Significance of muscle biopsies in neuronal ceroid-lipofuscinoses¹

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SYNOPSIS Muscle specimens obtained at necropsy from four cases of neuronal ceroid-lipofuscinosis (NCL), three of the juvenile and one of the late infantile type, and a muscle biopsy from a fifth patient with the juvenile type of NCL, all showed curvilinear bodies typical of NCL within the muscle fibres. The pigments were autofluorescent. It appears that skeletal muscle is a reliable tissue source for the diagnosis of these disorders by biopsy.

Muscle biopsy is now a widely accepted procedure for the investigation of neuromuscular problems. It is essential in establishing or confirming the diagnosis in disorders affecting the muscle and the lower motor neurone. Skeletal muscle is also involved in a variety of diseases that manifest themselves primarily in other organs—for example, rheumatoid arthritis or primary hyperparathyroidism and other metabolic or endocrine conditions. Recently, it has become apparent that striated muscle might exhibit pathological features in primary degenerative entities of the nervous system, such as Lafora's myoclonus epilepsy (Neville *et al.*, 1974), in Leigh's subacute necrotizing encephalomyelopathy (Crosby and Chou, 1974), and in Parkinsonism (Tomonaga and Tanabe, 1973).

In certain degenerative disorders of the central nervous system the lesion may not be accessible to biopsy. Since a brain biopsy is often deemed unwarranted, even in the case of a severe debilitation, clinicians and pathologists are searching for other tissues where biopsy procedures are less hazardous and costly but promise equally reliable results. In lysosomal disorders the rectum is often examined to identify storage

material, but ganglion cells must be present in the specimen for making the diagnosis.

Among the lysosomal disorders the neuronal ceroid-lipofuscinoses (NCL) are a somewhat heterogeneous group that has recently been separated from the loosely defined group of amaurotic familial idiocies (Zeman and Dyken, 1969). Within the various clinical subgroups of NCL, extraneural organs are involved to different degrees, among them skeletal muscle (Carpenter *et al.*, 1972). To our knowledge, NCL is the only lysosomal disorder other than Pompe's disease or type-II glycogenosis in which morphologically definable storage material occurs in the skeletal muscle fibres. It is true that skeletal muscles in Fabry's disease contain an increased amount of glycosyl-ceramide (Schibanoff *et al.*, 1969), but ultrastructurally definable lipids have been demonstrated only in smooth and cardiac muscle fibres from patients afflicted by this disorder (Sweeley *et al.*, 1972).

Since no light microscopic differences in tinctorial or autofluorescent properties exist between lipofuscin (also called 'wear and tear' or 'age' pigment) and the ceroid accumulating in NCL, the fine structure of the pigment in NCL, which is morphologically not uniform and probably different from lipofuscin, must be verified by electron microscopy.

Sampling of necropsy-derived muscle tissues from four cases of NCL and a muscle biopsy from a fifth case provided us with the oppor-

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TABLE
SUMMARY OF CLINICAL DATA

	Patient				
	1. B.W.	2. M.W. (sister of B.W.)	3. P.K.	4. A.F.	5. K.L.
Sex	F	F	F	M	M
Onset of symptoms (yr)	7	7	5	7 m	4
Death (yr)	16	22	18	7½	17
Visual disturbances (yr)	Early (7), now blind	Early (7), blind (10)	Early (5)	Blind (5)	Early (4), blind
Fundi	Retinal and optic atrophy, macular depigmentation	Retinal and optic atrophy, small vessels, pigment in macula	Retinal and optic atrophy, pigmentary degeneration	Retinal atrophy, pigmentary degeneration, small vessels	Pigmentary degeneration of retina, small vessels
Seizures (age, yr)	7	8	7	3	8
Dementia	Yes	Yes	Yes	Yes	Yes
EEG findings	Abnormal	Abnormal	Not known	Abnormal	Abnormal
Ataxia	Yes	Yes	Yes	Yes	Yes
Behavioural problems	Not certain	Yes	Yes	Yes	Yes
Azuropilic hypergranulation of neutrophils	Yes	Yes	Yes	Not known	Yes
Type of NCL	Spielmeyer-Sjögren, juvenile	Spielmeyer-Sjögren, juvenile	Spielmeyer-Sjögren, juvenile	Jansky-Bielschowsky, late infantile	Spielmeyer-Sjögren, juvenile

FIG. 1 Case 2. Deltoid muscle. Many subsarcolemmal autofluorescent granules are present. Unstained frozen section, $\times 700$.

FIG. 2 Case 2. Deltoid muscle. Most darkly stained type II and several type I fibres are smaller than normal. Some fibres display angulated cross-sections. ATPase (pH 9.3), $\times 100$.

tunity to test whether the assumption is valid that the morphological diagnosis of NCL can also be established by muscle biopsy.

METHODS

CLINICAL DATA Of the five patients, four belong to the Spielmeyer-Sjögren and one to the Jansky-Bielschowsky type as defined by Zeman *et al.* (1970). They all showed the hallmarks of this relentlessly progressive cerebral disease marked by dementia, ataxia, seizures, and a retinal pigmentary degeneration leading to blindness. Clinical findings are summarized in the Table. The patients afflicted by the

FIG. 3 Case 5. Deltoid muscle. Focal areas of acid phosphatase activity abound within the muscle cells. Gomori, $\times 400$.

FIG. 4 Case 5. Deltoid muscle. Many of the darkly stained type I fibres are smaller than normal. NADH-diaphorase, $\times 100$.

FIG. 5 Case 1. Deltoid muscle. Subsarcolemmal perinuclear aggregates of pigment granules. $\times 12\,250$.

FIG. 6 Case 5. Deltoid muscle. Clusters of pigment granules are located deep in the muscle cell, surrounded by abundant glycogen. $\times 22\,000$.

juvenile form of NCL had early visual disturbances and low seizure activity, whereas the patient with the late infantile form showed the converse. Although all the patients were emaciated terminally, none of them had signs or symptoms of a neuromuscular disorder during the course of the illness.

Classification of the five patients into one of the two subgroups was based on the age of onset of symptoms and on the course and duration of the illness. The clinical diagnosis was confirmed at necropsy in the first four patients, who had severely atrophic brains with numerous ceroid bodies in the neurones. The diagnosis of NCL in the fifth patient was supported by morphological findings in his skeletal muscle and recently confirmed by necropsy.

PROCEDURE For histochemistry, skeletal and extraocular muscles were obtained at necropsy from one patient and by biopsy from the fifth one of this series and quickly frozen in isopentane. Cryostat sections were prepared for H and E, modified trichrome, PAS, oil red O stains, and the following enzyme reactions:

NADH³, MAG⁴, ATPase⁵ at pH 9.3 (using additional preincubation at pH 4.6 and 4.2 in the biopsy specimen of case 5) and acid phosphatase (method after Burstone), according to routine procedures (Dubowitz and Brooke, 1973). Formalin-fixed skeletal and extraocular muscles of patients who had died were also embedded in paraffin.

For electron microscopy, tissue from skeletal, extraocular, and cardiac muscles, obtained at necropsy in four cases or by biopsy in the fifth patient, was immediately fixed in buffered glutaraldehyde, postfixed in OsO₄, dehydrated in graded series of ethanol and embedded in epon using standard procedures.

RESULTS

LIGHT MICROSCOPY Skeletal muscles sampled at necropsy showed green-yellow autofluorescent granules, chiefly packed beneath the sarco-

³ NADH-tetrazolium reductase (DPNH diaphorase).

⁴ Menadione-linked α -glycerophosphate dehydrogenase.

⁵ Adenosine triphosphatase.

lemma (Fig. 1). All extraocular muscles harboured autofluorescent pigment in larger amounts than the skeletal muscles, and the greatest amounts were present in the patients with the Spielmeyer-Sjögren type. The pigment showed tinctorial affinity to the PAS stain. Enzyme preparations of the autopsied muscle (case 2) revealed that many type II fibres and several type I fibres were atrophic (Fig. 2). A few scattered angulated fibres of both types were

FIG. 7 Case 4. Biceps muscle. Three curvilinear bodies are wedged between adjacent myofibrils. $\times 18\,400$.

FIG. 8 Case 1. *M. rectus abdominis*. Residual body bound by trilaminar membrane (double arrows) contains curvilinear profiles (arrow heads) and short straight lamellae (single arrows) alternately dark and light. V = vacuole. $\times 66\,000$.

FIG. 9 Case 3. Deltoid muscle. This atrophic muscle fibre, recognizable by remnants of myofibrils and basal lamina, contains large clusters of pigment bodies. $\times 18\,400$.

FIG. 10 Case 2. Extraocular muscles. A large subsarcolemmal pigment body is shown. $\times 20\,000$.

FIG. 11 Case 1. Extraocular muscles. The mesenchymal cell contains a residual body with curvilinear and fingerprint profiles, as well as some lipid vacuoles. $\times 32\,000$. The inset shows the fingerprint pattern at higher resolution. $\times 72\,500$.

also present. The biopsy specimen of the fifth patient displayed abundant activity of acid phosphatase (Fig. 3), but this reaction was not performed on the tissues of the other patients. A considerable number of type I fibres were reduced in diameter (Fig. 4).

ELECTRON MICROSCOPY Since the ultrastructural

features of all five cases were similar, the findings will be presented together. Although severe autolytic changes were encountered in those specimens obtained *post mortem*, the sarcomeres were fairly well preserved, confining the autolytic effects chiefly to the sarcoplasmic reticulum and the mitochondria. The cytoplasm between the myofibrils was often swollen and empty.

Many pigment bodies were found within the muscle fibres. They were mainly located beneath the sarcolemma, often close to the nucleus, forming either single granules or large clusters (Fig. 5). Infrequently, the residual bodies lay deeper inside the muscle fibres among the myofibrils (Fig. 6). All displayed the typical architecture of curvilinear bodies (Duffy *et al.*, 1968), irrespective of the clinical type (Fig. 7). The ultrastructural features of these curvilinear bodies consisted of small curved membranous lamellae. Occasionally, straight membranes of short length with alternating dark and light leaflets were present (Fig. 8). A trilaminar membrane around the curvilinear bodies could frequently be discerned but was often ruptured and segmentally absent, especially where swelling of the residual bodies had occurred which caused the individual curvilinear profiles to spread apart. The residual bodies only rarely contained clear lipid vacuoles, more frequently seen in the patients with the Spielmeyer-Sjögren than in the Jansky-Bielschowsky type. Sometimes electron-dense material was found incorporated within the curvilinear bodies.

In general, the sarcomeres of the striated muscle fibres did not show any intrinsic pathological changes. An occasional small degenerated muscle fibre, harbouring large cytosomes could be identified because of a few randomly oriented myofilaments and a surrounding basal lamina (Fig. 9).

The amount of pigment in the heart and extraocular muscles usually exceeded that of the skeletal muscles; the pigment granules were more numerous and the clusters were larger (Fig. 10); they, too, were composed of curvilinear profiles. Electron-dense material and lipid vacuoles were more frequent in cardiac and ocular muscles.

Mural cells of the vasculature—that is, pericytes and endothelial cells—as well as other mesenchymal elements contained bodies with curvilinear profiles, sometimes closely intermingled with fingerprint patterns (Fig. 11).

DISCUSSION

Our studies have shown conclusively that muscle biopsy is a reliable procedure for confirming a suspected diagnosis of neuronal ceroid-lipofuscinoses, at least for the Spielmeyer-

Sjögren and the Jansky-Bielschowsky types. In all five cases, autofluorescent lipopigments were readily discovered by fluorescence microscopy, and even by electronmicroscopy it was not difficult to find the equivalent in the form of pathognomonic residual bodies. The most interesting aspect of this study is that the ultrastructure of these residual bodies is uniform, regardless of the clinical type, and invariably consists of curvilinear profiles. These studies therefore suggest that, at least for the Spielmeyer-Sjögren and the Jansky-Bielschowsky types, the cell determines the fine architecture of the residual pigment body.

Our findings are in complete agreement with the observations of Carpenter *et al.* (1972) who additionally found that the smooth muscle fibres in the Spielmeyer-Sjögren type may contain residual bodies with fingerprint patterns. These authors distinguished curvilinear from rectilinear patterns, a differentiation which is not entirely clear and appears somewhat artificial to us. The Haltia-Santavuori type harbours granular osmophilic lipofuscin-like bodies in smooth and skeletal muscle cells (Haltia *et al.*, 1973; Rapola and Haltia, 1973). No pertinent studies have been performed on patients with the Kufs type, but in the canine form of neuronal ceroid-lipofuscinosis both fingerprint and curvilinear patterns are encountered in residual bodies located in cardiac and extraocular muscles (Koppang, 1973–74).

It follows that electronmicroscopic identification of the fine structure of the pigment bodies is required, not only for differentiation but also because other autofluorescent material may be present in the striated muscle fibres of man. Of particular significance is lipofuscin, especially in older individuals, which is also more densely present in the extraocular muscles where it occurs already during relatively early life (Rubinstein, 1961). Ultrastructurally, lipofuscin is endowed with a granular matrix and contains very prominent lipid vacuoles, which facilitates differentiation from the curvilinear and the fingerprint bodies (Carpenter *et al.*, 1972). Although we consider bodies with curvilinear and fingerprint profiles as ceroid, it should be pointed out that the ultrastructure of experimentally induced ceroid is different (Porta and Hartroft, 1969).

Further differential diagnostic considerations apply to human vitamin E deficiency, in which ceroid is found in the smooth and occasionally also in skeletal muscle cells. This process is associated with a myopathy (Binder *et al.*, 1965), a finding which serves as a useful differential diagnostic criterion. In fact, this myopathy may not be associated with the presence of pigment granules (Weinberg *et al.*, 1958). In experimentally induced tocopherol deficiency, residual bodies (Howes *et al.*, 1964) are commonly found in the muscle fibre which are also fluorescent (Telford, 1971) and associated with myopathic changes (Mason, 1973). Significantly, such myopathic changes were not present in our patients with NCL, indicating that the formation and accumulation of pigment is not causally linked to the myopathy.

Finally, it is well known that a variety of myotoxic drugs, especially chloroquine, produce a myopathy with deposition of autofluorescent material (Gérard *et al.*, 1973; Klinghardt, 1974). These autofluorescent substances represent also tertiary lysosomes containing debris and possibly also the drug that accumulates within the muscle (McChesney *et al.*, 1967). Membranous material which shows some similarity to curvilinear profiles is often encountered (Rewcastle and Humphrey, 1965; Gérard *et al.*, 1973) in addition to membranous cytoplasmic bodies as pointed out by Klinghardt (1974).

The mild histochemical abnormalities of the skeletal muscle fibres, type II atrophy in one and partial type I atrophy in another patient, may be interpreted as representing disuse atrophy or damage to the pyramidal tracts and the cerebellum respectively (Dubowitz and Brooke, 1973). These possibilities, of course, are implicit in the basic disease process which produces severe diffuse brain damage, leading to motor deterioration and immobility.

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