

## PAPS—CEREBROSIDE SULPHOTRANSFERASE ACTIVITY IN BRAIN AND KIDNEY OF NEUROLOGICAL MUTANTS

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### 1. Introduction

Three recessive mutations in mice result in grossly defective myelination in the central nervous system (CNS) while myelination of peripheral nerves is normal. Two of these mutants, the Jimpy (jp) and a myelin synthesis deficient mutation (msd) are sex-linked and may be alleles [1, 2], whereas the third one, the Quaking (qk) is autosomal recessive. Brain cerebroside and sulphatides are greatly reduced in all three mutants [2–5]. Moreover, a deficiency in the glycosyl-transferases involved in the biosynthesis of cerebroside has now been shown in the Jimpy [6, 7], Quaking [7, 8] and msd [9] mice. Sulphatide synthesis also appeared to be altered since there was reduced incorporation of  $^{14}\text{C}$ -galactose into this lipid [7, 10]. However this type of study does not distinguish between the possibility that sulphatide biosynthesis is decreased because the precursor cerebroside is greatly reduced, and the possibility that the activity of the 3'-phosphoadenosine-5'-phosphosulphate—cerebroside sulphotransferase (PAPS—CST), responsible for synthesizing sulphatides from cerebroside, is depressed. The results presented show that in the three mutants, when compared to normal animals, the PAPS—CST activities in the brains were greatly reduced while they were unchanged in the kidneys.

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#### Abbreviations:

msd : myelin synthesis deficiency;  
PAPS—CST: 3'-phosphoadenosine-5'-phosphosulphate—  
cerebroside-sulphotransferase;  
CNS : central nervous system.

A preliminary communication concerning Jimpy and Quaking mutants has been presented previously [11]. Since then, a paper has been published [12] demonstrating that the incorporation of  $^{35}\text{S}$ -sulphate into sulphatides was impaired in the brain of the Jimpy mutant, but remained normal in the sciatic nerve.

### 2. Materials and methods

The three mutant strains were originally obtained from the Jackson Laboratory (Bar Harbour, Maine, USA) and bred in our laboratory. The standard cerebroside and sulphatides were purified from crudely dissected human brain white matter, and their composition established as previously described [13]. PAPS was prepared enzymatically by the procedure of Robbins [14, 15] and was separated from  $\text{Na}_2^{35}\text{SO}_4$  by descending chromatography.

A 10% brain and kidney homogenate in 0.32 M sucrose containing 1 mM  $\text{Na}_2\text{EDTA}$  and 3 mM  $\text{Na}_2\text{HPO}_4$  was centrifuged at 18,000 g for 20 min at  $2^\circ$  in a Spinco model L centrifuge (rotor 30). The resulting supernatant was centrifuged at 100,000 g for 60 min. The pellet obtained was suspended in the above sucrose solution containing 50% glycerol, to give a final concentration of about 5 mg of protein/ml, as determined by the method of Lowry et al. [16]. This preparation was used as a source of enzyme. It could be kept unfrozen at  $-20^\circ$  for at least 10 days without apparent loss of the enzyme activity. In some experiments a 10% whole brain homogenate was also used as a source of enzyme.

The enzymic transfer of sulphate from  $^{35}\text{S}$ -PAPS to endogenous galacto cerebroside in a brain homo-

genate was carried out by the method of Balasubramanian and Bachhawat [17]. PAPS-CST activity was assayed in the presence of  $^{35}\text{S}$ -PAP and exogenous galactocerebrosides with a biosynthetic system similar to the one described by McKhann and Ho [18]. Untreated microsomes were used as a source of enzyme instead of a deoxycholate solubilized microsomal preparation. Addition of ATP to the incubation medium

resulted in a two-fold increase of incorporation of  $^{35}\text{S}$  into sulphatides from  $^{35}\text{S}$ -PAP.

The appropriate amounts of cerebroside and detergent solutions in chloroform-methanol (2:1, v/v) were mixed and dried in tubes before addition of other components in the incubation mixture. The reaction was stopped by addition of 2.5 ml chloroform-methanol (2:1, v/v) and thorough mixing on a

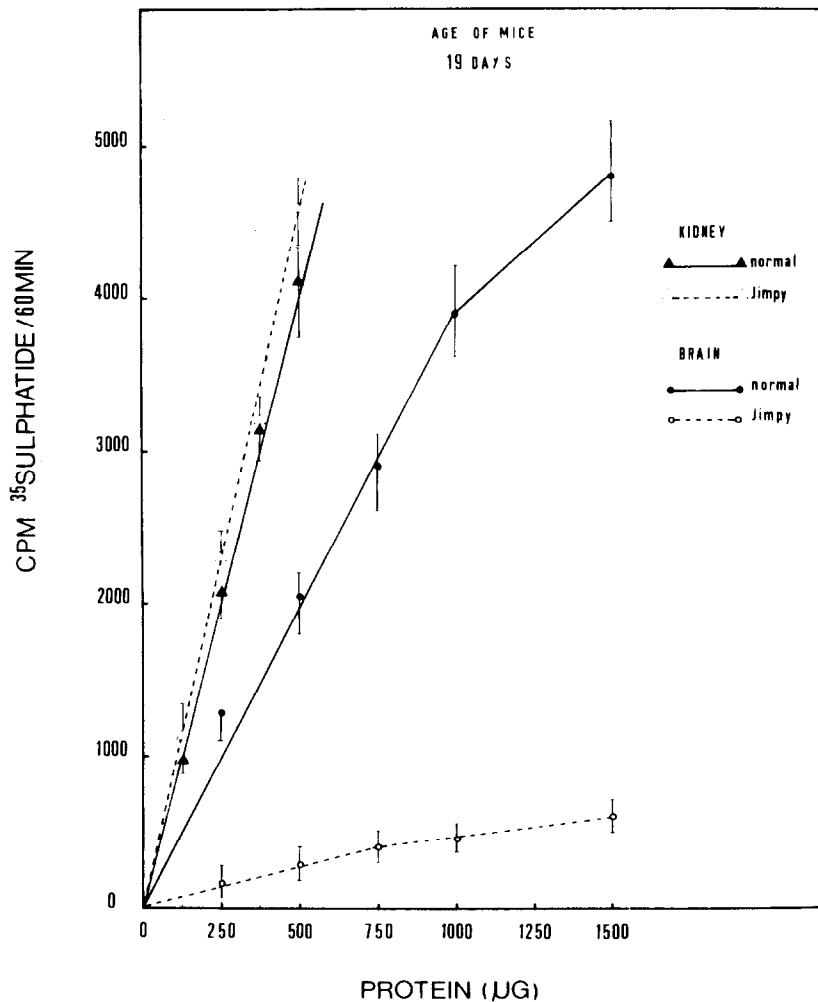


Fig. 1. *In vitro* formation of sulphatides from  $^{35}\text{S}$ -PAP in the presence of exogenous galactocerebrosides by the PAPS-CST of normal and Jimpy mice as a function of enzyme concentration. Incubations were performed in a vol. of 0.5 ml containing: 0.120  $\mu\text{mole}$  of galactocerebrosides; 4 mg of BRIJ 96; 50  $\mu\text{mole}$  of imidazole buffer (pH 7.0); 5  $\mu\text{mole}$  of ATP; 0.4  $\mu\text{mole}$  of  $\text{K}_2\text{SO}_4$ ; 2.5  $\mu\text{mole}$  of KCl; 130,000–150,000 cpm of  $^{35}\text{S}$ -PAP; and microsomal preparation, for 60 min at  $37^\circ$ . Assay of radioactivity and further experimental details as given in the text. Each point is the average of data from 3 experiments. Vertical bars represent the range of values.

rubber-tipped mechanical agitator. Carrier sulphatides were added. The samples were centrifuged briefly. The upper aqueous phase and the interfacial protein layer were discarded, while the lower phase was washed twice with 1 ml of "theoretical upper phase" [19] containing 0.88% potassium chloride to remove non-lipid contaminants. An aliquot of washed lower phase was dried in a counting vial. Scintillation fluid was added and the sample was counted in a liquid scintillation spectrometer (ABAC SL40, Intertechnique, France).

The labelled sulphatides were identified by subjecting the total lipid extract to thin-layer chromatography on silica gel G (Merck) using 5 different solvent systems. After chromatographic development, the thin-layer plate was exposed to X-ray film ("Kodirex", Kodak, France) for about 5 days, and the radioactive spots were identified by comparison with authentic sulphatides.

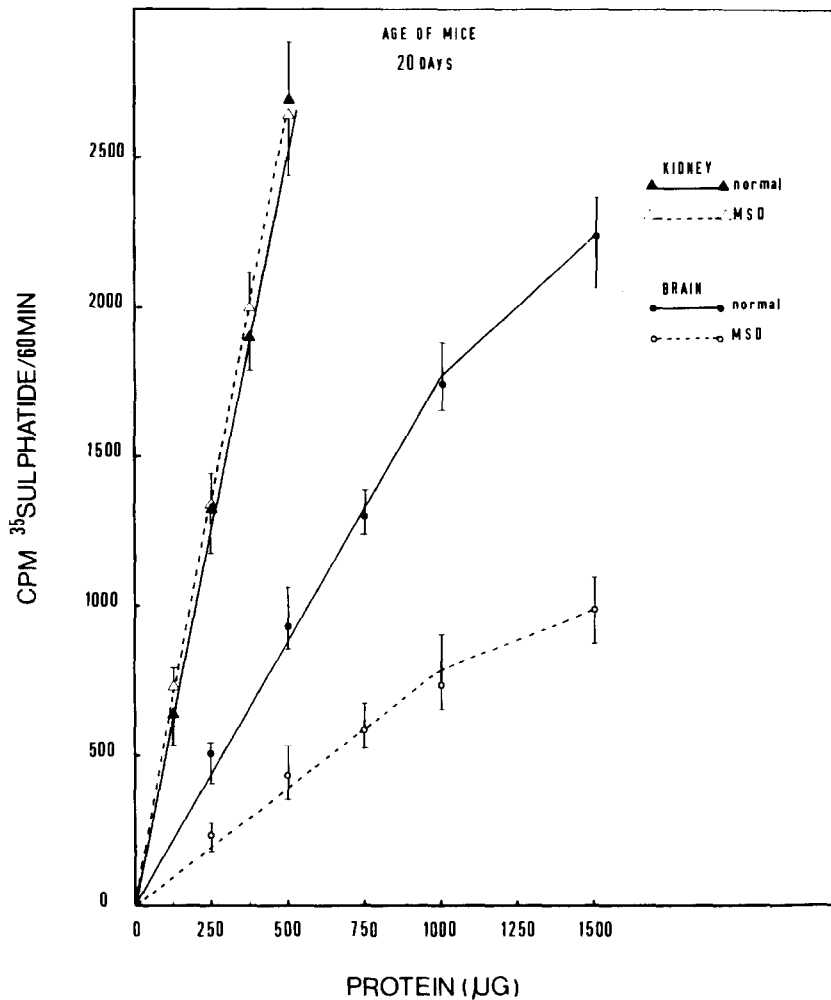


Fig. 2. Effect of the microsomal protein concentration on the PAPS-CST activity in normal and msd mice. The incubation system was similar to that described in fig. 1. Each point is the average of data from 3 experiments. Vertical bars represent the range of values.

### 3. Results and discussion

As a first step, we have demonstrated the enzymic transfer of sulphate from  $^{35}\text{S}$ -PAP to endogenous galactocerebrosides in brain homogenates. In experiments carried out with brain homogenates of 19 day old animals, the PAPS—CST activity was strikingly decreased (7 to 10-fold) in Jimpy mice compared to controls. As a second step, the formation of sulphatides from radioactive PAPS was studied using free exogenous galactocerebrosides as substrate.

The addition of galactocerebrosides in the incubation system containing normal or mutant brain microsomes increased the amount of synthesis of  $^{35}\text{S}$ -sulphatides 10 to 40-fold and even more, 32 to 52-fold with kidney microsomes.

Radioautographs demonstrated 2 well-labelled reaction products ( $R_f$  0.66 and 0.69 in the solvent system chloroform—methanol—2N ammonium hydroxide (60:35:8, v/v), corresponding to phrenosine sulphate and Kerasine sulphate, respectively. More than 90% of the total lipid radioactivity was found in the two

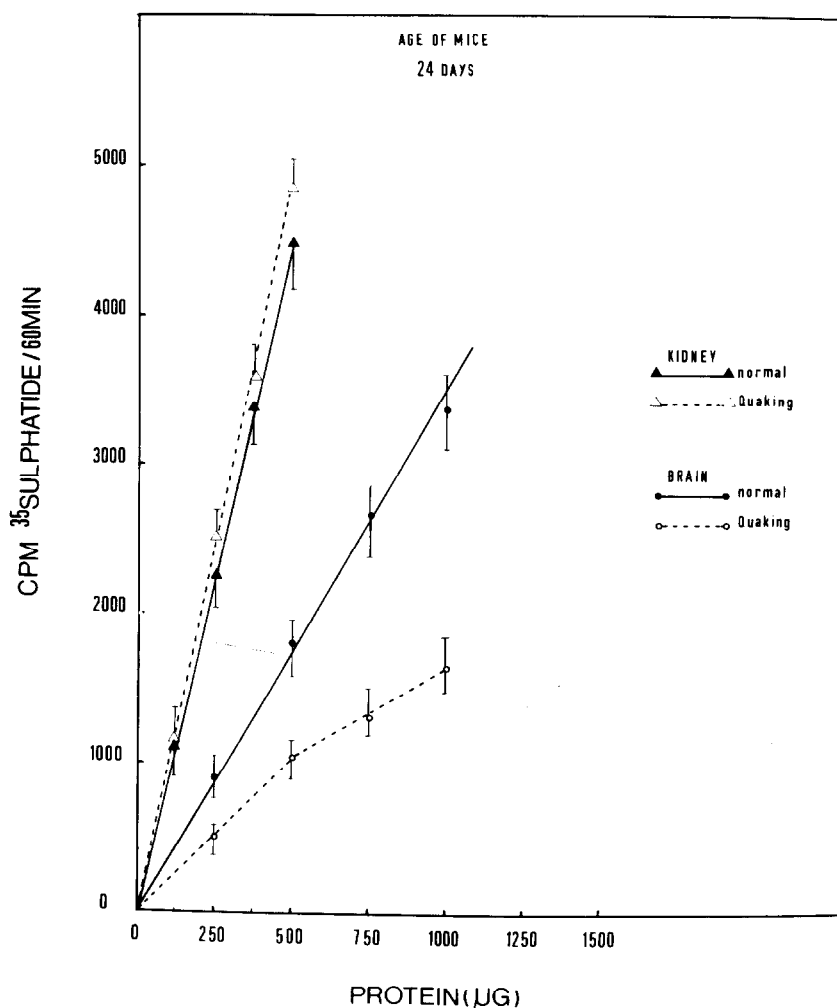


Fig. 3. Effect of the microsomal protein concentration on the PAPS—CST activity in normal and Quaking mice. The incubation system was similar to that described in fig. 1. Each point is the average of data from 3 experiments. Vertical bars represent the range of values.

fractions. The brain microsomal sulphotransferase activities were strikingly decreased in all three mutants, 4 to 7-fold in Jimpy (fig. 1), 2-fold in msd (fig. 2) and Quaking (fig. 3), as were brain sulphatides [2]. We assume that the observed decrease of sulphatide content is the result of a deficient biosynthesis rather than an increased catabolism. The latter alternative is rendered unlikely by the results of Bowen and Radin [20] and by our own results showing that the total arylsulphatase activity at 20 days of postnatal age was unchanged in msd mutant as compared to control brains. Crossed incubations performed with the normal and mutant microsomal fractions gave no evidence of activation or inhibition effects.

In normal mice, kidney PAPS-CST activity was about 2.5 times higher than in the brain. Using kidney microsomes as an enzyme source, no differences were found in PAPS-CST activity in any of the three mutants when compared to normal animals (figs. 1, 2 and 3). Therefore our findings suggest that the genetically determined disorder is limited to the CNS in all three mutants. This is in contrast to human and canine Globoid (Krabbe) leucodystrophy (GLD), where PAPS-CST activity is reduced in the kidney, although not as markedly as in GLD brain [21].

The results of this study provide strong evidence that the decrease of sulphatide synthesis in the mutant brains is not secondary to the lack of cerebroside available for sulphation, but results from a deficiency of the sulphotransferase activity. The observation that the deficiency of PAPS-CST activity is limited to the CNS, which is in accord with the results on other enzyme activities in mutant mice [7, 22], is of great interest considering the genetic control of enzyme biosynthesis. The data reported suggests an autonomic genetic control of enzyme synthesis in different cell types.

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