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Research Article

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STUDIES OF RED-CELL STROMAL LIPIDS IN TAY-SACHS DISEASE AND OTHER LIPIDOSES *

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The cherry-red spot in the fundus as a sign of what is now known as Tay-Sachs disease was first described by Tay in 1881 (1). The first clinical and pathological description of the condition was reported by Sachs in 1887 (2). The similarity between the morphologic and retinal changes in this condition and Niemann-Pick disease led Pick and Bielschowsky (3) to suggest that the conditions were different manifestations of the same disease. On the other hand, the work of Klenk (4–8), showing that the accumulated lipid in Tay-Sachs disease was a glycolipid (ganglioside), whereas that in Niemann-Pick disease was sphingomyelin, led to the view that these were different conditions (9). It has also been stated that the abnormalities in Tay-Sachs disease were confined to the nervous system (9).

The studies of Slome (10) and Aronson, Aronson, and Volk (11) showed that the inheritance of Tay-Sachs disease was by a recessive autosomal gene with complete penetrance. They considered that the heterozygous carriers showed no evidence of the disorder. The consanguinity rate among the parents of affected children was high in both series. Aronson and co-workers (11) showed that the incidence of the disease was highest among Ashkenazic Jews.

Balint, Nyhan, Lietmann, and Turner (12) reported reduction of the cephalin fraction of the phospholipids of the red-cell stroma in a child with Niemann-Pick disease. In a later study of the same patient, Balint and Spitzer (13) reported a reduction of red-cell sphingomyelin as well as cephalin. The same authors (14), in a preliminary communication, reported marked reduction of red-cell sphingomyelin and cephalin in Tay-Sachs disease. Rouser, Bauman, Nicolaides, and Heller (15) had reported suggestive evidence of reduction of sphingomyelin in the red-cell stroma in Niemann-Pick disease by paper chromatography. These findings suggested a more widespread disorder of lipid metabolism that appeared to be common to both of these conditions.

This report concerns our findings in six children with Tay-Sachs disease and their parents, who were clinically normal, as well as in normal children and adult subjects and in one patient with Gaucher's disease.

METHODS

Heparinized blood (8 to 20 ml) was obtained from six children with Tay-Sachs disease and from nine of the twelve apparently healthy parents, and from six normal children of similar age to those of the Tay-Sachs group and from four normal adults, two of them of Anglo-Saxon parentage, one of Greek, and one of Jewish. Clinical data on the six children with Tay-Sachs disease are presented in Table I, which shows that each child exhibited the characteristic features of the disorder. The red blood cells were morphologically normal. The red cells were immediately separated from the plasma by centrifugation at 3,000 rpm for 30 minutes, and the plasma was withdrawn. The cells were then washed three times with 0.9% saline. The saline and the buffy coat were withdrawn, and the red-cell volume was recorded. The red cells were then hemolyzed with distilled water and mixed with Celite, as previously described (12), and extracted with 20 vol of choloroform-methanol (2:1, vol:vol). The extract was then filtered and washed with an equal volume of 0.9% saline, and the chloroform layer was separated and dried over anhydrous sodium sulfate. Because saline washing may result in loss of glycolipids, the lipid extract was reduced to 1 to 2 ml, brought back to a volume of 50 ml with chloroform, and partitioned against 0.25 M $MgCl_2$ (16) in one patient with Tay-Sachs disease. The lipid extract was then evaporated under reduced pressure at 37 to 40° C to a small volume and made up to 25 ml

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TABLE I

Clinical data on children with Tay-Sachs disease

Patient	Age	Sex	Cherry-red spot	Optic atrophy	Convulsions	Blindness	Anemia	Nutrition
	months							
W.H.	15	F	+	+	+	0	0	†
L.S.	19	M	+	+	+	+	0	†
L.Schr.	15	F	+	+	+	+	0	†
L.C.	34	F	+	+	+	+	0	Tube
D.Sch.	21	F	+	+	+	+	0	†
R.S.	9	M	+	+	+	0	0	†

† Normal diet, spoon feeding.

with chloroform. Total lipid was determined gravimetrically on a suitable small sample. Total lipid phosphorus was measured by Bartlett's procedure (17) on a suitable sample.

In four subjects, the chloroform-methanol extraction procedure was performed on duplicate samples of red cells, with Celite in only one of these, to assess the effect of this material on the total recovery of lipid. The results are shown in Table II. In one subject (E.K.) the residue was re-extracted by Bloor's method (18) to check the completeness of extraction.

Lipid fractionation was performed on silicic acid [1] columns with column loads of less than 10 mg lipid per gram of silicic acid (usually 5 g silicic acid, 10 cm high, 1 cm i.d.). Neutral lipids were eluted with chloroform. The phospholipids were then fractionated with increasing concentrations of methanol in chloroform as described elsewhere (12). The individual peaks, as determined by monitoring 1-ml samples from alternate 10-ml fractions for phosphorus (17), were pooled and made up to suitable volumes. The weight of each peak was determined gravimetrically on a 1-ml sample. Each peak was analyzed for phosphorus (17), for ester by the hydroxamate procedure (19, 20), and for amino nitrogen (21). Samples containing 8 to 10 μg of phosphorus were further identified by chromatography on silicic acid-impregnated Whatman 1 paper in an ascending system of methanol-chloroform (1:4) with 2% water against appropriate pure standards. This procedure showed that the cephalins were free of contaminants. There was essentially no detectable contamination of the lecithin by sphingomyelin, or the reverse, in the patients with Tay-Sachs disease or their parents. In the normal controls, some contamination of both compounds by the other was demonstrated, as confirmed by the ester-phosphorus (E:P) ratios. By use of the paper chromatograms and the E:P ratios, the values for lecithin and sphingomyelin were recalculated. Where significant contamination of lecithin occurred, the E:P ratio was always less than 1.8:1. The ester value was assumed to be correct, and the phosphorus value was then adjusted so that E:P was 1.8:1. Where contamination of the sphingomyelin peak was noted, the E:P ratio of this peak was always higher than 0.4:1, and the appropriate correction was made.

In some instances, the cephalins were further fractionated on ammonium silicate columns as previously described (22) to determine the proportions of phosphatidyl ethanolamine and phosphatidyl serine. Total lipid extracts were also analyzed for hexose after hydrolysis by the anthrone method. The neutral lipid fraction was shown to contain no lipid phosphorus. It was also analyzed for cholesterol by a modification of Zak's method (23) (omitting the protein and precipitation, and reading at 530 mμ) and for esters (19, 20), and its weight was determined gravimetrically.

TABLE II

Effect of Celite on the yield of lipids extracted from red blood cells with chloroform-methanol (2:1)

Subject	With Celite		Without Celite	
	Total lipid P		Total lipid P	
	mg/100 ml rbc	*μmoles/ 100 ml rbc*	*mg/100 ml rbc*	*μmoles/ 100 ml rbc*
P.B.	521.0	253.4	448.0	288.6
R.D.	602.0	354.3	524.6	332.3
D.P.	572.0	277.7	566.0	329.3
E.K.	481.9	260.3	441.7	299.0
Mean	544.2	286.4	495.1	312.3

[1] Unisil, Clarkson Chemical Company, Williamsport, Pa.

RESULTS

Table II presents the data obtained in duplicate extractions of four samples of red cells, with and

TABLE III

Effect of Celite on the red-cell phospholipid pattern (Subject P.B.)

	Unknown	Cephalins	Lecithin	Sphingomyelin	Lysolecithin
	% total lipid phosphorus				
Celite	2.0	39.2	37.4	20.3	0.8
No Celite	3.6	36.0	37.8	23.8	0.7

without the addition of Celite. Although the mean recovery of phospholipids was slightly lower and that of total lipids slightly higher in the presence of Celite, the differences are inconstant and statistically not significant. In one subject (P.B.), the two extracts were fractionated by column chromatography and gave similar results (Table III). In another subject (E.K.), re-extraction of red-cell residue by Bloor's method yielded 20 μmoles of lipid phosphorus per 100 ml red blood cells from the cells mixed with Celite, and 10 μmoles per 100 ml without Celite.

The typical elution patterns for the normal red-cell phospholipids and for those of a child with Tay-Sachs disease are shown in Figure 1. Recoveries from the columns averaged 96.5%. Table IV shows the percentage distribution of the cephalins, lecithin, and sphingomyelin in the red-cell stroma of the normal children and those with Tay-Sachs disease. There is significant reduction of sphingomyelin (10.4 ± 1.8% as against 24.1 ± 1.1%) and increase in lecithin (48.8 ± 4.5% as against 36.4 ± 1.5%). Table V gives the corresponding data for normal adults and the parents of the Tay-Sachs children. The differences are similar to those observed in the children, but less pronounced (13.4 ± 1.2% and 45.9 ± 1.7%, respectively). Lysolecithin accounted for 1.0 to 1.5% of the total phospholipids in all subjects.

The full extent of the changes in lipid composition of the red-cell stroma are shown in Tables VI and VII, where the total cholesterol, total phospholipid, cephalin, lecithin, and sphingomyelin content of the red-cell stroma in normal sub-

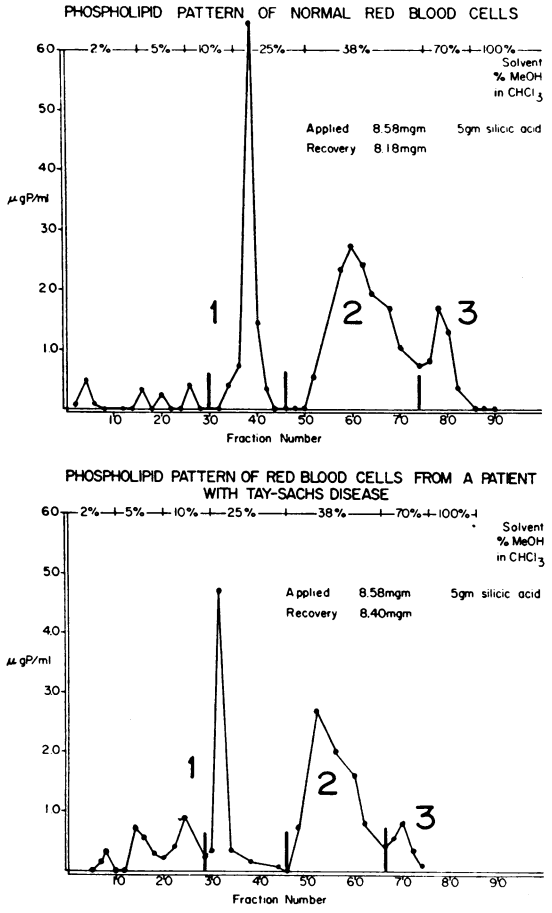

FIG. 1. ELUTION PATTERNS OF PHOSPHOLIPIDS OF RED CELLS OF A NORMAL CHILD AND OF A CHILD WITH TAY-SACHS DISEASE. Peak 1 contains cephalins, peak 2 lecithin, and peak 3 sphingomyelin in each case. The small peaks eluted before the cephalins could not be identified because of insufficient quantities of material. Vertical bars indicate the limits of each peak for purposes of pooling.

TABLE IV

Red-cell phospholipid patterns in normal controls and in children with Tay-Sachs disease

Normal				Tay-Sachs			
Subject	Cephalins	Lecithin	Sphingomyelin	Subject	Cephalins	Lecithin	Sphingomyelin
	% total lipid phosphorus				*% total lipid phosphorus*		
M.P.	33.9	42.1	22.5	W.H.	29.4	63.8	3.2
D.M.	27.4	33.1	25.6	L.S.	23.7	52.0	6.7
J.B.	36.4	34.4	26.3	L.Schr.	27.4	55.4	10.2
K.O'K	38.5	36.2	23.2	L.C.	28.8	52.0	13.8
S.S.	37.2	39.8	19.5	D. Sch.	34.9	39.2	11.6
D.S.	35.3	32.8	27.9	R.S.	38.6	30.4	16.6
Mean	33.1	36.4	24.1		30.5	48.4	10.4
±SEM	± 1.47	± 1.47	± 1.13		± 2.0	± 4.5	± 1.80
Significance of difference from normal					NS	<.025	<.001

TABLE V

Red-cell phospholipids in normal adult controls and in parents of children with Tay-Sachs disease

Normal				Tay-Sachs			
Subject	Cephalins	Lecithin	Sphingomyelin	Subject	Cephalins	Lecithin	Sphingomyelin
	% total lipid phosphorus				*% total lipid phosphorus*		
A.D.	37.7	36.2	22.6	F.S.	34.3	46.7	14.8
J.B.	35.3	40.3	21.3	M.S.	32.9	43.4	18.9
E.K.	38.2	37.6	22.8	F.C.	34.4	50.2	8.4
P.E.	37.5	37.1	24.4	M.C.	35.7	50.2	9.4
				F.Schr.	31.1	52.1	8.3
				M.Schr.	36.5	46.1	13.0
				F.H.	35.3	43.1	15.3
				F.Sch.	35.8	47.5	15.7
				M.Sch.	36.9	33.9	16.6
Mean	37.2	37.8	22.8		34.8	45.9	13.4
±SEM	±0.56	±0.76	±0.55		±1.83	±1.71	±1.21
Significance of difference from normal					NS	<.001	<.001

jects and children with Tay-Sachs disease and their parents is given in micromoles per 100 ml red cells, together with the values for total lipids in milligrams per 100 ml. These tables show that, both in children with the disease and in their parents, there is an increase in cholesterol content as compared to controls. In normal children and adults, the molar ratio of cholesterol to phospholipids was 0.9 and 1.3, respectively. In the affected children, this ratio was 1.82, and in their parents, 1.72. Cholesterol accounted for an average of 49% of the neutral lipids (range, 43 to 60%). Tables VI and VII also show a significant decrease of cephalins as well as of sphingomyelin in the children with Tay-Sachs disease and their parents. The concentration of the cephalins in red cells of the affected children was 61.6 ± 9.4 μmoles per 100 ml packed cells, and that of sphingomyelin was 23.3 ± 5.8 μmoles per 100 ml, compared with normal values of 116.9 ± 6.8 and 81.3 ± 4.5 μmoles per 100 ml, respectively. In the parents, the respective values were 84.0 ± 5.1 and 33.0 ± 4.1 μmoles per 100 ml, compared with normal adult values of 101.1 ± 4.4 and 61.7 ±1.7 μmoles per 100 ml, respectively. In contrast, concentration of lecithin was normal in both groups. There was no significant difference between normal and test subjects in terms of total lipid concentration.

Similar studies have been carried out on a single 38-year-old patient with Gaucher's disease. The lipid pattern of this patient's red cells closely resembled that seen in Tay-Sachs disease. Concentrations, in micromoles per 100 ml red cells,

TABLE VI

Red-cell lipids in normal children and in children with Tay-Sachs disease

Normal							Tay-Sachs						
Subject	Total lipids	Cholesterol	Phospholipids	Cephalins	Lecithin	Sphingomyelin	Subject	Total lipids	Cholesterol	Phospholipids	Cephalins	Lecithin	Sphingomyelin
	mg/100 ml		*μmoles/100 ml rbc*					*mg/100 ml*		*μmoles/100 ml rbc*			
M.P.	462.1		290.6	98.5	122.3	65.4	W.H.	371.4	406.0	160.0	47.0	102.1	5.1
D.M.	450.0		376.7	103.2	124.7	96.4	L.S.	400.0	310.0	138.1	32.8	71.7	9.3
J.B.	547.0	272.5	336.5	122.5	115.8	88.5	L.Schr.	439.7	355.0	197.5	54.1	109.4	20.1
J.O'K	466.2	291.9	300.0	115.5	108.6	69.6	L.C.	557.8	343.5	290.2	83.6	150.9	40.0
S.S.	448.5		405.2	149.8	161.2	79.0	D.Sch.	403.7	234.9	183.7	51.8	77.2	21.9
D.S.	523.5		317.9	112.1	104.3	88.6	R.S.	452.1	389.3	260.7	100.6	79.3	43.3
Mean	482.9	282.2	337.8	116.9	122.8	81.3		437.5	339.8	205.0	61.6	98.4	23.3
±SEM	±15.4		±16.7	±6.8	±7.6	±4.5		±24.5	±26.5	±21.9	±9.4	±13.0	±5.8
Significance of difference from normal								NS		<.001	<.001	Ns	<.001

TABLE VII

Red-cell lipids in normal adult controls and in parents of children with Tay-Sachs disease

Normal							Tay-Sachs						
Subject	Total lipids	Cholesterol	Phospholipids	Cephalins	Lecithin	Sphingomyelin	Subject	Total lipids	Cholesterol	Phospholipids	Cephalins	Lecithin	Sphingomyelin
	mg/100 ml		*μmoles/100 ml rbc*					*mg/100 ml*		*μmoles/100 ml rbc*			
A.D.	407.7	352.8	277.9	104.8	100.6	62.8	F.S.	429.1	393.4	269.8	92.5	125.9	39.9
J.B.	482.5	355.3	293.3	103.5	118.2	62.5	M.S.	403.3	316.2	253.8	83.5	110.2	48.0
E.K.	431.1	368.1	287.2	109.7	108.0	65.5	F.C.	511.4	481.9	263.6	90.7	105.9	22.2
P.E.	373.3	371.1	230.0	86.4	85.4	56.1	M.C.	436.7	330.2	200.6	71.6	80.9	18.8
							F.Schr.	398.4	416.1	198.1	61.5	103.2	16.5
							M.Schr.	316.0	333.7	168.5	61.5	77.7	21.7
							F.H.	506.2	473.8	268.6	94.8	115.7	41.2
							F.Sch.	366.7		255.2	91.4	121.3	40.0
							M.Sch.	424.1		295.0	108.9	100.1	48.9
Mean	423.7	361.8	272.1	101.1	103.1	61.7	Mean	421.3	392.3	241.5	84.0	104.5	33.0
±SEM	±42.9	±3.95	±12.5	±4.4	±5.9	±1.7	±SEM	±16.6	±24.04	±12.9	±5.1	±5.2	±4.1
Significance of difference from normal								NS	NS	>.05	<.01	NS	<.001

were: cholesterol, 317.4; cephalins, 65.2; lecithin, 111.3; and sphingomyelin, 32.5.

The relative proportions of phosphatidyl serine and phosphatidyl ethanolamine were normal in the pooled cephalin fractions from the parents of the affected children. This study was not performed on the samples from the children with Tay-Sachs disease, since the quantities of material available were not sufficient. P:E ratios for the cephalin fraction ranged from 1:1.55 to 1:1.90 (mean, 1:1.76), and phosphorus:amino nitrogen ratios varied from 1:0.81 to 1:1.09 (mean, 1:0.96). Only trace amounts of anthrone-positive material were detected in the one subject studied (D.Sch.). In this same subject, three samples of red cells were analyzed. In the first of these, the extract was washed with $MgCl_2$. The reason for the low total recovery in this instance is not clear. The values for the cephalins and sphingomyelin, however, were comparable in the three extracts (Table VIII). The results obtained in the second extract are presented in Tables IV and VI.

DISCUSSION

The proportions of the various phospholipids in the red blood cells of normal subjects in this study are in fair agreement with those previously reported (12, 13, 24–26). Reed, Swisher, Marinetti, and Eden (24) and Phillips and Roome (25) noted a higher percentage of cephalins (39.5% and 42.4%, respectively), but based their calculations on the results of paper chromatography of the phospholipids. Both groups reported lower percentages of lecithin (30.0 and 32.7%, respectively) than were observed in the present study. Farquahar (26), using a similar extraction procedure to ours and successive column chromatography of the lipids, found that cephalins accounted for 39% of the phospholipids, as compared with 33.1 ± 1.47% in our control children and 37.2 ± 0.56% in our normal adults. Farquahar (26) reported only 3% of the total phospholipids as unidentified, whereas in our series, 10 to 15% was in this category (Figure 1), a result that agrees well with the data of Weed, Reed,

TABLE VIII

Results of repeated studies of red-cell lipids in Patient D.Sch.

Study no.	Total lipids	Total phospholipids	Cephalins	Lecithin	Sphingomyelin
	mg/100 ml	*μmoles/100 ml*	*μmoles/100 ml*	*μmoles/100 ml*	*μmoles/100 ml*
1	287.4	160.9	56.2	63.1	18.7
2	403.7	183.7	51.8	77.2	21.9
3	464.3	225.1	59.0	108.5	21.4

and Berg (27). The small peaks of phosphorus-containing compounds eluted before the cephalin peak (A) in the system used in this study and possibly containing cephalin plasmalogens may have been included in the cephalins in the experiments just cited (24–26) and may account for the differences observed. Farquahar (26) reported total lipid of 510 ± 51 mg and total phospholipids of 384.5 ± 25.9 μmoles per 100 ml of red blood cells. These figures are slightly higher than those of Reed and co-workers (24) and Phillips and Roome (25) and appreciably higher than ours. As shown in Tables II and III, however, these differences cannot be attributed to the use of Celite in the extraction procedure. Furthermore, re-extraction of the residue after chloroform-methanol extraction by Bloor's method (18) in subject E.K. (Table II) yielded only small amounts of additional phospholipids. The most likely explanation of our lower figures would seem to be an overestimate of the red-cell volume.

An objection has recently been raised to the use of heparin as an anticoagulant in studies of the lipids of red cells because of the possible resultant loss of phosphatidyl ethanolamine (28). This possibility cannot be ruled out in the present study, but seems unlikely, since Phillips (29) obtained comparable results with heparin to those of Farquahar (26), who used EDTA, whereas Hanahan, Watts, and Pappajohn (28) obtained lower values for cephalins with citrate as an anticoagulant. Even if such a loss does occur, however, it is unlikely to have affected the results in regard to the differences between the normal subjects and the patients with Tay-Sachs disease and their parents in this study, since the same procedure was employed in all instances. Furthermore, such considerations do not apply to sphingomyelin. Our findings in regard to the cholesterol as a fraction of the neutral lipids agree with those of other investigators (26, 27, 29).

We have shown a highly significant decrease in the sphingomyelin content of the red blood cells in Tay-Sachs disease, and also, a significant decrease of cephalins in the red-cell stroma. These observations are similar to those we reported earlier in a patient with Niemann-Pick disease (12, 13) and to those in the one patient with Gaucher's disease reported here. These findings indicate a disturbance affecting the metabolism of cephalins as well as of sphingolipids in these conditions. Furthermore, the data presented show that the disturbances of lipid metabolism in Tay-Sachs disease affect tissues outside the nervous system.

The data on the red-cell lipids of the parents of children with Tay-Sachs disease are of great interest. Extensive family studies (10, 11) have shown that this disease is inherited as an autosomal recessive trait with complete penetrance. Hence the parents must be heterozygous carriers and might be expected to show some abnormality by analogy with the situation in phenylketonuria (30). This has in fact proved to be the case. Reduced levels of serum fructose-1-phosphate aldolase in children with Tay-Sachs disease, their parents, and some of their grandparents have recently been reported by Aronson, Perle, Saifer, and Volk (31). The changes observed in the parents in the present study are similar to those of the affected children, but appear to be less severe, and are thus consistent with the hypothesis of a recessive mode of inheritance.

The similarity of the phospholipid changes in the red cells of patients with Tay-Sachs disease to those observed in Niemann-Pick disease (12, 13, 15) and in one patient with Gaucher's disease suggests that there must be a factor common to all three conditions. Published studies of red-cell phospholipids in various other diseases have shown no comparable abnormalities. Thus, Phillips and Roome (25) showed that red-cell phospholipids are normal in a variety of hemolytic disorders, whereas Ways, Reed, and Hanahan (32) and Phillips (29) have reported increased sphingomyelin and decreased lecithin concentrations in the red cells of patients with acanthocytosis. There may be at least two traits involved in the inheritance of Tay-Sachs, Niemann-Pick, and Gaucher's diseases, one of which is common to all three diseases and which determines the changes we have observed in the red cells, whereas the other(s) determines the specific sphingolipid accumulating in the nervous system or the reticuloendothelial system, or the predisposition of these tissues for such accumulation. It is not possible with the limited data available to determine whether these traits represent two reactions at one locus. That all subjects studied thus far have shown the same changes is, however, con-

sistent with this view. Further studies to elucidate these problems are in progress in our laboratory.

SUMMARY

Analysis of the lipids of red cells by means of silicic acid column chromatography in six patients with Tay-Sachs disease, in nine of the twelve healthy parents, and in one patient with Gaucher's disease has revealed significant reduction in cephalins and sphingomyelin as compared with normal controls. These changes in the heterozygotes are likely to be a phenotypic expression of the single allele.

These observations are discussed in relation to the present concept of the inheritance of Tay-Sachs disease. Inheritance may be multifactorial, and the disorder of lipid metabolism in Tay-Sachs disease and related conditions may be more complex than hitherto believed.

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