Tissue Culture Techniques as an Aid to Prenatal Diagnosis and Genetic Counselling in Homocystinuria

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Summary. Cystathionine synthase activity was studied in skin fibroblasts from a mother with homocystinuria and her husband and newborn baby. Enzyme studies were also undertaken on a fibroblast cell line derived from amniotic fluid taken at 16 weeks' gestation. The enzyme activity was very low in the mother, within the normal range in the father, and at an intermediate level consistent with heterozygosity in the infant. The activity present in the amnion fibroblasts was similar to that found in the cell line cultured from the infant's skin biopsy.

Homocystinuria, first discovered in 1962 by Carson and Neill in Northern Ireland and Gerritsen, Vaughn, and Waisman in the United States, is a rare hereditary amino-acid disorder in which the deficiency or abnormality of the enzyme cystathionine synthase (EC 4.2.1.21) prevents the normal transsulphuration from methionine to cystine. There is present a classical clinical picture with mental retardation, ectopia lentis, thrombotic episodes, and a characteristic connective tissue disturbance resembling that present in the Marfan syndrome. Diagnosis is made on the basis of elevated levels of plasma homocystine and methionine associated with low levels of plasma cystine and excessive excretion of homocystine in the urine. Approximately 40% of patients with homocystinuria respond biochemically to large doses of the coenzyme pyridoxine (Carson, 1971); the remainder can be treated with a methionine restricted diet.

In 1964, Mudd *et al* established the nature of the defect by demonstrating a specific deficiency of hepatic cystathionine synthase in patients with the disorder. Subsequently, fibroblasts derived from skin and amniotic fluid from control subjects were found to have appreciable levels of cystathionine synthase activity but skin fibroblasts from homocystinuric patients were found to be deficient in this enzyme (Uhlendorf and Mudd, 1968).

Case History

In 1968, a 24-year-old woman of low intelligence (IQ < 70) was diagnosed as suffering from homocystinuria

when she was admitted to an ophthalmic unit with glaucoma secondary to ectopia lentis. The proposita married in 1969 and became pregnant in 1970 but had a complete abortion at 22 weeks. She again became pregnant in 1972 and this time gave birth to a normal infant in whom repeated examination of blood and urine showed no evidence of homocystinuria. The proposita was found to have the pyridoxine-responsive type of the disorder and treatment with pyridoxine was commenced immediately following diagnosis and continued throughout both pregnancies (Ritchie and Carson, 1973).

Methods and Results

Skin samples were taken by pinch biopsy from the forearm of the mother and father and from the base of the umbilical cord of the newborn. Amniotic fluid was collected by transabdominal amniocentesis at 16 weeks' gestation. The cell lines were grown in Minimal Essential Medium (Glasgow Modification) supplemented with 10% fetal bovine serum (Flow Laboratories). Penicillin and streptomycin were routinely incorporated in the medium.

TABLE I
RESULTS OF FIBROBLAST
CYSTATHIONINE SYNTHASE
ACTIVITY IN HOMOCYSTINURIC
FAMILY

Subject	Cystathionine Synthase (nM/mg protein/135 min)
Mother	1.6
Father	50.0
Infant	11.3
Amniotic fluid	12.6

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Cystathionine synthase assays were carried out according to the method of Mudd *et al* (1965) and protein was estimated by the technique of Lowry *et al* (1951). The results are shown in Table I and compared with values obtained in a series of homocystinuric patients, heterozygotes (parents of homocystinurics), and non-homocystinuric subjects (Table II).

TABLE II

SPECIFIC ACTIVITY OF CYSTATHIONINE SYNTHASE
(nM/mg PROTEIN/135 MIN) IN CONTROL SUBJECTS

	Non-homocystinurics	Heterozygotes	Homozygotes
Mean	47.5	21.6	1.6
Median	44.1	20.3	0
Number	9	5	8
Range	16.2-84.5	10.8-36.0	0-6.1

Discussion

Since homocystinuria has an autosomal recessive mode of inheritance (Finkelstein *et al*, 1964) a child born to a normal father and an homozygous mother will be an obligate heterozygote. The results presented in Tables I and II are in agreement with this hypothesis.

Few studies have been reported for cystathionine synthase activity in fibroblasts. Mudd in 1971 reported the results of an investigation into cystathionine synthase activity in extracts of cells from non-homocystinurics, homocystinurics, and parents of homocystinurics. In 36 normal subjects investigated (with one exception) the range was 10-65 nM/mg protein per 135 min; no values were quoted for heterozygotes. Lower specific activities were reported in healthy individuals by Seashore, Durant, and Rosenberg in 1972 on 17 cell lines (2.1-11.1 nM/mg protein per 135 min). The results for normal individuals quoted in the present study are in substantial agreement with those of Mudd (1971). Very few values have been documented for the activity of this enzyme in the amnion fibroblast. Uhlendorf and Mudd (1968) quoted

levels of four normal individuals with a range of 55.1 to 162.0 nM/mg protein per 135 min. No values can be found in the literature for heterozygotes or homozygotes.

This report is a preliminary communication of work in progress on the study of the activity of cystathionine synthase in homocystinuric patients using tissue culture techniques, and shows how these techniques may be used as an aid to genetic counselling and prenatal diagnosis.

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