A mouse model for Niemann-Pick disease: phospholipid class and fatty acid composition of various tissues

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Abstract Recently, a strain of mice bearing an autosomal recessive gene, *spm,* has been described. On the basis of clinical and pathological findings these mice have been suggested as a useful model of human Niemann-Pick disease. Phospholipids and their fatty acid compositions were analyzed for liver, spleen, whole brain, erythrocytes, and blood plasma from "Niemann-Pick" animals (*spm/spm*) and heterozygous controls (*spm/+*). Sphingomyelin and bis(monoacylglycero)phosphate accumulated in the liver and spleen of the affected mice, whereas no significant proportional change of phospholipids was observed in the whole brain. The phospholipid composition in erythrocytes and blood plasma of the homozygous mice was not different from that of the heterozygous controls. The fatty acyl chain profile of accumulated bis(monoacylglycero)phosphate was characterized by the high content (more than 80%) of unsaturated fatty acids; the main components were oleic acid, linoleic acid, and docosahexaenoic acid. A high unsaturation index of the fatty acyl chain was found in sphingomyelin accumulated in organs and in almost all phospholipids of brain, erythrocytes, and blood plasma of "Niemann-Pick" mice. It is conceivable that desaturation of fatty acids is enhanced in the "Niemann-Pick" mice.—**Nakashima, S., K. Nagata, Y. Banno, T. Sakiyama, T. Kitagawa, S. Miyawaki, and Y. Nozawa.** A mouse model for Niemann-Pick disease: phospholipid class and fatty acid composition of various tissues. *J. Lipid Res.* 1984. **25:** 219–227.

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Niemann-Pick disease is a hereditary lipid storage disorder of humans characterized by degenerative neuropathy and hepatosplenomegaly, with accumulation of sphingomyelin. However, the low frequency of this disease in humans has made detailed analyses difficult. Therefore, an animal model should contribute significantly to clarification of the underlying mechanism(s) of the disease and to application of new therapeutic trials such as enzyme replacement.

In 1975 a mutant strain of C57BL/KsJ mice with a lipid storage disease was found in the Research Laboratories, Nippon Shinyaku Co., Ltd. Clinical and genetic studies (1) revealed that a single autosomal recessive gene, *spm,* is responsible for the disease. Physical abnormalities in homozygous affected mice (*spm/spm*) begin at approximately 7 weeks of age in the form of a lack of motor coordination, coarse tremor of the body and extremities, and weight loss. They become progressively unable to walk and finally die at 12–14 weeks. Histopathological examination has revealed striking hepatosplenomegaly and marked enlargement of lymph nodes. Extensive infiltration of foamy cells has been found in the liver and spleen. Sphingomyelin and free cholesterol have been reported to accumulate in these two visceral organs (1). Sphingomyelinase activity is reduced to 30% of that in control liver, 50% in the spleen, and 70–80% in the brain (1, 2). These clinical and pathological features suggest that this mutant strain is a potentially useful animal model for human Niemann-Pick disease.

MATERIALS AND METHODS

Mice

Mice were reproduced by transplanting affected female ovarium into normal mice. The detection of affected mice was carried out by observance of splenomegaly through a laparotomy at 4 weeks of age in order to distinguish

Abbreviations: BMP, bis(monoacylglycero)phosphate; DPG, diphosphatidyl glycerol; FC, free cholesterol; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

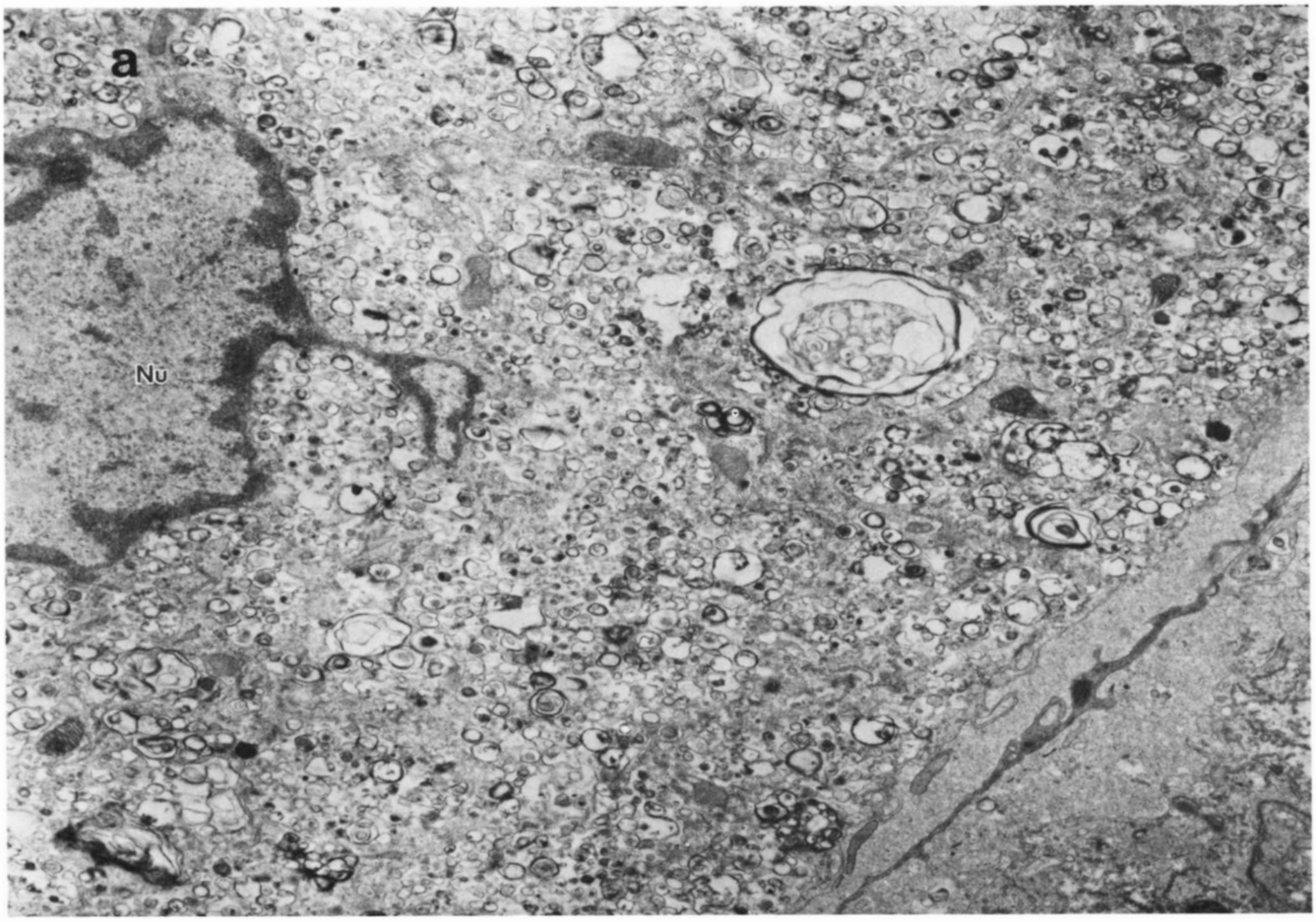

Fig. 1. Thin section (a) and freeze-fracture (b) electron micrograph of a foamy cell in "Niemann-Pick" mouse liver. Nu; nucleus. (a) ×14,400. (b) ×43,200.

affected mice (*spm/spm*) from carriers (*spm/+*). In this report, male homozygous-abnormal (*spm/spm*) and heterozygous-carrier (*spm/+*) mice as controls were used at 8 to 9 weeks of age. Heterozygotes are normal in both sphingomyelinase activity and lipid composition (1, 3). All mice were fed a standard laboratory diet and were maintained in the Research Laboratories, Nippon Shinyaku Co., Ltd.

Tissues

Mice were exsanguinated via the retro-orbital sinus by means of heparinized pipettes before tissue collection. Liver, spleen, and brain were removed immediately after the mice had been killed. The tissues were washed and perfused with isotonic saline. Plasma and erythrocytes were separated by centrifugation at 3000 rpm for 10 min and erythrocytes were washed three times with isotonic saline.

Electron microscopy

The liver specimens for thin section electron microscopy were fixed in a solution of 4% glutaraldehyde and 2% OsO_4 (1:1 v/v) in Milloning buffer (4). Dehydration was carried out in a graded ethanol series and specimens were embedded in Spurr's (5) low viscosity plastic. Sections were cut on a Porter-Blum Mt-2 ultramicrotome and stained with uranyl acetate and citrate. For freeze-fracture electron microscopy, samples were fixed with 2% glutaraldehyde and then suspended in a fresh solution of 30% glycerol in 0.85% NaCl (w/v). After freezing in Freon-12 and liquid nitrogen, these samples were fractured and shadowed with platinum-carbon at −110°C in a Hitachi HFZ-1 freeze-fracture apparatus. Thin sections and freeze-fracture replicas were examined in a JEM-100U electron microscope.

Lipid extraction

The lipids of erythrocyte membranes were extracted using isopropanol and chloroform according to the method of Broeckhuyse (6). The plasma lipids were extracted by the method of Folch, Lees, and Sloane Stanley (7). Individual tissues were homogenized in 4 ml of ice-cold water prior to lipid extraction, which was performed

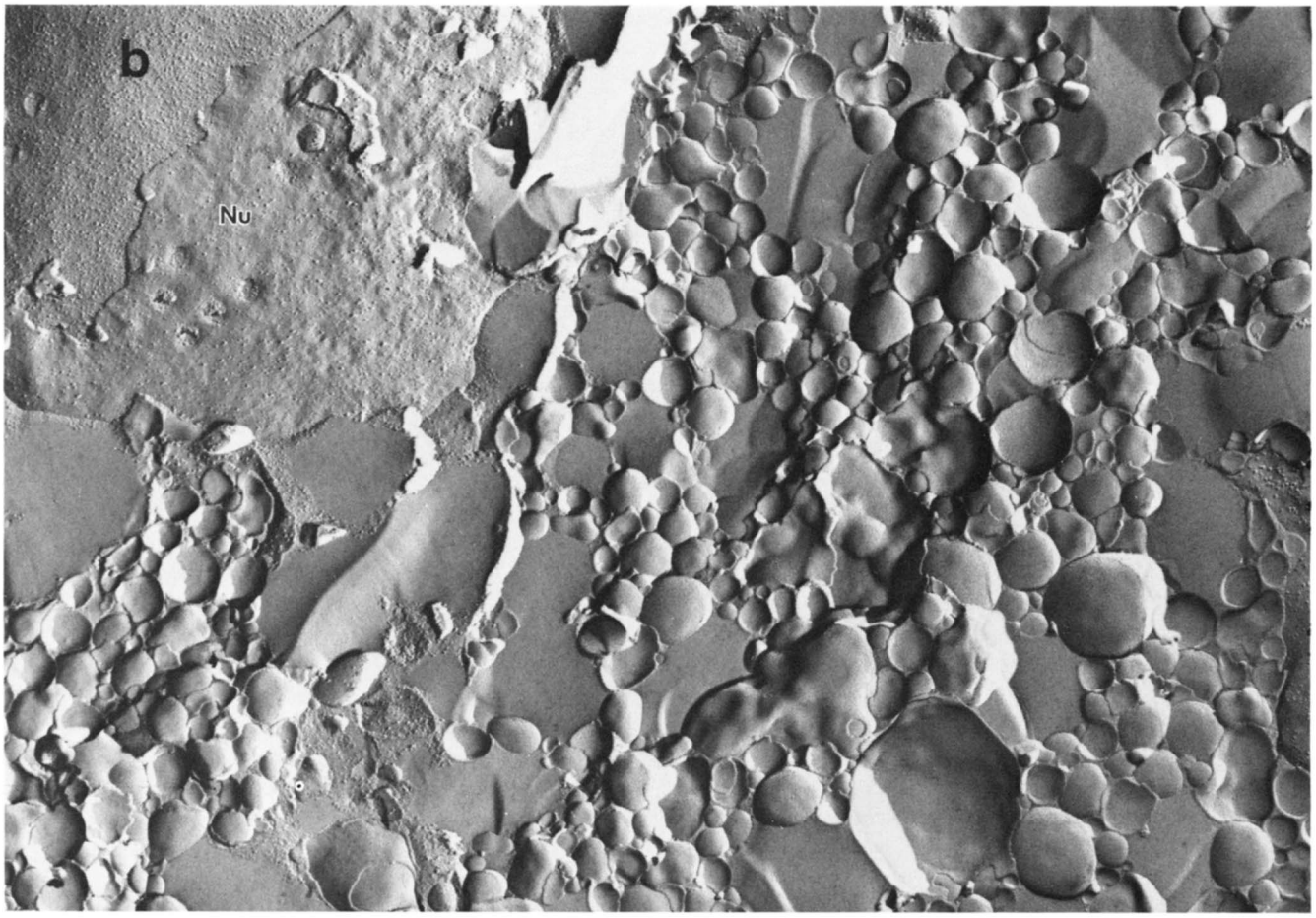

Fig. 1. (*Continued*)

according to the procedure of Bligh and Dyer (8). Lipids were dissolved in 1 ml of chloroform–methanol 6:1 and flooded with nitrogen gas, then stored at −20°C.

Lipid class analysis

The phospholipid classes of erythrocyte membranes and blood plasma were separated by one-dimensional thin-layer chromatography on Silica Gel H (Merck), in a solvent system of chloroform–methanol–acetic acid–water 60:30:8:4 (v/v) (9). Tissue phospholipids were analyzed by two-dimensional thin-layer chromatography on Silica Gel 60 (Merck), impregnated with magnesium acetate (2.5% w/v), using chloroform–methanol–13.5 N ammonia water 65:35:5.5 (v/v) in the first dimension, and chloroform–acetone–methanol–acetic acid–water 3:4:1:1:0.5 (v/v) in the second development (10). For quantitative analysis of individual phospholipids, plates were sprayed with 50% H_2SO_4 and charred. Spots corresponding to individual phospholipid classes were then scraped off the plates and analyzed for total phosphorus content as described by Rouser, Siakotos, and Fleischer (11).

Fatty acid analysis

The fatty acid composition of individual phospholipids was determined by gas–liquid chromatographic analysis of the fatty acid methyl esters (12). Analysis was carried out on a Shimazu GC6A gas chromatograph with a glass column packed with 15% DEGS on Chromosorb W 80–100 mesh (Nihon Chromato Industrial Co., Tokyo). The flow rate of carrier nitrogen gas was approximately 50 ml/min. The column temperature was maintained at 200°C and the injector and ion detector temperatures at 250°C. For fatty acid analysis of each phospholipid, one-dimensional thin-layer chromatography was used to separate individual phospholipids and plates were exposed to I_2 vapor to locate major phospholipids. The corresponding areas on Silica Gel H plate were then scraped into ampules and 1 ml of 10% BF_3–methanol was added. The ampules were flushed with nitrogen gas, sealed, and heated in boiling water for 1 hr. Methyl esters were then extracted with n-hexane and analyzed, and individual peaks were identified by comparing retention times with those of authentic standards.

RESULTS

Pathological feature

By 8 weeks *spm/spm* mice showed striking hepatosplenomegaly and liver weights became approximately twice those of heterozygous controls (2.0 ± 0.2 g for *spm/spm* mice versus 1.1 ± 0.1 g for *spm/+* mice). Large areas of the liver and spleen were occupied by numerous foamy cells that were filled with inclusions of lamellar structure (**Fig. 1a**). By freeze-fracture electron microscopy, these lamellar inclusions showed vesicle-like structures that were free of membrane protein particles (Fig. 1b). It has been suggested that this lamellar appearance indicates the deposition of lipids (13).

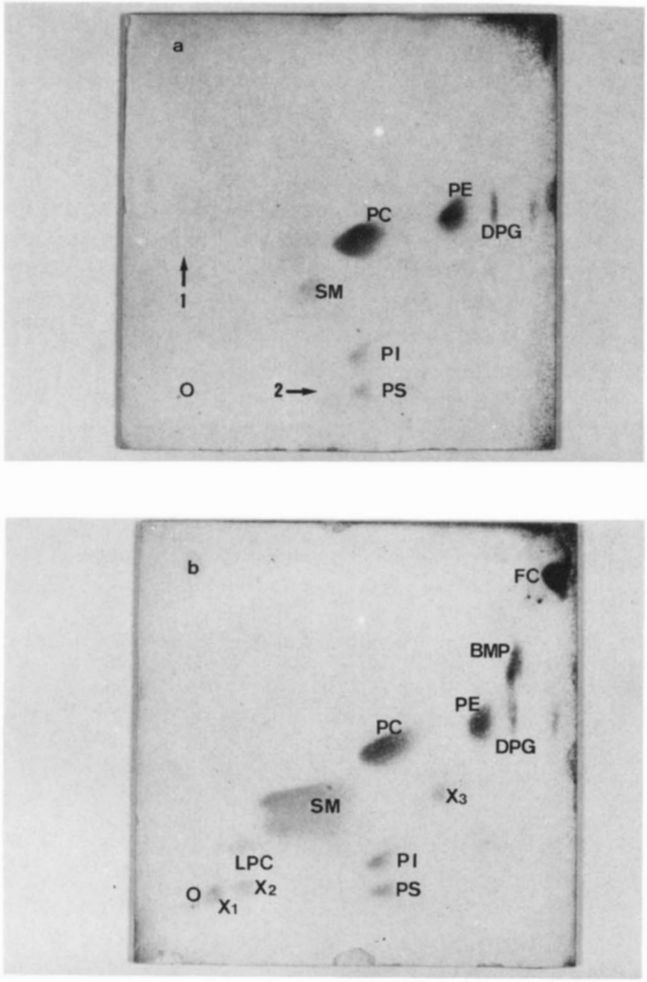

Fig. 2. Thin-layer chromatography of the liver lipids. The extracted lipids were separated using the solvent system as described under Materials and Methods. For visualization of the spots, the plates were sprayed with 50% H_2SO_4 and charred. (a) Control liver; (b) affected liver. Abbreviations used: BMP, bis(monoacylglycero)phosphate; DPG, diphosphatidyl glycerol; FC, free cholesterol; LPC, lysophosphatidylcholine; O, origin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; Xs, non-phosphorus-containing lipids.

TABLE 1. Phospholipid composition of various tissues in Niemann-Pick (*spm/spm*) and carrier (*spm/+*) mice[a]

Phospholipid	Liver		Spleen		Brain		Erythrocytes		Plasma	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
SM[b]	4.3 + 0.5	32.5 + 1.0[c]	7.5 + 1.6	32.1 + 0.9[c]	3.5 + 1.8	5.5 + 1.3	11.2 + 0.9	11.0 + 0.1	4.6 + 0.6	1.5 + 0.9
PC[b]	48.6 + 1.4	31.1 + 1.4[c]	43.8 + 1.7	31.5 + 1.1[c]	37.8 + 1.7	39.7 + 1.1	43.2 + 1.5	44.3 + 0.8	73.4 + 2.6	76.9 + 1.9
PE[b]	28.7 + 1.1	14.0 + 1.0[c]	27.8 + 1.9	14.9 + 0.9[c]	40.1 + 1.9	37.9 + 1.2	27.5 + 1.4	27.3 + 1.8	5.8 + 1.2	3.8 + 1.7
PS[b]	3.0 + 0.6	2.4 + 0.8	7.6 + 1.3	5.4 + 1.2	9.5 + 0.9	7.6 + 1.1	14.0 + 0.5	13.4 + 1.9	0.3 + 0.4	1.1 + 0.5[c]
PI[b]	4.7 + 0.7	4.2 + 0.5	5.3 + 1.2	2.3 + 0.4[c]	4.1 + 0.8	5.3 + 1.1				
LPC[b]	5.2 + 1.1	6.7 + 0.8	1.8 + 1.4	3.5 + 1.9	1.5 + 0.9	2.2 + 1.5	2.1 + 1.8	3.0 + 0.5	16.3 + 2.3	16.3 + 1.8
BMP[b]	0.8 + 0.7	7.9 + 0.7[c]	1.0 + 1.2	8.2 + 1.1[c]	0.6 + 0.4	1.6 + 1.2	0.3 + 0.4	0.4 + 0.7	n.d.[d]	n.d.[d]
Others	3.4 + 1.5	2.2 + 0.8	4.3 + 1.3	1.9 + 0.3	1.5 + 1.1	1.2 + 0.9	1.1 + 0.7	1.0 + 0.5	0.6 + 0.4	0.3 + 0.3

[a] The results are expressed as percent of total phosphorus and include the mean ± SD of seven (*spm/spm*) or four (*spm/+*) different animals.
[b] Abbreviations: SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; BMP, bis(monoacylglycero)phosphate.
[c] Significantly different ($P < 0.01$, paired *t*-test) from *spm/+* value.
[d] Not detected (<0.1%).

TABLE 2. Fatty acid composition of individual phospholipids in liver[a]

Fatty Acid	Sphingomyelin		Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol + Phosphatidylserine	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
				area percent				
16:0	12.9 ± 2.2	24.6 ± 1.2[b]	31.6 ± 0.5	36.7 ± 1.6[b]	18.8 ± 1.7	16.1 ± 1.1	9.0 ± 0.8	7.9 ± 0.7
18:0	5.6 ± 1.5	3.7 ± 0.8	12.1 ± 0.4	12.3 ± 0.9	21.0 ± 0.7	16.5 ± 1.8[b]	41.0 ± 0.6	42.0 ± 3.2
18:1 (n-9)	5.2 ± 0.9	0.8 ± 0.3[b]	7.3 ± 0.4	8.8 ± 1.8	6.8 ± 0.6	14.7 ± 2.9[b]	4.1 ± 0.4	4.7 ± 0.9
18:2 (n-6)	8.1 ± 2.0	0.9 ± 0.7[b]	26.5 ± 1.0	19.3 ± 1.6[b]	10.3 ± 0.8	9.5 ± 0.6	4.9 ± 0.4	4.9 ± 0.4
20:4 (n-6)			7.6 ± 0.6	9.4 ± 1.1	15.6 ± 0.9	14.0 ± 1.9	22.8 ± 2.9	23.1 ± 1.2
22:0	16.3 ± 2.7	13.2 ± 1.2						
22:2 (n-6)	5.6 ± 0.6	2.6 ± 1.2[b]						
22:5 (n-3)	1.0 ± 0.9	6.3 ± 0.7[b]						
22:6 (n-3)			9.6 ± 0.7	9.7 ± 1.1	20.2 ± 1.3	19.0 ± 2.7	9.2 ± 1.1	9.1 ± 0.7
24:0	15.1 ± 1.7	13.9 ± 2.3						
24:1 (n-9)	18.0 ± 1.3	29.4 ± 4.9[b]						

[a] The results are presented as mean ± SD of seven (*spm/spm*) or four (*spm/+*) different animals and minor components less than 2% are not included in this table.
[b] Significantly different ($P < 0.01$, paired *t*-test) from *spm/+* value.

Phospholipid composition

Thin-layer chromatography of the liver lipids from *spm/spm* mice showed a marked increase in sphingomyelin, bis(monoacylglycero)phosphate, free cholesterol, and other non-phosphorus-containing lipids (probably glycolipids) (**Fig. 2a** and **2b**). Similar findings were observed in lipids extracted from the spleen of the homozygous mice, whereas no compositional changes occurred in the whole brain (data not shown). **Table 1** summarizes the phospholipid composition of various tissues. The sphingomyelin contents of the liver and spleen of homozygous mice were elevated 7.5-fold and 4-fold, respectively, as compared to control animals. In both tissues, the level of sphingomyelin exceeded that of phosphatidylcholine, a main component in normal mice. In addition, a large amount of bis(monoacylglycero)phosphate (BMP) was found to accumulate in these visceral organs, with concomitant decreases in other glycerophospholipids. This unique phospholipid (BMP) is a compositional isomer of phosphatidylglycerol in which each glycerol is esterified with one fatty acid only. It is localized exclusively in lysosomes and comprises merely 1% or less of the total phospholipid P in normal tissues. On the other hand, no obvious changes were observed in the lipid composition of the whole brain, although the homozygous abnormal mice had severe neurological symptoms similar to those seen in human Niemann-Pick disease. Furthermore, there

TABLE 3. Fatty acid composition of individual phospholipids in spleen[a]

Fatty Acid	Sphingomyelin		Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol + Phosphatidylserine	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
				area percent				
16:0	31.0 ± 1.1	30.7 ± 2.2	41.0 ± 1.2	47.3 ± 1.5[b]	6.9 ± 0.4	7.7 ± 0.5	5.8 ± 0.2	7.9 ± 0.9
16DMA					9.4 ± 0.7	7.8 ± 0.8		
18:0	8.6 ± 0.6	4.6 ± 0.9[b]	9.1 ± 0.4	8.7 ± 2.6	16.6 ± 1.2	14.6 ± 0.9	42.5 ± 0.7	40.8 ± 1.9
18DMA					3.8 ± 0.6	2.7 ± 0.5		
18:1	2.4 ± 0.5	0.8 ± 0.5[b]	9.9 ± 0.4	9.4 ± 0.2	6.1 ± 0.3	12.4 ± 1.3[b]	7.0 ± 0.5	7.5 ± 1.4
18:2	2.1 ± 0.8	0.7 ± 0.6	16.4 ± 0.9	14.6 ± 1.6	6.4 ± 0.7	11.9 ± 0.9[b]	6.9 ± 1.3	8.2 ± 2.5
20:0	3.5 ± 0.3	1.9 ± 0.2[b]						
20:4			14.9 ± 0.3	14.4 ± 0.4	19.8 ± 0.5	17.8 ± 2.2	19.0 ± 0.7	17.1 ± 2.9
22:0	7.6 ± 0.1	9.0 ± 0.6[b]						
22:4					2.7 ± 0.4	2.6 ± 1.5	2.2 ± 0.2	2.1 ± 0.8
22:5	8.4 ± 0.2	12.1 ± 0.5[b]			4.8 ± 0.2	3.4 ± 0.4[b]	3.1 ± 0.1	2.7 ± 0.4
22:6			3.2 ± 0.1	2.6 ± 0.1[b]	18.1 ± 0.4	13.5 ± 2.2[b]	9.0 ± 0.4	8.8 ± 1.1
24:0	7.1 ± 0.4	11.7 ± 0.8[b]						
24:1	22.8 ± 0.8	24.9 ± 1.8[b]						

[a] The results are presented as mean ± SD of seven (*spm/spm*) or four (*spm/+*) different animals and minor components less than 2% are not included in this table.
[b] Significantly different ($P < 0.01$, paired *t*-test) from *spm/+* value.

TABLE 4. Fatty acid composition of bis(monoacylglycero)phosphate in affected liver and spleen[a]

Fatty Acid	Liver	Spleen
	area percent	
16:0	8.8 ± 2.0	6.2 ± 1.4
18:0	5.3 ± 0.7	5.5 ± 0.5
18:1	22.3 ± 2.6	21.8 ± 1.2
18:2	16.3 ± 2.8	20.0 ± 1.8
18:3	4.3 ± 0.6	3.7 ± 0.6
20:4	6.5 ± 0.8	9.8 ± 1.1
22:4	3.3 ± 0.3	5.2 ± 1.2
22:5	3.8 ± 0.5	4.4 ± 0.4
22:6	26.5 ± 4.7	19.8 ± 1.7

[a] The results are expressed as mean ± SD of seven different animals and minor components less than 2% are not included in this table.

were no differences in the phospholipid composition of erythrocytes and blood plasma between the homozygous and heterozygous animals.

Fatty acyl chain composition

The detailed fatty acyl chain composition of individual phospholipids from various organs was analyzed by gas–liquid chromatography. Each phospholipid class had a characteristic fatty acid pattern that differed from one organ to another, and profound differences were seen between affected and heterozygous control animals. Sphingomyelin of the affected liver showed decreases in 18-carbon fatty acids and docosadienoic acid and increases in palmitic, nervonic, and docosapentaenoic acids (**Table 2**). In the affected spleen, similar alterations were observed with the exception of the increased level of palmitic acid (**Table 3**). The compositional patterns of fatty acyl chains of phosphatidylserine plus phosphatidylinositol in these two affected organs were not significantly different from those of controls, whereas in phosphatidylcholine and phosphatidylethanolamine there were small but significant alterations in fatty acid composition. Bis(monoacylglycero)phosphate had a unique fatty acyl profile in both the liver and spleen, which was characterized by a high content of unsaturated long chain fatty acids, mainly oleic, linoleic, and docosahexaenoic acids (**Table 4**).

Sphingomyelin in the whole brain (**Table 5**) showed an altered fatty acyl chain composition, although this lipid did not accumulate in the affected mice. The major fatty acids of sphingomyelin from visceral organs were palmitic, nervonic, lignoceric, and arachidic acids, whereas stearic acid was the major fatty acid in brain sphingomyelin, greater than 50% of the total fatty acids in *spm/spm* mice. It was of interest to note that stearic acid was a relatively minor component in visceral sphingomyelin. Other glycerophospholipids in the *spm/spm* brain did not show any significant changes in fatty acyl composition.

Although the proportional distribution of phospholipids was not altered in the erythrocytes, the percent composition of total fatty acids was slightly modified; the polyenoic fatty acid content was higher for *spm/spm* erythrocytes compared with the unaffected animal erythrocytes (**Table 6**). A similar trend was also observed in plasma glycerophospholipids (**Table 7**). In contrast to the erythrocyte membranes, plasma sphingomyelin from affected mice contained more long chain fatty acids than that from unaffected mice.

It is evident that the unsaturation index of the fatty acyl chain was slightly but significantly higher in almost all phospholipid classes of the whole brain, erythrocytes, and blood plasma of the affected mice (**Table 8**). Furthermore, the accumulated bis(monoacylglycero)phos-

TABLE 5. Fatty acid composition of individual phospholipids in whole brain[a]

Fatty Acid	Sphingomyelin		Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol + Phosphatidylserine	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
				area percent				
16:0	16.4 ± 1.3	13.3 ± 3.4	46.3 ± 1.2	45.7 ± 1.7	6.1 ± 0.3	6.9 ± 0.6	4.3 ± 0.8	3.9 ± 0.5
16DMA					5.4 ± 0.4	5.7 ± 0.2		
18:0	43.0 ± 0.8	55.0 ± 3.4[b]	13.1 ± 0.8	11.6 ± 0.9	22.4 ± 0.4	21.6 ± 0.8	40.5 ± 1.8	40.8 ± 1.6
18DMA					8.5 ± 0.6	6.3 ± 1.0		
18:1	6.5 ± 0.9	3.6 ± 0.8[b]	26.5 ± 1.3	28.5 ± 1.5	12.6 ± 0.5	12.2 ± 0.4	16.2 ± 0.4	16.2 ± 0.6
20:2	4.0 ± 0.9	0.9 ± 0.5[b]						
20:4			4.8 ± 0.2	5.7 ± 0.4[b]	13.5± 0.4	14.0 ± 0.7	8.6 ± 0.4	11.0 ± 0.6[b]
22:0	4.8 ± 0.3	6.0 ± 0.6[b]						
22:2	3.2 ± 0.4	0.7 ± 0.3[b]						
22:4					4.6 ± 0.1	4.5 ± 0.3	2.5 ± 0.2	2.3 ± 0.4
22:6			4.1 ± 0.3	4.9 ± 0.2[b]	21.5 ± 0.8	24.3 ± 1.2[b]	20.5 ± 1.1	19.9 ± 3.1
24:0	2.8 ± 0.1	3.0 ± 0.5						
24:1	10.9 ± 0.8	13.2 ± 2.4[b]						

[a] The results are presented as mean ± SD of seven (*spm/spm*) or four (*spm/+*) different animals and minor components less than 2% are not included in this table.

[b] Significantly different ($P < 0.01$, paired *t*-test) from *spm/+* value.

TABLE 6. Fatty acid composition of individual phospholipids in erythrocyte membranes[a]

Fatty Acid	Sphingomyelin		Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol + Phosphatidylserine	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
				area percent				
16:0	17.2 ± 1.5	17.3 ± 0.7	49.5 ± 1.5	48.0 ± 2.6	13.2 ± 1.3	11.5 ± 0.3	12.2 ± 0.7	10.7 ± 1.5
16DMA					5.1 ± 0.7	4.4 ± 0.3		
18:0	8.3 ± 1.6	4.6 ± 0.7[b]	13.0 ± 0.4	12.6 ± 0.8	6.6 ± 0.2	6.9 ± 0.3	24.3 ± 0.7	21.8 ± 0.7
18DMA					2.0 ± 0.3	2.5 ± 0.2		
18:1	2.0 ± 0.3	1.6 ± 0.3	8.6 ± 0.3	9.1 ± 0.7	20.1 ± 1.6	18.5 ± 1.2	9.7 ± 0.6	8.9 ± 1.1
18:2	1.2 ± 0.3	1.5 ± 0.2	18.4 ± 0.8	17.7 ± 1.7	10.1 ± 0.4	9.7 ± 0.4	9.7 ± 0.3	9.4 ± 0.8
20:4			3.8 ± 0.4	5.7 ± 0.8[b]	18.4 ± 0.8	20.3 ± 2.0	26.2 ± 0.8	29.1 ± 1.4[b]
20:5					2.0 ± 0.1	2.0 ± 0.6		
22:0	11.5 ± 0.8	12.2 ± 0.3						
22:2	2.3 ± 0.2	1.4 ± 0.2						
22:4					2.2 ± 0.3	2.9 ± 0.6		
22:5	9.9 ± 0.7	11.1 ± 0.6			2.1 ± 0.2	2.3 ± 0.4		
22:6			2.5 ± 0.2	3.7 ± 0.4[b]	11.8 ± 1.4	15.4 ± 1.4[b]	8.5 ± 0.6	12.6 ± 2.2[b]
24:0	23.3 ± 1.1	25.2 ± 1.6						
24:1	20.5 ± 0.6	19.8 ± 1.3						

[a] The results are presented as mean ± SD of seven (*spm/spm*) or four (*spm/+*) different animals and minor components less than 2% are not included in this table.
[b] Significantly different ($P < 0.01$, paired *t*-test) from *spm/+* value.

phate was rich in unsaturated fatty acids (Table 4) and sphingomyelin in the affected visceral organs also exhibited a high unsaturation index.

DISCUSSION

Accumulation of sphingomyelin in various tissues, especially in liver and spleen, is the characteristic feature of human Niemann-Pick disease. The results presented in this paper demonstrate that there is a marked increase in sphingomyelin content of the liver and spleen in the affected mice, leading us to expect that this mouse lineage could be an animal model of human Niemann-Pick disease. Earlier investigations by Kitagawa et al. (1, 2) showed a reduced activity of sphingomyelinase and an accumulation of free cholesterol in affected mouse liver and spleen. Similar findings have been noted also in the human Niemann-Pick disease (14, 15). The relative content of sphingomyelin in the whole brain of the affected mice is

TABLE 7. Fatty acid composition of individual phospholipids in blood plasma[a]

Fatty Acid	Sphingomyelin		Phosphatidylcholine		Phosphatidylethanolamine	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
			area percent			
16:0	25.3 ± 2.0	24.4 ± 1.2	27.8 ± 1.3	30.6 ± 1.9	12.3 ± 2.4	17.6 ± 0.8[b]
16DMA					4.1 ± 0.9	4.0 ± 1.0
18:0	13.7 ± 1.7	7.0 ± 1.5[b]	14.2 ± 0.5	14.3 ± 0.6	12.9 ± 1.3	15.4 ± 2.0
18DMA					3.9 ± 0.6	3.3 ± 0.7
18:1	4.6 ± 0.6	3.4 ± 0.8	6.3 ± 0.4	7.1 ± 0.9	7.9 ± 0.6	8.8 ± 0.7
18:2	5.1 ± 0.7	2.8 ± 1.5[b]	34.4 ± 1.2	25.9 ± 0.8[b]	11.7 ± 0.6	10.6 ± 1.3
20:0	3.0 ± 1.1	1.8 ± 0.8			11.4 ± 2.6	4.5 ± 0.6[b]
20:2	5.4 ± 0.5	2.2 ± 1.2[b]				
20:4			5.6 ± 0.5	8.2 ± 0.9[b]	7.8 ± 1.6	11.9 ± 0.6[b]
20:5					3.0 ± 0.5	1.8 ± 0.2[b]
22:0	7.4 ± 0.7	7.3 ± 1.0				
22:2	4.8 ± 0.6	3.5 ± 0.8				
22:5	1.2 ± 0.3	6.7 ± 1.5[b]				
22:6	0.8 ± 0.4	3.6 ± 1.5[b]	5.4 ± 0.4	9.8 ± 0.9[b]	17.9 ± 1.8	18.5 ± 1.0
24:0	6.9 ± 0.4	11.9 ± 1.8[b]				
24:1	16.3 ± 0.5	25.1 ± 2.1[b]				

[a] The results are presented as mean ± SD of seven (*spm/spm*) or four (*spm/+*) different animals and minor components less than 2% are not included in this table.
[b] Significantly different ($P < 0.01$, paired *t*-test) from *spm/+* value.

TABLE 8. Unsaturation index[a] of fatty acid of phospholipids in various tissues[b]

Tissue	Sphingomyelin		Phosphatidylcholine		Phosphatidyl-ethanolamine		Phosphatidylinositol + Phosphatidylserine	
	spm/+	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*	*spm/+*	*spm/spm*
Liver	58.4	69.6	149.8	145.4	214.0	204.9	162.3	164.2
Spleen	72.8	89.8	105.5	98.9	244.2	217.6	178.1	169.8
Brain	32.4	21.2	72.2	79.8	192.1	218.4	168.4	174.8
RBC[c]	79.8	83.7	76.8	91.5	224.4	246.8	194.1	221.4
Plasma	63.9	101.3	131.4	154.3	187.2	194.5	n.e.[d]	n.e.[d]

[a] The summation of the percent of total times the number of double bonds for each unsaturated fatty acid.
[b] The results are presented as mean of seven (*spm/spm*) or four (*spm/+*) different animals.
[c] RBC, red blood cell (erythrocyte).
[d] Not examined.

not changed, despite the considerable neurological disorder. The neurological symptoms caused by the *spm* gene suggest a resemblance to juvenile Niemann-Pick disease (type C). However, the visceral impairments, especially the massive accumulation of sphingomyelin, do not exclude the possibility that this animal model may be relevant to human type A (1).

The accumulation of bis(monoacylglycero)phosphate in the liver and spleen has been described for human Niemann-Pick disease (16), other genetic storage diseases (17), and drug-induced lipidoses (18, 19). This unique phospholipid is known to localize in the secondary lysosomes (20). The elevation of its content in the affected organs may result from proliferation of secondary lysosomes as a cellular response to the accumulation of various lipids, sphingomyelin, free cholesterol, and glycolipid (13, 20). Indeed, lamellar structures were abundant in the cells as observed by electron microscopy (Fig. 1).

The finding that the phospholipid composition of erythrocytes does not differ from that of the control mice seems incompatible with data demonstrating a marked accumulation of sphingomyelin in the bone marrow of *spm/spm* mice (21). The enhanced level of sphingomyelin would lead one to expect an altered phospholipid composition of erythrocytes. Thus there are two possible explanations for the largely normal phospholipid composition in erythrocytes. Sphingomyelin deposition occurs mainly in the reticuloendothelial system and therefore erythrocyte lineage is free from the lipid accumulation. Alternatively, since the accumulated lipids are primarily localized in lysosomes in human Niemann-Pick disease, it is reasonable to suppose that sphingomyelin and other lipids are not deposited in erythrocytes which do not have lysosomes.

The fatty acid composition in the accumulated sphingomyelin was modified. Although sphingomyelin did not accumulate in the whole brain of the affected mice, the relative proportion of stearic acid increased, as has been observed in juvenile Niemann-Pick disease (22). The bis(monoacylglycero)phosphate is characterized by a high content of unsaturated fatty acids, especially docosahexaenoic acid, which amounted to 60–80% in drug-induced lipidoses (19, 23) and to 30% in a probable case of human Niemann-Pick disease (24). Our results from *spm/spm* mice agree with these findings. Harder and Debuch (19) have demonstrated that chloroquine treatment of rats stimulated the biosynthesis of docosahexaenoic acid which was abundant in bis(monoacylglycero)-phosphate and that this fatty acid was produced from linolenic acid via chain elongation and desaturation. The number of double bonds in fatty acids was observed to increase in most of phospholipids from the unaffected tissues (brain, erythrocytes, and plasma) and in sphingomyelin of the liver and spleen from *spm/spm* mice. These results suggest but do not prove that microsomal desaturation activity may be enhanced in the "Niemann-Pick" mouse.

Despite extensive studies of the metabolic pathway for bis(monoacylglycero)phosphate (25, 26), its exact synthetic pathway remains to be clarified. The underlying mechanism for multiple accumulation of other lipids (free cholesterol and glycolipids) as well as sphingomyelin is also unknown. Furthermore, more extensive work is necessary to understand the difference in sphingomyelinase activity in various types (A, B, C, D, and E) of human Niemann-Pick disease and also the organ specificity of this enzyme activity (14, 27).

Recently, some effective therapeutic approaches have been attempted for several genetic enzyme deficiencies, such as enzyme replacement and bone marrow transplantation. Animal models that resemble human disorders are very useful for these therapeutic trials. In fact, Sakiyama et al. (21) have reported the partial success of bone marrow transplantation in this "Niemann-Pick" mouse.

We propose that this mutant strain of mice is a potentially useful model of human Niemann-Pick disease for better understanding the precise molecular mecha-

nism of the disease and for applying new therapeutic approaches.

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