Hum. Genet. 50, 107—143 (1979)

Human Genetics

Review Articles

Biochemistry and Genetics of Gangliosidoses

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Summary. The gangliosidoses comprise an-ever increasing number of biochemically and phenotypically variant diseases. In most of them an autosomal recessive inherited deficiency of a lysosomal hydrolase results in the fatal accumulation of glycolipids (predominantly in the nervous tissue) and of oligosaccharides.

The structure, substrate specificity, immunological properties of and genetic studies on the relevant glycosidases, ganglioside G_{M1} β-galactosidase and β-hexosaminidase isoenzymes, are reviewed in this paper. Contrary to general expectation, only a poor correlation is observed between the severity of the disease and residual activity of the defective enzyme when measured with synthetic or natural substrates in the presence of detergents. For the understanding of variant diseases and for their pre- and postnatal diagnosis, the necessity of studying the substrate specificity of normal and mutated enzymes under conditions similar to the in vivo situation, e.g., with natural substrates in the presence of appropriate activator proteins, is stressed. The possibility that detergents may have adverse affects on the substrate specificity of the enzymes is discussed for the β-hexosaminidases. The significance of activator proteins for the proper interaction of lipid substrates and water-soluble hydrolases is illustrated by the fatal glycolipid storage resulting from an activator protein deficiency in the AB variant of G_{M2}-gangliosidosis. Recent somatic complementation studies have revealed the existence of a presumably post-translational modification factor necessary for the expression of ganglioside G_{M1} β-galactosidase activity. This factor is deficient in a group of variants of G_{M1}-gangliosidosis. Among the possible reasons for the variability of enzyme activity levels in heterozygotes and patients, allelic mutations, formation of hybrid enzymes, and the existence of patients as compound heterozygotes are discussed. All these may result in the production of mutant enzymes with an altered specificity for a variety of natural substrates.

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0340-6717/79/0050/0107/$ 07.40

Abbreviations

Cer, ceramide; Gal, D-galactose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranoside; Glc, D-glucose; MUF, 4-methylumbelliferone; MUF-β-Gal, 4-methylumbelliferyl-β-D-galactopyranoside; MUF-β-GalNAc, 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-galactopyranoside; MUF-β-GlcNAc, 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside; NeuAc, *N*-acetylneuraminic acid; PNP-β-Gal, *p*-nitrophenyl-β-D-galactopyranoside.

Variant B of infantile G_{M2}-gangliosidosis, Tay-Sachs disease; Variant 0 of infantile G_{M2}-gangliosidosis, Sandhoff disease, Sandhoff-Jatzkewitz disease; Variant 0 of juvenile G_{M2}-gangliosidosis, juvenile Sandhoff disease; Variant AB, Variant AB of infantile G_{M2}-gangliosidosis.

Introduction and General Considerations

In the early decades of this century the term amaurotic idiocy was used to describe a group of rather similar diseases leading to a dysfunction of neurons (Schettler and Kahlke, 1967; Minauf, 1975). From the 1960s onward, biochemical investigations of amaurotic idiocy revealed a variety of different molecular mechanisms all leading to similar clinical symptoms (Hers and Van Hoof, 1973). In one subgroup a recessively inherited lysosomal storage of gangliosides was demonstrated, whereas no primary biochemical abnormality has so far been shown in the second subgroup, i.e., in ceroid lipofuscinosis (Zeman and Siakotos, 1973). As time went on an increasing number of variants of the gangliosidoses were identified; these are clinically rather heterogeneous and extend well beyond the confines of the original description of amaurotic idiocy. Two main forms can be distinguished on the basis of the major storage compound in the nervous tissue: the G_{M1}- and G_{M2}-gangliosidosis, which are characterized by the neuronal accumulation of G_{M1}- and G_{M2}-ganglioside, respectively.

The classic clinical description of amaurotic idiocy is restricted to Tay-Sachs disease, whereas G_{M1}-gangliosidosis has different clinical signs. In 1881 Warren Tay was the first to draw attention to the clinical characteristics of infantile amaurotic idiocy when he observed a cherry-red spot in the retina of a year-old child. The children suffering from this disease develop normally at first, but later lose abilities, such as grasping and sitting up, that they had already learned in their first months of life. Blindness, paralysis of the limbs, and convulsions set in. An abnormal startle response is characteristic of this disease. Death comes within 2—3 years, after progressive mental and physical deterioration. Morphologically, a characteristic change of the ganglion cells is found. The cytoplasm of these cells is severely swollen, the cells being enlarged many times and their dendrites distended. Under the electron microscope many oval, multilaminated profiles can be seen in the cytoplasm of the neurons (Terry and Weiss, 1963). These bodies contain the storage substance of this disease—ganglioside G_{M2}: the disease is therefore referred to as G_{M2}-gangliosidosis. Whereas ganglioside G_{M2} only occurs in traces as an intermediate product in healthy brain tissue, it forms about 6%—12% of the dry brain weight of patients with this disease (Svennerholm, 1962; Sandhoff et al., 1971).

The enormous increase of ganglioside G_{M2} in Tay-Sachs disease leads to the destruction of the nerve cells and—in a way that is poorly understood and may possibly be related to axon degeneration—secondary demyelination, which is reflected in the lipid composition of the diseased nervous tissue (Sandhoff et al., 1971). A drastic decrease of all typical myelin lipids, such as cerebrosides, sulfatides and C_{24}-sphingomyelin is observed. Similar secondary changes also occur in G_{M1}-gangliosidosis.

In the late 1930s Ernst Klenk (1939/1940, 1942) discovered the gangliosides as a new group of glycolipids stored in Tay-Sachs disease. They are glycosphingolipids containing a hydrophobic ceramide (*N*-acylsphingosine) and a hydrophilic oligosaccharide chain bearing one or more *N*-acylneuraminic acids (sialic acid). The first ganglioside structure was established in 1963 by Kuhn and Wiegandt for the monosialoganglioside G_{M1} : Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc)β1→4Glcβ1→1′Cer. Gangliosides are typical components of cell plasma membranes anchored by their hydrophobic ceramide moiety (Ledeen, 1978). The hydrophilic oligosaccharide chain extends into the extracellular space. Gangliosides are concentrated in neuronal plasma membranes, especially in the regions of nerve endings and dendrites (Hansson et al., 1977). Although their function in these regions still remains obscure, specific gangliosides have recently been implicated as binding molecules for toxins and hormones (for literature see Yamakawa and Nagai, 1978). As specific cell surface markers they are likely to be involved in cell differentiation and cell-cell interaction (Roseman, 1970).

Biosynthesis of gangliosides is catalyzed by a group of membrane-bound transferases (for literature see Richardson et al., 1977; Landa et al., 1977). For catabolism (Fig. 1) they are transported in a poorly understood process from the membranes into lysosomes, where they are degraded in a stepwise manner by acid hydrolases starting at the hydrophilic end of the molecules (Gatt and Barenholz, 1973; Neufeld et al., 1975; Sandhoff, 1977). The recessively inherited deficiency of such a hydrolase will block complete degradation and may result in the accumulation of all substrates for whose degradation the missing enzyme activity is necessary. Many sphingolipid hydrolases are bond-specific and degrade several related substrates (e.g., oligosaccharides, glycoproteins and lipids) (Hers and Van Hoof, 1973). As a consequence the deficiency of such a hydrolase results in the intralysosomal accumulation of substrates. The water-insoluble lipid substrates precipitate and form pathologic storage bodies, which lead to the ballooning and finally to the destruction of the cells.

Since the enzyme deficiency is considered to be the primary cause for lipid accumulation and health impairment, a correlation between the extent of the enzyme deficiency and clinical signs and neuropathologic findings should be expected. Such a qualitative correlation has been observed. On the other hand, the deficiency of the same enzyme or isoenzyme may result in quite different phenotypes of G_{M1}- and G_{M2}-gangliosidosis. Indirect evidence suggests that allelic mutations at the same locus may be involved, resulting in different structural mutations for a polypeptide chain of an enzyme (O'Brien, 1975). As a consequence, the stability (e.g., against changes in pH, temperature or proteases) of the enzymic activity as well as the substrate specificity might be changed. Furthermore, the inheritance of several, presumably equally rare, allelic

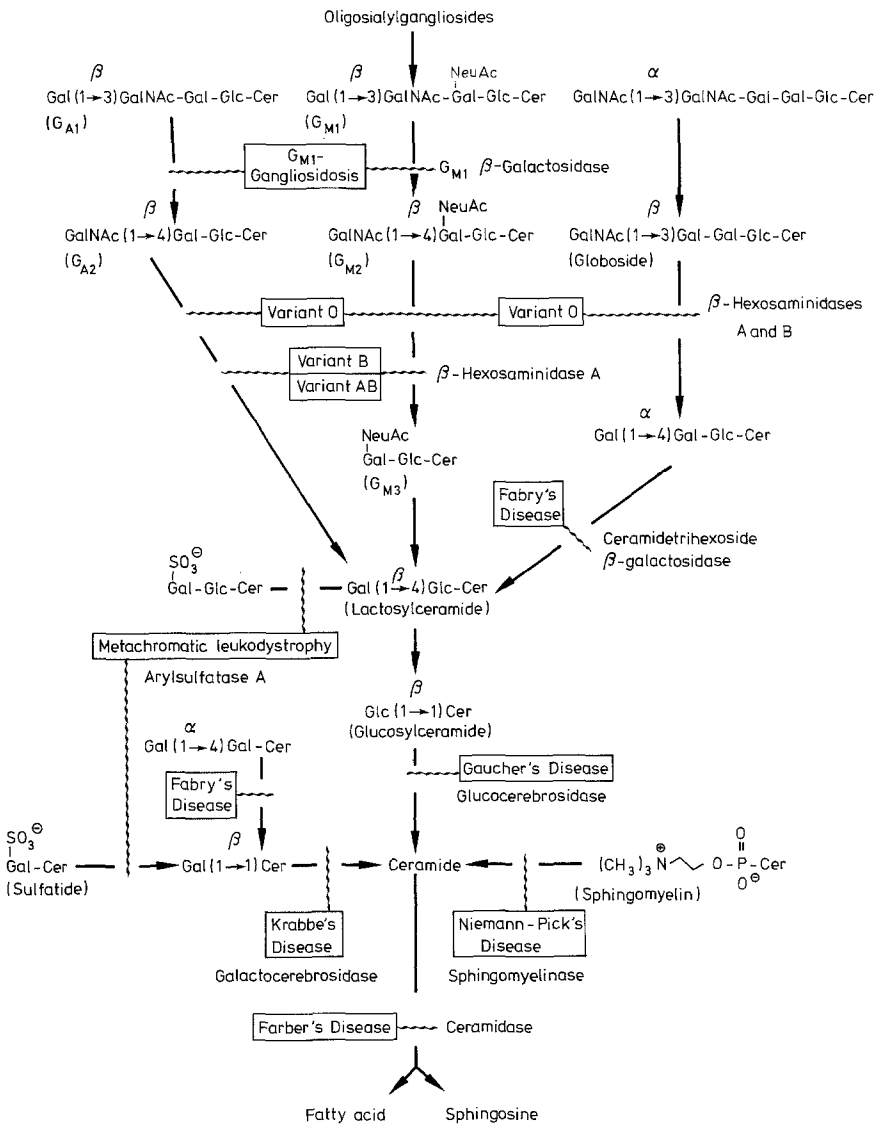

Fig. 1. Degradation scheme of sphingolipids denoting metabolic blocks of known diseases. For variants AB, B, and 0 see Abbreviations

mutations in the population should result in patients being 'compound heterozygotes' more often than homozygotes for a defective structural gene (Fig. 2). Thus most patients who are defective in a monomeric enzyme (as suggested for ganglioside G_{M1}-β-galactosidase (Norden et al., 1974) are heterozygotes with two different abnormal alleles and will therefore presumably produce a mixture of defective enzyme proteins. The situation might be even more complicated when homo- and heteropolymeric enzymes are involved. In this case (as will be discussed for G_{M2}-gangliosidosis) patients and even carriers of the disease may

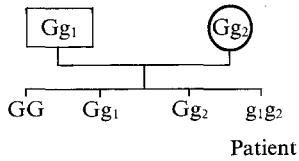

Fig. 2. Patient as compound heterozygote, being defective in a monomeric enzyme. G, wild-type allele for a monomeric enzyme; g_1, defective allele mutated at one site; g_2, defective allele mutated at another site. In the special case with identical mutations in g_1 and g_2, a homozygous patient, in the classic sense, would result.

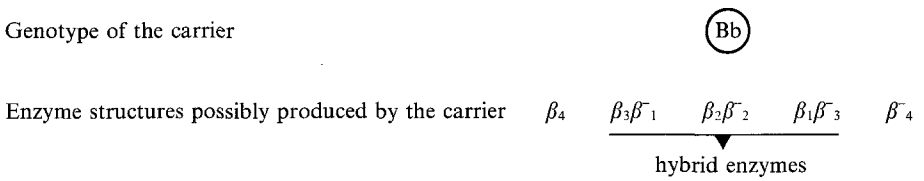

Fig. 3. Formation of hybrid enzymes in heterozygotes. Exemplified for an enzyme composed of four identical subunits β in an heterozygote carrying one defective allele resulting in the production of β^-. B, wild-type allele resulting in the production of normal polypeptide chain β; b, mutant allele resulting in the production of defective polypeptide chain β^-

have hybrid enzymes (Fig. 3); the carrier enzymes may contain mutated and normal polypeptides. This should result in the production in carriers of abnormal enzyme proteins. Further, the discussion of G_{M2}-gangliosidosis will emphasize the significance of isoenzymes for the development of different types of a disease and the importance of specific activator proteins allowing the interaction between micelle-forming glycolipid substrates and water soluble hydrolases.

G_{M1}-Gangliosidosis

A number of reviews are available on the biochemical and genetic aspects of G_{M1}- and G_{M2}-gangliosidosis (Neufeld et al., 1975; O'Brien, 1975, 1977, 1978; Volk and Schneck, 1975, 1976; Svennerholm, 1976a; Harzer and Benz, 1976; Sandhoff, 1977; Ho et al., 1977; Pentchev and Barranger, 1978; Robinson, 1978; Brady, 1978; Adachi et al., 1978). Recent biochemical and genetic findings have made major contributions to our knowledge of the gangliosidoses. Still, despite intensive research, major controversies remain unresolved. The pathologic accumulation of ganglioside G_{M1} and its asialo derivative G_{A1} was first recognized in a case of late infantile amaurotic idiocy (Jatzkewitz and Sandhoff, 1963). So far, a number of widely differing phenotypes of G_{M1}-gangliosidosis have been described in the literature, all of which are characterized by a deficiency of the degrading enzyme, ganglioside G_{M1}-β-galactosidase. In the more severe types I and II, the additional accumulation of a keratan-like material and of oligosaccharides and glycopeptides has been shown (Wolfe et al., 1974; Tsay et al., 1975; Tsay and Dawson, 1976; Lundblad et al.,

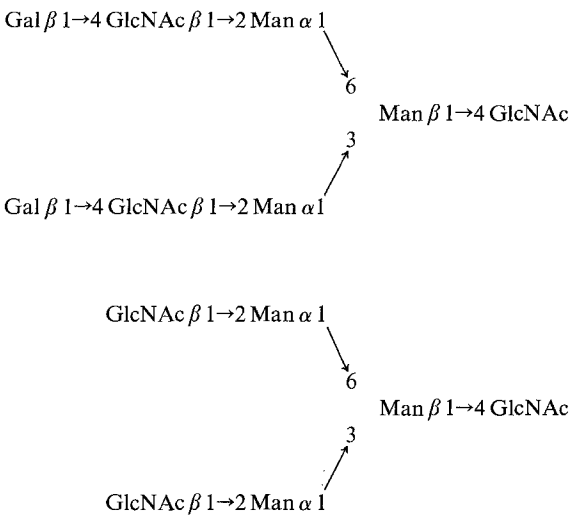

Fig. 4. Structures of two oligosaccharides stored and excreted by patients with G_{M1}-gangliosidosis *(above)* and with G_{M2}-gangliosidosis, Variant 0 *(below)*. (Data from Tsay et al., 1975; Wolfe et al., 1974; Tsay and Dawson, 1976; Lundblad et al., 1978; Strecker et al., 1977)

1978) (Fig. 4), which contain terminal galactose moieties bound by means of β-glycosidic linkages. These results imply that the degradation of such substances also requires the action of the missing enzyme. The enormous storage of ganglioside G_{M1}, above all in the neurons, leads to progressive damage of the nervous system, while the deposition of acid mucopolysaccharides, oligosaccharides, and glycopeptides causes enlargement of the liver and spleen and also changes in the bones.

1. Clinical Symptomatology

Patients with G_{M1}-gangliosidosis type I usually manifest symptoms during the neonatal period, have coarse facial features, visceromegaly, dysostosis multiplex, and progressive mental and motor retardation resulting in death by the age of 2 years (O'Brien, 1969, 1972). Cherry-red spots in the fundi of the eyes are present in about half the patients. Clonic-tonic convulsions are frequently observed. Ganglioside G_{M1}-β-galactosidase activity in tissues and in cells from patients varies from less than 1% to 5% of control values (Pinsky et al., 1974). While ganglioside G_{M1} is normally present in traces in liver and spleen it accounts for as much as 2% of the total lipids in G_{M1}-gangliosidosis (Robinson, 1978) and may comprise 70% of the total amount of ganglioside in the brain and 4% of the brain dry weight in this condition (Sandhoff et al., 1971).

In G_{M1}-gangliosidosis type II, mental and motor development is normal during the first year of life (O'Brien et al., 1972). Locomotor ataxia and muscular hypotonia are early symptoms. Progressive spasticity and seizures develop, and death occurs within 10 years. The viscera are not significantly enlarged and only mild bone changes are observed. Unlike the infantile form there is no macular

cherry-red spot. Besides the two classic G_{M1}-gangliosidosis phenotypes, numerous clinical presentations of other unusual variants with β-galactosidase deficiency exist, as described by Goldberg et al. (1971), Orii et al. (1972), Lowden et al. (1974), Pinsky et al. (1974), Yamamoto et al. (1974), Wenger et al. (1974a), Koster et al. (1976), Atsumi et al. (1976), O'Brien et al. (1976), Svennerholm (1976a), Suzuki et al. (1977), Stevenson et al. (1978), and Andria et al. (1978). These various phenotypes differ in the degree of intellectual deficit and skeletal involvement, age of onset, and course of the disease. At present it seems impossible to classify these heterogeneous cases of G_{M1}-gangliosidosis only on the basis of the clinical phenotype, since the number of patients described is still very small. More knowledge of the mutations underlying different β-galactosidase deficiencies in clinical variants of G_{M1}-gangliosidosis will permit a better understanding of their pathology.

2. *Biochemistry*

a) Subunit Structure of Ganglioside G_{M1}-galactosidase. β-Galactosidase exists in acid, heat-labile forms active at pH 4 to 5, and neutral ones that are less heat-labile and are active above pH 5 (for literature see Ho et al., 1977). The major acid form, referred to as G_{M1}-β-galactosidase A_1, has been purified from human liver and studied in detail (O'Brien, 1975; Miller et al., 1976). The purified enzyme is a monomeric protein with a molecular weight of 65,000 (Norden et al., 1974; Frost et al., 1978). Besides ganglioside G_{M1}-β-galactosidase A_1, another acid form of G_{M1}-β-galactosidase, A_3, is also deficient in G_{M1}-gangliosidosis. Apparently this enzyme is a multimeric aggregate of the A_1 enzyme (O'Brien, 1975; Frost et al., 1978); under appropriate conditions it can be transformed into the A_1 enzyme (O'Brien and Norden, 1977) and it precipitates with monospecific antisera against the A_1 enzyme (Norden et al., 1974). Recent studies (Frost et al., 1978; H. Galjaard et al., personal communication, 1979) with extracts of human liver and fibroblasts have revealed in addition a dimeric β-galactosidase, A_2, being the predominant and presumably enzymatically active form under the assay conditions at pH 4.5. Under these conditions both enzymes, A_1 and A_3, are converted to the dimeric form, exhibit a rather similar substrate specificity, and are activated by chloride ions.

b) Substrate Specificity of Ganglioside G_{M1}-β-galactosidase. The specificity of acid β-galactosidases for the multiplicity of putative substrates has not been fully investigated. Purified ganglioside G_{M1}-β-galactosidase is optimally active over an acidic pH range between 4 and 5, where the dimeric form prevails. It splits water-soluble β-galactosides such as PNP-β-Gal, MUF-β-Gal, lactose, and asialofetuin, but also MUF-α-L-arabinose and PNP-β-D-fucose (Norden et al., 1974). Its activity on amphiphilic substrates like ganglioside G_{M1} (Norden et al., 1974) and lactosylceramide (Tanaka and Suzuki, 1975) is negligible unless the appropriate bile salts, such as sodium taurode oxycholate are added to the incubation mixture.

It still remains unclear how the defect of a monomeric or homopolymeric enzyme, such as ganglioside G_{M1}-β-galactosidase, results in a variety of pheno-

typically different diseases that are characterized by different degrees of accumulation of the respective putative substrates, glycolipids, oligosaccharides, and glycopeptides. It has been suggested (O'Brien, 1975; O'Brien and Norden, 1977) that different allelic mutations of ganglioside G_{M1}-β-galactosidase produce abnormal enzyme proteins with different degrees of residual activity against the various substrates. A patient affected with G_{M1}-gangliosidosis may either be homozygous for a defective enzyme having a minor residual activity and a changed substrate specificity, or he may be a compound heterozygote producing two different defective enzyme proteins each low in residual activity but differing in their substrate specificities. Both notions can be verified by a careful biochemical analysis of the patient's enzymes. As an example of an altered substrate specificity a patient may be cited who showed only residual G_{M1}-β-galactosidase activity, but in whom the ratio of the activities measured against the lipid substrate, ganglioside G_{M1}, and the glycoprotein, asialofetuin, was five times higher than the norm (O'Brien et al., 1976). Correspondingly, the nervous system was only slightly affected. In another patient a mutant enzyme was demonstrated by an altered electrophoretic mobility (Norden and O'Brien, 1975). But the notion of allelic mutation cannot explain all the findings at present. So far no quantitative correlation between the extent of enzyme deficiency, the extent of storage, and the onset of the disease could be established. For example, Stevenson et al. (1978) published data on three patients aged from 27 to 39 years of age, who had only a minor residual activity of G_{M1}-β-galactosidase in fibroblasts, which is typical for the infantile form of G_{M1}-gangliosidosis. One can imagine, of course, that the in vivo activity of the enzyme might be even higher in some or all tissues than the activity measured in fibroblasts with the in vitro assays available. For example, the stability of an altered enzyme might be higher in vivo than in vitro. Furthermore, the in vitro assays may fail to some extent to represent the real, in vivo activity of a mutant enzyme, which might be enhanced by special factors not present in the in vitro assay.

The fundamental question as to the relevance of in vitro activities obtained with different lipid substrates in the presence of detergents for the in vivo specificity of the enzymes is still open. The controversy on the lactosylceramide-β-galactosidase activity of two different β-galactosidases clearly demonstrates that the substrate specificity of lipid hydrolases is greatly influenced by the detergents used, which are, of course, not present in the lysosomes of the living cells. Thus, Suzuki and Suzuki (1974a and b) showed that ganglioside G_{M1}-β-galactosidase, and not galactosylceramide-β-galactosidase, cleaves lactosylceramide in the presence of crude sodium taurocholate. In contrast, Wenger et al. (1974b) found that galactosylceramide-β-galactosidase, and not ganglioside G_{M1}-β-galactosidase, hydrolyzed lactosylceramide when pure sodium taurocholate and oleic acid were used as detergents. Tanaka and Suzuki (1975) resolved this controversy by demonstrating that both enzymes can hydrolyze lactosylceramide but require different incubation conditions, particularly in the presence of different types of bile acids, to expose their activity on lactosylceramide. The question as to which enzyme hydrolyzes lactosylceramide in vivo cannot be answered until enzyme specificities can be determined under conditions representative of the in vivo situation.

In recent years it has become clear that there are proteins in vivo that act like the detergents in the in vitro assay and facilitate the interaction between lipid substrate and water-soluble lipid hydrolase. Li, S. C., et al. (1973) and Li, Y.-T., et al. (1974) have isolated an activator protein, which stimulates the hydrolysis of ganglioside G_{M1} by human β-galactosidase when present in stoichiometric amounts with the substrate. The factor has a rather broad specificity and also stimulates ceramide trihexoside α-galactosidase, ganglioside G_{M2}-β-D-*N*-acetyl-galactosaminidase, and sulfatide-sulfatase (G. Fischer and H. Jatzkewitz, personal communication). The significance of activator proteins for the degradation of glycolipids will be discussed below (see G_{M2}-gangliosidosis).

A correlation between the extent of enzyme deficiency and the clinical course of the disease might be expected from determinations of enzyme activities performed with natural substrates in living cells under conditions similar to the in vivo situation. By loading fibroblasts derived from various types of G_{M1}-gangliosidosis with labeled G_{M1} ganglioside, Suzuki et al. (1978) obtained a good correlation between the rate of ganglioside degradation and the onset of the disease. A similar correlation between the extent of enzyme deficiency and the clinical onset of the disease had been obtained previously by determination of sulfatide degradation by sulfatide ^{35}S-loaded fibroblasts derived from different forms of metachromatic leukodystrophy (Kihara et al., 1973; Percy et al., 1977). An obligate heterozygote of metachromatic leukodystrophy exhibited arylsulfatase A deficiency in a fibroblast extract but showed essentially normal sulfatide clearance after loading of fibroblasts in culture with cerebroside sulfate (Fluharty et al., 1978).

c) Other β-Galactosidases. Neutral β-galactosidases—optimally active at pH 6.2 (Ben-Yoseph et al., 1977), inhibited by chloride ions, and heat-stable—do not split galactose from ganglioside G_{M1} or from asialofetuin (Norden and O'Brien, 1973; Ho et al., 1973) but, in contrast to ganglioside G_{M1}-galactosidase, cleave water-soluble aryl-β-D-galactosides and aryl-β-D-glucosides (Ho and O'Brien, 1970) as well as lactosylceramide (Ben-Yoseph et al., 1977). Immunologic studies have indicated that neutral β-galactosidases do not share a polypeptide with G_{M1}-β-galactosidase (Ben-Yoseph et al., 1977). Whereas Cheetham et al. (1978) described a widespread deficiency of neutral β-galactosidase assayed with a fluorogenic substrate in the human population without clinical and biochemical disturbances, in the case of lactosylceramidosis Nadler and co-workers (Burton et al., 1978) found a severe defect of the enzyme activity, which they regard as the primary defect of the disease. This enzyme deficiency was observed with fluorogenic substrate and with lactosylceramide. In human tissues there is another rather specific β-galactosidase, which cleaves galactose from galactosylceramide and galactosylsphingosine only. This enzyme is deficient in Krabbe's disease (Suzuki, K., and Suzuki, Y., 1970), but the mutation is not allelic with that in G_{M1}-gangliosidosis.

3. Genetic Studies

The notion of allelic mutation can hardly be reconciled with the results of genetic complementation studies performed between fibroblasts with various types of

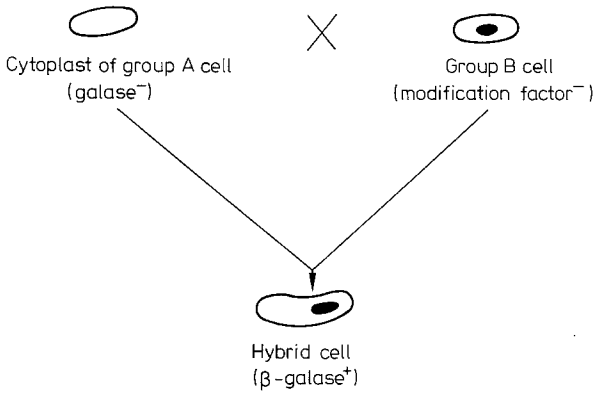

Fig. 5. Intergenic complementation groups in different variants of β-galactosidase deficiency. Model of Bootsma and Galjaard (1979) assumes that group A cells are defective in the structural gene of ganglioside G_{M1} β-galactosidase but still contain a modification factor in their cytoplasm necessary for the processing of the (presumably inactive) primary gene product. Group B cells are thought to be defective in this factor. Hybrids between cytoplasts of group A and cells of group B show intergenic complementation for ganglioside G_{M1} β-galactosidase activity.

Group A (galase⁻)	*Group B (modification factor⁻)*
Type 1	Type 3 (Pinski)
Type 2	Type 4
Lowden's variant	Andria's Italian variant
O'Brien's electrophoretic variant	Wenger's adult variant
Svennerholm's Scandinavian variant	

G_{M1}-gangliosidosis, all of which have been shown by immunologic methods to be structural gene mutations of the mono- or homopolymeric G_{M1}-β-galactosidase (Meisler and Rattazzi, 1974; O'Brien, 1975). Whereas cell hybrids between classic types I and II of G_{M1}-gangliosidosis did not result in any restoration of β-galactosidase activity, as expected, fusion of type I cells with type IV cells resulted in higher activities in 50% of the hybridized cells after fusion than in any of the parental cells before fusion (Galjaard et al., 1975). This shows a restoration of the β-galactosidase by genetic complementation, and suggests that the lesions in type I and IV are located in two different cistrons and that β-galactosidase, as tested with natural and synthetic substrates, needs at least two different polypeptide chains to express full activity. These studies have been extended to other variants of G_{M1}-gangliosidosis. Two intergenic complementation groups A and B have been distinguished (see Fig. 5) (Bootsma and Galjaard, 1979; Hoeksema et al., 1979; de Wit-Verbeek et al., 1978). Complementation is also obtained when cytoplasts of group A are fused with nucleated cells of group B. Fusion of nucleated cells of group A with cytoplasts of group B yielded no complementation indicating that the nucleus of group B is mandatory. Galjaard and coworkers (Bootsma and Galjaard, 1979; de Wit-Verbeek et al., 1978) proposed an intergenic complementation model, assuming that the enzyme defect in group A results from allelic mutations in the structural gene for ganglioside G_{M1}-β-galactosidase but that these cells still produce a modification factor present in the

cytoplasm and necessary for processing the presumably inactive primary gene product. On the other hand, group B cells contain a normal structural ganglioside G_{M1}-β-galactosidase gene but a second gene product, the modification factor, which is still present in the cytoplasm 2 days after enucleation is defective. Occurrence of a simultaneous neuraminidase and β-galactosidase deficiency in one patient raises the possibility that neuraminidase may be part of the modification factor (Wenger et al., 1978).

Contrary to an earlier assignment (Rushton and Dawson, 1977) of β-galactosidase activity to human chromosome 12, recent interspecies cell hybridization studies showed two loci for β-galactosidase activity on chromosome 22 (de Witt et al., 1977) and 3 (Bootsma and Galjaard, 1979), presumably one for the structural gene and the other for the modification factor mentioned above. However, in view of the methodological problems associated with such studies (see p. 000), alternative interpretations cannot be excluded; for example, there may be two structurally similar isoenzymes of β-galactosidase.

4. Animal Models

Mutants with β-galactosidase deficiency and G_{M1}-gangliosidosis have been reported in cats (Baker et al., 1971; Handa and Yamakawa, 1971; Cheetham et al., 1974; Blakemore, 1972; Farrell et al., 1973; Baker and Lindsey, 1974; Baker et al., 1976), in a 9-month-old dog (Read et al., 1976), and in calves (Donnelly et al., 1973). Clinical, morphologic and biochemical similarities appear to exist between G_{M1}-gangliosidosis in man and the disease in animals. Residual liver acid β-galactosidase from a case of feline G_{M1}-gangliosidosis showed an altered substrate specificity, an increased thermolability, and reduced molecular weight, and appeared to be genetically different from the normal enzyme (Holmes and O'Brien, 1978). The animal cases may be of value for study of the correlation between ganglioside accumulation in neurons and impairment of neurologic function. Furthermore, morphologic studies on mature cortical neurons in feline G_{M1}-gangliosidosis (Purpura and Baker, 1976, 1977; Purpura et al., 1978) suggest a role for gangliosides in neurite formation and synaptogenesis.

G_{M2}-Gangliosidosis

The genetic and biochemical background of some rare variants of G_{M2}-gangliosidosis still remains obscure, although a basic understanding of the classic forms has already been achieved. The prototype of this disorder is Tay-Sachs disease (variant B of infantile G_{M2}-gangliosidosis), in which ganglioside G_{M2} is the major storage compound in the nervous tissue (Svennerholm, 1962; Ledeen and Salsman, 1965). Its accumulation is correlated with a deficiency of hexosaminidase A (Okada and O'Brien, 1969; Sandhoff, 1969). In human tissue, three, presumably lysosomal, hexosaminidases can be distinguished: the main isoenzymes, A and B (Robinson and Stirling, 1968; Sandhoff, 1968) and the minor one, S (Sandhoff, 1969; Beutler et al., 1975a; Ikonne et al., 1975). In addition, a microsomal glucosaminidase (called hexosaminidase C) (Hooghwinkel et al.,

1972; Poenaru et al., 1973; Poenaru and Dreyfus, 1973; Braidman et al., 1974a and b) and three serum enzymes I_1, I_2 (Price and Dance, 1972), and P have been identified (Stirling, 1971, 1972).

1. Biochemistry

a) Subunit Structure of Hexosaminidases A and B. On the basis of chemical and immunologic analysis (Geiger and Arnon, 1976; Lee and Yoshida, 1976; Beutler et al., 1976), somatic cell hybridization studies (Lalley et al., 1974; Gilbert et al., 1974, 1975; Thomas et al., 1974), and conversion experiments (Sandhoff, 1973; Carmody and Rattazzi, 1974; Beutler et al., 1975b) a molecular model was proposed for hexosaminidases A and B (Geiger and Arnon, 1976; Lee and Yoshida, 1976; Beutler et al., 1976) (Table 1). According to this model, hexosaminidase B is a homopolymer, $\beta_2\beta_2$, built of two subunits, β_2, each of which is

Table 1. Comparison of *N*-acetyl-β-D-hexosaminidases A and B

Property	Hexosaminidase A	Hexosaminidase B	References
Isoelectric point	5.0	7.3	Sandhoff, 1968
Stability at 50° C	−	+	O'Brien, 1973
Precipitation with antibodies to hexosaminidase B	+	+	Carroll and Robinson, 1973; Beutler and Kuhl, 1975; Geiger et al., 1975
Precipitation with antibodies to hexosaminidase A	+	+	Carroll and Robinson, 1973; Beutler and Kuhl, 1975; Geiger et al., 1975
Precipitation with specific antibodies to hexosaminidase A[a]	+	−	Beutler and Kuhl, 1975; Geiger et al., 1975
Molecular weight	100,000	100,000	Geiger and Arnon, 1976
Proposed composition	$\alpha_2\beta_2$	$\beta_2\beta_2$	Geiger and Arnon, 1976
Activity in variant 0	−	−	Sandhoff and Harzer, 1973; Sandhoff et al., 1968; Sandhoff et al., 1971
Activity in variant B	−	+	Sandhoff, 1969; Okada and O'Brien, 1969; Hultberg, 1969

Hydrolysis of	K_m (mmol/liter)	V_{max} (μmol · min^{-1} · mg^{-1})	K_m (mmol/liter)	V_{max} (μmol · min^{-1} · mg^{-1})	
MUF-β-GalNAc	0.09	14	0.05	35	Sandhoff et al., 1977
MUF-β-GlcNAc	0.8	160	0.6	280	Sandhoff et al., 1977

[a] Antibodies to hexosaminidase A are freed from cross-reacting material by absorption with Sepharose-bound hexosaminidase B

composed of two identical, S-S linked β-chains. Hexosaminidase A is a heteropolymer, $\alpha_2\beta_2$, sharing one subunit with the B enzyme and containing a specific one, α_2, composed of two identical S-S linked α-chains (Geiger and Arnon, 1976). The minor hexosaminidase S is presumably a homopolymer with the structure $\alpha_2\alpha_2$ (Beutler et al., 1975a; Geiger et al., 1977a).

The model is in accordance with conversion studies and immunologic properties of the enzyme. Purified hexosaminidase A can be converted to hexosaminidase B under mild conditions (Sandhoff, 1973). This conversion is stimulated by merthiolate (Carmody and Rattazzi, 1974; Beutler et al., 1975b). In the reverse process, hexosaminidase B can be obtained from a mixture of hexosaminidases A and S under proper conditions (Beutler and Kuhl, 1975). Immunologic data indicate a common antigenic site (α_2) for hexosaminidases A and S (Geiger et al., 1977a) and another one (β_2) for hexosaminidases A and B (Carroll and Robinson, 1973; Srivastava and Beutler, 1974; Bartholomew and Rattazzi, 1974; Ben-Yoseph et al., 1975); on the other hand the homopolymers hexosaminidase S and hexosaminidase B cross-react only weakly with antisera raised against hexosaminidase B and hexosaminidase S respectively (Geiger et al., 1977a). This has been interpreted as meaning that the enzymes do not share a common subunit but that their subunits, β_2 and α_2, respectively, exhibit immunologic similarities. Both subunits are presumably derived from a common ancestor, since they are rather similar in size and aminoacid composition (Geiger and Arnon, 1976).

b) Substrate Specificity of Hexosaminidases A and B. The main isoenzymes, A and B, have been purified to apparent homogeneity (Srivastava et al., 1974; Geiger et al., 1974; Lee and Yoshida, 1976; Sandhoff et al., 1977); hexosaminidase S has been enriched several thousand-fold (Geiger et al., 1977a). Both isoenzymes, hexosaminidase A and hexosaminidase B (as well as the minor component hexosaminidase S), are active on a variety of water-soluble β-D-galactosaminides and -glucosaminides (Robinson and Stirling, 1968; Sandhoff, 1968; Sandhoff and Wässle, 1971; Geiger et al., 1977a), including oligosaccharides (Bearpark et al., 1977) [which also accumulate in G_{M2}-gangliosidosis (Cantz and Kresse, 1974; Strecker et al., 1977)], and sterol β-*N*-acetyl-glucosaminides (Tomasi et al., 1974). Hexosaminidase A preferentially cleaves *N*-acetylgalactosamine from a heptasaccharide derived from chondroitinsulfate (Thompson et al., 1973) and *N*-acetylglucosaminide or *N*-acetylgalactosaminide from trisaccharides isolated from hyaluronic acid and desulfated chondroitin 4-sulfate, both of which contain a subterminal negatively charged sugar (Werries et al., 1975; Bearpark and Stirling, 1978).

None of the purified enzymes, however, hydrolyzes the accumulating lipids, e.g., the ganglioside G_{M2}, to any significant extent (Sandhoff and Wässle, 1971; Li, S. C., et al., 1973; Li, Y.-T., et al., 1974; Srivastava et al., 1974; Tallman et al., 1974b; Sandhoff et al., 1977). This situation is typical of a general problem in lipid enzymology that has already been mentioned for the interaction between ganglioside G_{M1} and ganglioside G_{M1}-β-galactosidase. In aqueous solutions glycolipids form large, tightly packed aggregates called micelles, which render lipid molecules more or less inaccessible to water-soluble, catabolic hydrolases.

Only the rather small equilibrium concentration of monomers can be degraded. The in vitro degradation is greatly enhanced by the addition of suitable detergents, resulting in the formation of small mixed micelles, from which the oligosaccharide chains of the glycolipids protrude far enough to be attacked by the glycosidases. In the presence of 2 m*M* sodium taurodeoxycholate hexosaminidases A, B and S are active on glycolipid G_{A2} and globoside (Sandhoff and Wässle, 1971; Sandhoff et al., 1977; Geiger et al., 1977a); ganglioside G_{M2} is split by hexosaminidase A six times faster than by hexosaminidase B (Sandhoff et al., 1977). The minor isoenzyme hexosaminidase S also splits G_{M2} (Geiger et al., 1977a).

In vivo, lipids are incorporated into membranes, where they are also hardly accessible. In the membranes, activation might be accomplished by naturally occurring nonenzymic proteins, which have recently been demonstrated for a number of hydrolases (Mehl and Jatzkewitz, 1964; Ho and O'Brien, 1971; Li, S. C., et al., 1973; Li, Y.-T., et al., 1974; Li and Li, 1976; Mraz et al., 1976; Hechtman, 1977; Hechtman and Le Blanc, 1977; Fischer and Jatzkewitz, 1977, 1978). A similar activator protein can also be shown to exist for the degradation of ganglioside G_{M2} and glycolipid G_{A2} by hexosaminidase A (Conzelmann and Sandhoff, 1978). The activator shows a clear specificity for the A isoenzyme. Hexosaminidase B does not attack the glycolipids even in the presence of sufficiently high amounts of the activator protein. A similar isoenzyme specificity can, however, be shown for the ceramide-free residue of ganglioside G_{M2}, Des-G_{M2} (Sandhoff et al., 1977). This water-soluble derivative is hydrolyzed by the A isoenzyme 150-fold faster than by the B enzyme in the absence of activator or detergents. The degradation of water-soluble synthetic substrates such as MUF-β-GalNAc is not affected by the activator, indicating that it does not act upon the enzyme. It seems to function in a similar way to the activator for the degradation of sulfatides, which has been studied in detail by Fischer and Jatzkewitz (1978). This activator protein binds to the lipid substrate, thus solubilizing it, the resulting complex being the true substrate for the degrading enzyme. These activator proteins, like the detergent, sodium taurocholate, act primarily on the lipid substrates. But in contrast to the activator, the detergent forms small, mixed micelles with the glycolipid which can be attacked by the degrading enzyme. In the presence of sodium taurodeoxycholate, however, hexosaminidases A and B exhibit substrate specificities which differ from those seen in the presence of activator (see Table 2).

Whether or not human hexosaminidase A is homogeneous in its capability to degrade ganglioside G_{M2} is still an open question. Bach and Suzuki (1975) found that only the acidic slope of the crude, liver hexosaminidase A peak (isoelectric point at pH 5.0—5.1) obtained after isoelectric focusing is active on ganglioside G_{M2}. The G_{M2}-ganglioside hydrolyzing activity had its peak at an isoelectric point of pH 4.8—4.9, which coincides with that of the activator protein mentioned above at pH 4.7—4.8 (Conzelmann and Sandhoff, unpublished). This activator stimulates the hexosaminidase A-catalyzed degradation of G_{M2}-ganglioside (Conzelmann and Sandhoff, 1978) in the absence of detergents. Therefore, it appears reasonable that G_{M2}-ganglioside-hydrolyzing activity was found mainly in those fractions that contain both hexosaminidase A and activator protein. On the other

Table 2. Degradation of glycosphingolipids by hexosaminidases A and B in the presence and absence of stimulating factors

Lipid substrate	Hexosaminidase	Additions		
		–	Sodium taurodeoxycholate (2 m*M*)[a]	Activator[b]
			(μmol/h · mg)	
Ganglioside G_{M2}-^{3}H	A	0.009	0.40	0.97
	B	0.001	0.07	0.005
Glycolipid G_{A2}-^{3}H	A	0.008	6.3	2.48
	B	0.001	30.7	0.072
Globoside-^{3}H	A	0.004	14.4	0.42
	B	0.005	24.0	0.005

[a] This concentration of detergent leads to a time-dependent inactivation of the enzyme, so that the degradation rate measured depends very much upon the incubation times. Values given were obtained with short incubation times (< 1 h) (For methodology see Sandhoff et al., 1977). A similar situation has been described for α-galactosidase A (Schram et al., 1979)

[b] Under these conditions in the presence of a partially purified activator preparation (Conzelmann and Sandhoff, unpublished) the reaction rate depends on the amount of activator protein added, which was still not saturating in this case

hand, the peak of glycolipid G_{A2}-hydrolyzing activity did not differ from the hexosaminidase A peak, but coincided with it. The reason for this might be that activity against glycolipid G_{A2} was assayed in the presence of detergent, which substitutes in vitro for the function of the activator protein (Conzelmann and Sandhoff, 1978).

From human liver, Hechtman (1977) and Hechtman and Le Blanc (1977) isolated an activator protein enhancing the degradation of ganglioside G_{M2} by hexosaminidase A, which differed from the one mentioned above. The apparent molecular weight was higher (36,000—39,000 instead of around 25,000), and its specificity differed in that it did not stimulate the hydrolysis of glycolipid G_{A2} by hexosaminidase A; furthermore, the authors presented evidence in favor of the formation of an enzyme-activator complex rather than an activator-substrate complex. In view of the different properties, and of the fact that a deficiency of the activator protein mentioned before results in storage of glycolipids G_{M2} and G_{A2} in the AB variant (Conzelmann and Sandhoff, 1978), as discussed below, the function of the activator specific for ganglioside G_{M2} remains to be clarified.

c) Other Hexosaminidases. The microsomal glucosaminidase, referred to as hexosaminidase C, has a molecular weight greater than 200,000 and is active on MUF-β-GlcNAc with a pH optimum of 6—7 (Poenaru et al., 1973; Poenaru and Dreyfus, 1973; Braidman et al., 1974a and b). It does not split MUF-β-GalNAc, and remains active in G_{M2}-gangliosidosis. No natural substrate has so far been identified for this enzyme. Genetic and immunologic evidence indicates that this enzyme does not share a polypeptide with the acidic hexosaminidases A and B.

In serum, additional hexosaminidases, I_1 and I_2 (Price and Dance, 1972), and hexosaminidase P (Stirling, 1971, 1972), have been identified, which are active on

MUF-β-GlcNAc and MUF-β-GalNAc at an acidic pH (around 4.5). Natural substrates for these enzymes have not yet been found. The P enzyme increases during pregnancy and has a molecular weight of around 150,000. Immunologic evidence indicates that the P isoenzyme has an antigenic specificity identical to hexosaminidase B but does not contain A-specific antigenic determinants (Geiger et al., 1978a).

2. *Variants of Infantile G_{M2}-Gangliosidosis*

Biochemically three distinct forms of infantile G_{M2}-gangliosidosis (Fig. 6) can be distinguished (Sandhoff, 1969; Sandhoff et al., 1971):

a) Variant B (Tay-Sachs disease), caused by a complete lack of hexosaminidase A (Okada and O'Brien, 1969; Sandhoff, 1969) (and of the minor isoenzyme hexosaminidase S). The defect results mainly in neuronal storage of G_{M2} and to a lesser degree of G_{A2} (Svennerholm, 1962; Sandhoff et al., 1971). A minor accumulation of oligosaccharides, glycopeptides (Brunngraber et al., 1972, 1974), and other glycolipids (Iwamori and Nagai, 1978, 1979) containing terminal β-glycosidically bound *N*-acetylgalactosamine residues, such as gangliosides G_{D1a}-GAN and G_{D2}, has also been demonstrated. Immunologic studies with antisera specific against hexosaminidase A have so far failed to demonstrate the existence of enzymically inactive cross-reactive material (Carroll and Robinson, 1973; Srivastava and Beutler, 1974; Bartholomew and Rattazzi, 1974; Geiger et al., 1975). On the other hand, low-molecular-weight protein (mol. wt. around 20,000) cross-reacting with hexosaminidases A and B was identified in variants B and 0, presumably representing a common subunit of both hexosaminidases (Carroll and Robinson, 1974). In a recent study (Srivastava and Ansari, 1978), evidence for cross-reactive material was presented, presumably representing free defective α-chains, by the use of specific antibodies against glutaraldehyde cross-linked hexosaminidase A. Subunit structure of hexosaminidases and hybridization experiments (see below) indicate, for variant B, a mutation in the α-chain that is common to hexosaminidases A and S and does not affect hexosaminidase B, the level of which is normal in most organs and elevated up to six-fold in nervous tissue (Sandhoff, 1969; Okada and O'Brien, 1969; Sandhoff et al., 1971). However, in a $2\frac{1}{2}$-year-old child with hexosaminidase A deficiency, heat-lability of serum hexosaminidase B was increased (Momoi et al., 1978). This child's condition is probably due to a mutation of the β-chain, which would be allelic to variant 0 (see below), resulting in this particular case in defective hexosaminidase A and altered hexosaminidase B.

b) Patients with variant 0 are defective in isoenzymes hexosaminidase A and hexosaminidase B, and store globoside in extraneuronal tissues in addition to a neuronal storage of glycolipids G_{M2} and G_{A2} (Sandhoff et al., 1968; Sandhoff et al., 1971; Krivit et al., 1972; Okada et al., 1972; Sandhoff and Harzer, 1973). Furthermore, oligosaccharides and glycopeptides accumulate in various tissues (Cantz and Kresse, 1974; Brunngraber et al., 1974; Berra and Brunngraber, 1977), all of which are characterized by terminal *N*-acetylhexosamines β-glycosidically bound. Oligosaccharides are excreted in the urine (Strecker et al., 1977). Cross-reactive material to hexosaminidase A and B was identified in one patient

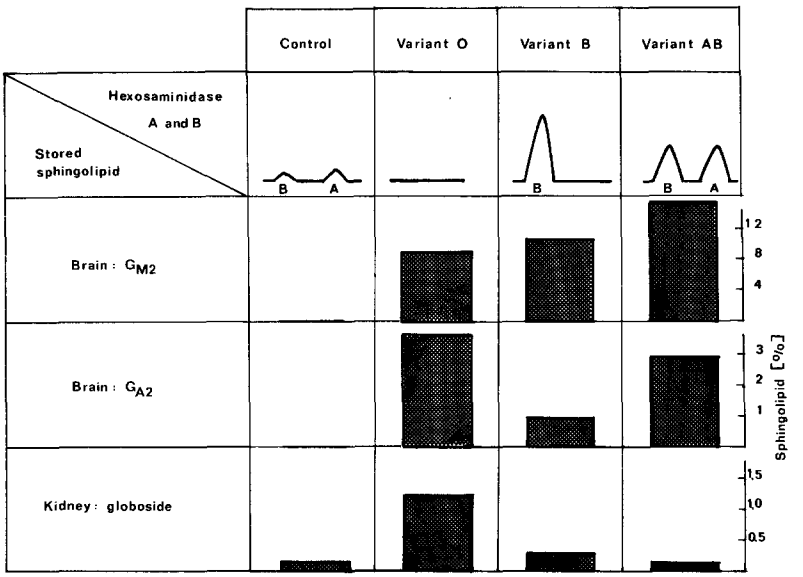

Fig. 6. Storage of glycolipids and hexosaminidase pattern in infantile G_{M2}-gangliosidosis. The pattern of hexosaminidase activities was measured with synthetic substrates after the isoelectric separation of isoenzymes A and B. The quantities of the stored sphingolipids are given as percentages of the dry weight of the storage organ. Sandhoff et al., 1971

(Srivastava and Beutler, 1973), but not in others (Carroll and Robinson, 1973; Geiger et al., 1977a). However, the recent finding of cross-reactive proteins in human liver with antibodies raised to apparently homogeneous preparations of hexosaminidases A and B (Carroll, 1978) casts some doubt on the nature of cross-reactive material found in the patient with variant 0, since some of these proteins were enzymically inactive and others were structurally unrelated to hexosaminidase. Hybridization studies yielded evidence for a mutation of the β-chain common to hexosaminidases A and B.

Residual hexosaminidase activity is mainly hexosaminidase S (Beutler et al., 1975a; Ikonne et al., 1975; Geiger et al., 1977a). In a black patient, Spence et al. (1974) found higher residual hexosaminidase activities in serum (20%—24%) and in fibroblasts and leukocytes (7%—11%) that were mostly heat-labile and therefore presumably the isozymes A and S.

The polymeric structure of hexosaminidases A and B theoretically allows for the appearance of hybrid enzymes in heterozygotes and in patients. A polymeric enzyme of a heterozygote might be composed of wild-type and mutant polypeptide chains, and that of a patient of two different mutated polypeptide chains. This notion suggests that heterozygotes may form hybrid enzymes (possibly as well as normal ones) with altered physical and kinetic parameters. Some evidence for this situation was recently presented in two families with variant 0 of infantile G_{M2}-gangliosidosis (Lane and Jenkins, 1978; Lowden, 1979). Hexosaminidase B of an obligate heterozygote was much more labile than the normal enzyme. The

authors suggested this enzyme to be a hybrid composed of wild-type and mutant β-chains.

c) The very rare variant AB exhibits an especially pronounced neuronal accumulation of glycosphingolipids G_{A2} and G_{M2}, even though no enzyme deficiency has yet been demonstrated (Sandhoff, 1969; Sandhoff et al., 1971; de Baecque et al., 1975; Brett et al., 1973). One patient's hexosaminidases A and B exhibited quite normal physical, kinetic and immunologic properties, and split the storage compounds in the presence of detergents (Conzelmann et al., 1978). Therefore the disease is not caused by an enzyme deficiency, like all the other storage diseases. It is rather due to a deficiency of the activator protein required for the normal degradation of ganglioside G_{M2} and its derivative G_{A2} (Conzelmann and Sandhoff, 1978). The disease demonstrates the importance of such activators for the normal interaction between lipid substrates and catabolizing hydrolases in lipid degradation.

The enormous increase in ganglioside G_{M2} caused by a deficiency of hexosaminidase A in variants B and 0, and by an activator deficiency in variant AB, leads to the destruction of nerve cells and to myelin degradation. The demyelinization is reflected in the lipid content of the diseased nervous tissue (Sandhoff et al., 1971). A drastic decrease of all typical myelin lipids, such as cerebrosides, sulfatides, and C_{24}-sphingomyelin, is observed. Similar secondary changes occur also in G_{M1}-gangliosidosis. Among secondary changes the increase of some lysosomal hydrolase activities is striking, e.g., β-glucosidase activity may increase up to ten-fold of the control level (Sandhoff et al., 1971).

3. Juvenile and Adult Forms of G_{M2}-Gangliosidosis

Besides the severe infantile G_{M2}-gangliosidoses, juvenile and adult forms (Jatzkewitz et al., 1965; Bernheimer and Seitelberger, 1968) have been described with a partial deficiency of hexosaminidase A (Suzuki, Y., and Suzuki, K., 1970; Suzuki et al., 1970; Okada et al., 1970; Buxton et al., 1972; Brett et al., 1973; Anderman et al., 1974; Rapin et al., 1976; Brandt et al., 1977) or hexosaminidases A and B (Spence et al, 1974; Wood and MacDougall, 1976; Johnson et al., 1977; Johnson and Chutorian, 1978; MacLeod et al., 1977; Goldie et al., 1977; Felding and Hultberg, 1978), respectively. In juvenile G_{M2}-gangliosidosis of variants B and 0, residual activities of hexosaminidase A or of hexosaminidase A and B, respectively, as measured with synthetic substrates may range from 5% up to 50% of control values for different patients. Quite variable residual activities can be obtained in serum and different tissues of the same patient. So far no correlation has been observed between extent of residual enzyme activity and extent of storage of glycolipids G_{M2} and G_{A2} (see discussion under G_{M1}-gangliosidosis). Some patients exhibit the enzyme levels typical for heterozygotes of the infantile diseases, while nevertheless suffering from fatal neuronal storage of ganglioside G_{M2}. In one case of juvenile G_{M2}-gangliosidosis, variant B, evidence for a mutant hexosaminidase A with a changed substrate specificity has been presented (Zerfowski and Sandhoff, 1974); the patient's enzyme was much more active on water-soluble substrates than on the stored lipid compounds G_{M2} and G_{A2} in the presence of detergents. Detailed studies on the hexosaminidases from other

juvenile and adult forms of G_{M2}-gangliosidosis are still lacking. Intergenic complementation studies showed that juvenile and infantile variant 0 are due to different allelic mutations, presumably both affecting the polypeptide chain β (Wood, 1978). Recently an adult onset form of AB variant has been described with storage of ganglioside G_{M2} and elevated activities of hexosaminidases A and B in cortical tissue (O'Neill et al., 1978).

4. Healthy Adults with Low Levels of Hexosaminidase Activity

In two families with variant B, obligate heterozygotes have been identified with rather low activity of hexosaminidase A on synthetic substrates in serum, leukocytes, and fibroblasts (Vidgoff et al., 1973; Navon et al., 1973, 1976). But their ganglioside G_{M2}-β-galactosaminidase activity in leukocytes was in the heterozygous range (Tallman et al., 1974a). It was proposed that these normal individuals are compound heterozygotes carrying the Tay-Sachs allele and a second allele whose gene product retains activity for ganglioside G_{M2} but lacks activity for synthetic substrates. Closely related findings have been obtained in two further adults (Kelly et al., 1976; Spence et al., 1977).

A similar situation has been described for a father of two children who died apparently with variant 0. A deficiency of hexosaminidases A and B was found in serum and leukocytes with synthetic substrates, whereas heterozygous activity levels were observed with glycolipid G_{A2} as substrate (Dreyfus et al., 1975, 1977). The authors suggest that the father is a compound heterozygote carrying the variant 0 gene and another allelic mutation whose gene product is inactive against synthetic but active against lipid substrates.

Enzyme levels tested with synthetic water-soluble substrates or with natural lipid substrates assayed in the presence of artificial detergents do not necessarily represent the in vivo activity of hexosaminidase isoenzymes. Heterozygotes may contain hybrid enzymes, as discussed in Figure 3, having a changed substrate specificity, e.g., their activity against synthetic substrates might be especially low. Their activity should be assayed under conditions similar to the in vivo situation, e.g., with lipid substrates in the presence of appropriate activator proteins instead of detergents. Furthermore, hybrid hexosaminidases, still active enough under in vivo conditions, might be more labile against proteases, extraction procedures, detergents, etc., and therefore escape proper determination in vitro.

5. Genetic Studies

Hexosaminidases A, B, and S are polymeric isoenzymes of which the A and S and the A and B enzymes contain one subunit, α_2 and β_2, respectively, in common (Geiger and Arnon, 1976; Geiger et al., 1977a). Their structure suggests that the α_2 subunit is defective in variant B and the β_2 subunit is defective in variant 0. Although the defective subunits have not so far been demonstrated biochemically in the affected tissues, hybridization experiments (Thomas et al., 1974; Galjaard et al., 1974a; Rattazzi et al., 1975; Ropers et al., 1975) between fibroblasts of variants 0 and B result in intergenic complementation supporting this notion. Thus, hybrid cells express hexosaminidase A ($\alpha_2\beta_2$), which is defective in both

parental cell lines. Intergenic complementation is obtained also in hybrids from fibroblasts with juvenile variant 0 and those with variant B, but not in hybrids from fibroblasts with juvenile and those with infantile variant 0, indicating that the mutations in infantile and juvenile variant 0 are allelic (Wood, 1978). Furthermore, hybridization experiments between human and mouse fibroblasts (Lalley et al., 1974; Gilbert et al., 1975; Chern et al., 1976, 1977; Hoeksema et al., 1977a; George and Francke, 1977) revealed that the gene for the polypeptide chain α is located in chromosomes 15 and that for the β chain on chromosome 5. Immunologic identification of enzymes appearing in interspecies hybrids turned out to be essential for proper interpretation, since hybrid enzymes may be formed as well as human and mouse enzymes (Chern et al., 1976; Hoeksema et al., 1977b). Formation of such hybrid enzymes in fused cells from variant 0 and mouse proves the existence of intact α_2 subunits in the disease (Hoeksema et al., 1977a).

6. Animal Models

G_{M2}-Gangliosidosis has been identified in German shorthaired pointers (Bernheimer and Karbe, 1970), but the gene was lost before a permanent breeding colony could be established. Yorkshire swine with G_{M2}-gangliosidosis have been reported (Pierce et al., 1976) but not yet fully characterized. Recently a recessively inherited G_{M2}-gangliosidosis has been discovered in domestic cats (Cork et al., 1977, 1978; Rattazzi et al., 1979). Morphologic, biochemical, and enzymatic analysis indicate that the feline disorder is homologous to human variant 0 of G_{M2}-gangliosidosis.

G_{M3}-Gangliosidosis

So far three cases of G_{M3}-gangliosidosis have been reported with a pronounced accumulation of ganglioside G_{M3} in the nervous system but differing greatly in their clinical and pathologic picture. The first case was that of a 29-month-old non-Jewish boy with enlargement of the spleen and deterioration of mental and motor function. This boy was first diagnosed as a case of Niemann-Pick disease due to the morphologic findings (Jørgenson et al., 1964). The brain contained high concentrations of ganglioside G_{M3} and lactosylceramide. The significance of the latter is uncertain, since the brain tissue had been fixed in formalin for about 6 years (Pilz et al., 1966).

Another patient was a 14-year-old Jewish boy who had macroglossia at birth and generalized seizures 2 days after birth (Max et al., 1974; Tanaka et al., 1975). Pathologically, the brain showed spongy changes. Brain and liver revealed a virtual absence of higher gangliosides, with a marked increase of ganglioside G_{M3}. This finding was thought to be due to a deficiency in ganglioside biosynthesis since no activity of ganglioside G_{M3}-*N*-acetylgalactosaminyltransferase was detected (Max et al., 1974).

The third case was that of a $4\frac{1}{2}$-year-old girl affected by severe growth failure, frequent myoclonic jerks, flaccid paralysis, and infrequent voluntary movements.

Brain tissue showed generalized storage of ganglioside G_{M3} and a lesser increase of ganglioside G_{M2} (Rose et al., 1977).

Diagnosis

An inherited deficiency of a lysosomal enzyme is present in all tissues and body fluids except erythrocytes, and will lead to serious accumulation of its substrates in the organs of their synthesis. A considerable increase may occur in the lysosomes of these organs even during early intrauterine life, as has been proven for the nervous tissue in aborted fetuses with G_{M2}-gangliosidosis (O'Brien et al., 1971; Schneck et al., 1972, 1975). The biochemical diagnosis of any lipid storage disease should include characterization of the accumulating lipids and determination of the underlying enzymic lesion. Identification of stored material such as oligosaccharides (Humbel and Collart, 1975; Strecker et al., 1977; Lundblad et al., 1978) or sulfatides (Harzer and Recke, 1975) can be made at first hand in some cases from urine with chromatographic methods or from biopsies, such as liver or brain specimens.

In the case of the gangliosidoses there is no increased excretion of the gangliosides in urine, but an increased excretion of oligosaccharides in G_{M1}-gangliosidosis (Lundblad et al., 1978) and in the 0 variant of G_{M2}-gangliosidosis (Strecker et al., 1977), which can easily be diagnosed. Cerebrospinal fluid is also useful for identification of the stored gangliosides (Bernheimer, 1968; Bernheimer et al., 1977). Usually lipid analysis in G_{M1}- and G_{M2}-gangliosidosis requires a percutaneous liver biopsy, which can be more easily performed than a brain biopsy. The latter should not be performed until late in the course of the disease. Since the enzymic defect is generalized, it can be demonstrated in most tissues and body fluids. Leukocytes and fibroblasts are among the very few easily accessible human tissues that can serve as optimal enzyme sources for the diagnosis of G_{M1}- and G_{M2}-gangliosidoses (Friedland et al., 1970; Harzer et al., 1971; Padeh and Navon, 1971; Kolodny, 1972; Young et al., 1972; Harzer, 1973; Kolodny and Mumford, 1976; Raghavan et al., 1977; Nakagawa et al., 1977; Pilz et al., 1978). Urine (Thomas, 1969; Navon and Padeh, 1972; Saifer et al., 1976) and recently tears (Goldberg et al., 1977; Tsuboyama et al., 1977) have also proved to be convenient enzymic sources for the detection of homozygotes and heterozygotes for Tay-Sachs disease and G_{M1}-gangliosidosis. Serum has been successfully used for the diagnoses of genotypes of Tay-Sachs disease and variant 0 of G_{M2}-gangliosidosis and for screening of Tay-Sachs disease genotypes (O'Brien et al., 1970; Kaback and Zeiger, 1972; Suzuki et al., 1973; Saifer et al., 1975; Molzer and Bernheimer, 1976; Kaback, 1977; Lowden et al., 1978). But one should keep in mind that serum from patients with diabetes mellitus (Belfiore et al., 1974) and from pregnant women (Stirling, 1972) has high hexosaminidase activities. Besides enzyme assays for G_{M2}-gangliosidosis with synthetic substrates, assays with radioactive natural substrates (O'Brien et al., 1977) have also been developed. It is useful to test any new index case with synthetic and natural lipid substrates, since the genetic heterogeneity of these disorders is great (Svennerholm, 1976). New mutant variants can be better differentiated by lipid analysis

and by enzyme assays with labeled substrates. If present, residual enzymic activity should be carefully investigated with various substrates and assay conditions.

The enzymic assays should be performed in laboratories with experienced personnel who are familiar with the limitations and pitfalls of various techniques such as heat inactivation, electrophoresis, and isoelectric focusing (see under substrate specificity) and have access to a large body of control data obtained from normal and pathologic cells. The normal range of levels of an enzyme in a given tissue may vary from one individual to another and with age and sex.

Besides biochemical demonstration of the accumulated lipids and the enzymic lesion, ultrastructural examinations of biopsies and other easily available tissues, such as fibroblasts, appear to be of value, since distinct morphologic changes in cultured fibroblasts have been observed in G_{M2}-gangliosidosis (Wyatt et al., 1978).

Detection of Carriers

Among the Ashkenazi Jews, the Tay-Sachs disease gene is quite common, occurring with a frequency of about 0.0167 (1:30 carriers) (Kaback et al., 1978), whereas outside this religious group the Tay-Sachs disease gene frequency is 0.0035 and that for the 0 variant 0.0012 (1:425 carriers) (Kaback et al., 1978). Detection of the carriers is an important part of genetic counseling in affected families. Although the number of allelic genotypes may be large for these diseases, affected members of the same family will usually show the same manifestation of the disease. When a patient having gangliosidosis has been identified, parents and other family members should be screened for the affected enzyme. Relatives of the patient have a much larger risk of carrying the abnormal gene and of bearing affected children themselves. Levels of affected enzymes vary considerably in heterozygotes of different families and may overlap with data found in homozygous normals or even with patients (e.g., with levels in patients with AB variant or with juvenile and adult forms of G_{M2}-gangliosidosis). Therefore, levels of the affected enzyme should be known in the index case and in obligate heterozygotes to facilitate a meaningful enzymic investigation of a proband.

Carrier detection of G_{M1}-gangliosidosis is well established by demonstration of intermediate β-galactosidase activity levels in leukocytes of fibroblasts (Young et al., 1972; Raghavan et al., 1977; Kolodny and Mumford, 1976). Carrier detection programs for Tay-Sachs disease are regularly carried out (see Lowden et al., 1976; Kaback, 1977). Identification of carriers has also been described for the 0 variant of G_{M2}-gangliosidosis (Harzer et al., 1971; Kolodny, 1972; Suzuki et al., 1973; Kolodny and Mumford, 1976; Molzer and Bernheimer, 1976; Lowden et al., 1978). However, the knowledge that there are unaffected individuals with decreased or absent hexosaminidase A activity on the one hand, and patients with normal hexosaminidase activity on the other, makes carrier detection quite difficult in many forms of G_{M2}-gangliosidosis.

Prenatal Diagnosis

In high-risk groups, e.g., when a couple has already had a child with gangliosidosis or a couple has been shown to carry the genes for G_{M1}- or G_{M2}-

gangliosidosis, prenatal diagnosis should be performed to avoid the birth of an affected child. Amniocentesis is usually performed in the 14th to 16th week of pregnancy. The cells obtained by centrifugation are cultured for 3—4 weeks before the relevant enzyme activities are assayed with labeled lipid or synthetic substrates (Schneck et al., 1970; O'Brien et al., 1971; Booth et al., 1973; Svennerholm, 1976b, 1979; Kudoh et al., 1978). The application of micromethods (Niermeyer et al., 1974; Galjaard et al., 1977; Hösli, 1977; D'Azzo et al., 1978; Kleijer et al., 1979) may reduce the length of the culture period to about 2 weeks. For G_{M1}- and G_{M2}-gangliosidosis, cell-free amniotic fluid can also be used for the enzyme assays (Portier et al., 1977; Christomanou et al., 1978; Geiger et al., 1978b), but cultured cells should always be tested to confirm the diagnosis (Desnick et al., 1973a). Results obtained with synthetic substrates can be considered relevant only if the same assay procedure has resulted in appropriate values for carriers and for the index case of the family under investigation. Levels of lysosomal hydrolase activities in cultured fibroblasts and amniotic cells fluctuate considerably, e.g., with the time of culture, culture conditions used, cell age, and cell density (Galjaard et al., 1974b). Therefore the cells of an index patient should always be cultured together with those of negative normal and positive pathologic cases under identical conditions.

Therapy

Prenatal diagnosis of neurolipidosis, especially of gangliosidosis in affected families, appears to be especially important since no therapy is available for these fatal inherited diseases. In model experiments it has been shown that the uptake of active enzymes by defective cells leads to the degradation of storage substances (Kihara et al., 1973; Cantz and Kresse, 1974; Suzuki et al., 1978; Figura, 1978; Halley et al., 1978; Ullrich et al., 1978; Brady, 1978). Defective fibroblasts can take up lysosomal enzymes from the culture medium. The enzymes are considerably stable in the cells and have a half-life of several days. On the basis of these experiments, pure enzymes have been infused to patients (see Brady, 1978). Short-term successes were obtained in Fabry's disease (Tallman et al., 1974c) and Gaucher's disease (Brady et al., 1975), where mainly visceral organs are affected by the storage process. In Fabry's disease and Gaucher's disease (Desnick et al., 1972, 1973b; Philippart et al., 1972); minimal improvement was also obtained by transplantation of a kidney; this is thought to have supplied the missing enzyme activity. In cases of gangliosidoses that affect mainly the brain, enzyme replacement has so far been unsuccessful. This is not unexpected, since the infused enzymes are taken up mainly by the liver, and degraded in the same hepatic cells (Stahl et al., 1976; Bearpark and Stirling, 1977; Brady, 1978). Apparently they are unable to penetrate the blood-brain barrier and to reach the nervous system. So far, clinical use of enzyme replacement is prevented by too many unsolved problems. The infused enzymes cannot be targeted at present to reach the nervous tissue, and they are inactivated too quickly. Repeated doses therefore appear to be necessary, which in turn might cause an undesirable immune response. It is

important to determine whether the patient's tissues contain cross-reactive material. If they do not, an immune reaction is probable. These difficulties cannot be overcome by methods in which the stability of the enzyme is increased by chemical modification (Snyder et al., 1974; Geiger et al., 1977b) or by packing of the enzymes in liposomes (Gregoriadis, 1978a and b; Reynolds et al., 1978), in immunologically neutral erythrocyte ghosts (Ihler et al., 1973; Fiddler et al., 1974) or in leukocytes (Cohen et al., 1976) from the patients.

Since the uptake of only small amounts of exogenous enzyme can normalize the catabolism of accumulating lipids (Desnick et al., 1976), therapeutic measures should be elucidated in appropriate animal models. In a feline model with the 0 variant of G_{M2}-gangliosidosis, it has been shown that minute amounts of infused hexosaminidase reach the brain tissue when the permeability of the blood-brain barrier is increased by the use of hyperbaric oxygen treatment (Rattazzi et al., 1979). A greater uptake of the enzyme by the brain may be obtained by blocking enzyme clearance through the liver. This might be achieved by the simultaneous injection of glycocompounds rich in terminal mannose and *N*-acetylglucosamine residues, which compete with the uptake of hexosaminidase by the liver receptors (Rattazzi et al., 1979). Studies with fibroblasts indicate that phosphorylated carbohydrates (e.g., mannose-6-phosphate) of some lysosomal enzymes are particularly responsible for their binding to cell surface receptors (Kaplan et al., 1977; Ullrich et al., 1978).

Final Remarks

Biochemical and genetic techniques have already provided considerable insight into the molecular neuropathology of gangliosidoses. Investigation of these rather rare inherited diseases has increased our knowledge about the normal metabolism and function of glycosphingolipids. Further analysis, especially of variant forms, may give us a better understanding of the structure and function of the normal and mutant enzymes involved, and may elucidate the molecular mechanism underlying the various clinical features and neuropathologic processes. Although no therapy seems to be possible in the near future, application of prenatal diagnosis has already reduced the frequency of birth of affected children quite efficiently. The future development of recombinant DNA techniques to probe the organization of human genes for β-galactosidases and β-hexosaminidases might allow a more sensitive antenatal diagnosis of gangliosidoses by the use of gene-specific probes, as has been achieved for sickle cell anemia (Kan and Dozy, 1978).

Acknowledgements. We thank Dr. Anzil, Dr. Carroll, and Dr. Reddington for their assistance with the correction of the English manuscript and Professor Tager for helpful comments. The authors' work presented in this review has been supported by grants (Sa 257/2, Sa 257/4, Sa 257/6 B 100/28) from the Deutsche Forschungsgemeinschaft.

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Received January 8, 1979