

**499** MULTIPLE ALLELES IN METHYLENETETRAHYDROFOLATE REDUCTASE DEFICIENCY. Richard W. Erbe and David S. Rosenblatt, Harvard Medical School, Massachusetts General Hospital, Genetics Unit, Boston.

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the formation of 5-methyltetrahydrofolate, the main tissue and serum form of folic acid, and methyl donor for conversion of homocysteine to methionine. Four patients, 2 of whom are sibs, have been identified. All 4 have neurologic abnormalities and one of the sisters has a folate-responsive, schizophrenialike disorder (Freeman et al. N. Engl. J. Med. 292:491, 1975). MTHFR activity is present at comparable levels in normal skin fibroblasts, amniotic fluid cells and lymphoblasts. MTHFR activities in extracts of both normal and reductase-deficient fibroblasts were low and quite variable during log growth, and were therefore studied at confluency. MTHFR activity in the patients' fibroblasts was 14-20% of normal. Activities in the parents of a patient with 20% of normal activity were 40% and 35% of normal suggesting autosomal recessive inheritance. When extracts were incubated at 55°C, residual MTHFR activity in the sibs showed normal thermal stability, decreasing to 22% and 38% of the initial values in 30 min. In contrast MTHFR from a 3rd patient was exponentially inactivated in 20 min, while that from a 4th unrelated patient was also completely inactivated but somewhat less rapidly. These results suggest that the reductase deficiency in these unrelated families results from at least 3 distinct mutant alleles.

**500** COMPARISON OF COLLAGEN FROM CULTURED HUMAN FIBROBLASTS DERIVED FROM NORMAL INDIVIDUALS AND THOSE WITH CONNECTIVE TISSUE DISORDERS. E. Feng, O.M. Rennert.

Dept. Pediatr. and Biochem., Univ. Fla., Gainesville, Florida. In 1973 Priest et al. indicated that collagen from cultured fibroblasts from patients with Marfan Syndrome contained more soluble collagen than that from normals. In vivo studies of patients with Ehlers-Danlos Syndrome, specifically types V, VI and VII, indicated abnormally soluble collagen. The copper cofactor requirement of lysyl oxidase, abnormal copper transport in Menkes Kinky Hair Syndrome (MKHS) and abnormal vascular collagen and elastic fibers prompted the study of collagen in MKHS fibroblasts. Acid-soluble collagen was extracted from the cell layer of cultured human fibroblasts and estimated by measuring hydroxyproline. The amount of hydroxyproline in the soluble fraction of fibroblast cultures from normal individuals, Ehlers-Danlos Syndrome (EDS), Marfan Syndrome (M) and Cutis Laxa (CL) were 68% ± 3%, 70% ± 7%, 77% ± 10% and 65% ± 10%, respectively. Fibroblasts from 2 MKHS patients gave values of 76% and 87%. There was no correlation between the percentage of soluble collagen and the passage number of the cultures. Total collagen, combined soluble and insoluble fractions, expressed with respect to DNA content indicated 1/3 - 1/2 the total collagen in EDS, CL and M fibroblasts as contrasted to normals. Studies of <sup>14</sup>C-proline incorporation into collagen in these fibroblasts as a function of passage number will be presented.

**501** ARGININOSUCCINIC ACIDURIA: PRENATAL DIAGNOSIS AND STUDIES OF AN AFFECTED FETUS. L.D. Fleisher, D.K. Rassin, P. Rogers, R.J. Desnick & G.E. Gaull. Div. of

Human Devel. & Genet., Inst. For Res. Ment. Retard., Staten Is., NY; Dept. Pediat., Univ. Minnesota, Minneapolis, Mn.; Dept. Pediat., Mt. Sinai Sch. Med., CUNY, NY. (Spon. by N.G. Beratis) Argininosuccinic acid (ASA) lyase deficiency, the basic defect in ASauria, can be studied in cultured skin fibroblasts and cultured amniotic fluid (AF) cells. We diagnosed a fetus affected with ASauria, after studying the obligate heterozygote parents and an affected child. The affected child had been thought to excrete cystathionine, which we showed to be an ASA anhydride. An increased ASA conc was found in AF from amniocenteses at 14, 15 and 17 weeks (fetus at risk = 0.3 μmoles/ml, normal=0). ASA lyase activity in cultured AF cells from the fetus at risk, as measured by the ratio of <sup>14</sup>C-citrulline/<sup>3</sup>H leucine uptake into TCA-precipitable protein, was 0.4% of that in normal AF cells. The pregnancy was terminated, and the in utero diagnosis was confirmed by analysis of fetal liver for ASA lyase (1.8% of normal). It was demonstrated also that all 8 fetal tissues studied, including brain, had significant accumulations of ASA (normal=0). These findings: demonstrate the possible pitfalls in the diagnosis of ASauria by urinary amino acid analysis; confirm earlier suggestions that ASauria may be diagnosed by determining ASA levels in amniotic fluid; cast doubt upon the value of "early" neonatal dietary therapy with arginine, as it appears that the enzymatic defect is already producing an accumulation of unmetabolized substrate in fetal tissues by the beginning of the second trimester; and provide evidence that the urea cycle is active prenatally.

**502** RANDOM X-INACTIVATION IN A FEMALE WITH AN INTERSTITIAL SHORT ARM DELETION OF THE X CHROMOSOME. Uta Francke. University of California San Diego, Dept. of Pediatrics, La Jolla, CA.

Structurally abnormal X chromosomes with a deletion (deletions, rings, isochromosomes) are usually late replicating (inactive) in female cells. An exception to this rule was found in a 19 yr old girl who had normal height and sexual development, a discordant pattern of mental deficiency, and lacked features of Turner Syndrome. In all metaphases examined from her lymphocyte and fibroblast cultures, most of the prominent band Xp21 was deleted in one of the X chromosomes. The late replicating X was identified by <sup>3</sup>H-thymidine labelling and autoradiography in combination with Giemsa-trypsin banding, and by BrdU labelling with subsequent acridine orange staining. The normal X was late replicating in 44% of PHA stimulated lymphocytes, and in 46% of cultured skin fibroblasts.

We concluded that in this case, the Xp interstitial deletion did not interfere with random X-inactivation and that, in the tissues studied, no selection had taken place against cells with the deleted X active, although these cells should be functionally nullisomic for the deleted segment if X-inactivation was complete. However, significance of these findings for the clinical problems was assumed. Testing for X-linked genes was not informative as the patient and both parents were negative for the Xy(a) blood group and the patient had normal color vision. Somatic cell hybridization with an HPRT-deficient Chinese hamster cell line is under way in order to isolate the deleted active X.

**503** ASSOCIATION OF TRIPHALANGLAL THUMBS, CAFE AU LAIT SPOTS AND LEUKEMIA. Lytt I. Gardner, Diane Cass and Tadashi Kajii. Depts. of Peds. and Med., SUNY, Upstate Med. Ctr., Syracuse, New York.

Triphalangeal thumbs (TrTh) have been linked with 3 syndromes involving bone marrow dysfunction: Fanconi pancytopenia and Blackfan-Diamond (both autosomal recessive) and Aase-Smith (? X-linked recessive). The present patient may represent a 4th such association, since the combination of findings is novel.

Studies have been made on an 18 8/12 year old girl with TrTh who showed café au lait skin lesions and acute lymphocytic leukemia. Heart is normal. Her first symptoms were fatigue and anemia (Hgb. 9.9 gm%; Hct. 29.9%) which improved on iron and Infeon therapy. Subsequent studies revealed a hypercellular bone marrow consistent with acute lymphocytic leukemia. There was 86% blasts which were Sudan black B negative and PAS positive. At this time peripheral blood was essentially normal, including platelets. Cytogenetic analysis of bone marrow cells showed two main cell lines: one with 46,XX karyotype and the other with a wide range of distribution (between 48 and 97). The latter cell line is believed to be derived from malignant bone marrow cells. There appeared to be no increase in chromosome breaks, nor were examples of quadriradial figures or endoreduplication seen.

The kindred has 7 persons in 4 generations with abnormal thumbs (2 with polydactyly). Pattern is autosomal dominant. The proposita's father (affected) died at a relatively young age of "cancer", and one of her affected aunts had "anemia".

**504** PLASMA INFUSION IN MENKES SYNDROME. A.D. Garnica, D.T. Sargeant, P.M. Desrosiers, O.M. Rennert. Dept. Ped., Univ. Fla. College of Medicine, Gainesville.

A 4 month-old male with Menkes Kinky Hair Syndrome (MKHS) was treated with plasma infusions [ceruloplasmin (Ce) oxidase activity 0.388±0.060], 10 ml/kg/day, after a course of subcutaneous CuSO<sub>4</sub>. Prior to plasma therapy, the serum copper (Cu), Ce, and liver Cu were low; urine Cu and amino acids were increased (Table).

Patient	Liver Cu	Serum Cu	Ceruloplasmin	Urine Cu
Before Rx	0.088	37.5	0.188	65 μg
Plasma Rx	0.142	62.5	0.364	28 μg
CuAc Rx	0.080	110.0	0.511	85 μg
Control	0.119	--	0.478±0.082	

Plasma infusion increased serum Cu, Ce, and liver Cu; urine Cu and aminoaciduria decreased (Table). The infusions were followed by a 14-day course of cupric acetate, 60 μg/kg/day, subcutaneously, during which serum Cu and ceruloplasmin were maintained; however, the urine Cu increased, the aminoaciduria increased, and the liver Cu decreased (Table).

Parenteral Cu salts are the accepted treatment for Menkes Syndrome. However, they are apparently not effective in improving liver Cu stores, and may cause aminoaciduria. In contrast, infusions of plasma, which contains Cu as Ce, increase serum Cu and Ce, increase liver Cu, decrease urine Cu, and do not cause aminoaciduria. The subcellular distribution of infused ceruloplasmin-bound Cu is known to be different from that of free Cu. This observation, together with these data, imply that protein-bound Cu, as ceruloplasmin may be a more effective treatment for MKHS.