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).

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.

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.

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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.

Pure cultures of *Chlorella pyrenoidosa,* Van Niel's strain Z 2.2.1, were cultured autotrophically and synchronously in Schmidt's medium (*2*). In some cases, cultures were exposed to P^{32}-phosphate, S^{35}-sulfate, or C^{14}-carbon dioxide for one life cycle of growth.

Samples of 100 g (wet weight) of *Chlorella* cells were stirred in 300 ml of dilute KOH (*p*H 11, at 25°C for 1 hour). An equal volume of 88 percent phenol was then added, and the mixture was shaken thoroughly. The phases were separated by centrifugation, and the upper, aqueous phase, which contained the glycopeptide, was treated two more times with phenol to free it completely of protein. The residual phenol was extracted with ether, the glycopeptide remaining in the aqueous fraction. Sodium acetate (2 percent dry weight) and three volumes of cold ethanol were added. The resultant precipitate was separated by centrifugation and dissolved in 0.01*M* $MgCl_2$. The sample was then treated with snake-venom phosphodiesterase (*p*H 8.8 at 35°C for 24 hours) to hydrolyze the bulk of the RNA present. The hydrolysate was eluted from a diethylaminoethylcellulose (DEAE) column with a gradient of sodium chloride. The glycopeptide, which eluted between 1.0 and 1.5*M*, was then hydrolyzed with 0.5*N* KOH for 18 hours at 35°C and rechromatographed on a DEAE column.

After this purification, the glycopeptide had no absorption in the visible or ultraviolet regions, and only one fraction, which was a symmetrical peak, was eluted from the DEAE column. Sialic acid was assayed in both the phenol-water extract and the final preparation by the thiobarbituric acid method (*3*). The yield, 10 μmole of sialic acid, amounted to 80 percent of the sialic acid found in the phenol-water extract. That no thiobarbituric acid reaction occurred unless the sample was first treated with 0.1*N* HCl at 80°C for 1 hour indicated a ketosidic linkage. The spectrum of the chromogen is identical with that produced in the reaction when known *N*-acetyl neuraminic acid (one of the sialic acids) is used. Further evidence that the unknown is indeed a sialic acid included a positive Partridge reaction for amino sugars (*4*) and a positive HCl test (compound turns dark in 0.1*N* HCl at 100°C but not at 80°C).

The glycopeptide gave an intense metachromatic reaction with toluidine blue at *p*H 7 but gave no uronic acid reaction (*5*). No incorporation in vivo of radioactive sulfate or phosphate into the glycopeptide was detected. Thus, the metachromasy and strong interaction with DEAE indicate that the carboxyl function of the sialic acid units are free in the polymer. The material was nondialyzable and was excluded by G-25 Sephadex (cross-linked dextran gel) which indicates a molecular weight of over 10,000.

The polymer contained 9.5 μmole of glucose equivalent of nonamino sugars per micromole of sialic acid as determined by the anthrone reaction (*6*), 1 μmole of reducing sugar before any acid treatment (*7*), and 875 μg of protein (*8*). After 0.1*N* HCl hydrolysis for 1 hour at 80°C, reducing functions were increased to 2.75 μmole.

A C^{14}-labeled sample having 15,000 count/min per micromole of sialic acid was isolated. This material was chromatographed on Whatman No. 1 paper by the descending method with ethyl acetate, water, and pyridine (5:7:2) (*9*). Spots were detected by autoradiography, also by spraying with silver nitrate then with alcoholic sodium hydroxide. The untreated polymer remained at the origin.

After mild acid hydrolysis another spot appeared at $R_{glucose}$ (movement relative to that of glucose) of 0.13, identical to the location of *N*-acetyl neuraminic acid. The material at this location reduced silver ion and gave positive Partridge (*4*) and ninhydrin tests. These are all expected properties of sialic acids. After more severe acid hydrolysis the sialic acid spot was no longer found, but spots at $R_{glucose}$ of 0.65 and 1.86 appeared. The materials at both of these locations were able to reduce silver ion. These mobilities coincide with those of glucosamine and L-rhamnose, respectively.

Three types of polymers containing sialic acid have been isolated: (i) glycoproteins—such as that from the ovine submaxillary gland—from which the moiety containing sialic acid is released by mild alkali (*10*); (ii) glycoproteins—such as that from equine erythrocytes—in which the protein-carbohydrate linkage is stable to mild alkali (*11*); (iii) colominic acid—obtained from *Escherichia coli*—a polymer which contains only sialic acid, none of which is released by mild alkali (*12*). Very mild acid hydrolysis cleaves the ketosidic linkage of the sialic acid in all three types of polymer.

The glycopeptide isolated from *Chlorella* has properties most closely resembling the second type of polymer. It is stable to mild alkali and contains both amino acids and carbohydrates.

More thorough identification of the components of the glycopeptide from *Chlorella* will require larger amounts of material or the application of more sensitive methods. However, it seems important to note that this glycopeptide, which contains sialic acid, does occur in *Chlorella.*

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Tobacco Mosaic Virus: Cytological Evidence of the Synthesis in the Nucleus

Abstract. *Living tomato hair cells were directly injected with tobacco mosaic virus or tobacco mosaic virus RNA with a fine glass needle. Use of a fluorescent tobacco mosaic virus antibody showed the presence of the virus protein in the nucleus 6 to 18 hours after the injection. As time of infection progressed, staining of the cytoplasm became more prominent.*

The nuclei of plant cells may play an important role in the formation of tobacco mosaic virus (TMV) (*1*). However, these observations have all been made on cells that became infected with TMV indirectly. This report shows that the TMV infective process may be induced by direct injection of TMV or TMV-RNA into living tomato hair cells. From observations on the course of the infection both by phase microscope and by the use