Pathology of the Neuromuscular Junction

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ABSTRACT

Diagnosis of neuromuscular disease by the study of motor endplate structure in the light and electron microscopes is probably one of the most specialized examinations in the field of neuropathology. The classical means of staining endplates using gold, silver or methylene blue are described as well as more modern techniques suitable for light microscopy. Specific abnormalities in patterns of terminal innervation after methylene blue staining are noted. Techniques for localizing endplates suitable for electron microscopic study are given and some of the typical changes that occur in endplate structure in some neuromuscular diseases are indicated.

Motor Endplate Pathology

Diagnosis of neuromuscular disease by study of motor endplate structure in the light and electron microscopes is probably one of the most specialized examinations in the field of neuropathology. This is hardly a problem for the general pathologist in the community hospital or, indeed, for many neuropathology laboratories.

The nerve terminus where it innervates a skeletal muscle has been difficult to study because of its small size and the lack of easily performed staining methods. This contribution is confined to a brief discussion of the classical means of staining motor endplates which are admittedly, with only few exceptions, generally useless for diagnostic purposes and to a brief consideration of the ultrastructure of normal and diseased endplates. The terms motor endplate and neuromuscular junction are equivalent in this discussion.

Staining Motor Endplates

In the classical literature, that is before 1947, staining of motor endplates was accomplished by means of gold and silver impregnation methods. For studying the general overall structure of the motor endplate but not the terminal axons, one of the methods using gold chloride and citric acid, such as that used by Cole or Carey, are generally successful. These require fresh muscle and are not suitable for paraffin embedded tissue. Silver staining methods, such as those of Cajal modified extensively by numerous investigators thereafter, are basically axon stains that demonstrate the neural components of motor endplates but not the postsynaptic apparatus. In general, these kinds of methods are capable of demonstrating the effects of denervation but generally are worthless for the study of abnormal endplates. In the 1950's, the older methods of methylene
blue staining were adapted to human muscle biopsy specimens particularly by Denz\textsuperscript{8} and extensively by Coërs\textsuperscript{2} and Evans.\textsuperscript{11} In one form of this staining technique, the dye is injected into the living muscle whereas other methods utilize the dye to stain excised muscle specimens. Very fine branches of the terminal axons can be demonstrated in a successful methylene blue preparation but, unfortunately, nuclear staining does not occur and the structure of the postsynaptic apparatus is not seen. A major contribution was made by Couteaux\textsuperscript{6} who used Janus Green B as a supravit stain to demonstrate both terminal axons and the subneural apparatus in unfixed motor endplates. When properly performed, this stain will clearly demonstrate the clefts of the subneural apparatus. The only methods that will stain motor endplates after fixation are the paraphenylene diamine method for glutaraldehyde fixed, plastic-embedded tissues for electron microscopy\textsuperscript{10} and the Spicer and Meyer\textsuperscript{22} method utilizing aldehyde fuchsin after previous sulfation. In this method, endplates may be demonstrated in formalin fixed paraffin embedded tissue. Demonstration of endplate structure is based upon the delicate and discrete staining of the muscle external lamina by aldehyde fuchsin.\textsuperscript{28} The structure of the subneural apparatus is demonstrated by the stained external lamina. Finally, the structure of the subneural apparatus and some information about the esterases present within it may be obtained by staining with histochemical methods for cholinesterases. This is a complex subject in itself and can not be reviewed at this time. However, a recent review of this subject may be consulted.\textsuperscript{27} The histochemical procedures recommended for maximum specificity are the basic acetylthiocholine methods originally described by Koelle\textsuperscript{16} and modified by various investigators including Couteaux,\textsuperscript{7} Karnovsky and Roots\textsuperscript{16} and Koelle and Gromadzki.\textsuperscript{17} Particularly for electron microscopy, the gold acetylthiocholine and thiolacetic acid methods are capable of demonstrating good localization. However, with the exception of denervation studies, these methods are infrequently used in diagnostic pathology.

**Endplate Ultrastructure**

Selection of the proper site to biopsy and handling of the biopsy specimen are important if the maximal amount of information is to be obtained. Firstly, the site of biopsy is most important. As a general rule, motor endplates are located in the mid-portion of the myofibers in skeletal muscles. This is the "innervation band." In those muscles where the myofibers run without intermediate insertion end to end from one tendon to another, biopsy in the mid-portion should yield motor endplates for study. The situation in multipennate muscles with complex internal tendons is more difficult and the site has to be chosen after consideration of a map of motor endplate location such as those provided by Coërs and Woolf.\textsuperscript{4} Favorite sites for biopsy include the inferior lateral mid-portion of the deltoid, the lateral border of the vastus medialis approximately one cm proximal to the patella and, in some circumstances, the mid-portion of the external intercostal muscles. Although some prefer to detect location of endplates by electrical stimulation of motor points before biopsy and others use intravital methylene blue staining, these procedures are probably unnecessary if a site rich in endplates is selected. Once selected, the skin should be infiltrated with anesthesia although the muscle belly should not, and some kind of muscle clamp is preferable to prevent severe contraction of the myofibers. It is possible to obtain adequate biopsy specimens without the use of a clamp. However, a clamp is especially desirable in the case of the large muscles. The pathologist should receive the specimen in the operating room and may then select pieces
of the muscle biopsy for various purposes. If enzyme histochemistry is desired, a piece is frozen in liquid nitrogen, a second piece is placed in buffered glutaraldehyde fixative for electron microscopy and the third piece may be used for routine paraffin embedding and staining.

**Endplate Pathology**

With respect to pathology that may be demonstrated by these methods, the results of methylene blue staining, the commonest technique used for light microscope studies, will be discussed. Included in the "catch basket" diagnosis of "amyotonia congenita" are entities such as polyneuritis and certain diseases that selectively involve the motor endplates. Abnormal terminal axons demonstrated by methylene blue staining have been reported in children with the "amyotonia congenita" syndrome. Coers and Woolf described overly elaborate and poorly formed neuromuscular junctions in these children. Single axons innervating several myofibers were found suggesting previous denervation and subsequent reinnervation and there was, in some instances, excessive branching of the intramuscular nerve fibers. Other neuromuscular junctions displayed shrinkage and abnormally small size with fine ultraterminal axon sprouting.

In contrast to these changes, neuromuscular junctions from patients with alcoholic polyneuritis and vitamin B deficiency were characterized by loss of axons and little evidence of reinnervation in some patients whereas in others, there were complex terminal arborizations and "ribbon-like" axon swellings. These authors also described peculiar fusiform swellings, lightly stained by methylene blue, that occurred on the terminal axons of neuromuscular junctions from patients with vitamin B_{12} deficiency. The significance of these particular axon swellings which, to the author's knowledge have not been studied by EM, is unknown. When such structures contain aggregates of abnormal mitochondria and electron dense bodies, they may represent significant lesions such as those that occur in the central nervous system in vitamin E deficiency.

Diabetic neuropathy according to Woolf and Malins is characterized by early swelling and pale staining of terminal axon arborizations in motor endplates followed by fusion to form balloon-shaped masses.

In later stages, irregularly swollen and weakly stained axons as well as some with sprouts were found. Also, abnormally large or abnormally small terminal arborizations occurred in patients with diabetes mellitus. In carcinomatous neuropathy, methylene blue staining of neuromuscular junctions has revealed fusion of the terminal arborizations with fusiform swelling of subterminal axons. These changes are accompanied by collateral reinnervation. The methylene blue technique has also been extensively used to study motor endplate pathology in myasthenia gravis. Detailed discussion of this prime example of motor endplate disease is beyond the scope of this brief review. Methylene blue staining has demonstrated abnormalities in the terminal arborizations of myasthenic neuromuscular junctions. Woolf et al reported that the changes in the terminal axons resemble those seen in myopathy and were beaded sprouts resembling those observed in patients with Werdnig-Hoffmann disease. Later reports by Coërs and Desmedt described two kinds of endings in myasthenia gravis, — "dystrophic" endings that had abnormally numerous ramifications of the terminal arborization and "dysplastic" endings that were characterized by marked elongation of motor endings in the axial direction without side branching. Coërs and Desmedt found 5 to 45 percent with the abnormality in the total number of neuromuscular junctions in each biopsy.

It should be stressed that in all of the studies, only the presynaptic portion (axon
branches) of the neuromuscular junction is demonstrated because the subneural apparatus is not stained by methylene blue. Thus ultrastructure studies are required to study significant changes in the subneural apparatus of neuromuscular junctions. In interpreting such changes, it is well to recognize that different kinds of neuromuscular junctions occur in fast and slow myofibers,21 that there is evidence that degeneration and regeneration of neuromuscular junctions occur under physiological conditions probably as a replacement mechanism or a response to incidental trauma of daily life22 and, therefore, examination of an individual neuromuscular junction may not be typical and diagnostic. With these warnings, the structure of the normal neuromuscular junction as seen in the electron microscope will be reviewed and several neuromuscular diseases in which they have been extensively investigated will be described.

Neuromuscular Junction

As the terminal axon approaches the myofiber surface, it is surrounded by a myelin sheath composed of multiple layers of Schwann cell cytoplasm peripheral to which is a thin tube of perineural epithelium. The latter ends approximately one μm from the muscle surface. The axon emerges from its Schwann cell sheath at a terminal node and extends into gutters lying in the muscle sarcolemma covered only by a small amount of Schwann cell cytoplasm which in turn is covered by a layer of external lamina material. The gutter (primary synaptic cleft) in which the terminal axon lies is filled with amorphous external lamina material which extends into the secondary synaptic clefts that project from the primary cleft into the underlying sarcoplasm. The terminal axon contains mitochondria, synaptic and coated vesicles. The underlying sarcoplasm of the soleplate is devoid of myofilaments but contains elements of the T system that extend to and connect with the base of the subneural apparatus. Ribosomes, glycogen granules and mitochondria are also present. The endplate region contains three kinds of nuclei, — Schwann cell, perineural epithelial cell and myofiber.

Myasthenic endplates have been studied most extensively with the electron microscope. Characteristically, neuromuscular junctions in myasthenia gravis in severely affected individuals show one of three patterns. They may appear normal without obvious pathology, they may appear atrophic or there may be evidence of both denervation and reinnervation. In the rare syndrome of canine myasthenia the appearance of the endplates is that of denervation atrophy. Also, on rare occasions evidence of reinnervation can be demonstrated. Changes of this sort have been put on a quantitative basis by the work of Engel and his associates.9 These investigators have also demonstrated that in the myasthenic syndrome, which occurs in some patients with bronchogenic carcinoma,18 the subneural apparatus differs significantly from that structure in endplates of patients with myasthenia gravis. In the myasthenic syndrome, there is considerable increase in the length and area of the subneural clefts whereas the myasthenia gravis endplate has decreased cleft area. Other investigators have also claimed that there are fewer synaptic vesicles in the myasthenic axon terminal.14 Abnormal subneural apparatus also occurs in the endplates of patients with muscle denervation resulting from porphyria or peripheral neuropathy. Striking changes resembling denervation and reinnervation changes occur in myotonic dystrophy. Ultrastructure studies of endplates from patients with muscular dystrophy have shown endplate changes that, in some cases, appear to arise from myofiber degeneration. However, Nara20 described large vacuoles in the subneural sarcoplasm in
biopsy specimens from patients with progressive muscular dystrophy. These changes were consistent with denervation or, in some cases, reinnervation changes. Thus, like myasthenia gravis, there is clinical as well as morphologic evidence that denervation and reinnervation occurs in some forms of muscular dystrophy.

Abnormal neuromuscular junction fine structure has also been observed in rare forms of myopathy such as nemaline myopathy.13 This kind of morphologic data has led some support to those who doubt the intrinsic etiology of muscular dystrophy and suggest that an abnormal trophic influence from the nerve is responsible for the myofiber pathology.

Summary

In summary, this brief review has stressed the non-routine nature of motor endplate study in diseases of the neuromuscular unit, has described the technical methods used and has attempted to outline briefly some of the pathologic abnormalities that may be observed. It is clear that none are highly specific, particularly those employing supravital methylene blue staining, but that useful information can be obtained concerning the nature of diseases such as myasthenia gravis by examining the fine structure of neuromuscular junctions. Currently, such studies are in the nature of research investigations rather than diagnostic procedures suitable for the average laboratory.

References


