TABLE I
Brain Lecithin Fatty Acids [a]

Fatty Acid	1 NAM	2 NAF	3 CAAF	4 AlzAF	5 NI	6 NP	7 TS	8 MLD
12:0	.....	.....	.....	0.78	.....	.....	.....	.....
14:0	.....	.....	0.80	0.88	.....	0.82	1.09	0.91
15:0	.....	.....	0.21	.....	.....	.....	0.18	.....
16:0	47.58	44.38	41.59	43.89	60.60	52.85	51.57	52.32
16:1	.....	.....	2.47	2.45	.....	3.48	3.59	1.80
17:0	.....	.....	0.51	.....	.....	.....	.....	.....
18:0	12.09	13.88	11.88	13.00	11.27	8.65	7.16	12.63
18:1	40.33	41.74	35.88	37.17	23.27	29.47	31.94	27.29
18:2	.....	.....	1.29	0.74	2.23	.....	1.06	.....
20:0	.....	.....	.....	.....	.....	0.57	.....	.....
20:1	.....	.....	1.06	.....	2.63	0.98	0.68	.....
21:0	.....	.....	0.86	.....	.....	.....	.....	.....
22:0	.....	.....	0.46	1.08	.....	3.18	1.83	5.05
22:1	.....	.....	2.36	.....	.....	.....	.....	.....
(?)	.....	.....	0.32	.....	.....	.....	0.89	.....

[a] Values expressed as relative percent. Blank spaces indicate the failure to detect a fatty acid or detection of a trace amt only.
NAM, normal adult male; NAF, normal adult female; CAAF, senile cerebral atrophy, adult female; AlzAF, Alzheimer's disease, adult female; NI, normal infant (5 months of age); NP, Niemann-Pick disease; TS, Tay-Sachs disease; MLD, metachromatic leucodystrophy.

TABLE II
Brain Sphingomyelin Fatty Acids [a]

Fatty acid	1 NAM	2 NAF	3 CAAF	4 AlzAF	5 NI	6 NP	7 TS	8 MLD
12:0	.....	.....	2.75	.....	.....	.....	.....	.....
14:0	.....	.....	0.34	0.67	0.75	.....	0.79	.....
15:0	.....	.....	.....	0.62	.....	.....	.....	.....
16:0	6.42	6.79	7.62	6.82	7.30	5.89	11.93	15.80
16:1	1.35	0.45	.....	0.61	.....	0.37	.....	0.57
18:0	47.04	45.80	42.78	42.40	70.33	85.63	64.84	79.58
18:1	3.23	.....	3.81	2.26	1.97	.....	3.21	2.32
18:2	.....	.....	0.59	.....	.....	.....	.....	.....
20:0	1.04	1.48	1.91	1.83	2.62	3.70	3.47	1.71
22:0	3.63	2.35	2.11	2.58	3.65	4.24	3.88	.....
22:1	1.89	.....	.....	.....	.....	.....	.....	.....
23:0	.....	3.10	1.89	4.37	2.67	.....	.....	.....
24:0	4.52	6.69	7.63	9.89	4.46	.....	4.12	.....
24:1	30.86	31.71	28.55	27.14	8.39	.....	7.78	.....
(?)	.....	1.62	.....	.....	.....	.....	.....	.....

[a] Values expressed as relative percents. Abbreviations as for Table I.

tween those of normal infant and normal adult brain in some respects. The C_{24} fatty acids characteristic of the normal brain are present only in the sample from Tay-Sachs disease. This large change in C_{24} acids is not a specific feature of one disease. Svennerholm (6) has reported a decrease in longer chain fatty acids of sphingomyelin in metachromatic leucodystrophy.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Research Grants NB-01847-06, NB-04116, NB-04277 from the National Institute of Neurological Diseases and Blindness; CA-03134-08 from the National Cancer Institute, and Grant DA-AMC-18-035-71(A) from the U.S. Army Chemical Research and Development Laboratories, Army Chemical Center, Maryland.

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Speculations on the Nature of the Metabolic Defects in Tay-Sachs, Niemann-Pick, Gaucher's and Alzheimer's Diseases, and Metachromatic Leucodystrophy

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Abstract

It is concluded that the defect in Tay-Sachs disease is for an enzyme degrading a monosialoganglioside, in Niemann-Pick disease for an enzyme degrading sphingomyelin or possibly ceramide, in chronic Gaucher's disease for an enzyme degrading a glucocerebroside, and in metachromatic leucodystrophy for an enzyme for degradation of sulfatide. Alzheimer's disease does not appear to involve any specific changes in lipid composition. An hypothesis to explain the findings in Alzheimer's disease is presented.

Introduction

PATHOLOGICAL PROCESSES affecting lipids can be classified as primary or secondary events. Secondary changes in lipids brought about by some other primary event such as an infectious disease, a disturbance in carbohydrate or amino acid metabolism, or a structural defect of the vascular system are important, but the primary changes involving lipids directly are particularly interesting. We have sought to avoid the inadvertent study of lipid changes secondary to other processes by studying hereditary metabolic diesases where histological

and/or chemical evidence exists to link the condition with lipid metabolism. Genetic analysis can indicate a single enzyme defect.

The primary defects of lipid metabolism can be on the biosynthetic or degradative enzyme pathways. A biosynthetic defect leads to a decreased amt of the lipid class or classes synthesized through the affected pathway, and a degradative enzyme deficiency or lack leads to an increase in amt of the lipid class or classes dependent upon the affected pathway. The major difficulty is, however, that secondary changes may be present and tend to obscure the primary defect. The most pronounced defects are to be expected in the stillborn and in children. Enzyme deficiencies that are less complete or involve less critical changes may be expected, however, in adults.

We believe that data should be presented along with suitable working hypotheses to stimulate and guide research. Bold and imaginative hypotheses are permissible if they do not conflict with reliable data and can be checked, preferably in some relatively simple manner. In this report some of the observations on several hereditary metabolic diseases are brought together and an effort is made to derive working hypotheses with regard to specific enzyme defects and methods for checking the hypotheses.

Assumptions and General Procedure

It is necessary to make some assumptions with regard to biosynthetic and degradative enzyme pathways for the sphingolipids since the data are inadequate, particularly with regard to significant in vivo pathways in humans.

Although turnover studies demonstrate the existence of various degradative enzymes, good in vitro demonstrations of these enzymatic activities have not been reported. It is therefore assumed that active degradative enzymes exist, particularly in the developing brain, for amide, ester, and acetal bonds of the sphingolipids. The available data indicate that cerebrosides are synthesized either through the ceramide or psychosine pathways and this is also assumed for sulfatide. It seems probable that gangliosides and sphingomyelin are synthesized via the ceramide pathway, although a pathway involving transfer of one or more polar groups prior to formation of the amide bond between sphingosine (or related base) and fatty acid cannot be excluded. It is assumed that there is essentially no turnover of myelin lipids in adult brain and that lipids of the cells of adult brain do turnover. Discussions of sphingolipid metabolic pathways and chemical findings in the sphingolipidoses were reviewed recently (1).

It must be assumed that the enzymes of sphingolipid metabolism are relatively nonspecific. The various phospholipases are not specific for one lipid class or for fatty acid composition. O'Brien and Rouser (2) demonstrated that ceramide, cerebroside, sulfatide and sphingomyelin of beef brain have very similar fatty acid compositions and this strongly indicates common precursors and relatively nonspecific enzymes for sphingolipid biosynthesis and degradation. Additional evidence of the relatively nonspecific nature of sphingolipid metabolism enzymes in human brain from studies of pathological specimens is summarized below. Generally it is found that changes in fatty acid composition may be large in pathological states, but the same large changes can be found in more than one disease and in more than one lipid class.

The simplest hypothesis, one involving the smallest number of assumptions, must be selected and only hypotheses that can be checked in some relatively simple manner are considered.

Lipid Class Distributions in Sphingolipidoses

Niemann-Pick disease, Tay-Sachs disease, chronic Gaucher's disease, and metachromatic leucodystrophy are well-defined conditions that have been widely studied from the genetic, clinical, pathological, and chemical standpoints and in each case have been linked by extensive data to abnormalities of lipid metabolism. The literature on these conditions has been reviewed recently in detail (1). The classical clinical features of each disease were present in the cases studied and pathological examinations confirmed clinical observations in each case. These conditions present interesting variations. Niemann-Pick disease was first linked to sphingomyelin metabolism by Klenk (3) who also linked Tay-Sachs disease with ganglioside metabolism (4). Gaucher's disease (chronic form) has long been linked with cerebroside metabolism (5) and metachromatic leucodystrophy has recently been linked with cerebroside sulfate (sulfatide) metabolism (6).

These disorders present interesting variations in terms of the affected organs. Niemann-Pick disease affects brain and other organs, and metachromatic leucodystrophy affects brain with changes in other organs also apparent. Tay-Sachs disease is localized to the brain. In classical chronic Gaucher's disease the brain is not involved but neurological involvement in the acute form is encountered.

The method of approach is clear. The lipid composition of brain in each pathological state must be compared with the composition of mature normal brain and immature normal brain specimens at different ages to test the goodness-of-fit so to speak, ie., to determine what differences can be observed. This can be accomplished by two-dimensional thin-layer chromatography (TLC) and the findings confirmed by the column-TLC approach to quantitative analysis (7).

The two-dimensional TLC comparison of the lipids of a mature brain and brain specimens from metachromatic leucodystrophy, Tay-Sachs disease, and Niemann-Pick disease (7) clearly show that there are marked alterations in disease. Sulfatide is increased in metachromatic leucodystrophy, one type of ganglioside is increased in Tay-Sachs disease, and sphingomyelin is increased in Niemann-Pick disease. Other differences are also apparent and the major conclusion from comparisons with adult brain is that the specimens from the disease states differ in many ways from the normal mature brain.

Since the specimens from these disease states have an abnormal myelin content, they are clearly not like mature brains and comparison with a normal but immature brain from an infant (before myelination is complete) is suggested. Comparison of a brain specimen from a 5-month-old child with a mature normal adult brain specimen shows that the brain contains less cerebroside and sulfatide in particular than does the mature brain (7). This is expected since these are "myelin" lipids.

Comparison of brain lipids of metachromatic leucodystrophy, Tay-Sachs, and Niemann-Pick diseases with those of the normal adult and 5-month-old in-

fant (7) shows that the lipid class distribution of each pathological state is much more similar to that of the normal infant than normal adult and that one sphingolipid is greatly increased in each pathological state. Many differences that might be attributed to a pathological process when the sphingolipidoses are compared with mature brain are less impressive when comparison is made to immature brain. These are the findings expected for diseases caused by a deficiency (or absence) of a degradative enzyme for either the particular lipid class that accumulates or its immediate precursor. The three diseases present a uniform picture in a general way. It appears that brain development and the myelination process in particular have not proceeded in the normal manner. The data are not compatible with biosynthetic defects as causes of the diseases since no specific decrease of one lipid class is observed for each disease. Definite conclusions on the nature of the enzymatic defects in the sphingolipidoses can be drawn from the chemical data.

Secondary Changes in the Sphingolipidoses

Two major problems requiring consideration are the means for differentiation of primary from secondary changes, and the means for arriving at explanations for the organ specificities in the diseases. Relatively reliable conclusions can be drawn from a comparison of the results of brain and other organs from the different diseases with each other and with results from normal organs.

Some features of the lipid class distributions in the sphingolipidoses appear to be secondary changes. A very low level of cerebroside characteristic of infant brain is seen in the three sphingolipidoses where the brain is affected (but not in classical, chronic Gaucher's disease where the brain is not affected) (7). The uniformity of this finding when the brain is affected indicates that this decrease of cerebroside does not represent a primary defect of cerebroside biosynthesis in any of the diseases. In keeping with this interpretation, Jatzkewitz (8) has reported nearly normal cerebroside values for several cases of metachromatic leucodystrophy.

The slight elevation of gangliosides in Niemann-Pick disease and metachromatic leucodystrophy is probably a secondary change not only because it is seen in two different diseases, but because the large elevation of ganglioside in Tay-Sachs disease is not accompanied by an elevation of sphingomyelin or sulfatide as would be expected if the slight elevation of ganglioside in Niemann-Pick disease and metachromatic leucodystrophy were to be assigned the role of a primary defect leading to even larger secondary increases in the other lipid classes.

There are several reasons why the increase of one type of ganglioside in Tay-Sachs disease must be considered the primary defect and not secondary to a biosynthetic defect for another type of ganglioside. First, it seems improbable that a biosynthetic defect for all but one type of ganglioside could exist since the ganglioside in Tay-Sachs disease appears to be of a type present in normal brain in small amt (17). Second, we have observed an increase of ganglioside in erythrocytes in Tay-Sachs disease (9) where there appears to be only one type of ganglioside, i.e., the Tay-Sachs disease type (10). While most of the ganglioside of the Tay-Sachs brain is one type, we have seen other types after cellulose column chromatographic recovery of total gangliosides and TLC, and Svennerholm and Raal (11) reported the presence of other gangliosides. Finally, a general biosynthetic defect should be apparent in other organs where gangliosides occur, but this has not been reported. The only conclusion in keeping with all of the findings is that the elevation of one ganglioside is related to the deficiency or absence of a degradative enzyme for one type of ganglioside.

There is no apparent alternative to the conclusion that in chronic Gaucher's disease a deficiency of a degradative enzyme for glucocerebroside exists.

It is not difficult to visualize how secondary changes can occur. The accumulation of one sphingolipid probably disrupts normal cellular structures with displacement of enzymes and substrates from their normal relationships. Such displacement could lead to decreases in biosynthetic and degradative rates for some lipid classes and can account for the relatively small increases and decreases in some lipid classes.

Specific Enzyme Defects in the Sphingolipidoses

The specific nature of the degradative enzyme defects in each of the sphingolipidoses can be judged with some certainty. The deficiency appears to be specific for sulfatide in metachromatic leucodystrophy since cerebroside is not increased. This suggests a defect in the removal of sulfate. Biosynthetic defects involving sphingosine, fatty acids, or ceramide cannot account for the observed changes in any simple manner since increased or decreased biosynthesis of any of these substances should result in large increases or decreases in more than one sphingolipid. The possibility that the basic defect is one of biosynthesis of cerebrosides resulting in an increase in sulfatide as postulated by Svennerholm (12) is unlikely because, as pointed out above, low cerebroside is seen in several diseases associated with the accumulation of different sphingolipids. Jatzkewitz (8) has reported higher cerebroside levels in several cases of metachromatic leucodystrophy.

The low content of longer chain fatty acids in cerebrosides in metachromatic leucodystrophy has been postulated to be the basic defect in this disease (13), but since decrease of longer chain fatty acids is also found in sphingomyelin in metachromatic leucodystrophy (12,19) and Niemann-Pick disease (19) the hypothesis seems unjustified. Berry et al. (14) have found that longer chain fatty acids decrease in cerebrosides and sphingomyelin of degenerating nerve, and Jatzkewitz and Mehl (15) have shown a decrease of C_{24} acids in adult demyelinating disease. These findings indicate that as long as sphingolipid is not a part of a stable myelin or other membrane structure and undergoes turnover that shorter chain fatty acids will predominate. This is an adequate explanation for the fatty acid composition of cerebrosides and sphingomyelins in metachromatic leucodystrophy, Niemann-Pick disease and Tay-Sachs disease.

The most obvious hypothesis to account for the accumulation of sphingomyelin in Niemann-Pick disease is failure of degradation of sphingomyelin to ceramide or ceramide phosphate since it would seem that either ceramide or ceramide phosphate should accumulate if either were not degraded in a normal manner. It is possible, however, that the defect could be in degradation of ceramide and that a rapid conversion of ceramide to sphingomyelin prevents accumulation of ceramide and results in the accumulation of sphingomyelin. The elevation of gangliosides in this disease could be explained by this

hypothesis, but it is difficult to see why cerebroside is not increased as well if this hypothesis is correct. A decision between these two hypotheses should be possible by study of other cell types and organs. It may be that the lower sphingomyelin content observed in erythrocytes of Niemann-Pick disease (9) is best explained by failure to degrade ceramide rather than sphingomyelin.

The defect in chronic Gaucher's disease is most probably for an enzyme for the degradation of glucocerebroside to ceramide or psychosine since one of these two lipids should accumulate rather than glucocerebroside if the defect were for another enzyme unless a rapid synthesis to cerebroside occurs. The latter seems unlikely since sphingolipids do not accumulate in the brain in chronic Gaucher's disease and such an accumulation would be expected if a failure to degrade ceramide (or psychosine) were the basic defect.

The defect in Tay-Sachs disease appears to be for the removal of carbohydrate from a monosialoganglioside. Other types of gangliosides can be degraded to the Tay-Sachs type with neuraminidase and it is clear that a deficiency of a degradative enzyme in Tay-Sachs disease could lead to the accumulation of ganglioside produced by degradation of other gangliosides as well as by direct biosynthesis. The isolation and characterization of a glycolipid (16,17) from Tay-Sachs brain with the structure of Tay-Sachs ganglioside without neuraminic acid indicates that the defective enzyme is for cleavage of the galactose-galactosamine linkage.

The organ specificities of the sphingolipidoses present interesting problems, but some rather definite conclusions can be drawn from general comparisons of the features of brain and other organs. The accumulation of sphingomyelin in other organs as well as brain in Niemann-Pick disease and an accumulation of sulfatide in other organs in metachromatic leucodystrophy has been reported. These findings are expected since sphingomyelin is present in all organs and sulfatide appears to occur in small amts in other organs (18). The simplest assumption for Tay-Sachs disease is that the degradative enzyme deficiency is most apparent for brain either because more enzyme is present in cells of other organs or because gangliosides, being less abundant in other tissues, do not accumulate to such an extent that obvious changes are evident. The choice here can be made when more data on the ganglioside content of organs other than brain are available.

The glucocerebroside of chronic Gaucher's disease could be formed by direct biosynthesis or by incomplete degradation of gangliosides or related glycolipids. The uniform findings of increased cerebroside in spleen in the acute (infantile) and chronic forms of Gaucher's disease and a decrease of cerebroside in brain in the chronic form demonstrates a marked difference in brain and spleen metabolism. A reasonable interpretation of the findings is that a failure to degrade glucocerebroside results in depression of galactocerebroside biosynthesis in brain and that glucocerebroside is converted in part to other lipids, presumably to gangliosides in brain. The spleen normally contains only a minute trace of cerebroside and a low level of ganglioside. The major difference in spleen and brain may then be that brain converts glucocerebroside to some other glycolipid (probably ganglioside) and spleen does not (or that the conversion is less extensive). Quantitative differences in the decrease of degradative enzyme are indicated by the findings in the acute and chronic forms of Gaucher's disease. The chronic form does not show brain involvement, but the acute form does. A slight reduction in cerebroside is noted in the chronic form. These findings can be explained if it is assumed that the same enzyme is involved but to a different extent. Thus, if there is a greater reduction of the enzyme degrading glucocerebroside in the acute form it would be expected that conversion to ganglioside would not be as extensive and inhibition of galactocerebroside biosynthesis would be more extensive. The presence of some glucocerebroside in brain in the acute form of Gaucher's disease may be expected. The ganglioside levels in the various forms of Gaucher's disease in spleen and brain must also be determined carefully.

Fatty Acid Composition of Lipids in the Sphingolipidoses

Several important conclusions can be drawn from the analysis of the fatty acid composition of the lipids in the sphingolipidoses. Sphingomyelin in Niemann-Pick disease lacks C_{24} fatty acids, but this is also seen in metachromatic leucodystrophy and hence the change cannot be assigned a specific role in either disease (19). Failure to degrade sphingomyelin cannot thus be attributed to an altered fatty acid composition. Similarly, Table I shows that the fatty acid composition of gangliosides of normal infant brain and Tay-Sachs brain are similar and thus failure to degrade ganglioside cannot be attributed to an abnormal fatty acid composition. Data reported for sulfatide in metachromatic leucodystrophy (13) also demonstate that failure to degrade sulfatide in this disease cannot be attributed to abnormal fatty acid composition.

Brain Lipid Class Distributions in Senile Cerebral Cortical Atrophy and Alzheimer's Disease

The study of senile cerebral cortical atrophy and the type of presenile atrophy known as Alzheimer's disease is of particular interest since the role of lipids in these conditions has not been investigated. Comparison with normal adult brain and the sphingolipidoses is instructive.

The data obtained from the brains of two cases of Alzheimer's disease, normal adult brains, and a brain showing senile cerebral cortical atrophy demonstrate that both fresh weight and total lipid are greatly reduced in Alzheimer's disease (7). This is not characteristic of cerebral atrophy per se since these changes are not seen in senile cerebral cortical atrophy.

Although the amts of grey and white matter of the brain in Alzheimer's disease are reduced, as is total lipid, the lipid class distributions of whole brain and grey and white matter are very similar to normal brain (7). The brain specimen showing senile cerebral atrophy differed from the normal specimens in several respects, but the reduction in sulfatide was most striking. No characteristic lipid

TABLE I

Fatty Acid Composition of Normal Infant and Tay-Sachs Brain Gangliosides

	Fatty acid								
	16:0 %	18:0 %	18:1 %	18:2 %	20:0 %	21:1 %	22:0 %	24:0 %	24:1 %
Normal infant	5.70	74.18	9.27	0.93	2.37	1.09	4.17	2.28	....
Tay-Sachs disease	2.10	78.31	6.26	0.79	6.91	....	1.83	....	3.80

class distribution changes are apparent for the two cases of Alzheimer's disease.

The reduction of sulfatide in the two atrophic brains from females (one senile cortical atrophy and one case of Alzheimer's disease) meets the criteria for a biosynthetic defect. Perhaps these lower sulfatide levels reflect a predisposition to cerebral cortical atrophy. Additional data from other specimens are required, however, to determine if this is the case.

Fatty Acids of Lecithin and Sphingomyelin in Senile Cerebral Cortical Atrophy and Alzheimer's Disease

The fatty acid compositions of whole brain lecithin and sphingomyelin in cerebral cortical atrophy and Alzheimer's disease (19) are essentially normal except that a greater variety of fatty acids is evident for lecithins in both pathological states.

Interpretation of the Findings in Alzheimer's Disease

The large loss of brain weight and total lipid must be explained. It is clear that with such large weight reduction, a large loss of water must have taken place. Whether or not water loss is accompanied by loss of other components including salts, proteins, lipids, etc. is not known. A general loss of water and other cellular components would be essentially a generalized autolysis. This is difficult to imagine from the lipid class distribution data that is very similar to the findings in the normal brain. It is difficult to visualize an autolytic process affecting all lipids classes to almost exactly the same extent in both grey and white matter, particularly when the amt of grey matter is greatly reduced. It is also difficult to understand why intermediate degradation products such as lysophosphatides are not present if autolysis is the main feature. Chromatographic techniques demonstrate the presence of many minor components, apparently intermediates of biosynthesis and degradation of major lipid classes, in the brain in the sphingolipidoses and the absence of these substances in Alzheimer's disease is thus particularly significant. Since the lipid class composition can be essentially normal in both grey and white matter in Alzheimer's disease and the ratio of lipid to brain weight is normal, it may very well be that generalized lipid degradation did not take place. If this is the case, it is evident that the brain in Alzheimer's disease prior to onset of symptoms and degradation must have had an abnormally high water content and an abnormally low total lipid content. This is the state characteristic of the brain in early life.

The fatty acid composition data of brain lipids in Alzheimer's disease does not disclose any abnormality. Degradation of myelin lipids does not appear to have taken place because the decrease of long chain fatty acids of demyelinating diseases (15) is not seen in Alzheimer's disease.

The defect in Alzheimer's disease, although present at birth, clearly does not prevent formation of myelin and development of normal brain function. If the brain fails to develop normally, total solids including lipids might remain abnormally low and water content remain abnormally high in brain before onset of the symptoms of the disease. Some trigger mechanism, e.g., change in hormonal balance, could initiate a process leading to water loss with weight reduction. This concept could explain the frequently used term "shrunken" in descriptions of the brain and its cellular elements in this disease. A reduction in whole brain water content from 85% to the measured value of 76% without loss of solids accounts for the weight loss observed in the two cases studied assuming an adult brain weight of about 1200 g.

A clear distinction between the general autolytic and water loss hypotheses can be drawn when specimens of brain early in the course of Alzheimer's disease are obtained. Determination of water and lipid in such specimens can be decisive.

ACKNOWLEDGMENTS

This work was supported in part by USPHS Grants NB-01847-06 from the National Institute of Neurological Diseases and Blindness and CA-03134-08 from the National Cancer Institute, Research Grant DA-AMC-18-035-71 (A) from the U.S. Army Chemical Research and Development Laboratories, Army Chemical Center, Maryland. The specimens from the sphingolipidoses nad normal infant brain were obtained from Dr. Alfred Knudson. Dr. Charles Altschuler provided the adult brain specimens including the two cases of Alzheimer's disease.

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