Virchows Arch. A Path. Anat. and Histol. 377, 329–338 (1978)

Virchows Archiv A

An Unusual Case of Phospholipidosis

M. Elleder[1], A. Jirásek[1], F. Šmíd[1], K. Harzer[2], and D. Schlegerová[3]

[1] First Department of Pathology, Faculty of General Medicine, Prague, CSSR
[2] Laboratory of Neurochemistry, Institute for Brain Research, University of Tübingen, Federal Republic of Germany
[3] Department of Pathology, Paediatric Faculty, Prague, CSSR

Summary. We present the results of a structural, histochemical and lipid-chromatographic study of tissues obtained at postmortem from an unusual case of phospholipidosis. A previous biopsy of the appendix and liver (Elleder et al., 1975a) had revealed a predominance of phosphoglyceride storage, principally of lysobisphosphatidic acid (LBPA) postmortem material showed that this lipid was stored exclusively in central neurons. In the spleen and the lymph node, however, sphingomyelin (SP) was shown, histochemically and chromatographically, to be the main lipid stored. Total sphingomyelinase (SPase) activity in the appendix was reduced to about 50% of normal. Neuroaxonal dystrophy (NAD) and a conspicuous discrepancy between the degree of distension of some neurons and their lipid content deserve special mention. The case is contrasted with classical sphingomyelinosis; the complexity of the Niemann-Pick group of diseases is discussed as an indication of the difficulties of classification of any atypical case.

Key words: Sphingomyelinosis – Lysobisphosphatidic acid – Sphingomyelinase – Neuroaxonal dystrophy.

Introduction

In 1975, we published (Elleder et al., 1975a) our observations on a rare form of phospholipidosis, differing from classical sphingomyelinoses of the Niemann-Pick group in that the main lipid stored in the liver and the appendix was a phosphoglyceride, lysobisphosphatidic acid (LBPA). This type of phospholipidosis, which can probably be added to the group reported by Baar and Hickmans (1956) and Wiedemann et al. (1972) was described by the working term "phospholipidosis type II (Baar-Wiedemann disease)". In the present communication we add more data to our original biopsy studies by reporting the findings made postmortem. These add significantly to the picture of this very rare form of storage disease.

Send offprint requests to: Dr. M. Elleder, 1st Department of Pathology, Faculty of General Medicine, 12800 Prague 2, Studničkova 2, ČSSR

0340-1227/78/0377/0329/$02.00

Material and Methods

All tissues obtained at autopsy (performed at the pathological department of the Faculty of Paediatrics in Prague, Section No. 100 B/75) which took place 24 h after the death, were examined by routine *histological* methods. The brain was examined by standard neuropathological technique. Formol fixed material taken from different parts of the brain, particularly the brain stem, was osmicated, dehydrated with acetone, embedded in Araldite and subjected to *electronmicroscopic* investigation. For methology and details of *histochemical* investigations, performed mainly on formol fixed material see our previous paper (Elleder et al., 1975a). *Chromatographic* examination of lipids (Dr. Šmíd) was made on unfixed tissues. The spectrum of phospholipids was estimated according to Rouser, Fleischer and Yamamoto (1970). The cholesterol: SP ratio was determined densitometrically from chromatograms after spraying with specific detection agents. The quantity of the two lipids and their molar ratio were ascertained using calibration with pure substances. Sphingomyelinase activity in the biopsy specimen was determined by the method of Harzer and Benz (1973). The values were compared with activity in a normal appendix obtained during diagnostic appendectomy in a child with an non-specified neurological disease.

Results

The basic *clinical* and *necropsy* data of the case are summarized in Table 1.

The *structural, histochemical* and *chemical* findings in the organs affected by storage were as follows:

Central nervous system. Macroscopically, the brain showed discernible, though moderate atrophy with narrowed gyri particularly in the frontal, parietal, temporal, and insular regions. The occipital cortex showed no macroscopic signs of atrophy. The pons and medulla oblongata were smaller and toughened, the cerebellum showing no visible changes. Basal arteries, leptomeninges and sinuses were found to be intact. A horizontal section through the brain revealed visible dilatation of all ventricles and narrowing and greying of the white matter in the centrum semiovale.

Microscopically, the cytoplasm of neurons was found to be variably distended or even ballooned (Fig. 1a), was finely granular, or typically foamy and almost deprived of Nissl substance. Neuronal atrophy and numerical reduction, neuronophagia and cellular and fibrous gliosis were also found. Neuroaxonal dys-

Table 1. Basic clinical and pathological data

Clinical data		
Duration of illness	–	3 years, 11 months
Onset	–	6 months
Biopsy	–	2 years, 3 months
Death	–	4 years, 5 months
Cause of death	–	bronchopneumonia
Symptomatology	–	psychomotorical retardation
		hypotonia
		mild visceromegaly
Postmortem findings		
Body weight	–	13 kg
brain	–	1050 g
spleen	–	280 g (pro 48 g)
liver	–	900 g (pro 421 g)

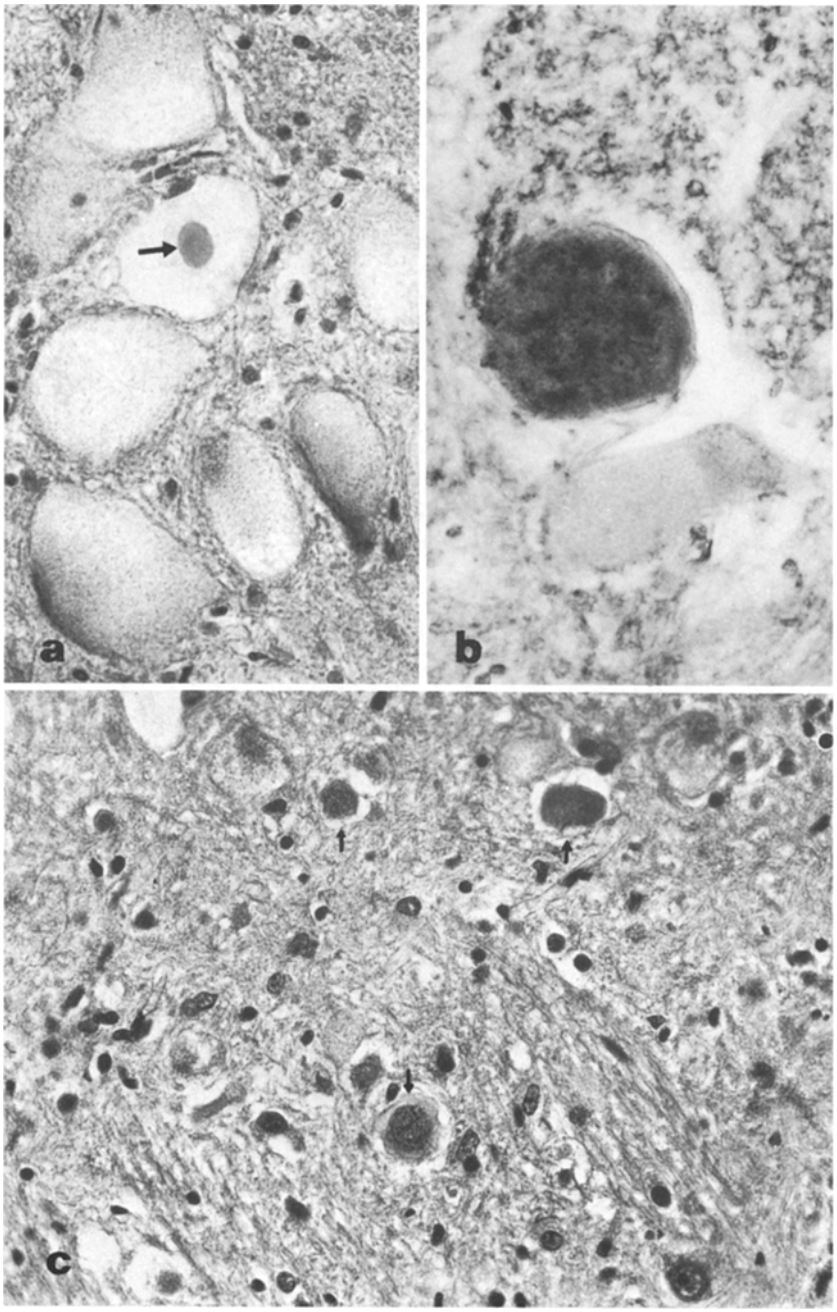

Fig. 1. a Ballooning of neurons of N XII with occasional intracellular oxyphil clod (arrow). Haematox. eosin. ×400. **b** Variable content of phospholipids in the ballooned neurons. Ferric haematox. method ×400. **c** Ventromedial thalamus. Neuronal storage, gliosis and neuroaxonal spheroids (arrows). Haematox. eosin. ×400

trophy (NAD) (Fig. 1c) with finely granular oxyphilic spheroids was seen and the latter bodies were found also in the roots of the facial nerve. The spinal cord was not investigated. The distribution of the changes and their severity are shown in Table 2.

Histochemical investigation revealed phosphoglyceride to be the main neuronal lipid stored, accompanied by cholesterol and small amounts of glycosphingolipid. Acid group detection revealed moderate orthochromasia or even β-metachromasia, the former being alkali-sensitive (LBPA?), the latter alkali-resistant (ganglioside?). The lipid stored was isotropic. There was a striking discrepancy between the cytological appearance of the neurons, clearly indicating storage and the low content of the lipids described above, whose presence in any appreciable quantity could only be demonstrated in a small portion of the abnormal neuronal population (Fig. 1b).

Table 2. Distribution and intensity[a] of neuropathological lesions

Localization	Storage	NAD	Atrophy	Neuron-ophagy	Gliosis	Remark
Cortex						
pariet., temp., front.	2	1	1	–	1	maximum in III., V. layer
insular	2	–	1	–	–	maximum in III., V. layer
hipocamp., amygdalar	1	–	2	–	1	
occipital	1	–	–	–	–	
centrum semiovale	–	–	–	–	2	
Thalamus ventromed.	–	–	3	–	3	
dorsolat.	3	3	2	2	2	
Striatum	2	–	–	–	–	in large neurons only
Pallidum	3	–	–	–	–	
Hypothalamus						
nc. paraventricularis	3	2	–	1	–	
nc. supraopticus	1	1	–	–	–	
Colliculus inf.	2	2	1	1	1	
Periaqueductal grisea	3	2	–	2	–	
Nucleus of III	3	2	–	2	–	
Red nucleus	1	2	–	–	–	
Substantia nigra						
compact zone	3	2	–	–	–	melanin absent
reticular zone	2	1	–	–	–	
Nucleus of IV	3	1	–	–	–	
Locus coeruleus	1	1	–	–	–	melanin present
Nuclei pontis	2	–	–	–	–	
Nc. of V, IX, XII	2	1	–	–	–	
Nc. of X	2	2	–	–	–	
Nc. olivaris inf.	1	–	–	1	–	
Nc. arcuati	1	–	–	–	–	
Cerebellum						
cortex	1	–	–	–	–	dendrites of Purkinje cells
nc. dentatus	1	–	–	–	–	
Nc. Golli et Burdachi	1	2	–	–	–	

[a] *Storage*: 1 mild distension of isolated neurons, 2 marked distension of most neurons, 3 ballooning; *NAD*: 1 occasional spheroids, 2 frequent spheroids, 3 status globosus; *Atrophy*: 1 discernible, 2 pronounced, 3 absence of neurons; *Neuronophagia*: 1 isolated, 2 frequent; *Gliosis*: 1 discernible increase in astroglial nuclei, 2 Kanzler-Arendt strongly positive, 3 glial cicatrization

Chromatographic analysis of the phospholipids taken from specimens of the cortex and basal ganglia showed LBPA accumulation in quantities of 0,86 and 1.55 μM of lipid Phosphorus/gr of wet tissue weight, respectively. The other phospholipids were not assesed quantitatively as they were not visibly altered and their quantity was in direct proportion to the admixture of myelin. In the cortex a moderate increase of GM_{1-3} gangliosides was found.

Non-lipid material or *lipopigment* were impossible to demonstrate in the neurons. Silver methenamine and aldehydefuchsin produced mild staining in paraffin sections, the latter after permanganate preoxidation only.

Electronmicroscopic examination performed in a limited number of specimens revealed a considerable number of oligomembranous, vesicle-like structures of an average size of 1 μ, present both in perikaryons and in neuronal processes. Typical membranous cytoplasmic bodies (MCB, see Terry and Weiss, 1963) were not seen. In the axons, there were dense, pleiomorphic, rudimentary membranous bodies, probably corresponding to spheroids. Lamellar formations, common in NAD (Jellinger and Jirásek, 1971) were not found.

Findings in the *peripheral nervous system* of the appendix were described elsewhere (Elleder et al., 1975a).

Of *the rest of the organs* examined postmortem, whilst a considerable degree of foam cell storage was seen in the lungs, the reticuloendothelial system (RES), i.e. the spleen, lymphnodes, bone marrow was found to be most affected. The *spleen* showed a picture of typical foam cell infiltration of the red pulp and an unaffected sinus lining. The white pulp was grossly reduced, with only isolated storage cells. The *lymph node* structure was practically obliterated by a multitude of enlarged cells. The results of an analysis of the *spectrum of phospholipids* in both these organs are shown in Table 3. These results suggest that, unlike other storage sites, the phospholipid spectrum differs in that SP is the main lipid stored. This is in agreement with the results of histochemistry which repeat-

Table 3. Phospholipid spectrum in samples of spleen and lymphnode

		Patient		Control spleen	
		Lymphnode	Spleen	♀1 year	♂1 year
Total lipid Phosphorus (μM lipid P/1 gr of wet weight)		30.0	95.9	40.0	29.6
Phosphatidylcholine	% of total lipid phosphorus	23.0	27.6	55.0	55.2
Phosphatidylethanolamine		11.0	10.4	25.9	20.4
Phosphatidylserine		9.5	7.4	7.5	7.1
Phosphatidylinositol		3.3	3.1	1.6	3.0
Diphosphatidylglycerol		ND[a]	ND	ND	ND
Lysophosphatidylcholine		6.9	6.2	2.5	1.1
Lysophosphatidylethanolamine		1.1	ND	ND	ND
Lysophosphatidylserine		1.1	ND	ND	ND
Lysobisphosphatidic acid		7.7	11.9	ND	ND
Lysophosphatidylinositol		0.6	ND	ND	ND
Sphingomyelin		34.0	33.4[b]	6.7	13.2

[a] ND = non detectable

[b] Ratio sphingomyelin:cholesterol 2.7:1

edly showed the clear predominance of alkali-stable phospholipid. Also demonstrable was storage of cholesterol and small amounts of glycosphingolipids. Anisotropy was poor, lipopigment participation minimal. Subsequent lipid chromatographical examination of the *liver*, only available as fixed tissue, is reported on in Table 4. The results confirm the histochemical finding in that SP accumulation was not demonstrable. Phosphoglycerides could not be demonstrated as they are destroyed by formalin fixation (Heslinga and Dierkauf, 1962). The following tissues showed no signs of storage demonstrable by light microscopy: myocardium, skeletal muscle, smooth muscle, kidneys, suprarenals, pancreas, thyroid gland, pituitary gland, ovaries and skin.

Table 4. Sphingomyelin content in the fixed liver specimen

	Patient	Control	Niemann-Pick (type B)
Sphingomyelin (μM P/gr wet w.)	3.36	2.84	55.17

Sphingomyelinase activity is given in Table 5. The results of stored lipid analysis obtained from biopsy and necropsy materials can be summarized as showing that the case was one of phospholipidosis with pronounced regional heterogeneity of stored lipid. Phosphoglyceride (LBPA) was practically the only lipid stored in the liver and central neurons, whereas SP was predominant in the RES, particularly in the lymph node and the spleen. In other parts of the RES (Kupffer cells and appendiceal macrophages) the two phospholipids were histochemically demonstrable in considerable quantities. Also stored were cholesterol and, in some areas glycolipid. Lipopigment was found in only limited parts of the RES.

Table 5. Sphingomyelinase activity in the appendix tissue

	Units of activity	% of activity
Control	2.9	100
Patient	1.2	41.4[a]

[a] activity about 40% of mean control liver value; β-hexosaminidase highly active

Discussion

The problem of precise nosological identification is the crucial point of the discussion of this case. It differs from classical types of sphingomyelinoses in that it shows pronounced regional heterogeneity of the lipid stored as well as neuroaxonal dystrophy of unusual extent and severity. It may represent evidence of genetic heterogeneity in the NPD complex, genetic pleiotropism,

or merely a non-genetic conditioned variation of one of the standard phenotypes. How is the case related to those with a strikingly similar "chemical" phenotype (see Baar and Hickmans, 1956; Wiedemann et al., 1972)?

Unlike the present approach to the mucopolysaccharidoses (McKusick et al., 1972; McKusick, 1973) in the NPD complex the phenotype traits continue to play a significant role in classification (see Frederickson and Sloan, 1972). In terms of the enzyme deficit, there are presently two basic groups in the NPD complex. The *first group*, characterised by a severe SPase deficit, appears to include two forms, usually referred to as A and B. According to McKusick (1973), these may be allelic disorders. The *second group*, with no demonstrable enzyme deficit, includes the present types C–E (see Brady and King, 1973). The first group are termed the "SPase deficit group", but a less precise term "sphingomyelinosis" is used to describe the latter group, acknowledging the possibility of heterogeneity particularly in type C.

As some communications suggest, phenotypically-defined groups (Crocker, 1961) are not homogenous since there are forms which do not fit in the scheme. This is the case for phenotype C, with a severe SPase deficit (Martin et al., 1972), and that of a relatively benign form with 18% residual activity (Schneider et al., 1973; Golde et al., 1975). It may be that studies which describe a deficit of one isoenzyme of SPase in cases with phenotype C (Callahan et al., 1974) could prepare the ground for a successful classification similar to that which was successful in the gangliosidoses (Sloan and Frederickson, 1972; van Hoof, 1973) and mucopolysaccharidoses (Kolodny, 1976), although recent studies (Harzer et al., 1977) provide evidence against such a view.

It seems clear that any relationship between phospholipidoses with a pronounced LBPA component and classical cases of the NPD complex, may only be approached by making multi-level comparisons of the phenotype, though it is clear that identity in phenotype does not exclude difference in genotype and vice versa. A clear *enzyme deficit* was not demonstrated in our case since a 50% drop in activity still might be within the normal range. A similar reduction of activity in a biopsy specimen of liver was described by Wiedemann et al. (1972), but subsequent leucocyte investigation showed normal values (Wiedemann, 1977). Variability in enzyme activity was reported by Callahan and Philippart (1971) and should obviously be considered in the analysis of a single tissue specimen. The finding suggests a high degree of probability of cases with partly reduced activity belonging to the group of sphingomyelinoses without SPase deficit, in type C, perhaps. The *spectrum of stored lipids* appears to be the most useful way of distinguishing the NPD cases studied. In our case LBPA is almost the only regionally stored phospholipid (in hepatocytes and central neurons) and resemble the findings of Wiedemann et al. (1972, see also Seng et al., 1971). In cases studied by Baar and Hickmans (1956), lipid analysis were not comparable with ours but rule out any major SP participation in storage. LBPA has so far been found to be a minor lipid in those isolated cases of NPD which have been adequately investigated (Kamoshita et al., 1969; Martin et al., 1972; Elleder et al., 1975b), though it is occasionally found in substantially larger quantities (Rouser et al., 1968; Karpati et al., 1977).

In our own series of six cases of NPD, SP was demonstrable as the main lipid histochemically and chromatographically in liver biopsies (Elleder, work in progress). The communications published so far do not suggest that the LBPA is a lipid which accompanies the main stored substance non-specifically in lysosomal enzymopathies (see Rouser et al., 1968; Kahma et al., 1976) as is often the case with cholesterol (Uzman, 1958; Suzuki et al., 1967, 1968).

A goal-oriented study of more cases may help to establish whether LBPA accumulation is directly determined genetically as in the case of mucosubstances in GM_1 gangliosidosis (van Hoof, 1973) or neuronal gangliosides in mucopolysaccharidoses (Dorfman and Matalon, 1972), or whether its accumulation is a phenocopy. In this connection the assumption that LBPA is the substrate for some of the phosphodiesterases (Callahan and Philippart, 1971) is worth mentioning.

The problem of cholesterol and glycolipid participation in storage in phospholipidoses has been discussed elsewhere (Seng et al., 1971; Frederickson and Sloan, 1972; Anzil et al., 1973; Kannan et al., 1974; Elleder et al., 1975a, b).

Another unique feature of the case is neuronal storage combined with *neuroaxonal dystrophy* (NAD), which has only been described in isolated cases of neurolipidosis (Martin et al., 1972; Klinghardt, 1974). By comparing the severity of each lesion, *i.e.* storage, NAD, atrophy and gliosis, it is possible to suppose that in some regions storage does not change into atrophy or gliosis directly or through storage neuronophagy but via NAD. This is particularly striking when comparing the ventromedial and dorsolateral thalamic nuclei. Another point to be stressed is that in our case NAD did not follow its usual pattern of distribution (Jellinger and Jirásek, 1971).

A further peculiarity of the case is the discrepancy between the cytological appearance of most of the central neurons, suggesting storage, and a minimal lipid content, for which there is no satisfactory explanation. Similar findings have already been described by Philippart et al. (1969) and Hassoun et al. (1975).

In conclusion, from our findings precise classification of this, undoubtedly peculiar form of phospholipidosis within the NPD complex remains a problem. Inclusion in group C is possible, but this group is itself far from being accurately defined. In future cases, analysis should not be limited to RES tissue specimens where differences in the spectrum of the stored lipids appear to be obliterated. Attention should be paid to the LBPA:SP ratio and the liver is probably the most important site as far as the detection of possible differences is concerned. This, coupled with determination of the deficient enzyme and its substrate specifity, will help in finding a solution to the problem of nosological individuality and the possibility of phenotype variability found. It may also show the true cause of LBPA accumulation.

To deal with those cases of sphingomyelinosis in which LBPA is stored in a generalized manner or shows clear-cut regional predominance, the authors would like to recommend the temporary working term "LBPA variant of sphingomyelinosis".

References

Anzil, A.P., Blinzinger, K., Mehraein, P., Dozic, S.: Niemann-Pick disease type C: case report with ultrastructural findings. Neuropädiatrie **4**, 207–225 (1973)

Baar, H.S., Hickmans, E.M.: Cephalin-lipidosis. A new disorder of lipid metabolism. Acta medica scand. **155**, 49–64 (1956)

Brady, R.O., King, F.M.: Niemann-Pick's disease. In: Lysosomes and storage diseases (H.G. Hers and F. van Hoof, eds.), p. 439. New York: Academic Press 1973

Callahan, J.W., Philippart, M.: Phosphodiesterases (including sphingomyelinase) in Niemann-Pick disease types A and C. Neurology **21**, 442 (1971)

Callahan, J.W., Khalil, M., Gerrie, J.: Isoenzymes of sphingomyelinase and the genetic defect in Niemann-Pick disease type C. Biochem. biophys. Res. Commun. **58**, 384–390 (1974)

Crocker, A.C.: The cerebral defect in Tay-Sachs disease and Niemann-Pick disease. J. Neurochem. **7**, 69–80 (1961)

Elleder, M., Šmíd, F., Kohn, R.: Lipidosis with a predominant storage of phosphoglycerides (Phospholipidosis type II-Baar, Wiedemann). Virchows Arch. A. Path. Anat. and Histol. **365**, 239–255 (1975a)

Elleder, M., Jirásek, A., Šmíd, F.: Niemann-Pick disease (Crocker's type C). A histochemical study of the distribution and qualitative differences of the storage process. Acta neuropath. **33**, 191–200 (1975)

Fredrickson, D.S., Sloan, H.R.: Sphingomyelin lipidoses: Niemann-Pick disease. In: The metabolic basis of inherited disease (J.B. Stanbury, J.B. Wyngaarden, D.S. Fredrickson, eds.), p. 783. New York: McGraw-Hill 1972

Golde, D.W., Schneider, E.L., Bainton, D.F., Pentchev, P.G., Brady, R.O., Epstein, Ch.J., Cline, M.J.: Pathogenesis of one variant of sea-blue histiocytosis. Lab. Invest. **33**, 371–378 (1975)

Harzer, K., Benz, H.U.: A simple sphingomyelinase determination for Niemann-Pick disease: Differential diagnosis of types A, B and C. J. Neurochem. **21**, 999–1001 (1973)

Harzer, K., Anzil, A.P., Schuster, I.: Resolution of tissue sphingomyelinase isoelectric profile in multiple components is extraction-dependent: evidence for a component defect in Niemann-Pick disease type C is spurious. J. Neurochem. (1977) (in press)

Hassoun, J., Pellisier, J.F., Gambarelli, D., Toga, M.: Niemann-Pick disease (Crocker's type C): an electron microscopic study. Proc. VIIth Int. Congr. Neuropath., p. 529–562 (1975)

Heslinga, F.J.M., Deierkauf, F.A.: The action of formaldehyde of human brain lipids. J. Histochem. Cytochem. **10**, 704–709 (1962)

van Hoof, F.: GM_1 gangliosidosis. In: Lysosomes and storage diseases (H.G. Hers and F. van Hoof, eds.), p. 305. New York: Academic Press 1973

Jellinger, K., Jirásek, A.: Neuroaxonal dystrophy in man: character and natural history. Acta neuropath. Suppl. **V**, 3–16 (1971)

Kahma, K., Brotherus, J., Haltia, M., Rennkonen, O.: Low and moderate concentrations of lysobisphosphatidic acid in brain and liver in patients affected by some storage diseases. Lipids **11**, 539–544 (1976)

Kannan, K., Hong-Boen Tjiong, Debuch, H., Wiedemann, H.-R.: Unusual glycolipids in brain cortex of a visceral lipidosis (Niemann-Pick disease?). Hoppe-Seyler's Z. Physiol. Chem. **355**, 551–556 (1974)

Kamoshita, S., Aron, A.M., Suzuki, K., Suzuki, K.: Infantile Niemann-Pick disease. A chemical study with isolation and characterization of membranous cytoplasmic bodies and myelin. Amer. J. Dis. Child. **117**, 379–394 (1969)

Karpati, G., Carpenter, S., Wolfe, L.S., Andermann, F.: Juvenile dystonic lipidosis: an unusual form of neurovisceral storage disease. Neurology **27**, 32–42 (1977)

Klinghardt, G.W.: Experimentelle Schädigungen von Nervensystem und Muskulatur durch Chlorochin: Modelle verschiedenartiger Speicherdystrophien. Acta neuropath. **28**, 117–141 (1974)

Kolodny, E.H.: Lysosomal storage disease. New. Engl. J. Med. **294**, 1217–1220 (1976)

Martin, J.J., Philippart, M., Hauwaert, J.V., Callahan, J.W., Deberdt, R.: Niemann-Pick disease (Crocker's group A). Late onset and pigmentary degeneration resembling Hallerworden-Spatz syndrome. Arch. Neurol. **27**, 45–51 (1972)

McKusick, V.A., Howell, R.R., Hussels, I.E., Neufeld, E.F., Stevenson, R.E.: Allelism, non-allelism and genetic componds among the mucopolysaccharidoses. Lancet **1**, 993–996 (1972)

McKusick, V.A.: Phenotypic diversity of human diseases resulting from allelic series. Amer. J. hum. Genet. **25**, 446–456 (1973)

Philippart, M., Martin, L., Martin, J.J., Menkes, J.H.: Niemann-Pick disease. Morphologic and biochemical studies in the visceral form with late central nervous system involvement (Crocker group A). Arch. Neurol. **20**, 227–238 (1969)

Rouser, G., Kritchevsky, G., Yamamoto, A.: Accumulation of a glycerolphospholipid in classical Niemann-Pick disease. Lipids **3**, 287–290 (1968)

Rouser, G., Fleischer, S., Yamamoto, A.: Two dimmensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids **5**, 494–496 (1970)

Schneider, E.L., Epstein, C.J., Brady, R.O., Pencher, P., Hibbert, S.: Sea-blue histiocyte syndrome: a family with sphingomyelinase deficiency. Amer. J. Hum. Genet. **25**, 66A (1973)

Seng, P.N., Debuch, H., Witter, B., Wiedemann, H.-R.: Bis (monoacylglycerin)–phosphorsäure-Vermehrung bei Sphingomyelinose (M. Niemann-Pick?). Hoppe-Seyler's Ztschr. f. Physiol. Chem. **352**, 280–288 (1971)

Sloan, H.R., Frederickson, D.S.: GM_2 gangliosidosis: Tay-Sachs disease. In: Metabolic basis of inherited disease (eds. J.B. Stanbury, J.B. Wyngaarden, D.S. Frederickson), p. 615. New York: McGraw-Hill 1972

Suzuki, K., Suzuki, K., Chen, G.C.: Isolation and characterization of metachromatic granules from a brain with metachromatic leukodystrophy. J. Neuropath. exp. Neurol. **26**, 537–550 (1967)

Suzuki, K., Suzuki, K., Chen, G.C.: Membranous cytoplasmic bodies from Tay-Sachs disease and GM_1–gangliosidosis (generalized gangliosidosis). J. Neuropath. ex. Neurol. **27**, 142–143 (1968)

Terry, R.D., Weiss, M.: Studies in Tay-Sachs disease. II. Ultrastructure of the cerebrum. J. Neuropath. exp. Neurol. **22**, 18–55 (1963)

Uzman, L.L.: The significance of the increase of non-specific lipid components in primary lipid storage disease. Arch. Path. **65**, 331–339 (1958)

Wiedemann, H.-R., Debuch, H., Lennert, K., Caesar, R., Blümcke, S., Harms, D., Tolksdorf, M., Seng, P.N., Korenke, H.-D., Gerken, H., Freitag, F., Dörner, Kl.: Über eine infantil-juvenile, subchronisch verlaufende, den Sphingomyelinosen (Niemann-Pick) anzureihende Form der Lipidosen – ein neuer Typ? Z. Kinderheilk. **112**, 187–225 (1972)

Received August 1, 1977