ZACKS

THE MOTOR ENDPLATE

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Dedication

This monograph is dedicated to my father, *David Zacks, M.D.*, for his encouragement past and present.

"General physiology is the basic biological science toward which all others converge. Its problem is to determine the elementary condition of vital phenomena. Pathology and therapeutics also rest on this common foundation. By normal activity of its organic units, life exhibits a state of health; by abnormal manifestation of the same units, diseases are characterized; and finally, through the organic environment modified by means of certain toxic or medicinal substances, therapeutics enables us to act on the organic units. To succeed in solving these various problems, we must, as it were, analyze the organism, as we take apart a machine to review and study all its works; that is to say, before succeeding in experimenting on smaller units we must first experiment on the machinery and on the organs. We must, therefore, have recourse to analytic study of the successive phenomena of life, and must make use of the same experimental method which physicists and chemists employ in analyzing the phenomena of inorganic bodies."

CLAUDE BERNARD, 1865

PREFACE

This monograph deals with the anatomy, ultrastructure, and physiology of the mammalian motor endplate and with its alterations resulting from laboratory experimentation and the processes of disease. Its purpose is to bring together information from numerous sources to encourage further study of this structure in several still obscure neuromuscular diseases. Another objective is to illustrate the great potential value of investigations of ultrastructure in pathology. Just as application of the Cell Theory by Virchow in the eighties of the last century revolutionized pathology, the electron microscope promises to open a new and richly productive era in ultramicroscopic pathology.

I have also included personal opinions and indeed some speculations for which I take sole responsibility. This perhaps is the privilege of the essayist, which may lighten what otherwise would be a dry recital of anatomic description and formal scientific analysis.

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INTRODUCTION

Although the history of the investigation of the terminal nerve ending on muscle can be traced for 137 years, I believe that the time is ripe for an attempt to bring together available information concerning the various aspects of the motor endplate. Such a review should be useful in indicating possibilities for further research, especially with regard to clinical problems. Pathological investigations of the motor endplate have been few until quite recently, perhaps because of technical difficulties or the tendency of both general and neuropathologists to ignore this borderline zone between the nervous system and the musculature.

In the early years of endplate studies, heated controversies were common concerning the detailed anatomy visible with the light microscope after the application of various staining methods, most of which involved the use of heavy metal precipitation. In retrospect, it is not difficult to understand why such arguments arose, considering the complexity and perversity of the staining methods used and the small size of the structure studied. We know now with all the wisdom of hindsight that many of the structures in the motor endplate are tantalizingly at or near the limit of resolution of the light microscope. Indeed, the controversies of the past might be taken as a lesson to teach us humility in establishing the verity of our present views. Perhaps in a similar period of time, even the most refined electron microscopic studies of this region obtained by current methods will be regarded as of mere historical interest, because new methods, some still to be invented, will be used further to unravel the chemical ultrastructure of the motor endplate.

However, not being equipped with high resolution foresight, but having the advantage of the combined experience of many investigators over the past ten years, we shall attempt to apply information obtained with the electron microscope to reinterpret some of the classical controversies of the past.

Even though many details of the function of the neuromuscular synapse are still uncertain, it is possible now to present a more or less detailed picture of the morphology of the motor endplate and some of its histochemical characteristics, to serve as a beginning toward studies of motor endplates experimentally altered by the laboratory investigator or by the natural experiments of spontaneous disease. This monograph is concerned with the present knowledge of structure of the motor endplate and will undertake an exploratory foray into the complex field of experimental evidence relating to the correlation of form and function of this structure. With knowledge of the fine structure of the human neuromuscular junction and some of the changes that occur in disease, it is hoped that these observations will stimulate further investigation of the important group of neuromuscular diseases which now have only begun to be studied.

It is well realized that morphologic study in itself is insufficient to explain the etiology of many of these neuromuscular diseases. However, since at the electron microscopic level of resolution, structure and function are inextricably associated, it is hoped that clues to possible chemical abnormalities may be suggested by changes in fine structure. These clues may then suggest appropriate biochemical experimentation. Furthermore, use of labeled substances in conjunction with electron microscopy as well as electron microscopic histochemistry offer additional possibilities for extending our knowledge of these disease entities.

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1

Anatomy of Motor Endplates

THE CLASSICAL PERIOD: 1840–1947

Credit for the first investigations, and incidently the source of a major misconception concerning the motor endplate, belongs to Valentin (1836) and Emmert (1836), who believed that the termination of the nerve fiber in striated muscle consisted of an arc-like structure with branches dividing and rejoining in a continuing network (Fig. 1). The concept of Emmert, which antedates the Cell Theory of Schleiden and Schwann, seems particularly quaint to the modern biologist. He said:

> The arc-like ("bogenformige") ending of the motor nerve fiber leads to the conclusion that primitive (nerve) fibers lead from the central part to the periphery and back again from the periphery to the central part (nervous system).
>
> The appearance of the muscle activity and the arc-like ending of the motor nerve fiber indicates that there is a continuous stream and circulation of the nervous fluid in the motor nerve.

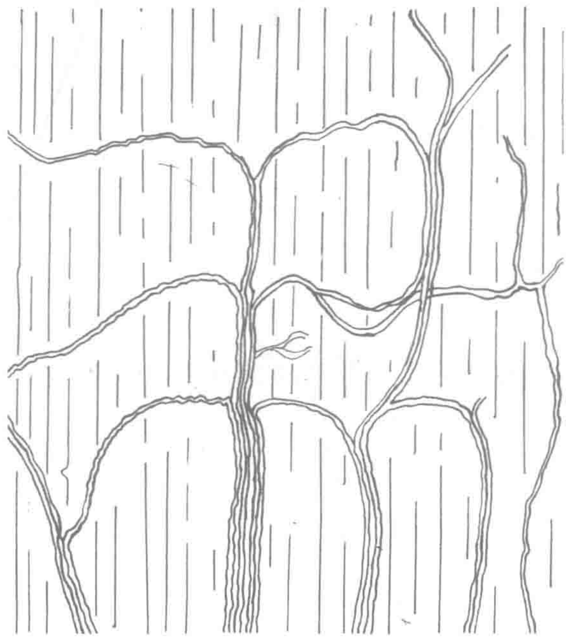

Figure 1. Line drawing of the arc-like endings of motor nerve fibers on skeletal muscle fibers according to the concept of Emmert. (After Emmert, 1836.)

This view was generally well established until 1840, when Doyère first proposed that the nerve fiber came to a complete termination on the surface of the muscle fiber. His studies were carried out in the invertebrate *Milnesium tardigardum*, a species chiefly containing smooth muscle. Figure 2 shows this first illustration of a neuromuscular junction. Three years later, Quartrefages (1843) confirmed Doyère's observations in *Elolidina parodoxa*. Figure 3 remarkably resembles the neuromuscular junction illustrated by Doyère. Careful observations by Wagner of frog muscles (1847) led him to deny the existence of terminal arcs and to recognize the terminal branching of the axon in the junctional region. He also recognized that the terminal nerve fiber lost its myelin sheath before entering the innervation site.

It was not until 1862 that Kühne, one of the outstanding early students of the motor endplate, published his first series of observations. He believed that after penetrating the sarcolemma, the nerve fiber branched and formed a complex terminal arborization. In the same year (1862), Rouget described a heap of granulated material at the level of the nerve termination in striated muscles of reptiles, birds, and mammals. Although he believed

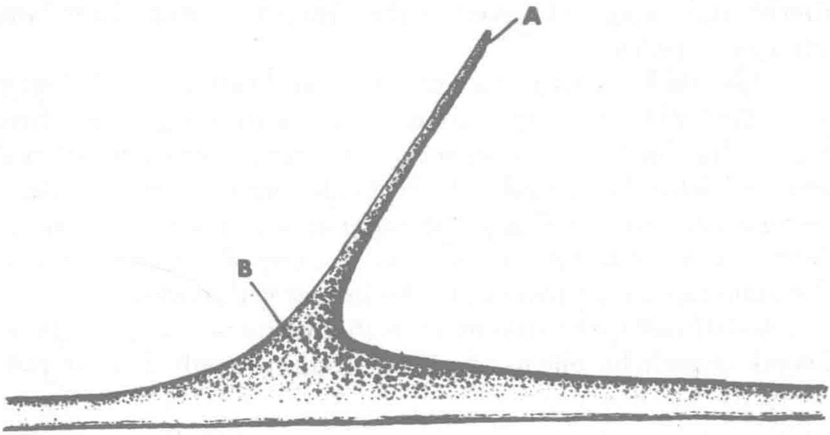

Figure 2. Motor innervation in *Milnesium tardigardum* as described by Doyère. A motor nerve (*A*) is seen to join the muscle fiber at an ill defined, cone-shaped eminence (*B*) on the surface. (After Doyère, 1840.)

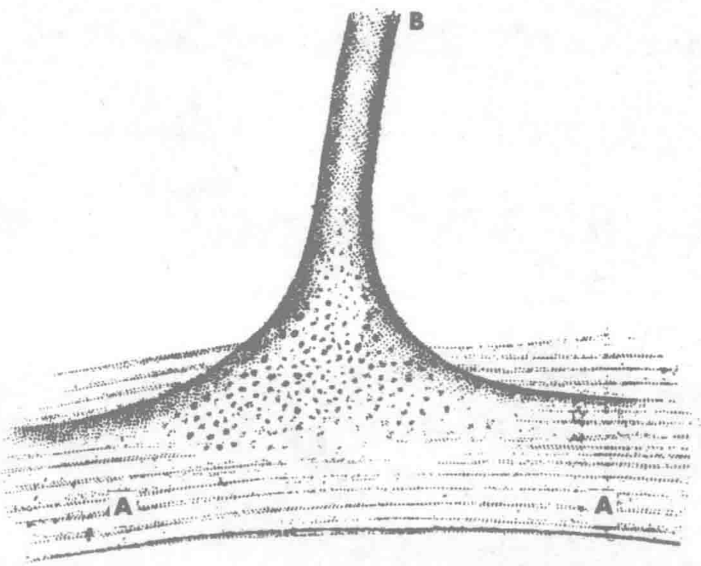

Figure 3. Drawing illustrating the connection between the muscle fiber (*A*) and the nerve fiber (*B*), according to Quartrefages. Note the similarity to the Doyère illustration. (After Quartrefages, 1843.)

that this was due to the spreading out of the terminal axis cylinders, he did recognize the presence of a specialized region in the neuromuscular junction. This he termed the "plaque terminale." Krause (1863) was the first to use the term "motorische endplatte." He used it to refer to the terminal nerve branching and altered muscle zone observed in the innervated area of cats' eye retractor muscles.

It should be pointed out that the terms "neuromuscular junction" and "motor endplate" are not freely interchangeable. "Neuromuscular junction" is a generalized term referring to all anatomic varieties of specialized junctional regions between nerve terminals and muscle fibers. "Motor endplate" refers more specifically to a particular anatomic form of arborization characteristic of certain animals, particularly the higher vertebrates.

Additional early studies of motor endplate morphology in several animals by means of various staining methods were pub-

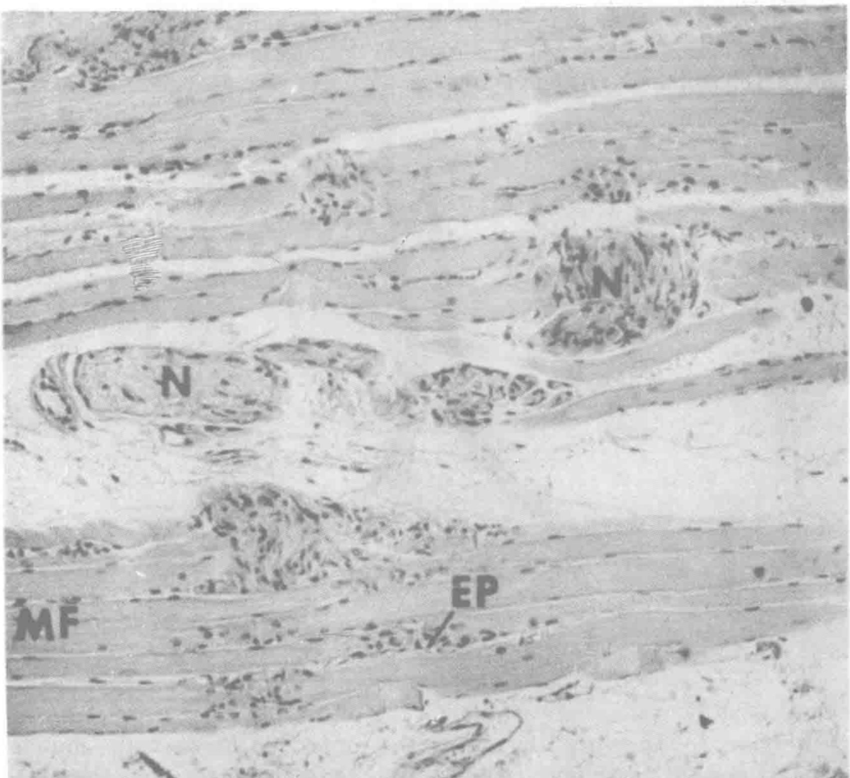

Figure 4. Photomicrograph of human skeletal muscle, showing large nerve bundles at *N* dividing into terminal branches that form areas of innervation on individual muscle fibers (*MF*). The area marked *EP* indicates a probable site of a motor endplate. Note that in this PAS–Alcian blue preparation, little structural detail in the motor endplates is visible. (× 100.)

lished by Ranvier (1878) and by Kühne (1864, 1883, 1886a, b, and 1887).

The cause of the intense scientific polemics that occurred as additional investigators added their observations on the structure of motor endplates can be understood today in terms of the several histologic methods that were used to demonstrate endplates. Furthermore, we now know that motor endplates differ considerably in structural details from one animal to another and indeed in different muscles within a given animal (Cole, 1957).

Before beginning a discussion of the controversial anatomy of the motor endplate, a few words must be said about the methods used during the Classical Period of endplate studies. It was soon apparent that routine staining methods with available dyes showed very few structural details in motor endplates. Figure 4 illustrates the appearance of sectioned paraffin-embedded endplates stained by the PAS–Alcian blue method. This illustration shows considerably more detail than a section stained with hematoxylin and eosin. Because the available dyes failed to differentiate structural details, other methods were employed.

Staining with Gold

One of the favorite techniques was gold impregnation (Ranvier, 1878). Multiple variations were employed by different investigators, although all had the same basic theme. Blocks of muscle tissue bearing nerve fibers were soaked for several hours in solutions of lemon juice. The specimen was then transferred to a solution of gold chloride ($AuCl_3$) for an additional period of time, following which a reducing bath was used. Formulas for several of these procedures are given in the appendix.

The chemical basis of gold-staining methods is unknown. Because gold-stained endplates tend to fade, especially if exposed to light for long periods of time, it is unlikely that the color is due to metallic gold. It is more probable that the red color results from a complex with some component of the endplate. Figure 5 illustrates the typical appearance of a gold-stained motor endplate.

In a typical gold-staining method such as that used by Kühne, two major components of the motor endplate could be distinguished. The first, representing the terminal nerve branches, stained dark violet and was called the "axialbaum," and the remainder of the junctional region, which appeared red, was called the "stroma." This zone enclosed the axial part. In some prepara-