Homocystinuria: Absence of Cystathionine in the Brain

Abstract. *Cystathionine was absent in the cerebrum of a patient with homocystinuria. This supports the theory that the enzymatic defect in homocystinuria is deficiency of cystathionine synthetase.*

Homocystinuria, an inborn abnormality of sulfur amino acid metabolism, characterized clinically by mental retardation, subluxated lenses, fine sparse hair, and convulsions, was described recently in the United States (*1, 2*) and in England (*3*). The syndrome was believed to be due to an enzymatic defect, namely a deficiency or absence of the enzyme cystathionine synthetase which catalyzes the formation of cystathionine from homocysteine and serine in the metabolic pathway which forms cysteine from methionine. Mudd *et al.* (*4*) have recently demonstrated the absence of cystathionine synthetase in the liver of a child with homocystinuria. The only known role of cystathionine in the body is that of an intermediate in the metabolism of methionine, and at the present time no means of formation of cystathionine is known other than from homocysteine and serine. While cystathionine is absent in fetal brain (*5*), occipital lobes of normal adult human brain contained 22 to 56 mg per 100 g of brain tissue of this amino acid (*6*). In a brain biopsy of a 3-year-old girl, 43 mg of cystathionine per 100 g of brain tissue was found (*7*). Various animal brains, with the exception of the monkey, contained 0.2 to 3.9 mg of cystathionine per 100 g of brain tissue (*6*). A difference was noted between the cystathionine content of different parts of the brain, the cerebrum having a higher cystathionine content than the cerebellum (*5*). Contrary to this, Hope (*7*) found that the cystathionine concentration in the cerebellum of pyridoxine deficient rats was five times higher than in the cerebral hemispheres.

The formation of cysteine from methionine by brain tissue proteins has never been proven. From the work of Gaitonde and Richter (*8*) and from Clouet and Richter (*9*) it is known that when methionine was introduced directly into the cerebral spinal fluid of rats, only 2 percent was metabolized in the brain after 30 minutes. The amount of methionine incorporated into brain protein was only 0.23 percent, and 97 percent passed from the brain into the blood, ultimately to be metabolized in the liver. Presumably the enzyme systems in the liver aid the formation of cystathionine, which is then transported to the brain. It was of obvious interest, therefore, to analyze the brain from a homocystinuric patient for its cystathionine content.

A small part of the cerebrum of patient R.S. (*2*) was removed several hours after death and kept for 19 months in an airtight plastic wrapping at −15°C. For comparison, the only samples similarly preserved were from the cerebra of three other patients. A brain extract for the determination of free amino acids was prepared according to a new procedure (*10*). One gram of brain tissue, containing both cortex and subcortex, was homogenized with 5 ml 0.2*M* citrate buffer, *p*H 1.5. The homogenate was centrifuged for 15 minutes at 78,500*g* in an ultracentrifuge and 1 ml of supernatant equivalent to 0.2 g of wet brain was placed on the chromatography column without further deproteinization. Separation of the amino acids was excellent and recoveries of added amounts of homocitrulline and α-amino-γ-guanidinobutyric acid (*11*) were between 98 and 102 percent. The moisture content of the brain tissue was determined, and the amino acid analyses were expressed in milligrams of amino acid per 100 g of brain (based on 90 percent water content).

Table 1. Free amino acid content of brain*, expressed as milligrams per 100 g of wet tissue, containing cortex and subcortex of different areas.

Amino acid	Patient			
	J.D. (1 yr 10 mo), acute leukemia	D.F. (14 yr 11 mo), phenylketonuria	C.L. (2 yr), "Failure to thrive"	R.S. (1 yr), homocystinuria
Methionine	2	7	28	34
Homocystine	0	0	0	0
Serine	11	63	13	28
Cystathionine	8	7	10	0
Cystine	<1	<1		<1
Taurine		16	27	12

* Complete analysis available on request.

Results on the most important amino acids in the metabolic pathway of methionine are given in Table 1. No homocystine was found in any brain extract, and no cystathionine was detected in the brain of R.S. in comparison with 8, 7, and 10 mg per 100 g in the three other brains. This observation is consistent with the absence of cystathionine synthetase activity in the liver of a homocystinuric patient (*4*). Because normal brain contains high concentrations of cystathionine (*5, 6*), it seems likely that this amino acid plays some important role in the metabolic activities of the brain. When L-serine was given to a homocystinuric patient alone (*11*) or with methionine (*2*) the homocystine excretion decreased, and a very small amount of cystathionine was excreted in the urine. In homocystinuria, therefore, it may be that the formation of cystathionine is strongly inhibited, but not completely blocked. It would seem appropriate to attempt to increase the cystathionine level of the brain in these patients either by feeding them cystathionine per se or by providing them with large amounts of L-serine.

THEO GERRITSEN
HARRY A. WAISMAN
Joseph P. Kennedy, Jr., Laboratories, Department of Pediatrics, University of Wisconsin Medical Center, Madison

References

1. T. Gerritsen, J. G. Vaughn, H. A. Waisman, *Biochem. Biophys. Res. Comm.* **9**, 493 (1962).
2. T. Gerritsen and H. A. Waisman, *Pediatrics* **33**, 413 (1964).
3. N. A. J. Carson and D. W. Neill, *Arch. Disease Childhood* **37**, 505 (1960); N. A. J. Carson, D. C. Cusworth, C. E. Dent, C. M. B. Field, D. W. Neill, R. G. Westall, *ibid.* **38**, 425 (1963).
4. S. H. Mudd, J. D. Finkelstein, F. Irreverre, L. Laster, *Science* **143**, 1443 (1964).
5. N. Okumura, S. Otsuki, A. Kameyama, *J. Biochem. (Tokyo)* **47**, 315 (1960).
6. H. H. Tallan, S. Moore, W. H. Stein, *J. Biol. Chem.* **230**, 707 (1958).
7. D. B. Hope, *J. Neurochem.* **11**, 327 (1964).
8. M. K. Gaitonde and D. Richter, *Proc. Roy. Soc. London, Ser. B* **145**, 83 (1956).
9. D. H. Clouet and D. Richter, *J. Neurochem.* **3**, 219 (1959).
10. T. Gerritsen and H. A. Waisman, in preparation.
11. ———, unpublished data.

25 June 1964

Sialic Acid–Containing Glycopeptide from Chlorella

Abstract. *A C^{14}-labeled polyanion was isolated from* Chlorella pyrenoidosa. *It contained amino acids, sialic acid, and other carbohydrates, but no uronic acids, phosphate, or sulfate. It was stable to mild alkali, but released sialic acid upon mild acid treatment.*

Sialic acids, which are derivatives of neuraminic acid, have only been reported from certain animals and bacteria. Warren (*1*) reported failure to demonstrate sialic acid in a number of algae and other lower plants.