in adrenal size and cholesterol content produced by this drug. Testosterone prevented the aminoglutethimide-induced production of hypospadias and the increase of testicular cholesterol content. Corticosterone reduced fetal adrenal enlargement, increase in fetal adrenal cholesterol content, and the increase of the anourethral distance in female fetuses produced by aminoglutethimide. These experiments provide evidence that the production of hypospadias by this drug is due to inhibition of fetal testicular cholesterol desmolase, and that the production of the paradoxic virilization of females by aminoglutethimide is due to androgens of aerenal origin.

78 *The Metabolism of Androgens by Human Fibroblasts.* THOMAS MOSHANG, Jr., ALFRED M. BONGIOVANNI and WALTER R. EBERLEIN, The Univ. of Pennsylvania Sch. of Med., The Children's Hosp. of Philadelphia, Pa.

In vitro studies have demonstrated that testosterone (T) is converted to dihydrotestosterone (DHT) by skin and male accessory organs, and it has been postulated that DHT may be the intracellular biologic androgen of the target organ. In order to study androgen metabolism at the cellular level, fibroblasts cultured from human skin were incubated with radioisotopically labeled steroids in a NADH generating system. Both intact cells and subcellular fractions were studied. The metabolites were separated by paper chromatography and identified by reverse isotopic dilution. DHT was found to be a major metabolite. Etiocholanolone was not found to be a metabolite, indicating no 5β reduction of ring A. Thus, the fibroblasts metabolized T and androstenedione ($\Delta 4$) in a manner similar to skin and male accessory organs. Preliminary data indicate that fibroblasts cultured from males and females formed comparable amounts of DHT in the same incubation time. However, there appeared to be a more rapid metabolism of T by female fibroblasts through the 17-oxo pathway to $\Delta 4$ and androsterone. The cytoplasmic fraction obtained after homogenization of fibroblasts and centrifugation at 800 g metabolized T to DHT, $\Delta 4$, androstanediol and androsterone. Fibroblasts offer a model for the study of cellular metabolism of androgens and will be useful in elucidating the causes of end organ resistance to androgens.

79 *Pseudoaldosteronism (Liddle's Syndrome): Evidence for Increased Cell Membrane Permeability to Na+.* HAROLD J. HELBOCK and JOHN W. REYNOLDS, Univ. of Minnesota, Dept. of Ped., Minneapolis, Minn.

A 10-month boy was found to have pseudoaldosteronism (Ps. Ald.) characterized by hypertension (178/116), alkalosis (HCO_3^-35 mEq/l, pH-7.50), hypokalemia (3.0 mEq/l) and very low aldosterone secretion rates (ARS) (0.9–5.8 μg/d). The hypertension and hypokalemic alkalosis were worsened by salt-loading and unchanged by treatment with spironolactone and dexamethasone. Treatment with triamterene, plus severe Na+ restriction (< 2 mEq/d) for 12 days, led to normal b.p., and serum electrolytes, and an elevation of the ASR (60 μg/d). Long-term therapy has been successful with triamterene, hydrochlorthiazide and moderate salt restriction. Because triamterene is a specific therapy for Ps. Ald. and because it reduces epithelial surface Na+ permeability of isolated frog skin, an increased permeability to Na+ of the luminal membrane of distal renal tubular cells is postulated in Ps. Ald. On no therapy, the patient's RBC Na+ uptake was increased (2.6 μEq/c.c. cells/h) compared to controls (1.9 μEq/c.c. cells/h, $p < 0.01$), and RBC [Na+] was 15.7 mEq/l (controls -10.1 ± 1 mEq/l, $p < 0.01$). After 2 weeks of severe Na+ restriction and triamterene therapy, RBC Na+ uptake was 2.35 μEq/c.c. cells/h (significant decrease, $p < 0.01$) and RBC [Na+] was 11.9 mEq/l. Thus, RBC's show the increased Na+ permeability and increased [Na+] postulated for the distal tubular cells, in which the increased [Na+] would stimulate the Na+-K+ exchange pump leading to Na+ retention and K+ excretion.