alignment, as given by the Langevin function, will be poor (*17*). Organization of the particles into chains similar to those in the magnetosomes of magnetotactic bacteria (*18*) will yield greater coupling energies and is consistent with the interactions between the particles detected in the dermethmoid tissue. The decay with warming of the IRM acquired by the dermethmoid tissue indicates that the particle groups are at least partially free to rotate. Taken together, these results suggest an association of the particles with a mechanoreceptor that detects the position or movement of the groups. Theoretical analyses (*19*) show that chains of 20 to 60 particles would provide ideal coupling energies with the geomagnetic field for use in magnetoreception. Assuming that the 8.5×10^7 particles detected in the dermethmoid tissue are arranged in such a fashion, a magnetite-based magnetoreception system in the yellowfin tuna could resolve magnetic field direction to within a few seconds of arc, or magnetic field intensity differences of 1 to 100 nT (*19*).

Gross dissection of the dermethmoid region of the yellowfin tuna revealed the supraophthalmic trunk nerve, which carries branches of the trigeminal, facial, and anterior lateral line nerves and which ramifies in the ethmoid region. Histological studies have suggested the presence of nerve axons in the dermethmoid tissue (*20*). A suitable physical and possible neural basis for previously demonstrated behavioral responses to magnetic fields has thus been demonstrated for the first time in one species. Our magnetometry results are consistent in phylogenetically distant fishes (*12*) and, along with similar results for other vertebrates (*1, 4, 5*), suggest that the ethmoid region of the skull is a likely site for a vertebrate magnetic sense organ.

Note added in proof: Magnetite crystals isolated from the dermethmoid tissue of chinook salmon, *Oncorhynchus tshawytscha*, are organized in chains when viewed in TEM (*21*).

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Progressive Accumulation of Toxic Metabolite in a Genetic Leukodystrophy

Abstract. *Progressive accumulation of a cytotoxic metabolite, galactosylsphingosine (psychosine), was found in the brain of the twitcher mouse, a mutant caused by genetic deficiency of galactosylceramidase. Similar abnormal accumulation was also found in the brain of the genetic galactosylceramidase deficiency disease in the dog and in human patients (globoid cell leukodystrophy or Krabbe disease). Galactosylsphingosine was absent in the brains of normal and heterozygous mice. The finding provides support for the psychosine hypothesis as the biochemical pathogenetic mechanism of globoid cell leukodystrophy. Analogous mechanisms may be important in the pathogenesis of other genetic lysosomal diseases.*

Genetic galactosylceramidase deficiency (globoid cell leukodystrophy or Krabbe disease) is known in several mammalian species, including the human (*1*), the dog (*2*), and the mouse (*3*). It is a rapidly progressive fatal disorder with clinical and pathological manifestations almost exclusively restricted to the nervous system, particularly to the white matter and the peripheral nerves. Conceptually the disease is related to the lysosomal storage disorder of Hers (*4*). Paradoxically, abnormal accumulation of galactosylceramide, the natural substrate of the missing enzyme, does not occur in the nervous system despite the genetic catabolic block (*2, 5, 6*).

Miyatake and Suzuki (*7*) in 1972 proposed a hypothesis for the biochemical mechanism that leads to the absence of galactosylceramide accumulation and to the devastating pathology of the white matter, particularly the rapid and total degeneration of the oligodendroglia. The hypothesis has become known as psychosine hypothesis, because a toxic metabolite, galactosylsphingosine (psychosine), which is also a substrate of galac-

Table 1. Galactosylsphingosine (psychosine) in the brains of dogs with globoid cell leukodystrophy. The brain of the 7-month-old affected dog was analyzed for gray and white matter separately. The other specimens were approximately equal mixtures of gray and white matter. The 1.8-month-old carrier and affected dogs were littermates.

Age (months)	Brain galactosylceramidase (nmole hour^{-1} mg^{-1})	Genetic status	Psychosine (ng/100 mg)
1.8	1.39	Carrier	Undetectable
> 2	5.14	Normal	Trace
18	1.93	Carrier	35
1.8	0.32	Affected	420
6	0.39	Affected	2500
7	0.33	Affected	100 (gray)
7	0.38	Affected	4310 (white)

tosylceramidase, is assumed to be responsible for the unusual pathological and analytical abnormalities of the disease. In 1980, Svennerholm *et al.* (*8*) found increased amounts of galactosylsphingosine in the white matter of five human patients. The human data were obtained by necessity at the terminal stage of the disease. The abnormality in the mouse mutant, the twitcher, is genetically equivalent to the human disease and allows controlled studies of the genetic galactosylceramidase deficiency throughout the course of the disease. Our results, which indicate an early and rapidly progressive accumulation of psychosine in the brain of affected mice, provide the strongest support to date for the psychosine hypothesis.

To analyze exceedingly small amounts of galactosylsphingosine in individual mouse brains of various ages, we developed a sensitive and specific analytical procedure involving extraction of lipids, a series of fractionation procedures, dansylation, and finally fluorescent densitometry of dansylated psychosine, which has been separated by thin-layer chromatography (*9*). We can reliably determine 50 ng of galactosylsphingosine in 100 mg of brain tissue, and the detection limit is estimated to be 5 ng per 100 mg.

A colony of twitcher mutant mice is maintained in our institution. The genetic status of individual mice—normal, heterozygous, and affected—was routinely determined by galactosylceramidase assays on clipped tips of the tail soon after birth (*10*). Affected mice and a similar number of control (normal or heterozygous) mice were killed at 7, 16, 25, 36, 37, and 38 days. Generally the whole brain was analyzed; however, brains of three 37-day-old mice were dissected to the cerebral cortex and the pons-medulla and analyzed separately to assess the distribution of psychosine between the gray and white matter. In addition, brain tissues from affected dogs and human patients were analyzed together with tissues from appropriate controls. Many of the canine and human specimens had been stored frozen at −30° or −80°C for several years and were defrosted at least once during the period. The undetectable or trace amounts of psychosine in frozen-stored control specimens assured that no inadvertent hydrolysis of galactosylceramide to galactosylsphingosine occurred during the storage.

Psychosine was undetectable in normal or heterozygous mouse brains at any age examined, except in one at 36 days, in which a trace amount (< 10 ng per 100 mg) was detected (Fig. 1). Even at 7 days, before the onset of any significant myelination, there were readily detectable amounts of psychosine in the brain of affected mice. At 16 days, galactosylsphingosine was already at least 20 times normal on the basis of the estimated detection limit of 5 ng per 100 mg. The accumulation was rapidly progressive, reaching more than 100 times the normal level near the terminal stage. Since psychosine was not detectable in control brains, these estimates relative to the normal value are underestimates. The results on the dissected brain of the three 37-year-old twitcher mice were 300, 260, and 210 ng per 100 mg for cerebral cortex and 1840, 1660, 1130 ng per 100 mg for the pons-medulla, a clear indication that psychosine accumulation occurs predominantly in the white matter.

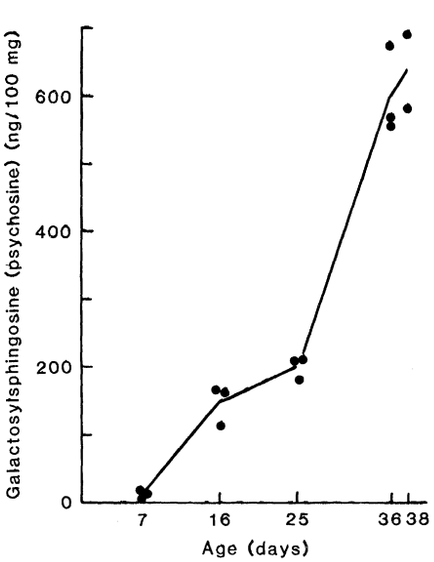

Fig. 1. Galactosylsphingosine (psychosine) in the brain of the twitcher mouse. Psychosine was undetectable in all normal and heterozygous control brains at all ages, except for one 36-day-old brain which showed a trace amount (< 10 ng per 100 mg).

Psychosine was undetectable in the brain of a 1.8-month-old carrier dog but was detectable in one young normal dog and one older carrier dog (Table 1). However, already at 1.8 months, the concentration of psychosine in the brain of the affected dog was very high. It increased to an enormous level by 6 months. The preferential accumulation in the white matter was more dramatic in the dog brain than in the mouse brain. We also confirmed the abnormal accumulation of psychosine in brains of human patients. Psychosine was undetectable in control brains, including one from a patient with Tay-Sachs disease. In four human patients, the psychosine values ranged from 120 ng per 100 mg in gray matter to nearly 2000 ng per 100 mg. We measured 1680 ng per 100 mg in the lipid extract of white matter from a patient included in the series of Svennerholm *et al.* (*8*).

The psychosine hypothesis for the pathogenesis of genetic galactosylceramidase deficiency includes the following steps. (i) Psychosine is a substrate for galactosylceramidase, which is genetically deficient in the disease. (ii) Since psychosine is chemically and metabolically related to galactosylceramide, and since galactosylceramide is almost exclusively localized in the myelin sheath and its parent cells—the oligodendroglia and the Schwann cells—psychosine accumulates within these myelin-generating cells. (iii) Psychosine is highly cytotoxic (*5*) and selectively kills the cells within which it accumulates. (iv) Since biosynthesis of galactosylceramide is restricted to the myelin-generating cells, their degeneration effectively terminates further synthesis at an early stage of myelination. (v) Myelination continues rapidly in normal brain and thus the amount of galactosylceramide in affected brain will be lower than normal, even though catabolism of galactosylceramide is genetically blocked.

Step (i) above has been experimentally demonstrated (*11*). Cytotoxicity of psychosine has been amply reported [see (*5*)] (step iii). That synthesis of galactosylceramide is restricted to the myelin-generating cells has been shown with

bulk-isolated cell preparations (*12*). Our results now clearly demonstrate that in genetic galactosylceramidase deficiency, rapidly progressive accumulation of psychosine in white matter occurs from very early stages of the disease. This finding establishes step ii of the psychosine hypothesis.

Although the fundamental genetic defects of most of the lysosomal storage disorders have been clarified, the molecular mechanisms that lead to the clinical and pathological manifestations remain largely obscure. While the psychosine hypothesis has by no means been proven, evidence accumulated in the past dozen years since its proposal has made it a probable explanation for the biochemical pathogenesis of globoid cell leukodystrophy. Similar mechanisms may play important roles in the pathogenesis of other lysosomal storage diseases. In an analogous disease, Gaucher disease, which is caused by a genetic defect of glucosylceramidase, presence of glucosylsphingosine in the spleen has been shown (*13*), and more recent data suggested a correlation between the amount of glucosylsphingosine in the brain of patients and the degree of neurological involvement (*14*). In other diseases also, abnormal accumulation of "normal" constituents may not be sufficient to account for clinical and pathological manifestations. Attention to possible involvement of "abnormal" constituents may prove rewarding.

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Is α_1-Protease Inhibitor Inactivated by Smoking?

Stone *et al.* (*1*) report that functional α_1-protease inhibitor (α_1-PI) in the lower respiratory tract is not decreased by cigarette smoking, whereas Gadek *et al.* (*2*) and Carp *et al.* (*3*) found decreased functional α_1-PI in the lower respiratory tract of smokers. Carp also found 4 mole of methionine sulfoxide per mole of inactive α_1-PI in lung lavage fluids of smokers but oxidized methionine was not found in α_1-PI of nonsmokers (*3*). Oxidation of two to four methionine residues in α_1-PI has been associated with loss of its inhibitory activity toward human neutrophil elastase (*4, 5*). The apparent contradictions between Stone's observations and the above findings are perplexing and suggest that further studies are needed. In future experiments, it may be helpful if the following points are borne in mind.

Ideally, focal areas of centrilobular emphysema would be sampled, rather than whole lung. This being impractical, some effort should at least be made to lavage superior segments of the lung, since lesions of centrilobular emphysema in smokers are more common and more severe in the upper than in the lower zones of the lung (*6*). Also, subject selection may be critical; heavier smokers of unfiltered high-tar cigarettes should be preferred, especially those with signs of airflow limitation.

Furthermore, as mentioned by Stone *et al.* in their report, the time interval between smoking and lavage may be an important variable. One of us (S.K.C.) recently explored the time course of lung α_1-PI inactivation in mice after short-term and long-term exposure to smoke. Inactivation of α_1-PI occurred much sooner after a standard smoke exposure if the animals had been repeatedly exposed to smoke beforehand. Recovery of normal values of lung α_1-PI activity after the standard smoke exposure also occurred earlier in mice that inhaled smoke frequently than in mice never previously challenged with smoke. Despite this, the extent of α_1-PI inactivation was the same or greater in the group repeatedly exposed to smoke. Although these results were obtained in an animal model and their direct extrapolation to humans may be unwarranted, humans who are heavy smokers could also have significant decreases in lung α_1-PI activity immediately after smoking, but these decreases may be transient.

We agree with Stone *et al.* that "additional studies of the effects of cigarette smoke on α_1-PI in the lower respiratory tract are needed" and look forward to an eventual resolution of this controversy.

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Stone *et al.* (*1*) recently reported that functional α_1-protease inhibitor (α_1-PI) in the bronchoalveolar lavage fluid of smokers is not decreased. They cited our study (*2*) in which we assayed α_1-PI in lung fluid from young smokers who were subjected to bronchoalveolar lavage before smoking, and again 10 to 60 minutes after smoking. We later studied another 18 smokers and found no significant decrease in α_1-PI activity in lung fluids of smokers after smoking, except in smokers who were subjected to lavage 1 hour after smoking two cigarettes; these smokers showed a statistically significant mean decrease of 10 percent in α_1-PI activity compared with the values before smoking.

Stone *et al.* reported that α_1-PI in the lung fluids of nonsmokers was only 47 percent active, whereas the data of Carp *et al.* (*3*) indicated a mean activity of 118 percent; we found it about 90 percent active. We had initially reported (*2*) that