A historical perspective of the glycosphingolipids and sphingolipidoses

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Glycosphingolipids are a polysaccharide chain between 1 and 40 carbohydrate residues long glycosidically linked to ceramide (a long-chain aliphatic amino-alcohol or sphingoid) that is embedded in the cell plasma membrane with the carbohydrate moiety on the outside. The sphingoid imparts rigidity to the membrane and the carbohydrate tails protect the cell surface and have functions in relation to cell adhesion, growth, regulation, differentiation, cell interaction, recognition and signalling. They provide adhesion sites for pathogens and change during oncogenic transformation. Ceramide is also a component of sphingomyelin. Glycosphingolipids are degraded by lysosomal hydrolysis. The sphingolipidoses are a series of diseases in which mutations affecting the enzymes catalysing the last 11 steps of this process causing abnormal compounds proximal to the metabolic block to accumulate intralysosomally. Thus, they are a sub-group of the lysosomal storage diseases. The degradation of sphingolipids containing three or less carbohydrate residues requires a sphingolipid activator protein and mutations affecting these proteins also cause abnormal glycosphingolipid storage.

With one exception (Fabry disease, which is X linked) the sphingolipidoses are inherited autosomally. The phenotypic manifestations of the individual sphingolipidoses are variable although the more severe variants are usually the better known. They have generally been regarded as untreatable but notable therapeutic advances are being made by enzyme replacement therapy and regulating the rate of glycosphingolipid synthesis by inhibiting UDP-glucose-*N*-acylsphingosine D-glucosyl transferase (CerGlcT), which is the first reaction on the pathway of glycosphingolipid synthesis. The compounds used are *N*-alkylated iminosugars whose glucose and galactose stereochemistries inhibit CerGlcT.

Prenatal and carrier state diagnosis, genetic counselling and the abortion of affected fetuses are reducing the incidence of some of the most severe sphingolipidoses in certain high-incidence populations.

Keywords: glycosphingolipids; sphingolipidoses; lysosomal diseases; enzyme replacement; glycosphingolipid synthesis inhibition; *N*-alkylated iminosugars

1. THE GLYCOSPHINGOLIPIDS

(a) *Chemistry*

The glycosphingolipids are essential, although quantitatively minor, components of cell membranes. They consist of a polysaccharide chain, between 1 and approximately 40 carbohydrate residues long, glycosidically linked to ceramide, which is a long-chain aliphatic amino-alcohol or sphingoid that is embedded in the plasma membrane lipid bilayer with the polysaccharide chain on the outside (Sandhoff & Kolter 2003).

(b) *Function*

The intercalated hydrophobic sphingoid contributes to the rigidity of the membrane. The carbohydrate portions protect the cell surface from chemical and mechanical damage as well as having important functions in relation to cell adhesion, cell growth, cell regulation, differentiation, cell–cell interaction, cell recognition, interaction with signal molecules and toxins. Pathogenic viruses and

bacteria adhere to specific carbohydrate residues as a prelude to their entry into the cell and pathogenesis. Changes in the expression of the carbohydrate tails of the glycosphingolipids also follow oncogenic transformation.

The development of knowledge of the intracellular trafficking of glycosphingolipids and their role as physiological components of cell plasma membranes has been particularly striking as shown by the work of Pagano *et al.* (2000), van Meer (2001), Harder (2003), Pagano (2003) and van Meer *et al.* (2003). This has opened up an entirely new field of knowledge in cell physiology.

(c) *Biosynthesis*

Ceramide biosynthesis involves the following four sequential reactions:

palmitoyl-CoA + serine → 3-ketosphinganine;
3-ketosphinganine → sphinganine;
sphinganine + fatty acyl-CoA → dihydroceramide;
dihydroceramide → ceramide.

The ceramide is either incorporated into sphingomyelin by combination with phosphorylcholine, or converted to galactosyl ceramide (Gal-Cer) under the catalytic influ-

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ence of β -galactosyltransferase or to glucosylceramide (Glc-Cer) by a ceramide-specific glucosyltransferase of which UDP-glucose is the co-substrate. The first committed step on the pathway of glycolipid synthesis is this UDP-glucose *N*-acylsphingosine β -glucosyltransferase-catalysed conversion of ceramide Glc-Cer, which is then converted to lactosylceramide (Gal-Glc-Cer). The carbohydrate chain is lengthened by a series of reactions on the endoplasmic reticulum and in the Golgi apparatus. Polymorphisms in the genes directing the synthesis of the enzymes catalysing these chain-lengthening reactions are not associated with any currently identified inherited human diseases.

(d) *Degradation*

Glycosphingolipids are degraded by a series of lysosomal acid hydrolases, which remove the carbohydrate residues sequentially from the non-reducing end. The monosaccharide and ceramide moieties are reused in the cytosol. Mutations in the genes directing the final 11 steps in this process are responsible for the production of catalytically inefficient enzymes so that the degradation process is arrested and macromolecules proximal to the metabolic block accumulate in the lysosomes and cause a series of lysosomal storage diseases, the sphingolipidoses (table 1).

C. de Duve (de Duve & Wattiaux 1966; de Duve 1983) identified lysosomes as subcellular particles containing a wide range of hydrolytic enzymes with acidic optimum pH values and termed them 'lytic bodies' whose function was to degrade macromolecules, either derived from the metabolic turnover of cell constituents or derived from material entering the cell by endocytosis.

(e) *The glycolipid activator proteins*

The degradation of sphingolipids containing three or less carbohydrate residues requires SAPs as well as the specific enzymes shown in table 1 (Sandhoff & Kolter 2003). These are encoded on two chromosomes, the GM2 activator protein on chromosome 5, and on chromosome 10 for the saposin precursor (prosaposin) which is processed to form homologous activator proteins or saposins: SAPA, SAPB, SAPC and SAPD. The mutations in the relevant stretch of the SAP precursor gene produce different diseases, which more or less resemble some of the lysosomal enzyme deficiency diseases. It may be that as more patients are studied we will see these analogies more precisely. It is already apparent that abnormalities in saposin A produce a Krabbe disease-like syndrome, with saposin B mutations resembling multiple sulphatase deficiency, saposin C deficiency resembling juvenile neuropathic Gaucher disease but with more macrophage involvement, and saposin D deficiency producing a syndrome reminiscent of ceramidase deficiency. Mutations that inactivate the whole prosaposin gene show that the absence of all four saposins produces an accumulation of a wide range of gangliosides with a clinical syndrome resembling Gaucher's disease type II rather than, as might have been expected, ceramidase deficiency (Farber's disease).

Mutations in the genes directing the synthesis of the activator proteins presumably account for those cases where the phenotypic manifestations occur but the cognate hydrolase has normal activity.

2. THE LYSOSOMAL STORAGE DISEASES IN GENERAL

The concept of lysosomal storage diseases was developed by Hers (1965, 1973) and his colleagues based on de Duve's identification of lysosomes and their physiological function. The work of these two investigators was crucial for all subsequent developments in this field. It is necessary to set the sphingolipidoses within the context of the whole class of lysosomal storage diseases because there are, in some cases, clinical and biochemical phenotypic overlaps.

Two other major groups of lysosomal storage diseases (reviewed by Watts & Gibbs 1986) are: (i) the mucopolysaccharidoses in which there is deficiency of either a lysosomal exoglycase or a lysosomal sulphatase which causes glycosaminoglycans (mucopolysaccharides) to accumulate in lysosomes and to be excreted in the urine; (ii) the glycoproteinoses in which there is a failure to cleave individual sugar residues sequentially from the carbohydrate moieties of glycoproteins. Here, there is some overlap with other macromolecules in which oligosaccharide links occur, such as the GM1 and GM2 gangliosides. Fucosidosis, mannosidosis and sialidosis are examples of the glycoproteinoses. The mucopolipidoses, the acid lipase deficiency diseases and glycogenosis type 2 are also lysosomal storage diseases. The mucopolipidoses are a heterogeneous group, clinically intermediate between the sphingolipidoses and the mucopolysaccharidoses. Mucopolipidoses II (I-cell disease) and III (pseudo-Hurler polydystrophy) are due to defective post-translational modification of lysosomal hydrolases so that they cannot be translocated to the lysosomes. Mucopolipidoses I and IV are due to lysosomal neuraminidase deficiency with respect to the cleavage of neuraminic acid residues from GM3 and GM2 gangliosides.

The designations GM3 and GM2 arise from a notation for the sialoglycosphingolipids (gangliosides) introduced by Svennerholm in 1963, where G is ganglioside, M is monosialo-, D is disialo-, T is trisialo and the arabic numerals indicate the sequence on thin-layer chromatograms (see Watts & Gibbs (1986) for a correlation of Svennerholm's designation with the recommendations of the International Union for Pure and Applied Chemistry—International Union of Biochemistry (IUPAC-IUB)).

There are two lysosomal storage diseases that are due to metabolic lesions involving specific efflux processes whereby low-molecular-weight products of macromolecular degradation cannot leave the lysosome, and not to a failure of enzymatic hydrolysis. These are cystinosis, in which cystine accumulates intralysosomally, and Salla disease, in which the storage product is *N*-acetylneuraminic (sialic) acid.

The study of the sphingolipidoses and the other lysosomal storage diseases and their biochemistry has often begun with the description of unusual clinical syndromes, for example the recognition of what we now know as the GM2 gangliosidosis by an ophthalmologist Tay (1881) and a neurologist Sachs (1887). Clinical observation obviously has its limitations and, retrospectively, one can see that much of the earlier work was confused by what we now recognize as being cross-substrate specificity, multiple allelism, double heterozygosity, the need for SAPs,

Table 1. The sphingolipidoses.
(Reproduced with minor modifications from Watts & Gibbs (1986), with permission.)

number in figure 1	systematic name	eponymous and/or other generally used name	main storage compounds		
			generally used name	structure	enzyme deficiency ^a
1	GM1 gangliosidosis	—	ganglioside GM1 N-acetyl glucosaminyl oligosaccharides	Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ 4Gal(3 $\leftarrow$ 2 α NeuAc) β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1Cer	GM1- β -galactosidase or β -galactosidase A (EC 3.2.1.23)
2	GM2 gangliosidosis (type II; variant O)	Sandhoff disease	ganglioside GM2 ganglioside GA2 globoside N-acetylglucosaminyl oligosaccharides	GalNAc β 1 $\rightarrow$ 4Gal(3 $\leftarrow$ 2 α NeuAc) β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1Cer GalNAc(β 1 $\rightarrow$ 4)Gal(β 1 $\rightarrow$ 4)Glc (β 1 $\rightarrow$ 1)Cer GalNAc(β 1 $\rightarrow$ 3)Gal(α 1 $\rightarrow$ 4)Gal(β 1 $\rightarrow$ 4)Glc(β 1 $\rightarrow$ 1)Cer	β -N-acetylhexosaminidase (A and B) (hexosaminidase A and B) (EC 3.2.1.30)
3	GM2 gangliosidosis (type I; variant B)	Tay-Sachs disease	ganglioside GM2	GalNAc β 1 $\rightarrow$ 4Gal(3 $\leftarrow$ 2 α NeuAc) β 1 $\rightarrow$ 4Glc β 1 $\rightarrow$ 1Cer	β -N-acetylhexosaminidase A (EC 3.2.1.30)
4	α -galactosyl-lactosyl ceramidosis	Fabry disease, Anderson-Fabry disease	α -galactosyl-lactosylceramide	Gal(α 1 $\rightarrow$ 4)Gal(β 1 $\rightarrow$ 4)Glc(β 1 $\rightarrow$ 1)Cer Gal(α 1 $\rightarrow$ 4)Gal(β 1 $\rightarrow$ 1)Cer	α -galactosidase A (ceramide trihexosidase) (EC 3.2.1.22)
5	lactosylceramidosis ^c	—	galabiosylceramide lactosylceramide	Gal(β 1 $\rightarrow$ 4)Glc(β 1 $\rightarrow$ 1)Cer ^b	ceramide-lactoside- β -galactosidase (EC 3.2.1.23)
6	mucosulphatidosis	multiple sulphatase deficiency syndrome	cerebroside sulphate sulphated glycosaminoglycans cholesteryl sulphate	Gal (3-sulphate) β 1 $\rightarrow$ 1Cer	multiple sulphatases including arylsulphatase A, B, C and steroid sulphatase (EC 3.1.6.1 and EC 3.1.6.2)
7	sulphatidosis	MLD	cerebroside sulphate	Gal (3-sulphate) β 1 $\rightarrow$ 1)Cer	arylsulphatase A (cerebroside sulphatase) (EC 3.1.6.8)
8	galactocerebrosidosis	Krabbe disease, global cell leukodystrophy	galactocerebroside (galactosylceramide)	Gal (β 1 $\rightarrow$ 1)Cer	galactosylceramidase (EC 3.2.1.46)
9	glucocerebrosidosis	Gaucher disease	glucocerebroside (glucosylceramide)	Glc(β 1 $\rightarrow$ 1)Cer	glucosylceramidase (β -glucocerebrosidase) (EC 3.2.1.45)

(Continued.)

Table 1. (Continued.)

number in figure 1	systematic name	eponymous and/or other generally used name	main storage compounds		
			generally used name	structure	enzyme deficiency ^a
10	sphingomyelinosis	Niemann–Pick disease	sphingomyelin	PCho → 1Cer	sphingomyelin phosphodiesterase (sphingomyelinase) (EC 3.1.4.12)
11	ceramidosis	Farber disease	ceramide	Cer	acylsphingosine deacylase (ceramidase) (EC 3.5.1.23)

^a The first name given is generally the IUPAC-IUB recommended name; the common, generally used name is given in parentheses followed by the IUPAC-IUB enzyme commission (EC) number. It is necessary to specify the substrate for the α- and β-, gluco- and galactosidases because these names and EC numbers refer to enzymes catalysing hydrolysis at a particular type of glycosidic linkage. Similarly, the term arylsulphatase (EC 3.1.6.1) refers to a group of enzymes. Cerebroside sulphatase and arylsulphatase A activities are properties of the same protein, and the arylsulphatase A activity against a synthetic substrate (nitrocatechol sulphate) is measured diagnostically.

^b Blood group B and AB patients also accumulate:

^c Lactosyl ceramidosis is not now considered to be a disease in its own right because there are two enzymes that hydrolyse lactosylceramide (Gal—Glc—Cer) to glucosylceramide (Glc—Cer) and no cases in which both enzymes are deficient have, to my knowledge, been reported. Cases previously reported as lactosylceramidosis are now thought to have been variants of neuronopathic Niemann–Pick disease.

parental imprinting, the effects of mutations in the non-coding regions of the gene directing the synthesis of a polypeptide chain in the enzyme concerned, and variable X inactivation in X-linked disorders. The complexities of genotype–phenotype correlations have recently been reviewed by Weatherall (2001) and Badano & Katsanis (2002).

It also has to be borne in mind that although the product proximal to the metabolic block is the primary or main abnormal storage compound, this accumulation affects the function of other macromolecule-degrading enzyme systems so that different macromolecules accumulate as a secondary effect (Walkley 2003).

3. THE SPHINGOLIPIDOSES

(a) *Definition and inheritance*

The sphingolipidoses are the series of inborn errors of metabolism in which there is failure to degrade glycosphingolipids containing three or less carbohydrate residues. Except for Fabry disease, which is X linked, they are all inherited as autosomal recessives. The sphingolipidoses are listed in table 1, together with their main storage compounds and enzyme deficiencies. The sites of the metabolic blocks are shown in figure 1.

(b) *GM1 gangliosidosis*

The terms infantile, juvenile and adult (types 1, 2 and 3, respectively) are used to describe the age at which the clinical manifestations appear. Infantile cases sometimes referred to as ‘generalized gangliosidosis’ or ‘infantile gangliosidosis’ die between birth and *ca.* 2 years of age. They are grossly retarded, unresponsive, blind, quadriplegic and dysmorphic with skeletal abnormalities. The juvenile type presents at 6–12 months with psychomotor delay, progressive neurological deterioration and seizures, and dies in an unresponsive state with decerebrate rigidity by the age of 10 years. The adult type presents as teenagers with signs of cerebellar and upper motor neuron damage but only mild intellectual impairment; vision is unimpaired and seizures uncommon.

(c) *The GM2 gangliosidoses*

There are two isoenzymes of β -N-acetylhexosaminidase which are termed Hex A and Hex B. Hex A is a trimeric protein containing two identical chains termed the β -chains with one different chain termed the α -chain, and its structure is designated $\alpha\beta_2$. The Hex B is a tetramer containing only β -chains and its structure is designated $\beta_2\beta_2$. Only mutations in the α -chain cause GM2 gangliosidosis. The subunit structure of the β -N-acetylhexosaminidases was largely established by the seminal work of Neufeld (see Neufeld *et al.* 1984). There are three forms of GM2 gangliosidosis:

- (i) Tay–Sachs disease and its variants, in which only Hex A is deficient;
- (ii) Sandhoff disease and its variants, in which both Hex A and Hex B are deficient;
- (iii) mutations in the gene directing the synthesis of GM2 activator protein, which leaves normal Hex A and Hex B activity but failure to form a functional

ganglioside GM2/GM2 activator complex. Pseudo-deficient benign changes in Hex A with functional activity towards ganglioside GM2 are known.

The gross pathology and clinical features of all three diseases are similar except for the involvement of viscera in Sandhoff disease. There are infantile-, juvenile- and adult-onset phenotypes. The psychomotor development of the juvenile cases is subnormal from birth onwards, reaches an abnormally low plateau at *ca.* 1 year then declines rapidly with death in a decerebrate state at 2–3 years.

The juvenile group present at 2–6 years with progressive neurological manifestation (e.g. spastic weakness, non-coordination, dystonia, dysarthria) and die blind and decerebrate by their mid-teens. The adult cases present in late childhood or early adulthood with motor disabilities but little intellectual impairment.

(d) *Fabry disease*

The deposition of α -galactosyl-lactosylceramide affects mainly the vascular endothelium and smooth muscle cells with manifestations mainly in the nervous system (particularly pain and paraesthesiae in the limbs, personality changes, psychotic behaviour, intellectual deterioration), predisposition to vascular disease, progressive renal failure with neutral glycosphingolipid ‘Maltese cross’ crystals in the urine, eyes, gastrointestinal tract, lungs and skeleton. The disease is only fully expressed in the male hemizygotes but some abnormalities occur in the female heterozygotes.

(e) *Multiple sulphatase deficiency*

This is another very rare inborn error of metabolism. The deficiency of all 12 known sulphatases leads to a clinical picture with features resembling mucopolysaccharidoses I and II (Hurler and Hunter diseases), skeletal abnormalities, coarse features and manifestations resembling metachromatic leukodystrophy. Motor weakness and psychomotor delay occur in infancy, with death in a vegetative state before 10 years of age.

(f) *MLD*

Three clinical types have been recognized: the first two are an infantile variant with death after a few months, and a late infantile variant presenting with developmental delay and progressive neuropsychiatric degeneration progressing to death in a totally unresponsive state within 2–3 years. The adult-onset group show personality changes with dementia, a frontal type psychosis and peripheral neuropathy; these signs are followed by progressive brain destruction. It is often misdiagnosed as a psychosis.

(g) *Krabbe disease*

The overall clinical spectrum is similar to that of MLD with an infantile type with progressive psychomotor delay and retrogression to death after *ca.* 1 year; seizures and spastic tetraparesis dominate the clinical picture. Later-onset cases (late infantile and juvenile cases) have been reported.

(h) *Gaucher disease*

The three clinical groups of Gaucher disease patients are: (i) type I (chronic non-neuronopathic); (ii) type II

Figure 1. Location of the metabolic lesions in the sphingolipidoses. 1, GM1 gangliosidosis; 2, Sandhoff disease (GM2 gangliosidosis, type II or variant O); 3, Tay-Sachs disease (GM2 gangliosidosis, type I or variant B); 4, Fabry disease (α -galactosyl-lactosyl ceramidosis); 5, lactosylceramidosis; 6, multiple sulphatase deficiency syndrome (mucopolysulphatidosis); 7, MLD; sulphatidosis; 8, Krabbe disease (global cell leukodystrophy; galactocerebrosidosis); 9, Gaucher disease (glucocerebrosidosis); 10, Niemann-Pick disease (sphingomyelinosis); 11, Farber disease (ceramidosis). Lactosylceramidosis is not now considered a disease in its own right because there are two enzymes that hydrolyse Gal-Glc-Cer to Glc-Cer and no cases in which both enzymes are deficient have been reported. Cases previously reported as lactosylceramidosis are now thought to have been variants of neuronopathic Niemann-Pick disease. (Reproduced from Watts & Gibbs (1986), with permission.)

(acute neuronopathic); (iii) type III (subacute neuronopathic). They cannot be distinguished from one another biochemically and the large (20–100 μ m diameter) glucosylceramide-laden histiocytes (Gaucher cells) are found in the bone marrow of all three variants.

Type I can present at any age with splenomegaly and hypersplenism (thrombocytopenia, anaemia and leukopenia) as the main features. The liver is grossly enlarged but liver failure is rare. Poor bone growth, mineralization and remodelling with pathological fractures are prominent features. Pulmonary involvement impairs gas exchange. There are also characteristic skin-pigment changes.

Patients with acute neuronopathic (type II) Gaucher disease have hepatosplenomegaly and show developmental delay and retrogression by the age of six months. There are fits, focal neurological signs and pulmonary infections due to bulbar paralysis as well as infiltration of the lung parenchyma by Gaucher cells. These patients die mainly in the first year of life.

The subacute neuronopathic (type III) cases present in later childhood and may survive into adult life with a variable degree of intellectual impairment, focal neurological

manifestations and seizures as well as the systemic manifestations of the disease. The Norrbottnian type of Gaucher disease occurs in a group of patients who represent a genetic isolate in Northern Sweden and it is a particularly well-defined group of patients with the type III variant of the disease. It usually initially becomes apparent during the first decade with splenomegaly, epistaxis, psychomotor retardation and neurological abnormalities; these are characterized by paralysis of conjugate gaze. Except for the neurological involvement, this variant runs a course more reminiscent of the severe cases of type I Gaucher disease with death, usually from the haematological complications, during later childhood or early adult life. Some patients survive through the fourth decade; vertebral collapse and crippling osseous manifestations are frequent.

(i) *Niemann-Pick disease*

There are five phenotypic variants: (i) type A, the acute neuronopathic form; (ii) type B, the chronic form without neurological involvement; (iii) type C, the chronic neuronopathic form; (iv) type D, the Nova Scotia variant; (v) type E, the adult non-neuronopathic form. They all show

abnormal lipid (sphingomyelin) storage in tissue macrophages except type C, in which cholesterol homeostasis is affected. The abnormal cells in the sternal marrow, and sometimes in the circulation (monocytes), are 50–90 μm in diameter and appear foamy.

Type A is the most common variety: hepatosplenomegaly, lymphadenopathy and neurological retrogression with epileptic attacks begin during the first 6–12 months of life and most patients die before the age of 2 years. The type B patients resemble type I Gaucher disease clinically. Type C patients show psychomotor delay and retrogression during early childhood and die in later childhood and adolescence. Type D patients resemble type C but have a well-defined common ancestry in Western Nova Scotia. Type E patients present in adult life with moderate hepatosplenomegaly but no neurological involvement; their exact taxonomic position is unclear.

(j) *Farber disease*

This is a very rare condition. The reported cases have presented with a characteristic hoarse cry, painful swollen joints and periarticular nodules. Feeding becomes difficult and the overall nutrition is impaired. Skin nodules develop, there are ocular, respiratory and cardiac complications, progressive neuropsychiatric deterioration and death within a few years. The skin nodules, kidney, liver and brain contain ceramide deposits.

4. PREVENTION OF THE SPHINGOLIPIDOSES

Although some progress is being made in the correction of genetic disease by the introduction of genetically corrected systemic cells (bone marrow stem cells in the case of adenosine deaminase deficiency), the prospect of replacing the abnormal gene in germline cells, as would be necessary to prevent the phenotypic expression in most genetic diseases such as the sphingolipidoses, appears to be remotely, if ever, attainable.

Increasing knowledge of the enzymology of glycosphingolipid degradation has led to progressively more refined diagnostic methods with early prenatal diagnoses based on enzymology and mutational analysis (Aerts *et al.* 2003). This, in turn, has led to the possibility of early termination of affected pregnancies, improved genetic counselling and, although I am not aware of it being applied to the glycosphingolipidoses, the possibility of diagnosis on a single cell removed from a four-cell embryo which has been produced by *in vitro* fertilization, so that only an unaffected embryo is implanted into the mother's uterus.

An example of what can be achieved by a modern clinical biochemical genetics programme is seen in the Ashkenazi Jewish population in New York City, where the incidence of GM2 gangliosidosis was *ca.* 1 : 2500 with a carrier frequency of *ca.* 1 : 25. An intensive educational and public health programme, supporting carrier-state diagnosis and prenatal diagnosis, has reduced the incidence of the disease by *ca.* 90%.

5. TREATMENT OF THE SPHINGOLIPIDOSES

Neurological damage is a feature of the severe variants of the lysosomal storage diseases. A major factor in

relation to their treatment is the impermeability of the blood–brain barrier except in a few small, specialized areas, such as the area postrema, the tuber cinereum, and the lymphatics that accompany the spinal and cranial nerve roots. The cerebral capillary endothelium has tight junctions and shows no micropinocytosis, and the ependymal cells covering the choroid plexus actively secrete cerebrospinal fluid of closely controlled composition. However, there is evidence that insulin, corticotropin and neuropharmacologically active peptides can cross the barrier from the bloodstream into the brain. Experimental procedures to temporarily increase the permeability of the blood–brain barrier, by either intracarotid infusion of hypertonic saline or oxygen embolization, have not led to any general therapeutic applications. It is hard to see how the permeability of the blood–brain barrier could be breached, either repetitively or permanently, without seriously damaging the internal environment of the brain cells and thus causing their dysfunction. We need to develop therapeutic agents that can penetrate the intact blood–brain barrier and can enter neurons without pinocytosis. The iminosugars offer promise in this direction.

Lysosomal enzymes undergo a cycle of exocytosis and re-uptake. This led to attempts to treat lysosomal storage diseases by systemic cell implantation. An early attempt using cultured histocompatible fibroblasts (Gibbs *et al.* 1983) in some of the mucopolysaccharidoses was unsuccessful. Bone marrow transplantation has been tried on the premise that engrafted bone marrow would produce large numbers of exocytic cells that would circulate and transfer their normal enzyme to enzyme-deficient cells (see Krivit & Paul 1986). This approach only led to some improvement in the non-neurological aspects, and made no impact on the neurological aspects due to the problems of the blood–brain barrier or the problems and risks of the bone marrow transplantation itself. Similar considerations apply to applications of the more recently developed technique of bone marrow stem cell implantation. The risk–benefit ratio requires very careful assessment with respect to the individual patient's clinical course and the known natural history of the disease concerned.

Approximately 5% of the neuroglia (non-neuronal cells of the central nervous system) are microglia and derived from blood macrophages (monocytes). Thus, provided the blood macrophages and microglia can interchange after the blood–brain barrier has formed, it was hoped that the microglia might carry enzymes to enzyme-deficient neurons after a successful transplant.

The possibility of transplanting normal foetal brain cells, or even multi-potential very early foetal cells, directly into the brain to overcome the problem of the blood–brain barrier should not be discarded, despite the uncertainty of cell–cell interaction and the apparent lack of endocytosis by mature neurons, although perhaps not by immature neurons. It should be borne in mind that, from a more favourable perspective (i) the central nervous system is an immunologically protected site, and (ii) possibly only relatively low levels of enzyme replacement may suffice to reduce abnormal storage.

Direct enzyme replacement, that is, the use of purified and targeted enzymes, has had real success in the non-neurological aspects of Gaucher and Fabry diseases. This approach holds out a measure of hope for other lysosomal

storage diseases such as Morquio disease (mucopolysaccharidosis IV) where the skeletal disease is devastating but intellect is unimpaired. I consider that with enzyme replacement we are at the stage of refining and extending the general method. It is a field in which Brady and his colleagues have made outstanding, pioneering and sustained contributions (see Barranger & Brady 1984; Schiffman & Brady 2002; Brady 2003). The situation with respect to Fabry disease was recently reviewed by Brady *et al.* (2001), Pastores & Thadhani (2001) and Weinreb *et al.* (2002).

In spite of the success and promise of direct enzyme-replacement therapy, an entirely new approach is needed. Such a development is being pioneered by the work of the Oxford Glycobiology Institute, which aims to regulate the rate of glycosphingolipid synthesis by inhibiting UDP-glucose: *N*-acylsphingosine D-glucosyl transferase (CerGlcT), which is the first reaction on the pathway of glycosphingolipid synthesis (Butters *et al.* 2003; Platt *et al.* 2003). The analogy with the competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the first specific step on the pathway of cholesterol biosynthesis in the treatment of hypercholesterolaemia, adds to the conceptual attraction of this approach. Clearly, one will need to balance the need for mature glycosphingolipids against the chances of excessive ceramide accumulation in long-term use. The compounds used are *N*-alkylated iminosugars whose glucose and galactose stereochemistries inhibit CerGlcT (Dwek *et al.* 2002; Butters *et al.* 2000). This approach offers real promise for the future, and one drug of this type OGT 918 (*N*-butyldeoxynojirimycin) is showing promise in Gaucher disease (Cox *et al.* 2000) although so far it has only been used in non-neuronopathic patients; thus its ability to cross the blood-brain barrier and produce pharmacological effects in the brain has not yet been explored at the clinical level (Moyses 2003; Zimran & Elstein 2003), although it has demonstrated this ability in the mouse models of the GM2 gangliosidoses. These drugs also inhibit virus replication where, because they are host-cell encoded and not virus encoded, drug-resistant strains of virus are less likely to occur (Dwek *et al.* 2002).

The development of knockout-gene animal models of the sphingolipidoses has been a major step forward in the development of the basic knowledge of the sphingolipidoses and their possible treatment (see Lullman-Rauch *et al.* 2001; Siatskas & Medin 2001; Proia 2003). Virus-mediated gene transfer as a potential treatment remains at a very basic level in this field and it has yet to make an impact on the treatment of any of the sphingolipidoses or other lysosomal storage diseases (Gieselmann *et al.* 2003). It remains something for the remote future, although Teixeira *et al.* (2001) have demonstrated retrovirus-mediated transfer and expression of hexosaminidase α -chain cDNA in human fibroblasts from GM2 gangliosidosis patients, and Siatskas & Medin (2001) have shown that α -galactosidase-A-transduced haemopoietic cells from Fabry disease mice showed enzymatic correction of recipient cells and dissemination of α -galactosidase-A upon transplantation leading to decreased sphingolipid accumulation.

It seems probable that future work will involve

- (i) the further exploitation of the work on saposins to provide a greater understanding of pathophysiology, further diagnostic refinement and the possibility of synthetic constructs which could be used where the activator proteins are lacking, or possibly to augment partly deficient enzyme activity;
- (ii) further developments in the field of blocking sphingolipid synthesis;
- (iii) further consideration of finding ways of manipulating the permeability of the blood-brain barrier that might be applicable in humans;
- (iv) possibly, finding some way of manipulating the function of the cytoskeleton to encourage exocytosis of abnormally stored products as was suggested by work in model systems (fibroblasts and isolated perfused rat liver) by Danpure (1982);
- (v) further developmental work on virus-mediated gene transfer.

The practice of medicine rests, in its broadest sense, on 'the scientific method'. Apart from this, medicine and science have wide areas of overlap at both the research and applied levels. This overlap is particularly well marked in relation to the inherited diseases with a combined chemical and clinical phenotype of which the sphingolipidoses are an example. The present plethora of work in biochemical genetics rests on the shoulders of such prescient combined clinical and chemical investigators as Garrod (1858–1936; see 1909 and 1931), Thannhauser (1885–1962) and Hofman & Zollner (2000), and this should not be overlooked.

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GLOSSARY

MLD: metachromatic leukodystrophy
SAP: sphingolipid activator protein