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-

ments clearly demonstrate that the metabolic lesion in Gaucher's disease is a deficiency of the enzyme glucocerebrosidase. The major source of the glucocerebroside that accumulates in peripheral tissues of patients with Gaucher's disease is believed to be cellular lipids from senescent leucocytes (Kattlove *et al.* 1969). The precursors of the glucocerebroside that accumulates in neuronal cells in patients with the infantile form of Gaucher's disease are probably the gangliosides, which undergo rapid turnover during the neonatal period.

Excessive quantities of sphingomyelin (ceramide-phosphorylcholine) accumulate in tissues of patients with Niemann-Pick disease. The nature of the enzymic defect in this condition was demonstrated in an analogous fashion to that employed for Gaucher's disease. [^{14}C]Sphingomyelin was chemically synthesized with the label in the choline portion of the molecule (Kanfer, Young, Shapiro & Brady, 1966). An enzyme is present in most mammalian tissues that catalyses the hydrolytic cleavage of the phosphorylcholine moiety of sphingomyelin. The activity of this enzyme was measured in control human tissue samples and those obtained from patients with Niemann-Pick disease. The sphingomyelinase activity in patients with the classic infantile form of Niemann-Pick disease was only 7% of that found in the control samples (Brady, Kanfer, Mock & Fredrickson, 1966). Patients with clinically less rapidly progressing forms of Niemann-Pick disease also have diminished sphingomyelinase activity in their tissues; however, the residual activity is higher than that in the patients with the classic infantile form of Niemann-Pick disease (Brady, 1969).

The ceramide trihexoside galactosylgalactosylglucosylceramide accumulates in various tissues of patients with Fabry's disease. This accumulation causes particular damage to the kidneys and impairment of renal function. The metabolic disturbance in Fabry's disease is transmitted on the X-chromosome. Thus females are carriers of the defect and only males show severe clinical manifestations of the disease. In order to demonstrate the enzymic defect in Fabry's disease, ceramide trihexoside was labelled throughout the molecule by exposure to tritium gas according to the Wilzbach procedure. The recovered radioactive compound was purified by dialysis, repeated recrystallization from organic solvents and preparative t.l.c. Through the use of this ^3H -labelled material as substrate, an enzyme was demonstrated and partially purified from rat intestinal tissue that catalyses the hydrolysis of only the terminal molecule of galactose of ceramide trihexoside (Brady, Gal, Bradley & Mårtensson, 1967a). The activity of this ceramide trihexosidase was determined in intestinal biopsy specimens from control human sources and from patients with Fabry's dis-

ease. Tissue specimens from hemizygous males with Fabry's disease had no ceramide trihexosidase activity (Brady *et al.* 1967b). Heterozygous female carriers of the Fabry trait had an intermediate ceramide trihexosidase activity in their tissues.

Still another labelling technique was employed to demonstrate the metabolic defect in Tay-Sachs disease. A glycolipid called Tay-Sachs ganglioside [*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide] accumulates in tissues of patients with this disease. This compound was labelled in either the *N*-acetylneuraminyl or *N*-acetylgalactosaminyl moiety through a combination of biosynthetic and selective enzymic degradation procedures (Kolodny, Brady, Quirk & Kanfer, 1969a, 1970). The enzymic hydrolysis of the *N*-acetylneuraminyl moiety of Tay-Sachs ganglioside in tissue specimens from patients with Tay-Sachs disease was found to be equal to that in the control tissue samples (Kolodny *et al.* 1969a). This observation indicated that the catabolism of Tay-Sachs ganglioside labelled in the *N*-acetylgalactosaminyl moiety should be investigated. Experiments performed with Tay-Sachs ganglioside labelled in the hexosaminyl portion of the molecule revealed that there is a complete absence of the hexosaminidase that catalyses the cleavage of the *N*-acetylgalactosaminyl residue of Tay-Sachs ganglioside in tissues obtained from patients with Tay-Sachs disease (Kolodny, Brady & Volk, 1969b). While these experiments were in progress, Okada & O'Brien (1969) clearly showed through the use of the artificial substrates *p*-nitrophenyl β -D-*N*-acetylgalactosaminide and 4-methylumbelliferyl β -D-*N*-acetylgalactosaminide that there was a missing hexosaminidase component in extracts of tissues obtained from Tay-Sachs patients in spite of an overall increase in total hexosaminidase activity as measured by these substrates (Sandhoff, Andreae & Jatzkewitz, 1968). Therefore, even though the nature of the specific metabolic defects in lipid-storage diseases must be conclusively established by experiments with authentic accumulating lipids, it seems likely that, through the proper selection of assay conditions, the use of artificial substrates may become an increasingly important adjunct to the clinical chemist's diagnostic armamentarium (Öckerman & Köhlin, 1968; Percy & Brady, 1968).

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Phospholipase A Activities in Nervous Tissues

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The phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) activities in snake venoms and in several mammalian tissues have been extensively studied in recent years, but relatively little is yet known about this group of enzymes in nervous tissues.

Evidence for the presence of phospholipase A in brain was first reported by Gallai-Hatchard, Magee, Thompson & Webster (1962), who showed that when aqueous-glycerol extracts of acetone-dried brain were incubated at pH 7.3 with diacylglycerophosphatides and deoxycholate there was substantial formation of lyso derivatives, but the structure of the formed lyso derivatives and the site(s) of action of brain phospholipase on the ester bonds at the 1- and 2-positions of the substrates was not known. Webster & Cooper (1968) incubated rat cerebrum homogenates at pH 7.4 with trace amounts of 2-[¹⁴C]acyl-labelled or [³H]choline-labelled lecithins (phosphatidylcholines). From the pattern of radioactive hydrolysis products it was concluded that both acyl ester bonds of lecithin had been split, forming a mixture of isomeric lysolecithins (1- and 2-acyl-glycerophosphorylcholine), thus indicating that brain contained two positionally specific types of phospholipase, A₁ and A₂ (see van Deenen & de Haas, 1966), operative at physiological pH.

A phospholipase A₁, splitting off only the 1-linked fatty acid of diacylglycerophosphatides, has been isolated from rat and calf brain (Gatt, Barenholz &

Roitman, 1966; Gatt, 1968); this enzyme had optimum activity at pH 4.0 and was inactive at pH 7.4. There is evidence therefore that nervous tissues contain a number of types of phospholipase A activity, differing in pH optima, positional specificity and possibly other properties; recent studies (M. F. Cooper & G. R. Webster, unpublished work) have been concerned with extracting and defining these enzymes more precisely.

Acetone- and butan-1-ol-treated tissue powders were extracted with cold 0.9% NaCl to give clear lipid-free extracts containing about 10mg of protein/ml. Extracts of rat cerebrum were incubated for 1 or 2h with (1-[³H]stearoyl)lecithin (2.5mm) in the presence of sodium taurocholate (3mg/ml) between pH 3.8 and 8.5. Release of [³H]stearic acid was optimum at pH 4.2 and at this pH only traces of labelled lysolecithin were formed. Production of [³H]lysolecithin, on the other hand, showed a peak at pH 5.5. At higher pH values production of both hydrolysis products fell off progressively and at pH 7.4 were only about one-sixth of the peak amounts. These extracts also exhibited lysophospholipase activity, with a broad pH optimum between 6 and 8, when incubated with 2.5mm-(1-[³H]stearoyl) lysolecithin. However, from control experiments with traces of labelled lysolecithin in the presence of unlabelled lecithin and taurocholate it appeared that secondary breakdown of lysolecithin formed by phospholipase A would be only slight at neutrality and negligible below pH 6.0. The patterns of [³H]stearic acid and [³H]lysolecithin formed from (1-[³H]stearoyl)lecithin by the extracts therefore indicated that they contain phospholipase A₁, with optimum activity at pH 4.2 and thus confirming Gatt's earlier finding, and, in addition, a phospholipase A₂ with a higher optimum at pH 5.5.

Extracts of human cerebral cortex and white matter showed similar patterns of activity, although differing from rat brain with regard to the pH optima of the two phospholipase A activities (see below).

To isolate the phospholipase A₁ activity saline extracts of rat cerebrum were first dialysed against 0.9% NaCl buffered to pH 4.2 with 20mm-sodium acetate. This step precipitated 60% of the protein, and a further 20% was then precipitated by pre-incubating the dialysed supernatant with sodium taurocholate (6mg/ml). The final supernatant contained 90% of the phospholipase A₁ activity (pH 4.2) in the crude extract, but was virtually free of phospholipase A₂ (pH 5.5) and lysophospholipase (pH 7.4) activities; phospholipase A₁ activity at pH 7.4 was also substantially decreased, to 25% of its original low value. This procedure for extracting and isolating the acid phospholipase A₁ of nervous tissue is simple and reproducible and could be applied to small samples of tissue. Extraction of the enzyme from the acetone-dried powder appeared essentially