Blocks in the Catabolism of Sulphatides and Gangliosides as a Cause of Human Neurological Diseases

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As far as is known, all the human storage diseases with a recessive mode of inheritance are due to a disturbed turnover of the accumulated substances caused by a deficiency in their catabolic enzyme systems. From a genetic point of view this deficiency can be expected in all cases showing the storage of a metabolic end product in a biosynthetic pathway. However, it can also be assumed in those cases with an excess of a metabolic intermediate. These two possibilities are realized in sphingolipidoses.

An example for the first possibility (accumulation of a metabolic end product) is metachromatic leukodystrophy. This demyelinating disease is marked by an excess of sulphatides, predominantly in brain white matter and kidney (Jatzkewitz, 1958, 1960; Austin, 1959). Sulphatides are cerebroside 3-sulphates biosynthesized from cerebroside and 'active sulphate' (adenosine 3'-phosphate 5'-sulphatophosphate) (McKhann, Levy & Ho, 1965). Conversely they are catabolized into cerebroside and sulphate (Mehl & Jatzkewitz, 1964). The enzyme responsible for the latter reaction is termed cerebroside sulphatase. It is this enzyme that is deficient in metachromatic leukodystrophy (Jatzkewitz & Mehl, 1969). On the other hand it was shown that this deficiency is not restricted to cerebroside sulphatase acting on a physiological substrate but also involves an enzyme, arylsulphatase A, that acts on an unphysiological substrate, namely 2-hydroxy-5-nitrophenyl sulphate (Austin *et al.* 1963). With the one gene-one enzyme (polypeptide chain) principle in mind and assuming only a single genetic defect in this disorder, it has been proposed that the physiological substrate of arylsulphatase A is cerebroside sulphate (Mehl & Jatzkewitz, 1968).

An example for the second possibility (accumulation of an intermediate) is infantile amaurotic idiocy. This condition, affecting grey matter in brain, is primarily due to the accumulation of gangliosides (and derivatives) in nervous tissue. Therefore it is also termed 'gangliosidosis'. Gangliosides are neuraminic acid (= sialic acid)-containing glycosphingolipids (Klenk, 1942). The 'glyco' moiety of the major monosialosylganglioside (ganglioside G_{M1}) is an oligosaccharide chain of the tetrahexosyl type with a single *N*-acetylneuraminic acid residue (Kuhn & Wiegandt, 1963) (Table 1).

The following considerations are restricted to the catabolism of ganglioside G_{M1} , since as far as is known all the gangliosidoses are caused by a deficiency of the individual enzymes involved in the stepwise

degradation of ganglioside G_{M1} and related substances. The degradation of ganglioside G_{M1} occurs via the lower ganglioside homologues G_{M2} and G_{M3} (Gatt, 1967) (Table 1). Accordingly a deficiency of the β -galactosidase (Dacremont & Kint, 1968; van Hoof & Hers, 1968; Okada & O'Brien, 1968) removing the final galactose of the tetrahexosyl chain leads to an accumulation of ganglioside G_{M1} (and its asialosyl residue) in brain. An increase of ganglioside G_{M1} in the visceral organs is accompanied by an increase of mucopolysaccharides (Suzuki, 1968), giving rise to a 'generalized G_{M1} gangliosidosis'.

The resulting degradation product of ganglioside G_{M1} is its homologue G_{M2} (and its asialosyl residue). This is strongly accumulated in brains of patients suffering from the conventional Tay-Sachs disease (Svennerholm, 1962) and also from an exceptional form of Tay-Sachs disease with visceral involvement (Sandhoff, Andreae & Jatzkewitz, 1968a,b). This visceral involvement (only histologically recognizable) is due to an additional storage of kidney globoside (Table 1). The exceptional case and the conventional one show a similar, only quantitatively different, accumulation pattern of substances. Thus the excess of the asialosyl residue of ganglioside G_{M2} is somewhat less and that of kidney globoside considerably less in the conventional cases than in the exceptional one. Since all three glycolipids have in common β -linked *N*-acetylgalactosamine in the terminal position of the oligosaccharide chain, it seems reasonable to assume a deficient β -*N*-acetylgalactosaminidase (β -*N*-acetylhexosaminidase) to be a cause of their accumulation. The human β -*N*-acetylhexosaminidase can be resolved by chromatography (Robinson & Stirling, 1968) and by electrofocusing (Sandhoff, 1968) into two main fractions representing the bulk of two multiple forms. Both fractions were absent in the exceptional case (Sandhoff, 1969) whereas only one was present in the conventional cases (Okada & O'Brien, 1969; Sandhoff, 1969).

In view of the similarity of the stored substances in these two conditions it can be supposed that the substrate specificity for the two multiple β -*N*-acetylhexosaminidase fractions is also very similar. Indeed (K. Sandhoff & W. Waessle, unpublished work) both highly purified preparations cleaved off the terminal hexosamine when *p*-nitrophenyl β -hexosaminide and the asialosyl residue of Tay-Sachs ganglioside were used as substrates. Both preparations also degrade Tay-Sachs ganglioside to give a substance with the R_F -value of monosialosyl-lactosylceramide (ganglioside G_{M3}) when thin-layer chromatography was employed. This activity, although very slight, was much more pronounced in that fraction lacking in the conventional cases of Tay-Sachs disease.

Surprisingly, both enzyme fractions were also present in brain tissue of a conventional Tay-Sachs

Table 1. *Gangliosides and related glycolipids, and their accumulation by deficient catabolic enzymes resulting in human diseases*

Abbreviations: IAI, infantile amaurotic idiocy; Glc, glucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; NeuNAc, *N*-acetylneuraminic acid. The deficient catabolic enzymes are indicated by broken lines.

$\begin{array}{c} \text{Glycosphingolipid} \\ \text{Ceramide (= N-acylsphingosine)} \\ \begin{array}{c} \\ \text{X} \end{array} \left(\begin{array}{c} \\ \text{X} \end{array} \right) \\ \text{X} \end{array}$	Disease caused by accumulation	Predominant localization of accumulated substances	Enzyme deficiency (where known)
$\begin{array}{c} \text{Ganglioside} \\ \text{NeuNAc-free residue} \\ \text{X = Glc-Gal-GalNAc-Gal} \\ \text{NeuNAc} \\ \text{Major monosialosylganglioside G}_{M1} \end{array}$	Exceptional cases of IAI with visceral involvement ('generalized gangliosidosis', 'pseudo-Hurler') (G _{M1} -gangliosidosis)	Brain (grey matter), visceral organs and bones (with excess of mucopolysaccharides)	β -Galactosidase
$\begin{array}{c} \text{---} \text{+} \text{---} \\ \downarrow \\ \text{= Glc-Gal-GalNAc} \\ \text{NeuNAc} \\ \text{Tay-Sachs ganglioside G}_{M2} \end{array}$	Conventional cases of IAI (Tay-Sachs disease) (G _{M2} -gangliosidosis)	Brain (grey matter)	Partial β -N-acetylhexosaminidase (hexosaminidase A)
$\begin{array}{c} \text{---} \text{+} \text{---} \\ \downarrow \\ \text{= Glc-Gal-Gal-GalNAc} \\ \text{NeuNAc} \\ \text{Kidney globoside} \end{array}$	Exceptional cases of Tay-Sachs disease with visceral involvement (G _{M2} -gangliosidosis with visceral storage of globoside)	Brain (grey matter), visceral organs (with storage of globoside)	Total β -N-acetylhexosaminidase
$\begin{array}{c} \text{---} \text{+} \text{---} \\ \downarrow \\ \text{= Glc-Gal} \\ \text{NeuNAc} \\ \text{G}_{M3} \text{ (haematoside)} \end{array}$	Neurovisceral lipidosis (G _{M3} -gangliosidosis?)	Brain (grey matter), visceral organs	
$\begin{array}{c} \text{---} \text{+} \text{---} \\ \downarrow \\ \text{= Glc-Gal} \\ \text{NeuNAc} \\ \text{G}_{M3} \text{ (haematoside)} \end{array}$	Infantile Gaucher's disease	Visceral organs, brain (grey matter)	Total β -glucosidase?

case (Sandhoff, 1969). Thus it seems as though in this condition the molecular organization of the catabolic enzymes involved plays an additional role.

Since the β -N-acetylhexosaminidase is lysosomal in origin and its partial or total lack in Tay-Sachs disease can be shown in all the tissues investigated and even in tissue culture from skin fibroblasts (Okada & O'Brien, 1969), the enzymic make-up of lysosomes seems to be species-specific rather than tissue-specific regardless of whether the substrate is present in large or minimal amounts.

Up to now it seems that the two conditions in Tay-Sachs disease are chiefly due to the different pattern of multiple enzymes. The same should be true for those conditions causing the infantile and adult forms of Gaucher's disease. The adult form with visceral involvement should only show a deficiency of some β -glucosidase, leading to an excess of glucosylceramide deriving from substance classes other than gangliosides, whereas the infantile form, which includes the ganglioside metabolism (Svennerholm, 1966) (Table 1), should be due to a more complete deficiency in β -glucosidase. The experimental findings support this assumption (Brady, Kanfer & Shapiro, 1965).

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Enzymic Defects in Lipid-Storage Diseases

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The nature of the metabolic abnormalities in all of the major sphingolipid-storage diseases has been established within the past 5 years. This remarkable progress is due largely to two factors. The first is the demonstration that the synthesis of the sphingolipid that accumulates in the various tissues in patients with two of the more common lipidoses, Gaucher's disease and Niemann-Pick disease, occurs at a normal rate (Trams & Brady, 1960; Crocker & Mays, 1961). These observations set the stage for the undertaking of experiments in which the catabolism of the involved lipid was examined in normal human tissues and in those from patients with lipid-storage diseases. The latter investigations postdated the synthetic studies by more than 5 years and they became truly feasible only after suitable techniques had been devised for labelling the accumulating complex lipids. Several procedures were developed for introducing radiotracer elements into these lipid molecules. Investigations with the labelled materials provided in rapid succession for the explicit identification of the enzymic defects in Gaucher's disease, Niemann-Pick disease, Fabry's disease, and very recently in Tay-Sachs disease. The procedures and the results obtained in these investigations are briefly summarized in the present communication.

Excessive quantities of the sphingolipid glucocerebroside (ceramide-glucose) accumulate especially in the peripheral tissues of patients with Gaucher's disease. The chemical synthesis of glucocerebroside with ^{14}C in the glucose portion of the molecule was achieved in 1964 (Brady, Kanfer & Shapiro, 1965a). With this material as a substrate, an enzyme was demonstrated in all mammalian tissues examined so far that catalyses the hydrolytic cleavage of the glucose portion of the molecule. The characteristics of this glucocerebroside were determined and the activity of this enzyme was measured in tissue specimens from control human sources and from patients with Gaucher's disease. The mean activity of glucocerebroside in tissue specimens from patients with the adult form of Gaucher's disease was decreased to 15% of that of the control series (Brady, Kanfer & Shapiro, 1965b). Glucocerebroside activity is virtually absent in tissues from patients with the infantile form of Gaucher's disease (Brady, Kanfer, Bradley & Shapiro, 1966). These experi-