This finding underlines the difference between the various tissues, as far as sterol synthesis or uptake of plasma circulating sterols is concerned.

Further study is required to determine whether the relevant levels of desmosterol found in human brain tumors after triparanol treatment arise by local synthesis or deposition of desmosterol from the blood. It is known that human brain tumors actively synthesize cholesterol from labeled precursors (10) in contrast to normal adult brain, but it is also known that the blood-brain barrier is less efficient at the level of the tumors (24). However, direct evidence is still lacking as to whether blood sterols can be taken up and accumulated in human normal and tumor nervous tissue.

The present investigations on brain sterols suggest that the desmosterol levels are correlated with the degree of maturation of the brain. Desmosterol is present in brain during maturation in different animal species, and from the data obtained with PTU and thyroxine, the desmosterol concn seems related with the degree of development. On the other hand, the appearance of desmosterol in pathological specimens such as experimental and human brain tumors confirms the significance of this sterol in immature nervous tissue.

ACKNOWLEDGMENT

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Lipid Class Composition of Normal Human Brain and Variations in Metachromatic Leucodystrophy, Tay-Sachs, Niemann-Pick, Chronic Gaucher's and Alzheimer's Diseases

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Abstract

Procedures suitable for obtaining representative samples of whole brain and of total grey and white matter of brain are presented and discussed. A procedure is described for the quantitative determination of lipid class distribution of human brain specimens utilizing in sequence: a cellulose column to separate gangliosides and nonlipid material from the remaining lipids, diethylaminoethyl (DEAE) cellulose column chromatography to separate the lipid classes into manageable groups, and finally quantitation of the lipid classes by thin-layer chromatography (TLC). TLC is made quantitative by correlating the amt of charring of spots on chromatograms with the amt of lipid present by means of transmission densitometry. The use of two-dimensional TLC for the analysis of brain lipids and its application to the study of pathological brain specimens is also described.

The application of these procedures to the study of metachromatic leucodystrophy, Tay-Sachs, Niemann-Pick, and Alzheimer's diseases and senile cerebral cortical atrophy is described and data are presented. In two cases of Alzheimer's disease, a large reduction in fresh weight and total lipid of brain were found; the lipid class distribution of whole brain in one case and of total grey and total white matter in another were essentially normal. The lipid class distributions of the brain in metachromatic leucodystrophy, Tay-Sachs disease, and Niemann-Pick disease were shown to be similar to that of normal infant brain except that one sphingolipid was greatly increased in each disease (sulfatide in metachromatic leucodystrophy, one ganglioside in Tay-Sachs disease, and sphingomyelin in Niemann-Pick disease).

Introduction

LIPIDS ARE OF SPECIAL importance to brain. About 50% of the dry weight of brain is lipid. A part of this lipid is derived from brain cell membranes, membranes of subcellular particles and other structures but by far the largest part is derived from the myelin sheath, a special structure about the axons of the neurons. For the past several years we have investigated the relationships between lipid composition of the human brain and developmental stages, the aging process, and specific pathological processes.

This report presents procedures for the study of brain lipid class distribution for accurate determination of the composition of whole brain and total grey and total white matter. Results and interpretations for several hereditary metabolic diseases are also presented.

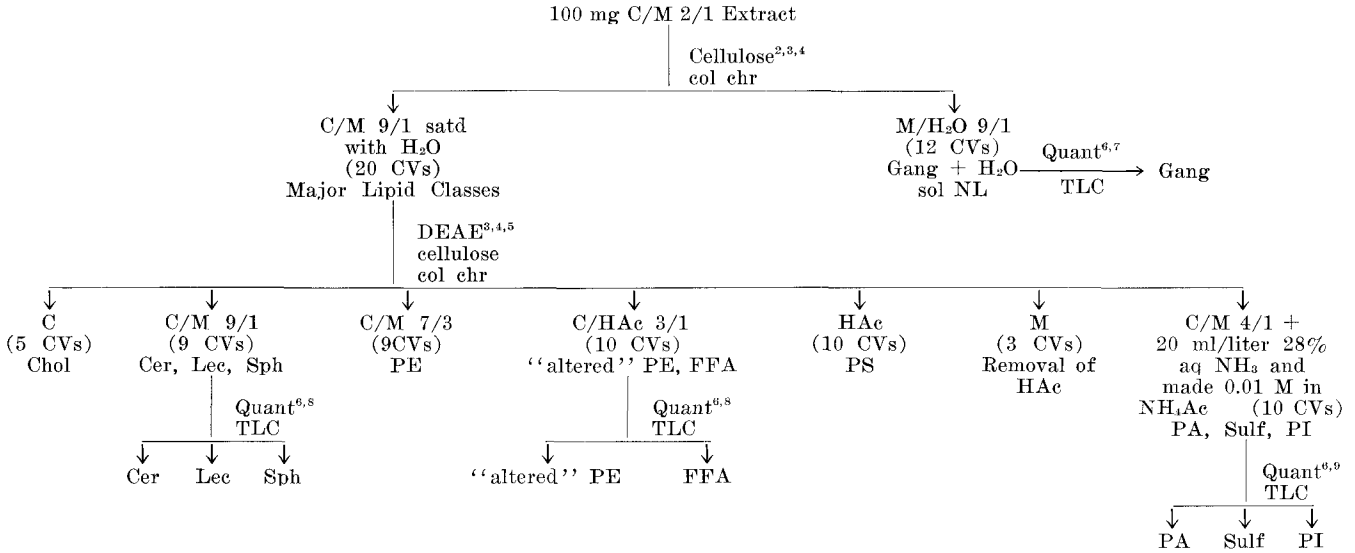
Materials and Methods

Brain Specimens

Fresh (unfixed) specimens obtained as soon as possible were frozen in liquid nitrogen or over dry ice, transported over dry ice, and kept in the frozen state (−20C) until extracted. Each brain was cut longitudinally into equal halves. One-half was used

Figure 1

Determination of Brain Lipid Class Distribution[1]



1. Abbreviations: C, $CHCl_3$; M, CH_3OH; col chr, Column chromatography; CVs, column volumes (1 CV = about 70 ml); Gang, gangliosides; NL, nonlipids; TLC, thin-layer chromatography; DEAE, diethylaminoethyl; Chol, cholesterol; Cer, cerebrosides; Lec, lecithin; Sph, sphingomyelin; PE, phosphatidyl ethanolamine; "altered" PE, incompletely characterized form of phosphatidyl ethanolamine; FFA, free fatty acids; PS, phosphatidyl serine; HAc, glacial acetic acid; NH_4Ac, ammonium acetate; PA, phosphatidic acid; PI, phosphatidyl inositol; Sulf, sulfatides.
2. Twenty grams Whatman *standard grade, ashless* cellulose powder, suspended in M/H_2O 1/1; packed into tube (Teflon stopcock, glass wool plug), washed with 7 CVs M/H_2O 1/1, 3 CVs C/M 1/1, 4 CVs C/M 9/1 saturated with water.
3. Column 2.5 (i.d.) × 20 cm, flow rate 3 ml./min.
4. Effluents checked for "fines" by evaporation of 1 ml in test tube to dryness in sand bath and observation of solids (solids test). Effluent checked for channels by application of about 20 mg of cholesterol in first solvent (C or C/M 9/1 + water) and elution with same solvent. First appearance of solids (solids test with 1 ml) should be 70-80 ml (10 ml fractions collected). If solids appear at 60 ml or less, repack column (significant channels present).
5. One hundred grams DEAE washed on filter (suction) with 1 N HCl, water to neutrality, 0.1 N KOH, water to neutrality (one cycle); cycle three times; air dry; dry to constant weight over KOH in desiccator; 15 gm DEAE treated with glacial HAc overnight, gently pressed free of lumps, packed (in HAc) into a column (Teflon stopcock, glass wool plug), then washed with 4 CVs each M, C/M 1/1, C/M 9/1, and C.
6. All quantitative TLC with heat activated (120C, 20 min) adsorbent composed of 9 parts silicic acid and 1 part magnesium silicate.
7. Solvent n-propanol/water 7/3, ascending in a chamber lined on *all sides with solvent saturated paper liner.*
8. Solvent C/M/H_2O 65/25/4; ascending as for (7).
9. Solvent C/Acetone/M/HAc/H_2O 5/2/1.5/1.5/0.75; ascending as for (7).

for pathological examination and the other half for chemical studies.

The cases of metachromatic leucodystrophy (Case 1, male, age 72 months), Tay-Sachs disease (Case 2, male, age 43 months), Niemann-Pick disease (Case 3, male, age 29 months), chronic Gaucher's disease (Case 4, female, age 57 months), and Alzheimer's disease (Case 5, female, age 49 years and a brother Case 6, male, age 57 years) all showed typical clinical features and the diagnosis in each case was confirmed by pathological examination. Normal brain specimens from males 5-months, 33 years, and 61 years of age and a normal specimen from a female 50 years of age were also examined. One brain specimen showing senile cerebral cortical atrophy was obtained from a 70-year-old female with rheumatic heart disease and myxedema for comparison with the presenile atrophy of Alzheimer's disease. The fresh weights, total lipid content, and percent water of the brain specimens are recorded in Tables I, II, and VI.

Separation of Grey and White Matter

One-half of a frozen brain specimen was cut into slices about 5 mm thick with an electric meat slicer, warmed to the point where grey matter was fluid and white matter solid, and grey matter aspirated under suction from a vacuum pump. Each slice was weighed before and after aspiration. The weight of white matter after aspiration was subtracted from the initial slice weight to give the weight of grey matter. Total grey matter and total white matter were determined as the sums of the weights from all slices.

Determination of Water and Total Lipid

Each specimen (i.e., all the aspirated grey matter, all the white matter, or one-half of the brain) was ground with a meat grinder to form a uniform homogenate. Three aliquots of about 0.5 gm were removed from each sample and the water content was determined by drying the sample to constant weight over KOH in a vacuum desiccator under a slight vacuum.

Total lipid was extracted from the remaining homogenate with chloroform/methanol 2/1 in a nitrogen atmosphere (1) and total crude lipid was determined by weighing the dry solids thus extracted.

Two-Dimensional Thin-Layer Chromatography

Two-dimensional TLC (2,3) was used routinely as the first step in examining each lipid mixture. A heat-activated (20 min, 120C) adsorbent composed of 9 parts of silicic acid and one part of magnesium silicate was used with chloroform/methanol/water 65/25/4 (by volume) as the first solvent. Chromatograms were then dried in air for 10 min and developed in the second dimension with n-butanol/acetic acid/water 60/20/20. After drying in air for several hours, the chromatograms were sprayed with alkaline Rhodamine 6G (1) or the sulfuric acid-potassium

TABLE I
Fresh Weight and Total Lipid of Normal Adult Brain

(Values for One-half Brain)			
Age	Sex	Fresh weight (gm)	Total lipid (gm)
33	M	563	69.8
50	F	582	71.6
70	F	598	70.1
61	M	668	73.0
Mean		603	71.1
Max. variation from mean		10.7%	2.7%

dichromate charring reagent (2) for development of spots. Another useful solvent pair is chloroform/methanol/conc ammonia (65/35/5) and chloroform/acetone/methanol/acetic acid/water (5/2/1/1/0.5).

Some lipid mixtures were compared using the following quantitative TLC technique (2). After development, chromatograms were sprayed with a sulfuric acid-potassium dichromate spray, heated at 180C for 30–60 min to develop spots, cooled, the back sides wiped clean, and the spots scanned with a TLC transmission densitometry apparatus (Photovolt Corp., 1115 Broadway, New York) equipped with a recorder and integrator. The integrator units obtained for each spot from each sample (spotted at known concentration) were used as a means of comparison of different samples, since these units were found to be proportional to amt of a given lipid class. Average values for each lipid class were determined from at least eight different chromatograms of each sample. Since some spots are too large and others too small to be read at any one conc, at least two sets of chromatograms must be prepared in order that all spots can be read. Thus, a minimum of 16 two-dimensional chromatograms are required for each sample. The chromatograms were run in pairs, i.e., one normal and one pathological, to avoid systematic variations.

Determination of Lipid Class Distribution

The percentages of the individual lipid classes in brain samples were also determined by a modification of the diethylaminoethyl (DEAE) cellulose column-TLC procedure (2). In this modification the crude lipid extract is first chromatographed on a cellulose column prepared with Whatman standard grade, ashless, cellulose powder in order to remove gangliosides and nonlipid water soluble substances. The major lipid classes are then separated into single classes or manageable groups by DEAE cellulose chromatography (1). The groups are then separated and quantitatively analyzed by the TLC-charring technique described above. The details of the entire procedure including sample size, column sizes, eluting solvents, eluting volumes, lipid classes eluted, and the TLC procedure are shown in Figure 1.

Results

Table I presents data that show that total lipid is less variable than fresh weight from normal adult brain specimens.

The data in Table II show that evidently both

TABLE II
Fresh Weight, Water, and Total Lipid of Pathological Brains[a]

	Normal[b]	Cerebral atrophy (female)	Alzheimer (female)	Alzheimer (male)
Fresh wt (gm)	604	598	310	381
Total lipid (gm)	71.3	70.0	40.6	44.2
% Water	78.2	78.4	76.0	77.5

[a] Values for one-half brain of adults.
[b] Average from 3 brains (2 male, 1 female).

TABLE III
Lipid Composition of Adult Whole Brain[a]

	NAM	NAF	AAF	AlzAF
Cholesterol	22.1	22.4	22.1	21.8
Cerebrosides	15.8	15.0	15.5	15.5
Lecithin	12.0	13.0	12.6	12.3
Sphingomyelin	5.4	6.5	6.6	6.0
Phosphatidyl ethanolamine	11.2	11.0	10.8	11.0
"Altered" PE	3.9	4.1	1.5	2.8
Free fatty acid	1.6	1.5	1.0	2.3
Phosphatidyl serine	4.0	3.7	3.7	6.6
Sulfatide	6.2	6.5	2.3	3.2
Phosphatidic acid	*	*	2.9	2.4
Phosphatidyl inositol	*	*	0.4	1.0
Gangliosides, water soluble nonlipids	10.6	10.4	11.5	10.2

[a] NAM, normal adult male (age 33 yr); NAF, normal adult female (age 50 yr); AAF, senile cerebral cortical atrophy, female (age 70 yr); AlzAF Alzheimer's disease, adult female (age 49 yr). See Tables I and II for fresh weights and total lipid content of each of the four brain specimens.
* Not determined.

fresh weight and total brain lipids are greatly reduced in Alzheimer's disease (a presenile atrophy), but this is not characteristic of senile cerebral cortical atrophy.

Table III shows the lipid class distribution of several adult brain samples expressed as percent of total lipid (determined by the column chromatography-quantitative TLC procedure). First note that most values for the four specimens are very similar. The very low level of sulfatide in the senile cerebral cortical atrophy specimen is distinctly abnormal. A low sulfatide value is also seen in this Alzheimer's specimen and is accompanied by an increase in phosphatidyl serine. No characteristic and specific change in lipid class distribution is evident for this Alzheimer's specimen.

The data in Table IV indicate a large reduction in total grey matter in Alzheimer's disease. Also, the percentages for total lipid (per gram fresh weight) of grey and white matter of the Alzheimer's specimen are very close to normal, but total lipid in one-half of the brain is greatly reduced.

The lipid class distributions shown in Table V are expressed as densitometer integrator units. It is clear that the lipid class distributions of grey and white matter of the Alzheimer's and normal specimens are quite similar.

Figures 2–7 show comparisons of two-dimensional TLC results from a normal mature brain (Figure 6), a normal 5-month-old infant brain (Figures 2 and 4) and specimens from metachromatic leucodystrophy (Figure 3), Tay-Sachs disease (Figure 5), and Niemann-Pick disease (Figure 7). The comparisons emphasize the much greater similarity of the three disease states to the normal infant brain rather than to the adult brain.

Table VI presents values for cerebroside in several diseases. From the data in Table VI it is clear that the total cerebroside content of the brain in metachromatic leucodystrophy, Niemann-Pick disease, and Tay-Sachs disease is very low and similar to the level of cerebroside in a normal infant brain (5

TABLE IV
Total Grey and Total White Matter of Adult Brain

	Normal		Alzheimer	
	Grey matter	White matter	Grey matter	White matter
Fresh wt ½ brain (gm)	304	364	122	259
Water (%)		76.4		75.9
Lipid, % fresh wt	7.0	14.4	6.3	14.1
Lipid in ½ brain (grey + white, gm)	73.0		44.2	

TABLE V

Lipid Class Distribution of Normal and Alzheimer's Disease Brain

(Densitometer Integrator Units per 50 μg Total Lipid)

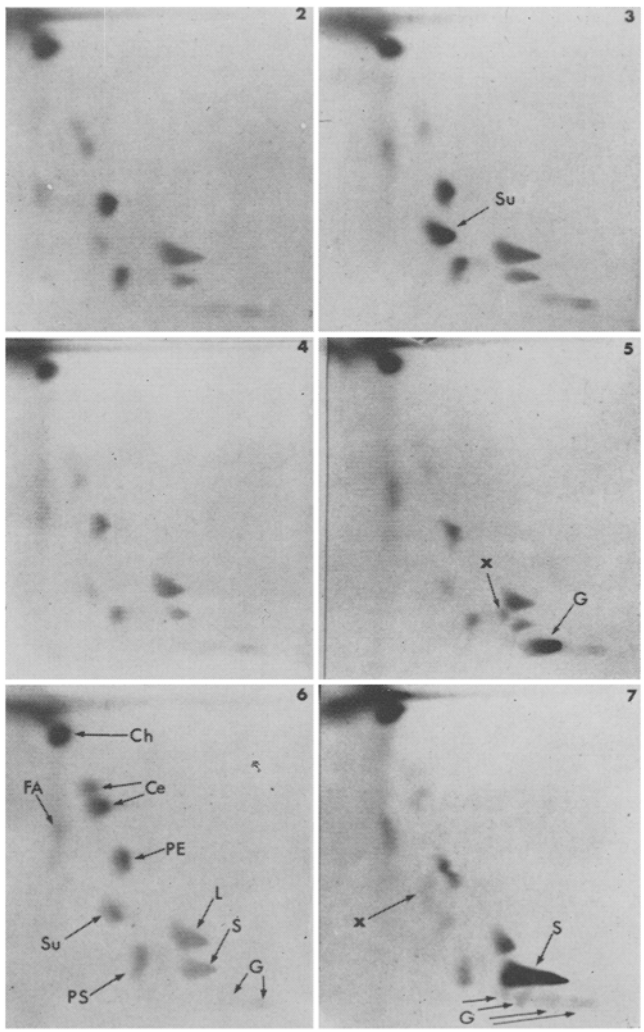
	Normal[a]		Alzheimer[b]	
	Grey matter	White matter	Grey matter	White matter
Cholesterol	211	221	173	210
Cerebroside	62	108	55	124
Lecithin	76	44	68	44
Sphingomyelin	30	37	29	40
Phospatidyl ethanolamine	51	35	45	34
Free fatty acid	10	4	10	6
Phosphatidyl serine	30	33	28	36
Sulfatide	23	37	24	38

[a] Sixty-one-year-old male.
[b] Fifty-seven year old male.

months of age) despite the age of the patients at the time of death. It is interesting that a case of chronic Gaucher's disease (no neurological involvement) showed about one-half the amt of cerebroside found in the normal adult brain, although the amt of total cerebroside of brain was much higher than found in infant brain. From the values presented it is clear that metachromatic leucodystrophy, Niemann-Pick disease, and Tay-Sachs disease are similar to the normal infant brain and not the mature brain in keeping with the two-dimensional TLC findings. Chronic Gaucher's disease appears to present a distinctly abnormal cerebroside level. The value of expressing results in terms of amt of a lipid class per brain is demonstrated by the values presented in Table VI. The changes in brain weight, water content, total lipid, and relative proportion of the total lipid as cerebroside alone do not disclose the true difference from normal clearly seen when results are expressed as amt of lipid in the brain, although all values given in Table VI are required for appreciation of the status of a pathological brain specimen as compared to normal brain.

Table VII shows the values for lecithin and sphingomyelin for the same brain specimens shown in Table VI. Several important points can be appreciated readily from these data. It is first to be noted that the lecithin content in Niemann-Pick disease is similar to that of normal infant brain, particularly when the values are expressed as total amt of lipid in brain. The total amt of sphingomyelin in Niemann-Pick brain is increased more than tenfold if comparison is made to infant brain and 2.5-fold when compared to adult brain. Evidently whole brain sphingomyelin is greatly increased despite the method of comparison to normal. Since the quantitative values of cerebroside and lecithin are similar to infant brain and the sphingomyelin value is greatly increased, it is apparent that the Niemann-Pick brain is similar to the normal infant except that sphingomyelin is greatly increased in agreement with the conclusions drawn from two-dimensional TLC comparisons.

The values for lecithin expressed as percent of the total lipid in metachromatic leucodystrophy and Niemann-Pick and Tay-Sachs diseases are similar to each other and resemble the value for normal mature brain, although the total amt of lecithin in brain for the three disease states is similar to the total amt of lecithin in normal infant brain. The sphingomyelin values in both Tay-Sachs disease and metachromatic leucodystrophy are quite similar to infant brain in keeping with the conclusions drawn from the two-dimensional TLC comparisons. The values for lecithin and sphingomyelin in Table VII along with the value for cerebroside (Table VI)



FIGURES 2–7 are two-dimensional TLC of total lipids of normal and pathological specimens extracted with chloroform/methanol 2/1. TLC with chloroform/methanol/water 65/25/4 and n-butanol/acetic acid/ water 60/20/20 as described in text. Spots developed with the sulfuric acid-potassium dichromate spray and heat (see text).

FIG. 2. 200 μg of brain lipids of a normal 5 month old infant (death from acute pulmonary tuberculosis). FIG. 3. 200 μg, from metachromatic leucodystrophy (age 42 months). FIG. 4. 100 μg, normal 5 month old infant (death from acute pulmonary tuberculosis.) FIG. 5. 200 μg, from Tay-Sachs disease (age 43 months). FIG. 6. 200 μg, normal adult human brain (male, age 33, death from acute pulmonary infection).

FIG. 7. 200 μg of lipids from Niemann-Pick disease (age 29 months).

Comparison of Figures 2 and 3 shows the similarity of normal infant brain lipids and lipids of metachromatic leucodystrophy except for a large increase in sulfatide (Su) in the pathological state. Comparison of Figures 4 and 5 shows the similarity of normal infant and Tay-Sachs brain lipids except for the large increase in one type of ganglioside and the presence of an uncharacterized substance (X). The similarity is best seen when 100 μg of normal and 200 μg of Tay-Sachs lipid is spotted since the large increase in proportion of ganglioside is associated with a reduction in the other lipids. Comparison of Figures 4 and 7 illustrates the similarity of normal infant and Niemann-Pick disease brain lipids except for the large increase in sphingomyelin (S) in the pathological state. The overall similarity is best appreciated when 100 μg of normal and 200 μg of pathological brain lipid were spotted since the large increase in sphingomyelin is associated with a decrease of other lipids. Several ganglioside spots (G) are increased in amt in Niemann-Pick disease and an uncharacterized substance (X) is present. Another uncharacterized lipid class migrates very close to phosphatidyl ethanolamine giving rise to a double spot in this region.

Abbreviations: Ch, cholesterol; Ce, cerebroside; FA, fatty acid; PE, phosphatidyl ethanolamine; Su, sulfatide; PS, phosphatidyl serine; L, lecithin; S, sphingomyelin, G, gangliosides; X, uncharacterized.

TABLE VI

Cerebrosides in Normal and Pathological Brains

	Age (yrs)	Sex	Wt ½ brain (gm)	% Lipid	% Water	Cerebroside			
						% Total lipid	% Fresh weight	% Dry weight	Gm in ½ brain
Normal infant	½	M	277	6.18	85.8	3.69	0.23	1.61	0.63
Niemann-Pick disease	2½	M	355	9.23	82.3	1.64	0.15	0.85	0.54
Metachromatic leucodystrophy[a]	3½	M	313	6.53	84.6	1.74	0.11	0.74	0.37
Tay-Sachs disease[a]	3½	M	633	4.34	88.0	1.84	0.08	0.65	0.51
Chronic Gaucher's disease[a]	4½	F	575	8.77	81.1	10.1	0.89	4.69	5.12
Normal adult	33	M	562	12.4	77.8	15.8	1.96	8.83	11.0

[a] Classical examples of these diseases.

indicate a specific reduction of cerebroside in brain in chronic Gaucher's disease without clinical signs of brain involvement.

The sulfatide levels in normal infant brain and brain of metachromatic leucodystrophy are shown in Table VIII. Sulfatide is greatly elevated in the pathological state and the significance of this change is emphasized by the fact that the amts of lecithin, sphingomyelin, and cerebroside in metachromatic leucodystrophy are similar to those of normal infant brain.

Discussion

Sampling Procedures

The most meaningful types of samples must be obtained for study. Most brain specimens are routinely fixed, usually in formalin, and are thus not suitable for precise chemical studies since there is general agreement that formalin fixation alters lipids. One change observed in this laboratory after formalin fixation is the destruction of phosphatidyl ethanolamine. Specimens should be obtained as soon as possible after death and frozen over dry-ice if liquid nitrogen is not available for more rapid freezing. To avoid autolytic changes specimens are maintained in the frozen state (−20C or lower) until extracted.

Most studies of brain lipid composition have utilized small specimens from one area of the brain, frequently the frontal lobe. This sampling procedure is widely used because such specimens are readily available. A small sample from the frontal lobe or any other area of the brain is not entirely satisfactory, however. Sizeable variations in lipid composition and water content are encountered with such samples. We have observed white matter water content from various parts of the normal adult brain to vary from 68.7–70.9% and grey matter from the same brain to vary from 80.0–83.5% water. The proportion of grey and white matter is different in different areas of the brain. Since white matter contains more lipid than grey matter, total lipid may be quite variable. Since both lipid class distribution and percent water are different for grey and white matter, variations in relative proportions of grey and white matter of samples gives rise to variations in lipid class distribution and water content of small samples.

Most investigators have sought to avoid the variations in proportions of grey and white matter in different parts of the brain by studying separated grey and white matter. A small specimen of grey matter is selected from an area rich in grey matter and a small specimen of white matter similarly removed from an area rich in white matter. This sampling procedure is not entirely satisfactory. It has been observed in this laboratory that both grey and white matter from different parts of the brain vary in water content, and hence the lipid content (per gram fresh weight) varies. Since histological stains indicate variations in the amts of different types of lipids in grey and white matter in different areas of the brain, variations in lipid class distribution must be expected. Variations in pathological states may be very large. Fatty acid composition varies in different regions of the brain (4).

The alternatives are clear. The most meaningful studies of whole brain require a representative sample of whole brain. This can be obtained by grinding one-half of the brain to a uniform state. Samples of grey and white matter that are representative of the whole brain are similarly obtained only by quantitative separation of total grey from total white matter of one-half of the brain as described above.

Analysis of samples representative of whole brain and total grey and total white matter are most meaningful for diseases affecting the brain as a whole as studied in the present investigations. Disease processes producing focal lesions require a different sampling procedure. Focal lesions should be studied separately and compared with a control specimen from the same region of normal brain if changes in disease are not to be obscured by inclusion of surrounding normal regions. The difficulties involved in obtaining suitable controls and the increased effort in analysis of diseases producing small focal lesions have led us to restrict our studies to those diseases producing large changes in the brain as a whole.

Useful information can be obtained from the study of grey and white matter. It must be appreciated, however, that while grey matter is richer in cells, it also contains myelin, and white matter, although richer in myelin, contains cellular material. The differences are quantitative and the differentiation is

TABLE VII

Lecithin and Sphingomyelin in Normal and Pathological Brains[a]

	Lecithin				Sphingomyelin			
	% Total lipid	% Fresh weight	% Dry weight	Gm in ½ brain	% Total lipid	% Fresh weight	% Dry weight	Gm in ½ brain
Normal infant	22.7	1.40	9.86	3.89	3.70	0.23	1.64	0.64
Niemann-Pick disease	9.38	0.87	4.89	3.07	28.1	2.59	14.6	9.19
Metachromatic leucodystrophy	13.4	0.88	5.68	2.74	6.83	0.45	2.90	1.40
Tay-Sachs disease	10.3	0.45	3.73	2.84	3.71	0.16	1.34	1.02
Chronic Gaucher's disease	11.7	1.03	5.45	5.92	5.57	0.49	2.59	2.81
Normal adult	12.0	1.49	6.71	8.37	5.40	0.67	3.02	3.76

[a] The cases are the same as those shown in Table VI where brain weight, etc., are given for each case.

at best a rather crude process, particularly in some pathological specimens where the clear line of demarcation between grey and white matter of normal specimens is lacking. Studies of whole brain, i.e., grey and white matter combined, are most meaningful in early life where white matter is less distinct and with specimens from adults when it is desired to compare total lipid, total water, and lipid class distribution of many samples.

Methods of Analysis

There are two types of quantitative chromatographic procedures applicable to the study of brain lipid composition changes. One type yields absolute quantitative values and with the other relative compositions can be determined. The procedures utilizing column chromatography alone or in combination with quantitative TLC (2) give results in terms of weight of each lipid class from which the percentages of the various lipid classes are calculated. These procedures require facilities and experience not available in all laboratories and are relatively time-consuming compared to the simple two-dimensional TLC procedure described above. Because of its speed and simplicity the two-dimensional TLC procedure is of real value, particularly when large numbers of samples are to be compared quickly prior to use of the more precise procedures.

Large differences in lipid class composition can be seen by eye with the two-dimensional TLC procedure (see Figures 2–7). This technique is useful for rapid semiquantitative comparisons. It can be termed semiquantitative because some idea of the amts of lipid classes is obtained. The relative amts of the lipid classes of various samples can be determined more precisely by quantitative TLC without conversion of the readings to absolute quantities of lipid (see Table V).

There are a number of practical reasons why comparison of brain samples by either optical density or recorder units is a useful routine procedure. Limitations exist with regard to lipid class standards and instrument variations. The extent of charring with the sulfuric acid-potassium dichromate spray under carefully controlled conditions is directly proportional to the amt of lipid in a TLC spot and can be determined accurately by transmission densitometry. The reaction is proportional to the carbon content of the lipid class and therefore conversion of optical density or recorder or integrator units to micrograms of lipid per spot requires either that the carbon content of the lipid class be known or that comparison be made directly with a weighed amt of a pure sample of the lipid class being determined. In either case the different lipid classes must be available in pure form if quantitative TLC values are to be converted to micrograms of lipid. It is further necessary for precise results to utilize a standard lipid preparation isolated from the same organ (e.g., brain) for comparison since variations in fatty acid composition (and hence carbon content) are found when the same lipid class is isolated from different sources. Clearly it is advisable to utilize optical density or recorder or integrator units for comparisons where suitable standards are not available and even when adequate standards are available this procedure is convenient and rapid.

Other restrictions exist on the conversion of values measured by transmission densitometry to micrograms of lipid. It is necessary to compare samples with standards at the same time. This procedure not only minimizes variations in sample application, TLC, and the charring reaction, but is necessary because the densitometer gives different values at different times. Changes in the electrical circuits and characteristics of the photocell are responsible for these variations. Marked changes in densitometry units are observed when vacuum tubes age and replacement of one or more vacuum tubes in the instrument may double the number of units recorded for the same amt of lipid.

Two-dimensional TLC, particularly when spot intensities are evaluated by transmission densitometry, is recommended as a routine procedure for comparing brain lipid samples and caution is urged in conversion of densitometry values to micrograms of lipid. Such conversions should not be attempted except under very carefully controlled conditions and with suitable standard preparations. Densitometry values can be used with confidence to demonstrate lack of change, increases, or decreases in lipid class distributions and can be used to determine whether the total amt of a lipid class in one brain is different from that in another brain when the amt of total lipid is known.

TABLE VIII

Sulfatide in Normal Infant and Metachromatic Leucodystrophy Brain

	% Total lipid	% Fresh weight	% Dry weight	Gm in ½ brain
Normal infant	1.76	0.11	0.77	0.30
Metachromatic leucodystrophy	9.30	0.61	3.94	1.90

Lipid Composition of Adult Brain

Several important features of lipid class composition of adult brain are clear from the data presented in Tables I–V. It is clear that fresh weight varies more than total lipid (Table I). Many brain weights much larger than those of Table I have been recorded (5) and it will be of interest to determine the contribution of lipid to the large brain weight. From the data available it is clear that larger brains generally contain more lipid, but the amt of total lipid in the brain is less variable than fresh weight.

The total amts of grey and white matter of the normal adult male brain are very nearly equal, but the high total lipid content (Table IV) and the high proportion of the myelin lipids (cerebroside and sulfatide) in grey matter (Table V) indicate the presence of a large amt of myelin in the grey matter or the presence of cerebroside and sulfatide in membranes other than myelin. With the same method beef brain was found to have a similar weight distribution for grey and white matter (60% white matter), but total lipid of grey matter was found to be about one-half the amt found in human brain and white matter of beef brain contained more lipid (17% of the fresh weight) than human brain white matter.

We became interested in Alzheimer's disease when a familial form of this presenile dementia was called to our attention. The disease has a history of three generations in the family under study, and most members of the affected family succumb to this disease. Its onset is usually past 40 years of age and typically between 50 and 60 years of age. The full clinical course is from 2 to 5 years and is characterized first by memory defects and hallucinations developing finally into a profound dementia. Pathologically the brain is quite small (atrophic), and

the presence of plaques in the cerebral cortex is characteristic as is the presence of the process known as Alzheimer's neurofibrillary degeneration affecting the neurons.

Large reductions in fresh weight, total lipid, and relative amt of grey matter are present in Alzheimer's disease (Table II and IV), but the fresh weight and total lipid of the brain of an elderly female showing cerebral cortical atrophy were near the normal values (Tables II and IV).

The lipid class distribution of normal adult male and female brains were found to be quite similar (Table III), but the amts of "altered" phosphatidyl ethanolamine and sulfatide in cerebral cortical atrophy were found to be lower than normal. Decreases of "altered" phosphatidyl ethanolamine and sulfatide were also found in one Alzheimer's disease brain (Table III) and were associated with an increase in phosphatidyl serine. The absence of similar changes in lipid class distribution in another Alzheimer's disease brain (Table V) makes it clear, however, that the changes seen in the first Alzheimer's brain specimen are not characteristic for the disease. This conclusion is supported by the similarities in composition of the cerebral cortical atrophy specimen and one Alzheimer's specimen (Table III). The findings point to the necessity for studying more than one brain specimen of a given disease, and the necessity for comparison with other pathological states before reliable conclusions can be drawn regarding lipid class changes characteristic for a particular pathological state.

It is indeed striking that despite large reductions in fresh weight, total lipid, and proportion of grey matter, the lipid class distribution of an Alzheimer's disease brain specimen and the percent of the fresh weight represented by lipid can be essentially normal. The possible significance of these findings is considered elsewhere (8).

Lipid Class Distributions of Brains in Children

The study of lipid class composition alterations in diseases beginning in early life is complicated by the occurrence of developmental changes. It has been known for many years that the immature brain has a high water content and less lipid than the mature brain. As the brain matures, the water content decreases and lipid increases. It has been appreciated for some time also that the amts of cerebroside and sulfatide increase as myelination proceeds and the brain matures. Data obtained in this laboratory indicate that lecithin, phosphatidyl inositol, and gangliosides decrease markedly, phosphatidyl ethanolamine decreases slightly, and sphingomyelin increases while cholesterol and phosphatidyl serine are changed very little during development. A comparison of the lipid patterns of the brain of an infant five months old and an adult male is shown in Figures 2 and 6. As expected the relative amts of cerebroside and sulfatide are greater in adult brain. The most important feature that is immediately apparent when the lipid class distributions of brains from metachromatic leucodystropy, Tay-Sachs disease, and Niemann-Pick disease (Figures 3,5,7) are compared by two-dimensional TLC with those of normal infant and a normal adult (Figures 2 and 6) is that the three diseases are much more similar to the normal infant brain despite the fact that the brain specimens from the three disease states were from children 24–72 months of age while the normal was only five months of age. One very large spot stands out in each of the three diseases: the sulfatide spot in metachromatic leucodystrophy (Figure 3); a ganglioside spot in Tay-Sachs disease (Figure 5); and the sphingomyelin spot in Niemann-Pick disease (Figure 7). The three lipid classes so outstanding on chromatograms of these disease states are all clearly present in amts above those found in the normal adult brain. It is only by comparison with infant brain, however, that the great overall similarity of the three disease states (except for the one predominant lipid class) to a normal specimen can be appreciated. These simple comparisons show that the lipid class distributions of brain in metachromatic leucodystrophy, Tay-Sachs disease, and Niemann-Pick disease are most similar to the immature brain. Table VI shows that the amt of cerebroside in these diseases is similar to that in infant brain. These findings suggest that the developmental process was interrupted.

It is clear that the present method of sampling and the TLC method of examination of brain lipids overcome a classical problem. Investigators have not always agreed that brain sphingomyelin was increased in Niemann-Pick disease (see ref. 6 for summary of earlier reports). Ivemark et al. (7) have recently reported sphingomyelin to be normal in grey matter and decreased in white matter in Niemann-Pick disease. This apparently comes in part from the sampling procedures (frontal lobe specimens) and in part from the methods of analysis and expression of results. Our results (Table VII) are clearly in disagreement with findings of low or normal brain sphingomyelin in Niemann-Pick disease since a large elevation was found.

The quantitative values for cerebroside, lecithin, and sphingomyelin (Tables VI and VII) in Niemann-Pick and Tay-Sachs diseases and metachromatic leucodystrophy are in full agreement with the conclusion drawn from TLC comparisons that these brains are similar to infant brain except for a large and specific increase of one sphingolipid in each disease. The findings in classical chronic Gaucher's disease are very interesting. Despite an increase in cerebroside in spleen, a decrease of cerebroside in brain is found. There are no clinical signs of neurological involvement in chronic Gaucher's disease. It appears that some change in cerebroside content can be tolerated without profound disturbance of brain function.

Data on the fatty acid composition of brain lecithin and sphingomyelin of adults and children and considerations of the possible significance of the lipid changes in diseases are presented in other communications (8,9).

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