Cerebral G_{M1}-Gangliosidosis: Chemical Pathology of Visceral Organs

Abstract. *The livers and spleens from three patients with cerebral G_{M1}-gangliosidosis contained greatly increased concentrations of a mucopolysaccharide tentatively identified as keratan sulfate. The concentration of a very soluble sialomucopolysaccharide was also increased. Concentrations of these compounds were not increased in the viscera of patients with Tay-Sachs disease (G_{M2}-gangliosidosis). G_{M1}-gangliosidosis appears to be a combined cerebral gangliosidosis and visceral mucopolysaccharidosis.*

G_{M1}-gangliosidosis is characterized chemically by an excess accumulation of a specific monosialoganglioside, G_{M1} [Svennerholm's ganglioside nomenclature (*1*)] in the central nervous system (*2*). Because of its clinicopathological features, the disease has been called neurovisceral lipidosis (*3*), a variant of Hurler's syndrome (*4*), or Tay-Sachs disease with visceral involvement (*5*). O'Brien *et al.* (*2*) detected G_{M1}-ganglioside in the liver and spleen of their patient and named the disease "generalized gangliosidosis." However, we found that the ultrastructure of the stored material in the liver is fibrillar and entirely different from the membranous cytoplasmic bodies found in neurons (*2*). This finding suggested that the stored material in the viscera of patients with G_{M1}-gangliosidosis, responsible for the gross histological abnormality, might not be ganglioside. We have investigated the livers and spleens from three patients with G_{M1}-gangliosidosis and three with Tay-Sachs disease (G_{M2}-gangliosidosis) to clarify the chemical pathology of the visceral organs of patients with G_{M1}-gangliosidosis.

The analysis for gangliosides was carried out as previously described (*6*). Crude mucopolysaccharides were prepared from that portion of the tissue which was insoluble in a mixture of chloroform and methanol (2 : 1, by volume). It was digested with papain (*7*) and α-amylase, and the mucopolysaccharides were precipitated from 80 percent ethanol as sodium salts. After the determination of the total amount of hexuronic acid, the crude mucopolysaccharide fraction was digested with testicular hyaluronidase (*8*) and reprecipitated. This precipitate was dissolved in water, dialyzed, and lyophilized. Samples of this fraction were analyzed for hexosamine by modified Elson-Morgan reaction (*9*), and for galactose by Galactostat reagent (Worthington Biochemical Corp.) after hydrolysis in 1*N* HCl at 100°C for 16 hours. The concentration of hexuronic acid was estimated by Dische's carbazole method (*10*) directly on the unhydrolyzed mucopolysaccharide fractions, both before and after its digestion with testicular hyaluronidase. Keratan sulfate was purified further by successive exhaustive digestion of the crude mucopolysaccharide fraction with α-amylase, testicular hyaluronidase, and then with Pronase (*8*). Keratan sulfate was then fractionally precipitated with ethanol as calcium salts (*11*).

The analysis for ganglioside showed that the normal control organs contain mostly hematoside (G_{M3}), whereas the organs of those with Tay-Sachs disease had increased amounts of Tay-Sachs ganglioside (G_{M2}), and those from patients with G_{M1}-gangliosidosis had increased amounts of G_{M1}-ganglioside (Table 1). However, the degree of the increase of the respective gangliosides, G_{M1} and G_{M2}, is similar in both diseases. The amounts of the multisialogangliosides were all normal. On the other hand, a spot with a large amount of sialic acid was noted at the origin of the thin-layer chromatogram of each G_{M1}-gangliosidosis sample. Its sialic acid content could be determined directly from the thin-layer plate in the same way as for gangliosides. This material was greatly increased only in G_{M1}-gangliosidosis, and not in Tay-Sachs disease. Paper chromatography (*12*) after preparative thin-layer chromatography and acid hydrolysis showed that this material contained galactose and glucosamine, much smaller amounts of galactosamine and mannose, and a trace of fucose. The compound could not be dialyzed, and it contained no fatty acid or sphingosine. In electrophoresis on cellulose polyacetate (Sepraphore III) in 0.1*M* phosphate buffer, *p*H 7.4, it migrated with the same mobility as that of keratan sulfate prepared from the same tissue. Therefore, this material appears to be an unusually soluble sialomucopolysaccharide possibly related to keratan sulfate.

The total amount of hexosamine in the acid polysaccharide fraction was greatly elevated in samples from patients with G_{M1}-gangliosidosis, and the increase in galactose roughly paralleled that of hexosamine (Table 1). Both before and after treatment with hyaluronidase, there was no significant difference in the concentration of total hexuronic acid among the three categories: the normal control, Tay-Sachs disease, and G_{M1}-gangliosidosis. Comparison of the quantitative data on the basis of wet weight is valid because the water content, residue insoluble in a mixture of chloroform and methanol (2:1, by volume), and total lipids in these pathological tissues were all normal. The sugar composition of the mucopolysaccharide fraction treated with hyaluronidase appeared almost identical to the sialomucopolysaccharide, galactose and glucosamine being the major components. As judged by the cellulose thin-

Table 1. Gangliosides, sialomucopolysaccharides, and acid polysaccharides in the viscera of patients with gangliosidoses.

Specimens	Gangliosides (μg sialic acid/g wet wt)			Sialomucopolysaccharide (μg sialic acid/g wet wt)	Constituents of acid polysaccharides (μg/g wet wt)	
	G_{M1}	G_{M2}	G_{M3}		Hexosamine	Galactose
			Liver			
Normal control	2.2	7.7	54.6	7.7	218	150
Tay-Sachs, case 1	7.5	83.2	69.0	3.7	181	56
Tay-Sachs, case 2	6.8	26.8	59.4	3.2	310	130
Tay-Sachs, case 3	9.5	81.5	79.8	3.9	314	71
G_{M1}-gangliosidosis, case 1	2.5	4.6	31.8	142.6	992	602
G_{M1}-gangliosidosis, case 2	39.4	4.3	53.1	34.7	2823	2670
G_{M1}-gangliosidosis, case 3	88.2	10.2	91.2	5.1	8213	7418
			Spleen			
Normal control	1.4	4.5	64.7	2.7	353	78
Tay-Sachs, case 1	8.9	80.9	70.7	3.4	448	87
Tay-Sachs, case 2	6.1	50.8	75.8	5.3	470	68
Tay-Sachs, case 3	6.0	52.7	50.3	6.3	507	103
G_{M1}-gangliosidosis, case 1	7.3	3.8	88.3	14.8	888	143
G_{M1}-gangliosidosis, case 2	32.2	6.6	43.6	90.0	741	126
G_{M1}-gangliosidosis, case 3	73.9	3.8	38.8	29.0	3534	2754

layer chromatography of Bischel *et al.* (*13*), metachromatic spots were present at the primary solvent front only in the G_{M1}-gangliosidosis samples. Similarly, only the G_{M1}-gangliosidosis samples contained a fast-moving component that stained pink with the orcinol-sulfuric acid spray in the plain silica gel thin-layer chromatography described by Wusteman *et al.* (*14*). The purified keratan sulfate migrated slightly faster than the standard skeletal keratan sulfate in electrophoresis at *p*H 7.4. A preliminary analysis suggested that the molar ratio of sulfate to hexosamine of this compound may be significantly greater than one. Such over-sulfation may account for its faster electrophoretic mobility. My findings justify the tentative, although by no means conclusive, identification of this compound as keratan sulfate.

I conclude that (i) the degree of increase in G_{M1}-ganglioside in the viscera of patients with G_{M1}-gangliosidosis is the same as that of Tay-Sachs ganglioside (G_{M2}) in viscera from those with Tay-Sachs disease; and (ii) there are specific increases of keratan sulfate and, to a lesser degree, a sialomucopolysaccharide with a similar sugar composition in the viscera of those with G_{M1}-gangliosidosis. The viscera of patients with Tay-Sachs disease have practically no histological abnormality, whereas those from patients with G_{M1}-gangliosidosis are characteristically affected. Therefore, the histological lesions of viscera in this disorder are more likely due to the accumulation of keratan sulfate and related compounds than to accumulation of ganglioside.

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References and Notes

1. L. Svennerholm, *J. Neurochem.* **10**, 613 (1963).
2. N. K. Gonatas and J. Gonatas, *J. Neuropathol. Exp. Neurol.* **24**, 318 (1965); J. S. O'Brien, *et al.*, *Amer. J. Dis. Children* **109**, 338 (1965); K. Suzuki, K. Suzuki, G. C. Chen, *J. Neuropathol. Exp. Neurol.* **27**, 15 (1968).
3. B. H. Landing, F. N. Silverman, J. M. Craig, M. D. Jacoby, M. E. Lahey, D. L. Chadwick, *Amer. J. Dis. Children* **108**, 503 (964).
4. J. M. Craig, J. T. Clarke, B. Q. Banker, *ibid.* **98**, 577 (1959).
5. R. M. Norman, H. Urich, A. H. Tingey, R. A. Goodbody, *J. Pathol. Bacteriol.* **78**, 409 (1959).
6. K. Suzuki, *Life Sci.* **3**, 1227 (1964); *J. Neurochem.* **12**, 629 (1965).
7. M. M. Szabo and E. Roboz-Einstein, *Arch. Biochem. Biophys.* **98**, 406 (1962).
8. N. Seno, K. Meyer, B. Anderson, P. Hoffman, *J. Biol. Chem.* **240**, 1005 (1965).
9. N. F. Boas, *ibid.* **204**, 553 (1953).
10. Z. Dische, *ibid.* **167**, 189 (1947).
11. K. Meyer, A. Linker, E. A. Davidson, B. Weissmann, *ibid.* **205**, 611 (1953).
12. F. G. Fischer and H. J. Nebel, *Z. Physiol. Chem.* **302**, 10 (1955).
13. M. D. Bischel, J. H. Austin, M. D. Kemeny, C. M. Hubble, R. K. Lear, *J. Chromatogr.* **21**, 40 (1966).
14. F. S. Wusteman, A. G. Lloyd, K. S. Dodgson, *ibid.*, p. 32.
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Bracken and Locust Ecdysones: Their Effects on Molting in the Desert Locust

Abstract. *Bracken contains ecdysone derivatives that are active when injected into locusts. However, when fed to the desert locust as its sole or chief diet, it does not affect molting, growth, or development. There is evidence that, in locusts, the active ecdysones are dehydroxylated to* α*-ecdysone and passed out through the gut in the feces. There is no evidence for any uptake of ecdysones from the gut.*

Substances which either are active in inducing puparium formation in Diptera (*Calliphora* and *Musca*) or have chemical structures similar to those of the insect ecdysones have been isolated from plants (*1*). Ecdysone, 20-hydroxyecdysone, and three related materials of unspecified structure are present in the pinnae of bracken [brake fern, *Pteridium aquilinum* (Linn.)]. Kaplanis and his co-workers (*2*) tentatively agreed with the supposition (*3*) that "perhaps these biologically active substances have been elaborated by the plant to interfere with the growth processes of insect predators" (by predators they meant plant-eating insects). This hypothesis is open to the simple test of feeding insects upon bracken.

The desert locust *Schistocerca gregaria* (Forskål) differs from most other Acridids in that it thrives better on a mixed diet of herbs than on grass alone. For this reason it was selected for these tests. The insects were reared individually in gauze-topped, glass jars under standard conditions (*4*). The controls were fed wheat seedlings. The experimental animals were fed air-dried fronds of autumnal bracken that were moistened immediately before being placed in the jars. At weekends, each locust fed upon bracken was given three wheat seedlings. Although the locusts were reared on these diets from 1 day after hatching until they became adult, molting was entirely normal and mortality was insignificant; less than 8 percent in any one group died.

Staal (*5*) implanted extra prothoracic gland tissue (as a source of extra molting hormone) into second-instar locusts (*Locusta migratoria*) and obtained fully formed adults after only three further molts in about 50 percent of his animals. The individuals that passed through the normal five instars produced adults with extra-long wings. In our work, all the locusts passed through the normal five instars, and the wings were shorter, not longer, in bracken-fed animals. This was related to an overall reduction in size. The difference in wing lengths was significant in the case of females: the mean for wheat-fed locusts was 4.70 $\pm$ 0.013 cm for males and 5.06 $\pm$ 0.009 cm for females; the mean for bracken-fed males was 4.42 $\pm$ 0.010 and for females 4.56 $\pm$ 0.018 cm. The weights of bracken-fed adults were significantly less than those for wheat-fed ones; for males the means were 0.832 $\pm$ 0.049 g and 1.094 $\pm$ 0.026 g, respectively; for females the means were 0.926 $\pm$ 0.018 g and 1.395 $\pm$ 0.025 g, respectively. The bracken-fed locusts

Table 1. Mean length (and standard error of the mean) of instars of locusts given different diets (based on results for at least 20 locusts per group).

Diet	Instar length (days)			
	2	3	4	5
Wheat	5.01 $\pm$ 0.322	7.84 $\pm$ 0.341	8.08 $\pm$ 0.292	11.90 $\pm$ 0.582
Bracken	9.23 $\pm$ .610	10.90 $\pm$ .791	11.63 $\pm$ .500	18.12 $\pm$ .463
		Probability		
	$< .01$	$< .05$	$< .01$	$< .01$