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Adult neurovisceral lipidosis compatible with Niemann-Pick disease type C

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Summary. The authors present a case of neurovisceral storage disease with the whole of its clinical course confined to adult life (symptoms from 26 to 46 years of age) and marked by mainly neurological symptomatology with dystonia, vertical supranuclear ophthalmoplegia and progressive mental deterioration as the dominant features. From the results of postmortem structural histochemical and chemical analysis the case was diagnosed as Niemann-Pick disease type C. This case, together with sporadic observations reported by other authors, represents a significant shift in our view of the incidence of NPD type C in older age groups.

Key words: Niemann-Pick disease type C – Adult lipidosis – Sphingomyelinosis

The original clinical and pathological definition of Niemann-Pick disease type C (Crocker 1961) can now be described as classical since we have now had reports on a number of variants regarding the nature of their clinical course. These include infantile forms with fulminant neurovisceral symptomatology (Lake 1983; Phillippart 1983), hepatic forms of the early type (Guibaud et al. 1979; Gautier et al. 1983; Vanier et al. 1983), protracted forms marked by mild visceral symptomatology lasting until adolescence or adulthood (Gautier et al. 1983; Vanier et al. 1983) and compatible with NPD type E according to Fredrickson and Sloan (1972). However, there have as yet been no independent reports on the primary adult forms with neurovisceral symptomatology even though the case described by Horoupian and Yang (1978) does, in fact, belong in that category. There are a few scattered references (Callahan and Khalil 1975) one of which gives some facts about two siblings who developed a neurovisceral disease in

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adulthood with chemical findings compatible with type C of NPD (Wherrett and Rewcastle 1969; Wherrett et al. 1983).

Our own case which has all the morphological and chemical characteristics of type C is unusual in that the clinical symptomatology did not develop until adulthood, which makes it yet another case of what is, for the time being at any rate, one of the rarest variants of NPD type C.

Case report

Patient B.M. was born in 1934. Her parents were cousins with no history of neurological disease. The patient was never seriously ill in her youth, she gave birth to two healthy children (1960, 1962), her two brothers had died in infancy, reportedly of pneumonia. There had been no post mortem examinations. She has one living healthy sister, born 1929, whose children are healthy. In 1970, she had an operation for extrauterine pregnancy.

In 1966, she had her first neurological examination for tremor of the left hand which, she reported, had started in 1960 when she was 26. At that time, the objective finding was minimal. In view of her slight mental deficiency, an under-developed form of perinatal encephalopathy was suspected.

However, the tremor of the left upper extremity deteriorated so that the patient was unable to carry anything in her hand. Later on, her gait also started deteriorating, her left foot stumbling. A neurological examination performed in the spring of 1973 established a central lesion of the left facial nerve, incomplete left spastic hemiparesis, and a dyskinetic extrapyramidal syndrome in both upper extremities, more pronounced on the left.

A more detailed investigation was made during hospitalization in the autumn of 1973. Objective findings included signs of dementia with scanning speech and a mask-like smile. In addition there was hypomimia, increased postural reflexes, athetoid dyskinesis in the upper extremities, and, to a noticeable degree, in the lower extremities as well, slightly increased reflexes in the left-sided extremities where positive pyramidal irritative (spastic) signs were also present.

Sedimentation rate, haemogram, BWR, liver function tests, urine mineralogram, cholesterol levels and lipaemia were normal. The fundus of the eys and the retinal perimeter were normal, EEG was slightly diffusely abnormal, X-ray of the skull revealed hyperostosis interna. Psychological tests showed the patient's intellectual capacities to have deteriorated to oligophrenia with a better quality of the verbal score. This was, however, not primary oligophrenia; rather, the patient showed signs of demented personality with progressive loss of mental abilities.

Proceeding from the above symptomatology, a vaguely defined degenerative disease of the basal ganglia was suspected. Treatment with Triphenidyl and Disipal was started, though irregularly and in minimum doses.

More progression was noted in 1975 when the patient's dementia grew worse, spastic symptomatology in the extremities became more pronounced, and, in particular, ballistic dyskinesia developed in addition to multiple extrapyramidal choroathetoid dyskinesia. Abdominal areflexia was discovered, too. In 1975 the patient was placed in a home with the diagnosis of perinatal encephalopathy with severe spasticity. In May 1980, she was admitted for hospitalization in a moribund condition. Objective signs were found of athetoid hyperkinesis of the upper extremities, the liver was enlarged 1 cm below the costal arch level, the lungs bore signs of severe left-sided bronchopneumonia which proved to be the cause of death. Typical extension of the head indicating an effort to overcome the unability to look up on volition was noted in retrospect as a prominent symptom.

Autopsy performed 27 h after death revealed a minimum of macroscopic changes. The brain, apart from mild atrophy (1030 g), exhibited no other changes, the spleen was slightly enlarged (140 g), the liver was brown and atrophic (1,000 g), coalescing patches of bronchopneumonia were seen in the left lung. Histological examination revealed a storage process, which was subjected to further analysis.

Material and methods

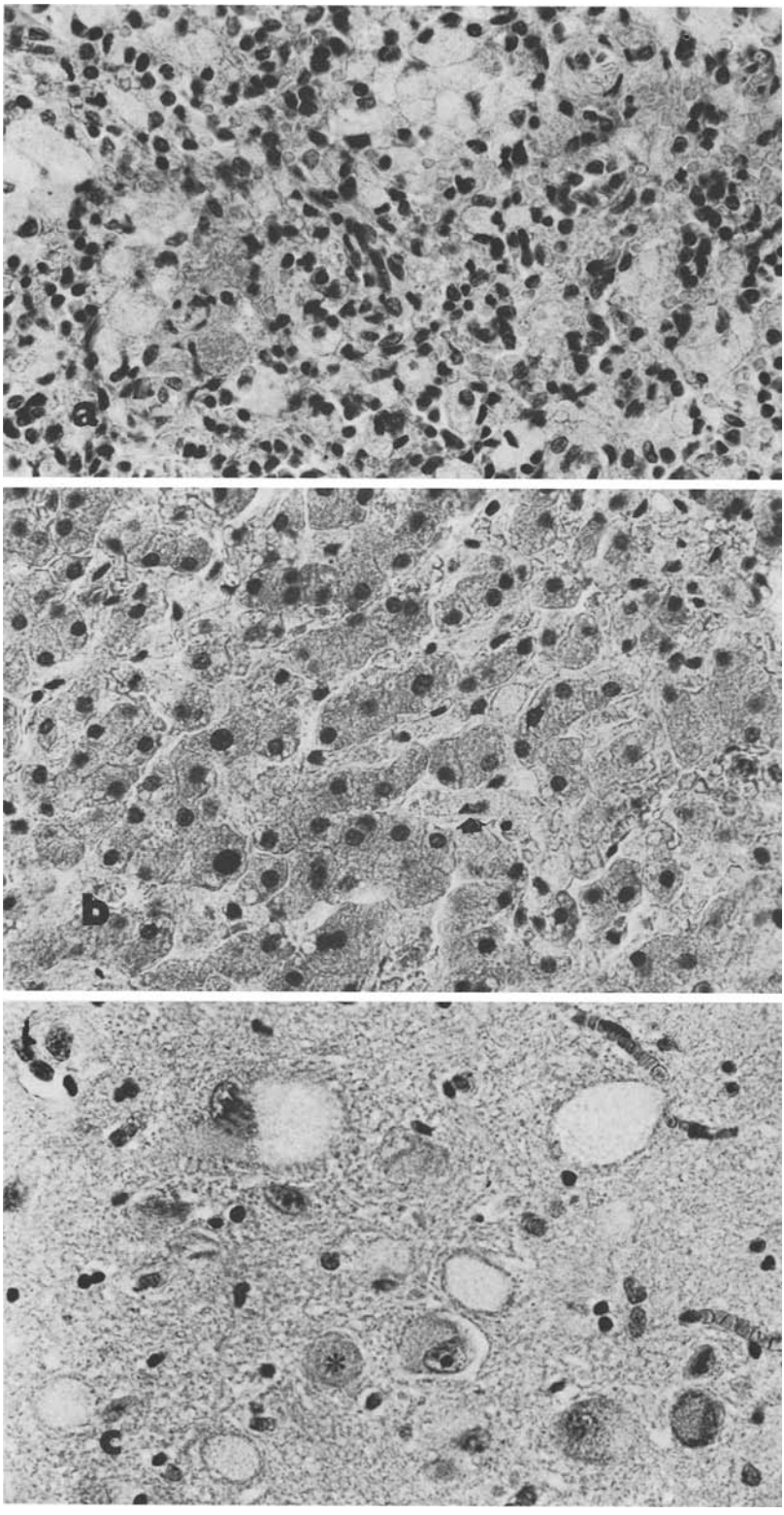
Practically the whole of the brain, and parts of the liver, spleen, kidney and lungs, all formaldehyde-fixed, were examined using standard histological methods. Specimens of the liver, spleen and some regions of the brain were examined electronoptically following osmication, dehydration with acetone, and embedding in Araldite. The following histochemical methods were used to test lipids in frozen sections: the iron hematoxylin method for the detection of phospholipids and sphingomyelin (Elleder and Lojda 1973a and b), the PAS reaction for the detection of glycolipids, and cresyl violet as the Hirsch-Peiffer test for the detection of sulphatides and other acidic lipids (for details see Adams 1965). Pre-extraction with chloroform-methanol (2:1 v/v 1 h, room temperature) was used for distinction form non-lipid substances. Sudan Black was employed for total lipid staining as it also stains pigments of the lipopigment type (Elleder 1977). Lipid deposits were also studied for autofluorescence and birefringence.

The liver, spleen and brain were examined by lipid chromatographically. Sphingomyelin was assayed following two-dimensional phospholipid TLC and phosphorus determination in the sphingomyelin zone (Rouser et al. 1970). Neutral glycolipids were isolated from the total lipid extract ofter moderate, alkaline-catalyzed methanolysis of phospholipids, and chromatographed on HPTLC silicagel plates (Merck, Darmstadt, FRG) in a mixture of chloroform:methanol:H_2O – 65:25:4. Detection: Orcinol-H_2SO_4 (Robert and Rebel 1975). Gluco- and galactocerebrosides were separated on borate-impregnated plates with silicagel (Kean 1966).

Results

Histology revealed a storage process particularly pronounced in the spleen and the brain, much less so in the liver. In the *spleen*, there was typical infiltration of the red pulp with foam cells bearing variable amout of ceroid (Fig. 1a). The white pulp was reduced with occasional foamy macrophages. The sinus epithelium was unaltered. Histochemically the foam cells showed large quantities of alkali-resistant phospholipids, i.e. sphingomyelin, and moderate quantities of glycolipids of the neutral type. The lipid deposits were crystalline with Maltese cross type birefringence. The non-extractable residuum was made up of ceroid. Chromatography revealed only ceramidemonohexosides (glucocerebrosides) as moderately elevated. There was a prominent increase in sphingomyelin (6.45 μM/g wet weight against 1.83 ± 0.6 μM/g in controls). Electronoptic examination showed the storage lysosomes to be translucent with loose membranous structures with no conspicuous arrangement. In some of the macrophages relatively dense coarse lamellar structures were seen corresponding to ceroid (unpublished). Smaller dense inclusions of the lipofuscin type were present in minor quantities in the endothelia of sinuses.

The *liver* (Fig. 1b) showed few scattered small foamy cells in the sinuses with variable admixtures of ceroid. The hepatocytes were solid with relatively many lipofuscin granules with the usual centroacinous gradient. As for the peribiliary granules, histochemical tests revealed small quantities of glycolipids, apolar lipids, and small amounts of sphingomyelin in distanded Kupffer's cells. Birefringence was restricted solely to Kupffer's cells. Chromatographically, sphingomyelin was slightly increased (2.17 μM/g wet weight against 1.24 ± 0.28 μM/g in controls), glycolipids were moderately increased in all the three main groups: ceramidemono (gluco), di-, tri- (in


a
b
c

the latter three fractions were present) and tetrahexosides and, very probably, GM_3 ganglioside too. The only elements to have been preserved in the ultrastructure were numerous dense peribiliary deposits corresponding to lipofuscin, probably in combination with other lipids. There were residua of quite translucent storage vacuoles in the same region.

The *brain,* macroscopically unaltered, showed signs of moderate degree storage as distinct from the early forms of the disease. The difference was mainly in the smaller number of massively storing cells in our particular case. Microscopically, the severely affected cells were typically ballooned, often even in their processes. The intensity of storage proved highly variable with great differences seen not only between neurons of the same nucleus (Fig. 1c) but also between different regions; the large pyramidal neurons of the cortex were involved, particularly in the lower layers. In the basal ganglia, conspicuous changes were seen in the large neurons of the striatum, pallidum, thalamus and hypothalamus. Only parts of the brain stem and the oblongata were available for study. The degree of storage could only be estimated at the level of the locus coeruleus where it was quite prominent. The pontine neurons were normal. In the cerebellar cortex, the dendrites of some of the Purkinje cells were only slightly affected. In the spinal cord, storage was noticeable in all parts of the grey matter but prominently in only some of the neurons. The vascular endothelium showed no signs of storage. Parallel to the storage, there was considerable deposition of neurolipofuscin in indirect proportion to the process of storage. There was also evidence of neuroaxonal dystrophy in the form of granular sphenoids, a situation moderately marked in the thalamus but very prominent in the posterior horns of spinal cord and in the Golli nucleus. Its overall intensity, however, was lower than in the early forms of the disease. Histochemistry was able to demonstrate but a moderate degree of storage of neutral glycolipid in the neurons. Chromatography provided evidence of a moderate excess of glucocerebroside in the white as well as in the grey matter. Electron microscopy revealed oligolamellar inclusions in the plasma of all the neurons, similar to those described by Anzil et al. 1973), Harzer et al. (1978), Horoupian and Yang (1978) and Pellissier et al. (1976). There was an increased amount of neurolipofuscin composed of the dense microtubular and homogeneous globules of moderate density.

Discussion

Considering our persisting lack of knowledge of the biochemical nature of the defect in NPD type C diagnostic procedures should make use of positive approaches. Smears of bone marrow may be available, since this

Fig. 1. a Collections of foamy and ceroid containing macrophages in the spleen red pulp. HE × 600. **b** Dispersed small foamy Kupffer cells (*arrows*) without any discernible changes in the hepatocytes. HE × 600. **c** Thalamus. Variable affection of neurons by storage. The fine granules in the cytoplasm of unaffected neurons is neurolipofuscin. A small granular axonal spheroid is marked by asterisk. HE × 600

is where histochemistry can identify special phospholipid storage pattern in macrophages (Elleder et al. 1983b); next comes liver biopsy with its relatively characteristic chemical (mild increase in bis (monoacylglyceryl) phosphate, sphingomyelin and glycolipids), histochemical (SM storage restricted to macrophages), and electron-microscopic changes (Elleder et al., manuscript in preparation). Persistent normal SMase activity in parenchymatous organs and in peripheral blood is an important negative finding distinguishing it from the SMase deficiency group. Recently changes were found in the isoenzyme SMase spectrum restricted to the cultured type C fibroblasts characterized by decrease in or complete disappearance of the cathodic component, very often accompanied by acid beta-glucosidase activity depression (Besley 1977; Besley and Moss 1983). Post mortem material offers still more findings such as the familiar sphingomyelinosis of the spleen and lymph nodes, a complex of neuropathological changes (Elleder and Jirásek 1981; Jirásek et al. 1983), SM storage restricted to macrophages, as well as a spectrum of organ involvement (summarized in Elleder 1983b), all of which were utilized in our case.

Our diagnosis proceeded as follows: Marked sphingomyelinosis of the spleen which was infiltrated by foam cells as found in cases with neurovisceral symptomatology, practically gave the NPD group diagnosis since in this particular constellation this characteristic feature has never been described in any other genetically conditioned or acquired lipid metabolism disturbance. Consequently, distinction had to be made between two basic groups in the NPD complex: between SMase deficiency (encompassing types A and B) and group of the C type according to recently revised Crocker's model (Elleder 1983a; Phillippart 1983). In the case concerned, we used our own criteria – invariably useful in our practical work so far – namely the existence of generalized sphingomyelinosis for SMase deficiency as distinct from SM storage limited to macrophages in type C (Elleder 1983b). In our case, SM was detectable solely in macrophages, not in hepatocytes or in neurons (this is where a small amout of glycolipid was present). Nor there was any storage in the suprarenals, macroscopically obviously normal, thus practically ruling out SMase deficiency since in our series of A, B types suprarenal cortical storage was always detectable macroscopically and histologically, as in the literature (unpublished results summarized in Elleder (1983b). Neuroaxonal dystrophy was somewhat less pronounced than in early cases of NPD type C where, in our own series, it was invariably quite prominent (Elleder and Jirásek 1981; Jirásek et al. 1983).

From the morphological and chemical points of view, the present case met the cirteria for NPD type C. Clinical symptomatology, too, concurred with this. There was, primarily, the symptom of vertical supranuclear ophthalmoplegia, the incidence of which in type C is so high that some authors chose to coin the term ophthalmoplegic lipidosis to label the disease (Neville et al. 1973; Lake 1981 and 1983). Then there were signs of dystonia which are very frequent mainly in juvenile cases of type C (Horoupian and Yang 1977; Karpati et al. 1977; Neville et al. 1973). The original cases reported by authors who introduced the name (Elfenbein 1968, de Leon et al. 1969),

if evaluated in retrospect, reveal symptomatology and findings quite compatible with type C (Elleder and Jirásek 1983; Elleder 1983b).

Our case has a precedent, namely the case of two brothers first briefly described by Wherrett and Rewcastle in 1969, and presented in more detail at a recent symposium on NPD (Wherrrett et al. 1983). Once again, there was dystonic symptomatology with VSO, mild sphingomyelinosis restricted to the spleen, and relatively prominent storage of bis (monoacylglyceryl) phosphate in the brain and viscera. The spleen was tested for glycolipids, the level of which was found to be increased (mainly glucocerebroside, less so lactosylceramide and trihexosylceramide). SMase and glucocerebrosidase activities were normal. Morphological changes were essentially identical with our own case. A similar case was briefly described by Callahan and Khalil (1975) in a biochemically orientated study. Interestingly enough, tissue culture of fibroblasts from this latter case showed demonstrable alterations of the isoenzyme SMase spectrum like those in a simultaneously examined case of type C. Younger patient with much the same personal history and similar symptomatology limited to early adulthood, was described by Horoupian and Yang (1978) and by Cogan et al. (1981a and b).

The significance of our case appears to be that it points to a very wide age range for the onset of clinical symptomatology in NPD type C, a feature which only adds to the already markedly pleiomorphic symptomatology of this type (summarized in Elleder and Jirásek 1983). Cases of the adult type fit in well with the revised classification of NPD. They also emphasize its advantages since it includes adult cases as subvariants of the two basic groups: SMase deficiency and group C (Elleder 1983 a).

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