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Focal deficiency of cytochrome-c-oxidase in skeletal muscle of patients with progressive external ophthalmoplegia

Cytochemical-fine-structural study

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Summary. In skeletal muscle biopsies of 8 patients with progressive external ophthalmoplegia combined light and fine structural cytochemical studies of cytochrome-c-oxidase revealed the absence of the enzyme in single fibres with or without accumulation of abnormal mitochondria. However, some fibres showed abnormal mitochondria without any deficiency of the enzyme. In one case with only slight mitochondrial proliferation the existence of the enzyme defect was the most remarkable finding. The occurence of the enzyme defect obviously does not depend on concomitant structural alterations of the chondriom. The results are consistent with an acquired mitochondrial injury leading to a gradual loss of enzyme activity either earlier (with or without a minimal reactive mitochondrial proliferation) or later (after a phase of mitochondrial proliferation) in the course of the disease. Focal lack of cytochrome-c-oxidase activity is apparently a constant feature of the syndrome; it therefore may be not only of pathogenetic but also of diagnostic importance and in this connection cytochemical-fine-structural demonstration of cytochrome-c-oxidase is a valuable method. In contrast to the biochemical approach it allows not only the detection but also the exact anatomical localization of single fibre defects.

Key words: Progressive external ophthalmoplegia – Mitochondrial myopathy – Cytochrome-c-oxidase

Progressive external ophthalmoplegia (PEO) is a complex disorder with multiorgan involvement. External ocular and skeletal muscles are regularly affected. In addition retinal, cerebral and cardiac manifestations are known to occur, among other symptoms (Bastiaensen 1978). The changes in skeletal muscle are best described as mitochondrial myopathy with accumulation of enlarged, structurally abnormal mitochondria and with storage of lipid

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and some glycogen. These structural alterations strongly point to a defective mitochondrial function. However biochemical studies of isolated mitochondria in PEO so far have failed to reveal any consistent or specific abnormalities (Berenberg et al. 1977; Carafoli and Roman 1980). This may be related to the heterogeneity of the disorder, or to the fact that usually only a small number of fibres is structurally affected (Morgan-Hughes 1982), being hardly detectable with biochemical methods. To solve this problem an enzyme histochemical approach is best suited, since it allows the demonstration of single fibre alteration. We therefore undertook a cytochemical and fine structural investigation of cytochrome-c-oxidase, the final enzyme of the respiratory chain, in order to obtain deeper insight into mitochondrial function of individual muscle fibres in PEO.

Materials and methods

Nine muscle biopsies (stored deep frozen up to 2 years, −20° C or −80° C) of eight patients with PEO were investigated by:

a) Histological methods (Staining with Azan, Trichrome, Sudan black, v. Gieson);

b) Routine electron microscopy;

c) Enzyme histochemistry: NADH-Tetrazoliumreductase, myofibrillary ATPase, and cytochrome-c-oxidase on frozen sections and for ultrastructural study as previously described (Müller-Höcker et al. 1983). KCN-inhibition of cytochrome-c-oxidase was used as control.

Results

In all but one case light microscopy revealed a variable number of muscle fibres with a coarsened fibrillary pattern, subsarcolemmal spaces free of fibrils, activated nuclei and focal accumulation of lipids and some glycogen (ragged red fibres in trichrome stain), (Fig. 1a, b). The capillary network around these fibres appeared to be more pronounced.

Electron microscopy of ragged red fibres showed subsarcolemmal aggregates of abnormally structured enlarged mitochondria with a high content of cristae, often with paracristalline inclusions, as well as accumulation of lipid droplets in the sarcoplasma (Fig. 2a, b).

Enzyme histochemistry of NADH-Reductase demonstrated an increased amount of reaction product mainly in the subsarcolemmal zone of the affected fibres (Fig. 3) which belonged predominantly to Type I fibres (confirmed by myofibrillary ATPase).

Demonstration of cytochrome-c-oxidase revealed, as a constant finding, varying numbers of fibres with complete absence of enzyme activity (Fig. 4). In addition some fibres showed a diminished intensity of the reaction. In longitudinal sections the enzyme defect was often confined to a segment of the muscle fibre (5a). Apparently the enzyme defect occured not only in fibres with increased NADH-reaction product but also in fibres with a normal reaction pattern. These findings were confirmed by ultracytochem-

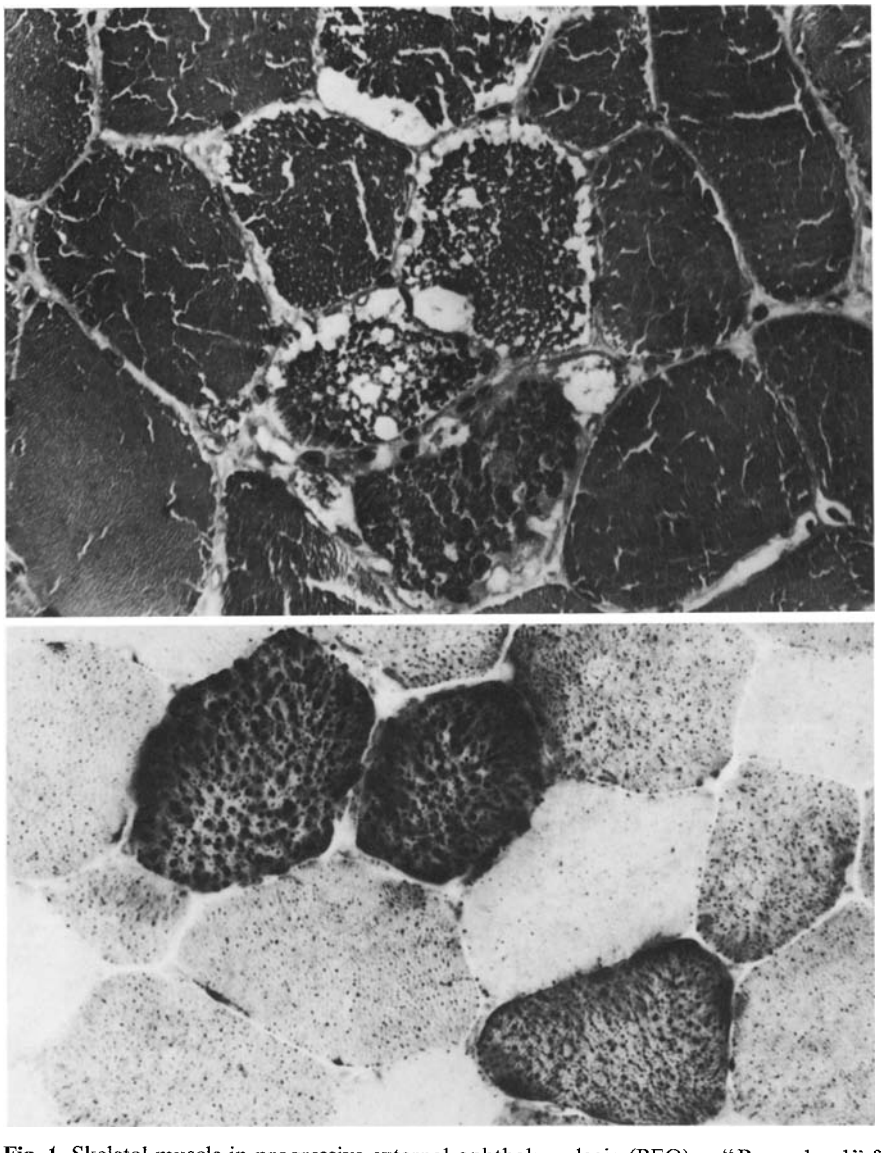


Fig. 1. Skeletal muscle in progressive external ophthalmoplegia (PEO). **a** "Ragged red" fibres with a loose arrangement and subsarcolemmal loss of myofibrils, as well as vacuolar degeneration. In the altered fibres increased lipid is stored. **a** Trichrome, ×350, **b** Sudan black ×350

istry: Fine structurally the enzyme defect was present in various muscle fibres, irrespective of the magnitude of structural alterations. It could be detected in quite normally appearing fibres (Fig. 5a), in others with only slight subsarcolemmal aggregates of mitochondria (Fig. 5b) and in typical ragged red fibres (Fig. 5c).

Abnormal fibres with a high content of enzyme reaction product in

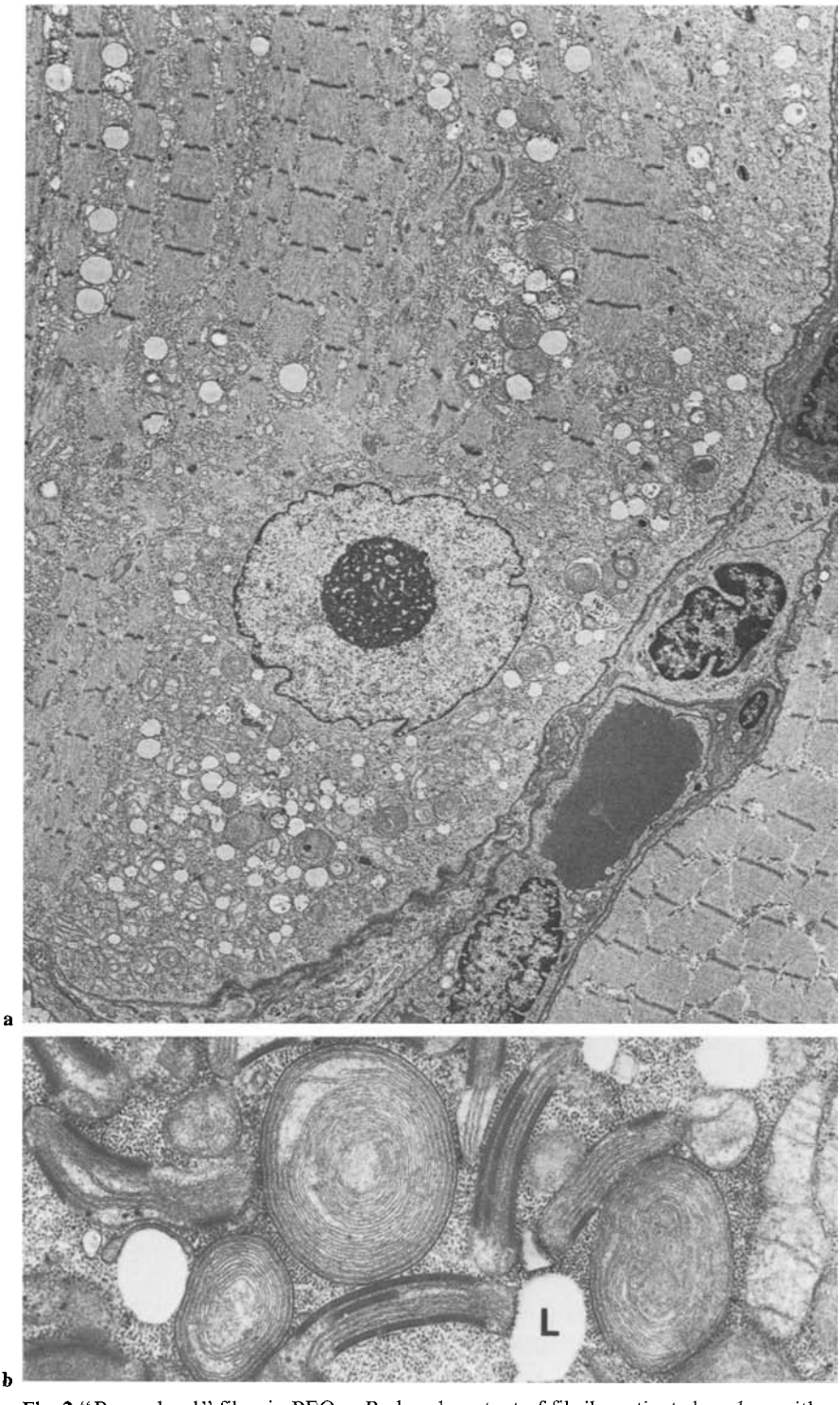
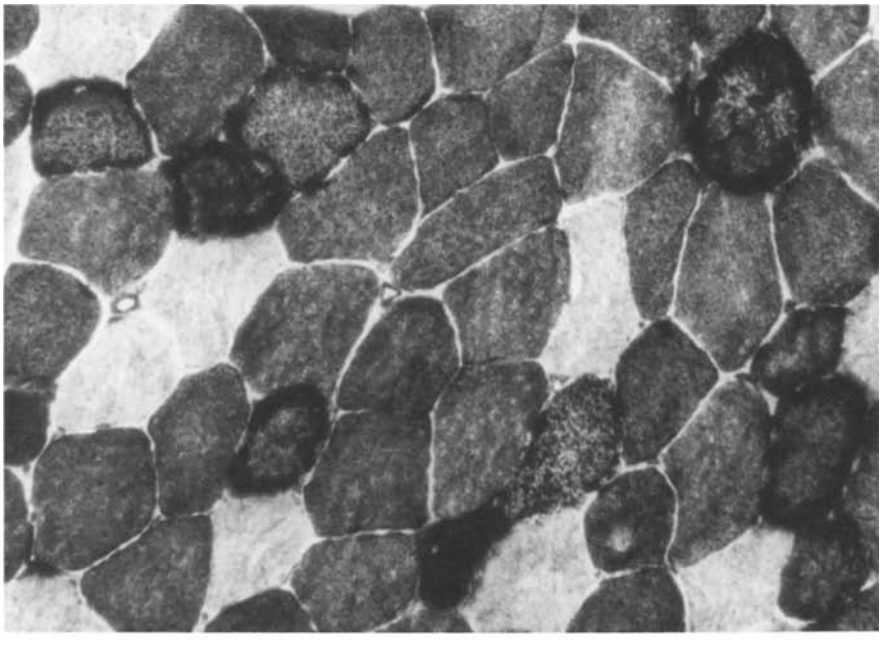


Fig. 2 "Ragged red" fibre in PEO. **a** Reduced content of fibrils, activated nucleus with prominent nucleolus, subsarcolemmal accumulation of enlarged abnormal mitochondria and multiple lipid droplets. **b** Higher magnification of abnormal mitochondria with concentric cristae, paracristalline inclusions and intermingled lipid droplets (*L*). **a** ×4,000, **b** ×21,000



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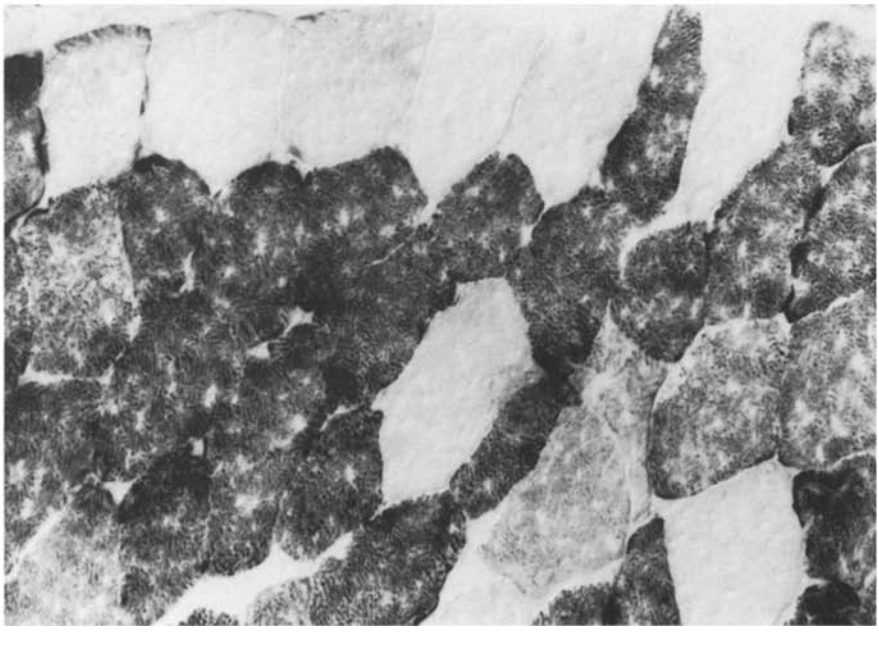

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Fig. 3. NADH-Reductase reaction to show increased activity in scattered fibres, particularly near the sarcolemma. × 225

Fig. 4. Cytochrome-c-oxidase reaction in PEO. In a considerable number of fibres the activity of the enzyme is lacking. × 225

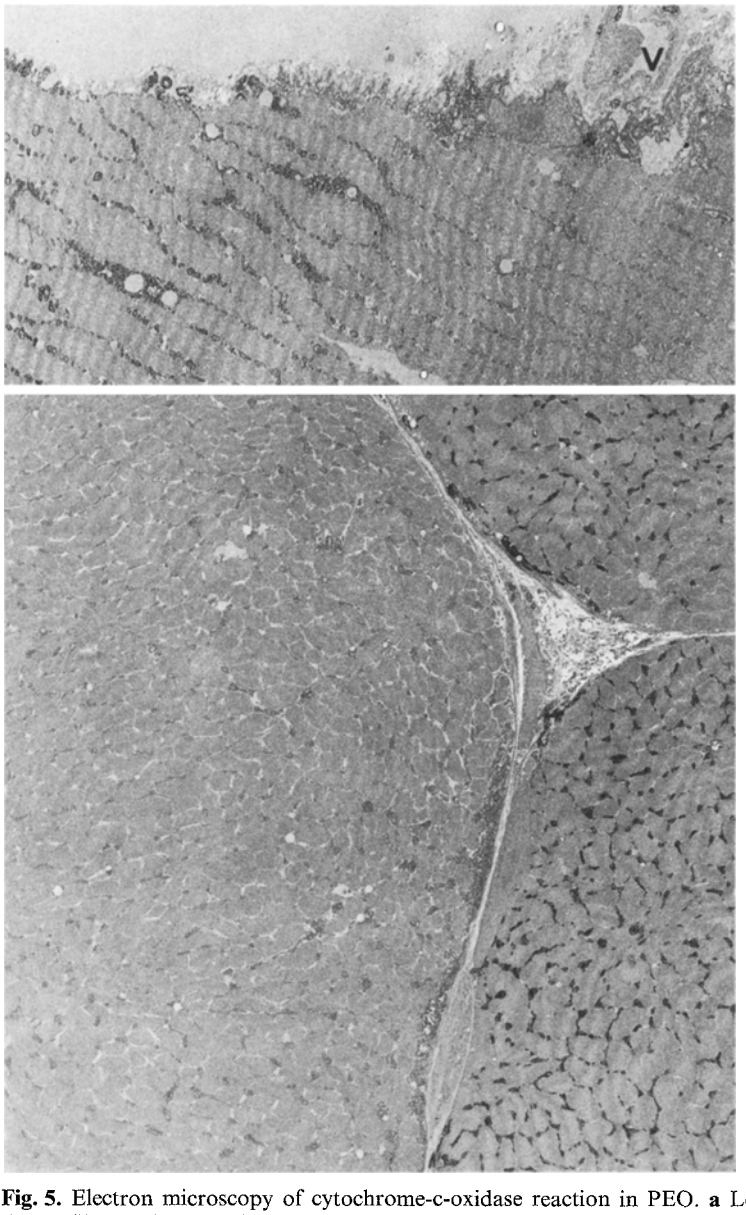
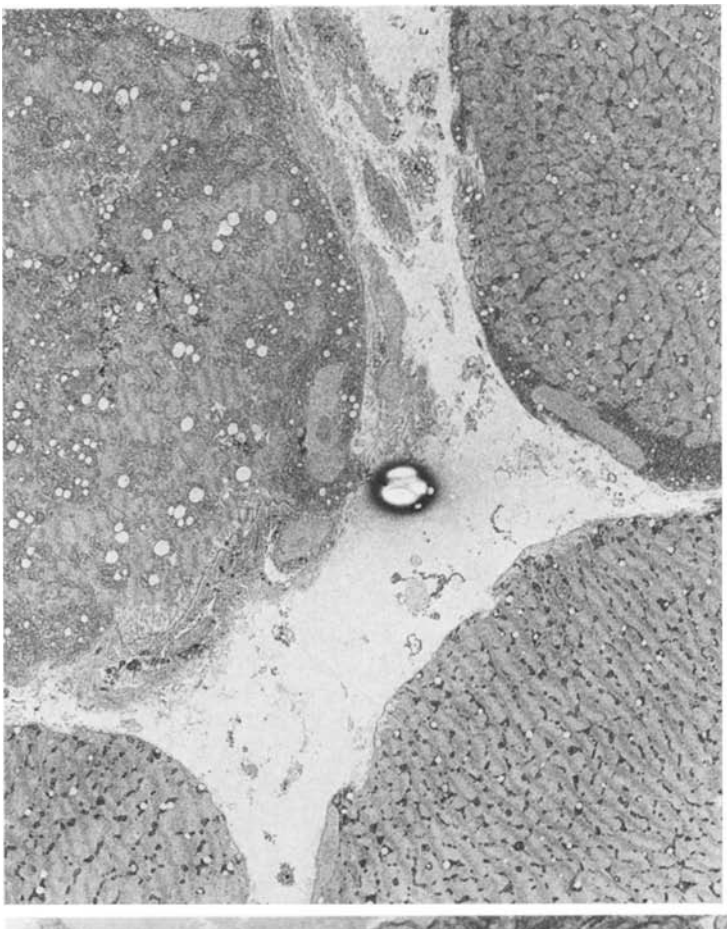


Fig. 5. Electron microscopy of cytochrome-c-oxidase reaction in PEO. **a** Longitudinally sectioned fibre without obvious mitochondrial abnormalities, but with failure of enzyme activity confined to a fibre segment (right). Note normal reaction in mitochondria of the adjacent blood vessel wall (*V*). **b** Enzyme defect in a fibre (left) with only slight subsarcolemmal proliferation of mitochondria. **c** Lack of enzyme activity in one "ragged red" fibre (upper left) and reduced reaction intensity in another one (upper right). At the bottom 2 morphologically unchanged fibres with normal reaction intensity. **d** Subsarcolemmal accumulation of abnormal mitochondria with high enzyme activity of the cristae (*mf* myofibrils). **a** ×2,640, **b** ×1,840, **c** ×2,140, **d** ×12,800



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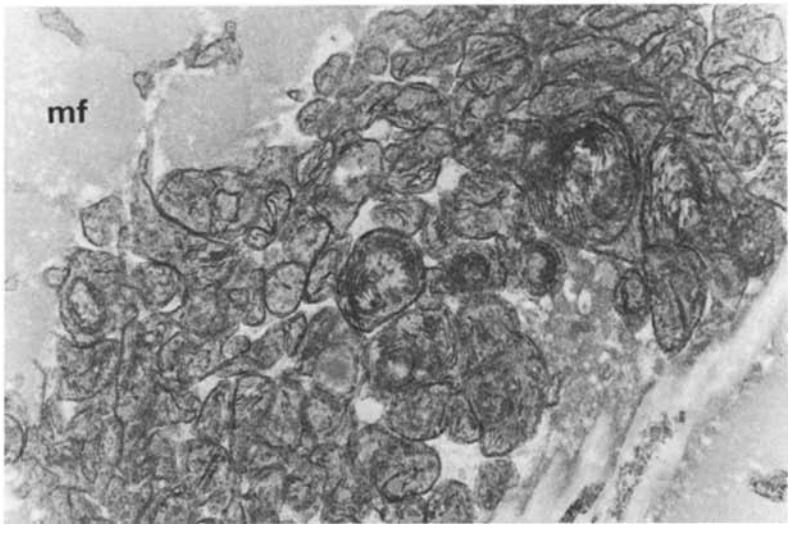

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clusters of mitochondria were also detected (Fig. 5d) but paracristalline inclusions were always free of enzyme activity.

The defect of cytochrome-c-oxidase was present in every biopsy specimen. In one case, with typical clinical manifestation where no ragged red fibres nor an abnormal NADH-pattern could be found, the existence of the enzyme defect was the most conspicuous finding. In this case only slight focal mitochondrial proliferation was visible in the electron microscope.

Discussion

Despite its varying degree of multiorgan involvement progressive external ophthalmoplegia (PEO) is a well established entity. Concerning its pathogenesis no basic defect has yet been demonstrated. Biochemical studies have revealed derangements of lactate-pyruvate metabolism (DiMauro et al. 1973; Reske-Nielsen et al. 1976; Hyman et al. 1977; Scarlato et al. 1978). In addition the function of oxidative phosphorylation has been investigated in some cases, but with conflicting results (DiMauro et al. 1973; Berenberg et al. 1977; Carafoli and Roman 1980). Nevertheless the morphological findings of different studies (Pongratz et al. 1979; Ringel et al. 1979; Kamieniecka and Schmalbruch 1980; Morgan-Hughes 1982) fit best with the assumption of a primarily mitochondrial disorder.

Our cytochemical-fine-structural investigation establishes that cytochrome-c-oxidase is focally lacking in skeletal muscle mitochondria of patients with PEO.

This defect apparently is not due to a destructive or degenerative process. It was present in various fibres of every muscle biopsy, irrespective of the concomitant morphological alterations. At the ultrastructural level it could be demonstrated that the enzyme defect did not involve every fibre containing clusters of abnormal mitochondria, but was occasionally present in normal appearing fibres. In addition longitudinal sections showed that mitochondrial damage, i.e. absence of enzyme activity, does not necessarily involve the fibre as a whole, but may be confined to a segment of the fibre.

From the results presented it can be concluded that deficiency of cytochrome-c-oxidase in single skeletal fibres is obviously a constant feature of PEO. It probably arises very early in the pathological process and therefore seems to be not only of pathogenetic, but also of diagnostic importance.

With regard to etiology and pathogenesis, no firm conclusions can yet be drawn. Our findings are consistent with the concept of an acquired mitochondrial damage which starts in the normal appearing fibre and leads to cytochrome-c-oxidase deficiency. As a consequence of the injury, compensatory mitochondrial hyperplasia may develop with accumulation of structurally abnormal mitochondria. These at first show normal cytochrome-c-oxidase activity which deteriorates later in the course of the disease.

Loss of cytochrome-c-oxidase appears to be a nonspecific reaction to mitochondrial damage: It is known to occur in such varying disorders as Leigh's disease (Willems et al. 1977), Trichopoliodystrophy (French et al. 1972, but see Zellkowitz et al. 76, cited from Carafoli and Roman 1980),

in Wilson's disease (Shokeir and Shreffler 1969) and in propionic or methylmalonic acidemia (Hayasaka et al. 1982).

Deficiency of cytochrome-c-oxidase is a constant feature of a fatal congenital syndrome with severe muscular hyoptonia, kidney dysfunction (van Biervliet et al. 1977; DiMauro et al. 1980; Heiman-Patterson et al. 1982; Stansbie et al. 1982; Müller-Höcker et al. 1983) and may be present in some other cases of floppy infants (Rimoldi et al. 1982). In addition there apparently exist more benign variants of cytochrome-c-oxidase deficiency (Monnens et al. 75, DiMauro et al. 1981). As cytochrome-c-oxidase is coded by nuclear as well as mitochondrial DNA (Schatz and Mason 1974), and is associated with haem groups and copper atoms (Whittaker and Danks 1978) a variety of defects may be at work. In any case more subtle studies are necessary to disclose the probably different causes of cytochrome-c-oxidase deficiency in the various clinical entities.

Our findings are corroborated by a previously presented short communication (Johnson et al. 1982). They are at variance with earlier cytochemical-fine-structural studies of two cases, where, perhaps due to sampling error no such defects were described (Engel et al. 1973; Bonilla et al. 1975). These authors however confirm our results, that no enzyme activity is found in paracristalline inclusions.

At present it is still premature to correlate the extent of the enzyme defect with the severity of clinical symptoms. In addition it is still unclear whether the enzyme defect is present in other organs, e.g. in liver, in sweat glands, in the cerebellum, where abnormal mitochondria have been described (Gonatas et al. 1967; Karpati et al. 1973; Schneck et al. 1973), or even in neurogenic or inflammatory muscular disease with abnormal mitochondria, (Shafiq et al. 1967; Swash et al. 1978; Pongratz et al. 1979; Schiffer et al. 1979).

From a practical point of view it must be stated that in our experience cytochrome-c-oxidase is a very stable enzyme, which is still demonstrable in longterm frozen tissue. Even in autopsy muscle of a previously published case of unexplained mitochondrial cardiomyopathy (Hübner et al. 1983) enzyme activity was sufficiently well preserved after two years of storage. With regard to the present results cytochemical demonstration of cytochrome-c-oxidase can be an essential diagnostic measure in progressive external ophthalmoplegia, and may reveal early or latent variants of the disease. Furthermore it leads to a better understanding of this complicated disorder.

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Note added in proof

Since submission of this manuscript the results of the cytochemical light microscopical study of Johnson MA et al. cited in our publication as "short communication" have recently been reported in detail (Johnson MA, Turnbull DM, Dick DJ, Sherratt HSA, 1983, A partial deficiency of cytochrome-c-oxidase in chronic progressive external ophthalmoplegia. J Neurol Sci 60:31–53).