in areas of Colorado (*5*) and California (*6*). Squirrels dying of WEE virus infection were found during 1968 in the same area as was the opossum. It thus seems possible that the opossum was infected by mosquito bite, or by eating an infected animal. Our findings indicate that there was active infection of the brain and not merely an incidental viremia.

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Generalized Gangliosidosis: Impaired Cleavage of Galactose from a Mucopolysaccharide and a Glycoprotein

Abstract. *We have demonstrated a tenfold (or greater) reduction of the cleavage of galactose from a mucopolysaccharide and a glycoprotein in generalized gangliosidosis, a lysosomal β-galactosidase deficiency disease. The bony abnormalities in this disease may be due to defective catabolism of mucopolysaccharides in connective tissue.*

Generalized gangliosidosis is an inborn error of metabolism involving the accumulation of ganglioside GM_1 in brain (*1*, *2*) and viscera (*1*). The disease is characterized by (i) progressive cerebral degeneration leading to death within the first 2 years of life; (ii) accumulation of glycolipid in neurons and of glycolipid and mucopolysaccharide in hepatic, splenic, and other histiocytes, and in renal glomerular epithelium; and (iii) the presence of skeletal abnormalities resembling those seen in Hurler's syndrome (*3*). Okada and O'Brien (*4*) have demonstrated a 10- to 30-fold deficiency of a β-galactosidase (*p*H 5.0), an enzyme that cleaves the terminal galactose from the stored ganglioside, GM_1—{galactosyl-(1 → 3)-*N*-acetyl-galactosaminyl-(1 → 4)-[(2 → 3)-*N*-acetylneuraminyl]-galactosyl-(1 → 4)-glucosyl-(1 → 1)-[2-*N*-acyl]-sphingosine}. Generalized gangliosidosis can be classified as a glycosphingolipid hydrolase deficiency, the ganglioside accumulation resulting from an impairment of catabolism.

Mucopolysaccharides also accumulate in the viscera in generalized gangliosidosis (*5*). One of these has (*6*) a structure similar to that of keratan sulfate; it contains nearly equimolar proportions of glucosamine and galactose with much smaller proportions of other sugars. The mechanisms leading to the storage of the mucopolysaccharide is unknown. The possibility exists that β-galactosidase participates in the degradation both of ganglioside GM_1 and of mucopolysaccharides containing galactose and that accumulation of the latter may also be due to an impairment in catabolism.

To test this possibility, we isolated the "keratan sulfate-like" mucopolysaccharide from the liver of a patient who died at 2 years of age from generalized gangliosidosis. The isolation procedure (*6*) involved extraction of the tissue with a mixture of chloroform and methanol (2:1), digestion of the solvent-insoluble residue with papain for 24 hours at 60°C, and isolation of mucopolysaccharides by precipitation as their sodium salts with 80 percent ethanol. The preparation was then digested with α-amylase for 2 hours at 37°C and precipitated from 80 percent ethanol. The precipitate was then dissolved in water, dialyzed, and lyophilized.

The isolated mucopolysaccharide contained galactose and hexosamine in nearly equimolar amounts (galactose : hexosamine 0.9). This was determined by hydrolyzing the compound in 1*N* HCl at 100°C for 18 hours, and, after purification on a charcoal column (*7*), estimating galactose by galactose oxidase assay (*8*). Hexosamine was determined by the method of Boas (*9*). The hexosamine was identified as glucosamine by chromatography (*10*). Only small amounts of galactosamine were present. The intact mucopolysaccharide migrated in a way similar to keratan sulfate, with an R_F of 0.8 in a silica-gel thin-layer chromatography system (*11*). The stored mucopolysaccharide stained pink with the orcinol sulfuric acid spray, also characteristic of keratan sulfate (*11*). It was resistant to degradation by testicular hyaluronidase.

A portion of this mucopolysaccharide was then incubated in the presence of β-galactosidase partially purified from equal amounts of liver tissue from normal patients and that from generalized gangliosidosis. The protein and water contents of the patient's tissue were nearly identical to normal. The enzyme was purified (*12*); the particulate enzymes and free galactose present in the tissue were removed, and the enzyme was obtained in soluble form. Small losses of β-galactosidase activity occurred in preparing the enzyme from the normal and diseased livers, but

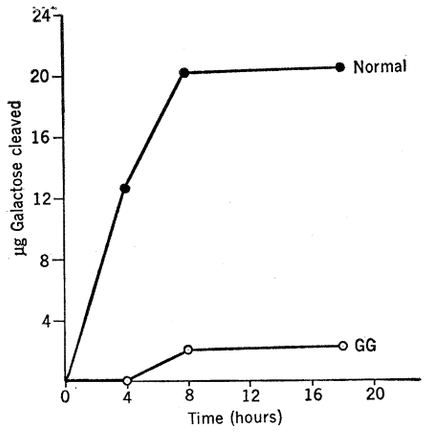

Fig. 1. Cleavage of galactose from 1.5 mg of "keratan sulfate" from the liver of a patient with generalized gangliosidosis. β-Galactosidase was prepared from 35 mg of liver from a normal patient or from a patient with generalized gangliosidosis (*GG*). The enzyme and the mucopolysaccharide were incubated over an 18-hour period in acetate buffer (0.1*M*), *p*H 5.0, at 37°C, and the galactose released was determined by galactose oxidase assay.

the losses were nearly identical for both preparations.

The rate of cleavage of galactose from the stored mucopolysaccharide was tenfold lower in generalized gangliosidosis; after 18 hours, 38.9 percent of the galactose in the mucopolysaccharide was released by the preparation from a normal patient, whereas 3.8 percent was released by the preparation from patients with generalized gangliosidosis (Fig. 1). The same result was obtained in a second patient with the disease.

One objection to the use of partially purified β-galactosidase preparations is that the enzyme may not fractionate the same in diseased tissue. This objection is removed by our previous demonstration (*4*) with whole liver homogenates that β-galactosidase activity (both for *p*-nitrophenyl-β-D-galactopyranoside and for ganglioside GM_1) is 20- to 30-fold lower than normal in this disease. Our previous work (*4*) also demonstrated that inhibitors of β-galactosidase activity (for *p*-nitrophenyl-β-D-galactopyranoside and for ganglioside GM_1) are not responsible for the lowered enzymic activity. We have assumed here that endogenous inhibitors do not account for the impaired cleavage of galactose when the mucopolysaccharide serves as enzyme substrate.

In another experiment we measured the rate of cleavage of galactose from sialic acid–free fetuin. This glycoprotein contains an oligosaccharide chain with a terminal galactose linked to hexosamine, and a sialic acid moiety linked to the galactose. Sialic acid–free fetuin was prepared by mild acid hydrolysis (*13*) to give a glycoprotein with a free terminal galactose. This glycoprotein (2 mg) was then incubated with purified preparations of β-galactosidase obtained from normal (two patients) and generalized gangliosidosis (two patients) livers. The preparations from normal liver liberated small (average, 5.5 μg) but readily detectable amounts of galactose after 18 hours of incubation at 37°C, whereas no detectable galactose was liberated by the generalized gangliosidosis preparation.

Our results demonstrate an impaired cleavage of galactose from a mucopolysaccharide in generalized gangliosidosis. It appears likely that the normal degradation of these and similar macromolecules containing galactose involves the participation of lysosomal β-galactosidase and that their accumulation is the result of a block in their catabolism. The bony abnormalities so characteristic of generalized gangliosidosis may be explained by defective catabolism of mucopolysaccharides in connective tissue.

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N-Formylseryl-Transfer RNA

Abstract. *The reactions between serine and transfer RNA from baker's yeast and from* Escherichia coli *have been investigated. Results obtained from in vitro, in vivo, and chemical syntheses and from electrophoretic, chromatographic, and radioautographic analyses demonstrate that* N*-formylseryl-transfer RNA is formed in these systems.*

Formation of *N*-formylmethionyl-tRNA in *Escherichia coli* and in yeast was reported by Marcker and Sanger (*1*), and the importance of this particular transfer RNA (tRNA) derivative on the mechanism of protein synthesis has been elucidated (*2*). I now present evidence of the formation of *N*-formylseryl-tRNA.

The tRNA's of baker's yeast and *E. coli* B, ^{14}C-serine, ^{35}S-methionine, ^{14}C-formate, and ^{14}C-adenosine triphosphate (ATP) were purchased (Schwarz BioResearch, Inc.) and stored at −20°C. Occasionally tRNA's were prepared from yeast according to the method of Zubay (*3*). The purchased tRNA's gave a single absorption peak when chromatographed in water on a Sephadex G-25 column, but some nuclease was present; and in the ribonuclease or alkaline hydrolyzates of both tRNA's fewer than 60 percent of the terminal groups were adenosine while more than 40 percent were cytosine, a fact that has been reported (*4*). Accordingly, in critical experiments the commercial tRNA's were treated with phenol, extracted in aqueous phase, washed twice with peroxide-free ether, dried, and recharged with adenosine monophosphate (*5*). Radioactive chemicals were generally purified with ion-exchange resins and subsequent electrophoresis or chromatography until they gave a single band on the radioautograph. Strains of *E. coli* B and A19 were

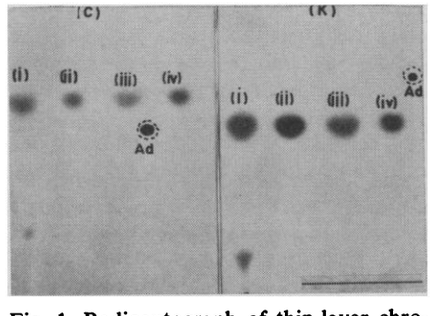

Fig. 1. Radioautograph of thin-layer chromatography of *N*-formylseryl-adenosine isolated from ribonuclease digests of tRNA of various treatments on two plates, cellulose (*C*) and kieselgel (*K*). Compounds were isolated as B bands of electrophoretogram and purified by passing them through Dowex I. They were isolated from (i) yeast tRNA charged with ^{14}C-serine in vivo, (ii) and in vitro, (iii) yeast tRNA terminally labeled with ^{14}C-adenosine and subsequently charged with unlabeled serine, (iv) *N*-^{14}C-formylseryl-tRNA synthesis in the manner described in the text. *Ad*, position of unlabeled adenosine located under ultraviolet light. The R_F values were as follows: on cellulose plate: adenosine, 0.62; *N*-formyl compounds, 0.70; kieselgel: adenosine, 0.86; *N*-formyl compounds, 0.60. Scale, 5 cm.