ENZYMATIC SYNTHESIS OF PSYCHOSINE IN "JIMPY" MICE BRAIN

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The previous studies [1–4] have provided the basic data on a biochemical disorder in the central nervous system (CNS) of "Jimpy" mouse, a neurological mutant characterized by a severe myelin deficiency. The most marked feature of "Jimpy" brain was a striking reduction of cerebrosides and sulfatides. It was suggested that a disturbance of galactolipid synthesis may be the primary cause of cerebroside and sulphatide reduction leading to a defective myelin formation [2, 3].

Experiments conducted *in vitro* [5–7] with brain tissue indicated that UDP-galactose is the precursor of the galactose moiety of cerebrosides, and that cerebrosides are formed from sphingosine via psychosine. Although the findings suggesting that cerebrosides may be formed directly from ceramide have been reported [8], psychosine seems to be more likely precursor.

The study described in this communication was undertaken in order to elucidate whether the psychosine pathway is involved in the metabolic error of "Jimpy" mice. The results presented show that the incorporation of radioactivity into psychosine following incubation of UDP-galactose-^{14}C and sphingosine with brain homogenate is significantly decreased in "Jimpy" brain.

UDP-galactose-^{14}C (uniformly labelled in the galactose moiety) was obtained from New England Nuclear Corp. (Boston). Natural sphingosine was prepared by hydrolysis of rat brain sphingolipids [9] and purified by silicic acid column chromatography. Psychosine was obtained after hydrolysis of rat brain cerebrosides [10] and purified by silicic acid column chromatography.

The freshly removed brains of 17-day-old "Jimpy" mice and the corresponding normal phenotypes were homogenized with 9 vol of cold 0.075 M Tris buffer containing 0.5 mM EDTA in a Potter-Elvehjem homogenizer. An incubation system similar to that described by Cleland and Kennedy [6] was used. Labelled products were identified by co-chromatography with carrier lipids with several solvent systems. As it is shown in fig. 1, after 60 min of incubation virtually all of the radioactivity detected was associated with the fraction corresponding to psychosine (91 and 96% in normal and "Jimpy" mice, respectively). In a considerably wide range (from 1–3 mg of protein), psychosine formation was proportional to the homogenate concentration (fig. 2). With normal brain homogenates the synthesis of psychosine was 5–8 times as high as that observed with "Jimpy" homogenates.

The possibility that an inhibitor is present was examined by the following experiment. To a complete incubation mixture containing 0.15 ml of normal brain homogenate, 0.15 ml of "Jimpy" homogenate was added. No decrease of enzymatic activity was observed as shown in table 1.

These findings, in addition to those previously reported [2, 3], support the statement that low cerebroside and sulphatide levels in mutant brain may result from an impairment of the galactolipid synthesis and may be interpreted as an evidence for the possibility that the metabolic error is located in the stage at which psychosine is formed. If this is so, the

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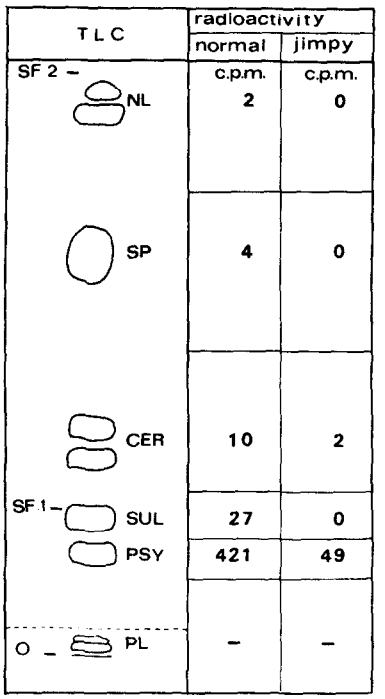

Fig. 1. Illustration of thin-layer chromatographic separation of radioactive lipids after incubation of UDP-galactose-^{14}C with normal and "Jimpy" brain homogenates. The reaction mixture contained 0.05 ml of 0.01 M UDP-galactose-^{14}C, 0.1 μC per μmole; 0.025 ml of 0.1 M $MgCl_2$; 0.05 ml of a 0.02 M solution of sphingosine in 2.5% Tween; 0.075 ml of 0.075 M Tris; 0.3 ml of a 10% brain homogenate. Incubation was 60 min at 37°. Reaction was terminated with 5 ml of chloroform-methanol (2:1, v/v) and carrier cerebrosides, sulphatides and psychosine were added, followed by 0.5 ml of H_2O. After mixing, the upper phase was discarded and the lower phase washed twice with 1 ml of chloroform-methanol-H_2O (3:47:48, v/v/v). The lipids in the lower phase were chromatographed on plates (20 X 24 cm) covered with a layer (0.5 mm) of silica gel H + 10% of finely ground Florisil using a two-step development technique [11]. The plates were first developed to the level 7 cm from the bottom with chloroform-acetone-pyridine-20% aq. ammonia-H_2O (20:30:60:2:2, v/v). The area of adsorbent including phospholipids (a 2.5-cm band starting at the bottom of the plate) was removed and chromatogram developed with chloroform-acetone-methanol-H_2O (65:30:12:2) to the top of the plate. Spots were revealed with 2',7'-dichlorofluorescein, scraped into counting vials and radioactivity determined by liquid scintillation techniques. *Identification of fractions: NL, neutral lipids; SP, sphingosine; CER, cerebrosides; SUL, sulphatides; PSY, psychosine; PL, phospholipids.* O = origine; SF1 = first solvent front; SF2 = second solvent front.

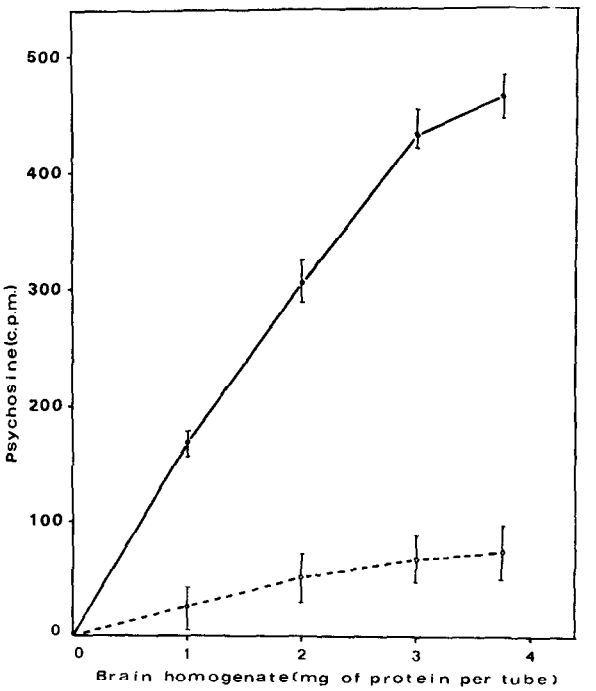

Fig. 2. Incubation of UDP-galactose-^{14}C with normal and "Jimpy" brain homogenates: formation of psychosine as a function of enzyme concentration. The incubation system was similar to that described in fig. 1. Each point is the average of data from two or three experiments. Vertical bars represent the range of values.

Table 1
Effect of "Jimpy" homogenate addition on the enzyme activity in normal brain

Brain homogenate in the incubation mixture *		Psychosine formed (cpm)
Normal (ml)	Jimpy (ml)	
0.15	0.15	202
0.15	–	137
–	0.15	37

* Other conditions for incubation and assay of radioactivity were those described in fig. 1.

results presented above are consistent with the view that the inborn metabolic defect of "Jimpy" mice may be related to the reduced activity of an enzyme (galactosyl-sphingosine transferase) catalyzing the formation of psychosine. The presence of this enzyme has been reported in guinea-pig and rat brain microsomes [6].

In this connection, it is of interest to note that in "Quaking" mouse, another myelin deficient mutant with similar biochemical and histological characteristics [1, 12], our preliminary results revealed a markedly lowered activity of psychosine formation.

Further studies are necessary to find if the results presented in this paper may be related to the histochemical observations [13], suggesting a serious alteration of the glial cells which are essential for myelination of CNS.

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