Sphingolipidoses

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Sphingolipidoses are inborn errors of metabolism, characterized by the accumulation of sphingolipids. These lysosomal diseases (Hers, 1965) are due to a deficiency in the degradative pathway (for references see Hers and Van Hoof, 1973). So far, neither changes in the extent of sphingolipid biosynthesis, ie, an overproduction, nor a deficiency of a transferase activity have been observed. The latter could result in the absence of the enzyme product and/or accumulation of its substrates.

Recessive inherited deficiencies of sphingolipid degrading enzyme activities have been found in all tissues that have been tested. The sphingolipid hydrolases studied thus far have been shown to be of lysosomal origin (for references, see Vaes, 1973). Most of these enzymes display an optimal activity in an acidic pH range. With the remarkable exception of Krabbe's disease, the storage material is found in vacuoles or storage granules which represent profoundly modified lysosomes incapable of degrading the accumulating lipids (fig 1; for references, see Hers, 1973). Some of these storage granules, eg, the membranous cytoplasmic bodies in Tay-Sachs disease, have been shown to contain lysosomal-like enzymatic activities (Tallman, Brady, and Suzuki, 1971). The amount of stored material is highest in tissues which exhibit the greatest biosynthetic rates for the accumulated lipids. The pathogenesis of this process seems to be especially severe when the nervous tissue is involved.

The physiological role of sphingolipids is still poorly understood, and almost no knowledge exists about the mechanism by which the lipid accumulation finally causes dysfunction and death of the affected cells. It seems obvious, however, that an elevated concentration of a single sphingolipid may cause a variety of changes in the cells, including changes in incorporation of lipid into membranes as well as mechanical distortion of the cell due to the deposition of the storage compound in granules and vacuoles (fig 1) which finally may occupy an abnormally large portion of the cytoplasm.

Nomenclature

As clinical entities, many of these diseases have long been known by eponyms (Tay, 1881; Gaucher,

1882; Sachs, 1896; Fabry, 1898; Anderson, 1898; Alzheimer, 1910; Niemann, 1914; Krabbe, 1916; Scholz, 1925). With progress in the analysis of lipids these diseases have been more accurately defined as specific lipid storage diseases, and it has become feasible to classify them more systematically on the basis of the main accumulating lipid (Aghion, 1934; Klenk, 1934, 1942; Jatzkewitz, 1958; Svennerholm, 1962). However, these nomenclatures, the first based on clinical and pathomorphological descriptions and the second based on the main accumulating lipid, are not always congruent. For example, diseases such as infantile GM_2 -gangliosidosis (Tay-Sachs disease) are ganglioside storage diseases, while no ganglioside accumulation has been found in neuronal ceroid-lipofuscinoses (Batten-Vogt disease), even though these diseases have been classified in the same group of familial amaurotic idiocies (for references, see Zeman and Siakotos, 1973). Different biochemical defects can therefore result in a similar clinical and pathological picture. Furthermore, biochemical analysis has shown that infantile amaurotic idiocy (Tay-Sachs disease), defined by clinical and pathomorphological criteria, includes at least two closely related but nevertheless biochemically different diseases: the variants O and B of infantile GM_2 -gangliosidosis (Sandhoff, Harzer, Wässle, and Jatzkewitz, 1971).

The classification of the sphingolipidoses based on the accumulation of lipid appears to be more precise than the one based on clinical and pathomorphological criteria but is also unsatisfactory. In many of these diseases, several lipids accumulate simultaneously, often concomitant with the accumulation of polysaccharides, as in GM_1 -gangliosidosis (for references, see Van Hoof, 1973). The main reason for this is believed to be the broad substrate specificity of certain sphingolipid hydrolases. Many of these enzymes catalyze the breakdown of several substances that have one chemical feature in common, and the deficiency of such an enzyme can easily result in the accumulation of several compounds. Therefore, the enzymatic deficiencies should be characterized in order to obtain a final classification of the storage diseases based on primary metabolic defects.

Fig 1 *Neurone from cortex of a child with Tay-Sachs disease. Tightly packed membranous cytoplasmic bodies (MCB) $\times 56\,000$ (electron micrograph; courtesy of B. W. Volk, Isaac Albert Research Institute of the Kingsbrook Jewish Medical Center; New York).*

Fig 2 Structure of ceramide and ganglioside G_{M1} . G_{A1} , asialoganglioside G_{M1} ; G_{A2} , asialoganglioside G_{M2} ; Cer, ceramide (= N-acyl-sphingosine); FA, fatty acid; Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine, G_{M1} , ganglioside G_{M1} ; G_{M2} , ganglioside G_{M2} ; Glc, D-glucose; NeuAc, N-acetylneuraminic acid; sialic acid, N-acylneuraminic acid; Sph, sphingosine.

Catabolism of Sphingolipids and Enzyme Deficiencies in Sphingolipidoses

Sphingolipids have in common a hydrophobic ceramide (ceramide = N-acylsphingosine) residue bound to either a hydrophilic mono- or oligosaccharide chain (fig 2), or in the case of sphingomyelin, phosphorylcholine. Figure 3 shows schematically the degradative pathway of sphingolipids. The degradation always starts from the hydrophilic end of the molecule (for references, see Gatt and Bahrenholz, 1973; Leeden and Yu, 1973; Sugita, Dulaney, and Moser, 1972). Each step is catalyzed by a lysosomal hydrolase that removes the terminal non-reducing sugar moiety in the case of ceramide oligosaccharides, sulphate in the case of sulphatides, or phosphorylcholine in the case of sphingomyelin. It is of special interest to note that there is a disease known for each degradation step in which the respective hydrolase activity is deficient (fig 3), with one important exception: a neuraminidase deficiency, which should result in a primary accumulation of

oligosialo-gangliosides or of ganglioside G_{M3} (fig 3), has never been observed. It may be that a neuraminidase deficiency is incompatible with life and therefore lethal at very early stages of development.

Variant Forms of Sphingolipidoses

The schematic representation of sphingolipidoses given in figure 3 is complicated by the fact that many sphingolipid storage diseases exist as variant forms. Analysis of the enzymes involved in the breakdown of sphingolipids has shown that many of them occur as multiple forms, or isoenzymes, of which one or more can be deficient in different forms of a lipid storage disease. A further heterogeneity seems to be due to the fact that different degrees of enzyme deficiency have been observed in different families affected with a given storage disease. Patients with higher residual enzymatic activity appear to have a longer life span than those with almost complete enzyme deficiency.

Fig 3 Catabolism of sphingolipids and enzyme deficiencies in sphingolipidoses. Abbreviations as in figure 2.

Number	Disease	Major Storage Compound	Deficient Enzyme Activity
1	Farber's disease (ceramidosis)	Ceramide	Ceramidase
2	Niemann-Pick disease (sphingomyelinosis)	Sphingomyelin	Sphingomyelinase
3	Gaucher's disease (glucocerebroside)	Glucocerebroside	Glucocerebroside β -glucosidase
4	Krabbe's disease (galactocerebroside)	Galactocerebroside	Galactocerebroside β -galactosidase
5	Metachromatic leucodystrophy (sulphatidosis, 'variant B')	Cerebrosidesulphates	Cerebrosidesulphatase, (arylsulphatase A)
6	Metachromatic leucodystrophy (sulphatidosis, 'Variant O')	Cerebrosidesulphatases and steroid sulphates	Arylsulphatases A, B, C and steroid
7	Lactosylceramidosis	Lactosylceramide	Lactosylceramide β -galactosidase
8	Fabry's disease (α -galactosyl-lactosyl-ceramidosis)	α -Galactosyl-lactosylceramide	α -Galactosidase
9	Tay-Sachs disease (G_{M2} -gangliosidosis, variant B ¹)	Ganglioside G_{M2}	N-Acetyl- β -D-hexosaminidase A
10	G_{M2} -gangliosidosis, Variant O ¹	Ganglioside G_{M2} and globoside	N-Acetyl- β -D-hexosaminidases A and B
11	G_{M1} -gangliosidosis	Ganglioside G_{M1}	β -Galactosidase

¹Synonyms used for these variants:

Variant O = G_{M2} -gangliosidosis, type A₁B₀ (Young, Ellis, Lake, and Patrick, 1970).

= G_{M1} -gangliosidosis, type II, eponymous classification

= Sandhoff's disease or Sandhoff-Jatzkewitz-Pilz disease (O'Brien, Okada, Ho, Fillerup, Vaeth, and Adams, 1971)

Variant B = G_{M2} -gangliosidosis, type A₁B_H (Young *et al*, 1970).

= G_{M2} -gangliosidosis, type I, eponymous classification

= Tay-Sachs disease (O'Brien *et al*, 1971).

The Role of Isoenzymes in Sphingolipidoses

In human tissue there are two main N-acetyl- β -D-hexosaminidases (hexosaminidase), the hexosaminidase A with an isoelectric point at pH 4.9 and the hexosaminidase B with an isoelectric point at pH 7.3 (Robinson and Stirling, 1968; Sandhoff, 1968). Both enzymes have been demonstrated in all tissues studied, such as liver, spleen, kidney, and brain. In brain tissue, hexosaminidase A activity stays nearly constant throughout life, whereas hexosami-

nidase B activity in adults is about four times that in the fetus (Harzer and Sandhoff, 1971). Both enzymes exhibit both N-acetyl- β -D-glucosaminidase and -galactosaminidase activity, and cleave a variety of synthetic N-acetyl- β -D-glucosaminides and N-acetyl- β -D-galactosaminides such as the p-nitrophenyl- and the 4-methylumbelliferyl-N-acetyl- β -D-hexosaminides. Furthermore, it has been shown that the total hexosaminidase activity from calf brain hydrolyses the storage compounds in Tay-Sachs disease—ganglioside G_{M2} and asialoganglioside

Fig 4 Hexosaminidase pattern and glycolipid accumulation in four variants of GM_2 -gangliosidosis. Glycolipid content is expressed as percentage of dry weight (Sandhoff et al, 1971).

+Level of
N-acetyl- β -D-hexosaminidase B activity is normal, level of N-acetyl- β -D-hexosaminidase A activity is about half normal.

*Calculated from data of Suzuki, Suzuki, Rapin, Suzuki, and Ishii (1970) and Menkes, O'Brien, Okada, Grippio, Andrews, and Cancilla (1971).

GA_2 —by removing the terminal non-reducing N-acetylgalactosamine residue (Frohwein and Gatt, 1967). Therefore, it seemed reasonable to study the role of these enzymes in Tay-Sachs disease.

We found three different variations of the normal hexosaminidase pattern in the three cases of Tay-Sachs disease we had available at that time. The enzymes in tissue extracts were separated by isoelectric focusing and their activity was tested with both synthetic substrates and tritium-labelled storage compounds—the sialic acid-free ganglioside, asialoganglioside, GA_2 , and globoside (Sandhoff, 1969; Sandhoff *et al*, 1971). In the first patient, there was an almost total loss of both enzyme activities as well as storage of ganglioside GM_2 and asialoganglioside GA_2 in the nerve tissue and accumulation of globoside in the visceral organs (Sandhoff, Andreae, and Jatzkewitz, 1968). In the second patient a loss of hexosaminidase A activity was observed in all tissues studied as well as storage of ganglioside GM_2 and asialoganglioside GA_2 in the nerve tissue. No hexosaminidase deficiency was observed in the third patient who had a storage pattern almost identical to that of the second patient. Therefore, the relationship between hexosaminidase A deficiency and the storage of ganglioside GM_2 as found in the second patient was in doubt, but both were observed later in two additional patients with Tay-Sachs disease (Sandhoff, 1969). Furthermore, the deficiency of hexosaminidase A associated with Tay-Sachs disease was reported by Okada and O'Brien (1969) who used gel electrophoresis for the separation of the enzymes, and also by Hultberg (1969) who used the isoelectric focusing technique. Hexosaminidase A and total hexosaminidase deficiencies in other patients have been confirmed in many further reports, but a second case of the variant AB

has not yet been reported (for references, see O'Brien, 1973; Sandhoff and Harzer, 1973; Okada, McCrea, and O'Brien, 1972; Krivit, Desnick, Lee, Moller, Wright, Sweeley, Snyder, and Sharp, 1972).

Based on these enzymatic defects, the following nomenclature is used in the present paper: the classical Tay-Sachs disease is called variant B of infantile GM_2 -gangliosidosis, since the B enzyme is still active in all tissues studied (fig 4); the variant with additional globoside accumulation and a generalized total hexosaminidase deficiency is called variant O of infantile GM_2 -gangliosidosis, since both isoenzyme activities are deficient (fig 4); the variant represented by the third case is named variant AB, since both hexosaminidases A and B were active in several postmortem tissues such as brain, liver, kidney, and spleen using both synthetic substrates and the asialoganglioside GA_2 (fig 4).

The hexosaminidases A and B have been purified several thousand-fold from postmortem normal human liver tissue using conventional protein fractionation procedures (Sandhoff and Wässle, 1971) in order to study the correlation between the different hexosaminidase deficiencies and the storage pattern in the three enzymatic variants of infantile GM_2 gangliosidosis. Both enzymes exhibited an apparent molecular weight of about 130 000 daltons. Their substrate specificity was found to be very similar, as shown in table I. Both isoenzymes A and B hydrolyze the neutral storage compounds globoside and asialoganglioside GA_2 at about the same rate in the presence of a detergent mixture. But in our experiments, only the hexosaminidase A exhibited a definite ganglioside GM_2 -N-acetyl- β -D-galactosaminidase activity, degrading the ganglioside GM_2 to ganglioside GM_3 (Sandhoff, 1970; Sandhoff and Wässle, 1971). However, the ganglioside GM_2

Substrate	Hexosaminidase A		Hexosaminidase B	
	K_m	V_{max}	K_m	V_{max}
p-Nitrophenyl-N-acetyl- β -D-glucosaminide	0.67	117	0.67	73
p-Nitrophenyl-N-acetyl- β -D-galactosaminide	0.16	9.5	0.15	4.8
Asialoganglioside G_{A1}	0.2	1.1	0.2	1.1
Kidney globoside	0.15	0.2	0.2	0.2
Ganglioside G_{M1} split in 20 h $\left(\frac{\mu\text{mol}}{\text{mg protein}} \right)$		0.12		—

Table I Michaelis constants (K_m [mmol/l] and maximum velocities (V_{max} [$\mu\text{mol}/(\text{min} \times \text{mg protein})$]) of N-acetyl- β -D-hexosaminidases A and B (Sandhoff, 1970; Sandhoff and Wässle, 1971)

hydrolyzing activity of the hexosaminidase A was very low compared to its activity against asialoganglioside G_{A2} and globoside (table I), and it appeared doubtful whether this small hydrolysis rate had any physiological significance. Therefore, the recent finding of Li, Mazzotta, Wan, Orth, and Li (1973), who showed that a heat-stable and non-dialyzable factor from human liver stimulates the ganglioside G_{M2} degrading activity of the hexosaminidase A about five fold, is of great interest.

Based on these *in-vitro* studies it appears reasonable that the ganglioside G_{M2} accumulation in the variants B and O is a result of the hexosaminidase A deficiency, and that the additional storage of globoside and asialoganglioside G_{A2} in the variant O is a result of the simultaneous deficiencies of both hexosaminidases. Indeed, tissue extracts from variant B (Kolodny, Brady, and Volk, 1969) and variant O (Sandhoff *et al*, 1971) showed no ganglioside G_{M2} -N-acetyl- β -D-galactosaminidase activity, whereas the hexosaminidase B extracted from variant B tissues hydrolyzed asialoganglioside G_{A2} and globoside (Sandhoff *et al*, 1971). The minor accumulation of asialoganglioside G_{A2} in the nerve tissue of patients with variants B and AB (fig 4) may have two explanations: (1) the asialoganglioside G_{A2} in the storage granules (MCBs) is not fully accessible to the hexosaminidase B; (2) the inhibition of the hexosaminidase B by the main storage compound, ganglioside G_{M2} ($K_i = 0.2 - 0.4$ mmol/l, Sandhoff and Wässle, 1971), reduces its activity against asialoganglioside G_{A2} . In the case of the variant AB, both hexosaminidases were active against synthetic substrates and the minor storage compound asialoganglioside G_{A2} . But the ganglioside G_{M2} -N-acetyl- β -D-galactosaminidase activity of a crude hexosaminidase preparation from the respective liver tissue was almost as low as in similar preparations from variant O and B tissues (Sandhoff *et al*, 1971). Among possible explanations are: (1) the hexosaminidase A of the variant AB tissues is a mutated enzyme that has specifically lost its ability to recognize the ganglioside G_{M2} ; (2) an activating factor

such as found by Li *et al* (1973) for the hydrolysis of ganglioside G_{M2} is missing in the variant AB tissues.

The molecular relationship between hexosaminidase A and B is still obscure. Both enzymes exhibit a very similar substrate specificity (table I) and their activity is susceptible in a parallel manner to a wide range of inhibitors (Robinson and Stirling, 1968; Sandhoff and Wässle, 1971). Their immunological properties are very similar (Carroll and Robinson, 1973; Srivastava and Beutler, 1972, 1973), and both enzymes cross react with their respective antibodies. There is suggestive evidence that hexosaminidase A may be converted into hexosaminidase B: heating a purified, neuraminidase-free hexosaminidase A preparation to 50°C at pH 6 results in the formation of a new enzyme activity that has the same heat stability, isoelectric point, electrophoretic mobility, and molecular weight as hexosaminidase B (Sandhoff, 1973). On the other hand, van Someren and Beijersbergen van Henegouven (1973) reported evidence that both isoenzymes behave as independent markers in somatic fusion experiments between human and Chinese hamster fibroblasts. These results do not support the possibility that one isoenzyme is merely the precursor of the other. However, the data accumulated so far are compatible with the hypothesis that both isoenzymes have a polypeptide chain in common.

Isoenzymes play a similar role in other sphingolipidoses, such as late metachromatic leucodystrophy (see fig 3). The classical form of late infantile metachromatic leucodystrophy is characterized by a deficiency of arylsulphatase A (Austin, Armstrong, and Shearer, 1965; Mehl and Jatzkewitz, 1965) and an accumulation of cerebrosulphatides (sulphatides) (Jatzkewitz, 1958), especially in the brain and kidney. Arylsulphatase A cleaves the sulphate ester bond of sulphatides, which have been shown to be the natural substrates for the enzyme (Mehl and Jatzkewitz, 1964, 1968; Jatzkewitz and Mehl, 1969). This sulphatase activity is stimulated about three-fold by a heat stable non-dialyzable factor

(Mehl and Jatzkewitz, 1964; Jatzkewitz and Stinshoff, 1973).

In a variant form of metachromatic leucodystrophy, Austin (1963) and Murphy, Wolfe, Balazs, and Moser (1971) reported the simultaneous deficiency of the arylsulphatases A, B, C and two steroid sulphatases, with the concomitant accumulation of both sulphatides and steroid sulphates. However, considerable residual arylsulphatase B activity was found in the liver tissue. This simultaneous enzyme deficiency appears to represent a situation similar to the variant O of infantile G_{M2} -gangliosidosis, whereas the classical form of metachromatic leucodystrophy seems to be analogous to the variant B of the infantile G_{M2} -gangliosidosis (Harzer, Stinshoff, Mraz, and Jatzkewitz, 1973). Again, the exact relationship between the different sulphatases remains to be clarified.

Different Degrees of Enzyme Deficiencies

Many sphingolipidoses occur in different age-related forms with a clinical onset during infantile, juvenile, or adult life. It has been shown for some of them, such as Gaucher's disease and metachromatic leucodystrophy, that the infantile forms show a higher degree of enzyme deficiency than the adult forms. In Gaucher's disease, for instance, Brady, Kanfer, Bradley, and Shapiro (1966) found that the residual glucocerebroside β -glucosidase activity ranges from 0 to 9% in the infantile form (Brady, Kanfer, and Shapiro, 1965), from 10 to 17% in the juvenile form, and may be as high as 40% in the adult form, of the activity found in control human tissues. The degree of enzyme deficiency appeared

to be fairly constant within individual families. Therefore, it was presumed that the varying degree of enzyme deficiency in various families is caused by differences in an underlying genetic mutation (Brady and King, 1973a).

A similar situation has been found in metachromatic leucodystrophy. The activity of the arylsulphatase A is usually measured in tissue extracts with synthetic substrates such as nitrocatecholsulphate. Such assay techniques yielded no detectable arylsulphatase A activity in urine or in extracts from leucocytes and fibroblasts with any of the different age-related forms—late infantile, juvenile, and adult (Austin, Armstrong, Fouch, Mitchell, Stumpf, Shearer and Briner, 1968; Percy and Kaback, 1971). Based on the almost complete arylsulphatase A deficiency in urine and leucocytes and on a high level of sulphatide excretion in the urine, Pilz and Hopf (1972) and Pilz (1972) were able to diagnose metachromatic leucodystrophy in adults without obvious clinical symptoms. Despite these very profound enzyme deficiencies in all age groups of metachromatic leucodystrophy, Porter, Fluharty, Trammell, and Kihara (1971) demonstrated different levels of residual sulphatase activity for different age groups by using an *in-vivo* activity assay (fig 5). Fibroblasts derived from different patients were plated in a ^{35}S -sulphatide-containing medium. The authors followed the uptake of sulphatide into the cells and the sulphatase activity by measuring the release of labelled sulphate into the medium. The rate of release of labelled sulphate into the medium increased with the age of clinical onset. On the other hand, the intracellular ^{35}S -sulphatide retention was inversely proportional to the age of onset of clinical

Fig 5 Correlation of intracellular cerebroside sulphatase activity in fibroblasts with age of clinical onset of metachromatic leukodystrophy. Petri dishes (60 mm) were plated with fibroblasts (300 000/dish) in 3 ml growth medium containing ^{35}S -cerebroside sulphate (23 nmol/ml; 3.6×10^3 cpm/nmol). On the days indicated one dish of each cell strain was analyzed for intracellular ^{35}S -sulphatide and extracellular ^{35}S -sulphate. The cells were from patients with metachromatic leucodystrophy whose age of onset of clinical symptoms are indicated by the numbers in years opposite the plots

(From Kihara, H.; Porter, M., and Fluharty, A.; Enzyme Replacement in Cultured Fibroblasts from Metachromatic Leukodystrophy. In Birth Defects: Orig. Art. Ser., ed. D. Bergsma. Enzyme Therapy in Genetic Diseases. Published by Williams & Wilkins Co., Baltimore, for The National Foundation-March of Dimes, White Plains, N.Y. Vol. IX (2); 22, 1973 with permission of the author and copyright holder, the National Foundation).

symptoms. Electron micrographs and radioautography have shown that the accumulating ^{35}S -sulphatide resides in structures resembling lysosomes (Kihara, Porter, and Fluharty, 1973).

Different residual activities of hexosaminidase A have been observed in the age-related forms of GM_2 -gangliosidosis. In general, *in vitro* measurements with synthetic substrates showed higher levels of residual hexosaminidase A activity (5-50% of control) in juvenile cases than in infantile cases with variant B (0-7% of control) (for references see O'Brien, 1973; Brett, Ellis, Haas, Ikonne, Lake, Patrick, and Stephens, 1973). But Brett *et al* (1973) could find no correlation between the age of onset of symptoms and the degree of hexosaminidase deficiency.

It was, therefore, desirable to repeat such activity measurements using the natural substrates to see if they would yield different results. In the case of juvenile GM_2 -gangliosidosis described by Suzuki and Suzuki (1970), the hexosaminidase A activity of the liver tissue was lowered only to about 50% of the control value when tested with p-nitrophenyl-N-acetyl- β -D-glucosaminide as substrate. Using the minor storage compound asialo-ganglioside GA_2 as substrate, the activity of this enzyme appeared to be even less, 29% of the control value (Zerfowski and Sandhoff, 1974). But using the main storage compound as substrate, no ganglioside GM_2 -N-acetyl- β -D-galactosaminidase activity could be detected in the extracts of the liver tissue within the rather limited sensitivity of the test used.

Diagnosis and Genetics

The diagnosis of sphingolipidoses with similar clinical symptoms can be achieved by lipid analysis of the affected tissues and by the determination of the specific enzyme defect in tissues and body fluids. The enzymatic approach seems to be more useful and convenient than lipid analysis, since it can be made not only with extracts of solid tissues but also with those of leucocytes, cultured fibroblasts, and amniotic cells (for references see Kaback and Howell, 1973). Therefore, the enzymatic approach allows the diagnosis of mutant homozygotes and the detection of heterozygotes (fig 6) which is important for genetic counselling and for the analysis of the mode of inheritance of these diseases. Heterozygotes usually appear to have levels of activity of the relevant enzyme intermediate between those of controls and homozygotes (fig 6).

Unfortunately, there is usually an overlapping between the ranges of normal and heterozygous enzyme levels, which makes it impossible to identify with certainty all of the carriers. But by relating the

Fig 6 Statistical data (Mean (—●—) $\pm$ 2 SD) of leucocyte hexosaminidase A activities obtained by acrylamide gel electrophoresis for: adult (normal) controls (49), pregnant controls (40), Tay-Sachs parents (34), Tay-Sachs children (13), and normal (*non-neurological) children (11) (From Saifer, Perle, Valenti, and Schneck (1972) with permission of the author and copyright holder, Plenum Press).

enzyme activity involved in a disease to the mean relative activity of three or four other lysosomal reference enzymes, Harzer (1973) found no overlapping between the activity ranges of normal persons and heterozygous carriers. He could therefore detect, with a very high probability, the heterozygotes in nine families with GM_2 -gangliosidosis (variant B and variant O) and metachromatic leucodystrophy, by measuring the respective enzyme activities in leucocytes.

Using enzyme measurements for the detection of carriers, it has been shown that Gaucher's disease, metachromatic leucodystrophy, Krabbe's disease, Niemann-Pick disease, infantile GM_2 -gangliosidosis, variant B and variant O (fig 7) and GM_1 gangliosidosis are autosomal recessive inherited diseases, whereas Fabry's disease is an X-linked recessive inherited disease (for references see Kaback and Howell, 1973).

Most of the lysosomal enzyme activities involved in sphingolipidoses can be tested conveniently with synthetic substrates, but no synthetic substrates are available for the determination of galactocerebroside β -galactosidase in Krabbe's disease (Bowen and Radin, 1969; Suzuki and Suzuki, 1973) or sphingomyelinase in Niemann-Pick disease (Brady and King, 1973b). Therefore, these assays are usually performed with labelled galactocerebrosides or labelled sphingomyelin as substrates. This limits the effectiveness of these assays in routine clinical analysis, since labelled lipids have to be prepared by laborious procedures. To avoid this problem a sen-

Fig 7 Pedigree of a patient with infantile GM_2 -gangliosidosis, variant O. The values indicate the total hexosaminidase activity in the blood (leucocytes and serum) as a percentage of the normal control (From Harzer, Sandhoff, Schall, and Kollmann (1971) with permission of the author and the copyright holder, Springer-Verlag).

sitive assay for sphingo-myelinase was recently developed by Harzer and Benz (1973), using unlabelled sphingomyelin as substrate.

Based on the assay of specific enzyme deficiencies in amniotic cells, prenatal diagnosis has been achieved for infantile GM_2 -gangliosidosis variant O (Desnick, Krivit, and Sharp, 1973) and variant B, GM_1 -gangliosidosis, Fabry's disease, Gaucher's disease, Krabbe's disease, metachromatic leucodystrophy, and Niemann-Pick disease (for references see Kaback and Howell, 1973).

But it remains questionable whether the deficiency of a lysosomal enzyme activity determined with synthetic substrates provides enough evidence for the unequivocal diagnosis of a lipid storage disease. For example, Navon, Padeh, and Adam (1973) and Vidgoff, Buist, and O'Brien (1973) could not detect any significant hexosaminidase A activity in blood samples or cultured fibroblasts of healthy adult members of two Tay-Sachs families using synthetic substrates. On the other hand, in the AB variant of infantile GM_2 -gangliosidosis (Sandhoff, 1969; Sandhoff *et al*, 1971), both hexosaminidases A and B were active against synthetic substrates, although a larger accumulation of ganglioside GM_2 was found in the nerve tissue (fig 4). However, only a minor residual enzymatic activity could be measured using the ganglioside GM_2 as substrate.

Therapeutic Attempts

The evidence collected so far indicates very strongly that accumulation of lipids in sphingolipidoses results from specific degradative enzyme deficiencies. Indeed, some model experiments have shown that the uptake of active enzyme preparations from the medium by enzyme-deficient cultured fibroblasts results in the breakdown of the storage material. Dawson, Matalon, and Li (1973) showed the uptake of purified plant α -galactosidase from the medium by Fabry fibroblasts and the concomitant degradation of the storage material α -galactosyl-lactosyl-ceramide. However, the α -galactosidase activity in both cells and medium diminished rapidly with time and became negligible after three to four days.

Fig 8 Incorporation of exogenous arylsulphatase A by fibroblasts from patients with metachromatic leucodystrophy. A crude preparation of arylsulphatase A derived from 70% ammonium sulphate precipitate of human urine was added to growth medium to give a final concentration of 2 units of enzyme activity per ml of medium. (A unit of enzyme catalyzes the hydrolysis of 1 μmole of p-nitrocatechol sulphate per hour.) The medium was sterilized by filtration and fed to confluent cultures of cells from patients with late infantile metachromatic leukodystrophy. On day 7, the cells were washed, trypsinized with 0.25% trypsin, replated at the same cell density, and incubated with normal medium. After four days, the cells were trypsinized and subcultured at one-third the original density to permit growth. For arylsulphatase A activity cell extracts were prepared and assayed. T = trypsinization; S = subculture (from Porter, Fluharty, and Kihara (1971) 'Correction of Abnormal Cerebroside Sulfate Metabolism in Cultured Metachromatic Leukodystrophy Fibroblasts', Science, Vol. 172, pp. 1263-1265, Fig. 1; with permission of the author and copyright holder, American Association for Advancement of Science).

In contrast to this, Porter, Fluharty, and Kihara (1971) reported stability for at least two weeks of human arylsulphatase A taken up from the medium by fibroblasts derived from patients with metachromatic leucodystrophy (fig 8). These arylsulphatase-A-treated fibroblasts were subcultured after trypsinization and showed almost the same rate of sulphatide degradation as that of control cells (fig 9, cf fig 5) (Kihara *et al*, 1973). In similar experiments with cultured fibroblasts from patients with variant B and O of infantile G_{M2} -gangliosidosis, Kolodny, Milunsky, and Sheng (1973) found no significant uptake of human hexosaminidase A from the medium by the cells.

Enzymatic measurements in cultured amniotic cells have shown that the specific enzyme deficiencies, as expected, are already evident in early fetal life allowing the prenatal diagnosis of sphingolipidoses. Furthermore, Schneek, Adachi, and Volk (1972) demonstrated the accumulation of ganglioside G_{M2} in the brain tissue of 16- to 22-week-old fetuses affected with Tay-Sachs disease. This

Fig 9 Enzyme replacement therapy in fibroblasts from patients with metachromatic leucodystrophy (MLD). Late infantile MLD cells were cultured in arylsulphatase A containing medium for four days as described in figure 8. They were subcultured into 60 mm Petri dishes. At the same time normal cells and untreated MLD cells were also subcultured into 60 mm Petri dishes. After one day, 3 ml of growth medium containing ^{35}S -cerebroside sulphate (20 nmol/ml; 1.16×10^5 cpm/nmol) was added to each dish. On the days indicated one dish of each set was analyzed for intracellular ^{35}S -sulphatide and extracellular ^{35}S -sulphate (from Kihara, H., Porter, M. and Fluharty, A.: *Enzyme Replacement in Cultured Fibroblasts from Metachromatic Leukodystrophy*. In *Birth Defects: Orig. Art. Ser.*, ed. D. Bergsma. *Enzyme Therapy in Genetic Diseases*. Published by Williams & Wilkins Co., Baltimore, for The National Foundation-March of Dimes, White Plains, N.Y. Vol. IX (2): 20, 1973; with permission of the author and copyright holder, The National Foundation).

finding indicates that the damaging process is already detectable very early in development. Despite this fact, therapeutic attempts have been limited so far to postnatal life. Thus far, two major attempts to correct the deficiencies have been made: (1) enzyme replacement by enzyme infusion, and (2) replacement by transplantation of a healthy organ. At present, the latter attempt appears more successful. Philippart, Franklin, and Gordon (1972), Philippart (1973) and Desnick, Allen, Simmons, Woods, Anderson, Najarian, and Krivit (1973) reported improvements in patients with Fabry's disease three to 15 months after kidney transplantation. In the treated patients, the plasma level of α -galactosidase rose from almost 0 to 8-22% of the normal level. Furthermore, the concentration of the storage compound in the plasma dropped to almost normal levels. Enzyme replacement by infusion of α -galactosidase also yielded a drop in the plasma level of the storage compound (Mapes, Anderson, Sweeley, Desnick, and Krivit, 1970) but attempts at therapy for sphingolipidoses with major involvement of the nervous system have been thus far unsuccessful. Johnson, Desnick, Long, Sharp, Krivit, Brady, and Brady (1973) found no increase in the enzyme level in the cerebrospinal fluid after infusion of human hexosaminidase A into a patient with variant O of G_{M2} -gangliosidosis. The infused enzyme disappeared from the plasma within six hours, but did not reach the brain, probably due to the blood brain barrier.

Today no specific therapy for sphingolipidoses is available. Early diagnosis and genetic counselling are the simplest and most effective means for preventing the conception and birth of affected children.

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