A Cerebroside Sulphotransferase Deficiency in a Human Disorder of Myelin

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An unusual human disease begins during myelination. Children develop this neurological disorder (globoid leucodystrophy) at about 4 months of age. They usually die before the age of 2 years with a widespread degeneration of white matter. There is a marked decrease in the concentration of cerebroside sulphates (sulphatides) both in white matter (Austin, 1963*a,b*; Svennerholm, 1963) and in cerebral cortex (Svennerholm, 1963) of patients with globoid leucodystrophy. On the other hand, concentrations of neutral cerebroside are proportionately elevated in white matter (Austin, 1963*c*) of patients with this disorder.

The present findings suggest a reason for this distinctive change in proportions between cerebroside and their sulphated conjugates in globoid leucodystrophy. The evidence suggests that in globoid leucodystrophy a metabolic block may be interposed between cerebroside and cerebroside sulphate (Austin, 1963*b*). This block appears to involve an enzyme that normally helps to conjugate cerebroside with sulphate to form cerebroside sulphate. This enzyme system, cerebroside sulphotransferase, was demonstrated in animals by Balasubramanian & Bachhawat (1965*a,b*) and by McKhann, Levy & Ho (1965). The sulphate donor in the system is 'active sulphate' (adenosine 3'-phosphate 5'-sulphatophosphate). The reaction (in greatly oversimplified form) is as follows:

Evidence presented below indicates that (a) this enzyme activity does occur in normal control children, but (b) there is a characteristic deficiency of cerebroside sulphotransferase activity in post-mortem tissues of children with globoid leucodystrophy. A deficiency of this enzyme *in vivo* would imply a deficiency of myelin sulphatides that could lead to defective myelination.

Materials and methods. Enzyme activities in two unrelated globoid-leucodystrophy patients were contrasted with those in five relevant controls. All samples were assayed simultaneously. Controls were carefully selected so that they and the leucodystrophy patients were similar in terms of age at

death, cause of death, interval between death and autopsy and length of time frozen (in the same freezer).

For example, globoid-leucodystrophy patient no. 1 was 1 year 7 months old at death. Tissues were frozen at -20° within 8 hr. of death and were assayed 1 month later. Globoid-leucodystrophy patient no. 2 was 1 year 11 months old at death. Tissues were frozen within 2 hr. of death and were assayed 5 months later. Three normal control children (patient nos. 5-7) ranged from 1 year 4 months to 2 years 1 month old at death. Tissues were frozen within 5-18 hr. of death and were assayed 1-21 months later. One abnormal control child had a slightly different kind of myelin breakdown (metachromatic leucodystrophy). This child (patient no. 3) died at 6 years 11 months of age. Tissues were frozen 1 hr. later and were assayed 11 months later. A comparable normal control (patient no. 4) died at 6 years 10 months of age. Tissues were frozen 8 hr. later and were assayed 27 months later.

Methods of assay for cerebroside sulphotransferase activity were essentially as cited by Balasubramanian & Bachhawat (1965*b*). However, 0.16M-phosphate buffer, pH 7.4, was used instead of tris, and the glutathione concentration was increased to 0.01M (V. R. Bhandari & B. K. Bachhawat, unpublished work). Incubations were for 2 hr. at 38° . Each reaction mixture contained 180000 counts/min. of carrier-free adenosine 3'-phosphate 5'-[^{35}S]sulphatophosphate prepared as described by Balasubramanian & Bachhawat (1964).

All cerebroside sulphotransferase assays were performed with whole homogenates as the enzyme source. The quantity of tissue used for assay was approx. 0.150g. wet wt. Kidneys comprised 50% cortex and 50% medulla. After extraction and preliminary fractionations, ^{35}S -labelled conjugates were separated by thin-layer chromatography on silica gel G (Austin, 1963*c*). One solvent system was chloroform-methanol-water (28:13:2, by vol.). The ^{35}S -labelled products were shown by radioautography on Royal Blue film after periods of 4-60 days in comparison with spots of known sulphated standards. Control hydrolytic enzymes

Table 1. *Cerebroside sulphotransferase specific activities in two globoid-leucodystrophy patients and five controls*

One unit of cerebroside sulphotransferase activity represents the incorporation of 1 count/min. Cerebroside sulphotransferase specific activity is given as units/10 mg. of protein.

Patient and diagnosis	Cerebral white matter			Cerebral cortex			Kidney		
	Cerebroside sulphotransferase	Sulphatase A	Acid phosphatase	Cerebroside sulphotransferase	Sulphatase A	Acid phosphatase	Cerebroside sulphotransferase	Sulphatase A	Acid phosphatase
1. Globoid leucodystrophy	4	35.4	1.4	2	15.5	0.9	9	26.2	1.7
2. Globoid leucodystrophy	0	16.8	0.9	9	15.6	1.3	15	16.2	0.8
3. Metachromatic leucodystrophy*	40	0.2	0.9	56	0.3	1.0	252	0.3	2.8
4. Normal	69	32.8	0.5	58	21.0	0.7	723	46.5	1.8
5. Normal	70	31.8	0.4	47	12.9	0.6	41†	16.3	1.8
6. Normal	368	28.7	0.3	877	14.2	0.5	294	60.4	1.5
7. Normal	594	12.6	0.5	180	8.7	0.6	188	14.5	0.8

* Low sulphatase A in patient no. 3 confirms the histological diagnosis of metachromatic leucodystrophy (Austin *et al.* 1963, 1965).

† The patient received nitrogen-mustard therapy 1 week before death. This may explain the relatively low cerebroside sulphotransferase activity.

and proteins were assayed and expressed in specific activities as described by Austin, Armstrong & Shearer (1965).

Results. The results in Table 1 indicate that globoid-leucodystrophy patients have low cerebroside sulphotransferase activity. Globoid-leucodystrophy patient no. 2 had no detectable cerebroside sulphotransferase activity in white matter. In globoid-leucodystrophy patient no. 1, the cerebroside sulphotransferase activity of white matter was one-tenth that of the lowest control. In globoid-leucodystrophy patient no. 2, the cerebroside sulphotransferase activity of cerebral cortex was one-fifth that of the lowest control. Kidneys from globoid-leucodystrophy patients were also low in cerebroside sulphotransferase activity. Radioautographs demonstrated two well-labelled reaction products (R_f 0.60 and 0.56). These corresponded to those of reference standards of cholesterol sulphate and kersin sulphate. Dihexose sulphatides were not detectable.

Total proteins in all samples were essentially the same and do not account for the low specific activity in globoid leucodystrophy. In common with other synthetic enzyme activities, cerebroside sulphotransferase activity in tissues does slowly decrease with time in the freezer. However, the autopsy information and duration of frozen storage do not in themselves explain the low cerebroside sulphotransferase activity in globoid leucodystrophy. Appro-

priate additions of samples from globoid-leucodystrophy patients to normal samples showed no evidence of any inhibition of cerebroside sulphotransferase activity.

Samples from globoid-leucodystrophy patients showed no selective deficiency of the other categories of enzyme activity assayed. Thus neither sulphatase A nor acid phosphatase activities were decreased in globoid leucodystrophy below control values (Table 1).

Discussion. The low cerebroside sulphotransferase activity could be more of a primary event (i.e. genetically determined), or it could be only secondary to the pathological changes in white matter associated with globoid leucodystrophy. Weighing against a simple secondary explanation is the finding that the cerebroside sulphotransferase deficiency extends to tissues that have not obviously degenerated. For example, cerebral cortex and kidney from globoid-leucodystrophy patients each have low sulphotransferase activity, but do not show histological abnormalities that would explain the deficiency. Moreover, cerebroside sulphotransferase activity is still present in a different leucodystrophy (metachromatic leucodystrophy) in which white matter is also destroyed (Table 1). This control finding would make it difficult to attribute the low cerebroside sulphotransferase activity of globoid leucodystrophy to a simple secondary degeneration of white matter. Further,

despite the obvious degeneration of white matter in globoid leucodystrophy, other transferases were within normal limits in brain from a globoid-leucodystrophy patient in previous studies (e.g. UDP glucose-glucosyltransferase) (Austin *et al.* 1963).

An enzymic disorder of sulphate conjugation has not hitherto been reported. The expected result *in vivo* of a cerebroside sulphotransferase activity deficiency would be a deficiency of sulphatides. Because sulphatides are normally most concentrated in myelin, a deficiency of sulphatides could interfere with the proper development of myelin in this human disease.

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Hydroxylamine and a ³²P-Labelled Intermediate in Sodium-plus-Potassium Ion-Activated Adenosine Triphosphatase

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An acid-stable ³²P-labelled phosphoprotein complex can be recovered after incubation of membrane preparations exhibiting (Na⁺ + K⁺)-activated ATPase* activity with [γ -³²P]ATP (Charnock & Post, 1963a; Gibbs, Roddy & Titus, 1965; Rodnight, Hems & Lavin, 1966). Formation of this complex is stimulated by Na⁺ but not K⁺; when both ions are present the steady-state concentration of ³²P-labelled phosphoprotein complex is greatly decreased whereas the hydrolysis of [γ -³²P]ATP is enhanced. Most investigators have concluded that the ³²P-labelled phosphoprotein complex recovered in these experiments is an acyl-phosphate-enzyme derivative that functions as an intermediate (E ~ P) during the hydrolysis of ATP by (Na⁺ + K⁺)-activated ATPase (Charnock, Rosenthal & Post, 1963; Nagano *et al.* 1965; Bader, Sen & Post, 1966).

However, this concept has been challenged by Schoner, Kramer & Seubert (1966), who reported

that the Na⁺-stimulated formation of ³²P-labelled phosphoprotein complex was greatly decreased when the reaction was conducted in the presence of hydroxylamine, but that hydroxylamine did not affect the rate of hydrolysis of ATP by this enzyme in the presence of Na⁺ and K⁺. From these findings these workers concluded that the ³²P-labelled phosphoprotein complex was not an intermediate in the hydrolysis of ATP by (Na⁺ + K⁺)-activated ATPase.

The present experiments are a further examination of the interaction of hydroxylamine with (Na⁺ + K⁺)-activated ATPase.

Materials and methods. Preparation of guinea-pig kidney microsomal (Na⁺ + K⁺)-activated ATPase and the assay conditions for the colorimetric determination of ATP hydrolysis by liberation of inorganic phosphate have been described previously (Charnock & Post, 1963b).

The ³²P-labelling experiments were carried out

* Abbreviation: ATPase, adenosine triphosphatase.