Abnormal gangliosides in Tay-Sachs disease, Niemann-Pick's disease, and gargoylism

D. A. BOOTH, H. GOODWIN, and J. N. CUMINGS

Institute of Neurology, The National Hospital, Queen Square, London, England

ABSTRACT The molar ratios of *N*-acetyl neuraminic acid, hexose, hexosamine, and sphingosine have been determined for the abnormal ganglioside in Tay-Sachs disease that was previously detected as a fast-moving band in thin-layer chromatography, and in two abnormal fast-moving bands of gangliosides from the cortex and white matter of the brain in cases of gargoylism and Niemann-Pick's disease. The fastest-moving ganglioside band in these two conditions contains neither hexosamine nor glucose.

KEY WORDS thin-layer chromatography · fast-moving band · gangliosides · white matter · cortex · Niemann-Pick · gargoylism · Tay-Sachs · molar ratios · *N*-acetyl neuraminic acid · hexosamine · hexose · sphingosine

SEPARATION OF THE various gangliosides containing *N*-acetyl neuraminic acid (NANA) has been greatly assisted by the use of thin-layer chromatography. A band specific for Tay-Sachs disease has been described by Müldner, Wherrett, and Cumings (1) and by Svennerholm (2); it is a fast-moving band obtained from lipid extracts of the cerebral cortex and also from the white matter of infants affected with this disease. Wherrett and Cumings (3) found that more than 80% of the total NANA present in the cortex was in this band.

A number of workers have determined the relative molar proportions of NANA, hexosamine, hexose (glucose and galactose), and sphingosine in both this Tay-Sachs band and in one with slightly lower R_f in the chromatogram of the gangliosides of the normal cerebral cortex.

In gargoylism and in Niemann-Pick's disease there are in addition to the normal bands two faster-moving fractions, as has already been demonstrated in Niemann-Pick's disease (4). These features are illustrated in Figs. 1 and 2 for Tay-Sachs disease, gargoylism, and Niemann-Pick's disease. The three bands investigated here are arbitrarily indicated by the letters A, B, and C. Band C corresponds to G_{M2} of Svennerholm (2, 5), or G_0 of Kuhn and Wiegandt (6).

We report here the relative molar amounts of the component parts of these three ganglioside bands, which from the figures can be seen to be present in both white matter and cortex.

MATERIALS AND METHODS

Samples of fresh, unfixed brain from two cases each of Tay-Sachs disease, Niemann-Pick's disease with cerebral lesions, and gargoylism were obtained at biopsy or autopsy. The white matter and the cerebral cortex were in every case at once separated and from each portion a lipid extract was prepared as previously described [Wherrett and Cumings (3)]. The lipid was subjected to thin-layer chromatography on Silica Gel G (Merck) with the apparatus of Stahl (Camlab, Ltd., Cambridge) (1). The solvent mixtures used were: chloroform–methanol–10% aqueous ammonia 60:35:8 (v/v/v) (1) as well as chloroform–methanol–3.5 M NH_4OH 55:40:10 (v/v/v) (7). The various bands were made visible by means of the resorcinol spray of Svennerholm (8). The bands shown as A, B, and C (Figs. 1, 2) amongst the faster-moving gangliosides are those that stained purple; other bands visible in the photograph in these regions were yellow and appear not to contain NANA.

Each original lipid extract was then chromatographed on single plates and the position of each band was determined by spraying with water or with bromothymol blue (10 mg/100 ml of 9 M NH_4OH) and outlining with the point of a needle. When water was used the plates were subsequently dried in a stream of air. The gel containing each band was removed separately from ap-

Abbreviation: NANA, *N*-acetyl neuraminic acid.

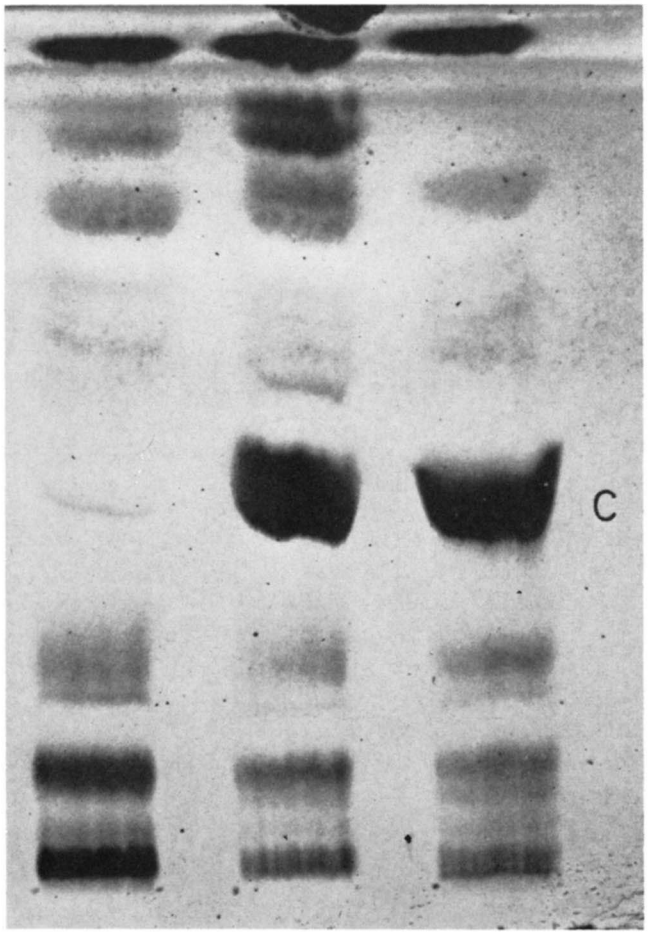

FIG. 1. Thin-layer chromatographic patterns of lipid extracts (from left to right) of normal cortex, white matter, and cortex from a case of Tay-Sachs disease. Developing solvent: chloroform–methanol–3.5 M NH_4OH 55:40:10 (v/v/v). Detected by resorcinol spray of Svennerholm (8). Only C, of the fast-moving bands, stained purple (NANA present).

proximately 7/8ths of the plate and the remainder of the gel on the plate was sprayed with the resorcinol spray to make sure that each band removed did correspond to bands A, B, and C. Bromothymol blue was removed by stirring and washing the gel with 10 ml of acetone, allowing the gel to settle for 5 min, and washing twice more with acetone. Gangliosides were then extracted from the gel with pure methanol (7), the methanol washings were pooled, taken to dryness at 60°C, and rechromatographed for final purification. The dried preparations were white and could be stored without deterioration. Each compound was pure, as judged by chromatography with both solvent systems.

Portions of each sample so prepared were analyzed by the methods indicated: NANA [Svennerholm (8)], hexosamine [Rondle and Morgan (9)], hexose [Roe (10)], sphingosine [Lauter and Trams (11)]. Hexose was also determined after separation of hexosamine from hexoses on a Dowex-X8 column as described by Wolfe and Lowden (12), and the identity of the hexose present was established by using anthrone and glucose oxidase in the manner they describe.

RESULTS AND DISCUSSION

Molar ratios calculated for the various bands are recorded in Table 1. In preliminary experiments on the fast band in the normal cortex [G_{M1} of Svennerholm (2, 5)] results similar to those of other workers were obtained. Our results in the abnormal band in Tay-Sachs disease are almost identical with those of other workers. It should be noted that care should be taken not to remove and examine the lowest quarter of band C, for the normal fast band may slightly overlap the abnormal fast band in Tay-Sachs disease, and hexose ratios of 2.3–3.05 were obtained in the lowest portion because of the consequent admixture.

Several workers (13–17) have confirmed Svennerholm's analyses (2, 5) of the ganglioside in the fast-moving band in Tay-Sachs disease and have shown that equimolar amounts of glucose and galactose are present. Whereas most of these workers have used thin-layer chromatography, some [Makita and Yamakawa (17)] have obtained similar results with column chromatography. All the earlier results relating to the Tay-Sachs band [G_{M2} of Svennerholm (2, 5)] were obtained on extracts of cerebral cortex; the present work shows its presence in white matter as well. It might be noted that some compounds not containing NANA have also been described in Tay-Sachs disease (17).

There are fewer reports of the presence of faster-moving bands (such as A and B) in other diseases. The analysis of bands A and B demonstrated that virtually no hexosamine was present in either; no glucose, only galactose was detected in the hexoses. Svennerholm (18) demonstrated a fast and faint small band (G_{M3}) in normal white matter but not in the cortex. An abnormal pattern in

TABLE 1 MOLAR DISTRIBUTIONS IN BANDS A, B, AND C (FIGS. 1 AND 2)

	NANA	Hexosamine (as base)	Hexose	Sphingosine
Tay-Sachs disease				
Band C	0.98	0.95	2.1	1
Niemann-Pick's disease				
Band B	0.9	0.07	1.95*	1
Band A	1.0	0.1	2.5*†	1
Gargoylism				
Band A	1.05	0.05	2.4*†	1

* The hexose present was galactose only.
† Further purification of these bands has resulted in a hexose molar figure of 2.14 in each case.

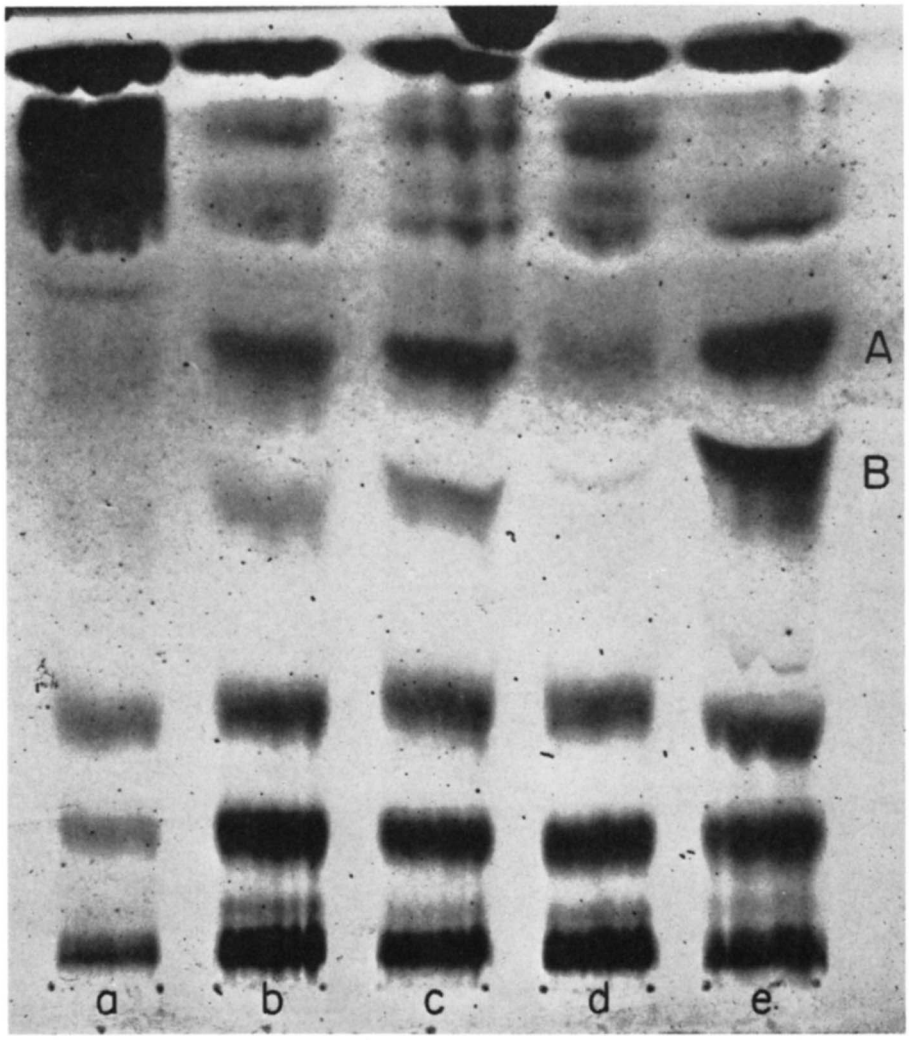

FIG. 2. Thin-layer chromatographic pattern, from left to right, of lipid extracts from: (*a*) cerebral white matter from a case of gargoylism, (*b*) cerebral cortex from same case of gargoylism, (*c*) cerebral cortex from another case of gargoylism, (*d*) normal cerebral cortex, (*e*) cerebral cortex from a case of Niemann-Pick's disease. Development and detection as in Fig. 1. Only A and B, of the fast-moving bands, stained purple.

Niemann-Pick's disease was recorded by one of us earlier (4); Jatzkewitz, Pilz, and Sandhoff (19) record an increase, for this disease, in the amounts of "Tay-Sachs ganglioside" present (3). Ledeen and coworkers (16, 20) have reported two fast-moving bands in a case of gargoylism, but they examined whole brain extract only. They found no hexosamine in the fastest-moving band G_6 (our A), although they also discussed a band which migrated at the same rate as the Tay-Sachs band (our C) and which contained hexosamine. Molar ratios were not given for the fastest-moving band.

It is of considerable interest that these abnormal fast bands are practically devoid of hexosamine. Although Svennerholm (18) demonstrated in extracts of normal white matter a faint fast band (G_{M3}) containing a very small amount of hexosamine, he did not find it in the normal cerebral cortex. The absence of glucose from band A is also noteworthy. These fast bands (A and B) appear to represent new ganglioside compounds containing NANA, sphingosine, and galactose in molar ratios of approximately 1:1:2. This ganglioside has been found by us in four diseases; apart from the two mentioned here it has been seen in globoid body diffuse sclerosis (Krabbe's disease) and in a form of amaurotic family idiocy similar to Tay-Sachs disease but occurring in older children, some of whom had visceral as well as cerebral manifestations.

Among the formulae that have been suggested for gangliosides are two by Klenk (21) in one of which hexose was present instead of an hexosamine. The ganglioside demonstrated here could be the one without the hexosamine. Whether in the diseases in which this gan-

glioside is present an abnormal ganglioside is synthesized or whether the normal metabolic processes are shifted in such a way as to increase the concentration of a ganglioside normally present at the expense of other gangliosides is not at present known.

We suggest that the nomenclature used by Svennerholm be employed in regard to these fast bands. The three bands A, B, and C would then be named G_{M4}, G_{M3}, and G_{M2}.

Manuscript received 16 August 1965; accepted 17 December 1965.

References

1. Müldner, H. G., J. R. Wherrett, and J. N. Cumings. *J. Neurochem.* **9:** 607, 1962.
2. Svennerholm, L. *Biochem. Biophys. Res. Commun.* **9:** 436, 1962.
3. Wherrett, J. R., and J. N. Cumings. *Trans. Am. Neurol. Assoc.* **88:** 108, 1963.
4. Cumings, J. N. In *Diseases of Metabolism, Fifth Edition*, edited by Garfield G. Duncan. W. B. Saunders Company, Philadelphia, 1964, pp. 1405–1439.
5. Svennerholm, L. *J. Neurochem.* **10:** 613, 1963.
6. Kuhn, R., and H. Wiegandt. *Chem. Ber.* **96:** 866, 1963.
7. Wherrett, J. R., J. A. Lowden, and L. S. Wolfe. *Can. J. Biochem.* **42:** 1057, 1964.
8. Svennerholm, L. *Biochem. Biophys. Acta* **24:** 604, 1957.
9. Rondle, C. J. M., and W. T. J. Morgan. *Biochem. J.* **61:** 586, 1955.
10. Roe, J. H. *J. Biol. Chem.* **212:** 335, 1955.
11. Lauter, C. J., and E. G. Trams. *J. Lipid Res.* **3:** 136, 1962.
12. Wolfe, L. S., and J. A. Lowden. *Can. J. Biochem.* **42:** 1041, 1964.
13. Klenk, E., U. Liedtke, and W. Gielen. *Z. Physiol. Chem.* **334:** 186, 1963.
14. Johnson, G. A., and R. H. McCluer. *Biochim. Biophys. Acta* **70:** 487, 1963.
15. Gonatas, N. K., and J. Gonatas. *J. Neuropathol. Exptl. Neurol.* **24:** 318, 1965.
16. Ledeen, R., K. Salsman, J. Gonatas, and A. Taghavy. *J. Neuropathol. Exptl. Neurol.* **24:** 341, 1965.
17. Makita, A., and T. Yamakawa. *Japan. J. Exptl. Med.* **33:** 361, 1963.
18. Svennerholm, L. In *Metabolism and Physiological Significance of Lipids*, edited by R. M. C. Dawson and D. N. Rhodes. John Wiley & Sons, New York, 1964, pp. 553–567.
19. Jatzkewitz, H., H. Pilz, and K. Sandhoff. *J. Neurochem.* **12:** 135, 1965.
20. Taghavy, A., K. Salsman, and R. Ledeen. *Federation Proc.* **23:** 128, 1964.
21. Klenk, E. *A.M.A. J. Diseases Children* **97:** 711, 1959.