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GLYCOSPHINGOLIPIDS AND AMINES IN NEOPLASTIC MAST CELLS*

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Abstract—Glycosphingolipids containing neuraminic acid (gangliosides) have been isolated from two lines of mouse neoplastic mast cells. Thin-layer chromatography and chemical analysis of these lipids suggest the presence of at least three different types of gangliosides: *N*-acylneuraminosyl-monoglycosylceramide, *N*-acylneuraminosyl-monaminoglycosyl-triglycosylceramide and N-acyl-dineuranminosyl-monoaminoglycosyldiglycosylceramide. The intracellular distribution of the gangliosides differs from cerebroside sulfate in that the neuraminic acid lipids are primarily found in small-particle fractions, but the cerebroside sulfate occurs mainly in the amine containing large-particle fractions. Density gradient centrifugation of this large-particle fraction has demonstrated that the cerebroside sulfate is retained in a subfraction showing high fumarase activity, characteristic of mitochondria, and separable from the amine containing granules. The chemical analysis of purified mast cell cerebroside sulfate showed an approximately equimolar ratio of sphingosine, hexose and sulfate.

WE HAVE previously described a multistep, coupled morphological–biochemical, granule maturation process that occurs in mouse neoplastic mast cells.[1] During the course of this work, we noted that the incorporation of 5-hydroxytryptamine (5-HT) into the granules preceded the deposition of heparin and histamine, suggesting the presence of a 5-HT binding component during the early stages of granule maturation. Acidic lipids were considered as possible participants in this 5-hydroxytryptamine binding process, since they possess certain electrochemical properties that are similar to those of heparin. Two types of acidic lipids have been identified in tumor mast cells. The acidic glycosphingolipid, cerebroside sulfate, has been demonstrated in lines of mastocytoma cells‡ by Green and Robinson[2,3] and the occurrence of the acidic phospholipid, phosphatidyl serine, has been identified in another cell line§ by Riley.[4]

The present paper reports the occurrence of gangliosides and cerebroside sulfate in two lines of neoplastic mast cells and describes the intracellular location of these acidic glycosphingolipids in relation to the intracellular distribution of amines.

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‡ Derived from the Dunn–Potter tumor.

§ Derived from the Furth–Hagen–Hirsch tumor.

MATERIALS AND METHODS

Mast cells. Two lines of neoplastic mast cells[1] were used for this study [HC derived from the Furth–Hagen–Hirsch mastocytoma,[5] P-815Y derived from the Dunn–Potter mastocytoma].[6] The HC line was carried in LAF_1, female mice and the P-815Y line was carried in AKD_2F_1 female mice that were obtained from the Jackson Laboratories (Bar Harbor, Me.). The cells were harvested from the peritoneal cavity of each mouse 6–8 days after inoculation and suspended in 0·9% NaCl. The suspension was centrifuged at 500 *g* for 2 min at 25°. The cells were washed once and resuspended in saline. Approximately 4–12 $\times$ 10^6 HC or P-815Y cells, in a volume of 0·1–0·3 ml, were reinjected into the peritoneal cavities of new mice for propagation of the tumor lines.

Cell fractions. Cells were sonicated in 0·34 M sucrose at 0° for 2 min at 2 amp using a "Sonifier" (Heat Systems-Ultrasonics, Inc.). Differential centrifugation was performed at 4° with fractions obtained by spinning at 3000, 5500 and 30,000 *g* for 10 min. A Beckman/Spinco ultracentrifuge with an SW-40 rotor was used to obtain the 204,000 *g*, 30-min pellet and supernatant fractions.

Density gradient fractionation. The 5500 *g* fraction was resuspended in 3 ml of 0·34 M sucrose and 1-ml aliquots were layered on a discontinuous gradient, 0·60–1·50 M sucrose in each of three tubes (see Fig. 2). The separation was performed in the Spinco ultracentrifuge using an SW-65 rotor at 204,000 *g* for 60 min at 0°. Fractions designated F_1–F_5 [1·25, 0·5, 2·0, 1·25 and 2·0 ml (F_5, after resuspension)] were collected from the top of the tube (see Fig. 2) and assayed as described in subsequent sections.

Lipid extraction and chromatography. The acidic glycosphingolipids were extracted from the cells and separated as shown in Fig. 1. Scheme A of Fig. 1 describes the simultaneous isolation of cerebroside sulfate and gangliosides by the method of Svennerholm.[7] Scheme B describes the procedure for the isolation of cerebroside sulfate based upon the methods of Folch *et al.*,[8] Radin *et al.*[9] and Rouser *et al.*[10]

Column chromatography. The separation of cerebroside sulfate from the total lipid extract (scheme B) was performed on magnesium silicate (Floridin Company, Tallahassee, Fla.) columns by the method of Rouser *et al.*[10] The lipids were eluted from the column with the following solvents, in the order listed: chloroform, chloroform–methanol (19:1), chloroform–methanol (2:1), methanol, and chloroform–methanol (2:1) plus 7% water. Five percent (v/v) 2,2-dimethoxypropane (Eastman) was added to all solvents except the last one. All eluates were collected, concentrated in a flash evaporator and tested for their content of sulfate, phosphorus and hexose (see Analytical methods). These determinations showed that 93·4 per cent of the total cerebroside sulfate was present in the chloroform–methanol (2:1) eluate.

The isolation of the gangliosides from the lipid extract (scheme A) was performed on silicic acid (Mallinckrodt) columns as described by Svennerholm.[7] The columns were first washed with chloroform–methanol (3:1) to remove cerebroside sulfate and the gangliosides were then eluted with chloroform–methanol (1:4). The eluate was evaporated and the residue was dissolved in 8 ml chloroform–methanol (2:1). The solution was centrifuged, the undissolved residue was re-extracted with 1 ml chloroform–methanol (2:1), and the two solutions were combined.

Thin-layer chromatography. A slurry of silica gel G (Merck) was applied to glass plates with an AO (American Optical Company) adjustable applicator to give a coat

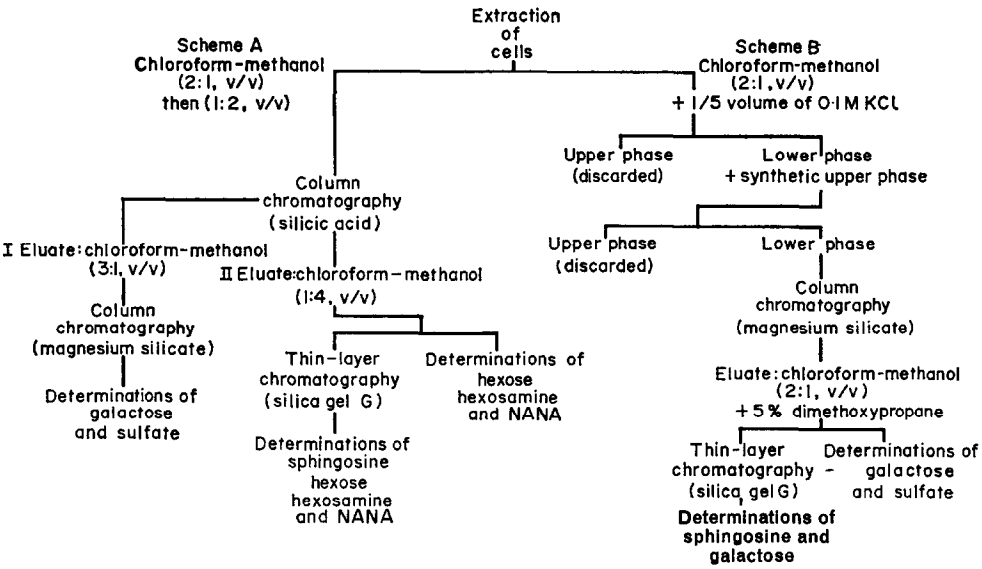

FIG. 1. Isolation of acidic glycosphingolipids.

approximately 250 μ in thickness. The plates were activated by heating for 1 hr at 120°. Three solvent systems were used for the chromatography of cerebroside sulfate: chloroform–methanol–water (65:25:4)[11] chloroform–methanol–water–pyridine (40:10:1:1),[12] and chloroform–methanol–ammonium hydroxide (80:20:0·4).[13] Chromatography of the ganglioside was carried out in *n*-butanol–pyridine–water (46:31:23),[14] and in *n*-propanol–water (70:30).[15] The plates were dried in air and sprayed with 20% perchloric acid to locate the compounds.[7] Resorcinol reagent spray was used to identify neuraminic acid-containing material.[16] Bands designated for elution were located by exposure to iodine vapor, marked, and the iodine was sublimed prior to elution.

Analytical methods. Total lipids were determined as described by Brante.[17] Phospholipids were measured in the total washed-lipid extract[8] by the phosphorus assay of Fiske and Subbarow.[18] Phospholipid content was calculated by multiplying the phosphorus value by a factor of 25.[7] Lipid sulfate was determined by the method of Green and Robinson[2] and the value obtained was multiplied by 9·45 to give the cerebroside sulfate level.[19] Gangliosides were assayed by determining *N*-acylneuraminic acid (NANA) after the lipid extracts were hydrolyzed in 0·05 N sulfuric acid at 80° for 1 hr.[20] The samples of cerebroside sulfate eluted from columns or preparative thin-layer chromatography plates were divided into two aliquots. One aliquot was hydrolyzed in 2 ml of a 1:1 mixture of glacial acetic acid and 6 N HCl for 48 hr, in a boiling water bath and the hydrolysate was assayed for its sulfate content as described above.[2] The second aliquot of the sample was hydrolyzed in 1 ml of a 5% methanol–2 N HCl mixture for 6 hr at 100° in a sealed tube. After removal of the methyl esters of the fatty acids with petroleum ether, the hexose[9] and sphingosine[21] contents were determined. The ganglioside samples obtained by thin-layer chromatography were divided into three aliquots. One aliquot was hydrolyzed with 0·05 N H_2SO_4 at 80° for 1 hr and *N*-

acylneuraminic acid was determined.[22] The second aliquot was hydrolyzed with 2 N HCl at 100° for 6 hr and the carbohydrates were separated into hexose and hexosamine fractions on a Dowex 50W-X8 column.[23] Hexose[9] and hexosamine[24] were determined by chemical methods. The third aliquot was hydrolyzed with 1 ml of 5% methanol in 2 N HCl for 6 hr at 100°. After removal of methyl esters of fatty acids with petroleum ether, the hydrolysate was assayed for its content of sphingosine by the methyl orange method.[21] Fumarase was assayed spectrophotometrically.[25]

Amine assays. The histamine levels in the mast cells were determined spectrophotofluorometrically by the method of Shore *et al.*,[26] and 5-hydroxytryptamine was determined by the modified method of Bogdanski *et al.*[27] (as described by Carlini *et al.*[28]). 5-Hydroxytryptamine creatinine sulfate (Calbiochem) and histamine dihydrochloride (Eastman Chemical Company) were used as standards.

Electron microscopy. Density gradient fractions were mixed with an equal volume of fixative, centrifuged at 30,000 *g* for 10 min and the pellet was overlaid in the same fixative, which consisted of 2% glutaraldehyde in 0·08 M cacodylate buffer, pH 7·2. The material was processed for electron microscopy as previously described.[1]

RESULTS

The lines of neoplastic mast cells used in these experiments differed in their amine content. The level of 5-HT and histamine in the HC cells was 0·57 ± 0·15 and 0·27 ± 0·02 μg/10^6 cells respectively. The amine content of P-815Y cells was 0·18 ± 0·03 μg 5-HT and 0·10 ± 0·01 μg histamine 10^6 cells.

The total lipid and phospholipid content of both lines of mast cells were approximately equal (Table 1), but the level of the acidic glycophingolipids, cerebroside sulfate and gangliosides in the HC line was about twice that in the P-815Y line.

TABLE 1. LIPID CONTENT OF MURINE NEOPLASTIC MAST CELLS*

Cell line	Total lipids (%)	Total phospholipids (%)	Cerebroside sulfate (%)	Gangliosides (Lipid–NANA) (%)
HC (Furth†)	14·3 ± 0·22	9·9 ± 0·27	1·00 ± 0·025	0·10 ± 0·002
P-815Y (Dunn–Potter†)	13,7 ± 0·16	9·3 ± 0·21	0·45 ± 0·033	0·50 ± 0·002

* Values represent the mean of five experiments expressed as per cent of dry weight (± standard error of the mean).
† Tumor of origin.

To examine the intracellular distribution of the lipids and amines, washed HC cells were sonicated and a number of subcellular fractions were collected by differential centrifugation. Table 2 shows that the total lipid, the phospholipids and the gangliosides were primarily distributed in the small-particle fractions (30,000 *g* plus 204,000 *g*). The bulk of the cerebroside sulfate, on the other hand, was found in the histamine, 5-HT-containing, large-particle fraction (5500 *g*).

To aid in the further identification of the acidic glycosphingolipid-containing particles, the 5500 *g* fraction was resuspended in 0·34 M sucrose and layered on a sucrose

TABLE 2. INTRACELLULAR DISTRIBUTION OF LIPIDS AND AMINES IN HC CELLS*

Fraction	% Total Lipids	RSC†	% Total Phospholipids	RSC	% Cerebroside Sulfate	RSC
3000 *g*	6·9 ± 0·61	0·61	7·4 ± 0·40	0·66	6·6 ± 0·26	0·59
5500 *g*	19·5 ± 0·31	1·19	16·1 ± 0·46	0·98	42·7 ± 0·77	2·62
30,000 *g*	26·8 ± 0·40	1·31	26·2 ± 1·62	1·28	27·4 ± 0·32	1·34
204,000 *g*	29·2 ± 2·01	1·40	32·7 ± 1·90	1·57	11·0 ± 0·63	0·53
Supernatant	17·6 ± 2·68	0·56	17·6 ± 2·86	0·56	12·1 ± 0·40	0·38

Fraction	% Gangliosides (Lipid-NANA)	RSC	Histamine	RSC	% 5-Hydroxy-tryptamine	RSC
3000 *g*	10·5 ± 0·37	0·93	13·7 ± 0·57	1·22	10·2 ± 0·29	0·92
5500 *g*	15·4 ± 0·45	0·94	34·2 ± 0·50	2·09	41·7 ± 0·60	2·49
30,000 *g*	26·2 ± 0·75	1·28	21·7 ± 0·32	1·06	26·7 ± 0·47	1·30
204,000 *g*	35·4 ± 1·08	1·70	18·8 ± 0·32	0·90	9·2 ± 0·28	0·44
Supernatant	12·5 ± 0·17	0·39	13·6 ± 0·14	0·37	12·2 ± 0·24	0·38

* Values represent the mean of three experiments (± standard error of the mean) expressed as per cent of distribution of total.

† Relative specific concentration[29] (RSC) = $\frac{\%\text{ of lipid, histamine or fumarase}}{\%\text{ of total protein}}$.

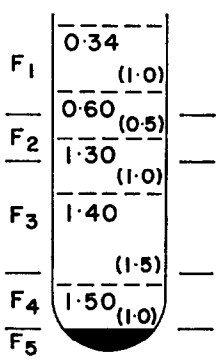

FIG. 2. Sucrose density gradient. The fractions collected from the top of the tube were designated F_1–F_5 and each corresponded to a visible band between the two layers (bands occurred between 0·6 and 1·3, 1·3 and 1·4 and 1·5 M sucrose). The large numbers (in the tube) indicate the sucrose molarities of the gradient layers. The numbers in parentheses (1·0) etc., are the volumes of the layers.

density gradient (see Methods and Fig. 2). Five fractions were collected and each was assayed for cerebroside sulfate, phospholipids, gangliosides and amines (Table 3). In addition, each fraction was assayed for the mitochondrial enzyme fumarase. Almost complete separation of the amine-containing fraction (F_5) from the fumarase activity was achieved. The bulk of the acidic glycosphingolipids, cerebroside sulfate and gangliosides, and the phospholipids appeared in a fraction (F_2) that showed high fumarase activity and low amine content. A considerable portion of the gangliosides (30·7%)

TABLE 3. DISTRIBUTION OF LIPIDS, AMINES AND FUMARASE (OF THE 5500 *g* FRACTION OF HC CELLS*) IN A DENSITY GRADIENT

Density gradient fractions	% Cerebroside sulfate	RSC†	% Phospholipids	RSC	% Gangliosides	RSC
F_1	26·1 ± 2·04	0·98	33·2 ± 1·30	1·19	14·6 ± 0·50	0·52
F_2	58·7 ± 5·06	2·70	43·9 ± 2·40	1·95	37·7 ± 1·35	1·67
F_3	2·1 ± 0·20	0·17	5·6 ± 1·12	0·46	7·8 ± 1·40	0·64
F_4	5·6 ± 0·84	0·55	5·4 ± 0·82	0·45	9·2 ± 0·50	0·76
F_5	7·5 ± 0·70	0·29	11·9 ± 3·20	0·46	30·7 ± 1·50	1·15

Density gradient fractions	% 5-Hydroxy-tryptamine	RSC	Histamine	RSC	Fumarase	RSC
F_1	14·6 ± 0·50	0·52	14·8 ± 1·18	0·53	34·8 ± 1·52	1·26
F_2	14·1 ± 0·49	0·62	17·4 ± 0·47	0·77	37·3 ± 1·77	1·64
F_3	8·8 ± 0·33	0·71	4·9 ± 0·29	0·40	17·3 ± 1·82	1·42
F_4	6·5 ± 0·38	0·54	5·4 ± 0·55	0·44	8·0 ± 1·49	0·66
F_5	55·8 ± 1·87	2·16	57·5 ± 1·26	2·23	2·7 ± 0·15	0·10

* Values represent the mean of three experiments (± standard error of the mean) expressed as per cent distribution of total.

† Relative specific concentration (RSC) = $\frac{\%\text{ of lipid, amines or fumarase}}{\%\text{ of total protein}}$.

was also found in the amine-containing fraction (F_5). The relative specific concentration (RSC)[29] values accentuate these differences in distribution.

Electron microscopic examination of the density gradient fractions of the HC cells showed a very good separation of the particles of the homogenate (Figs. 3–7). Particularly clean were fractions F_1 and F_3, while all of the remaining fractions contained mitochondria; most of these were in fractions F_2 and F_3. Fraction F_5 contained multivesicular, relatively electron-translucent granules and markedly electron-opaque granules, as well as granules of intermediate density between these two types (Fig. 7). All of these granules were previously shown to be mast cell granules in varying stages of maturation.[1]

To further characterize the acidic glycosphingolipids (see Methods and Fig. 1 for details), the choloroform–methanol (2:1) eluate from a magnesium silicate column and the chloroform–methanol (1:4) eluate from a silicic acid column were examined by thin-layer chromatography. Three different solvent systems were used for the thin-layer separation of cerebroside sulfate. One spot was detected. A typical chromatogram is shown in Fig. 8. Samples of cerebroside sulfate from thin-layer plates were eluted and the ratio of the cerebroside sulfate components was determined. The components were present in essentially equimolar proportions (Table 4), having a ratio of sphingosine:hexose:sulfate of 1·00:1·08:0·95. These values are close to the theoretical ratio of 1:1:1.

The ganglioside-containing eluate from a silicic acid column was examined by thin-layer chromatography in two solvent systems. Four resorcinol positive spots

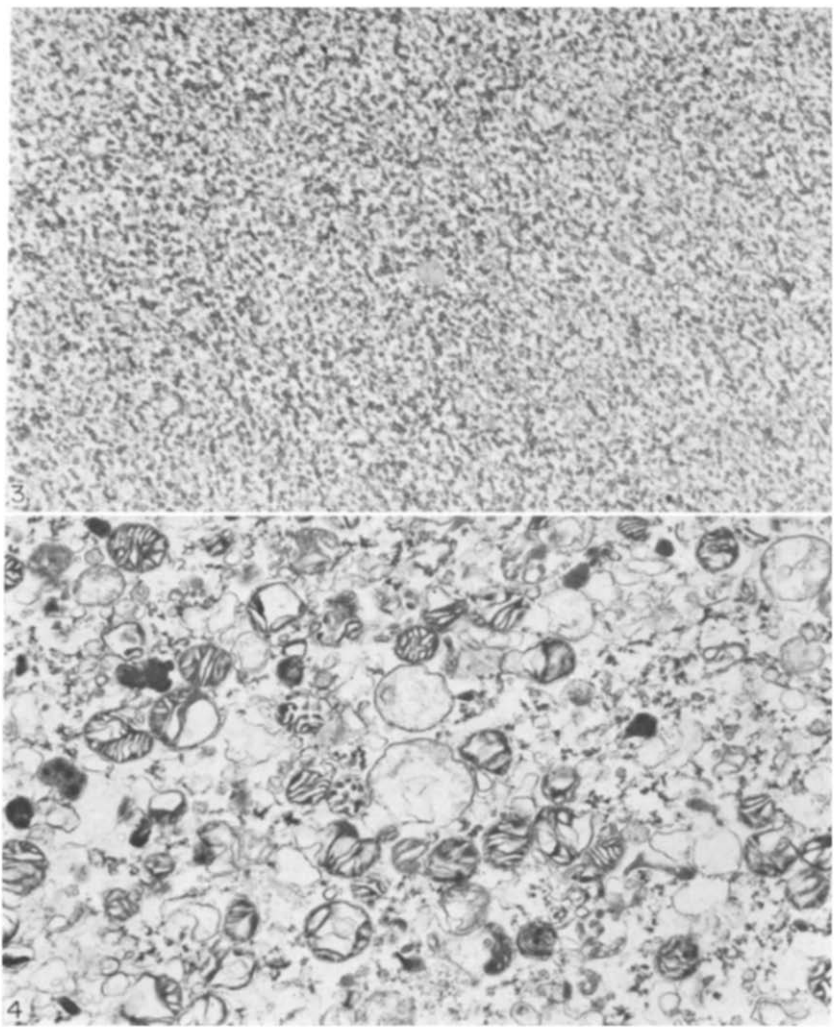

FIG. 3. The remarkably homogeneous fraction F_1 consisted of finely granular material suggestive of ribosomes. Magnification: 20,000×.

FIG. 4. Mitochondria predominated in this fraction (F_2); in addition, clumped fine granular material was observed, as well as a few vesicles. Magnification: 17,000×.

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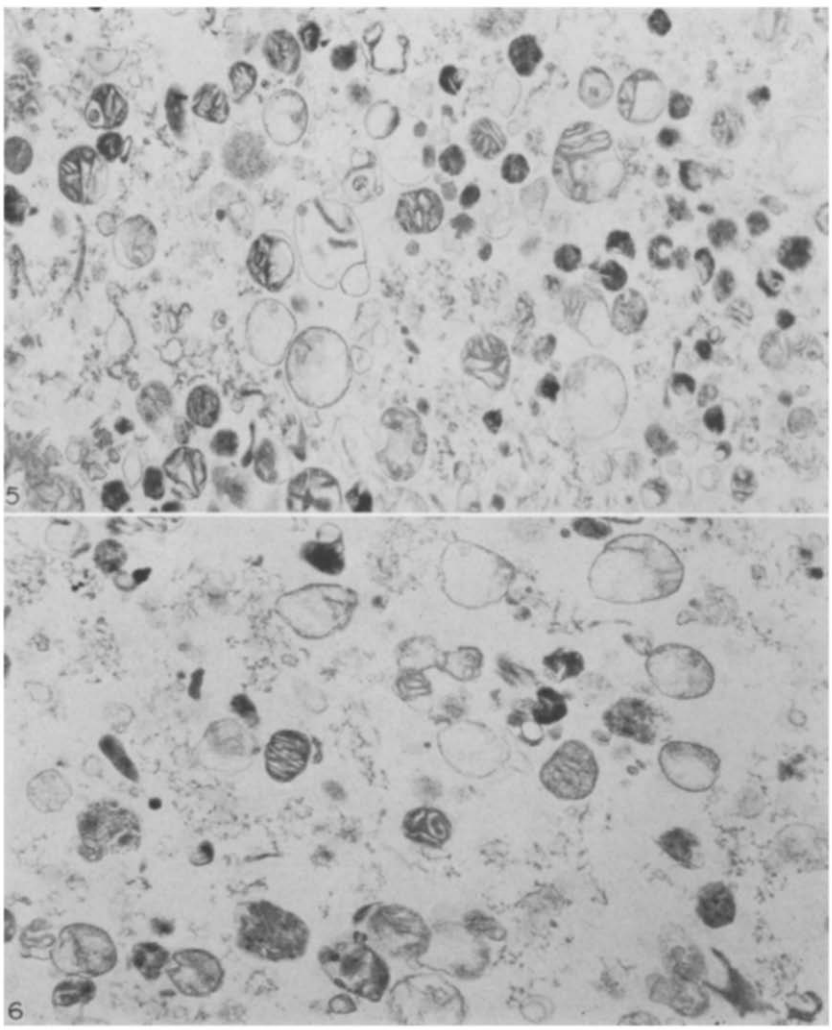

FIG. 5. Here again, mitochondria were the main constituents of the fraction (F_3); some of the mitochondria had a relatively electron-opaque matrix which, by a cursory examiner, might be mistaken for dense bodies. Magnification: 18,000×.

FIG. 6. Fraction F_4 consisted of mitochondria and loosely aggregated granules. Magnification: 20,000×.

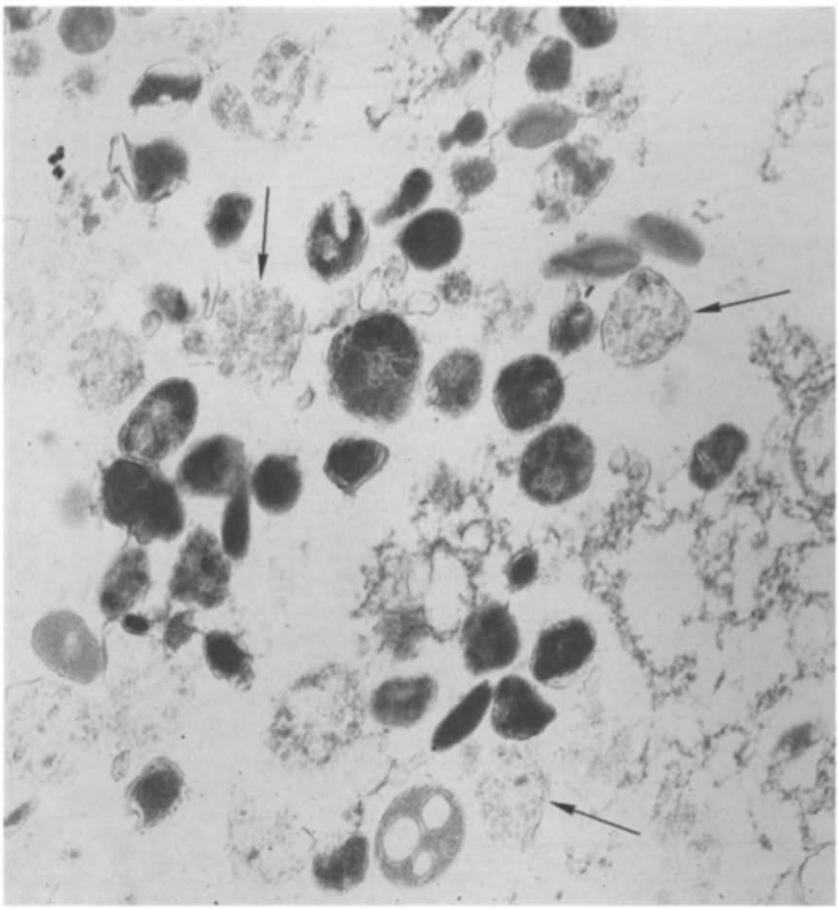

FIG. 7. Structures typical of mast cell granules for this cell type were ubiquitous in fraction F_5; these ranged from multivesicular bodies to irregularly shaped, very dense granules. Present in this photograph are about a dozen multivesicular bodies, three of which are marked by arrows. Irregular granular aggregates of moderate electron opacity, also present, are thought to be proteinaceous. Magnification: 38,000×.

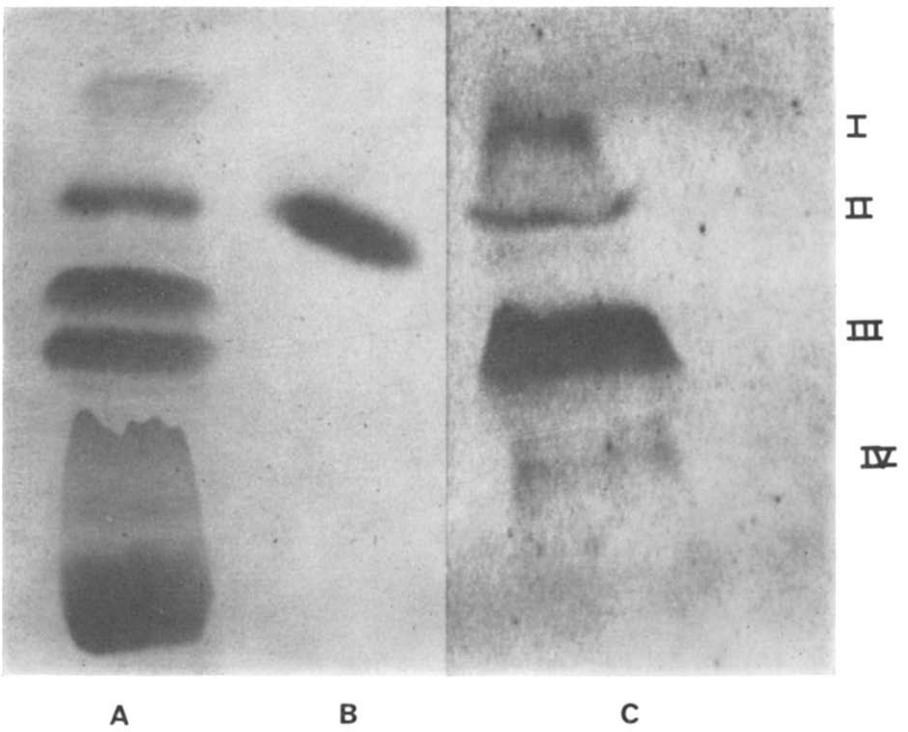

FIG. 8. Thin-layer chromatogram of mast cell lipids. (A) Total crude lipid extract. (B) Cerebroside sulfate purified by magnesium silicate column chromatography. Thin-layer chromatography solvent: chloroform–methanol–water (65:25:4, v/v); spray = 20% aqueous perchloric acid. (C) Gangliosides purified by silicic acid column chromatography. Thin-layer chromatography solvent: *n*-butanol-pyridine–water (46:31:23, v/v); spray = aqueous resorcinol–HCl–Cu^{2+} reagent (I) *N*-acylneuraminomonoglycosylceramide (II) *N*-acylneuraminosyl-monoaminoglycosyl-triglycosylceramide (III) *N*-acyldineuraminosyl-monoaminoglycosyl-diglycosylceramide (IV) unidentified.

TABLE 4. MAST CELL CEREBROSIDE SULFATE COMPOSITION

Preparation No.	Sphingosine (μmoles)	Hexose (μmoles)	Sulfate (μmoles)
1	2·64	2·84	2·44
2	2·03	2·25	2·32
3	3·12	3·39	2·69
Mean	2·60	2·83	2·48
Molar ratio*	1·00	1·08	0·95
Theoretical molar ratio*	1·0	1·0	1·0

* Calculated by using sphingosine as reference standard.

were seen, suggesting the presence of four different neuraminic acid containing compounds. The compounds were separated by preparative thin-layer chromatography, eluted and hydrolyzed. Their composition was determined by chemical analysis. The gangliosides have been designated as I, II, III and IV on the basis of their chromatographic mobility (Fig. 8). Ganglioside IV was present in trace amounts so that the ratio of its components could not be accurately determined. The results of the chemical

TABLE 5. MAST CELL GANGLIOSIDES COMPOSITION

Preparation No.	Sphingosine (μmoles)	Hexose (μmoles)	Hexosamine (μmoles)	NANA (μmoles)
Ganglioside I				
1	0·84	0·74	0·15	0·78
2	0·67	0·92	0·13	0·97
3	0·45	0·52	0·09	0·51
Mean	0·65	0·73	0·12	0·75
Molar ratio*	1·00	1·12	0·18	1·15
Theoretical molar ratio*	1·0	1·0		1·0
Ganglioside II				
1	0·52	1·48	0·48	0·53
2	0·66	1·81	1·61	0·66
3	0·33	1·07	0·33	0·36
Mean	0·50	1·45	0·47	0·52
Molar ratio*	1·00	2·90	0·94	1·04
Theoretical molar ratio*	1·0	3·0	1·0	1·0
Ganglioside III				
1	1·12	2·92	1·04	3·07
2	1·41	2·37	1·30	2·46
3	0·75	1·63	0·70	1·48
Mean	1·09	2·31	1·01	2·34
Molar ratio*	1·00	2·11	0·92	2·14
Theoretical molar ratio*	1·0	2·0	1·0	2·0

* Calculated by using sphingosine as reference standard.

analyses (Table 5) and chromatographic mobility suggest the following composition: (I) *N*-acylneuraminosyl-monoglycosylceramide, (II) *N*-acylneuraminosyl-monoaminoglycosyltriglycosylceramide, (III) *N*-acyl-dineuraminosyl-monoaminoglycosyl-diglycosylceramide.

DISCUSSION

The acidic glycosphingolipids, cerebroside sulfate and ganglioside, have been reported to occur as minor components in a number of human and animal tissues[30,31] and to accumulate in certain pathological conditions.[32,33] The present study has demonstrated the occurrence of two types of acidic glycosphingolipids, cerebroside sulfate and ganglioside, in two lines of neoplastic mast cells which contain histamine, serotonin and heparin. The highest level of both acidic glycosphingolipids was observed in the HC line derived from the Furth–Hagen–Hirsch mastocytoma. Each glycosphingolipid had a characteristic intracellular distribution. The distribution of cerebroside sulfate differed from that of the gangliosides in that the former was found mainly in a large-particle (5500 *g*) fraction, while the bulk of the gangliosides was found in small-particle fractions (30,000 *g* and 204,000 *g*).

The highest level of cerebroside sulfate was found in a cell fraction which showed high amine content, suggesting the possibility that both compounds were localized in the same intracellular structures. However, in the sucrose density gradient experiments, cerebroside sulfate occurred in a subfraction (F_2) separable from the amine-containing granules (F_5) and showing high fumarase activity that was characteristic of mitochondria. Electron microscopic examination of the density gradient fractions confirmed the biochemical findings by demonstrating that fraction F_5 contained the characteristic multivesicular amine storage granules with negligible contamination of mitochondria (Fig. 7). Therefore, cerebroside sulfate is probably not involved in the binding of biogenic amines in the mast cell's storage granules.

Analysis of mast cell cerebroside sulfate showed that sphingosine, hexose and sulfate were present in essentially equimolar proportions. This result is in accord with the findings of Green and Robinson[2] and suggests that mast cell cerebroside sulfate is similar in composition to cerebroside sulfate isolated from a number of mammalian tissues.[30,31]

Thin-layer chromatographic separation of the ganglioside-containing column eluate (Fig. 1, scheme A) demonstrated the presence of four resorcinol positive compounds (Fig. 8, C). Three of these compounds were present in sufficient quantity to permit chemical analysis. Two of them were monosialogangliosides and one was a disialoganglioside. The monosialoganglioside I (band I) had a ratio of NANA:hexose of 1:1. A measurable amount of hexosamine was found in all the preparations of ganglioside I. This was probably the result of contamination, but the possible occurrence of an additional hexosamine-containing ganglioside, similar in composition to I, has not been excluded. Monosialoganglioside II (band II) had a ratio of NANA: hexose:hexosamine of 1:3:1. Ganglioside III (band III) is a disialoganglioside having an NANA:hexose:hexosamine ratio of 2:2:1. These results suggest that the gangliosides present in the neoplastic mast cell may be structurally similar to gangliosides found in other normal and pathological mammalian tissues.[31,34–38] However, it is interesting to note the presence of at least three different gangliosides in a single cell type. The intracellular localization of gangliosides in plasma membrane has been

demonstrated in several cell types.[39–41] In brain tissue, consisting of many cell types gangliosides appear to be high in microsomal,[29,42–44] synaptosomal,[29,43] and myelin[43,45,46] fractions and low or absent in mitochondria,[43,45,47] nuclei,[48] and lysosomes.[44] The presence of gangliosides in synaptic vesicles[49] has not been confirmed by recent reports.[29,41] The metabolic relationship, function and distribution of gangliosides in the mast cell are not known.

An interesting characteristic of acidic glycosphingolipids is their capacity to form complexes with positively charged, biologically important organic compounds like acetylcholine, histamine and norepinephrine.[50–53] This has led to the suggestion that the acidic glycosphingolipids may play a role in the binding and transport of ions,[54] biogenic amines,[51] and the maintenance of electrical conductivity in excitable tissues.[55,56]

The results presented in this paper suggest that cerebroside sulfate does not participate in amine binding in mast cell granules, but its possible role in extragranular amine binding has not been eliminated. There also remains the possibility of ganglioside–amine interaction in these granules.[57]

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