529 IMMUNOCHEMICAL ANALYSES IN ORNITHINE TRANSCARBAMYLASE DEFICIENCY. John W. McReynolds and Leon E. Rosenberg. Dept. Human Genet., Yale U. Sch. Med., New Haven, CT

Ammonia intoxication is the clinical hallmark of X-linked ornithine transcarbamylase (OTC) deficiency. Hemizygous affected males have less residual OTC activity and more serious clinical consequences than do heterozygous females. We now describe some immunochemical features of mutant OTC in affected males and females. A monospecific antiserum against purified bovine hepatic OTC has been prepared in rabbits. Immunotitration shows that the antiserum can completely precipitate human OTC and that a given amount complexes bovine and human activity comparably. We have employed this antiserum in an Ouchterlony double diffusion system and in a classical immune precipitation inhibition assay. This latter method consistently detects <1% of control cross-reacting material (CRM). Livers from four affected males (OTC activities <0.1-10% of control) and two females (8 and 20%) have been studied with these techniques. Whereas only the female with 20% of residual activity shows a precipitin line by the Ouchterlony technique, both females and the two males with the greatest residual activities (10 and 0.3%) have detectable CRM by immune precipitation inhibition. In these four cases the percent of residual CRM approximates that of enzymatic activity. In the two males with enzymatic activity <0.1%, no CRM has been found. The observed agreement between residual enzymatic activity and CRM may reflect structural gene mutations which alter the rate of degradation and/or synthesis of OTC protein. The findings are equally consistent, however, with regulatory mutations controlling OTC synthesis.

530 SERUM LIPOAMIDE DEHYDROGENASE IN FRIEDREICH'S ATAXIA Serge B. Melançon, Michel Potier, Louis Dallaire, Guy Geoffroy, Bernard Lemieux and André Barbeau. Depts. of Ped. and Med., Univ. of Montreal and Univ. of Sherbrooke, Canada.

Friedreich's Ataxia patients were recently shown to have reduced activity of pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGD) in disrupted skin fibroblasts. As part of the Quebec Cooperative Study of Friedreich's Ataxia we have measured the third step in the dehydrogenase complex of PDH and OGD, namely lipoamide dehydrogenase (LAD) in the serum of 18 patients with Friedreich's Ataxia and 12 normal subjects.

Results in kUnits/L were as follows:

Subjects	Mean±SEM	Range
Friedreich's patients	2.15±1.23 (p<0.001)	0-4.51
Normal controls	4.56±1.25	2.25-6.76

When taken as a group, Friedreich's patients show 47% of control activity. This value is comparable to 43% PDH and 50% OGD activities reported by Blass et al.(N.Engl.J.Med.,295:62-67, 1976) in cultured fibroblasts. LAD values under the lowest control figure of 2.25 kU/L were observed in 9 patients. A LAD deficiency in tissues of Friedreich's Ataxia patients could account for low oxidation of pyruvate and 2-oxoglutarate as well as the major clinical findings in this severely debilitating autosomal recessive familial disorder. Further enzymatic studies are currently under way in our laboratories using cultured skin fibroblasts from the above patients.

531 METABOLIC STUDIES IN ARGININEMIA Virginia V. Michels and Arthur L. Beaudet (Spon. by George W. Clayton) Baylor College of Medicine, Department of Pediatrics, Houston, Texas.

A Mexican-American female, G.M., was seen at 4 years of age with spasticity, hypertonicity, loss of ability to walk, decreased weight and length, severe microcephaly, marked EEG abnormalities without seizures, and normal blood ammonia. Blood arginine levels ranged from 390 to 1074 μmol/l (normal 61 to 173). Increased arginine and argininosuccinic acid were present in the urine. Arginase activity in red blood cells was 24 nmol/hr/mg hemoglobin with a mean of 1784 and a range of 825 to 2446 in 10 control samples. Decreased activity consistent with a heterozygote state was present in the mother and in 5 of 6 siblings. Arginase activity in a percutaneous liver biopsy was 1580 nmol/hr/mg protein with a range of 89,000 to 158,000 in 4 controls. Arginase was <100 nmol/hr/mg protein in skin clippings with a range of 18,000 to 103,000 in 7 controls. Arginase activity was measured as ornithine production in extracts of cultured skin fibroblasts and found to be 22.5 nmol /hr/mg protein in the propositus with a control range of 14.9 to 43.8. We are evaluating whether control fibroblast arginase activity is lower when serum of the propositus is substituted for fetal calf serum in the growth medium. The low level of fibroblast arginase was also measurable as production of [^{14}C] urea from [^{14}C-guanido] arginine. Therapy was attempted by oral administration of up to 500 mg/kg/d of L-lysine and subsequently by infusion of 8 ml/kg packed red blood cells (post-transfusion red cell arginase, 695 nmol/hr/mg hemoglobin), but neither attempt had convincing effects on serum arginine or clinical parameters.

532 HYPERPHENYLALANINEMIA DUE TO PHENYLALANINE HYDROXYLASE COFACTOR DEFICIENCY. Sheldon Milstien, Sheldon Orloff, Stephen Spielberg, Stanley Berlow, Joseph D. Schulman, and Seymour Kaufman. NIMH and NICHD, NIH, Bethesda, MD., and Waisman Center, Univ. of Wisconsin, Madison, Wisconsin.

A child with phenylketonuria diagnosed early developed severe hypotonia with minimal signs of spasticity and retardation in all developmental patterns despite adequate dietary management. At 4 years of age, a liver biopsy was performed and the components of the phenylalanine hydroxylase system were measured in vitro. Normal levels of phenylalanine hydroxylase, dihydropteridine reductase and dihydrofolate reductase were found. However, there was only 5% of the normal level of active phenylalanine hydroxylase cofactor. This cofactor deficiency was also demonstrable in plasma and urine. Since the phenylalanine hydroxylase cofactor (tetrahydrobiopterin) is probably also necessary for the hydroxylation of tyrosine and tryptophan, the rate limiting steps in the biosynthesis of dopamine and norepinephrine and serotonin, this child may be suffering from a lack of these neurotransmitters.

The child's phenylalanine hydroxylase activity has also been measured with a new in vivo method. In this procedure, hydroxylase activity is assayed by following the rate of formation of tritiated water from ring tritium labelled phenylalanine. Phenylalanine hydroxylase activity 1-5% of normal was detected in vivo confirming the incomplete block in the conversion of phenylalanine to tyrosine predicted by the in vitro assays on the liver biopsy.

533 THREE GENERATIONS OF HEREDITARY COPROPORPHYRIA (HC). Ursula Muller-Eberhard, Ann Smith, Irene Bossenmaier, and Ruth Cardinal, Scripps Clinic and Research Foundation, Department of Biochemistry, La Jolla, Ca. 92037 and University of Minnesota, Minneapolis, Minn. 55407.

Hepatic porphyrias, disorders of heme synthesis, are rarely diagnosed in childhood. Two of three sons, ages 15 and 17 years, of a woman with longstanding neuromuscular disabilities eventually recognized to be associated with high urinary and fecal coproporphyrin (copro-P) exhibited similar biochemical findings. One son, DT, shows the typical porphyrin excretion pattern, whereas WT has only coproporphyrinuria.

Subject	Fecal Porphyrins μg/g dry weight			Urinary Porphyrins μg/24 h	
	copro-P	uro-P	proto-P	copro-P	uro-P
Mother, JT	1120	25	50	1520	90
Son, WT 12y	30	2	30	580	90
Son, DT 15y	1350	90	10	730	60
Son, MT 17y	10	10	2	250	50
Grandfather, ER	170	60	3	500	65
normal, upper limit	30	4	110	280	50

The mother took oral contraceptives over 10 years. How hormonal, nutritional and pharmacological factors contribute to the onset and clinical severity of the enzymatic defects in the porphyrias is not known. HC is rarely recorded (∿ 30 reported cases); to our knowledge the youngest was 10 years old. Thus, the incidence of latent carriers at risk is unknown.

534 THE "ACID AND "NEUTRAL" α-GLUCOSIDASES OF HUMAN LIVER Allen K. Murray and Ronald R. Townsend, (spon. by Thos. L. Nelson),Univ. of Calif., Irvine, Dept. of Pediatrics, Div. of Developmental Disabilities and Clinical Genetics,Irvine.

The relationship between the two α-glucosidases is of interest since the lysosomal and microsomal β-glucuronidases are products of the same structural gene (Owens, et. al., Arch. Biochem. Biophys. 166:258, 1975) while the lysosomal, Golgi and cytosolic α-manosidases appear to be genetically distinct (Tulsiani, et.al. Fed. Proc. 35:1727, 1976). The acid (lysosomal) α-glucosidase from human liver has been purified 2400-fold and its carbohydrate composition has been determined. The Con A binding of the acid α-glucosidase is prevented by D-mannose or α-methyl-D-glucoside. At half saturation 12.5 moles of Con A are bound per mole of enzyme. The binding is demonstrable both with the purified enzyme and crude homogenates. The neutral (soluble) α-glucosidase has been specifically assayed in homogenates. In contrast to the acid glucosidase, Con A does not bind the neutral glucosidase which suggests that its carbohydrate moiety may differ from that of the acid glucosidase, if the neutral glucosidase is in fact a glycoprotein. Further, antiserum to the acid α-glucosidase inhibits its activity but in contrast has no effect on the activity of the neutral α-glucosidase. We are isolating the neutral α-glucosidase to further investigate the relationship between the two α-glucosidases.