METABOLISM OF SULFATIDES. I. THE EFFECT OF GALACTOCEREBROSIDES ON THE SYNTHESIS OF SULFATIDES*

G. M. McKhann[1], R. Levy and W. Ho
Departments of Pediatrics and Medicine (Neurology)
Stanford University School of Medicine
Palo Alto, California

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Sulfatides, the 3'-sulfate esters of galactocerebrosides, are components of the mammalian myelin sheath. The synthesis of these lipids can be followed by measuring the incorporation of S^{35}-sulfate into the lipid fraction of brain (Davison and Gregson, 1962). In the rat cerebrum, the period of active synthesis of sulfatides *in vivo* (Davison and Gregson, 1962) and *in vitro* (McKhann, et al, 1965) coincides with the onset of the histological appearance of myelin.

Despite the close chemical similarity of galactocerebrosides and sulfatides, their metabolic relationships have not been established. The studies reported in this paper indicate that galactocerebrosides are sulfated by a soluble enzyme obtained from the microsomal fraction of rat brain.

Methods

The brains of 16-18 day old Sprague-Dawley rats were quickly removed and homogenized in 0.25 M sucrose. The sulfating enzyme was solubilized from the 100,000 x g sediment by resuspending the pellet in desoxycholate (5 mgm/ml) and sonicating for two minutes in a Ratheon sonicator. The sonicated particles were centrifuged at 100,000 x g for 45 minutes, and the resulting supernate used as

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[1]Joseph P. Kennedy, Jr. Senior Scholar in Mental Retardation and John and Mary R. Markle Scholar in Academic Medicine.

a source of enzyme. Solubilization of the enzyme resulted in a 27-fold increase in specific activity.

In the present studies, the activity of the sulfate-transferring system was assayed by the incorporation of S^{35}-sulfate into sulfatide. The incubation medium contained 100 μM Tris buffer, pH 8.0; 10 μM ATP; 0.8 μM K_2SO_4; 5 μM $MgCl_2$; 50 μC $Na_2S^{35}O_4$; 0.2 ml of 100,000 x g supernate (as a source of PAPS-generating system) and 0.2 ml of enzyme. Cerebrosides, and other lipids, were emulsified in 0.1 ml of 1% BRIJ-96® and added to a final volume of 1.0 ml. After two hour incubation at 37°, 19 volumes of chloroform-methanol, 2:1, were added and the suspension allowed to stand for one hour at room temperature. 0.2 volumes of 0.74% KCl were added, and the suspension clarified by centrifugation. The aqueous upper phase was removed, and the lower phase washed six times with aqueous "theoretical upper phase" (Folch, et al, 1957) to remove contaminating S^{35}-sulfate. The galactolipids were separated from the lipid extract by column chromatography with Florisil and the galactocerebrosides and sulfatides separated by column chromatography with DEAE cellulose, in the acetate form (Rouser, et al, 1961). Final identification of the sulfatides was by thin layer chromatography on Silica Gel G , using either n-propanol-12% aqueous ammonia, 4:1, or chloroform-methanol-water, 65:35:5, as solvents. In either system, 85-90% of the total radioactivity of the lipid extract could be accounted for in the sulfatide fraction.

Cerebrosides were purified from beef spinal cord. Palmitylgalactocerebroside and palmitylglucocerebroside were synthesized (Brady, et al, 1965) and were a gift of Dr. Roscoe Brady.

Results

When the microsomal fraction is used as a source of enzyme, addition of exogenous cerebrosides results in a three-fold increase

Table 1

Effect of Exogenous Cerebrosides on S^{35}-sulfate Incorporation into Sulfatides

Enzyme Source	Addition	cpm/mgm protein
A) Microsomes	Detergent	27
	Detergent + 2 mgm cerebrosides	89
B) Soluble Enzyme	Detergent	16
	Detergent + 0.5 mgm natural cerebrosides	132
	Detergent + 0.5 mgm palmitylgalactocerebroside	106
	Detergent + 0.5 mgm palmitylglucocerebroside	11

Table 2

Effect of Other Lipids (Soluble Enzyme)

Addition	cpm/mgm protein
Detergent	9
Detergent + 2 mgm natural cerebrosides	193
Detergent + 2 mgm cholesterol	7
Detergent + 2 mgm sulfatides	2
Detergent + 2 mgm sphingomyelin	20

in S^{35}-sulfate incorporation into sulfatides. (Table 1). With the soluble enzyme, there is a decrease in endogenous activity, and no significant S^{35}-sulfate incorporation unless exogenous galactocerebrosides are added. P h natural cerebrosides and synthetic palmitylgalact cerebroside are effective in stimulating incorporation into sulfatides. The enzyme appears to be specific for galactocerebrosides, because palmitylglucocerebroside has no significant effect. (Table 1).

The addition of other lipids, known to be components of the myelin sheath, has no significant effect on incorporation into sulfatide. (Table 2).

Prolonged incubation of S^{35}-sulfatide, in the presence of unlabeled sulfate, results in no significant release of S^{35}-sulfate into the aqueous upper phase of the lipid extract, indicating that no significant exchange reaction or sulfatide sulfatase in associated with the sulfating enzyme.

Discussion

On the basis of the pattern of *in vivo* incorporation of labeled hexoses into rat brain, Radin, et al, (1957) and Hauser (1964) have suggested that galactocerebrosides are the precursors of sulfatides. In an *in vitro* system, Goldberg (1961) has shown that the sulfate donor in the synthesis of sulfatides is phosphoadenosinephosphosulfate (PAPS). On the basis of the findings reported in this paper, we would suggest that the synthesis of sulfatides proceeds as follows:

$$\text{galactocerebrosides} + \text{PAPS} \longrightarrow \text{sulfatides} + \text{PAP}$$

The sulfate-transferring enzyme, "galactocerebroside sulfokinase", is involved in the conversion of one lipid which is enriched in myelin into another lipid enriched in myelin. The use of this enzyme as a biochemical marker for the study of myelinization is under investigation.

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