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SPECIAL TECHNIQUES IN DIAGNOSTIC MYOPATHOLOGY*

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While muscle biopsy has been standardized since the introduction of electron microscopy, histochemistry, and morphometry into diagnostic myopathology, additional diagnostic techniques have recently been added. Immunomorphologic methods-firmly established in oncology long ago-are beginning to be applied in diagnostic myopathology. These methods concern the evidence of intermediate filaments in regenerating and denervated fibers as well as in certain myopathies marked by intermediate filament-related inclusions such as cytoplasmic bodies, spheroid bodies, or Mallory body-like inclusions. The connotation of the immunomorphologic approach is further emphasized by the discovery of dystrophin, a normal protein within muscle fibers which is deficient in Duchenne's and Becker's muscular dystrophies resulting in the now-standard examination of dystrophin in muscle biopsy specimens. It is to be expected that immunomorphologic techniques will rapidly develop further, depending on the isolation of muscle-and disease-specific proteins or defective proteins and the availability of antibodies against these proteins. It is conceivable that immunohistological diagnostic methods in myopathology may, one day, render currently established techniques obsolete. *Key words: myopathology, morphologic diagnosis, electron microscopy, histochemistry, morphometry, immunomorphology, dystrophin, intermediate filaments.*

Muscle biopsy is a diagnostic procedure, not a curative one, as removal of tissue alone does not represent therapy but only may occasionally lead to treatment, e.g., in the case of inflammatory myopathy. On the other hand, postmortem myopathologic studies have become extremely rare and are usually confined to employing old-fashioned techniques, i.e., fixation of muscle tissue and subsequent embedding in paraffin for a very limited number of histological stains.

Contrary to and in accordance with the diagnostic importance of a muscle biopsy, a spectacular armamentarium of morphologic techniques have been developed over the past 20 years to examine a biopsied muscle specimen as thoroughly as possible. As a matter of fact, it may safely be stated that no other tissue obtained by biopsy is or can be subjected to such a large variety of morphologic methods as muscle tissue. These methods comprise regular histology; histochemistry to

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detect amounts of fat, glycogen, calcium or iron; enzyme histochemical preparations to determine fiber types and structural abnormalities as well as specific enzyme defects; morphometry; immunohistochemistry; and finally, electron microscopy. For all methods, muscle tissue must be prepared at the time of biopsy. Part of the tissue must be left unfixed for frozen preparations, while another part must be fixed for light microscopy, i.e; semithin plastic embedded sections and ultrathin sections for electron microscopy. Histological studies may be amplified when abundant tissue is available, by fixing the tissue in formalin and subsequently embedding it in paraffin to examine inflammatory infiltrates and vessel walls, including step and/or serial sections.

The basic procedure involves freezing the unfixed muscle tissue in liquid nitrogen for enzyme histochemical preparations that may also reveal fiber types as well as for morphometric studies, assuming that muscle tissue in the unfixed frozen state most closely resembles in vivo tissue in terms of the size and contours of myofibers. These must be mounted for cross-sectioning rather than longitudinally.

Unfixed frozen muscle tissue is also necessary for a number of immunohistological techniques, foremost of which is the determination of dystrophin. Furthermore, if metabolic myopathy is suspected, unfixed frozen tissue, retrieved separately or from the tissue block, may be utilized for biochemical studies.

Finally, electron microscopy is a technique integrated into the so-called "muscle biopsy program" requiring fixation and embedding of muscle tissue from each patient biopsied, even if subsequent ultrastructural examination is not warranted.

Beginning in the early sixties[1], these numerous myopathologic techniques led to reliable and reproducible standardized results of high quality, now documented in an impressive number of references and textbooks[2,3].
In view of the muscle tissue being affected (primarily or in association with other conditions) in well over 600 nosological entities, the spectrum of myopathologic lesions is rather limited. Therefore, a myopathologic diagnosis must always be incorporated into a final nosological diagnosis. On the other hand, in spite of the numerous techniques employed in a standardized "muscle biopsy program", new ways are consistently being explored to enhance diagnostic interpretation of diseased human muscle tissue. A few such technical aspects and trends are described below.

Conventional Histology: The regular histological stains comprise hematoxylin-eosin, the modified trichrome preparation[2], the standard method for routine examination of unfixed frozen muscle, and Elastica van Gieson to determine the amount of connective tissue and the state of the intramuscular vessel walls. Other stains used in general or neuropathology have been rarely employed.

Conventional techniques to ascertain myelinated and unmyelinated nerve fibers may be added.

The Azur B stain reveals regenerating fibers staining ribonucleic acids engaged in protein synthesis. In particular, groups of regenerating fibers, frequent in Duchenne's, Becker's and autosomal recessive (the "Arabic" type) muscular dystrophies are conspicuous. Disseminated regenerating fibers following bouts of rhabdomyolysis may sometimes be the only evidence of preceding muscle fiber necrosis.

While lysosomal enzymes (the acid phosphatase technique) may reveal enzyme activities, the autofluorescent nature of certain abnormal lysosomes attests to their lipopigment character, as seen in regular lipofuscin or accretion of abnormal amounts and abnormally structured lipopigments in vitamin E deficiency[4] and in childhood form of neuronal ceroid-lipofuscinosis. The amount of lipofuscin bodies within muscle fibers may vary considerably among individuals, even of the same age; lipofuscin appears increased in adult denervated muscle fibers, while denervated muscle fibers in childhood display small amounts of lipopigments.

Histochemistry: A muscle specimen should be checked regularly for the amount of lipid droplets and PAS-positive material, usually glycogen. PAS-positive inclusions may further be defined, characterized and differentiated by enzyme digestion, particularly amylase/diastase, but also by others such as glucosidase, mannosidase or even trypsin, e.g., when countering polyglucosan bodies.

Calcium may be revealed by several techniques, e.g., Alizarin red. Calcium is usually transported into the muscle fiber through plasmalemmal defects from the systemic circulation. Calcification of necrotic fibers may occur other wise, but seems to be a rare event. Likewise, iron found within a muscle specimen, though rare, usually indicates an earlier hemorrhage, and iron may be seen in interstitial macrophages as well as in muscle fibers.

Enzyme Histochemical Preparations serve three purposes:

1. Typing of fibers which is usually based on the ATPase techniques after alkaline and acid preincubations revealing types I, IIA, IIB, and IIC. The oxidative enzyme preparations also illustrate fiber types, even subtypes IA and IB[5].
2. Revealing of abnormalities, such as "cores" or "target" phenomena.
3. Revealing of metabolic abnormalities such as deficiencies of amylophosphorylase, phosphofructokinase, myadenylate deaminase, cytochrome c oxidase, or structural abnormalities indicating metabolic lesions, such as the enzyme histochemical equivalent of "ragged red fibers", abnormal mitochondria.

In the wake of studying enhanced enzyme activities in mitochondrial myopathies, additional enzyme histochemical preparations have become available, such as

cytochrome c oxidase or mitochondrial ATPase. The importance of the latter enzyme histochemical preparations is that they may reveal biochemical defects that can subsequently be confirmed or detailed by biochemical analysis of the same muscle tissue.

While the spectrum of oxidative enzymes may be further enhanced by adding succinate dehydrogenase, lactate dehydrogenase or malate dehydrogenase to the standard program, rarer enzyme histochemical preparations allow the disclosure of carboanhydrase or triosephosphate isomerase activities.

Acid phosphatase activity may be enhanced in disorders with activated lysosomal metabolism such as vitamin E deficiency[4], in the neuronal ceroid-lipofuscinoses[6], mucolipidosis IV[7], and foremost in the different clinical forms of acid maltase deficiency. Macrophages within nerotic muscle fibers as well as within the interstitium display acid phosphatase and non-specific esterase activities. Preparations of the latter enzyme further supported by that of acetylcholine esterase may demonstrate the presence, density and distribution of motor endplates.

Alkaline phosphatase activity may outline endothelial cells, but not consistently, and may be histochemically absent in lethal hypophosphatasia[8]. Degenerating-regenerating fibers may also have alkaline phosphatase activity.

The actual number of fruitfully employed enzyme histochemical preparations in diagnostic myopathology may disclose additional diagnostic features. However, this area has not been explored systematically as to muscle morphology and diagnostic myopathology. Lectins may be applied to muscle tissue, especially in the case of inclusions within muscle fibers, such as polglucosan bodies which stain with concanavalin A (Fig. 1) and PSA (Pisum sativum) (Fig. 2). On the contrary, endogenous neoglycoproteins as receptors for certain sugars, so-called endogenous lectins, may also be revealed by respective antibodies[9]. This recent technique may at least differentiate fiber types to a certain degree.

Morphometry : Morphometric analysis in diagnostic myopathology has utilized the smaller diameter technique[10] or the square area method to determine subtle deviations in muscle fiber size from the normal range of age and sex-dependent muscle fiber diameters. Certain pathomorphometric patterns correspond to certain groups of neuromuscular conditions such as the double-peak pattern in neurogenic atrophy. The congenital fiber-type disproportion is one such condition that is based on morphometric analysis. Capillary density, usually determined in plastic-embedded 1 µm-thick sections, has been studied in neuromuscular disorders such as inflammatory myopathies or muscular dystrophies. An increased vascular density is also revealed, particularly in dermatomyositis, in ATPase preparations after acid (pH 3.9) preincubation.

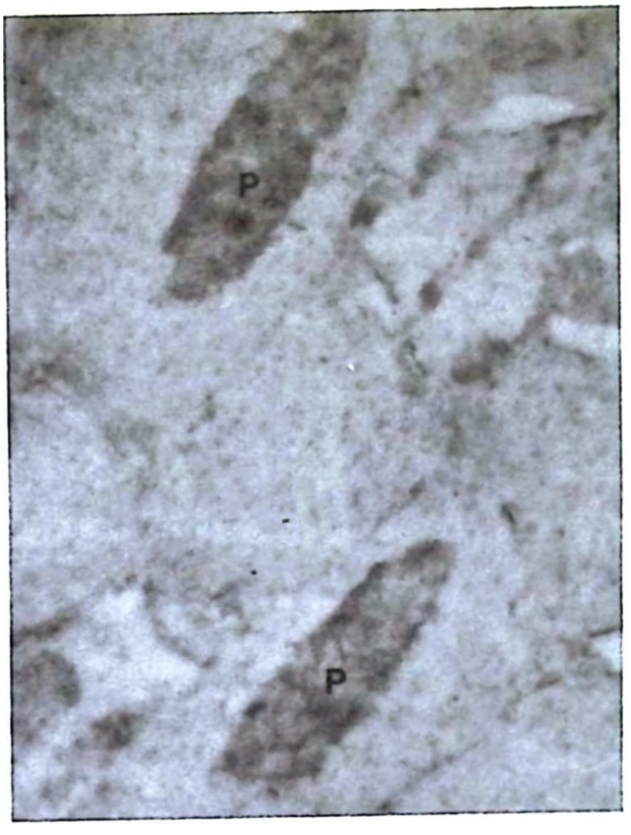

Fig. 1: Polyglucosan (P) bodies react with the lectin concanavalin A, X 220.

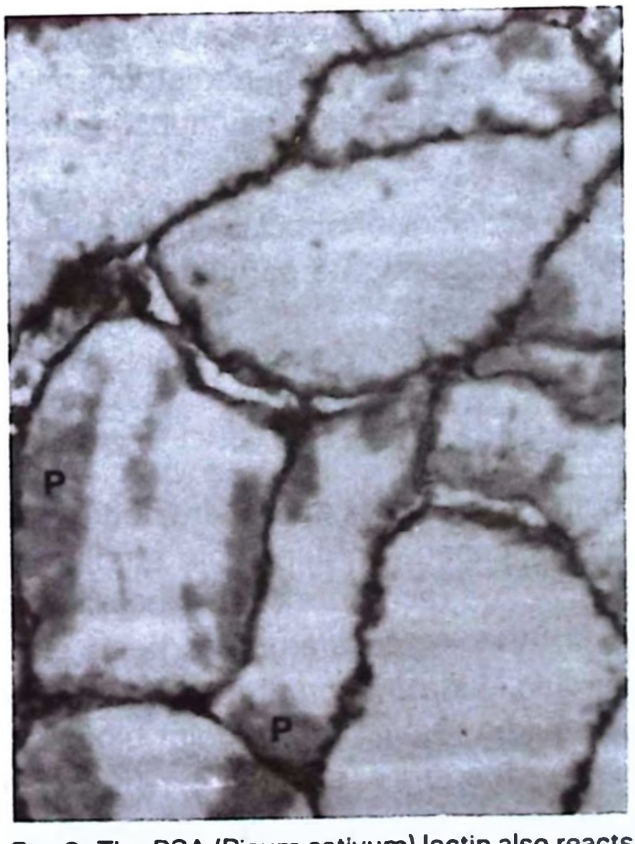

Fig. 2: The PSA (Pisum sativum) lectin also reacts with polyglucosan (P) bodies, X 228.

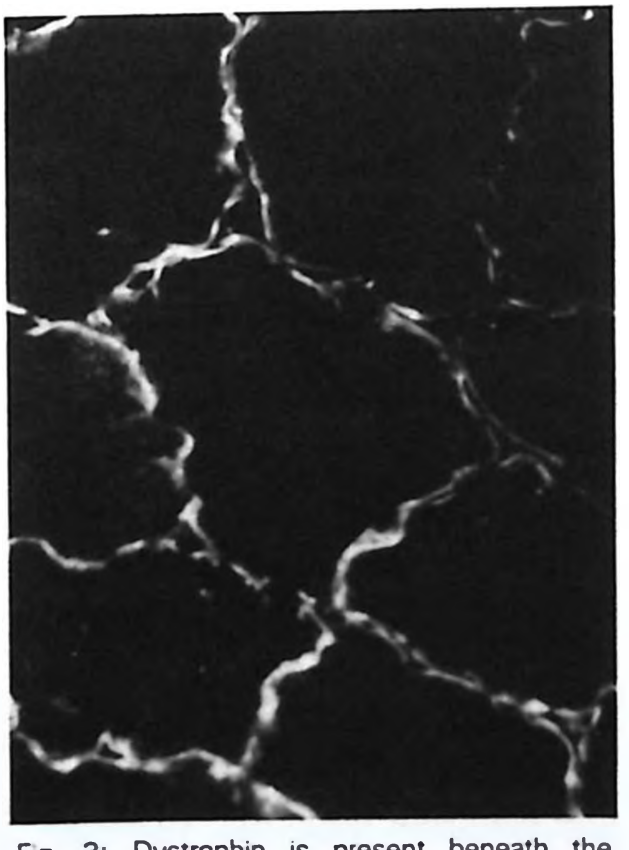

Fig. 3: Dystrophin is present beneath the plasmalemma of each muscle fiber in spite of ice crystal artifacts. Biopsy specimen was retrieved 6 years prior to dystrophin preparation, X 416.

An extremely cumbersome technique combines morphometry and electron microscopy, i.e., ultrastructural morphometry of organelles and other cellular constituents. Nuclear size, mitochondrial volume and histograms of mitochondria may reveal subtle respective defects such as mitochondrial lesions, provided age, sex, muscle-specific and possibly type and state of activity-related control data are available. As this is usually not the case, due to the time-consuming aspect of ultrastructural morphometry, only few such studies have been undertaken.

Whether automatized image analysis will improve the situation and reduce the time spent in morphometric electron microscopy remains to be tested.

Immunomorphology: During the past decade, immunohistological techniques have been firmly ensconced in the diagnostic histopathology of tumors. The presence of viral antigens in tissues may also be more easily detected by using antibodies that visualize viral infections. As regards diagnostic myopathology, the application of immunomorphological methods is still in its infancy. Such an immunohistochemical spectrum may entail several categories of neuromuscular disorders. As many of the immunomorphological methods are performed on praffin-embedded material it is also conceivable that, with the future spread and advance of immunohistochemical techniques in diagnostic pathology, the currently somewhat restrictive procedure of not fixing muscle tissue aside from electron microscopic examination may be altered or expanded by regularly incorporating old-fashioned formalin fixation for eventual immunohistochemical studies. On the other hand, primarily unfixed frozen sections may subsequently be fixed for immunomorphology.

When studying inflammatory myopathies, immunological subclassification of B and T-lymphocytes as well as other cytological components participating in the inflammatory process has repeatedly been performed[11-16]. While this immunological cell-typing largely concerns polymyositis, in dermatomyositis, which is now considered an inflammatory vascular disease[17], immunoglobulins and complement may be demonstrated in diseased vessel walls[18,19].

The myasthenic syndromes, the foremost being myasthenia gravis, are defined as conditios in which the motor endplates are diseased. Immunologic studies of such abnormal motor endplates as to acetylcholine receptors or the localization and identification of antibodies and other immunologic parameters been published in the literature[20,21]. However, systematic incorporation of motor endplate examination into diagnostic myopathology requires regular motor-point biopsies. Identification of the motor point prior to biopsy, therefore, should be included in the complete diagnostic evaluation of a patient's neuromuscular condition.

Detection and analysis of the gene for Duchenne's muscular dystrophy (DMD)[22] have created a completely new approach to diagnostic morphology. The protein

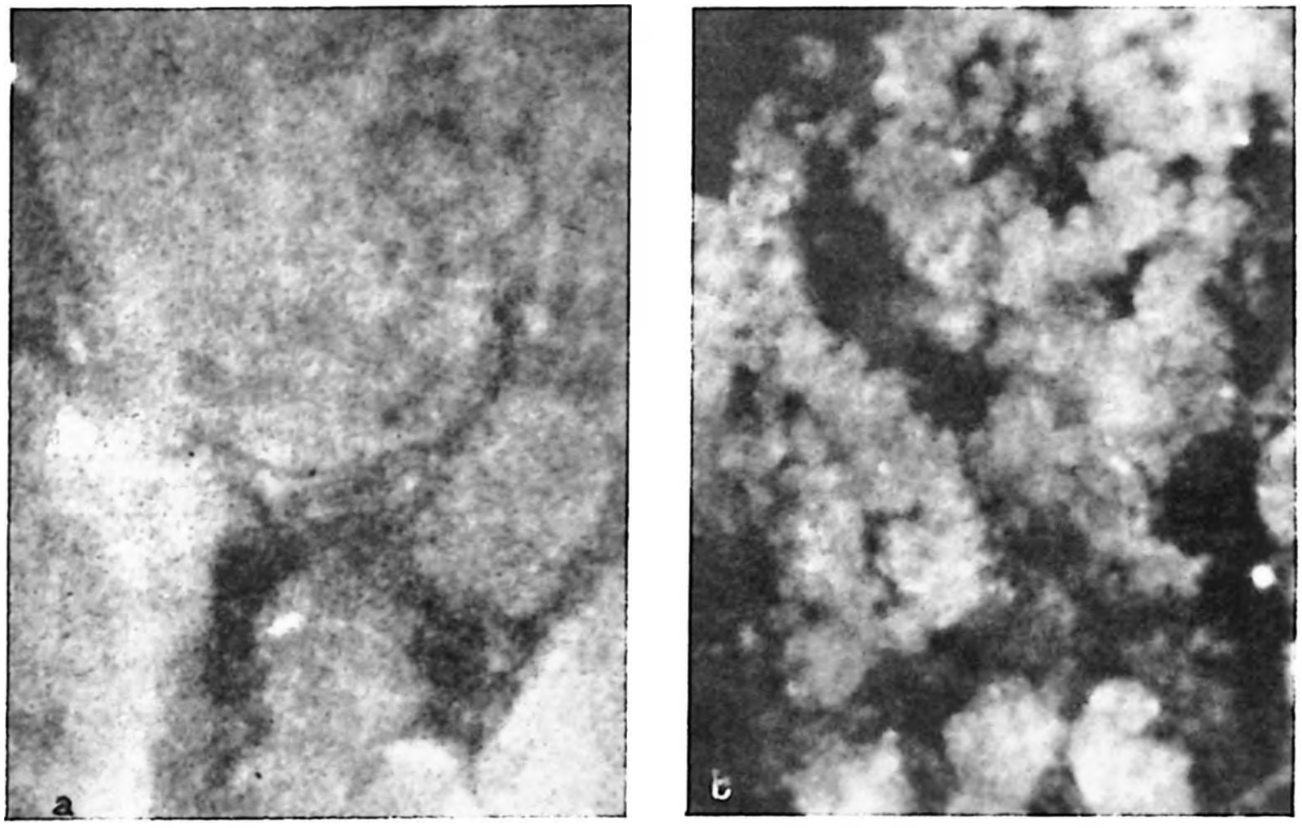

Fig. 4: Absence of dystrophin from muscle fibers reveals background staining, but no subsarcolemmal dystrophin.
a) Boy with Duchenne muscular dystrophy (DMD), X 228.
b) Fetal muscle at 22 weeks gestation from a DMD carrier, X 800.

dystrophin is the coded product of the respective normal gene and is located beneath the plasma membrane of the muscle fiber (Fig. 3). In DMD, dystrophin is either completely absent (Figs. 4 a, b) or present in only trace amounts. In Becker's muscular dystrophy (BMD), dystrophin is segmentally deficient, due to either too little formation or production of a molecularly abnormal dystrophin. In carriers of DMD and BMD, only disseminated muscle fibers may show respective deficiencies of dystrophin (Fig. 5)[23]. Thus, immunohistology and the Western blot technique for analysis of dystrophin now represent standard procedures[24] in evaluating DMD and BMD as well as respective biopsied tissues from carriers. As a result, diagnostic myopathology has closed ranks with other molecular biological techniques. All this may be just the beginning of a trend to revolutionize diagnostic myopathology of hereditary neuromuscular diseases by identifying immunological defects as they are discovered. As genetic and immunologic analyses of DMD and BMD have modified the nosological and clinical spectra of these disorders, examination of muscle tissue for dystrophin now goes well beyond the DMD and BMD muscle tissues (Table I). Conservation of unfixed frozen tissue also enables retrospective studies and there by renewed genetic counselling. Furthermore,

Table I: Diagnostic Examination of Dystrophin

– Confirmation or elimination of DMD
– Confirmation or elimination of BMD
– Carriers for DMD or BMD
– Fetal muscle tissue
– Pathological male muscle tissue specimens without any disease-specific pathology
– Pathological female muscle tissue specimens without any disease-specific pathology
– Congenital muscular dystrophy
– Severe myopathology

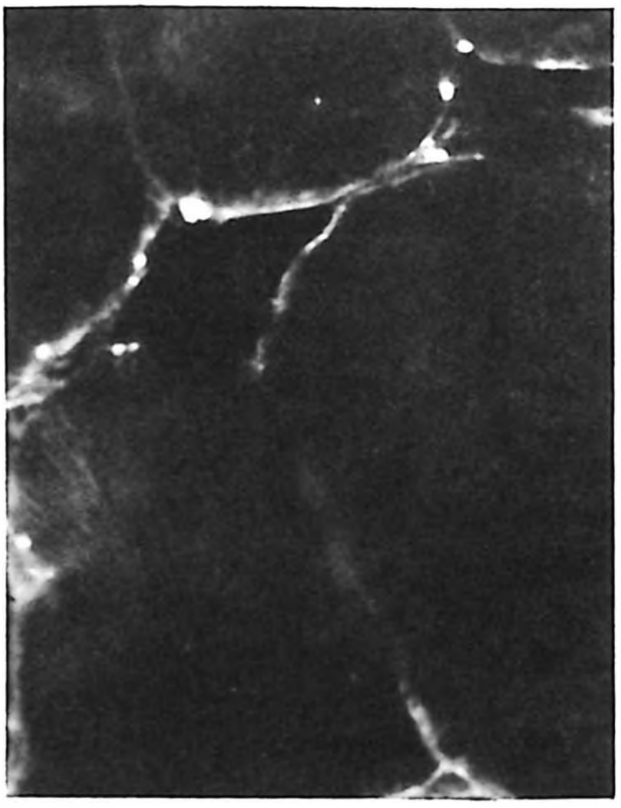

Fig. 5: There are several segments within muscle fibers free of dystrophin; mother of the patient with Becker muscular dystrophy, X 528.

modification of the current immunofluorescent technique of demonstrating dystrophin and application to paraffin-embedded tissue may even enable examination of muscle tissues retrieved by biopsy or at autopsy many years or decades ago. In this context, it would be tempting, as a medico-historical vignette, to reexamine still-available blocks of original patients studied and described by Duchenne himself.

As the number of proteins detected and defined in skeletal muscle fibers increases, their immunological evaluation as regards diagnostic myopathology of neuromuscular diseases will also grow. Nebulin[25], titin[26], alpha actinin[27] and certain myofilamentous proteins such as the myosins[28,29] and actin have already been studied, but not in a very systematic fashion concerning the entirety of neuromuscular diseases.

Among the skeletal muscle proteins, the intermediate filament proteins of muscle, the foremost of which is desmin, and vimentin in undifferentiated muscle cells, play an increasing diagnostic role. In regenerating fibers, coexpression of desmin and vimentin may occur within the same muscle cell (Figs. 6 a, b). Furthermore, atrophic fibers in neurogenic processes or in myotonic dystrophy may similarly coexpress desmin and vimentin (Figs. 7 a, b). Whether these fibers are actually atrophic fibers undergoing reinnervation remains to be seen.

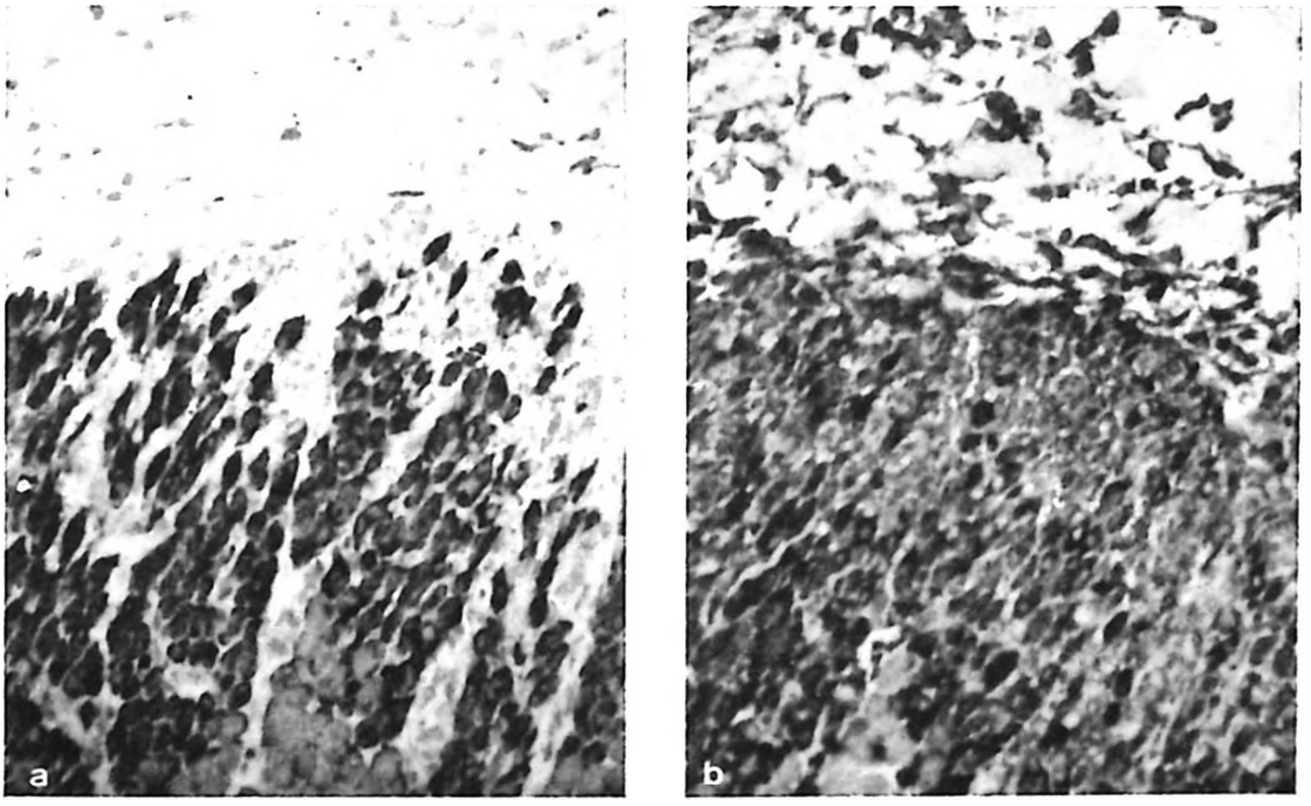

Fig. 6: Degenerating-regenerating fibers in the periphery of muscle fascicle express: a) desmin, X 140, b) vimentin, X 208, within the same muscle fibers.

On the other hand, desmin may be abnormally aggregated in certain inclusions within muscle fibers, such as cytoplasmic bodies[30], spheroid-cytoplasmic bodies, or Mallory body-like inclusions[31]. A number of patients with muscle tissues marked by abnormal aggregates of desmin intermediate filaments have now been described, and even abnormal aggregates of desmin have been seen in cardiac myofibers in certain cardiomyopathies[32,33].

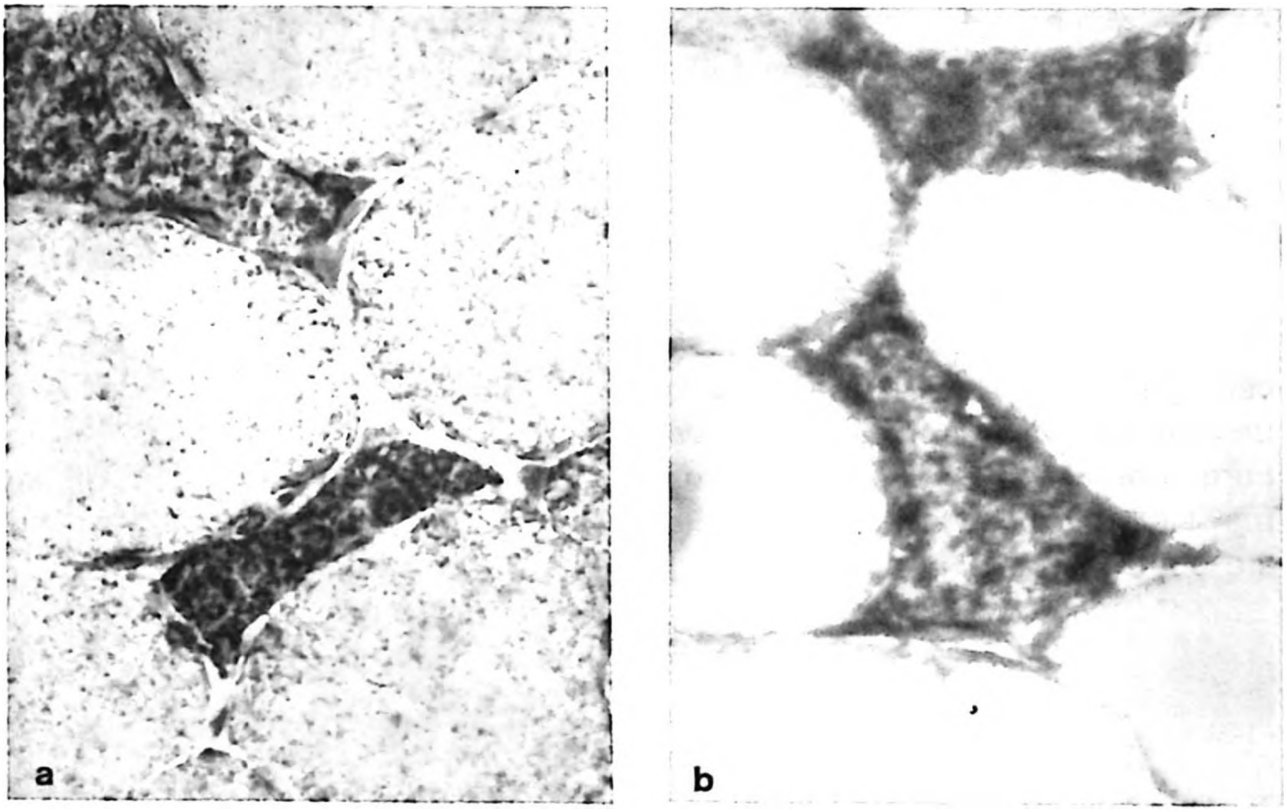

Fig. 7: Coexpression of a) desmin, X 400, and b) vimentin, X 360, within two denervated muscle fibers in neurogenic atrophy.

Denervated muscle fibers are marked by atrophy and are present within the respective muscle tissue in either scattered or grouped arrangements. As denervation in man if not traumatic, is a chronic process of varying length, the dynamics of which within the motor neuron, at the motor endplate and within the respective muscle fiber are largely unknown, the spectrum of denervated muscle fibers is not uniform, as the period of denervation is of different lengths within a group of denervated muscle fibers in both spinal muscular atrophies and neuropathies. The neural cell adhesion molecule (N-CAM) is expressed in denervated fibers[34] (Fig. 8 a), but not every denervated fiber expresses N-CAM. Characterization of N-CAM-positive fibers is still incomplete. Likewise, denervation results in resurfacing of extrajunctional acetycholine receptors during a certain period after denervation, and the presence and extent of extrajunctional acetylcholine receptors may follow different patterns in different forms of neurogenic atrophy. It is further conceivable, as denervation in chronic neurogenic disorders most likely is not a temporal event of very brief duration, that meticulous examination of the motor endplate regions of such patients' muscle tissues may also be rewarding when a) motor point biopsies are regularly performed and b) more detailed morphologic parameters of diseased motor endplates may become known. N-CAM is also expressed in regenerating muscle fibers (Fig. 8 b)[35].

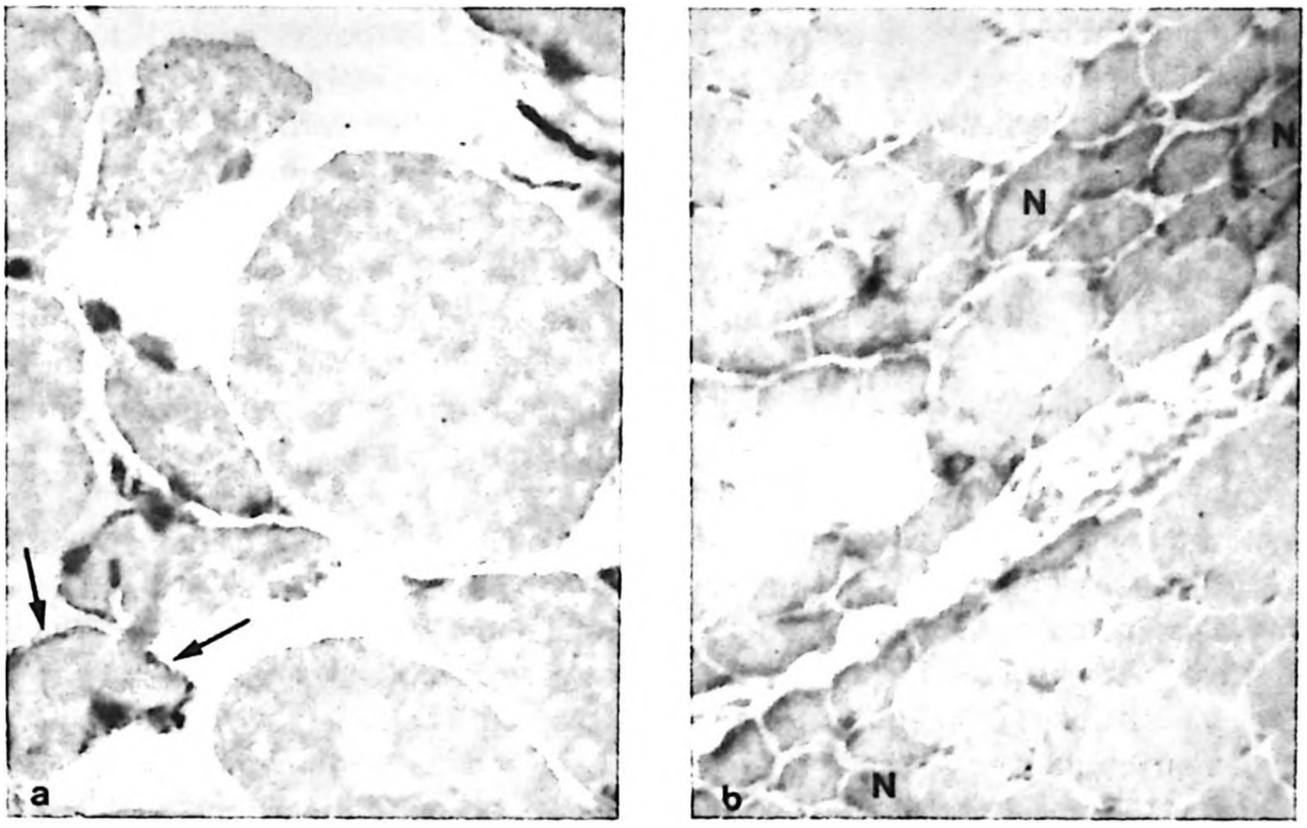

Fig. 8: Presence of the neural cell adhesion molecule (N-CAM), evidenced by antibodies against Leu-19:
a) Superficial location of N-CAM (arrows) in denervated fibers, X 530.
b) Diffuse presence of N-CAM (N) in regenerating myofibers arranged in groups, X 220.

Although the era of immunohistochemical myopathology is rapidly expanding, the full scope of this new field is far from being defined. However, one may speculate that it is this area within diagnostic myopathology, immunomorphology, which may modify, alter or even revolutionize our myopathological diagnostic approach in future years and thereby even render current techniques, such as those employed in enzyme histochemistry, somewhat obsolete.

Molecular Pathology : In situ hybridization is currently applied to tissues affected by viral diseases. However, it is certainly conceivable that morphologic demonstration within tissues, even using the polymerase chain reaction for amplification, may reveal chromosomal deletions in DMD and BMD myofibers and respective muscle fibers in carriers. Localization of abnormal genes in other neuromuscular diseases, such as infantile spinal muscular atrophy, and demonstration of chromosomal deletions in other neuromuscular conditions already portend an additional new era in diagnostic myopathology.

Electron Microscopy : Since the beginning of the modern time of diagnostic myopathology, and even before the introduction of enzyme histochemical

techniques, the electron microscope has been applied to the systematic study of neuromuscular conditions. Actually, the group of congenital myopathies largely emerged as a separate group of neuromuscular entities with the advent and introduction of electron microscopy into diagnostic myopathology.

Apart from systematic ultrastructural studies of neuromuscular disorders so far described in small numbers or even newly defined conditions as well as the evaluation of hitherto unrecognized lesions, employment of the electron microscope in myopathologic examination henceforth will probably involve combined techniques, i.e. morphometry and electron microscopy, as mentioned above; electron microscopy and histochemistry, e.g., the Thiéry technique to identify glycogen at the ultrastructural level such as in polyglucosan bodies or inclusions in Lafora disease; electron microscopy and enzyme histochemistry, e.g., ultrastructural activity and localization of cytochrome c oxidase[36]; or electron microscopy and immunomorphology, e.g., localizing dystrophin or desmin. These combined techniques, however, will further complicate the organization of the "muscle biopsy program", because at the time of biopsy, sophisticated cytochemical studies will have to be set up in advance in order to utilize the tissue within a reasonable time after the biopsy has been performed, thus avoiding a second biopsy of the same patient for such intricate studies when a preoperative diagnosis is not yet known or strongly suspected.

Conclusion

Unlike neoplastic diseases, which are defined by the histological diagnosis of the respective tumors, i.e., are nosologically detailed in the individual patient by the histopathologist, neuromuscular diseases are nosologically diagnosed by the concerted effort of the clinician (often including the geneticist), the electrophysiologist, the clinical chemist, and the myopathologist, and often the biochemist, among whom importance of the individual contribution by each of these experts rests upon the type of the individual neuromuscular condition. To ascertain a congenital myopathy or an inflammatory myopathy, myopathologic examination of a biopsied muscle specimen is essential. The myotonias are the domain of the electrophysiologist, while the clinician and geneticist identify the different hereditary forms of muscular dystrophy. However, each of the participating diagnosticians should be aware of the findings of the others within the concerted diagnostic work-up. As the muscle biopsy usually occurs at the end of the diagnostic regimen, it is the myopathologist who initiates and executes all necessary studies to be performed on the biopsied tissue is responsible for seeing that all essential techniques are readily and optimally applied to the tissue. However, the results of his studies may require additional endeavors by his diagnostic colleagues, such as family studies or further follow-ups if a diagnosis cannot be achieved at the time of the muscle biopsy. Therefore, more frequently

and more intensely than in other concerted enterprises, close cooperation and mutual information among the participants in the diagnostic program of a patient's neuromuscular condition appear mandatory. As the overwhelming majority of recently detected neuromuscular conditions have been delineated by biopsy rather than by postmortem studies, a muscle biopsy, always intended to aid in the diagnosis, should also leave room for additional scientific studies, among which sophisticated studies such as mitochondrial and nuclear DNA analyses of mitochondrial and dystrophic myopathies, respectively, only loom on the diagnostic horizon.

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REFERENCES

1. Engel WK. The essentiality of histo and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. Neurology 1962; 12: 118-94.
2. Dubowitz V, Brooke HH. Muscle biopsy: A modern approach. In: Major Problems in Neurology Vol.2. London: WB Saunders; 1973.
3. Pearson CM, Mostofi FK (eds). The Striated Muscle. Internat Acad Pathol Monograph. Baltimore: Williams and Wilkins; 1973.
4. Burck U, Goebel HH, Kuhlendahl HD, Meier C, Goebel KM. Neuromyopathy and vitamin E deficiency in man. Neuropediatrics 1981; 12: 267-78.
5. Askanas V, Engel WK. Distinct subtypes of type I fibers of human skeletal muscle. Neurology 1975; 25: 879-87.
6. Goebel HH, Zeman W, Pilz H. Significance of muscle biopsies in neuronal ceroid-lipofuscinoses. J Neurol Neurosurg Psychiatry 1975; 38: 985-93.
7. Goebel HH, Kolschütter A, Lenard HG. Morphologic and chemical biopsy findings in mucolipidosis IV. Clin Neuropathol 1982; 1: 73-82.
8. Goebel HH, Schile M, Burck U. Fetal congenital lethal hypophosphatasia: histochemical absence of alkaline phosphatase activity in endothelial cells of intramuscular capillaries. Acta Neuropathol (Berl) 1982; 57: 236-8.
9. Bardosi A, Dimitri T, Wosglen B, Gabius HJ. Expression of endogenous receptors for neoglycoproteins, especially lectins, that allow fiber typing on formaldehyde-fixed, paraffin-embedded muscle biopsy. A glycohistochemical, immunohistochemical, and glycobiochemical study specimens. J Histochem Cytochem 1989; 37: 989-98.
10. Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types. I. Adult male and female. Neurology 1969; 19: 221-33.
11. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. Ann Neurol 1984; 16: 193-208.

12. Engel AG, Arahata K. Monoclonal antibody analysis of mononuclear cells in myopathies. II: Phenotypes of autoinvasive cells in polymyositis and inclusion body myositis. Ann Neurol 1984; 16: 209-15.

13. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. III: Immunoelectron microscopy aspects of cell-mediated muscle fiber injury. Ann Neurol 1986; 19: 112-25.

14. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. IV: Cell-mediated cytotoxicity and muscle fiber necrosis. Ann Neurol 1988; 23: 168-73.

15. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. V: Identification and quantitation of $T8^+$ cytotoxic and $T8^+$ suppressor cells. Ann Neurol 1988; 23: 493-9.

16. Botet JC, Grau JM, Casademont J, Urbano-Márguez A, Rozman C. Characterization of mononuclear exudates in idiopathic inflammatory myopathies. Virchows Archiv [A] 1988; 412: 371-4.

17. Banker BQ. Dermatomyositis of childhood: ultrastructural alterations of muscle and intramuscular blood vessels. J Neuropathol Exp Neurol 1975; 34: 46-75.

18. De Visser M, Emsile-Smith AM, Engel AG. Early ultrastructural alterations in adult dermatomyositis. Capillary abnormalities precede other structural changes in muscle. J Neurol Sci 1989; 94: 181-92.

19. Whitaker JN, Engel WK. Vascular deposits of immunoglobulin and complement in idiopathic inflammatory myopathy. N Engl J Med 1972; 286: 333-8.

20. Engel AG, Lambert EH, Howard FM. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations. Mayo Clin Proc 1977; 52: 267-80.

21. Engel AG, Arahata K. The membrane attack complex of complement at the endplate in myasthenia gravis. Ann NY Acad Sci 1987; 505: 326-32.

22. Brown RH Jr, Hoffman EP. Molecular biology of Duchenne muscular dystrophy. Trends Neurosci 1988; 11: 480-4.

23. Arahata K, Ishihara T, Kamakura K, et al. Mosaic expression of dystrophin in symptomatic carriers of Duchenne's muscular dystrophy. N Engl J Med 1989; 320: 138-42.

24. Arahata K, Hoffman EP, Kunkel LM, et al. Dystrophin diagnosis: comparison of dystrophin abnormalities by immunofluorescence and immunoblot analyses. Proc Natl Acad Sci 1989; 86: 7154-8.

25. Bonilla E, Miranda AF, Prelle A, et al. Immunocytochemical study of nebulin in Duchenne muscular dystrophy. Neurology 1988; 38: 1600-3.

26. Osborn M, Hill C, Altmannsberger M, Weber K. Monoclonal antibodies to titin in conjunction with antibodies to desmin separate rhabdomyosarcomas from other tumor types. Lab Invest 1986; 55: 101-8.

27. Hashimoto K, Shimizu T, Nonaka I, Mannen T. Immunochemical analysis of alpha-actinin of nemaline myopathy after two-dimensional electrophoresis. J Neurol Sci 1989; 93: 199-209.

28. Biral D, Scarpini E, Angelini C, Salviati G, Margreth A. Myosin heavy chain composition of muscle fibers in spinal muscular atrophy. Muscle Nerve 1989; 12: 43-51.

29. Sawchak JA, Lewis S, Shafiq SA. Coexression of myosin isoforms in muscle of patients with neurogenic disease. Muscle Nerve 1989; 12: 679-89.

30. Osborn M, Goebel HH. The cytoplasmic bodies in a congenital myopathy can be stained with antibodies to desmin, the muscle-specific intermediate filament protein. Acta Neuropathol (Berl) 1983; 62: 149-52.

31. Fidzianska A, Goebel HH, Osborn M, Lenard HG, Osse G, Langenbeck U. Mallory body-like inclusions in a hereditary congenital neuromuscular disease. Muscle Nerve 1983; 6: 195-200.

32. Porte A, Stoeckel ME, Sacrez A, Batzenschlager A. Unusual familial cardiomyopathy with storage of intermediate filaments in the cardiac muscle cells. Virchows Archiv 1980; 386: 43-58.

33. Voit T, Krogman O,Lenard HG, et al. Emery-Dreifuss muscular dystrophy: disease spectrum and differential diagnosis. Neuropediatrics 1988; 19: 62-71.

34. Cashman NR, Covault J, Wollman RL, Sanes JR. Neural cell adhesion molecule in normal, denervated, and myopathic human mucle. Ann Neurol 1987; 21: 481-9.

35. Schubert W, Zimmermann K, Cramer M, Starzinski-Powitz A. Lymphocyte antigen Leu-19 as a molecular marker of regeneration in human skeletal muscle. Proc Natl Acad Sci USA 1989; 86: 307-11.

36. Müller-Höcker J, Johannes A, Droste M, Kadenbach B, Pongratz D, Hübner G, Fatal mitochondrial cardiomyopathy in Kearns-Sayre syndrome with deficiency of cytochrome-c-oxidase in cardiac and skeletal muscle. Virchows Arch [B] 1986; 52: 353-67.