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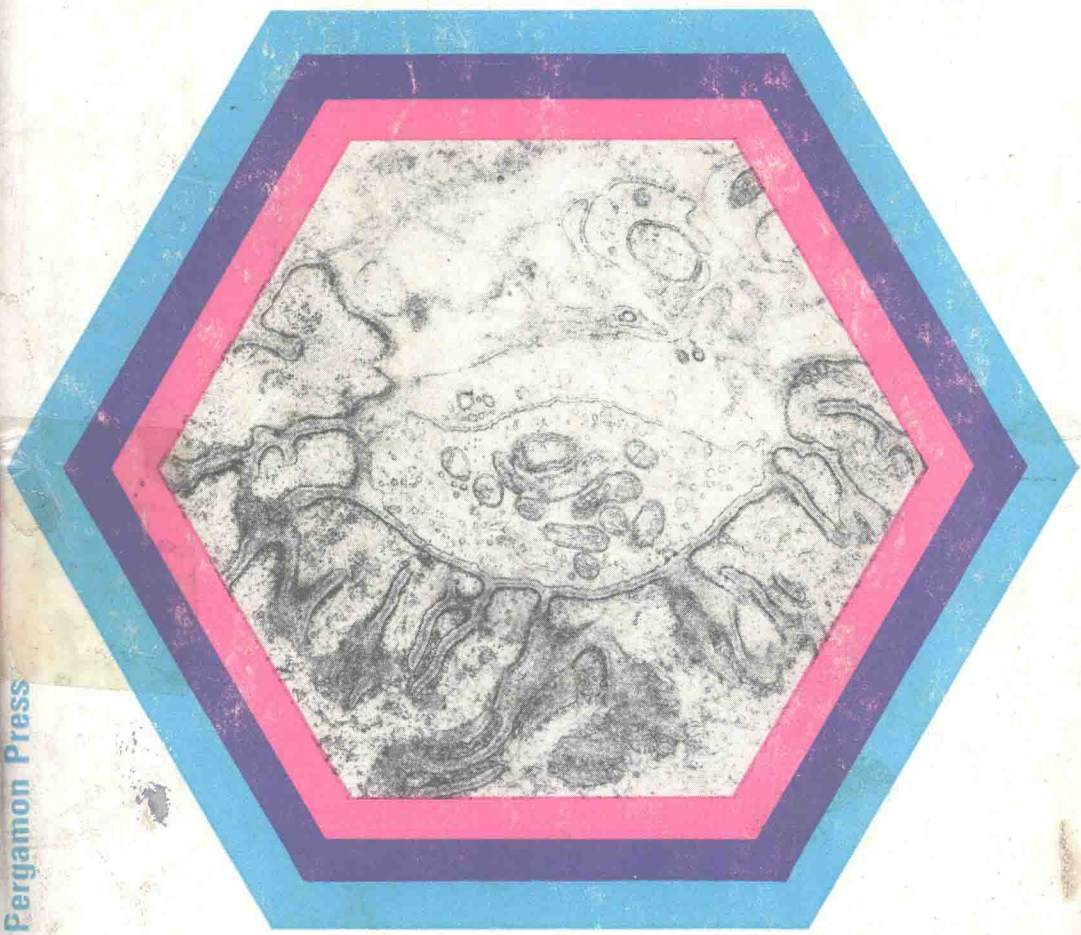
J. Cheymol *Paris*



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INTERNATIONAL ENCYCLOPEDIA OF
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NEUROMUSCULAR BLOCKING AND STIMULATING AGENTS

VOLUME II

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Section 14

NEUROMUSCULAR BLOCKING AND STIMULATING AGENTS

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VOLUME II

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† It is with regret that we learned of the recent death of Professor Osserman.

CONTENTS

CONTENTS OF VOLUME I vii

LIST OF CONTRIBUTORS ix

PREFACE xi

SUBSECTION III

PERIPHERAL STIMULANTS OF THE NEUROMUSCULAR JUNCTION

CHAPTER 16. ACETYLCHOLINE AND ANTICHOLINESTERASE DRUGS 427
W. C. Bowman and Sandra N. Webb, Glasgow, U.K.

CHAPTER 17. ACTIVATION OF NEUROMUSCULAR TRANSMISSION: CALCIUM, POTASSIUM, VERATRINE, GUANIDINE 503
J. Debecker, Brussels, Belgium

SUBSECTION IV

CLINICAL AND THERAPEUTIC STUDY

CHAPTER 18. DEPRESSANTS AND STIMULANTS OF NEUROMUSCULAR TRANSMISSION. A CLINICAL AND THERAPEUTIC STUDY IN ANESTHESIA 517
G. Vourc'h, Paris, France

CHAPTER 19. CLINICAL APPLICATIONS OF PERIPHERAL INHIBITORS AND STIMULANTS OF NEUROMUSCULAR TRANSMISSION 545
F. F. Foldes and D. Duncalf, New York, U.S.A.

CHAPTER 20. MYASTHENIA GRAVIS 561
K. E. Osserman, F. F. Foldes and G. Genkins, New York, U.S.A.

SUBSECTION V

CHAPTER 21. NEUROMUSCULAR TRANSMISSION IN ARTHROPODS 621
H. Grundfest, New York, U.S.A.

AUTHOR INDEX xiii

SUBJECT INDEX xxxv

CONTENTS OF VOLUME I

LIST OF CONTRIBUTORS ix

PREFACE xi

CHAPTER 1. INTRODUCTION 1
J. Cheymol, Paris, France

SUBSECTION I

THE NEUROMUSCULAR JUNCTION

CHAPTER 2. STRUCTURE AND CYTOCHEMICAL CHARACTERISTICS OF THE NEUROMUSCULAR JUNCTION 7
R. Couteaux, Paris, France

CHAPTER 3. PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF THE NORMAL NEUROMUSCULAR JUNCTION 57
J. I. Hubbard, Evanston, Ill., U.S.A.

CHAPTER 4. PHYSIOPATHOLOGY OF NEUROMUSCULAR TRANSMISSION 119
J. E. Desmedt, Brussels, Belgium

CHAPTER 5. POINTS OF IMPACT OF MODIFIERS OF NEUROMUSCULAR TRANSMISSION 135
J. Cheymol and F. Bourillet, Paris, France

SUBSECTION II

INHIBITORS OF THE NEUROMUSCULAR JUNCTION

A. NATURAL INHIBITORS

CHAPTER 6. VENOMS: THEIR INHIBITORY ACTION ON NEUROMUSCULAR TRANSMISSION 145
O. Vital-Brazil, Campinas, Sao Paulo, Brazil

CHAPTER 7. THE ACTION OF BOTULINUM TOXIN ON NEUROMUSCULAR TRANSMISSION 169
A. S. V. Burgen, London, U.K.

CHAPTER 8. TETRODOTOXIN AND SAXITOXIN 175
J. Cheymol and F. Bourillet, Paris, France

CHAPTER 9. HISTORY OF CURARE 187
A. R. McIntyre, Omaha, Nebraska, U.S.A.

CHAPTER 10. CHEMISTRY AND PHARMACOLOGY OF NATURAL CURARE COMPOUNDS 205
P. G. Waser, Zürich, Switzerland

B. SYNTHETIC INHIBITORS

CHAPTER 11. SYNTHETIC INHIBITORS OF NEUROMUSCULAR TRANSMISSION 243
D. Bovet, Rome, Italy

C. PHARMACODYNAMICS OF NEUROMUSCULAR BLOCKING AGENTS

CHAPTER 12. INHIBITORS OF POST-SYNAPTIC RECEPTORS 297
J. Cheymol and F. Bourillet, Paris, France

CHAPTER 13. INHIBITORS OF ACETYLCHOLINE SYNTHESIS 357
W. C. Bowman and I. G. Marshall, Glasgow, U.K.

CHAPTER 14. INHIBITOR ION: MAGNESIUM 391
Lise Engbaek, Copenhagen, Denmark

D. METABOLISM

CHAPTER 15. SYNTHETIC NEUROMUSCULAR BLOCKING AGENTS: ABSORPTION—DISTRIBUTION—METABOLISM—EXCRETION 409
G. Chagas, L. Sollero and G. Suarez-Kurtz, Rio de Janeiro, Brazil

AUTHOR INDEX xiii

SUBJECT INDEX xxxv

CHAPTER 16

ACETYLCHOLINE AND ANTICHOLINESTERASE DRUGS

W. C. Bowman and Sandra N. Webb

Glasgow

16.1. ACETYLCHOLINE

The mechanisms underlying the synthesis, storage and release of acetylcholine at the vertebrate neuromuscular junction, and the evidence pointing to its physiological role in transmitting the excitation wave from the membrane of the fine nerve terminals to that of the muscle fiber at this site, are comprehensively reviewed in the chapter by J. I. Hubbard in Subsection I of this Section. Hubbard has necessarily restricted his description of the actions of acetylcholine to those involved in the most widely accepted view of the physiological transmission process, namely, that acetylcholine, after being released from the nerve endings by nerve impulses, acts on the postjunctional membrane to excite the muscle fiber. Nevertheless, acetylcholine exerts additional actions at the neuromuscular junction, at least one of which—its action on motor nerve endings—has been incorporated into modified, but as yet inconclusively supported, theories of neuromuscular transmission (Koelle, 1962; Riker, 1960, 1966). This subsection is concerned with the *pharmacological* actions of acetylcholine on striated muscle and its associated structures, and an attempt has here been made to summarize all of its effects in this region.

The character of the response of vertebrate striated muscle to applied acetylcholine (or to substances with a similar action) is dependent on the area of each muscle fiber membrane that is highly sensitive to its action. The site of action of such substances on the muscle fiber membranes is mainly restricted to the region of the neuromuscular junctions and their immediate surroundings (Langley, 1907; Buchtal and Lindhard, 1937; Kuffler, 1943; Miledi, 1960b). Hence, the extent of the highly sensitive area of each fiber membrane is related to the number of nerve terminals

that impinge upon it. Some muscle fibers, for example, those of the slow-contracting rat soleus (Miledi and Zelená, 1966), are sensitive to acetylcholine over the whole of their lengths, exhibiting peaks of high sensitivity at the neuromuscular junctions and again, but of lower intensity, at the musculo-tendinous junctions. However, the maximal sensitivity at musculo-tendinous junctions is still several hundred times less than at neuromuscular junctions and, unless the effects of very large doses of acetylcholine are being studied, its action at sites other than the neuromuscular junction may be ignored. In other muscles, for example, the rat diaphragm (Miledi, 1960b) and extensor digitorum longus (Miledi and Zelená, 1966), sensitivity to acetylcholine is undetectable except in the region of the neuromuscular junctions.

Muscle fibers may be focally or multiply innervated. Each multiply innervated fiber is supplied by many small diameter myelinated motoneurones of the A gamma group which terminate on the muscle fiber surface as clusters of small grape-like ("en grappe") bodies (Tasaki and Mizutani, 1944; Günther, 1949; Couteaux, 1953 a and b, 1955; Ginsborg, 1960 a and b; Ginsborg and Mackay, 1961). The distance between adjacent junctions is at the most of the same order of magnitude as the space constant of the fiber. Focally innervated fibers are supplied by branches of large diameter alpha motoneurones which end in discrete ("en plaque") end-plates (Krause, 1863; Thanhoffer, 1892, 1894; Ginsborg, 1960 a and b; Hess, 1961 a and b; Ginsborg and Mackay, 1961). Focally innervated fibers may receive more than one nerve ending, but if this is so the distance between any two end-plates is very much greater than the distance between neuromuscular junctions on the densely multiply innervated fibers. Different muscles of the frog (Kuffler and Vaughan-Williams, 1953b; Csillick, 1965), domestic fowl and pigeon (Krüger and Günther, 1958; Ginsborg and Mackay, 1961; Hess, 1961 a and b, 1967; Page and Slater, 1965) contain different proportions of the two types of fiber. The iliofibularis muscle of the frog is composed of a large number of focally innervated fibers and a smaller number of multiply innervated fibers, the latter being grouped together in a compact bundle. Other frog muscles (e.g. rectus abdominis, gastrocnemius, semitendinosus) also contain a mixture of both types of fiber but neither type forms a compact bundle. The frog sartorius muscle, on the other hand, is largely composed of focally innervated fibers. In the domestic fowl, both focally and multiply innervated fibers are present in the biventer cervicis, semi-spinalis cervicis, gastrocnemius and sartorius muscles. The anterior latissimus dorsi of the domestic fowl and the latissimus dorsi of the pigeon are composed almost entirely

of multiply innervated fibers, whereas the posterior latissimus dorsi and the pectoral muscles of the fowl are composed mainly of focally innervated fibers.

Frog muscles now known to be composed of a predominance of multiply innervated fibers were shown to respond to excitation with a slow and maintained shortening, which was in contrast to the rapid and brief contractile response of muscles now known to be composed largely of focally innervated fibers (Sommerkamp, 1928; Wacholder and von Ledebur, 1930, 1931; Wacholder and Nothmann, 1932). The two types of fiber in the frog are described as slow or tonic fibers and fast, twitch, tetanic or phasic fibers respectively.

Nerve impulses in the γ-motoneurones innervating the tonic fibers of frog muscles evoke graded, relatively long-lasting junctional potentials which cannot give rise to propagated action potentials in the muscle fiber membranes (Kuffler and Gerard, 1947; Kuffler and Vaughan-Williams, 1953a). The mechanical response of these muscle fibers, like the electrical response, is confined to the junctional regions, but, because these junctional regions are densely scattered over a wide area, the non-propagated depolarizations can activate the contractile mechanism over most of its length. In contrast, the localized end-plate potentials evoked by nerve impulses in the α-motoneurones innervating a twitch fiber initiate propagating action potentials in the surrounding membrane, and these in turn activate the contractile mechanism (Kuffler and Vaughan-Williams, 1953 a and b, and see also the chapter by Hubbard in Subsection 1 of this Section).

The multiply innervated fibers in the fowl and the pigeon resemble the tonic fibers of frog muscles in that their contractile response to nerve impulses may be triggered by a non-propagated depolarization of the fiber membranes. However, unlike the tonic fibers of the frog, multiply-innervated avian muscle fibers are also capable of propagated electrical activity (Ginsborg and Mackay, 1960), and under certain *in vitro* conditions may even respond to a single nerve volley with a propagated twitch.

Most mammalian muscles contain only focally innervated fibers, but the four rectus and the two oblique extraocular muscles (Gerebtzoff, 1959; Kupfer, 1960; Hess, 1962; Hess and Pilar, 1963; Bach-y-Rita and Ito, 1966), the internal ear muscle (Erulkar and Szentagothai, quoted by Csillik, 1965) and the striated muscle in the oesophagus (Csillik, 1965) are exceptions in that many of their fibers are densely multiply innervated by small diameter γ-motoneurones with "en grappe" terminations, and they respond to nerve impulses with small junctional potentials which may not give rise to propagated action potentials. These muscle fibers, at

least those of the eye, resemble avian muscle fibers, but they differ from frog tonic fibers in that they are also capable of propagated electrical activity (Bach-y-Rita and Ito, 1966). The retractor bulbi muscle of the eye, at least in the cat, contains mainly focally innervated fibers (Bach-y-Rita and Ito, 1965).

In general, the two types of fiber differ in their ultra-structure (Krüger, 1950; Kuffler and Vaughan-Williams, 1953b; Krüger and Günther, 1958; Hess, 1961 a and b; Csillick, 1965; Page and Slater, 1965; Page, 1969). Fibers with focal innervation, usually, but not invariably, have a distinct fibrillar (Fibrillenstruktur) appearance under the light microscope owing to the regular arrangement and uniform width of the myofibrils which are discretely separated one from another by areas of sarcoplasm. Fibers with multiple innervation usually have a more granular and indefinite (Felderstruktur) appearance owing to the irregular width of the myofibrils and the fact that they are not discretely separated. In addition, the arrangement of the sarcoplasmic reticulum differs and this seems to be the main difference that determines the different contraction velocities; fibers with a Fibrillenstruktur contract more rapidly than fibers with a Felderstruktur. The sarcoplasmic reticulum is more regularly arranged in fibers with Fibrillenstruktur, and there are many more triads.

In birds the iris muscle is composed of striated fibers, and in the pigeon these have been shown to be multiply innervated yet to possess a Fibrillenstruktur (Hess, 1966, 1969). These fibers can respond either with a twitch to a single shock or by maintained depolarization and contracture to prolonged stimulation. In accordance with their Fibrillenstruktur their contraction velocity is rapid (Pilar and Vaughan, 1969 a and b).

Muscle fibers with a Felderstruktur have been identified in some other mammalian skeletal muscles (e.g. the diaphragms of rat and rabbit), and this type of structure may therefore be characteristic of the red slow-contracting muscle fibers (Krüger, 1950; Günther, 1952). Although the nerve fibers supplying these muscle fibers are thinner than those innervating the fast-contracting pale (Fibrillenstruktur) fibers (Haggqvist, 1938), they still belong to the alpha group and do not resemble the gamma motoneurones innervating frog tonic fibers. In fact, with the exceptions of the striated muscle fibers in the special sense organs and the viscera referred to above, all mammalian muscles studied, including all of the limb muscles in the cat (Brown and Matthews, 1960b), contain mainly focally innervated fibers, even though some of them (e.g. some of those in the gracilis muscle of the rat) may receive more than one nerve ending (Jarcho *et al.*, 1952; Hunt and Kuffler, 1954).

Thus the ability to respond to nerve impulses by non-propagated electrical activity is not a consequence of a Felderstruktur but rather of a dense multiple innervation, although in many instances these two properties go together.

Mammalian muscle spindles contain miniature muscle fibers (intrafusal fibers) which regulate the sensitivity of the afferent nerve endings to stretch (see Cooper, 1960). The intrafusal fibers are innervated by small, high-threshold myelinated fusimotor fibers (the γ-efferents), and all available evidence suggests that these fibers are cholinergic (for refs. see Smith, 1963). Two types of intrafusal fibers have been recognized; the smaller, innervated by γ_2 fibers with "en grappe" terminations, resemble the tonic fibers of the frog, and the larger, innervated by γ_1 fibers, appear similar to twitch fibers (Hunt and Kuffler, 1951; Boyd, 1958, 1959, 1962).

For further details of the patterns of innervation of striated muscles, articles by Tiegs (1953), Cole (1955), Couteaux (1958, 1960), Coers and Woolf (1959) and Koelle (1963) may be consulted.

16.1.1. FOCALLY INNERVATED MUSCLE FIBERS

Irregular contractions of muscle in response to intravenous or distant arterial injections of large doses of acetylcholine were described during the early 1930s (Feldberg and Minz, 1931; Feldberg, 1933; Simonart and Simonart, 1935; Simonart, 1935). The poor reproducibility of the effect and the large doses of acetylcholine required were taken by some as evidence against the hypothesis of cholinergic transmission from somatic motor nerve to striated muscle. However, soon afterwards techniques were described in which blood flow was temporarily restricted to a particular muscle of the cat and injections were then made directly into the arterial supply, close to the muscle (Brown *et al.*, 1936; Brown, 1937a, 1938). Using this technique of close-arterial injection, doses of acetylcholine as small as 2 mcg were found to produce muscle contractions of about the size of a maximal twitch (Brown *et al.*, 1936; Bacq and Brown, 1937). The apparent insensitivity of skeletal muscle, compared with smooth muscle, to a distant injection of acetylcholine is explained by the smallness of the area of a focally innervated striated muscle fiber membrane that is chemo-sensitive, and by the relative brevity of the mechanical responses of the individual fibers. For measurable tension to be produced, a large number of muscle fibers must be activated synchronously and this requires that an effective concentration of acetylcholine should reach many fibers simultaneously. For this reason, for a given dose, contractions produced

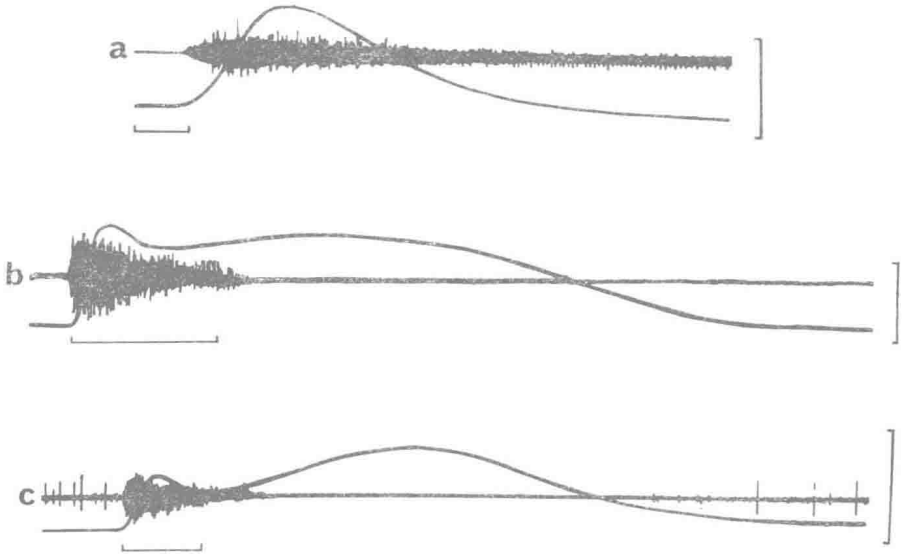

FIG. 1. Responses of skeletal muscles to close-arterially injected acetylcholine. In each response the upper trace is electrical activity recorded with a concentric needle electrode and the lower trace is isometric tension. (a) Normal tibialis anterior muscle of a cat under chloralose anesthesia; 5 mcg acetylcholine injected close-arterially. (b) Normal gastrocnemius muscle (lateral head) of a domestic fowl under chloralose anesthesia; 5 mcg acetylcholine injected close-arterially. (c) Chronically denervated (14 days) tibialis anterior muscle of a cat under chloralose anesthesia; 2 mcg acetylcholine injected close-arterially. Note the biphasic contraction-contracture response in (b) and (c). Vertical lines: 0.5 kg; horizontal lines: 20 msec in (a), 1.0 sec in (b) and (c).

by close-arterially injected acetylcholine are smaller, the greater the volume of injected solution and the more slowly it is injected (Dale *et al.*, 1936). Electrical recording shows (Fig. 1a) that the response of a muscle to close-arterially injected acetylcholine differs from a twitch evoked by a motor nerve impulse in that it is a brief asynchronous tetanus (Brown, 1937a). It is asynchronous because the acetylcholine, carried by the blood stream, reaches the muscle fibers one after the other instead of synchronously as it does when released from nerve terminals; it is repetitive because, no matter how quickly the injection is made, it is bound to last longer than the refractory period of the muscle fibers. Consequently, muscle fibers stimulated at the beginning may respond many more times as the injection continues. The magnitudes of effective intra-arterial doses of acetylcholine, used by various workers in experiments on skeletal muscle, vary over a

very wide range, and are largely dependent on the injection techniques used. Their absolute size is unimportant except that when very large doses are needed to produce an effect, complications may arise from additional effects produced by the choline set free by enzymatic hydrolysis. The actions of acetylcholine and choline are not identical in every respect (Hutter, 1952a; Blaber and Bowman, 1959, 1962b). Small doses of acetylcholine are effective when the close-arterial techniques of Dale *et al.* (1936) and Brown (1938) are followed, and the amount of choline set free from such doses is probably too small to exert additional actions.

The techniques of microelectrode recording and iontophoretic application of ionized drugs to the junctional region have allowed much greater insight into the action of acetylcholine. Iontophoretic release, onto the end-plate region, of amounts of acetylcholine as small as 10^{-17}–10^{-16} mole (about the amount released by a nerve impulse, according to Krnjević and Mitchell, 1961) produce a localized depolarization (Krnjević and Miledi, 1958) which, in its graded, non-propagated nature and ability to initiate a propagating action potential (Nastuk, 1951; del Castillo and Katz, 1955) resembles the end-plate potential evoked by a nerve impulse (Gopfert and Schaefer, 1937; Eccles *et al.*, 1941 a and b, 1942; Eccles and Macfarlane, 1949).

Acetylcholine depolarizes the muscle fiber membrane in the end-plate region by causing a localized increase in the membrane permeability to Na^+ and K^+ ions and, to a lesser extent, to Ca^{++} ions (Fatt and Katz, 1951; del Castillo and Katz, 1954; Takeuchi and Takeuchi, 1960). The receptor sites with which acetylcholine reacts are situated on the outside of the muscle fiber membrane, since intracellular application of acetylcholine from a micropipette does not produce a depolarization (del Castillo and Katz, 1955).

Only fragmentary knowledge of the nature of the acetylcholine receptor is available. It is likely to be polypeptide or protein (Gill, 1965), and this is supported by the demonstrated presence, near the active site, of a disulphide bond, reduction of which leads to a reversible decrease in sensitivity to acetylcholine (Turpajev *et al.*, 1963). [It is of interest that hexamethonium depolarizes this reduced receptor although it is an antagonist at the normal one (Karlin and Winnik, 1968).]

The phosphoryl groups of phosphatidyl ethanolamine or phosphatidyl serine may be components, as suggested by the nature of the pH dependence of the sensitivity of single chronically denervated muscle fibres to acetylcholine (Sokoll *et al.*, 1968). It can be deduced, from the structures of potent stimulant and blocking drugs, that the receptor includes an

anionic site, and the fact that uranyl ions, which have a high affinity for phosphate groups, compete with acetylcholine at the receptor, suggests that the anionic group could be phosphate (Nastuk, 1967).

Clearly there must be close similarities between the postjunctional acetylcholine receptor that mediates depolarization, and the receptor sites on acetylcholinesterase that mediate hydrolysis, and it has been suggested that in fact both may be functions of the same protein (Roepke, 1937; Wurzel, 1959, 1967; Župančič, 1963, 1967; Belleau, 1964; Changeux, 1966; Ehrenpreis, 1967). Although such a unified hypothesis has a number of attractive attributes, it remains difficult to explain the fact that some acetylcholine receptors may be detected in the region of the normal endplate outside the area in which cholinesterase is located. Some workers (Karlin, 1967; Podleski, 1967) claim that there is a chemical distinction between the two receptors and that they must therefore be separate proteins, as was originally assumed to be the case. The matter remains an open question (Župančič, 1969; Belleau, 1970).

Prolonged high-frequency nerve stimulation, or the application of acetylcholine after inhibition of cholinesterase, produces a disorientation of the regular longitudinal arrangement of protein particles, accompanied by a rearrangement of lipids, in the muscle fiber membrane at the junctional region. These changes, which have been demonstrated by polarization microscopical examination and imbibition experiments (Csillik, 1965), are prevented by tubocurarine. It seems likely that these demonstrable molecular alterations in the postjunctional membrane are those responsible for the increased ionic permeability occurring in the presence of acetylcholine. Drugs that depolarize the junctional region of the muscle fiber membrane have also been shown, by histochemical techniques, to release free (ionized) calcium in the postjunctional cytoplasm of the endplate region (Csillik, 1965).

The fall in membrane potential induced by acetylcholine at the junctional region of a muscle fiber causes this part of the membrane to act as a current sink for adjacent parts of the muscle fiber membrane. When the end-plate depolarization reaches a critical level (10–20 mV in mammalian muscles, Boyd and Martin, 1956b; Liley, 1956a) the local currents set up increase the conductance of the surrounding membrane, initially for Na^+ ions, and subsequently for K^+ ions, so that an action potential propagates in all directions away from the end-plate region around the muscle fiber membrane. The propagating action potential in the muscle fiber membrane activates the contractile mechanism and contraction results (for further details and references see Hubbard's chapter in Subsection 1 of this Section).