

# The lipid composition of the electric organ of the ray, *Torpedo marmorata*, with specific reference to sulfatides and Na<sup>+</sup>-K<sup>+</sup>-ATPase

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**Abstract** The lipids from the electric organ of the ray, *Torpedo marmorata*, have been isolated and characterized. The major lipids were cholesterol, choline phospholipids, ethanolamine phospholipids, and sphingomyelins. The major fatty acids of ethanolamine phospholipids were 18:1, 18:0, 22:6, and 20:4. More than 50% of the acids in choline phospholipids were 16:0. The sphingomyelins consisted of five major ceramide species, all with sphingosine and the fatty acids 14:0, 15:0, 16:0, 22:1, and 24:1. The fatty acid 15:0 was mostly branched (n-2), a fatty acid earlier identified in sphingomyelins of the rectal gland of spiny dogfish. All long-chain bases were dihydroxy bases with a small percentage of branched chains. Sulfatides (cerebroside sulfate) made up the largest glycolipid fraction. The polar moiety was galactose-3-sulfate. The fatty acids were normal and 2-hydroxy; the homologue 24:1 was the most abundant in both types of fatty acids. Most fatty acids were higher homologues of mono-unsaturated acids, but normal 18:0 fatty acid was also found. The long-chain bases were both dihydroxy and trihydroxy, with very small amounts of branched chains. The two major ceramide species of sulfatides were sphingosine combined with normal and hydroxy 24:1 fatty acids, respectively. Smaller amounts of trihydroxy base (18:0) were found linked to hydroxy 24:1 fatty acid, but not to its normal homologue. The cerebroside contained the two major species mentioned above but lacked the trihydroxy base-hydroxy fatty acid species. The ratio of the activity of Na<sup>+</sup>-K<sup>+</sup>-dependent ATPase (EC 3.6.1.3) and the concentration of sulfatides was similar to ratios found for other tissues with normal and increased Na<sup>+</sup> and K<sup>+</sup> transporting capacity. The significance of this finding is discussed.—**Hansson, G. C., E. Heilbronn, K.-A. Karlsson, and B. E. Samuelsson.** The lipid composition of the electric organ of the ray, *Torpedo marmorata*, with specific reference to sulfatides and Na<sup>+</sup>-K<sup>+</sup>-ATPase. *J. Lipid Res.* 1979. **20**: 509–518.

**Supplementary key words** phospholipids · sphingolipids · mass spectrometry

A quantitative relationship between the concentration of sulfatides (ceramide galactose-3-sulfate) and the activity of Na<sup>+</sup>- and K<sup>+</sup>-dependent adenosine triphosphatase (Na<sup>+</sup>-K<sup>+</sup>-ATPase, EC 3.6.1.3) has been

shown for different organs with increased ion transport like the kidney (1), the rectal gland of dogfish (2), and the nasal salt gland of birds (3). Parallel increases of sulfatide concentration and Na<sup>+</sup>-K<sup>+</sup>-ATPase activity have been shown for the salt gland of domestic duck after adaptation to a salt water load (4). No other lipids resemble sulfatides in this respect. Recently very small amounts of sulfatides were found in human erythrocyte membrane which have the same stoichiometric relation to Na<sup>+</sup>-K<sup>+</sup>-ATPase as those found earlier for other tissues (5, 6).

The electric organ with its specialized function has been the focus of many research interests associated with membranes, such as electrophysiology (7), isolation and characterization of acetylcholinesterase (8) and the acetylcholine receptor (9), isolation of synaptic vesicles (7, 10), and purification of the Na<sup>+</sup>-K<sup>+</sup>-ATPase (11). From the electric organ of the electric eel, *Electrophorus electricus*, a protein with lectin agglutinability has been isolated (12). Some studies have been devoted to lipids, like phospholipids of *Electrophorus* (13) and the ray, *Torpedo marmorata* (10, 14), or gangliosides of *Electrophorus* (15).

The present investigation was undertaken because of the lack of adequate data on the overall lipid composition, including sphingolipids, of this interesting tissue, and to test for the possible existence of sulfatides in the same ratio to Na<sup>+</sup>-K<sup>+</sup>-ATPase activity as

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Abbreviations: In the shorthand designations for long-chain bases, h means hydroxy, d means dihydroxy, t means trihydroxy, and br means a methyl branched chain. The number before the colon indicates chain length and the number after the colon is the degree of unsaturation. The designation n-2 means a methyl branch in position 2 from the end of the paraffin chain. Thus br(n-2)d18:1 is 1,3-dihydroxy-2-amino-16-methylheptadecene.

found for the other tissues mentioned. The relatively high  $\text{Na}^+\text{-K}^+\text{-ATPase}$  of the electric organ (16) is obviously connected with the high  $\text{Na}^+$  and  $\text{K}^+$  transport needed for the generation of electric current (7). The organ is derived embryologically from muscular tissue (7, 17) and thus has a different origin than the other tissues studied by us (1–5).

## MATERIALS AND METHODS

### Extraction and separation of lipids

The electric organ was obtained in the frozen state from the place of the ray's capture. A part of it was withdrawn for  $\text{Na}^+\text{-K}^+\text{-ATPase}$  assay as described earlier (4). The rest was lyophilized and extracted seven times in chloroform–methanol 2:1 (v/v) (2). Twelve g of dry tissue weight was used for this study.

The total lipid extract was analyzed by thin-layer chromatography (chloroform–methanol–water 65:25:4, by vol). The extract was separated on two silicic acid columns into a nonpolar fraction, a cerebroside fraction, a sulfatide fraction, and a fraction containing all other lipids, i.e., mostly phospholipids (2). From the nonpolar lipid fraction cholesterol was obtained (2). The sulfatide fraction and the phospholipid fraction were each run through DEAE-cellulose (1). After DEAE-chromatography the sulfatides were purified from the acidic fraction by silicic acid column chromatography. The cerebroside fraction was further purified on several Florisil columns (1). The non-acidic lipids were separated further on silicic acid columns (2) into a phosphatidylethanolamine fraction and a phosphatidylcholine–sphingomyelin fraction. The last fraction was treated with phospholipase C (18) and separated into ceramides and diglycerides on a silicic acid column (3).

### Characterization and quantitative determination of sulfatides and cerebroside

The cerebroside and sulfatide fractions obtained after the two initial chromatography steps were separated by preparative thin-layer chromatography (chloroform–methanol–acetone–water 50:30:25:5, by vol). The components were scraped off and measured by an anthrone reaction as described elsewhere (4).

The purified sulfatides were analyzed by thin-layer chromatography and infrared spectroscopy in KBr pellets by a Perkin-Elmer Model 157 instrument (2).

Evidence for the sulfate group position was obtained, after acetylation and trimethylsilylation of sulfatides, by mass spectrometry (19) (LKB 9000 instrument). From the purified sulfatides the sulfate group

was removed by acid treatment (4). The monoglycosylceramides obtained were analyzed on thin-layer chromatograms (see Fig. 2) and as trimethylsilyl ethers by mass spectrometry (20) on an MS 902 instrument, AEI. The carbohydrate residues in the sulfatide and the cerebroside were determined by gas–liquid chromatography of the alditol acetates obtained after acetolysis and aqueous hydrolysis (21). The gas chromatograph (a Perkin-Elmer 900 instrument) was equipped with an open tubular capillary column that had OV-1 (Applied Science Lab.) as stationary phase. Identification was made by comparing retention times with known standards.

For the detailed characterization of the fatty acids and long-chain bases, sulfatides were hydrolyzed in boiling 2 M HCl (22), followed by extraction and separation (3). The fatty acids were methylated (23) and two-thirds were separated into normal and hydroxy fatty esters. Normal esters, the trimethylsilyl ethers of hydroxy esters, and the same derivatives of the original mixture were analyzed by gas–liquid chromatography on a Hewlett-Packard 402 F and M and a Perkin-Elmer 900 instrument and by gas–liquid chromatography–mass spectrometry (LKB 9000 instrument). The long-chain bases were converted into dinitrophenyl derivatives (22), purified, oxidized (24), and analyzed as aldehydes by gas–liquid chromatography with Reoplex-400 and XE-60 as stationary phases (22). Quantification of the peaks was done with an integrator (model 3370 B, Hewlett-Packard) or by cutting out and weighing.

### Characterization and quantitative determination of sphingomyelins

The total nonacidic phospholipid fraction was separated by preparative thin-layer chromatography (chloroform–methanol–acetic acid–water 65:25:5:4, by vol) into sphingomyelins, phosphatidylcholines, and phosphatidylethanolamines; each component was scraped off and assayed by phosphorus analysis (4).

The purified ceramides from the sphingomyelins were analyzed by arsenite-impregnated thin-layer chromatography (25). The ceramides were converted to their trimethylsilyl ethers and analyzed by gas–liquid chromatography (24) with 3% OV-1 as the stationary phase and by direct inlet mass spectrometry (MS 902). The ceramides were also hydrolyzed in alkali (24) and separated into fatty acids and long-chain bases on silicic acid columns, and the fractions were characterized as described for sulfatides above. The aldehydes were also analyzed by gas–liquid chromatography–mass spectrometry. The fatty acids were further reduced by  $\text{LiAlH}_4$  to alcohols,

methylated by  $\text{CH}_3\text{I}$ , and analyzed by gas-liquid chromatography-mass spectrometry (26).

### Characterization and quantitative determination of glycerophospholipids

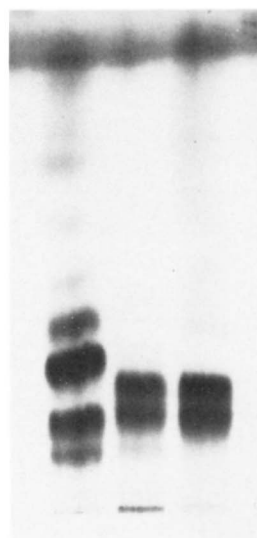
Phosphatidylethanolamines and phosphatidylcholines were quantitated as described above. Phosphatidylserines and phosphatidylinositols were separated from a total lipid mixture by thin-layer chromatography, scraped off, and phosphorus analyses were carried out (4). The sphingomyelin-phosphatidylcholine sample was hydrolyzed in 2 M HCl at 120°C (2), the lipid-soluble products were extracted with diethylether, the water phase was evaporated, and the redissolved sample was applied on silica gel G thin-layer plates with defined amounts of choline as reference. The plates were developed in methanol-water-25%  $\text{NH}_3$  60:35:5 (by vol) and detection was by iodine vapor. The phosphatidylethanolamines and the acidic phospholipids were treated in the same way. Ethanolamine and serine were detected by ninhydrin and inositol by a copper acetate-phosphoric acid reagent (1). Inositol was separated as described earlier (2). The amount of water-soluble products was estimated by visual comparison to known amounts of reference standards.

The phosphatidylcholines and phosphatidylethanolamines were methanolized in 9%  $\text{H}_2\text{SO}_4$  in dry methanol at 65°C overnight (27). After addition of one volume of water the products were extracted three times with heptane. The fatty acid methyl esters and the dimethyl acetals were analyzed and prepared by thin-layer chromatography using xylene as solvent and copper acetate reagent (analytical) or water (preparative) for detection (27). The methyl esters were analyzed by gas-liquid chromatography on both polar (10% EGSS-X) and nonpolar (3% OV-1) columns and by combined gas-liquid chromatography-mass spectrometry.

### Characterization and quantitative determination of other lipids

The nonpolar lipid fraction was analyzed by thin-layer chromatography (4). The pure cholesterol was quantitated by gravimetry. The cholesterol was analyzed by mass spectrometry as its trimethylsilyl ether derivative.

The acidic fractions, after the sulfatides had been removed, were subjected to mild alkaline hydrolysis (1) to degrade phosphoglycerides, and then dialyzed. The gangliosides obtained were analyzed by thin-layer chromatography (developed in chloroform-methanol-2 M ammonia 50:40:8, by vol) and detected by an anisaldehyde reagent (2).



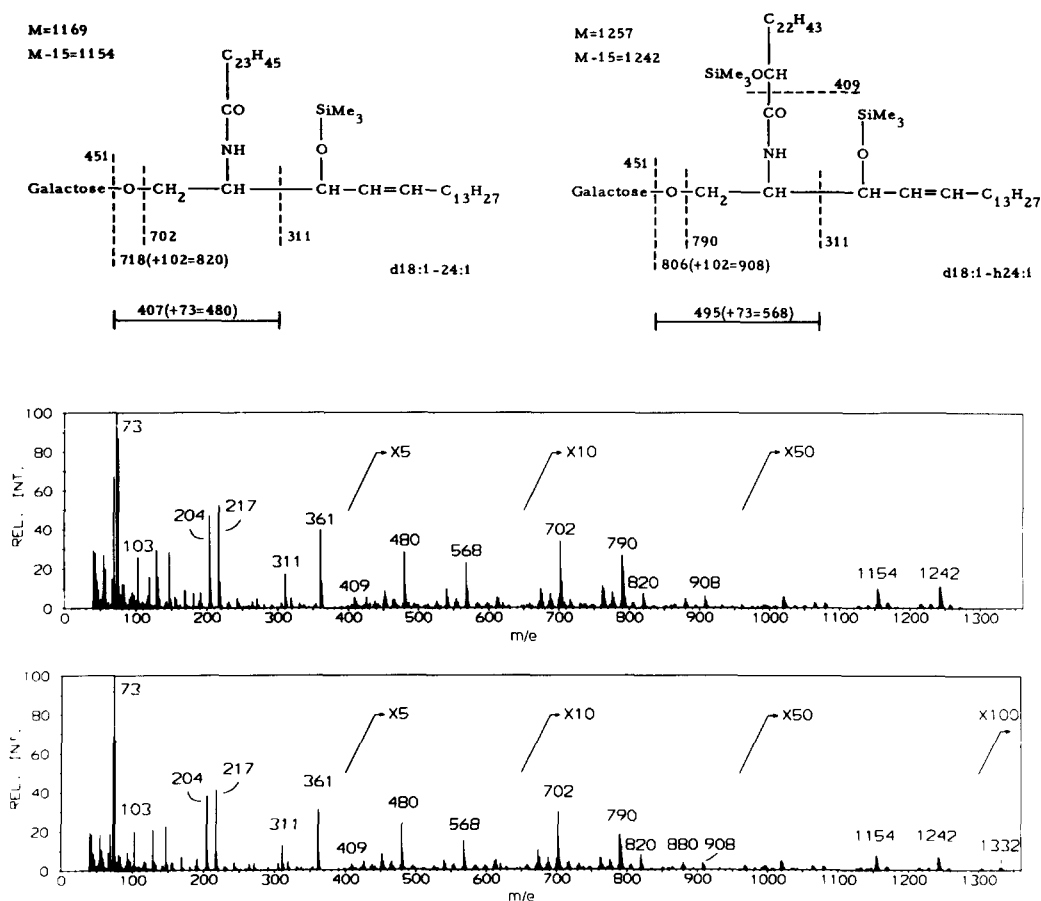
**Fig. 1.** Thin-layer chromatogram of reference bovine kidney monoglycosylceramides (28) (left), desulfated sulfatides (middle), and cerebrosides (right) from the electric organ of *Torpedo marmorata*. The layer was silica gel G with borate, the solvent was chloroform-methanol-water 100:30:4 (by vol), and the detection was with copper acetate. In the reference cerebrosides, the upper group of spots contain glucosylceramides and the lower group galactosylceramides.

## RESULTS

### Sulfatides

In the thin-layer chromatogram (not shown) of the total lipids of the electric organ, the nonpolar lipids were found in the solvent front and then, in descending order as separate spots, ethanolamine phospholipids, choline phospholipids, sphingomyelins (double spot), and serine phospholipids. A large spot of urea was identified below choline phospholipids. Sulfatides or cerebrosides were not detected in the total lipid chromatogram. Thin-layer chromatography of the intact purified sulfatides showed two main components, similar in mobility and color (anisaldehyde reagent) to human brain sulfatides (2). The acidic nature of the lipid was evident from its retention on DEAE-cellulose. The infrared spectrum was identical to previously published spectra of sulfatides (2), with amide absorption at 6.1 and 6.5  $\mu\text{m}$ . A strong absorption at 8.2  $\mu\text{m}$  due to  $\text{S}=\text{O}$  stretching vibrations indicated the presence of a sulfate group. The carbohydrate residue was only galactose as determined by gas-liquid chromatography of the alditol acetate derivative.

Evidence for the sulfate in position 3 of the hexose was obtained by mass spectrometry after substitution of the sulfate group of acetylated sulfatides with a trimethylsilyl group. The presence of an ion at *m/e* 361 was evidence for a monotrimethylsilylated tri-



**Fig. 2.** Mass spectrum of the trimethylsilyl derivative of cerebrosides (upper) and desulfated sulfatides (lower) of the electric organ of *Torpedo marmorata*. The two formulas represent the two major components in both spectra with a fragmentation pattern indicated. The conditions of analysis for both spectra were: ion source temperature 290°C, probe temperature 180°C, electron energy 70 eV, ionization current 500  $\mu$ A, and acceleration voltage 8 kV. Peaks below  $m/e$  40 were not reproduced.

acetylated hexose, and the ion at  $m/e$  169 indicated a 3-substitution with sulfate of the original lipid (19).

After mild acidic degradation, cerebrosides were obtained which were analyzed by thin-layer chromatography (Fig. 1), together with a reference of total

TABLE 1. Long-chain bases of sulfatides and sphingomyelins of the electric organ of *Torpedo marmorata*<sup>a</sup>

Aldehyde	Parent Base	Sulfatides	Sphingomyelins
14:0	d16:0	3	2
15:0	t18:0(d17:0)	1	
15:1	d17:1	trace	1
16:0	d18:0	16 (+ t19:0)	3
16:1	d18:1	80	88
br16:1	brd18:1	trace	1 br(n-2)
br(n-2)17:1	br(n-2)d19:1	} trace	1
br(n-3)17:1	br(n-3)d19:1		3

<sup>a</sup> The relative amounts in the two sphingolipids are not directly comparable due to different preparation methods.

bovine kidney monoglycosylceramides (28). All three bands moved less than the slowest reference of glucosylceramide and appeared in the region of galactosylceramides. The mass spectrum of the trimethylsilylated desulfated sulfatides is shown in Fig. 2 together with the chemical formula of the two most abundant species. The interpretation was based on spectra of synthetic monoglycosylceramides discussed in detail elsewhere (20). The hexose substituent is evident at  $m/e$  361 (451 minus trimethylsilanol) and other hexose fragments are  $m/e$  217, 204, and 103. The two major molecular species were composed of sphingosine (fragment  $m/e$  311) and normal 24:1 or hydroxy 24:1 fatty acids (fragments at  $m/e$  480 and  $m/e$  568, respectively). Additional evidence for this hydroxy fatty acid is seen at  $m/e$  409. The corresponding ceramide fragments are seen at  $m/e$  820 and 702 for the normal 24:1 fatty acid and at  $m/e$  908 and 790 for the hydroxy 24:1 fatty acid. Molecular weight ions ( $M - 15$ ) are shown at  $m/e$  1154 and  $m/e$  1242,

TABLE 2. Fatty acids from sulfatides, sphingomyelins, phosphatidylcholines, and phosphatidylethanolamines of the electric organ of *Torpedo marmorata*

Fatty Acid	Sulfatides		Sphingo- myelins Normal	Phosphatidyl- cholines Normal	Phosphatidyl- ethanolamines Normal
	Normal	Hydroxy			
14:0			10 <sup>a</sup>	1 <sup>a</sup>	trace
br14:0			trace		
15:0			1 <sup>a</sup>	trace <sup>a</sup>	trace <sup>a</sup>
br(n-2)15:0			6 <sup>a</sup>		
16:0	6 <sup>a</sup>	trace	26 <sup>a</sup>	56 <sup>a</sup>	7 <sup>a</sup>
16:1			3	4 <sup>a</sup>	5 <sup>a</sup>
17:0	2		trace	trace <sup>a</sup>	trace <sup>a</sup>
br17:0			trace	2 <sup>a</sup>	5 <sup>a</sup>
18:0	26 <sup>a</sup>	2	4	3 <sup>a</sup>	18 <sup>a</sup>
18:1	trace		7	22 <sup>a</sup>	25 <sup>a</sup>
18:2				trace	trace
19:0	trace				
20:0	3 <sup>a</sup>	2 <sup>a</sup>	1		
20:1		1		4 <sup>a</sup>	3 <sup>a</sup>
20:4				2 <sup>a</sup>	15 <sup>a</sup>
20:5				trace	trace
21:0	1	trace			
22:0	5 <sup>a</sup>	14	1		
22:1	9 <sup>a</sup>	11	9		
22:6				5 <sup>a</sup>	18 <sup>a</sup>
Unidentified polyunsaturated 22				1	4
23:0	1	5			
23:1	2	3	1		
24:0	2 <sup>a</sup>	4	1		
24:1	32 <sup>a</sup>	43 <sup>a</sup>	30		
25:1					
26:0	} 11 <sup>b</sup>	} 15 <sup>b</sup>			
26:1					
Fraction of total sulfatide fatty acids	56–63%	37–44%			

<sup>a</sup> Identity shown by mass spectrometry.

<sup>b</sup> Some peaks were found for 26:0 and 26:1 by mass spectrometry, but the results are not conclusive.

respectively. Smaller amounts of lower and higher homologues with saturated and monounsaturated fatty acids were also present. The molecular weight region ( $M - 15$ ) shows  $m/e$  1332, probably due to the combination of trihydroxy base (t18:0) and hydroxy 24:1 fatty acid. Other ceramide-containing fragments of this species are at  $m/e$  880 and 998. The long-chain base fragment at  $m/e$  299 was not detected due to the low concentration of this species and the usually low intensity of this peak (20).

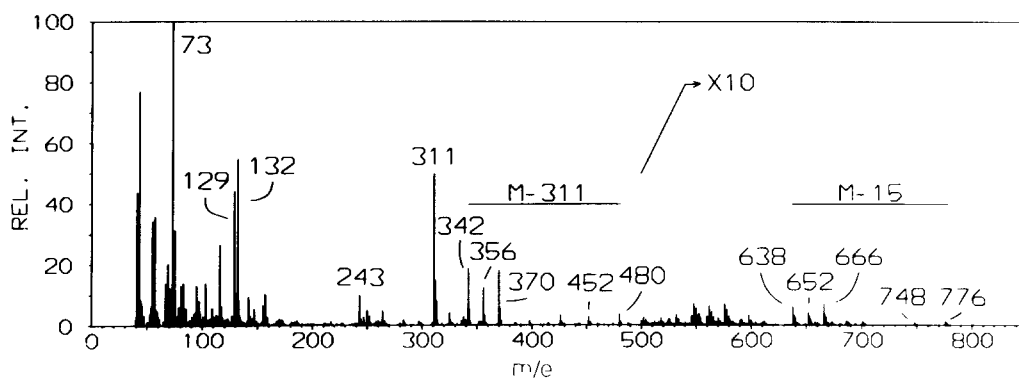
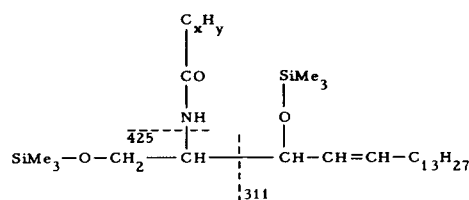
The long-chain base fraction contained only small amounts of trihydroxy bases (see **Table 1**), in agreement with thin-layer chromatography (the third faint band in Fig. 1) and mass spectrometry. Because of the acid degradation used there was a selective loss of monounsaturated bases and trihydroxy bases (22); therefore the amount of d18:0 is too high as given in Table 1, but the common sphingosine (d18:1) is nevertheless the largest component. Small amounts of branched-chain bases were also apparent.

The fatty acid fraction contained about 60% normal acids and 40% hydroxy fatty acids (**Table 2**). The major acids were 24:1 for both types, confirming the results from mass spectrometry of the galactosylceramide derivatives. High amounts of hydroxy 24:1 fatty acid have also been found in the rectal gland of spiny dogfish (2) but its normal homologue was not in such high concentrations as in this study. This relatively high amount of longer monounsaturated fatty acids was not found either for sulfatides of bovine kidney (1) or for salt glands of marine birds (4).

### Monoglycosylceramides

A thin-layer chromatogram of total monoglycosylceramides is shown in Fig. 1. The two main components were identical in mobility to the desulfated sulfatides and therefore contained galactose as the sugar moiety. The ratio of galactose to glucose was 16 to 1 as analyzed by gas-liquid chromatography. No third band con-

Ceramide	x	y	M
d18:1-14:0	13	27	653
d18:1-br15:0	14	29	667
d18:1-16:0	15	31	681
d18:1-22:1	21	41	763
d18:1-24:1	23	45	791



**Fig. 3.** Mass spectrum of trimethylsilylated ceramides derived from sphingomyelins of the electric organ of *Torpedo marmorata*. A table is shown above with the five major species together with a formula to aid the interpretation of the mass spectrum. The conditions of analysis were: electron energy 70 eV, ionization current 500  $\mu$ A, acceleration voltage 8 kV, ion source temperature 290°C, and probe temperature 150°C. Peaks below  $m/e$  40 were not reproduced.

taining trihydroxy base-hydroxy fatty acid as found for sulfatides was seen.

The mass spectrum of the total monoglycosylceramide fraction after trimethylsilylation is shown in Fig. 2. This mass spectrum was nearly identical to that of the desulfated sulfatides and showed the main species to be sphingosine (d18:1) combined with normal fatty acid 24:1 or hydroxy fatty acid 24:1. Nothing was found of the trihydroxy base-containing species, which is in accordance with the thin-layer chromatogram.

### Sphingomyelins

The sphingomyelin-phosphatidylcholine fraction was shown to contain choline in about the expected amount after hydrolysis (50–150% of theoretical, see ref. 3). Thin-layer chromatography on arsenite-impregnated plates of ceramides derived from sphingomyelins showed two major bands in the region for ceramide with dihydroxy bases and normal fatty acids (25). No spots with ceramides containing trihydroxy base or hydroxy fatty acids were detected. The mass spectrum of the trimethylsilylated total ceramides is shown in Fig. 3 together with a simplified formula for interpretation. The interpretation was based on earlier work (29). The only detectable long-chain base fragment is that of sphingosine (d18:1) at  $m/e$  311 and  $m/e$  426. Five intense fatty acid fragments ( $m/e$  M - 311) are seen, which correspond to the fatty acids

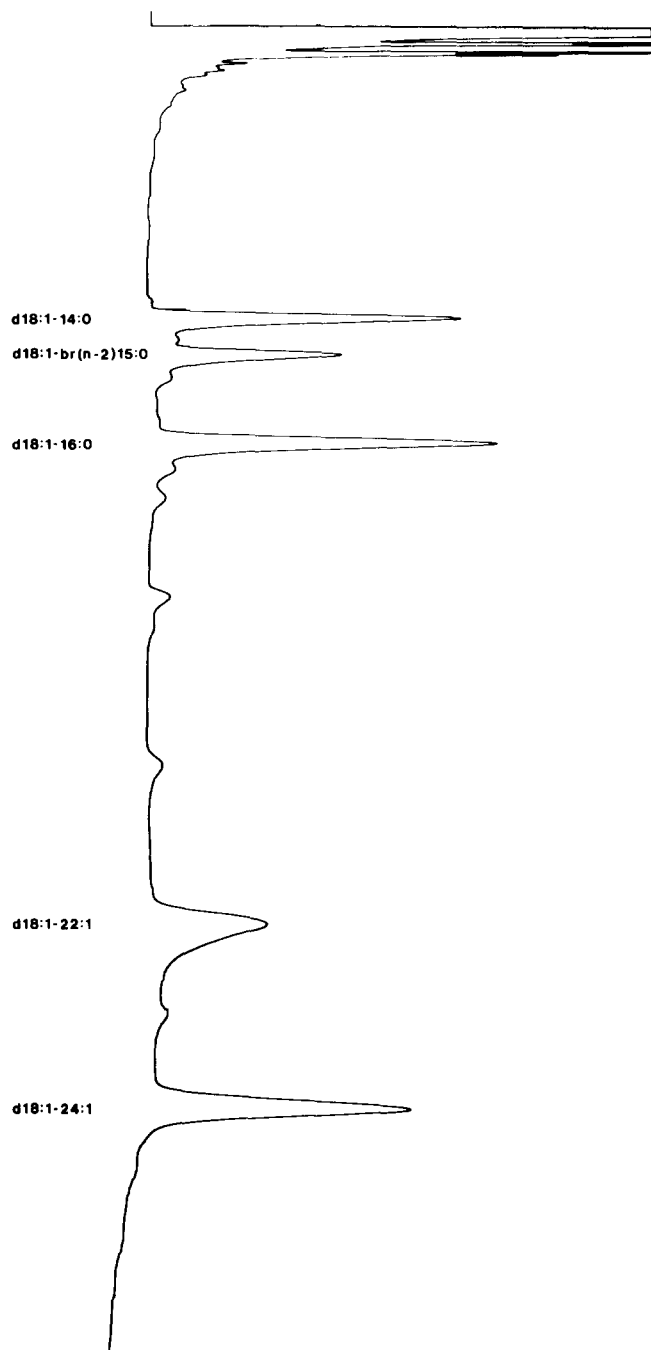
14:0, 15:0, 16:0, 22:1, and 24:1. In the molecular peak region (M - 15), peaks are seen that correspond to sphingosine and these five fatty acids. For the ceramides with fatty acids 14:0, 15:0, and 16:0 there are also peaks for M - 103 at  $m/e$  550, 564, and 578, respectively. This mass spectrum was recorded at a low temperature, and spectra taken at higher temperatures showed relatively more of the higher homologues and less of the shorter ceramide species.

These five ceramides were also found in the gas chromatogram (see Fig. 4) of the same derivative. They were eluted in two groups, which probably correspond to the two bands seen on the thin-layer chromatogram. The peak for the ceramide containing 15:0 fatty acid did not have the expected retention time for a straight-chain acid (see also below).

The ceramide fatty acids are shown in Table 2. All acids were normal and were divided in two groups, one with shorter chains (14–18 carbon atoms) and one with longer chains (22–24 carbon atoms). The shorter fatty acids were mainly saturated while the longer fatty acids were mainly monounsaturated. The fatty acids 15:0 were shown by mass spectrometry of the methyl ethers (26) to be mostly a branched fatty acid (n-2), which explains the gas chromatogram of ceramides discussed above.

Thin-layer chromatography of the dinitrophenyl derivatives of the long-chain bases (22) showed the

presence of only dihydroxy bases, all with *erythro* configuration. The result from analysis of the long-chain bases by gas-liquid chromatography is shown in Table 1. The dominating base was sphingosine but small amounts of branched-chain bases were also found.



**Fig. 4.** Gas chromatogram of trimethylsilylated ceramides derived from sphingomyelins of the electric organ of *Torpedo marmorata*. The stationary phase was 1% OV-1 (Applied Science Laboratories). The column temperature was kept isothermal at 260°C for 6 min and then raised to 310°C (0.5°C/min). The argon gas flow was 22 ml/min.

**TABLE 3.** Lipid composition<sup>a</sup> and Na<sup>+</sup>-K<sup>+</sup>-ATPase activity<sup>b</sup> of the electric organ of *Torpedo marmorata*

Cholesterol	26
Choline phosphoglycerides	29
Ethanolamine phosphoglycerides	20
Sphingomyelins	5.9
Sulfatides	0.90 (± 0.07) n = 6
Galactosylceramides	0.15
Glucosylceramides	trace
Serine phosphoglycerides	9.7
Inositol phosphoglycerides	3.9
Gangliosides	present
Na <sup>+</sup> -K <sup>+</sup> -ATPase	1.33 (± 0.05) n = 4
Ratio Na <sup>+</sup> -K <sup>+</sup> -ATPase/sulfatide	1.5 (2,0) <sup>c</sup>

<sup>a</sup> μmol/g dry tissue weight.

<sup>b</sup> mmol of P<sub>i</sub>/g dry tissue · hr<sup>-1</sup>.

<sup>c</sup> The value in parentheses was obtained by using the enzyme activity found in an earlier work, 1.79 mmol P<sub>i</sub>/g dry tissue · hr<sup>-1</sup> (16).

The three branched-chain bases were the same as in sphingomyelins from the rectal organ of the spiny dogfish (2).

### Glycerophospholipids

The sphingomyelin-phosphatidylcholine fraction was shown to contain choline (see above), and the pure phosphatidylethanolamine fraction, ethanolamine, in about the expected amounts (70–105% of theoretical, see ref. 3). The thin-layer chromatogram, after methanolysis, showed methyl esters and dimethyl acetals in both lipids. The amount of dimethyl acetals was very small (less than 0.5% in phosphatidylcholines and about 3% in the phosphatidylethanolamines). The methyl esters were analyzed by gas-liquid chromatography and the results are shown in Table 2. More than half of the fatty acids from the phosphatidylcholines were 16:0. The phosphatidylethanolamines contained more than one third polyunsaturated fatty acids. Small amounts of branched fatty acids were detected.

In the total acidic fraction phosphatidylinositols and phosphatidylserines were detected by thin-layer chromatography. The spots were both gray with anisaldehyde reagent and had the identical mobility and shape of reference compounds (2). After acid degradation serine and inositol were found but no choline or ethanolamine.

### Other lipids

The nonpolar lipid fraction was analyzed by thin-layer chromatography, and cholesterol was the major component. Small amounts of free fatty acids, triglycerides, and cholesteryl esters were also detected. The pure cholesterol was analyzed by mass spectrometry as the trimethylsilylether derivative. The mass spectrum was identical with earlier published spectra (30) with the molecular ion at *m/e* 458.

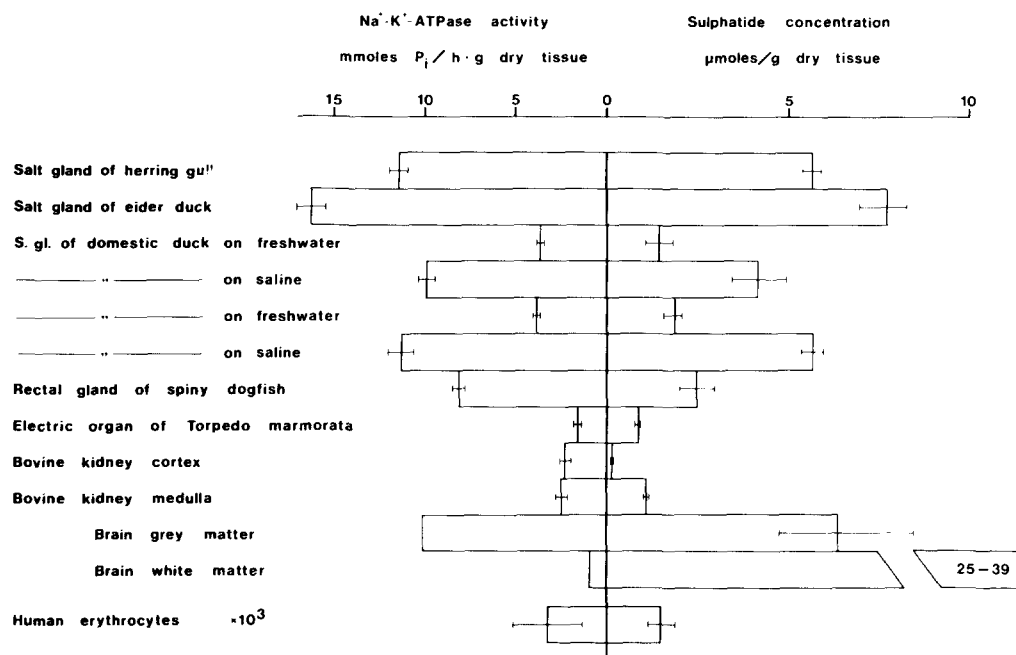


Fig. 5. Diagram showing the relation of Na<sup>+</sup>-K<sup>+</sup>-ATPase activity and sulfatide concentration in several animal cells. Note that the figures for erythrocytes have been multiplied by 10<sup>3</sup>.

In the acidic fraction gangliosides were tentatively identified by thin-layer chromatography, giving a green color with the anisaldehyde reagent. Many different gangliosides were visualized, with the major component having a mobility similar to that of the common brain disialoganglioside (31).

### The quantitative lipid pattern

Quantitative lipid data and Na<sup>+</sup>-K<sup>+</sup>-ATPase activity for the electric organ are shown in Table 3. The values based on phosphorus and carbohydrate determination were the mean of two or three estimates.

An Na<sup>+</sup>-K<sup>+</sup>-ATPase value determined by Glynn et al. (16) is included. Although our tissue was frozen before analysis, the two values are almost the same. The Na<sup>+</sup>-K<sup>+</sup>-ATPase/sulfatide ratio is in accordance with earlier published ratios for other tissues (1-5) (Fig. 5).

## DISCUSSION

Except for the presence of sulfatides, there is nothing unusual concerning the lipid composition of the electric organ (Table 3) compared with tissues in general. The fatty acid composition of the two major phospholipids was almost identical to that of the rectal gland of spiny dogfish (27). The low body temperature of these two animals may explain the higher unsaturation of ethanolamine phospholipids compared with

salt gland of birds (27). The maintenance of an optimal membrane fluidity has been shown to involve an adaptation of lipid structure to environmental temperature (32). The phospholipid composition of synaptic vesicles of the electric organ has recently been analyzed (7) and the results are similar to those of the whole organ, which suggests a relation of the vesicles to the cellular membranes.

Sphingolipids are surface membrane components and are probably located exclusively in the outer half of the membrane bilayer (see ref. 5). As a rule they are more saturated and have longer paraffin chains than glycerolipids, and the electric organ is no exception. The branched 15:0 fatty acid in sphingomyelins was also found in the rectal gland of dogfish (2). This acid has not been detected in sphingolipids of other higher animals. Compared with the rectal gland (2), sulfatides of the electric organ were less hydroxylated (i.e., contained lower amounts of hydroxy fatty acids and trihydroxy bases). The reason for such structural difference is not known, but it has been suggested (5) that the number of hydroxyl groups in this part of a sphingolipid may determine a system of laterally oriented hydrogen bonds in the membrane, of importance for optimal stability and permeability. This may indicate different needs for these properties in the two tissues in relation to cation translocation (see below). Concerning gangliosides, these are more complex than those of the rectal gland, a result in agreement with the situation in the electric organ of *Electrophorus* (15).



Of particular interest was the finding of sulfatides, not shown before in this organ, and their presence in the same stoichiometric relation to  $\text{Na}^+\text{-K}^+\text{-ATPase}$  (Table 3) as shown by us before for several other tissues with an increased transport capacity, like avian salt gland (3–5), rectal gland (2), and kidney (1). Recently, sulfatides were shown to exist in the membrane of human erythrocytes, again in the same relation to the enzyme in spite of an absolute level  $10^3$  times lower (5, 6). These data are gathered in Fig. 5. The only important deviation from the symmetry in Fig. 5 is shown for white matter of brain. This is obviously due to the presence of myelin membranes, which have a specialized insulation function and lack enzymes, but have typical surface membrane lipids due to their origin in the glia cell plasma membrane. No other membrane lipid shows this relation to  $\text{Na}^+\text{-K}^+\text{-ATPase}$ . According to a detailed model formulated (see 5 for details and references), sulfatides are located as an annulus around the transport unit. The known selection of  $\text{K}^+$  over  $\text{Na}^+$  for a sulfate group improves the protein-based translocation of  $\text{K}^+$  from a  $\text{K}^+$ -low and  $\text{Na}^+$ -high outside medium. In the absence of sulfatides, or if the sulfate group is blocked by certain amines, the pump becomes more electrogenic, that is, less  $\text{K}^+$  is translocated in relation to  $\text{Na}^+$ . Although this function is still hypothetical, it may be tested by appropriate reconstitution experiments with purified enzyme and lipid vesicles of defined composition.

The cellular unit of the electric organ, the electroplax, is a bipolar cell with two types of plasma membrane. One is an innervated membrane, which is excitable and contains the acetylcholine receptor, whereas the other is not excitable and contains the  $\text{Na}^+\text{-K}^+\text{-ATPase}$  (7). It would be of interest to analyze the two types of plasma membrane (33, 34) to learn if sulfatides are only occupying the ATPase pole. ■

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