Abstract: Analysis of lipid storage in postmortem brains of patients with amaurotic idiocy led to the recognition of five lysosomal ganglioside storage diseases and identification of their inherited metabolic blocks. Purification of lysosomal acid sphingomyelinase and ceramidase and analysis of their gene structures were the prerequisites for the clarification of Niemann-Pick and Farber disease. For lipid catabolism, intraendosomal vesicles are formed during the endocytotic pathway. They are subjected to lipid sorting processes and were identified as luminal platforms for cellular lipid and membrane degradation. Lipid binding glycoproteins solubilize lipids from these cholesterol poor membranes and present them to water-soluble hydrolases for digestion. Biosynthesis and intracellular trafficking of lysosomal hydrolases (hexosaminidases, acid sphingomyelinase and ceramidase) and lipid binding and transfer proteins (GM2 activator, saposins) were analyzed to identify the molecular and metabolic basis of several sphingolipidoses. Studies on the biosynthesis of glycosphingolipids yielded the scheme of Combinatorial Ganglioside Biosynthesis involving promiscuous glycosyltransferases. Their defects in mutagenized mice impair brain development and function.

Keywords: endocytosis of membrane lipids, lysosomal digestion, lipids regulate endolysosomal proteins, lipid storage diseases, lipid binding proteins, ganglioside metabolism

1. Lipid analysis of post-mortem brain tissues with infantile amaurotic idiocy and the discovery of GM1 gangliosidosis

As a student my special areas of interest were natural sciences and mathematics. In the early 1960s, I studied chemistry at the Ludwig-Maximilians-University in Munich. For a training in neurosciences I joined the neurochemical group of Prof. Horst Jatzkewitz at the Max-Planck-Institute of Psychi-
loration of lipid-containing cells in the viscera and in the nervous system, mental retardation and impaired vision or blindness. The classical infantile form of amaurotic idiocy had been known as Tay-Sachs disease (TSD). There had been little progress in its understanding until the development of chemical, biochemical and refined ultrastructural means of investigations. In the late 1930s, the biochemist Ernst Klenk from the University of Cologne, detected a new group of acid glycosphingolipids as storage material in the brain of TSD patients. They were also abundant in normal ganglion cells and named “Ganglioside” (gangliosides). The first ganglioside structure, that of ganglioside G1 (GM1), was elucidated by Kuhn and Wiegemann, the main storage compound in TSD was identified by Svennerholm, and its structure elucidated by Makita and Yamakawa and Ledeen and Salsman. In the electron microscope, Terry and co-workers observed storage granules (membranous cytoplasmic bodies) in the cytoplasm of the distended neurons that resemble pathologically modified lysosomes incapable of degrading their storage material.

2. Search for the metabolic defects in Tay-Sachs and related diseases

In the early 1960s, I collected post-mortem tissues of several cases of IAI with neuronal storage of ganglioside GM2 and its sialic acid free derivative GA2. Storage compounds were isolated and radiolabeled for enzymatic studies by catalytic reduction of the double bond in the sphingoid base. Since 1966, I also obtained fresh and deep-frozen autopsy material from several cases with IAI, suitable for metabolic studies to search for the defective enzymatic step causing the pathological lipid storage in the patients. Different from all cases of TSD studied so far, one of them contained an additional accumulation of globoside in its visceral organs. Most importantly, all three storage compounds—GM2, GA2 and globoside—had a structural feature in common, a terminal \( \beta \)-glycosidically linked N-acetylgalactosamine residue (Fig. 1). This observation suggested a \( \beta \)-N-acetylgalactosaminidase deficiency in the patients’ tissues, blocking the catabolism of the storage compounds. At that time, however, no N-acetylgalactosaminidase was known in human tissues, only N-acetylgalactosaminidases. Studies on the purification and substrate specificities of human N-acetylgalactosaminidases proved that they also hydrolyze N-acetyl-\( \beta \)-galactosaminids though with different kinetic constants and that their two major forms, the hexosaminidases A and B, could easily be separated by ion exchange chromatography and isoelectric focussing. Indeed, the case with an additional storage of globoside in the visceral organs exhibited complete deficiencies of hexosaminidases A and B (0-variant), another case a deficiency of hexosaminidase A (B-variant), and a third case no hexosaminidase deficiency at all (AB-variant) (Fig. 1).

3. Structure and function of the lysosomal hexosaminidases

In order to clarify the stunning and apparently contradictory findings on the first three cases of IAI described above, we purified the human hexosaminidases A (HexA) and B (HexB), and later also S (HexS) to apparent homogeneity for closer characterization. A historical view is given in. Here, some major observations are presented.

Both isoenzymes hydrolyzed the radiolabeled neutral storage compounds, GA2 and globoside, and released their terminal N-acetylgalactosamine residues in a detergent based assay using a crude sodium taurocholate preparation. On the other hand, only HexA had a minor but significant activity on the anionic storage compound, the radiolabeled ganglioside GM2 releasing its terminal N-acetylgalactosamine residue. Heating of HexA under appropriate conditions generated HexB, an observation which was explained by the finding of Srivastava and Beutler, that both isoenzymes had a subunit in common, HexA being a heteropolymer \((\alpha/\beta)_n\) and HexB a homopolymer \((\beta/\beta)_n\). This view was substantiated and refined by several groups (for review see) and was eventually proven by the observation that mutations in the \( \beta \)-subunit gene caused the loss of HexA and HexB activity in variant 0 of GM2-Gangliosidoses (Sandhoff disease), whereas mutations in \( \alpha \)-subunit gen caused the loss of HexA and HexS activity in TSD (Fig. 2).

The minor and labile HexS has a specificity similar to that of HexA. It hydrolyzes anionic and neutral substrates, oligosaccharides and glycosphingolipids including the sulfated SM2 and ganglioside GM2.

4. Search for the missing cofactor in AB variant of GM2 gangliosidoses

HexA and B purified from the brain tissue of our first case with AB variant (Fig. 1) exhibited normal activities and specificities against water-soluble synthetic substrates and hydrolyzed also the neutral
The biochemical analysis of brains with infantile amaurotic idiocy revealed puzzling results in 1967 and spring of 1968 (summarized in: ref. 249).

- The tissue of case 1 with TSD (called "B"-variant) was deficient in HexA activity and had increased HexB activity, a finding which was confirmed in 1968 by an additional case and the observation by Okada and O'Brian.251) This one we named "0"-variant of GM2-gangliosidosis and it was later called Sandhoff disease.
- Apparently contradicting the above findings, the brain tissue of case 3 contained both, HexA and HexB activity, even at elevated levels despite a huge accumulation of GM2 and GA2 (later called AB-variant after the defect of a cofactor —GM2AP— was discovered, see below).

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**Fig. 1.** Biochemical analysis of brains with infantile amaurotic idiocy. Lipid and hexosaminidase analysis in the brain tissue of the three first cases revealed puzzling results in 1967 and spring of 1968 (summarized in: ref. 249).

- The tissue of case 1 with TSD (called "B"-variant) was deficient in HexA activity and had increased HexB activity, a finding which was confirmed in 1968 by an additional case and the observation by Okada and O'Brian.251) This one we named "0"-variant of GM2-gangliosidosis and it was later called Sandhoff disease.
- Apparently contradicting the above findings, the brain tissue of case 3 contained both, HexA and HexB activity, even at elevated levels despite a huge accumulation of GM2 and GA2 (later called AB-variant after the defect of a cofactor —GM2AP— was discovered, see below).

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**Fig. 2.** The GM2 Gangliosidoses and the \(\beta\)-hexosaminidase system (modified after ref. 25). Three polypeptides, each encoded in a different gene, are needed for the degradation of ganglioside GM2: the \(\alpha\)- and \(\beta\)-subunit of the A isoenzyme and the activator protein which binds the ganglioside and presents it to the enzyme. GAGs, glycosaminoglycans.
storage compound GA2 in the presence of detergents.\textsuperscript{28} Also the main storage compound GM2 was hydrolyzed by a HexA preparation from the patient’s liver in the presence of detergents.\textsuperscript{28} It remained an enigma why the patient’s tissues stored glycolipids in the presence of their functional catabolic enzyme. Therefore, we looked for a missing factor in human tissues that might substitute for the detergent in the \textit{in vitro} assay and stimulate the HexA catalyzed hydrolysis of radiolabeled ganglioside GM2. Such a protein factor, now named GM2 activator protein, GM2AP, was found by Ernst Conzelmann\textsuperscript{29} and purified to homogeneity.\textsuperscript{30} Its activity was completely missing in AB variant tissues and its cellular uptake restored the catabolism of ganglioside GM2 in AB variant fibroblasts.\textsuperscript{31}

The GM2AP is a small glycolipid binding protein which can form water-soluble stoichiometric complexes (1:1) with ganglioside GM2\textsuperscript{30,32} and presents the lipid substrate to the water soluble HexA. In the presence of GM2AP, nice Michaelis-Menten kinetics were obtained for the hydrolysis of GM2 and GA2 by HexA (Fig. 3), whereas detergent assays always suffered from nonlinearities and poor reproducibility.

The mature GM2AP is a small glycoprotein of about 20 kDa generated from a precursor protein in the endolysosomal compartment of the cell.\textsuperscript{33} It carries an N-glycosylation site and contains eight cysteins that form four disulfide bonds\textsuperscript{34}. Its crystal structure shows a unique spacious hydrophobic \(\beta\)-cup for binding of the ceramide moiety.\textsuperscript{35}

A flexible hydrophobic loop adjacent to the cavity is supposed to interact with membranes and allows the extraction of the lipid at acidic pH values.\textsuperscript{36} A HexA binding region of the GM2AP facilitates the formation of the Michaelis-Menten complex\textsuperscript{37} (Fig. 3).

\textit{In vitro} and \textit{in vivo} data obtained until 2000 suggested that GM2AP might be quite specific for binding and solubilizing glycolipids like GM2 and GM1 from micelles and lipid vesicles, and transferring them to other membranes or enzymes like HexA,\textsuperscript{38} and \(\beta\)-galactosidase, respectively.\textsuperscript{39} However, expression of recombinant human GM2AP in insect cells revealed that it also recognizes phospholipids\textsuperscript{40} and fuses lipid vesicles at acidic pH values.\textsuperscript{41}

5. Variant B1 of GM2 gangliosidosis

In 1980, two cases with apparent variant AB of GM2 gangliosidosis were described.\textsuperscript{42} In one of them, however, Li and co-workers could not find an activator protein deficiency.\textsuperscript{43} They suggested that the patient might have a structural gene mutation rendering HexA nonresponsive to stimulation by the GM2 activator for the hydrolysis of GM2 ganglioside.\textsuperscript{44} To clarify this point, we studied the substrate specificity of human hexosaminidases. Competition experiments between different substrates showed that normal human HexA carries two active sites to hydrolyze both, anionic and neutral substrates, respectively, whereas HexB hydrolyzes neutral substrates only.\textsuperscript{45} Then Kytzia analyzed two patients with clinical TSD and extensive neuronal storage of GM2, still having apparently both, HexA and GM2 activator protein activity. HexA of both patients cleaved neutral N-acetylgalcosaminides and -galactosaminides with almost normal kinetics, but were almost inactive to hydrolyze an anionic sulphated and water soluble glucosaminide (MUGlcNAc-6-S, a newly described substrate for HexA\textsuperscript{46}) and ganglioside GM2 in the presence of the GM2 activator protein.\textsuperscript{45,47} The new form of the disease was named variant B1 of GM2 gangliosidoses since their HexA was still active against neutral substrates but had lost its capacity to cleave anionic substrates such as GM2.

Cellular hybridization experiments between patient fibroblasts demonstrated variant B1 being allelic to variant B.\textsuperscript{48} They indicated that the \(\alpha\)-subunit of HexA contributes to the hydrolysis of anionic substrates, MUGlcNAc-6-S and GM2 (as well as GA2) in the presence of the GM2AP and that this function is inactivated by mutations in the \(\alpha\)-subunit in patients with variant B1. Kinetic experiments showed furthermore that the free GM2 activator competes with the sulphated substrate MUGlcNAc-6-S for the \(\alpha\)-dependent site of HexA and HexS.\textsuperscript{45} Indeed, GM2AP recognizes the \(\alpha\)-subunit,\textsuperscript{37} an observation that is in line with an earlier finding\textsuperscript{39} that glycolipids GM2, GA2 and globoside in the presence of the GM2 activator protein were almost exclusively degraded by HexA and not by HexB. Even the precursor, proHexA degrades GM2 in the presence of the GM2 activator protein.\textsuperscript{49}

6. Threshold theory and the development of different clinical forms of GM2 gangliosidoses

Deficiencies of hexosaminidase activities of various extent caused by allelic mutations of the \(\alpha\)- and \(\beta\)-subunits, respectively, have been observed in a variety of patients with different clinical forms (infantile, juvenile, adult and chronic) of GM2 gangliosidoses representing a wide range of clinical
Fig. 3. Figure legend is on the next page.

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[S]_{eq}/K_m = 1/((v_{max}/v_i)-1)
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Fig. 4. Figure legend is on the next page.
symptomatology.\textsuperscript{25} Whereas the infantile forms show a very uniform clinical picture, that of juvenile and adult forms is quite variable and ranges from spinocerebellar degeneration, muscular atrophy- and amyotrophic lateral sclerosis-like syndromes, to psychiatric symptomatology which hardly resembles the clinical symptoms of the infantile form of the disease.\textsuperscript{51} The severity of the clinical symptoms correlates inversely with the residual activities of the deficient enzymes.\textsuperscript{55–57}

Fig. 3. Mechanism of GM2AP-Liftase. Model for the interaction of GM2-activator protein with luminal lysosomal membranes in the degradation of ganglioside GM2 (modified after ref. 36). GM2AP interacts with the membrane by dipping the two exposed hydrophobic loops, V90-W94 and V153-L163, into the apolar part of the membrane. Ganglioside GM2 is recognized by specific sites at the rim of the cavity. In the open protein conformation, the large hydrophobic area reaching from the apolar phase of the membrane to the activator’s cavity lowers the energy barrier for lipids leaving the membrane in an upward direction. After the ceramide tail has moved inside the activator’s cavity, the inward pointing orientation for the hydrophobic loop V153-L163 is favoured and the conformation changes to the closed form.\textsuperscript{25} This folding in of the hydrophobic loop leaves a more polar patch close to the membrane. The activator is then anchored only by the loop V90-W94. It may now rotate slightly upwards to expose all polar patches more fully to the solvent, and it may also leave the membrane and interact with the degrading enzyme. The photoaffinity label analogues C\textsubscript{(n=5,1)}-TPD-GM2 were photoincorporated specifically into the region V153-L163.

These observations can be understood qualitatively on the basis of a greatly simplified kinetic model\textsuperscript{51–53}. Considering an influx rate ($vi$) of the substrate (\textit{e.g.} ganglioside) into the lysosomal compartment of an individual cell, the steady-state substrate concentrations $[S]_{eq}$ and the turnover rate were simply calculated as a function of the residual catalytic enzyme activity (Fig. 4). Since the degradative capacity ($V_{max}$) in normal control cells exceeds the influx rate $vi$ severalfold (for the GM2-degrading system a factor of about 20-fold was estimated), $[S]_{eq}$ will be far below the $K_{M}$ value of the degrading enzyme. As discussed before,\textsuperscript{21} substantial reductions of the probands’ degrading enzyme activity $\nu$ to values still above $vi$ will lead to a moderate increase of $[S]_{eq}$ and not to an irreversible accumulation, since the remaining enzyme activity is still sufficient to cope with the substrate’s influx rate. However, when the residual activity of the patients’ variant enzyme falls below a critical threshold the turnover of the substrate will be reduced and it will accumulate. The rate of accumulation will be propor-
glycoproteins that bind and solubilize lipids. The discovery of sphingolipid activator proteins, small factors substituting for detergents resulted in the by Horst Jatzkewitz when his co-workers purified arylsulfatase A. Its deficiency causes sulfatide storage in MLD. Its deficiency causes sulfatide storage in MLD.62) Saposin B (Sap-B) is a small glycoprotein forming water soluble stochiometric complexes with sulfatides presenting them to the water soluble enzyme. Its inherited deficiency causes different clinical forms of MLD depending on the mutational profile.62–67}

In the absence of detergents or Sap-B, arylsulfatase A (ASA) has almost no activity on its lipophilic substrates, sulfatide and seminolipid. To clarify the mystery we synthesized years later sulfatides with fatty acid residues of different chain lengths and studied their hydrolysis by arylsulfatase A in the absence and presence of Sap-B.68)

As shown in Fig. 5, the water-soluble short chain sulfatides were readily hydrolyzed by ASA in the absence of any detergent or activator protein. Even more, the addition of Sap-B did not stimulate the hydrolysis of soluble short chain sulfatide analogs. However, to achieve substantial cleavage rates of the less soluble, lipophilic sulfatides with long acyl chains, the addition of Sap-B was needed. Similar studies were performed with soluble and less soluble lipophilic analogs of ganglioside GM1 and GM2. The hydrolysis of ganglioside GM1 micelles by β-galactosidase was achieved only by the addition of detergents, or GM2AP or Sap-B. However, the more soluble short acyl chain analogs of GM1 were readily hydrolyzed by β-galactosidase, even in the absence of these additions.69–70)

The GM2AP is an essential cofactor for the hydrolysis of GM2 ganglioside by HexA, but not for its more water-soluble analogs with short acyl chains.71) It is also not needed to cleave off the terminal N-acetyl-galactosamine residue from the extended and more exposed oligosaccharide chain of membrane-bound ganglioside GD1a-GalNAc.71)

These experiments showed that the soluble enzymes used can cleave water soluble substrates readily, but hardly recognize insoluble substrates forming micelles in aqueous solution or being components of liposomes or biological membranes. Glycosphingolipids with short oligosaccharide chains (four or less sugars) have to be complexed, lifted from their membranes or solubilized by SAPs to be efficiently degraded by water-soluble lysosomal hydrolases. This view is supported by in vivo findings in SAP deficient mice and human patients. The catabolism of all GSL with short oligosaccharide chains requires the action of SAPs.72) Their inherited deficiency causes fatal storage diseases.

7. Sphingolipid activator proteins and the lipid phase problem

The in vitro analysis of enzymatic sphingolipid degradation revealed early on that water-soluble lysosomal hydrolases can cleave their sphingolipid substrates effectively only in the presence of appropriate detergents.62–67) The search for in vivo factors substituting for detergents resulted in the discovery of sphingolipid activator proteins, small glycoproteins that bind and solubilize lipids. The first activator protein was discovered in the early 60s by Horst Jatzkewitz when his co-workers purified arylsulfatase A. Its deficiency causes sulfatide storage in MLD.62) Saposin B (Sap-B) is a small glycoprotein forming water soluble stochiometric complexes with sulfatides presenting them to the water soluble enzyme. Its inherited deficiency causes different clinical forms of MLD depending on the mutational profile.62–67)

8. The complex cellular functions of SAPs

Sap-B and GM2AP were discovered as essential cofactors for the catabolism of few glycolipids. Their inherited defects cause fatal sphingolipidoses with a rather limited number of storage compounds.67) Based on these observations and early biochemical tests,67) I expected rather limited lipid binding
specificities of these proteins. The first surprise came during an attempt to identify further cofactors involved in GSL-catabolism. Sap-B turned out to be quite unspecific as a lipid binding protein and enhanced the catabolism of several GSLs. Sap-B not only stimulated the hydrolysis of sulfatides by ASA, but also that of globotriaosylceramide by α-galactosidase, ganglioside GM1 by β-galactosidase, lactosylceramide by β-galactosidase and others. Li and co-workers even used the human Sap-B successfully to stimulate the hydrolysis of several bacterial glycolipids by bacterial hydrolases.

GM2AP, which is specifically needed for the catabolism of the GSLs, GM2 and GA2, also effectively stimulates the degradation of ganglioside GM1 by β-galactosidase and binds in its hydrophobic cavity a bunch of unspecific phospholipids. The identification of inherited SAP deficiencies causing variant forms of known sphingolipidoses and corresponding knock-out mice models (review see refs. 21, 67, 76–80) triggered a more detailed analysis of their formation and functions (for review see refs. 67, 81–85).

Unexpected was the finding of Werner Fürst, that the precursor of the sulfatide activator protein, now called saposin B, codes for a big polyprotein and is processed to at least 4 different proteins, including the well-known activators Sap-B, and -C. The galley proof of our paper was mailed to three authors of a later Science paper 4 months before they published a conceptually similar finding. No reference was given to our work.

A great help for the following studies on the genomic structures, the expression of recombinant proteins and the identification of mutations in patients, was the collaboration between our laboratory and that of Kunihiko Suzuki who also stayed in Bonn for several periods in the late 80s and early 90s.

The analysis of the precursor protein, prosaposin, revealed that it is generated as a complex glycoprotein and transported from the ER through the Golgi and TGN to the endolysosomal compartment where it is proteolytically processed to four homologous saposins (A, B, C and D) of about 80 amino acids each.

Each of their sequences contain a conserved N-glycosylation site (Sap-A contains two) and six conserved cysteins forming disulfide bonds. Inherited defects (Fig. 6) of individual saposins and constitutionally generated knockout mice (for review see refs. 67, 79, 80, 105) demonstrated the essential life supporting functions of these lipid binding and activator proteins and helped to determine their in vivo functions which are still a matter of debate.

A key observation was the discovery of a patient with a total deficiency of prosaposin and therefore also of all four saposins A-D caused by a homooligal mutation in the start codon of the prosaposin gene. Patients with this perinatally fatal disease store all those sphingolipids which need a saposin cofactor for their catabolism (ceramide, glucosylceramide, lactosylceramide, sulfatides, ganglioside GM3, globotriaosylceramide etc.) and most importantly they accumulate them together with intraendolysosomal vesicular membranes. A mouse model substantiated these findings. Storage of multiple sphingolipids and accumulation of internal membranes (IMs) in the endolysosomal organelles could be reversed in cultured prosaposin deficient fibroblasts by a receptor mediated cellular uptake of the missing prosaposin from the culture medium. The prosaposin deficiency turned out to be a “proof of principle” experiment by nature supporting our hypothesis on the cell biology of sphingolipid catabolism in mammalian cells. Luminal vesicles are formed within the endolysosomal compartment as platforms for the degradation of sphingolipids and membranes.

Saposins A, B, C and D are essential for the sphingolipid- and membrane degradation at inner lysosomal membranes. They bind lipids and transfer them as water-soluble complexes from vesicular membranes to catabolizing enzymes or acceptor membranes. GM2AP forms a soluble 1:1 stochiometric complex with ganglioside GM2 which is recognized by HexA as real Michaelis-Menten complex. The dimer of saposin B also binds and solubilizes sulfatides (and other lipids) stochiometrically, presenting them to catalytic hydrolases as substrates.

In plasm resonance studies, saposins A and B were even able to solubilize most of the membrane lipids – including cholesterol and PC – from BMP (bis[p- monoacylglycerol]phosphate) rich and cholesterol poor vesicles at acidic pH values efficiently within few minutes. Saposin C even transfers bacterial lipids to the CD1b immuno-receptors of the lysosomal membrane. Unexpectedly, membrane fusion assays identified GM2AP and the saposins A, C and D – the latter stimulates the hydrolysis of ceramides by acid ceramidase also as fusogens for lipid
vesicles at lysosomal pH values. Saposins are also essential for the generation and maintenance of the water permeability barrier in the skin of land dwelling mammals. Two different phenotypes occur in prosaposin knock-out mice, a neonatally fatal one, and another one with later onset surviving up to about 5 weeks. The latter one shows a complex pathology featuring ichthyosiform dermatitis. Prosaposin knock-out mice fail to generate a proper water permeability barrier in the skin. Detailed analysis uncovered the formation of glucosylceramides with very long acyl chains of 30 up to 36 C-atoms, \(\omega\)-hydroxylated and esterified with linoleic acid, in mice and in human keratinocytes. With linoleic acid as leaving group, these glucosylceramides can be covalently bound to surface proteins of the keratinocytes in the stratum corneum of the epidermis (Fig. 7).

After removal of their terminal glucose residue by lysosomal \(\beta\)-glucocerebrosidase in cooperation with saposin C, they form a hydrophobic protein-bound surface layer of ceramides which is essential for the generation of extracellular lipid stacks (mainly composed of long chain ceramides, fatty acids and cholesterol) forming the water permeability barrier of the skin. Mutagenized mice deficient in prosaposin or in \(\beta\)-glucocerebrosidase cannot remove the terminal

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**Fig. 5.** Sap-B stimulates the enzymatic hydrolysis of micellar sulfatides with long acyl chains. Derivates of sulfatide were hydrolyzed by human arylsulfatase A in the absence or presence of Sap-B (0.1 nmol) in a total incubation volume of 50 µl. The degradation rates given are means from six experiments. Experimental values were all within a range of 20 per cent of the mean. Sulfatides contain different acyl residues in their ceramide moieties: C18-stearoyl, C8-octanoyl, C6-hexanoyl and C2-acetyl-residues.

**Fig. 6.** Human Prosaposin. The cDNA of human prosaposin codes for a sequence of 524 (or 527) amino acids including a signal peptide of 16 amino acids. The precursor is proteolytically processed in the endolysosomal compartment to 4 domains, saposin A, B, C and D. Cystein residues are marked by vertical bars, N-glycosylation sites by arrow heads. Disease causing mutations affecting human Sap-A, Sap-B, Sap-C and prosaposin have been described. Murine models of prosaposin deficiency and deficiencies of individual saposins have been investigated: Sap-A, Sap-B, Sap-C and Sap-D.
glucose residue properly and fail to complete the permeability barrier in the skin. They die soon after birth due to a permanent water loss. This observation also explains instant death of so called Collodion babies who suffer from a complete loss of β-glucocerebrosidase activity.

9. Topology of endocytosis and lysosomal digestion of membrane lipids

Macromolecules and membrane components reach the lysosomal compartment for digestion by endocytic pathways, by autophagy and by phagocytosis. They are degraded by lysosomal enzymes at acidic pH values. Their components (amino acids, fatty acids, monosaccharides, gangliosides bases and others) are released to the cytosol and reused for biosynthetic pathways and/or cellular energy production. Complex lipids and proteins of the plasma membrane reach the lysosomal compartment mainly by a vesicular membrane flow though early and late endosomes. In cultured fibroblasts, up to 5% of the endocytosed gangliosides GM1 and GM2 are diverted to the Golgi membranes and are used directly for the biosynthesis of more complex gangliosides. The original view that the majority of the endocytosed membrane lipids will become part of the limiting lysosomal membranes poses a severe topological problem for their degradation and the stability of the lysosomal perimeter membrane. Newly endocytosed components reaching the limiting lysosomal membrane should be selectively degraded without endangering its essential permeability barrier. This was a quite unlikely hypothesis. Therefore, we proposed an alternative pathway based on the formation of multivesicular bodies in normal cultured cells and the deposition of storage material in lamellar arrays within the lysosomal lumen in sphingolipidoses.

A key observation for our hypothesis was the accumulation of intralysosomal vesicles and inner membranes (IMs) in patients with an inherited prosaposin deficiency and in its mouse model. Since the accumulation of luminal vesicles and IMs could be reversed by feeding the missing prosaposin at nanomolar concentrations to the patients’ cultured fibroblasts, they were identified as transient platforms for membrane- and lipid degradation. Cellular uptake and lysosomal delivery of prosaposin are efficiently facilitated, e.g. by LRP (low density lipoprotein receptor-related protein). Immunelectron microscopy revealed that lipids of the plasma membrane, e.g. biotinylated ganglioside GM1 indeed reach the IMs of the lysosomal compartment as a transient pool for degradation of sphingolipids and other membrane components. The major part of lipid degradation was supposed to take place at their surface with the help of lipid binding and transfer proteins, the SAPs.

Our present view on the topology of lysosomal digestion is given schematically in Fig. 8. During endocytosis, transient IMs are formed by successive steps of vesicle budding and fission controlled by ESCRT proteins along the endocytotic pathways. They are prepared for digestion by a lipid sorting process beginning at the level of endosomes.

Plasma membrane stabilizing lipids are removed from the luminal vesicles. The egress of cholesterol is mediated by two sterol binding proteins, NPC-2 and NPC-1 (summarized in 114), and sphingomyelin is degraded by acid sphingomyelinase (ASM) (Breiden et al., 2012, in preparation).

Anionic BMP stimulating most of the catabolic processes in the lysosome is exclusively formed in the IMs, but not in the lysosomal perimeter membrane, which seems to be quite resistant against lysosomal digestion. It is protected by a thick glycocalix on its luminal leaflet from the attack by membrane degrading enzymes present in the lumen of the lysosome. The glycocalix is formed by membrane proteins which are highly glycosylated with polylactosamine units highly resistant toward lysosomal digestion. As most biological membranes, the lysosomal perimeter membrane is supposed to have a lateral pressure of at least 31 mN/m, too high to be attacked by GM2AP. The lysosomes are also stabilized by HSP70.

Indeed, ganglioside GM3 of the limiting lysosomal membrane is in a stable pool protected from degradation whereas it is rapidly degraded during the constitutive process of membrane turnover. The major part of membrane degradation has to proceed on the surfaces of these transient IM structures. During endocytosis, there is a lipid sorting process to prepare IMs as platforms for sphingolipid and membrane degradation (see section 12). Apparently, acid sphingomyelinase plays a major role in the maturation of IMs for lysosomal digestion.

10. Acid sphingomyelinase and Niemann-Pick disease, type A and type B

Until recently, three main clinical forms of NPD were distinguished, the infantile neuropathic...
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Fig. 8. Figure legend is on the next page.
form type A, the non-neuropathic form type B and the rather variable form type C. As an early hallmark of the disease Ernst Klenk discovered an accumulation of the membrane lipid sphingomyelin,\textsuperscript{143} known later on to be caused in type A and B patients by a deficiency of its catabolic enzyme, the lysosomal acid sphingomyelinase (ASM).\textsuperscript{144} ASM is very special among the many soluble sphingolipid degrading enzymes of the lysosome, since it does not need an activator protein for the physiological turnover of sphingomyelin. The inherited defect of prosaposin and its splice variants (Fig. 6) does not cause a substantial SM accumulation. Purification of human ASM turned out to be rather difficult and attempts failed in many laboratories including ours. A breakthrough was achieved by Lothar Quintern in his thesis work where he realized that the urine from patients with peritonitis or severe organ trauma was a rich source of the enzyme.\textsuperscript{145,146} The enzyme was purified 25000 fold before peptide sequencing, the search for its cDNA and its sequencing were possible in collaboration with Edward Schuchman.\textsuperscript{147,148} This allowed us to early identify the molecular basis of acid sphingomyelinase deficiency in a patient with NPD type A\textsuperscript{149} in parallel to Levran \textit{et al.}\textsuperscript{150} and also in patients with type B.\textsuperscript{101} The generation of ASM deficient knockout mice\textsuperscript{151} paved the way to study the molecular and cellular pathology of NPD type A and the development of therapeutic approaches for NPD type B.\textsuperscript{152}

Studies on biosynthesis and intracellular processing of the lysosomal ASM\textsuperscript{153} identified several molecular forms including secreted proforms with high enzyme activity.\textsuperscript{158} Functional characterization of its N-glycosylation sites\textsuperscript{154} and protein domains\textsuperscript{155,156} revealed that it carries a saposin like domain, binds at acidic pH values to anionic surfaces of BMP containing vesicular membranes and catalyzes membrane bound SM to ceramide and phosphorylcholine,\textsuperscript{155} even in the absence of SAPs.

In the presence of detergents, it also exhibits some phospholipase C activity cleaving PG and PC,\textsuperscript{145} an observation which became important for recent studies, using detergent free assay systems with liposomal vesicles carrying the radio labelled SM substrate (or other lipid substrates) as a model of the IM of the late endosomes.\textsuperscript{157} It became clear that ASM is a nonspecific phospholipase C and that its lipid specificity and enzyme activity are regulated by membrane lipids of the vesicular surface (see section 12). ASM is a very active but labile enzyme having a fast turnover in the lysosomal compartment,\textsuperscript{153} which is tremendously increased by cationic CADs (cationic amphiphilic drugs) like desipramine.\textsuperscript{158} \textit{In vitro} studies at endolysosomal pH values show a strong electrostatic binding of cationic ASM to negatively charged vesicles doped with BMP.\textsuperscript{159} Desipramine is endocytosed by cultured cells and compensates the negative surface charge of IMs, reducing the electrostatic binding of proteins to the vesicular surfaces. ASM is released and proteolytically degraded.\textsuperscript{156,158} In general, CADs will weaken the electrostatic interaction between lysosomal proteins and luminal lysosomal vesicles and thereby induce a phospholipidosis.\textsuperscript{160,161}
11. Lysosomal acid ceramidase and Farber disease

The last big protein contamination, which was separated by Lothar Quintern from ASM during its purification, turned out to be acid ceramidase (AC)\textsuperscript{145}. This allowed purification, structural analysis and characterization of AC,\textsuperscript{162} the cloning of its cDNA and for the first time the identification of the molecular defect in a patient with Farber disease.\textsuperscript{162–164} The enzyme appears to be rather slow and cleaves ceramide to sphingosine and fatty acid at the surface of lipid vesicles mimicking IMs of the lysosomes. The surface reaction is reversible\textsuperscript{164,165} and interfacially regulated by membrane lipids and saposins.\textsuperscript{113} The cloning of the cDNA and genomic sequences encoding the murine AC\textsuperscript{156} allowed its insertional mutagenesis of mouse AC.\textsuperscript{167} It led to early embryonic lethality, demonstrating the importance of AC for embryonic development. At that time, we already knew that sphingosine is not an intermediate in de novo sphingolipid biosynthesis.\textsuperscript{168,169} Mammalian cells generate ceramide by dehydrogenation of dihydroceramide which is formed by de novo biosynthesis at the ER. After lysosomal degradation of sphingolipids to ceramide, the latter is hydrolyzed by AC to fatty acid and free sphingosine. The latter is the direct precursor of sphingosine-1-phosphate, an important regulator and stimulator of cellular growth, mobility and survival (reviewed in ref. 170). It may well be that a deficiency of sphingosine-1-phosphate contributes to the early embryonic lethality observed in AC deficient mice embryos.\textsuperscript{167}

12. NPC and an emerging role of ASM in endosomal lipid sorting

Niemann-Pick disease type C (NPC) is a complex neurodegenerative lipid storage disease characterized in systemic tissues by the accumulation of unesterified cholesterol, sphingomyelin, some glycolipids and a broad range of other lipids in the late endolysosomal compartment.\textsuperscript{171} The disease is caused by mutations in either of the genes of the cholesterol binding proteins, Niemann-Pick C1 (NPC-1) and Niemann-Pick C2 (NPC-2).\textsuperscript{174} NPC-1 is a late endolysosomal glycoprotein with 13 transmembrane domains\textsuperscript{175} and NPC-2 is a glycosylated, soluble protein.\textsuperscript{176} In a proposed model,\textsuperscript{114,130} soluble NPC-2 removes cholesterol from inner endosomal/lysosomal vesicles and delivers it to NPC-1 in the limiting membrane of endosomes/lysosomes for cholesterol egress from the late endocytic compartments\textsuperscript{177–179} (Fig. 8). Inherited defects of NPC-1 or NPC-2 can cause an accumulation of cholesterol in the late endosomes and lysosomes. Endosomal cholesterol accumulation triggers an increase of SM and GSL in NPC disease whereas ASM deficiency triggers SM storage and a cholesterol accumulation in Niemann-Pick disease types A and B.\textsuperscript{180}

As increasing cholesterol levels have a substantial inhibitory effect on the activity of some lysosomal lipid binding and transfer proteins such as Sap-A and -B,\textsuperscript{110,111} their increase in the lysosomes may well attenuate the turnover of other membrane lipids such as sphingomyelin, neutral glycolipids (e.g., glucosyleceramide, lactosylceramide), gangliosides GM3 and GM2,\textsuperscript{181} BMP,\textsuperscript{182} and sphingosine,\textsuperscript{183} causing a traffic jam in the endolysosomal compartment of NPC diseased cells.

Cellular plasma membranes (PMs) are enriched in membrane stabilizing lipids like cholesterol and sphingomyelin. They form short-lived microdomains in cellular membranes,\textsuperscript{184} reduce the permeability for sodium ions and keep many receptors and ion pumps, e.g. the ATP dependent Na\textsuperscript{+} and K\textsuperscript{+} pump at optimal activity.\textsuperscript{185} As stabilizing lipids they would impede sphingolipid and membrane degradation in the endolysosomal system\textsuperscript{110,111,114,186} and, therefore, have to be removed during the endocytic pathway.

For digestion of membrane components, IMs are formed along the endocytic pathway. They are prepared for degradation by a lipid sorting process, removing membrane stabilizing lipids like cholesterol and sphingomyelin and generating the degradation-stimulating BMP\textsuperscript{82,84,114}

Major proteins involved in endosomal lipid sorting at the IMs are NPC-2 and ASM. According to recent studies, both, enzyme activity of ASM and
cholate transfer activity of NPC-2, are regulated by membrane lipids.\textsuperscript{114,187} ASM turned out to be a rather nonspecific phospholipase C. Besides its known substrate sphingomyelin, it also cleaves PG, PC, PA and more than 15 other phospholipids including BMP in a detergent-free liposomal assay system.\textsuperscript{187} Surprisingly, its activity against vesicle bound lipid substrates is effectively regulated by the lipid composition of the vesicles. Whereas cholesterol inhibits PC hydrolysis, anionic phospholipids, PG and PA derived from the plasma membrane stimulate sphingomyelin hydrolysis by ASM, and BMP—which is generated after IMs are formed—specifically enhances the degradation of PC, PG and other phospholipids.\textsuperscript{187} These observations suggest a sequence of events for the removal of stabilizing lipids from the IMs of the late endosomes and their final degradation in the lysosomes: Firstly, in the late endosomes, ASM, stimulated by plasma membrane-derived PG, PI, PA and by BMP, degrades SM, PG and other phospholipids of the IMs to ceramide and DAG. Secondly, the disappearance of SM in combination with the generation of Cer, DAG and the anionic BMP enhances the removal of cholesterol from IMs.\textsuperscript{114,187} Along the further pathway of endocytosis, phospholipase A\textsubscript{2} and other lipases will transiently generate substantial amounts of free fatty acids and lysophospholipids. During these processes in the late endosomes, IMs and their bilayer structures will collapse and lose their topology and barrier function. Micellar-like lipid and glycosphingolipid-rich aggregates—still containing hydrophobic protein domains—will be presented to the luminal juice of the lysosomes for further degradation. Complex lipids and other macromolecules are degraded in the lysosomal compartment and their components are released to the cytosol for biosynthetic pathways and energy metabolism. In differentiated cells, glycosphingolipids will be generated mainly by such salvage pathways.\textsuperscript{188,189}

13. Ganglioside biosynthesis

Glycolipid pattern of cellular surfaces are cell type specific and depend on the stage of differentiation.\textsuperscript{190–192} They result from coordinated processes involving biosynthesis of these compounds in the ER and Golgi membranes, intracellular transport processes and the constitutive degradation of glycolipids mainly in the endolysosomal compartment.\textsuperscript{193} De novo biosynthesis of the hydrophobic membrane anchor of sphingolipids takes place in the ER and is controlled by 6 different ceramide synthases.\textsuperscript{194} The pathway of ceramide formation was mainly worked out in the late 60s.\textsuperscript{195,196} Unresolved, however, remained the origin of sphingosine formation either by direct dehydrogenation of sphinganine (dihydroceramide) or after dehydrogenation of dihydroceramide followed by ceramide hydrolysis. Our analysis showed that sphingosine is not an intermediate of de novo sphingolipid biosynthesis but a product of ceramide hydrolysis.\textsuperscript{168,187} This implies that most of the sphingosine is generated by the lysosomal acid ceramidase, released from the lysosome and used in the ER for biosynthesis of sphingolipids and sphingosine-1-phosphate, a major signaling molecule for cellular growth and migration.\textsuperscript{170} In differentiated cells, e.g., neurons, about 70–90% of newly formed sphingolipids are generated by such salvage pathways,\textsuperscript{188,189} illuminating the importance of lysosomal digestion for cellular biosynthetic pathways. Ceramide and dihydroceramide are the biosynthetic precursors of sphingomyelin, GSL and gangliosides, the major storage compounds in gangliosidoses.\textsuperscript{193,198}

The first biosynthetic steps leading to the formation of ceramide are controlled by membrane-bound enzymes at the cytosolic face of the ER.\textsuperscript{169,199} Formation of lactosylceramide, which is the precursor of the higher gangliosides, occurs on the luminal face of the Golgi membranes.\textsuperscript{200,201} Pathways for the biosynthesis of the main neuronal gangliosides had been worked out by the group of Saul Roseman.\textsuperscript{202–204} (Fig. 9). We wanted to know if the glycosyltransferases of these pathways also need cofactors for full activity as the catabolic enzymes in the lysosome and how the glycosyltransferases of the a- and b-pathways differ in their specificities. We could not find any evidence for the involvement of essential protein cofactors for the biosynthetic steps. However, in detergent free assays, tunicamycin inhibited and anionic membrane lipids, e.g., PG stimulated the biosynthetic rates substantially,\textsuperscript{205–207} suggesting a regulation of these biosynthetic steps by membrane lipids.

The interaction of glycosyltransferases and their glycolipid substrates—both bound to the same membrane—however, appeared to occur by diffusion within the plane of the membrane. Therefore, it does not follow the classical Michaelis-Menten kinetics for the interaction of water-soluble substrates and enzymes, but rather a model for 2-dimensional kinetics which we developed for the interaction of
membrane-bound enzyme (sialidase) and substrate (ganglioside GD1a).208,209)

Our main interest was to clarify the differences between the glycosyltransferases involved in the biosynthesis of the α-series of gangliosides on one hand, and of the β-series on the other hand (Fig. 9).

In his thesis work, Gottfried Pohlentz could not detect any significant differences between the two GalNAc transferases, converting the precursors of the α- and β-pathways into gangliosides GM2 and GD2 (Fig. 9). Therefore, I asked him to test if gangliosides GM2 and GD2 are formed by one and the same biosynthetic enzyme. In fact, both substrates, GM3 and GD3, competed for the same active site of one and the same GalNAc transferase210) (Fig. 10).

Using the same experimental approach given in Fig. 10 for the other glycosyltransferases established the scheme of Combinatorial Ganglioside Bio-
synthesis (Fig. 9). First, LacCer is sialylated to form the small ganglioside precursors (GM3, GD3, GT3), which are then used by a small number of promiscuous glycosyltransferases to generate a multitude of complex gangliosides. This scheme allows the generation of cell type specific patterns on cell surfaces depending on the availability of precursors, expression of glycosyltransferases and their regulation.

Since enzyme kinetic evidence is usually not accepted as final proof, I contacted Rick Proia at the NIH for a collaboration to test the validity of the scheme by a genetic approach. The generation of mutant mice models with defects in the biosynthetic pathways of ganglioside biosynthesis and their morphological analysis in collaboration with Kimiko Suzuki validated the scheme given in Fig. 9 (review in refs. 211, 212). Mutant mice carrying glycosyltransferase defects often suffered from severe and
Fatal diseases. Whereas a defect of GD3-synthase still allows a normal life span, a defective GM3-synthase causes an early fatal disease: The absence of all the major neuronal gangliosides triggers the formation of 0-series gangliosides, which are not observed in wild type brain tissue. Their additional synthesis, however, cannot compensate the loss of all the major neuronal gangliosides. A defect of the biosynthetic enzyme, GalNAc transferase, blocking the synthesis of complex glycolipids, causes the myelin sheets to detach from axonal membranes and triggers male infertility.

On the other hand, a block in the catabolism of complex gangliosides also causes a fatal neuronal storage disease in men and mice. However, if the mutant mice with ganglioside storage carry an additional defect in the biosynthesis of complex gangliosides, their neurological functions improve and they have a prolonged life span, suggesting that substrate deprivation may be a helpful treatment for sphingolipid storage disease.

A block in glucosylceramide synthesis is lethal already at embryonic day 6. It is still unclear why, since cell type specific defects of glucosylceramide synthase in neurons, hepatocytes, or keratinocytes still allow embryogenesis and birth of an animal. On the other hand, cultivated mutant teratoma cells with glucosylceramide deficiency have a normal growth rate in cell culture. Apparently, glucosylceramide and some of its derived complex glycolipids of the cellular surfaces including the SSEAs are involved in cellular adhesion and are therefore needed for the development of a multicellular organism.

Another surprising finding was the almost unchanged level of total gangliosides in the brains of the various mutant mice. It was still in the range of the wild type (Fig. 11). Apparently, mice with defects in the synthesis of major gangliosides try to keep the overall ganglioside concentration on neuronal surfaces constant. The level of the minor compound GM3 increases in the GM3-only mouse.
14. Analysis of sphingolipids

At the beginning, our studies were enabled by the development of analytical procedures for the separation and identification of (glyco-) sphingolipids pioneered by Horst Jatzkewitz.2,220,221 For quantification by thin-layer chromatography we developed a sample applicator which became the forerunner of the Linomat now commercially available (from Camag, Switzerland).6,220–223 Later we also used NMR techniques to establish the stereochemical D-erythro configuration of the sphingoid bases in some storage compounds224 and improved mass spectrometric procedures for the analysis of ceramides and some neutral glycosphingolipids.225,226

15. Chemical biology and the synthesis of sphingolipid analogs

For metabolic and cell biological studies we needed suitable probes and markers which often were not commercially available at the time. Therefore, we developed methods to synthesize labelled sphingolipids for metabolic and enzymatic studies.

- Radiolabeled glycosphingolipids and their use: e.g. refs. 22, 227–233.
- Lysogangliosides as key precursors for the synthesis of labeled or tagged gangliosides.128,234,235
- Spin labeled sphingolipid analogues carrying NO groups in their acyl residues for ESR measurements to study insertion of gangliosides into membranes of cultured cells.236–238
- C-Glycosides as hydrolysis resistant analogues.239,240
- Photosensitive ligands241 to label HexB,242 and GM2AP.243
- Fluorescent labelled gangliosides were developed to study their biophysical behaviour in lipid bilayers and cellular membranes.184,244,245
- Hydrolysis resistant analogues of GSL and double radiolabeled gangliosides GM1 and GM2 were used for metabolic studies in cultured wild type and mutant cells derived from patients with blocks in ganglioside catabolism. The ganglioside micelles were adsorbed by the cultured human fibroblasts,31 and ganglioside molecules were slowly inserted into the bilayer of the plasma membrane, endocytosed and most of them catabolized.31 However, a portion of about 5% of the endocytosed gangliosides GM1 and GM2 apparently reached the TGN intact and were glycosylated to more complex gangliosides, mainly ganglioside GD1α.31
- Membrane spanning labeled lipids were synthesized for an uncompromised monitoring of membrane fusion, intermembrane lipid transfer and solubilization of membrane lipids.246 They are needed to analyse the complex functions of saposins (see chapter 8). Besides their role in lysosomal catabolism of sphingolipids and biological membranes and the generation of the water permeability barrier in the
skin of land dwelling mammals, they can mediate intermembrane lipid transfer and vesicle fusion. During the development of \textit{in vitro} assays for membrane fusion, intermembrane lipid transfer and lipid solubilization, it became necessary to develop vesicle markers which cannot be extracted by saposins, as the commercially available ones (\textit{e.g.} Biotin-PE, NBD-PE and Rhodamine-PE). Therefore, we developed extraction resistant membrane spanning lipids carrying fluorescent labelled headgroups for FRET assays and biotinylated headgroups for magnetic separation of lysosomal vesicles.

\begin{itemize}
  \item General anaesthetics and the turnover of SL, SM and GD1a in plasma membranes
  \end{itemize}

\textit{In vitro}, amphiphilic ganglioside GD1a inserted into isolated neuronal plasma membranes is readily hydrolyzed by the membrane-bound sialidase.\textsuperscript{209} Surprisingly, its degradation rate was enhanced substantially by lipophilic general anaesthetics such as laughing gas (N\textsubscript{2}O) or halothane, but not that of water-soluble substrates, \textit{e.g.} of sialylactose. Membrane fluidity also correlated quantitatively with an increased GD1a hydrolysis when a series of primary alcohols, n-pentanol to n-octanol was used. Interaction of membrane-bound GD1a substrate and the enzyme sialidase occurred mainly within the plane of the membranes, presumably by lateral diffusion.\textsuperscript{208,209} Modified Michaelis-Menten kinetics for the 2-dimensional interaction of substrate and enzyme were developed which allowed a proper calculation of reaction rates.\textsuperscript{208}

The increased degradation rate of the lipophilic GD1a paralleled an increase of membrane fluidity.\textsuperscript{217} General anaesthetics like halothane and Xenon also enhanced membrane fluidity and sphingomyelin degradation by membrane-bound neutral sphingomyelinase of synaptosomal plasma membranes from calf brain.\textsuperscript{248} A significant increase of SM degradation was already observed at clinical concentrations, increased halothane concentrations stimulated SM hydrolysis up to 50 fold.\textsuperscript{248}

The molecular basis of these observations—possibly changes in the microdomain structure of the membranes—was never clarified. However, new biophysical methods became available recently to approach this interesting phenomenon.

\textbf{16. Acknowledgements and outlook}

I like to thank my students and coworkers who worked enthusiastically and hard to uncover some of the secrets surrounding the role of sphingolipids in the life of cells and men. During our long trip, some biochemical and cell biological concepts became visible, \textit{e.g.} the lipid phase problem involving the complex functions of lysosomal activator proteins, the role of intralysosomal membranes in lipid and membrane degradation, and metabolic principles such as the combinatorial ganglioside biosynthesis.

We are grateful for the productive collaborations with external groups, whose expertise and endeavour allowed to tackle these and other questions (Kuni and Kinuko Suzuki, Rick Proia, Al Merril, Marie Therese Vanier, Ed Schuchman, Heinz Egge, Wolfram Saenger, Walt Holleran, Richard R. Schmidt, Klaus Harzer, Derek Marsh, Volker Herzig and others). These collaborations contributed to the discovery and analysis of several rare inherited sphingolipid storage diseases (gangliosidoses, Niemann-Pick and Faber disease and activator protein deficiencies). However, during our journey more questions came up than answers, \textit{e.g.} on the regulation and function of sphingolipids in biological membranes. Also their cellular metabolism, intracellular trafficking and the development of therapeutic approaches for sphingolipidoses remain wide open areas for future research.

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\begin{center}
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Profile

Konrad Sandhoff was born in Berlin, Germany, in 1939. He studied Chemistry at the Ludwig-Maximilians-University in Munich, where he received his PhD in 1965.

His research career started at the Max-Planck-Institute for Psychiatry in the lab of Horst Jatzkewitz, Dept. of Neurochemistry, with short stops in the labs of Saul Roseman (Johns Hopkins University in Baltimore) and Ruth Arnon (Weizmann Institute in Rehovot). In 1979 he became a full professor for Biochemistry at the Kekulé-Institute in Bonn, Germany.

He gave the first description of GM1 gangliosidosis, and identified the molecular defects in Tay-Sachs disease and in three other newly discovered GM2-gangliosidoses: variant 0 (now known as Sandhoff disease), variant B1 and variant AB. He proposed intraluminal vesicles in endosomes and lysosomes as sites of cellular membrane digestion and established the so-called threshold theory as basis for the different clinical forms of lysosomal storage diseases. Synthesis of fluorescent, radiolabelled and biotinylated (glyco-) sphingolipids was used to study their biosynthetic pathways and cellular turnover.

He discovered omega hydroxylated and acylated glucosylceramides with acyl chains of up to 36 carbon atoms as essential factors for the formation of the water permeability barrier of the human skin. The purification and characterization of human acid sphingomyelinase and acid ceramidase was achieved in his laboratory, together with the characterization of the first mutations as cause for Niemann-Pick disease, type A, and Farber disease.

He was Dean of the Science Faculty (1992–1994), and since 2007 Senior Professor at the LIMES Institute of the University of Bonn. He is a member of different scientific organizations, for example the Gesellschaft Deutscher Naturforscher und Ärzte (President 2005–2006) or the Society for Biochemistry and Molecular Biology, GBM (President from 1999–2001), EMBO, the German Academy, Leopoldina, and was chairman of the Advanced Research Group (SFB) on Glycoconjugates and Structures of Cell Surfaces from 1991–2002.

Konrad Sandhoff was laurelled among others with the Shield of the Medical Faculty, University of Tokyo (1973); the Carl-Duisberg-Prize (1976) and the Richard-Kuhn-Medal (1992) of the GDCh, Chemical Society of Germany; the Heinrich-Wieland-Prize 1979; Honorary Membership of the ASBMB (1992); the K. J. Zülch Prize (1998) and the Research Award (1999) of the Max-Planck-Society; the Mendel-Medal of the Leopoldina (2001); Johns-Hopkins Society of Scholars Membership (2001); the Robert-Pfleger-Prize (1994); the International Glycoconjugate Organization Award 2005; the Otto-Warburg Medal of the GBM (2006); and the European Lipid Science Award (2008).