REVIEW ARTICLE

Pathology of Mitochondrial Encephalomyopathies

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ABSTRACT: Muscle biopsy provides the best tissue to confirm a mitochondrial cytopathy. Histochemical features often correlate with specific syndromes and facilitate the selection of biochemical and genetic studies. Ragged-red fibres nearly always indicate a combination defect of respiratory complexes I and IV. Increased punctate lipid within myofibers is a regular feature of Kearns-Sayre and PEO, but not of MELAS and MERRF. Total deficiency of succinate dehydrogenase indicates a severe defect in Complex II; total absence of cytochrome-c-oxidase activity in all myofibres correlates with a severe deficiency of Complex IV or of coenzyme-Q10. The selective loss of cytochrome-c-oxidase activity in scattered myofibers, particularly if accompanied by strong succinate dehydrogenase staining in these same fibres, is good evidence of mitochondrial cytopathy and often of a significant mtDNA mutation, though not specific for Complex IV disorders. Glycogen may be excessive in ragged-red zones. Ultrastructure provides morphological evidence of mitochondrial cytopathy, in axons and endothelial cells as well as myocytes. Abnormal axonal mitochondria may contribute to neurogenic atrophy of muscle, a secondary chronic feature. Quantitative determinations of respiratory chain enzyme complexes, with citrate synthase as an internal control, confirm the histochemical impressions or may be the only evidence of mitochondrial disease. Biological and technical artifacts may yield falsely low enzymatic activities. Genetic studies screen common point mutations in mtDNA. The brain exhibits characteristic histopathological alterations in mitochondrial diseases. Skin biopsy is useful for mitochondrial ultrastructure in smooth erector pili muscles and axons; skin fibroblasts may be grown in culture. Mitochondrial alterations occur in many nonmitochondrial diseases and also may be induced by drugs and toxins.

RÉSUMÉ: Anatomopathologie des encéphalomyopathies mitochondriales. La biopsie musculaire est le meilleur tissu pour obtenir confirmation d'une cytopathie mitochondriale. Il existe souvent une corrélation entre les caractéristiques histochimiques et un syndrome spécifique, ce qui facilite le choix d'études biochimiques et génétiques. La présence de ragged-red fibres indique presque toujours un défaut dans les complexes respiratoires I et IV. On observe une augmentation de coloration ponctuée de lipides dans les fibres musculaires dans les syndromes de Kearns-Sayre et PEO, mais non dans les syndromes MELAS et MERRF. Un déficit total en SDH indique qu'il existe un défaut sévère du Complexe II et il existe une corrélation entre une absence totale d'activité COX dans toutes les fibres musculaires et un déficit sévère en Complexe IV ou en coenzyme Q10. La perte sélective d'activité COX dans des fibres musculaires éparses, surtout si elle est accompagnée d'une forte coloration SDH dans ces mêmes fibres, est fortement en faveur d'une cytopathie mitochondriale et souvent d'une mutation importante de l'ADN mitochondrial, bien que non spécifique des maladies du Complexe IV. La concentration en glycogène peut être excessive dans les zones de ragged-red fibres. L'examen de l'ultrastructure démontre des changements morphologiques d'une cytopathie mitochondriale dans les axones et dans les cellules endothéliales ainsi que dans les myocytes. Les mitochondries axonales anormales contribuent probablement à l'atrophie musculaire neurogénique, une manifestation secondaire chronique. Des évaluations quantitatives des complexes enzymatiques de la chaîne respiratoire, utilisant la citrate synthase comme contrôle interne, confirment les impressions histochimiques ou peuvent être les seules observations en faveur d'une maladie mitochondriale. Des artefacts biologiques et techniques peuvent fournir des niveaux d'activité enzymatique faussement bas. Des mutations ponctuelles fréquentes de l'ADN mitochondrial peuvent être détecté par des études génétiques. Au niveau du cerveau, on retrouve des altérations histopathologiques caractéristiques dans les maladies mitochondriales. La biopsie cutanée est utile pour l'examen de l'ultrastructure mitochondriale des muscles lisses érecteurs des poils et des axones; les fibroblastes obtenus à partir de la peau peuvent être cultivés. Des changements mitochondriaux peuvent être observés dans plusieurs maladies non mitochondriales et peuvent également être induits par des médicaments et des toxines.

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A decade ago, it was unusual for pathologists to receive a muscle biopsy for the expressed diagnosis of mitochondrial disease. Today, as many as 25-50% of muscle biopsies performed in children, and an increasing number in adults, are taken for just this reason. The development of new histochemical, biochemical and genetic techniques now allows the more efficient study of tissue for mitochondrial cytopathy than a decade ago, and with more precise criteria, but the

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justification to pursue a comprehensive and expensive workup often remains problematic.

Mitochondrial diseases are metabolic disorders affecting every cell in the body, but are most frequently expressed, clinically and pathologically, in three organ systems: striated muscle, brain and heart, in any combination. The clinical manifestations are extremely variable, even with a known pathogenic point mutation and even amongst affected members of the same family. Phenotype/genotype correlations often are poor for the clinical identification of specific mitochondrial deoxyribonucleic acid (mtDNA) point mutations or even specific mitochondrial syndromes at times.[1-4]

Lactic acidosis also is variable, elevated in some and not in other mitochondrial cytopathies, so that high serum lactate, accompanied by normal to high serum pyruvate, is strong evidence in favor, but a normal serum lactate is by no means reassuring. It is important to measure pyruvate simultaneous with lactate because an elevated lactate with low pyruvate usually reflects physiological anaerobic metabolism. (For example, if the tourniquet constricts the arm too long during the search for a good vein.) Increased lactate due to mitochondrial disease nearly always is associated with normal or high pyruvate levels. In mitochondrial encephalopathies, cerebrospinal fluid lactate may be elevated though the serum lactate is normal.

Several distinctive mitochondrial syndromes are now recognized. Examples are the Kearns-Sayre syndrome, MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy with ragged-red fibers), PEO (progressive external ophthalmoplegia), periventricular necrotizing encephalopathy of Leigh, LHON (Leber hereditary optic neuropathy) and the mitochondrial DNA depletion syndrome. Each of these syndromes has some constant clinical features that allow the diagnosis to be suspected and justify investigations to confirm or refute this provisional diagnosis, but some patients fit a classical clinical and pathological phenotype for one, such as MELAS but with a mtDNA point mutation more typical for MERRF or there may be a mixed MELAS/MERRF phenotype[5,6] or MERRF/Kearns-Sayre phenotype.[7] Many mitochondrial cytopathies cannot yet be classified. Even within the examples cited above, each has several different associated genetic mutations, deletions or duplications, so that they are true syndromes and not single diseases.

The pathological expression of these mitochondrial cytopathies also varies, though less than the clinical picture. Muscle is the tissue best suited to investigate mitochondrial diseases, if the specific genetic defect is not known. If the defect is known from another affected member of the same family, genetic confirmation may be feasible from a blood sample. Muscle is a useful tissue to biopsy because it is less invasive than a myocardial or cerebral biopsy, a larger sample of tissue can be taken for studies and, because it can provide valuable diagnostic information in most cases, even if the primary clinical symptoms involve brain or heart with few clinical signs of myopathy. The mitochondria of muscle are abundant in the subsarcolemmal and intermyofibrillar spaces and they are larger than in most other tissues. At times, the muscle biopsy also provides an opportunity to examine the ultrastructure of mitochondria in endothelial cells and in axons, in addition to myofibres, to confirm involvement of multiple tissues of different origin. Several previous pathological studies of both muscle and brain confirm the reliability of histopathological observations between various authors.[8-16] These studies are in agreement with findings derived from our personal experience and described here. We have emphasized a few aspects infrequently considered, such as nucleic acid and glycogen stains in ragged-red fibres.

This paper attempts to systematically identify the diverse features of the muscle biopsy that not only may be used to make a diagnosis of mitochondrial cytopathy, but also narrows the differential diagnosis amongst the possible mitochondrial disorders. The role of histochemistry, electron microscopy, quantitative measurement of respiratory chain enzymes and genetic studies of mtDNA point mutations and deletions are discussed; criteria are suggested for proceeding with each of these studies, also considering economic limitations. The criteria applied to decide how extensively to study any given muscle biopsy is a complex issue that deserves to be addressed.[17]

Histopathological and histochemical features of mitochondrial cytopathy in the muscle biopsy

Selection of the muscle to be sampled. Whereas all muscles show similar findings in these systemic diseases, the quadriceps femoris muscle (vastus lateralis) is generally the most suitable muscle for study because a relatively large sample can be taken without the risk of damaging major nerves or blood vessels or causing functional impairment.

Gross handling of the tissue. The sample should be measured and described, though no macroscopic features are specific enough to suggest mitochondrial disease except that extensive fatty or collagenous connective tissue proliferation are not characteristic. The muscle should be divided into four unequal portions, taking care not to crush or stretch the tissue excessively. One portion is freshly frozen in isopentane (2-methylbutane) cooled to –160°C in liquid nitrogen for cryostat sections for histochemistry. A second portion is frozen directly in liquid nitrogen (or may be wrapped in aluminum foil) and stored directly in the –80°C freezer for possible biochemical studies. The frozen tissue left on the block after the histochemical sections are cut is not suitable for biochemical determination of respiratory chain enzymes because isopentane interferes with the measurement of Complexes I, II and III (but not IV or V or citrate synthase) and may give falsely low activities.[18] Another portion of the biopsy is fixed in 10% buffered formalin for paraffin embedding; a small, finely minced portion is fixed in glutaraldehyde, Karnovsky solution or some other fixative suitable for electron microscopy.

Histochemical studies. The routine battery of histochemical stains applied to frozen sections of muscle in most modern histopathology laboratories should be performed; it generally includes modified Gomori trichrome stain; periodic acid-Schiff reaction (PAS); oil red O or sudan black B for neutral lipids; myofibrillar adenosine triphosphatase, calcium-mediated (ATPase) preincubated at 2 or 3 pH ranges, usually 9.8, 4.6 and 4.3; total myophosphorylase and an oxidative enzymatic stain, usually nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR; Respiratory Complex I). For suspected mitochondrial disease, two other oxidative enzymatic stains are

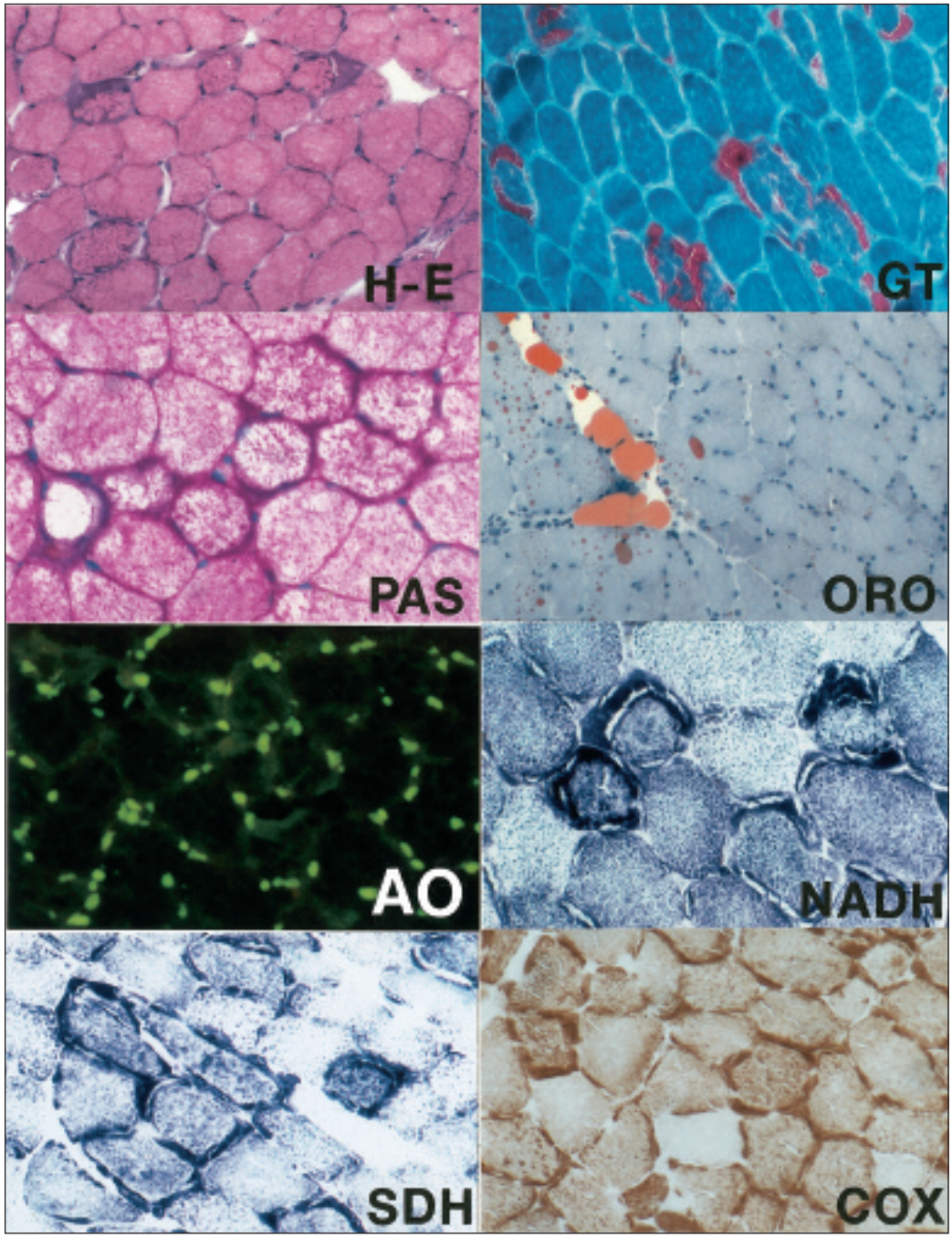

Figure 1: *Quadriceps femoris muscle biopsy of a 34-year-old woman with right spastic hemiplegia since infancy, due to a large left middle cerebral artery infarct and porencephaly. She also has several smaller cerebral infarcts bilaterally, demonstrated by MRI. She has acquired microcephaly, persistent lactic acidosis and generalised weakness that has progressed over the past two years, hence the muscle biopsy at this time. The clinical diagnosis is MELAS syndrome. Haematoxylin-eosin (H-E) stain shows variation in myofibre diameter and several fibres with basophilic sarcoplasmic masses, corresponding to ragged-red fibers clearly identified with modified Gomori trichrome (GT) stain because these subsarcolemmal zones are irregular in shape and intensely red in colour, whereas the normal myofibrils are green. Glycogen (PAS) is abundantly stained in these subsarcolemmal masses and is digested by diastase (not shown); one fibre shows loss of PAS staining except for its ragged-red margins. Neutral lipid, demonstrated by oil red O (ORO) is not increased within myofibres, but is globular in the perimysium. Acridine orange (AO) fluorochrome shows no orange-red fluorescence in the ragged-red zones or within myofibrils. The oxidative enzymatic stains NADH, SDH and COX all exhibit intense mitochondrial enzymatic activity in the subsarcolemmal zones corresponding to ragged-red fibres. Two myofibres show no COX activity and a few others show weak activity, strong evidence of a mitochondrial defect, though nonspecific for which mitochondrial disease. Frozen sections. X250 (H-E, GT, ORO, AO). X400 (PAS, NADH, SDH, COX).*

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Table 1: Mitochondrial respiratory chain enzymes of patient whose muscle biopsy is shown in Figure 1

	Specific Level	Range of 8 Controls
Complex I (NADH)	<0.003	0.014-0.055
Complex II (SDH)	0.022	0.003-0.035
Complex III (cytochrome b)	0.005	0.013-0.060
Complex IV (COX)	0.039	0.075-0.225
Complex V (ATP synthase)	0.020	0.060-0.300
Citrate synthase	0.235	0.090-0.262

There is significantly decreased activity in Complexes I, III, IV and V. The defects in Complexes I and IV correlate well with ragged-red fibres. Citrate synthase is normal, an internal control demonstrating that the number of functional mitochondria is satisfactory to render the values valid, and the specific deficiencies demonstrated were each confirmed when calculated as a ratio of citrate synthase. All units are expressed as µmol substrate/min/mg protein. None of the described mtDNA mutations or deletions were identified, hence the patient must have a novel mutation, but her clinical, pathological and biochemical profile clearly are consistent with the MELAS syndrome.

essential: succinate dehydrogenase (SDH; Complex II) and cytochrome-c-oxidase (COX; Complex IV). There are no reliable and easy histochemical techniques to demonstrate Respiratory Complexes III or V, hence these remain biochemical determinations. Formalin-fixed, paraffin-embedded sections of muscle are not essential for mitochondrial studies per se, but if sufficient tissue is available, they usually provide a larger sampling field if inflammation or other changes such as myofiber degeneration are diagnostic considerations.

Microscopic features of mitochondrial cytopathies

1) Ragged-red fibres. Several well-defined mitochondrial diseases are characterized by "ragged-red fibres". This name is derived from the irregular subsarcolemmal zone of many altered myofibres that stains red with the modified Gomori trichrome stain in frozen sections. The reason for the red color is that these zones contain an abundance of proliferated mitochondria and often show abundant glycogen as well. Mitochondrial membranes are stained red with this trichrome because one of the ingredients is chromotrome-2R, which has a strong affinity for phospholipids; mitochondrial membranes have a great deal of sphingomyelin, a complex phospholipid, which appears red with this stain. The term does not denote type I myofibres that predominate in "red muscles" of animals, such as the leg of the chicken as opposed to the white chicken breast. The standard Gomori trichrome stain applied to paraffin sections does not identify ragged red fibers easily because normal myofibrils also stain red after formalin fixation. Hematoxylin-eosin (H&E) stain often identifies the ragged-red zones of affected myofibers in both frozen and paraffin sections, however, because of the basophilic, amorphous appearance and absence of myofibrils in these zones. Oxidative enzymatic stains confirm the validity of ragged-red fibres, and also help distinguish them from "sarcoplasmic masses" and ringbinden, as occur in myotonic dystrophy but are of an entirely different nature, and also from degenerating and regenerating peripheral zones of myofibres. An example of a ragged-red mitochondrial myopathy is shown in Figure 1. Ragged-red fibres rarely are seen in muscle biopsies of children less than five years of age, so that their absence in myofibres of infants and toddlers is not evidence that these diseases are not present.

2) Neutral lipids. Increased neutral lipid within myofibers (but not perimysial or endomysial fat) is characteristic of some, but not all, mitochondrial cytopathies, presumably because of impaired

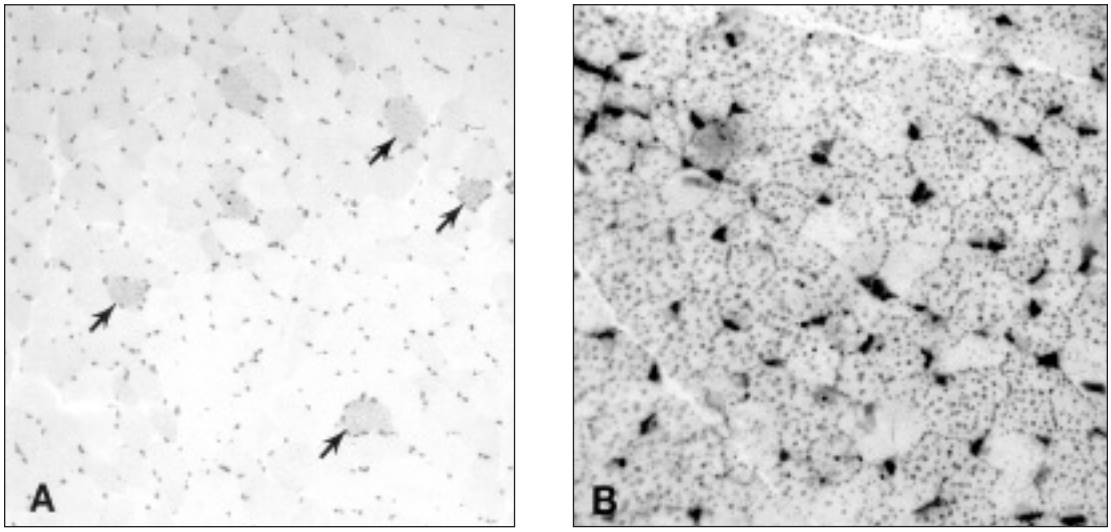

Figure 2: *Two patterns of increased punctate lipid within myofibres of patients with mitochondrial cytopathies. (A) Scattered myofibres show increased lipid in their sarcoplasm (arrows) in an infant with Leigh encephalopathy. (B) Nearly every myofibre has course droplets of lipid throughout the sarcoplasm in a 14-year-old boy with Kearns-Sayre syndrome. Figure 1 provides an example of absence of increased lipid in all myofibres in MELAS syndrome. Frozen sections. Oil red O. (A) X100. (B) X250.*

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lipid utilization. The presence of increased punctate lipid droplets in the sarcoplasm, seen with oil red O or sudan stains, is not, however, random and unpredictable in ragged-red fibre myopathies. Increased lipid is a constant feature in Kearns-Sayre syndrome (Figure 2) and PEO, but not in MELAS or MERRF. It is found in some, but not all, genetic forms of Leigh encephalopathy.

3) Glycogen. PAS-positive material digested by diastase may be abundant in myofibers of patients with mitochondrial myopathies, particularly in the subsarcolemmal zones of ragged-red fibres, but is also found in the intermyofibrillar sarcoplasm. This finding is confirmed by EM. Glycogen may be so abundant that the initial impression is that of a glycogenosis, and even single membrane-bound glycogen granules may be demonstrated. Not all myofibers in mitochondrial cytopathies have excessive glycogen, however, so that this is not a reliable criterion. The occasional finding of paracrystalline inclusions in muscular mitochondria of both paediatric and adult patients with acid maltase deficiency (glycogenosis II) raises speculation about a possible secondary mitochondrial defect in some glycogenoses and perhaps conversely contributes to increased glycogen in muscle with dysfunctional mitochondria.

4) Oxidative enzymes: NADH-TR; SDH; COX). Oxidative enzymatic stains, particularly NADH-TR (formerly called diphosphopyridine nucleotide reductase or DPNH) have been used since the introduction of muscle histochemistry into routine diagnostic myopathology in the 1960s. For decades, it was thought of in terms of antagonistic equilibrium with glycolytic enzymes, such as myophosphorylase and phosphofructokinase, as if an antagonism or opposition between oxidative (aerobic) and glycolytic (anaerobic) metabolism occurs, a perspective reinforced by the predominance of oxidative enzymes in type I myofibres and of glycolytic enzymes in type II myofibres. With the new knowledge about mitochondrial functions, we now recognize that each of these oxidative enzymes is a specific marker of a respiratory chain complex, and should be viewed as such when interpreting a muscle biopsy. NADH-TR is Complex I; SDH is Complex II; COX is Complex IV. Specific and reliable histochemical stains are still not available to demonstrate Complex III (ubiquinol; cytochrome-b-oxidase) and Complex V (ATP synthase), though these can be measured quantitatively in muscle homogenates or purified mitochondria. NADH-TR is expressed in sarcoplasmic reticulum as well as in mitochondria, by contrast with SDH and COX that are present only in mitochondria. This explains why NADH-TR stain is so much stronger in sections of muscle than SDH, even though both utilize tetrazolium reduction for their microscopic demonstration. However, in brain tissue, SDH is conversely stronger than NADH-TR. All of these oxidative enzymes appear as multiple points of activity in the sarcoplasm, each point corresponding to a mitochondrion. In longitudinal sections, this punctate activity appears to identify the Z-band of the sarcomeres because EM demonstrates that most intermyofibrillar mitochondria are found as pairs on either side of the Z-band, often closely adherent to a lipid droplet. Specific reductions or increases in these oxidative enzymes by histochemistry provide useful criteria of mitochondrial cytopathy, and some findings are rather specific for the complexes they represent:

a) Total reduction or absence of SDH activity indicates a severe defect in Complex II.
b) Total absence of COX activity may indicate a defect in coenzyme Q10 and defective electron transport from Complex I to Complexes III and IV.
c) The finding of scattered myofibers showing absence of COX activity is not specific for Complex IV disorders, but occurs in defects of other respiratory chain enzymes as well; it is strong evidence of mitochondrial cytopathy, and is one of the most important features of muscle biopsy in the investigation of mitochondrial disorders. The significance of absent COX activity is further enhanced if these same fibres show strong or increased SDH activity. This determination requires careful examination of serial sections stained with COX and SDH to identify the same fibres, using landmarks such as perimysial blood vessels. This important histochemical finding in scattered individual myofibres is not reflected in quantitative biochemical measurements of total COX activity per volume of muscle tissue.
d) In ragged-red fibres, the subsarcolemmal red zones generally show strong oxidative enzymatic activity of all types; NADH-TR; SDH; COX because mitochondria, both normal and abnormal, are numerous in those zones. Degenerating myofibres, by contrast, show loss of oxidative enzymatic activities.

5) Ribosomal RNA. Acridine orange fluorochrome is a sensitive means of identifying ribosomal ribonucleic acid (RNA) in the cytoplasm of cells, by the highly fluorescent complexes it forms with nucleic acids. It is much more sensitive than methyl green pyronin and other older RNA stains. Regenerating myofibrils and fetal myotubes have many ribosomes in their sarcoplasm and thus show a highly fluorescent orange-red color when viewed under ultraviolet-blue light. The peripheral zones of ragged-red fibres and myofibres altered in mitochondrial cytopathies do not show increased ribsomes ultrastructurally and do not fluoresce with acridine orange. The basophilia seen in ragged-red zones of H&E-stained myofibres are not, therefore, due to ribosomal proliferation.

6) Fibre-type ratios and distribution. Each muscle has a characteristic profile of the ratio of fibre types: the quadriceps femoris usually exhibits a 65% type I predominance in children and a similar mild type II predominance in adults; the deltoid may normally show up to 80% type I predominance. In various muscular dystrophies and congenital myopathies, this ratio often is altered in a characteristic fashion. In mitochondrial cytopathies, it also may be altered, usually toward a greater type I fiber predominance, but this is not a reliable enough diagnostic feature to be a criterion of mitochondrial cytopathy. In a minority of cases of mitochondrial cytopathy, particularly in infants and young children, an excessive incidence of congenital muscle fibre-type disproportion is found, with selective smallness and numerical predominance of type I myofibers. We have observed this phenomenon, also reported by other authors, but have not been able to correlate it with a specific mitochondrial syndrome or defective respiratory enzyme complex.

Mitochondria are more abundant in type I myofibres, and these fibres usually appear to be selectively more involved, but in a minority of cases type II fibres are more affected.[19]

7) Neurogenic atrophy of muscle. Chronic mitochondrial cytopathies in older children and adults often show areas of histochemical type-grouping indicative of denervation and

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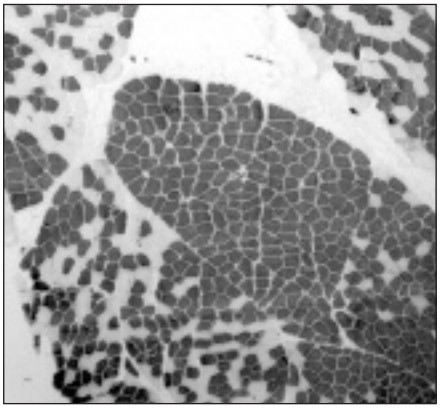

Figure 3: *Histochemical type-grouping in the muscle biopsy of the patient illustrated in Figure 1 with MELAS syndrome. Entire fascicles are composed of type I myofibres (dark), and smaller groups of type I fibres are seen in other fascicles. Other areas of this same biopsy showed extensive grouping of type II fibres. Denervation with reinnervation is common in chronic mitochondrial myopathies. Frozen section. Myofibrillar ATPase, preincubated at pH 4.6.*

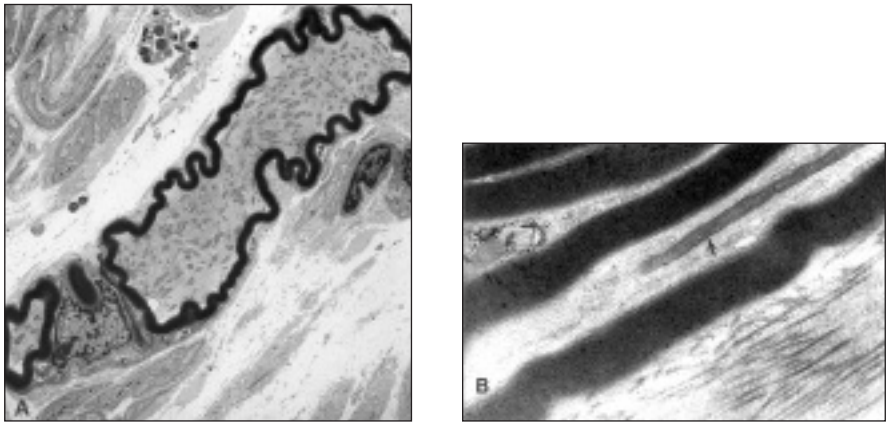

Figure 4: *Sural nerve biopsies of two young adult patients with mitochondrial myopathies, showing alterations in axoplasm. (A) The number of axonal mitochondria, in relation to neurofilaments and microtubules, is greatly increased, but the myelin sheath and adjacent Schwann cell are well preserved and the axoplasm shows no degenerative changes. (B) An extremely long mitochondrion (arrow) within the axon has irregular cristae but does not exhibit stacking or whorling of cristae or paracrystalline structures. Similar long mitochondria may be seen in muscle, extending over many sarcomeres. Though the sural is a sensory nerve, similar mitochondrial alterations in motor nerves may contribute to the pattern of denervation/reinnervation that is commonly found in chronic mitochondrial myopathies. Transmission electron microscopy. Lead citrate and uranyl nitrate (A) 8000X; (B) 20,000X.*

reinnervation of muscle (Figure 3). This finding should not mislead the pathologist to making a diagnosis of primary neurogenic atrophy of muscle. The reason for this neurogenic pattern is probably axonal alterations in peripheral nerve, as axonal mitochondria frequently show ultrastructural distortion (Figure 4). Despite early recognition, in the history of histopathology of mitochondrial diseases, of the presence of neuropathic findings in the muscle biopsy,[20,21] denervation with reinnervation is still not generally recognized as a predictable chronic finding. Grouped atrophy indicates an even more chronic condition of many more cycles of denervation-reinnervation over years, and is not commonly demonstrated in mitochondrial diseases.

8) Features not characteristic of mitochondrial cytopathies. Extensive myofibre degeneration or necrosis, regeneration, and inflammatory cell infiltrates are not features of mitochondrial myopathies, though they may occur in some acquired myopathies in which mitochondrial function is impaired, such as drug-induced myopathy with the hypocholesterolemic statin drugs. A mutation in subunit II of COX (complex IV) is a rare cause of rhabdomyolysis.[22] Proliferation of connective tissue in the endomysium or perimysium are not typical features of mitochondrial cytopathies. Selective type I or type II myofiber atrophy are not typical of mitochondrial diseases as they are in myotonic dystrophy, nemaline myopathy or congenital muscle fibre-type disproportion.

Table 2 summarizes histochemical differences between different mitochondrial myopathies that provide clues to the more specific diagnosis.

Correlations of deficiencies in specific respiratory complexes with histochemical findings.

Many attempts have been made to correlate clinical patterns with specific respiratory complex deficiencies, but none have

Table 2: Correlation of histochemical findings with specific defects in mitochondrial respiratory complexes

Ragged-red fibers:	Combination defects in Complexes I and IV
Increased lipid in ragged-red fibers:	Kearns-Sayre, PEO (not MELAS, MERRF)
Increased lipid in non-ragged-red myopathy:	Some, but not all, mitochondrial myopathies; specific, constant correlations not determined
Absent SDH in all fibers:	Severe Complex II defect
Absent COX in all fibers:	Severe Complex IV defect; defective CoQ10
Absent COX in scattered myofibers (often associated with increased SDH in those fibers):	Nonspecific for complexes, but reliable finding indicating mitochondrial myopathy
Increased glycogen in ragged-red fibers:	Nonspecific for complexes

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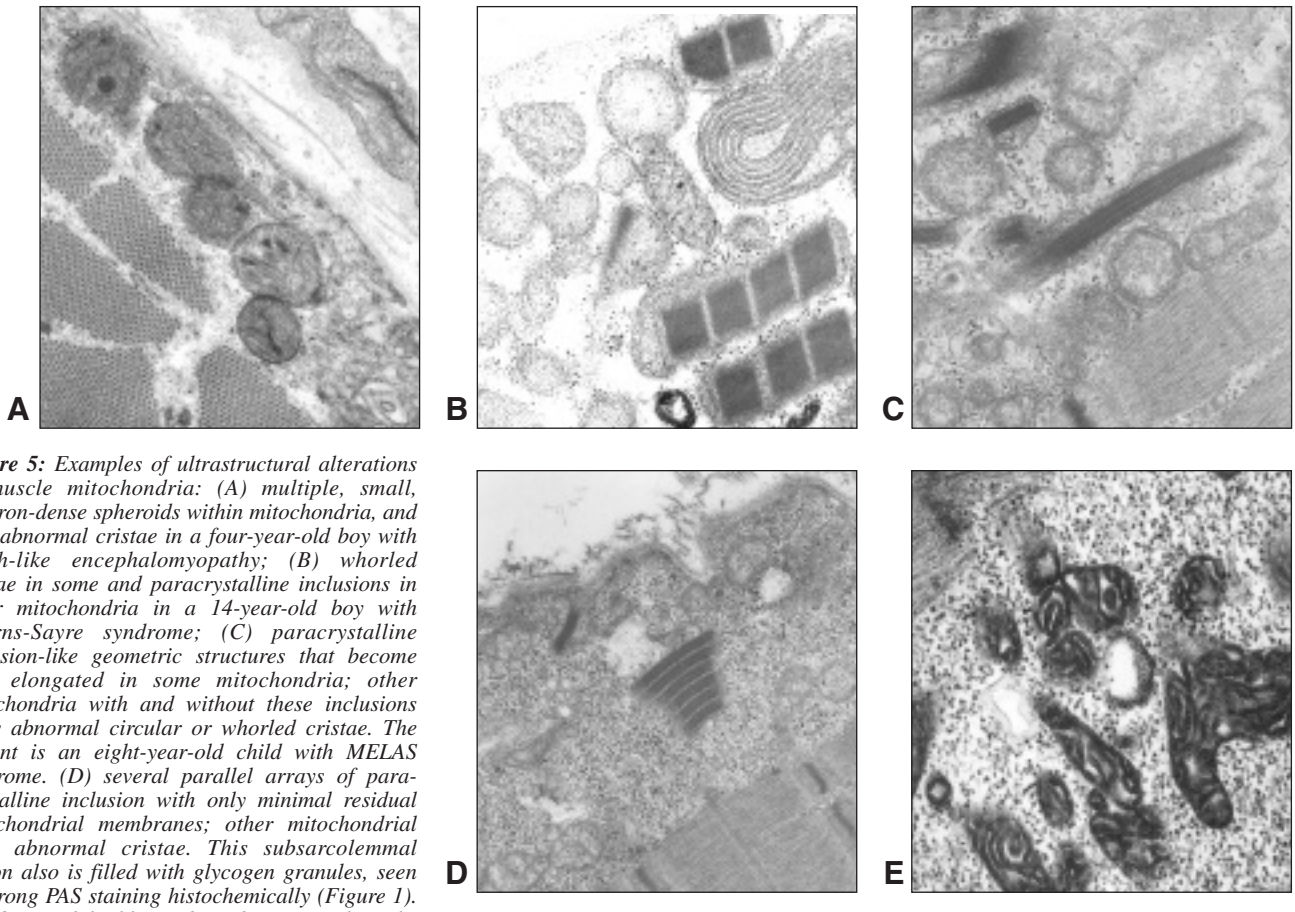

Figure 5: *Examples of ultrastructural alterations of muscle mitochondria: (A) multiple, small, electron-dense spheroids within mitochondria, and also abnormal cristae in a four-year-old boy with Leigh-like encephalomyopathy; (B) whorled cristae in some and paracrystalline inclusions in other mitochondria in a 14-year-old boy with Kearns-Sayre syndrome; (C) paracrystalline inclusion-like geometric structures that become very elongated in some mitochondria; other mitochondria with and without these inclusions show abnormal circular or whorled cristae. The patient is an eight-year-old child with MELAS syndrome. (D) several parallel arrays of paracrystalline inclusion with only minimal residual mitochondrial membranes; other mitochondrial have abnormal cristae. This subsarcolemmal region also is filled with glycogen granules, seen as strong PAS staining histochemically (Figure 1). (E) abnormal, highly condensed cristae without the formation of paracrystalline structures in the subsarcolemmal region of a one-month-old full-term neonate with Leigh encephalopathy (same patient whose brain is shown in Figure 6). Lead citrate and uranyl acetate. (A,B) X20,000; (C,E) X25,000; (D) X16,000.*

been very successful. Muscle pathology offers a better correlation, though it, too, is not definitive. Ragged-red fibre myopathies nearly always involve a combination of complexes I and IV, whereas single defects in either I or IV or deficient activity in other complexes, such as III or V, do not produce ragged-red fibres. Rare case reports suggest that Complex V myopathy may be associated with ragged-red fibers as well, but most Complex V myopathies do not have them. The constant increase in lipid in myofibers in Kearns-Sayre and PEO syndromes, but not in other ragged-red syndromes, has already been mentioned. The specificity of the oxidative enzymes, each of which corresponds to a particular respiratory chain complex, and the significance of increased or decreased activity in all or of scattered myofibres, is discussed above. Noncorrelations also occur and must be interpreted with caution. Some biochemical deficiencies in respiratory enzymatic activities are secondary to other nonmitochondrial conditions.[23] Respiratory chain defects may be nonspecific features in children.[18,24]

Correlations of phenotype (both clinical and pathological) with genotype.

Though in the majority of mitochondrial myopathies, there is a general correlation of clinical phenotype and presentation with the type of mitochondrial defect, the correlation is often poor and may even change depending on the extent of the deletion or point mutation. For example, in the nt3243 point mutation ($tRNA_{leu}$), a mild defect is associated with diabetes mellitus, type 1 or 2 and no myopathy; a more severe defect causes the MELAS syndrome in addition. The nt8296 point mutation ($tRNA_{lys}$) is nearly always associated with MERRF, but in rare patients this same mtDNA mutation has resulted in MELAS. The ntT8993G-tRNAarg point mutation causes nystagmus, ataxia and retinitis pigmentosa if less than 70% of the gene is mutated, but results in Leigh encephalopathy if more than 90% mutation occurs. A lower incidence of the nt-T9176C point mutation causes familial bilateral striatal necrosis, whereas a larger incidence produces a full picture of Leigh encephalopathy. A novel $tRNA_{leu}$, (UUR) mutation produces clinical features of both MERRF and Kearns-Sayre syndrome.[7] The clinical phenotype is not, therefore, reliably predictive of the genotype, but the histopathological and histochemical profile in the muscle biopsy shows much stronger correlation.

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Electron Microscopy

Characteristic ultrastructural alterations in mitochondria are demonstrated by EM in the majority of cases of mitochondrial cytopathy (Figure 5). These can be summarized as: a) excessive numbers of mitochondria in subsarcolemmal and intermyofibrillar spaces, beyond the expected greater number in type I than in type II myofibres; b) excessively bizarre shapes of mitochondria; c) excessively large size or length of mitochondria; normal mitochondria of striated muscle should not exceed 3-4 sarcomeres in length; d) irregularities of the cristae, ranging from deficient cristae, sometimes only one or two in an entire mitochondrion, to abnormal stacking or whorling of cristae, usually associated with other areas of the same mitochondrion in which cristae are absent with only an amorphous granular material present; e) multiple small, electron-dense spherical granules; f) paracrystalline structures with a regular geometrical periodicity. Some of these inclusion-like structures are very long and may even extend beyond the confines of the mitochondrial membrane. They consist of highly compressed, crystallized cristae, hence are not true "inclusions" of foreign proteins or metabolic products. Paracrystalline structures are the most pathognomonic of the various ultrastructural alterations, and mainly occur in ragged-red fibre diseases; however, these structures are not universal or specific diagnostic markers, as they also occur in some patients with acid maltase deficiency (see below). Paracrystalline structures in the mitochondria of muscle were identified early in the series of investigations that defined mitochondrial diseases.[25] However, they are not unique in muscle, and may be demonstrated at times in liver[26] and in the brain, as in the spongy periventricular region of Leigh encephalopathy.

In addition to the mitochondrial changes, increased lipid and glycogen demonstrated histochemically by light microscopy may be confirmed by EM. Mitochondria in other cells within the muscle also show alterations, particularly endothelial cells of capillaries and mitochondria within the axoplasm of intramuscular nerves, both of which normally have much smaller mitochondria than myocytes. These alterations are easily overlooked if they are not specifically sought.

Quantitative studies of respiratory chain complexes

The respiratory complexes are located on the inner mitochondrial membrane and each consists structurally of several subunits. Complexes I, III, IV and V contain subunits encoded in either mtDNA and nDNA (see below), whereas Complex II subunits are entirely encoded in nDNA.

Citrate synthase as an internal control.

Citrate synthase is a Krebs cycle enzyme located in the mitochondrial matrix and present in all mitochondria. It may be used as a type of internal control to determine whether the number of functional mitochondria are sufficient to validate the results of the respiratory chain enzymes. If the citrate synthase is very low, by comparison with normal controls, all other low enzyme levels are suspect as artifactual. If citrate synthase is very high, indicating an abnormally large number of mitochondria, the results of respiratory chain enzymes may be artifactually inflated and appear normal when actually the activities are low. It is, therefore, important to look first at the citrate synthase in the report and, particularly if less or greater than the control range, to correct for this biological artifact by calculating the respiratory complexes as a ratio of citrate synthase activity.

False causes of apparently low respiratory enzymes.

Just because quantitative studies provide specific numbers does not signify that they are reliable. There also are many artifactual causes of apparently low respiratory chain enzymatic activities that must be considered: a) delay in freezing the biopsy (>30 minutes); b) postmortem autolysis (>8 hours); c) technical laboratory errors (compare with controls); d) tissue frozen with isopentane (affects Complexes I, II and III); e) "biological artifacts" induced by certain drugs or toxins that affect mitochondrial function; examples are the statin drugs for hypercholesterolemia that inhibit coenzyme Q10, valproic acid, chemotherapeutic and immunosuppressive drugs and other antimetabolites including anti-AIDS drugs.

Interpreting reports of respiratory enzyme complexes.

An important difference between the histochemical demonstration of COX activity in frozen sections of muscle tissue and quantitative biochemical assay is that the scattered COX-deficient fibers with strong SDH activity seen in tissue sections represent too small an amount of total COX activity in the muscle, hence are not reflected in a low total COX activity. The histochemical finding is of primordial importance in the diagnosis of mitochondrial myopathy, and clinicians often do not understand that quantitative analysis is not a substitute or better simply because precise numbers are reported. Some respiratory chain deficiencies are potentially reversible or produce only mild, benign clinical manifestations, particularly in children.[27-29] The prognosis of all mitochondrial encephalomyopathies is not, therefore, uniformly that of a progressive, degenerative disease, and one should exercise caution in predicting outcome early in the course.

Previously, there was a tendency for high standard laboratories in academic institutions to be purchased wholly or in part by commercial laboratories. They often reduced their costs by combining complexes. Reporting "Complex II + Complex III activity" and "Complex I + Complex III activity" may miss significant deficiencies in Complex III activity.[30] The elimination of the more difficult (and costly) determinations altogether, such as Complex V, is inadequate and may overlook significant deficiencies. Despite exaggerated high or low values of citrate synthase, only raw data of specific activities is sometimes reported, rather than also providing a calculation of activity as a ratio of citrate synthase. This simple calculation should not be left to be performed by the physician receiving the report.

Genetic studies

The genetics of mitochondrial cytopathies are complex. The mitochondrion has its own DNA (mtDNA) on a single, circular structure (i.e. chromosome) of about 16.5 kilobases. The mtDNA has an intimate relation with the cell's nuclear DNA (nDNA), and for each of the five respiratory complexes, the majority of the subunits are encoded by nDNA, not mtDNA. Complex I

consists of 41 subunits, of which only seven are encoded in the mtDNA and the other 34 are encoded in the nDNA. Complex II has only four subunits, all of which are encoded in nDNA. Complex III has 10 subunits, one encoded by mtDNA and nine by nDNA. Complex IV has 13 subunits, three by mtDNA and 10 by nDNA. Complex V has 12 subunits, two by mtDNA and 10 by nDNA. The nDNA encodes the vast majority of the mitochondrial proteins, including all proteins present in the outer membrane and the matrix. The respiratory complexes are located on the inner membrane.

Since the nuclear genome contributes subunits to the respiratory complexes, some mitochondrial diseases may follow a Mendelian pattern of inheritance, in which the metabolic defect is due to defective subunits encoded by mutation in nDNA, with preservation of normal mtDNA. Inheritance in these mutations is nearly always autosomal recessive. Some cases of Leigh encephalopathy provide a good example of this phenomenon. Some nine different genetic defects have now been documented in this syndrome, five of which involve mtDNA and four involve nDNA. If a point mutation in mtDNA is involved, the obligatory transmission is maternal, though not involving the X-chromosome of nDNA. Most pathogenic mtDNA mutations are heteroplasmic (i.e. a mixed population of mutant and normal alleles). The proportion of specific mtDNA mutant alleles within that mixture also influences the clinical expression of the mitochondrial disease, not just its degree of severity. In the nt8993T➔C mutation, which substitutes leucine for arginine, if less than 70% of the mtDNA shows this point mutation, the patient has the nystagmus, ataxia and retinitis pigmentosa syndrome; if the mutation involves 90% or more, the patient presents with Leigh encephalopathy. Between 70 and 90% mutated mtDNA produces mixed or variable clinical expression.

Another class of mtDNA abnormalities is large-scale mtDNA rearrangements, such as kilobase (kb) deletions of the mitochondrial chromosome. Patients may harbour a 5 or 7.5 kb mtDNA deletion (the most common varieties), with striking differences in the proportion of deleted mtDNA molecules within the total mtDNA population. Sometimes as little as 2% mitochondria with kb deletions may be enough to be symptomatic. High levels of these large-scale mtDNA deletions frequently occur in patients with Kearns-Sayre and PEO syndromes.[31] Approximately one-third of Kearn-Sayre patients harbour the same kind of mtDNA deletion, often designated the common 5 kb deletion, but occasional patients with Kearns-Sayre syndrome have larger, 11 kb deletions, with correspondingly more severe neurological, muscular and cardiac manifestations.[32,33]

Many of the common point mutations are now known, particularly in mitochondrial myopathies with ragged-red fibers, in mitochondrial cardiomyopathies, and in some mitochondrial encephalopathies, especially Leigh encephalopathy, but new mutations are published weekly in this rapidly expanding field of genetics. A table summarizing the known mtDNA point mutations and deletions in mitochondrial encephalomyopathies is updated quarterly in the journal *Neuromuscular Disorders*.[34] Many mitochondrial point mutations are expressed in both striated muscle and CNS. For example, Kearns-Sayre syndrome is clinically mainly a myopathy, but also involves the visual system of the brain, demonstrated during life by abnormally slow visual evoked potentials.

Most genetic laboratories that study mtDNA employ batteries of several point mutations that they screen for different classes of mitochondrial diseases (e.g. panels for the ragged-red fibre myopathies, hypertrophic cardiomyopathies, LHON, another for Leigh-like encephalopathies, etc). These panels test the common, well-documented mutations in each category, but cannot test the entire mitochondrial genome. At times, point mutations are found in nucleotide sequences that are not evolutionarily conserved, do not specify highly conserved amino acid residues and/or are not associated with an amino acid substitution. The interpretation of such defects or polymorphic variants, in the context of clinical and pathological presentation, often is problematic and uncertain.

In addition to its principal function in energy metabolism, mtDNA has additional functions during development. It is important for neuroblast polarity by modulating calcium homeostasis in microtubules, and also regulates the *bcl2* gene for apoptosis.

Damage to mtDNA and loss of the mitochondrial membrane potential is demonstrated in apoptotic cell death.[35]

Criteria for the extent of investigation in muscle biopsies taken for suspected mitochondrial cytopathy

Each of the studies discussed above complements the others by supplementing rather than duplicating information, and together provide a comprehensive profile of the mitochondrial disease, particularly if clinical criteria are integrated as well. Nevertheless, these laboratory studies are expensive and often are only partially covered by health insurance plans. A decision must be taken in each case, therefore, regarding how extensively to investigate a muscle biopsy for a suspected mitochondrial cytopathy. This decision may require discussion between the clinician (usually a neurologist or a metabolic specialist) and the pathologist, to determine the index of clinical suspicion and the evidence suggesting a mitochondrial disorder, by contrast with the anxiety of not having a definitive diagnosis in a patient with a progressive myopathy or encephalopathy and "grasping at straws".

All muscle biopsies, in our opinion, should have a routine battery of histochemical studies of frozen sections that includes the modified Gomori trichrome, a lipid stain such as oil red O or sudan black, and oxidative enzymatic stains for NADH-TR, SDH and COX. The latter two enzymes used to be regarded as special purpose supplementary stains in selected cases, but most good muscle pathology laboratories now include them in their routine battery for both children and adults.

The criteria for proceeding with mitochondrial workup beyond the level of histochemistry depends upon 1) strong clinical evidence of mitochondrial disease, such as unexplained ophthalmoplegia not due to myasthenia gravis or brainstem lesions or persistent lactic acidosis; 2) a documented family history of mitochondrial disease and unexplained neurological or neuromuscular symptoms; or 3) histochemical findings in the muscle biopsy that suggest mitochondrial cytopathy, such as scattered myofibres with absent COX and strong SDH activities or ragged-red fibres.

Electron microscopy is regarded as the next level of complexity in investigating a muscle biopsy for mitochondrial

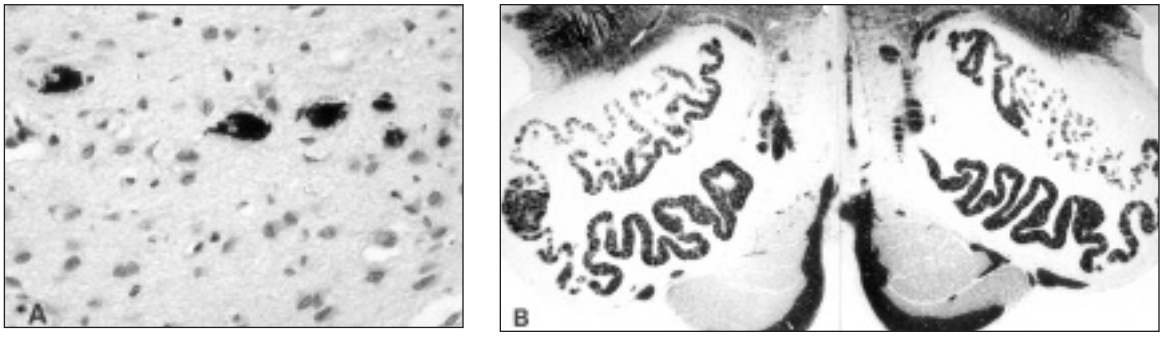

Figure 6: *Characteristic cerebral lesions in mitochondrial cytopathy of a three-month-old infant girl, born at term, with Leigh encephalopathy. (A) mineralized individual neurons in hypothalamus; (B) inferior olivary nuclei showing preserved convolutions of the olive, but the superior lip bilaterally shows irregular loss of synaptic vesicle immunoreactivity. Normal activity is preserved in the inferior lip, in the dorsal and medial accessory olives and in the arcuate nucleus at the medial and inferior margins of the pyramids. This finding is characteristic and almost pathognomonic of mitochondrial encephalopathies of infancy and is not typical in other cerebral malformations. It may be difficult to detect, however, in sections stained with haematoxylin-eosin. Paraffin section. Synaptophysin immunocytochemistry. X40. Other important findings in this brain (not illustrated) included periventricular encephalomalacia with mitochondrial paracrystalline structures seen by electron microscopy, delayed myelination for age, focal dysgeneses of the cerebellar cortex and cerebral microinfarcts.*

disease, because the demonstration of abnormally stacked or whorled cristae, electron-dense mitochondrial granules and paracrystalline inclusions provide strong supportive evidence, even if the histochemical findings are negative or equivocal. Some pathologists take the position that if quantitative respiratory chain enzyme and mtDNA analysis are to be performed, the EM in this situation is interesting but not essential to the diagnosis and is an unnecessary expense. We believe that the ultrastructural information is an important correlate that complements other data, and that the trouble and expenses are justified. Whereas occasional cases are cited that show no histochemical or ultrastructural alterations but have abnormal biochemical analyses, these cases are rare and may actually pose questions about the validity of the biochemical studies, particularly if a genetic mutation in mtDNA is not proved. By contrast, abnormal mitochondrial ultrastructure alone provides valid justification to proceed with the third, and most expensive, aspect of the workup, the quantitative analysis. At each stage, the total data collected must be reassessed to decide whether to proceed further. Economic considerations should not outweigh scientific merit in the selection of biopsies to submit for ultrastructural study.

Detection of mtDNA deletions in blood.

Southern blot hybridization analysis demonstrates mtDNA deletions in muscle tissue, but not always in blood in a subgroup of patients with myopathic signs.[36,37] The highly sensitive polymerase chain reaction can be used in blood for the detection of mtDNA deletions in several mitochondrial myopathies, and panels have been developed for screening the common mutations in categories, such as for MELAS, Kearns-Sayre, PEO or LHON. If the specific defect is suspected on clinical grounds, this approach may be diagnostic and save the patient the discomfort of a muscle biopsy, but it is by no means comprehensive and many mitochondrial disorders will be missed by blood studies alone. These screening panels in blood also are expensive. There is a tendency in some centers to be "economical" by simply screening blood for the common mtDNA point mutations instead of doing a muscle biopsy. Whereas this approach could be an initial step, together with a serum lactate and pyruvate, it should be understood that negative (normal) results by no means exclude all mitochondrial cytopathies. Moreover, several heteroplasmic pathogenic mtDNA point mutations and deletions have been reported as highly abundant and readily detectable in muscle tissues while at very low levels (often undetectable) in blood.[10]

PATHOLOGICAL FINDINGS IN BRAIN

The mitochondria of neurons, glial cells and endothelial cells all may be affected, and endothelial cells sometimes show the greatest alterations, leading to cytoplasmic swelling, decreased pinocytotic vesicles and impaired blood flow. This is the basis of both stroke-like episodes (i.e. transient ischemia) and true microinfarcts and macroinfarcts in the brain in MELAS syndrome and sometimes in other mitochondrial encephalopathies as well. Additional characteristic alterations demonstrated at postmortem examination in patients with mitochondrial cytopathies, particularly infants and children, are illustrated in Figure 6. They include: a) individual mineralized neurons in the thalamus, hypothalamus and sometimes the basal ganglia (i.e. neuronal calcinosis); b) white matter gliosis, particularly in the brainstem and cerebellum; c) dysmyelination or demyelination of white matter in the cerebral hemispheres; d) focal dysplasias and neuronal loss in the inferior olivary, red and dentate nuclei and the cerebellar cortex; e) periventricular necrosis or spongiform changes in Leigh encephalopathy.[13,38,39] Many additional details of mitochondrial defects in the CNS are available, such as mitochondrial alterations in ependymal cells[40] and the protective effect of mitochondrial uncoupling protein-2 against excitotoxic neuronal death in the immature brain.[41] Mitochondrial activity is a major factor in neuronal death in many conditions.[42] These aspects are beyond the present scope of this review.

Whereas the changes in brain described above are consistent with and may be strongly suggestive of mitochondrial disease,

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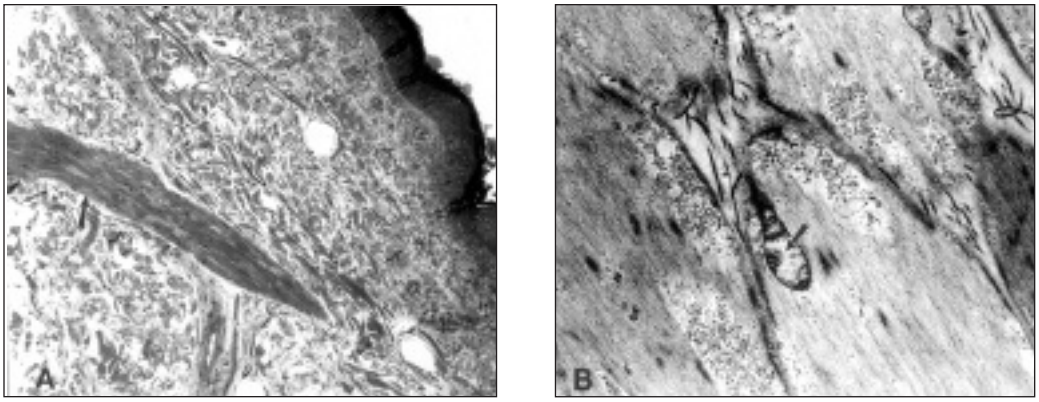

Figure 7: *Skin biopsy of a young adult. (A) Semithin section embedded in epoxy resin and viewed in the light microscope shows erector pili muscle (arrow) of hair follicle. (B) Electron micrograph of this smooth muscle demonstrates a large, swollen mitochondrion with incomplete cristae (arrow). (A) toluidine blue. X400; (B) Lead citrate and uranyl acetate. X6000.*

none of these alterations individually are pathognomonic and all may be seen at times in other diseases. The combination of neuropathological findings, supported by clinical and imaging data, allows as confident a diagnosis as is possible without confirmation by a mtDNA point mutation or deletion that is definitive. The role of the diagnostic brain biopsy for mitochondrial disease in living patients is not established, and at this time muscle remains the tissue of choice for biopsy.

Skin biopsy for mitochondrial studies

Punch biopsy of the skin seems an attractive alternative to muscle biopsy because it is simple, less invasive, and can be performed by any nonsurgical clinician. Epidermal cells are a poor source of mitochondria, but the smooth muscle of the pili erecti muscles and the axoplasm of cutaneous nerves have mitochondria suitable for ultrastructural examination (Figure 7). Because of the small size of the biopsy, the most practical management of these specimens is to fix the entire biopsy in glutaraldehyde or Karnovsky solution and prepare semithin sections stained for light microscopy (toluidine blue or methylene blue). If these sections show nerves or smooth muscle (arrector pili muscles attaching at the base of the hair follicle), EM may be performed to examine the mitochondria.[43]

Fibroblasts also sometimes show good mitochondria, but they are not as large as those in the smooth muscle. Only limited information concerning mitochondria can be obtained from skin biopsies using frozen sections or paraffin sections. The tissue is insufficient for biochemical studies of respiratory chain complexes or mtDNA analysis, though the ultrastructural findings may provide further evidence to justify a muscle biopsy for more definitive studies. One additional possibility with the skin biopsy is to culture fibroblasts, which can then be used in biochemical studies of respiratory complexes. This approach has been used for patients with proved point mutations of MELAS and MERRF complexes.[44] Experimentally, fibroblasts can even be converted *in vitro* to myoblasts by the myogenic gene *MyoD*. Occasionally, there are even clinical cutaneous manifestations in mitochondrial diseases, such as MELAS syndrome[45] and the mtDNA depletion syndrome (see below).

Mitochondrial depletion syndrome of early infancy

Mitochondrial disease may present in the neonatal period or even be clinically evident in fetal life. The two most frequent mitochondrial syndromes of early infancy are Leigh encephalopathy and the mitochondrial depletion syndrome. The latter leads to multisystemic failure in liver, kidneys and heart, as well as edema or bullous cutaneous lesions or even epidermolysis, generalized muscle weakness and encephalopathy.[46-53] As with Leigh encephalopathy, most infants have

Table 3: Mitochondrial respiratory chain enzymes of a two-year-old girl with mtDNA depletion syndrome

	Specific Level	Range of 8 Controls
Complex I (NADH)	0	16.8 ± 5.6
Complex II (SDH)	6	14.9 ± 5.9
Complex III (cytochrome b)	0	9.6 ± 3.4
Complex IV (COX)	43	111.6 ± 29.3
Complex V (ATP synthase)	21	86.5 ± 24.8
Citrate synthase	125	113.4 ± 33.5

This child has significantly low activities of all five respiratory enzymes, with normal citrate synthase, the latter indicating a normal number of mitochondria; the activity defects were confirmed as ratios to citrate synthase. No mtDNA point mutations or deletions were demonstrated. All units are expressed as nmol substrate/min/mg protein (compare with µmol expression used in Table 1). The muscle biopsy showed no ragged-red fibres (rarely seen at this age) or myofibre degeneration, only mild histochemical changes of scattered fibres with absent COX activity and subtle ultrastructural alterations. Clinically, she had global developmental delay, hypotonia, hyperreflexia, hepatomegaly and persistent lactic acidosis with normal serum pyruvate levels. She required a gastrostomy because of dysphagia since birth. A neonatal MRI of the brain revealed pachygyria and mild ventriculomegaly; the latter was detected by fetal ultrasound at 24 weeks gestation. She was born at 37 weeks gestation with intrauterine growth retardation; birth weight was 2300g. Her mother is mentally retarded since early infancy, of unknown cause.

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persistent lactic acidosis, but this is not an obligatory criterion and some affected infants have normal serum lactate. Cerebrospinal fluid lactate might be elevated in some cases with normal serum lactate. Infants may die in the neonatal period or occasionally survive several weeks or even months. Clinical suspicion of the syndrome is raised by unexplained multisystemic metabolic disease in the absence of a history of hypoxia or ischemia or other metabolic diseases. Confirmation is by muscle biopsy, but the histopathological and histochemical findings may be normal or subtle and quantitative analysis of respiratory chain enzymes is required. Ultrastructural alterations of mitochondria usually are demonstrated, but also may be subtle. An example of the mtDNA depletion syndrome is demonstrated in Table 3.

The diagnostic findings of this condition are supported by the quantitative studies of mitochondrial respiratory chain enzymes. The four respiratory complexes with subunits encoded by mtDNA (i.e. Complexes I, III, IV, V) exhibit abnormally low activities, but point mutations of mtDNA are not demonstrated, while the level of mtDNA is markedly reduced. Citrate synthase is low or normal. Normal levels of Complex II (succinate ubiquinone reductase) activity in conjunction with reduced Complex I, III and IV activity levels is suggestive of the mtDNA depletion syndrome.[51] It has been demonstrated in rats that mtDNA can be totally depleted by the administration of drugs and toxins, such as zidovine (AZT). In these cases the number of mitochondria and their replication are not affected, though the ultrastructure is abnormal.[54] Alper syndrome, an autosomal recessive neurodegenerative disease of childhood, is now known to be a mtDNA depletion syndrome.[55]

Mitochondrial participation in the pathogenesis of other diseases of the CNS and muscle that are not primary mitochondrial cytopathies

It is becoming increasingly evident that mitochondrial disorders are associated with a large number of diseases that are not primary mitochondrial cytopathies, but in which a disturbance in mitochondrial function may contribute to pathogenesis or clinical manifestations. The involved disorders fall into nearly all categories of disease: metabolic and degenerative, inflammatory, developmental malformations and neoplastic.

1. Both infantile (Pompe disease) and adult-onset acid maltase deficiencies have well-documented structural and functional abnormalities of mitochondria, including paracrystalline inclusions in myofibers (Figure 8), though these diseases are primary glycogenoses due to a defective lysosomal enzyme.[56]
2. In spinal muscular atrophy, the progressive motor neuron degeneration is associated with abnormal mitochondrial function.[57,58]
3. In infantile-onset spinocerebellar ataxia (IOSCA), two distinct point mutations are identified in the autosomal recessive IOSCA gene at the 10q24 locus that programs the synthesis of a mitochondrial protein.[59,60] Some cerebellar ataxias are associated with coenzyme-Q10 (CoQ10) deficiency resulting in functional mitochondrial defects.[61]
4. In inflammatory necrotizing myositis (i.e. "polymyositis"), an associated mitochondrial myopathy is documented and at times produces scattered ragged-red fibres in the muscle biopsy.[62,63]

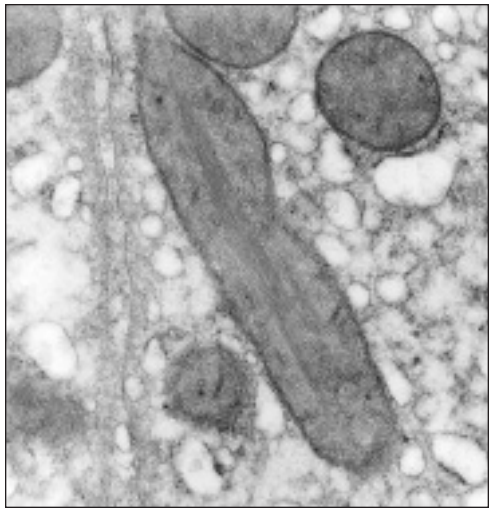

Figure 8: *Ultrastructure of muscle mitochondrion with paracrystalline structures in a 10-month-old girl with infantile acid maltase deficiency (Pompe disease). EM. Lead citrate and uranyl acetate. X30,000.*

5. Amongst cerebral dysgeneses, respiratory Complex III dysfunction (cytochrome-b) is demonstrated in septo-optic-pituitary dysplasia.[64] Mitochondrial structural and functional defects have not been systematically studied in most cerebral malformations.
6. Cerebro-hepato-renal disease (Zellweger syndrome) is fundamentally a systemic peroxisomal disorder associated with cerebral malformation as well. Though normal striated muscle contains few peroxisomes, involved infants have an associated mitochondrial myopathy with ultrastructural alterations of cristae.[65] Biochemical studies of mitochondria of muscle and brain have confirmed functional deficiencies in this disease.[66]
7. Neoplastic cells frequently show chromosomal and genetic abnormalites, and mitochondrial genetic mutations also occur in some cells.[67]
8. Antiepileptic medications administered to pregnant women may interfere with the placental carnitine transporter and, at least theoretically, may impair fetal mitochondrial function by inducing a relative deficiency of carnitine for long-chain fatty acid transport across the mitochondrial membrane.[68]

Toxic and drug-induced mitochondrial cytopathies

Toxic mitochondrial cytopathies are becoming increasingly common in patients taking immunosuppresive and antimetabolic drugs, including chemotherapy and antiviral drugs (Figure 9).[69] Other important group of pharmaceuticals that potentially may impair mitochondrial function are the statin drugs used to control hypercholesterolemia (Figure 9);[70] the mechanism is interference with CoQ10, resulting in impaired electron transport between Complexes I and III.[71] Some antiepileptic drugs, particularly valproic acid,[72] inhibit mitochondrial function or deplete mtDNA. Valproate is, therefore, contraindicated in patients with proved or suspected mitochondrial cytopathies. Long-term steroid therapy rarely may produce symptoms and signs,

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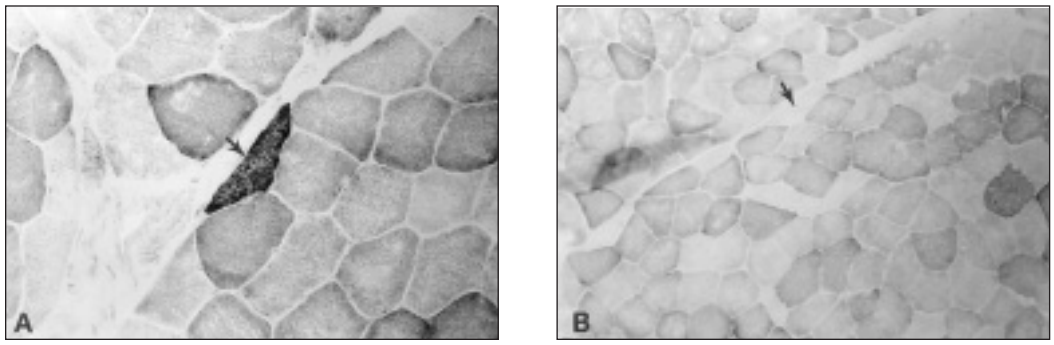

Figure 9: *Quadriceps femoris muscle biopsy of a 44-year-old man with HIV infection and progressive generalized weakness, who has taken numerous antiviral drugs over several years. (A) intense SDH activity (arrow). (B) Many scattered myofibres lack COX activity. A fibre with absence of COX activity (arrow) is the same fibre indicated by the arrow in (A). This reciprocal relation between COX and SDH histochemical activities is characteristic of mitochondrial myopathies of many types and is not specific for Complex IV defects. Ultrastructural alterations of mitochondria, with whorling of cristae and electron-dense spheroids, also were found in this biopsy (not illustrated). Frozen sections. (A) X 100; (B) X 250.*

including ophthalmoplegia, suggestive of mitochondrial cytopathy.[73] Other drugs capable of impairing mtDNA in humans include chemotherapeutic antimetabolites, immunosuppressive drugs, and chloramphenicol.

Mitochondrial alterations in aging

Both structural and functional abnormalities frequently are demonstrated in the muscle mitochondria of normal elderly individuals.[62,74-78] By contrast, mitochondrial respiratory chain function was the same in 12 elderly athletes as in nine young athletic subjects.[77] These alterations are regarded as a physiological change of aging, so that the demonstration of a single ragged-red fiber in the muscle biopsy in patients more than 60 years of age may not have the same pathological significance as might be inferred in children or young adults. The COX-deficient myofibres seen histochemically have very high levels of mutant mtDNA and different DNA mutations are present in different fibres of the same biopsy.[77] Some patients with late-onset of well-characterised mitochondrial syndromes, such as MELAS, also have been described.[79] Whether these patients represent latent genetic mitochondrial cytopathies or whether they are one extreme of the normal aging process is uncertain, but they also pose implications for dementia and other degenerative processes in the brain.[78]

Treatment of mitochondrial cytopathies

There is no definitive treatment of mitochondrial diseases at this time. Pharmacological substances that provide an improved substrate for mitochondrial function are used and include a "cocktail" of mainly CoQ10, L-carnitine, the antioxidant α-tocopherol (vitamin E)[80] and creatine monohydrate.[81] Alpha-tocopherol is theoretically more effective than ascorbic acid (vitamin C) because it helps regulate superoxide generation in mitochondria,[77] but some authors find it ineffective.[82] Alpha-tocopherol, crucial for mitochondrial integrity, is localized in the outer mitochondrial membrane, unlike the respiratory chain complexes at the inner membrane.[83] CoQ10 not only serves an important function in electron transport, but subserves membrane polarity of many subcellular organelles and is a gene regulator that upregulates some genes and downregulates others.[84,85] Other substances suggested as useful treatment in mitochondrial defects, but of less well established value, include quinones as substitutive electron carriers or antioxidants,[85] niacin, thiamin and the B-complex of vitamins in general, but good evidence of the efficacy of water-soluble vitamins is lacking.[82]

No longitudinal pathological studies are available to date that prospectively compare muscle biopsies before and after treatment, either in humans or animals. Controlled clinical trials of the various advocated treatments also are wanting. Reports of responses to agents such as CoQ10 are encouraging,[86,87] but still anecdotal and require systematic objective study.

Recommendations for laboratory investigation of suspected mitochondrial diseases

If the clinical course, neurological findings and imaging features in a patient at any age are suggestive of a particular mitochondrial disorder for which genetic testing in blood (leukocytes) is available, such as the most common mtDNA point deletions in MELAS or LHON syndromes, this is the next least invasive procedure to attempt to confirm the diagnosis. If these results are nondiagnostic, the muscle biopsy is the best approach in the living patient. The case should be discussed beforehand with the pathologist, to ensure that the tissue is handled promptly and properly to arrange for the necessary special studies as discussed above. Skin biopsy may be a supplementary procedure and provides a source of fibroblasts for cell culture, but is not histopathologically definitive.

Postscript thought from the cell, from the early days of understanding of mitochondrial diseases

I am obliged to do a great deal of essential work for my mitochondria. My nuclei code out the outer membranes of each, and a good many of the enzymes attached to the cristae must be synthesized by me. Each of them, by all accounts, makes only enough of its own materials to get along on, and the rest has to come from me.

L. Thomas, 1974[88]

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