PHARMACOLOGY OF THE NEUROMUSCULAR JUNCTION

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This paper, rather than attempting to be an inclusive review, will be confined to several problems which seem relevant to many current studies. Such an approach, although it omits a number of important contributions, seems timely, since many aspects of the pharmacology of the neuromuscular (n-m), system have recently been considered in several excellent reviews (51, 78, 105, 126, 137, 142, 162, 168). Specific problems relating to clinical, pharmacological, and electrical aspects have been covered in a recent symposium (1, 85, 115). Furthermore, the discussion will be biased towards electro-physiological studies relating to skeletal muscle.

Studies of the pharmacology of the n-m junction and progress in the physiology of n-m transmission, and of synaptic transmission in general, are closely linked. Indeed, the first physiological demonstration of the n-m junction dates from the studies on curare by Claude Bernard. His classical experiments showed that this agent blocked the transmission of impulses from nerve to muscle without apparent effects on either the nerve trunk or the muscle fibers themselves. Then Langley's experiments showed that nicotine caused twitch responses of muscle only when applied to specific areas (122). Later, evidence indicated a chemical transmitter in parasympathetic neuroeffector systems, particularly that obtained by Loewi and by Dale. The productive work in this field by Brown, Dale, Feldberg and others, pointed to a chemical mediator for n-m transmission in skeletal muscle. Interest in clinical studies of nerve-muscle disorders such as myasthenia and myotonia provided a further stimulus to investigations of the nerve-muscle system. A more detailed knowledge of events at the n-m junction during activity, however, was still needed. Investigations of the potential changes at the junction led to the detection of the endplate potential (e.p.p.) which provided a link between the nerve impulse and the muscle impulse (55, 71). Studies on the e.p.p. are only gradually being combined with other more established methods of investigation of n-m transmission, although the recording technics are relatively simple.

EVENTS OF THE NEUROMUSCULAR JUNCTION

Even fifteen years ago little was known about the mechanism by which a nerve impulse affects the post-junctional muscle region and there causes a propagated muscle impulse. It was widely assumed that the transmission process was caused in some way by the action currents of the nerve impulse. Later, considerable evidence was advanced that a chemical transmitter was involved, probably identical to acetylcholine (ACh). When the motor nerve to a perfused muscle

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was stimulated, there appeared in the perfusate a substance which had the pharmacologic characteristics of ACh (46). Further, when ACh was injected into the arterial supply close to the muscle, a contractile response consisting of a brief tetanus developed (22, 27). The effects of inhibitors of cholinesterase (ChE) on the response of muscle, stimulated through its nerve, were advanced as additional evidence. There remained difficulties in closer identification of processes and of their time course and sequence in transmission. In a complex event like transmission, a substance which appears in a perfusate may play a role in many of the phases of excitation or recovery. Studies on the e.p.p. clarified many of these points. These potentials are set up by every nerve impulse on reaching the junction and are recorded as a focal depolarization of the post-junctional muscle membrane. When this depolarization reaches a critical level, it sets up propagated impulses in the muscle membrane surrounding the junction. The e.p.p.'s in normal muscle are largely obscured by these impulses but can be made evident when n-m block is produced by curarization or fatigue. Then they can be detected in a reduced form, strictly localized to the junctional region and decrementing spatially along the muscle fibers in a characteristic fashion, depending on the electrical time constant of the tissue. The e.p.p.'s of curarized muscle have been studied most extensively (52, 53, 71). They rise to a peak in somewhat over 2 msec. in the frog sartorius at 20° C., and have a total duration of about 10–15 msec., while in the cat these values are approximately half. It became clear that the e.p.p. represents the electrical sign of the events which are set up by the nerve impulse and link it to the subsequent muscle impulse. Processes underlying and giving rise to the e.p.p. may be called the "transmitter" action of the nerve impulse. Analyses of the time course and intensity of this action can be made with fair accuracy and are independent of the actual nature of the "transmitter." They are based on simple assumptions, namely, that the "transmitter" intensity is approximately proportional to the rate of rise and degree of depolarization at the endplate and that its duration determines the length of the potential change which it sets up. According to calculations based on such assumptions, the "transmitter" effect at the junction rises to its maximal intensity in about 2 msec. and then gradually decays in a further 3 msec. in frog at 20° C. (52, 53). This could be shown more directly in experiments where a nerve impulse was fired into the junction at a time when that region was "occupied" by a muscle impulse which had been set up "antidromically" elsewhere. The nerve impulse, although it had arrived at the endplate region, was then quite ineffective for a period up to 1 msec. However, as the endplate region recovered from the antidromic muscle impulse traversing it, the effect of the nerve impulse became evident, and it built up a potential change of its own. This type of experiment demonstrates then that the "agent" or "transmitter" can survive for certain periods after its production. It also indicates that the membrane at the "endplate" and the muscle membrane is physiologically continuous (see later). The "transmitter" survival time could be shortened by agents like curare or lengthened by anticholinesterases (see below). Effects which are analogous to the "transmitter" action could also be produced artificially by applied drugs and current pulses under well-

controlled conditions (for details see 108, 109). When the motor nerve is stimulated, even within a few hundred micra of the endplate, a minimal latency is always observed (about 0.5 msec.) before the onset of the e.p.p.; this delay has been regarded as one of the arguments against the action current nature of transmission (115). The latency is unchanged by curare or anticholinesterases. When recording is done from multifiber preparations, change in latency of the onset of the muscle action potential may be observed with the addition of curare. This increased latency results from the difficulty of recording from the exact endplate site (107).

The present picture of the sequence of events at the n-m junction is as follows: nerve impulse → "transmitter" → e.p.p. → muscle impulse. The latter then initiates the activation of the contractile processes as it passes along the muscle fiber. In terms of membrane changes the excitation process between "endplate" and surrounding muscle fiber is now known. The e.p.p. depolarizes the region around the junction and thus sets up the muscle impulse when a critical potential level is reached (52, 107). If the transmitter action has been reduced, for instance by fatigue or by curarization, then the critical depolarization for muscle fiber excitation will not be reached and an e.p.p. alone, without a muscle impulse (and twitch) will be detected at the junction. Before such paralysis is reached, however, the e.p.p. may be reduced to about $\frac{1}{3}$ of its original height. Because of this safety factor, the effects of sub-paralytic doses of drugs like curare can be detected electrically in single units well before block occurs. E.p.p.'s in paralyzed muscles, if set up at appropriate intervals, can sum and attain the critical level of depolarization at which transmission is restored.

While the endplate region of the muscle fiber shows a striking specific excitability to applied drugs like ACh or nicotine, there is no detectable difference in electrical excitability between the endplate and other regions of the muscle (112). It is possible that the lizard is different in this respect (33).

The primary question in the transmission studies now is not how the muscle impulse is set up, but how the nerve impulse gives rise to the transmitter and what its nature is. Most of all one wants to know the events that take place between the arrival of the nerve impulse at the terminals and the setting up of the e.p.p. The main obstacle in this direction is our scant knowledge of the activity in the nerve terminals. Since there seems to be good evidence that this is the location where the transmitter arises and not in the post-junctional region of the endplate (52), progress in this direction is particularly desirable. At present it is often assumed that the activity in the nerve fibers and terminals is unchanged during most drug effects on the junction. This may be questionable in many instances (see below).

ACTION OF DRUGS ON THE NEUROMUSCULAR JUNCTION

Anticholinesterases: Studies of the effects of cholinesterase inhibitors, particularly eserine, provided some of the earliest evidence for the existence of a cholinergic mediator at n–m junctions (27, 44, 62). By the use of mechanical recording methods, it has been shown that the principal effects of anticholine-

sterases are to: (1) cause an increase in the height of the twitch response to a single nerve stimulus, the increased response being due to repetitive firing of the muscle; (2) increase the depression of response to high frequency stimulation of the nerve; and (3) alter the response to injected acetylcholine. In contrast to the above, the response to direct stimulation of the muscle is not altered. These effects have been found for a variety of cholinesterase inhibitors of divergent chemical structure such as a number of carbamates (8) and fluorophosphates (26, 41, 91, 147). Since the enzyme inhibition produced by a given anticholinesterase varies according to the particular tissue esterase studied, attempts to relate pharmacologic effect and antiesterase potency have more meaning if the inhibition is tested on the cholinesterase involved. Muscle cholinesterase has been found most concentrated in the endplate containing regions of muscle (135) and recent histochemical studies have shown a striking localization of this enzyme at the junction (104). Of further interest is the ability of cholinesterase inhibitors to correct the functional defect in transmission occurring in myasthenia gravis (85).

With progress in electrical recording technics the actions of anticholinesterases were studied on the e.p.p. (53, 66). The latter, reflecting the activity of the "transmitter," should be changed in a characteristic fashion according to the ACh hypothesis. In keeping with the view that the destruction of ACh is slowed or prevented, the antiesterases were found to lengthen the depolarizing action of the transmitter. After eserine, repetitive nerve stimulation caused junctional potential changes which could last for seconds. It seems most likely that in muscles treated with anticholinesterase the delay in decay of the e.p.p. is largely due to a small remainder of the transmitting agent, lingering on and supporting the depolarization of the junction. In curarized muscle the anticholinesterases also increased the time of rise and the magnitude of the e.p.p. and hence increased the intensity of the transmitter action, thereby acting as decurarizing agents. These findings alone show strikingly the usefulness of such an analysis, since they localize the site of action and give evidence of the time sequence of the processes affected by the anticholinesterases. If, for example, anticholinesterases did not act during the early stage of the e.p.p. time course, when the muscle impulse is set up, one might conclude that ACh was not concerned with the normal process of excitation, since the ACh hypothesis, as generally proposed, implies that the whole e.p.p. is set up by the liberated substance. The effects of prolonged and increased junctional potentials of eserinized muscle show a striking resemblance to the actions of applied catelectrotonic potentials which may either excite and produce repetitive muscle discharges, or, if strong enough, eventually lead to depression and block of transmission (53). This would explain the dual and opposite effects produced by eserine as noted by many workers. The action of eserine and other anticholinesterases appears to be confined to the nerve endings and endplate, since in concentrations sufficient to modify junctional transmission, conduction in muscle and nerve is not affected. Discharges from nerve terminals during eserine action in the cat are now well established (53, 66, 136). That the above actions of anticholinesterases are due to inhibition

of cholinesterase, rather than some adventitious effect, is shown by a recent study of Eccles and MacFarlane in which seven cholinesterase inhibitors (neostigmine and three quaternary ammonium analogs, two tertiary amines, and di-isopropyl fluorophosphate) were tested on the e.p.p. of curarized frog muscle (54). As judged by several criteria, all these inhibitors produced virtually identical changes on the e.p.p. and its slow component when the concentrations were suitably adjusted. Data are not available on the relative potency of these compounds in inhibiting frog muscle cholinesterase.

In studies involving anticholinesterases, adventitious effects must be considered before the data can be interpreted as referring to the ACh system alone. Certain quaternary ammonium compounds, in addition to inhibiting cholinesterase, may have an additional excitatory effect on muscle as described by Riker and Wescoe for neostigmine (152) and an interesting neostigmine analog (152a).

In *crustaceans*, the effect of anticholinesterases on the neuromuscular junction is either not striking or absent (57, 171), and this is also true for synaptic transmission in the central nervous system (155, 156).

Acetylcholine: ACh is capable of exciting the endplate region of muscle, as are a number of other choline esters, nicotine and onium salts. ACh is effective in very small concentrations, and the application of 1×10^{-6} to frog muscle or the intra-arterial injection of as little as 1 μgm to cat muscle causes a train of muscle discharges (22, 27, 33). There is now agreement on the point that ACh, when injected intra-arterially in humans, causes similar motor responses (discussed by Acheson *et al.*, 2). ACh is, however, practically ineffective on the muscle fiber proper even at concentrations 1000 times threshold for the endplate (109). Accordingly the depolarizing effect of ACh on muscle detected by Cowan (43) has been shown to be confined to the endplates. The lack of muscle depolarization has its parallel in the peripheral axons of frogs which are not appreciably affected by as much as 1% ACh (128). Langley long ago noted the specificity of receptive areas of muscle in the response to nicotine (122). When recording is made from the endplate region, the application of ACh is seen to produce a depolarization which, if sufficiently intense, excites a muscle impulse. The latter causes a collapse of the depolarization, but this is built up again by the continued action of ACh. In such a manner a train of muscle impulses results. However, in spite of the persisting depolarization the muscle impulses soon fail to be set up, some adaptive processes having occurred (109). In contrast, muscles which respond to the depolarization by ACh with a local contraction, such as those innervated by the small-nerve system in the frog, show a continued activation of the contractile elements when ACh is applied (see below). In innervated mammalian muscles, contractures to injected ACh developed after chronic reduction of ChE, and to a smaller extent after acute administration of anticholinesterases (26, 92).

Following the application of ACh, in particular during the effect of an anticholinesterase, a block of junctional transmission occurs. The muscle fails to respond first to repeated addition of ACh and then to nerve stimulation. This block may be due at least in part to excessive reduction in the resting potential

at the junctional region, thus acting similarly to a strong applied catelectrotonic potential (see also contracture). It is puzzling that when the membrane resting potential at the endplate region is reduced by a strong background concentration of ACh a nerve impulse can still produce a relatively large e.p.p., in theory by the liberation of further ACh (68).

In *crustaceans*, ACh appears to have a negligible effect on the n-m junction (see below) although it does have an accelerating action on the decapod crustacean heart and inhibits the molluscan heart. These aspects have recently been reviewed by Prosser (146) and by Welsh and Schallek (167).

Curare: The interest in curare and curare-like compounds has greatly increased during the past few years. This was undoubtedly due to a large extent to its introduction into clinical use following the availability of standardized preparations. It is used in conjunction with anaesthesia, in shock treatment, for "spasticity," in states of increased excitability, etc.

The effects of curare and n-m block have so long been associated that the word *curarizing* has been applied to a variety of agents causing junctional block. This is undesirable in that it implies a mechanism of action similar to that of curare. Prior to 1935 little was known of the composition of the active principles of curare except that quaternary ammonium compounds were involved. Since that time important advances have been made in the understanding of the chemical structure of the curare alkaloids, particularly by King and others (101, 102). The active substances have been shown to be bis-quaternary ammonium benzylisoquinoline structures, some of which exhibit optical isomerism. *d*-Tubocurarine may be considered as representative of the group. This material is commercially available in crystalline form and is the active principle of the standardized preparation "Intocostrin." Its optical isomer has considerably less paralytic potency (103). Prior to the availability of pure compounds many studies were made on curare of unknown composition, and some of the older observations appear to have been due to impurities. Among these was the evidence that curare inhibited cholinesterase (81); crystalline *d*-tubocurarine (132) and King's curarine (53) lack such inhibitory properties. For further and better treatment of the curare problem, consult Paton (142); also Craig's extensive review (45), and the text by McIntyre (131).

In physiological investigations on curare-like compounds it is necessary to separate the effects on: (i) completely isolated preparations, like those on frog muscle where the drug reaches the n-m junctions by diffusion, in which the simplest conditions for evaluating the curare action are obtained; (ii) circulated nerve-muscle preparations with the nerve cut; and (iii) preparations with intact central connections.

It appears from most studies that the nerve-muscle twitch system in frogs and mammals is, on the whole, comparable (see, however, the small-nerve system), and for the time being it seems justified to transfer conclusions from one to the other. Circulatory effects should be kept in mind (see below).

Several preparations of curare have been found to have no effect on the electrical excitability of the nerve axon or of the frog twitch-producing muscle fiber

(77, 112, 154), and curarine was found to have no appreciable effect on the membrane resting potential of normal muscle (43, 109).

The principal actions of curare on the junctional processes can be observed on the e.p.p., particularly in single nerve-muscle fiber preparations in frogs, or the "strip" preparation in the cat (55). The earliest curare effects comprise the reduction of the late portion of the e.p.p. and a gradual depression of its size. When the e.p.p. is reduced to about 30% of normal, it fails to set up muscle impulses. Thus subthreshold effects of curare can be detected electrically before any paralysis is evident. Curare has no effect on the latency of the e.p.p. which may be interpreted as indicating that conduction in the terminals as well as axonal conduction is not affected (107).

When the e.p.p. is reduced below the level causing excitation of the muscle fiber, it may sum with a second e.p.p. to attain the threshold for setting up a muscle impulse. This appears to be the basis for the well-known facilitation effect in curarized muscle described by Bremer (16). If a second stimulus is given outside the nerve refractory period but not late enough to fire the same muscle fibers twice, then it will excite some or all of the muscle fibers which received subthreshold excitation from the first stimulus. Such a test will usually reveal the existence of partial n-m block but not impairment of transmission which is caused by block along the axon or nerve terminals. A difference, affecting the facilitation phenomena, between frog and cat preparations, is the endplate response to a second nerve impulse. A second frog e.p.p. is larger than the first when the nerve impulse reaches the endplate within about 50 msec. The cat's second e.p.p., however, is reduced for several seconds (52). Accordingly, facilitation phenomena may vary.

The effects of curare can be accounted for by its action on the post-junctional membrane to reduce or prevent the depolarization from the transmitter effect of a nerve impulse.

A striking analogy to the e.p.p.-curarine relationship exists in the antagonism between curarine and ACh. Cowan (43) detected that curarine tends to reverse the ACh depolarization of muscle, and, since the latter is confined to the endplate (109), the two drugs act on the same specialized junctional region in an antagonistic fashion. Curarine, however, always prevails in this competitive set-up, since it can prevent or reverse the ACh effect if given in sufficient concentration. The analogy to the curare-e.p.p. relationship is even closer when ACh is directly applied to the endplate, where it sets up membrane changes resembling those produced by the nerve impulse. By suitably adjusting the curarine-ACh concentrations, excitation can be made subthreshold, just threshold, or superthreshold (109). A similar relationship also exists between nicotine and curarine and probably many other agents of different chemical structure (122).

The paralytic action of curare is antagonized by any agent which (i) increases and prolongs the e.p.p.; anticholinesterases act in this manner as also does guanidine which, however, only augments the e.p.p. size without affecting its time course (49, also unpublished); (ii) increases the muscle or endplate ex-

citablity. This latter effect may be obtained with many drugs which depolarize the membrane partially so that the e.p.p. may sum with the existing depolarization. Stronger depolarization will have the opposite effect, as can be seen with ACh or K^+ (see below). An anticurare action of applied currents, clearly due to summation of the e.p.p. and a catelectrotonic potential so that excitation threshold is attained, was described by Katz (97) before the junctional potential changes were known. An additional mechanism for an anticurare action is suggested by recent studies on congo red and related dyes (99). These azo dyes appear to combine chemically with curare and it is likely that their antagonism is effected in this manner. It is apparent that anticurare effects need not necessarily involve the ACh mechanism. However, with certain series of compounds a high degree of correlation does exist between anticurare effect and inhibitory action on "specific" cholinesterase (12).

d-Tubocurarine and allied preparations have been observed to block synaptic transmission in central sensory pathways and to diminish or abolish spontaneous cortical activity (141, 145). It appears, however, that doses which block n-m transmission are practically ineffective on the central nervous system. A striking and extraordinary observation on a human subject by Smith, Brown, Toman and Goodman (159) showed that there is no significant alteration of the conscious state or sensory perception during complete n-m paralysis. Apparent depression of spinal reflex arcs by curare in dogs has been shown to be due to circulatory changes (7); when hypotension following the rapid injection of curare was avoided, no change in synaptic function was observed.

A striking effect of curare on "tonus" was demonstrated some years ago by Bremer, Titeca and Vandermeiren (18). In this connection the possibility of an effect of curare on the proprioceptive spindle mechanism should be kept in mind. The cat's lumbosacral ventral root outflow contains about 25–30% of small diameter fibers which innervate the muscular elements of spindles (intrafusal muscle fibers) and not the twitch-producing extrafusal muscle fibers. Efferent small-nerve activity regulates, in conjunction with external stretch, the discharges from proprioceptors and presumably plays a role in the reflex mechanism serving posture (124, and unpublished results; for preliminary report see 118). The possibility of some difference in drug effects on this apparatus and the twitch system exists. Curare also blocks sympathetic ganglia in relatively high concentrations. Electrical events at the ganglionic synapses during block and after administration of eserine show many striking parallels to those at the n-m junction (50).

The effects of curare and ACh on the *crustacean neuromuscular junction* seem to be negligible, according to several authors (57, 96). Wright (171), however, reports that the crustacean n-m system is less immune to block by dihydro-beta-erythroidin and *d*-tubocurarine than had been assumed. The blocking action of these drugs was not antagonized by neostigmine. The peripheral inhibitory system in crab and crayfish shows a good parallel for the liberation of a curare-like substance. The inhibitory impulses can act on the muscle membrane without changing its potential, but prevent the excitatory effects of impinging motor im-

pulses. This shows a marked similarity to the action of curare in vertebrates, which, without changing the membrane polarization, prevents the excitatory effects of ACh. While these two substances do not play a significant role in the crustacean mechanism, some similar agents may do so. Another effect of the inhibitory impulses is their ability to interfere between the processes linking the membrane and contractile system (119, 134). On the whole it is difficult to compare the drug action in vertebrates and arthropods. Comparative studies are rare, and one feels that further development in arthropod, particularly crustacean, pharmacology would be of considerable general value since these species show a great number of interesting phenomena of excitation and inhibition at various synapses. Some notable effects of protecting crayfish synapses against drug action, particularly nicotine, have recently been described by Wiersma and Schallek (169).

Curare produces a number of important side-effects which are apparently due to the release of histamine. Anrep *et al.* (5) found that curare and curarine chloride caused a release of histamine from skeletal muscle as well as from heart, but not from lung. After such release the histamine content of the muscle was diminished, an effect which also occurred in chronically denervated muscle. This histamine release has no obvious relationship to the paralytic action of curare, but the observed side-actions of curare, such as fall in blood pressure, skin reactions and bronchospasm, can be so explained. Accordingly antihistamine agents can prevent certain vascular effects following curare (75). In evaluation of the actions of curare on the central nervous system such effects must be considered.

Other Quaternary Ammonium Compounds: While the testing of large numbers of compounds has been of considerable practical importance, sufficient data are not yet available to relate effects on n-m transmission to specific chemical properties. Several difficulties are involved in such analyses, one of the most important being the lack of information as to the physical-chemical properties of many of the agents tested. Another difficulty is the complexity of the test situation. Even in isolated tissues the relative potencies in a homologous series may be dependent on physical differences such as diffusion, dissociation, etc., rather than upon a difference in the reaction of the "receptor" itself. In the intact animal the complexities are multiplied, and such factors as distribution, metabolism, excretion and other effects of the compound come into play. This, of course, does not lessen the value of the data so obtained, but requires caution in their interpretation in relation to the behavior of the "receptor." Furthermore, with many of these compounds the exact site of action, *i.e.*, terminals, post-junctional membrane or muscle membrane proper, is not known. This information would be particularly important in considering the interaction of more than one agent on n-m transmission. Also, closer studies reveal more and more pharmacologic differences between muscles of various species and between "striated" muscle components of the same animal, such as frog small-nerve and twitch systems (see below), thus making interpretation of structure-activity relationship more difficult.

Several reviews (45, 142) and discussions (94, 144) of onium compounds have

recently appeared, and Ing's earlier paper (93) still serves as a valuable treatise covering the older literature (see also 148). The clarification of the structure of the active principles of curare (101, 102), as bis-quaternary ammonium benzylisoquinolines, led to the synthesis of various bis-quaternary ammonium compounds by several workers. The erythrina compounds have also received attention (123) and are of interest as tertiary amines causing n-m block. The structure-activity relationship of cholinesterase inhibitors has recently been considered by Koelle and Gilman (105). The present review is limited to a discussion of a few of the newer developments in this field.

Barlow and Ing (9) and Paton and Zaimis (143) have studied a number of polymethylene bis-quaternary ammonium salts. Among the most interesting of these is 1,10 bis-trimethylammonium decane (C10) which is highly potent in causing n-m block. The resultant paralysis is not antagonized by anticholinesterase agents but is antagonized by a lower homolog of the series 1,5 bis-trimethylammonium pentane (C5). C10 also causes a contracture of the frog's rectus which is prevented by the prior application of C5. Studies on this series indicate a maximal paralytic effect on muscle when the chain length is 10 (C10), while the greatest blocking action on sympathetic ganglia occurs with a chain length of 5 (C5). C10 was also reported to reduce the demarcation potential of cat skeletal muscle and to cause an initial contractile response on intraarterial injection (32). These findings indicate a qualitative difference between C10 and curare effects.

Bovet and associates (13) have studied the n-m blocking effects of a number of synthetic aromatic bis-quaternary ammonium compounds. The paralyzing dose, head drop dose and duration of action were compared among members of homologous series. Several compounds were produced whose potency was near that of *d*-tubocurarine. In compounds where symmetrical quaternary ammonium aromatic groups were linked by a polymethylene chain, the chain length influenced the potency of the compound. Thus, in several bis-quinolinepolymethylene series maximal activity occurred when the chain length was 5. Substitution of trimethyl ammonium ethyl ethers on a benzene ring yielded the mono or 1,3 diether which had the same paralytic potency (4 mgm./kgm. rabbit). In contrast, substitution of triethyl ammonium ethyl ethers on a benzene ring gave compounds of the following paralytic potency (rabbit): monoether, 50 mgm./kgm.; 1,3 diether, 1 mgm./kgm.; and 1,2,3 triether, 0.5 mgm./kgm. The same chemicals were also studied by Depierre (see 35) who found the n-m blocking potency to increase in the order of mono-, di- and tri-substituted compounds. The effectiveness in blocking sympathetic ganglionic transmission was in the inverse order, *i.e.*, the mono-substituted compound was the most potent.

If one assumes that these compounds have the same site of action as curare, it appears that the spatial configuration and distance between the two nitrogen groups can be one determinant in the reaction of such compounds with the receptor. This certainly is a feature in some homologous series. However, other factors certainly influence their activity, such as variations in the cationic group itself. One of the most puzzling phenomena is the striking difference in potency

between *d*- and *l*-tubocurarine (103). Attempts have been made to relate activity to intramolecular distances between specific groupings, as in tubocurarine and certain diatropine derivatives (100); these attempts are bound to uncover exceptions.

While the onium compounds, in particular the quaternary ammonium salts, show stimulatory and/or depressant effects on n-m transmission, certain alkaloids containing tertiary nitrogen groups are also very potent. Among the latter are nicotine, and the erythrina alkaloids, the potency of which is actually reduced by converting them to quaternary nitrogen compounds (45).

It is not clear whether a number of onium compounds cause n-m block by depolarizing the post-junctional membrane, as does ACh, and/or act like curare in preventing the depolarization caused by the nerve impulse or applied drugs. The possibility of effects on other aspects of n-m transmission, such as conduction in terminals, etc., must also be considered.

Procaine: Most work on procaine and allied compounds relates to nerve conduction, some to muscle and less to n-m transmission. It has been shown by Bishop (11), and later by Bennett and Chinburg (10), that nerve conduction can be blocked without changing the resting potential and the same is true of muscle (114). Procaine seems to have an ill-defined "stabilizing" or "freezing" action on the membrane. This action may be due to an increased muscle membrane resistance, as found by Guttman (79) with several other narcotics such as iso-amyl carbamate. High concentrations, presumably toxic, decreased the resistance. The electric time constant of muscle, as measured by relatively long current pulses, is not appreciably affected by procaine, and a constant current or agents like KCl have a similar depolarizing action before and after this drug. It is mainly the quick component of the action potential associated with the "breakdown" that is impeded by procaine. The selective blocking of nerves of small diameter by cocaine and procaine is well known (70), a fact which has been used to abolish the small-nerve effect in frogs (see below; 117).

Feng studied the procaine action on n-m transmission in frog and found that a block occurred while the nerve impulse still set up a relatively large and prolonged e.p.p. (66). The fact that the e.p.p. still occurs implies that the nerve or its terminals are not blocked. It seems that block of transmission is largely due to an increased threshold of the muscle fibers for propagated impulses. In curarized muscles procaine prolongs the e.p.p., not unlike eserine, a fact which is obscure at present; however, the drug may inhibit ChE (48). At this stage addition of eserine, in such concentration as would be effective by itself, does not further prolong the e.p.p. (unpublished observations). Procaine greatly reduces the endplate response to applied ACh and also prolongs the nerve refractory period.

In the cat Harvey (83) produced n-m block with procaine without appreciably changing the contraction of muscle to direct stimulation. At the same time ACh sensitivity was reduced, indicating an effect on the post-junctional membrane. He also found that a short tetanus, by itself hardly effective, could restore transmission for a subsequent nerve impulse, an effect seen also after curare (14).

Cocaine may also increase the muscle response in certain stages of fatigue (129). In the superior cervical ganglion, Harvey also measured the ACh output during progressive block and found it reduced. Recording of synaptic potentials in such preparations would give significant information.

Botulinus: One of the most interesting substances causing n-m block is botulinus toxin. Guyton and McDonald (80) working on guinea pigs found that this toxin, while producing n-m block, did not interfere with nerve conduction and left the muscle excitable to direct stimulation. In a recent report Burgen, Dickens and Zatman (36) studied effects of botulinus toxin on the isolated rat diaphragm and found an irreversible paralysis to indirect stimulation which developed slowly and progressed in spite of removal of the toxin from the bath. During partial paralysis to nerve stimulation the remaining units showed a normal tension response to a nerve tetanus, and no facilitation was observed with closely spaced stimuli. These observations are in contrast to curare effects. The response to direct muscle stimulation remained unimpaired, and even when the muscle was completely inexcitable to indirect stimulation, the contractile response to the intravascular injection of ACh showed no diminution from the control. Addition of immune serum to the bath, prior to the toxin, prevented the paralysis, and preparations from immunized rats showed a striking resistance to botulinus effects. Estimation of ACh, following nerve stimulation, showed a marked reduction in paralyzed muscles. In contrast to a previous report, no influence of botulinus toxin on choline acetylase or on cholinesterase was found. Botulinus toxin appears to affect either conduction in the nerve terminals and/or production of transmitter, and does not seem to act on the post-junctional muscle membrane. The absence of facilitation in either case is surprising.

Quinine: Interest in the effects of quinine on n-m transmission and on muscle largely dates from clinical observations that this compound relieves the symptoms of myotonia (30, 170). Certain earlier contradictory observations about quinine's effect on the contractile response to nerve stimulation were clarified by later studies of Harvey (82), Oester and Maaske (139) and Ravin (149). The main effect of the drug is to increase the tension response of muscle stimulated by single shocks to its nerve. This is accompanied by an increase in the amplitude and duration of the muscle action potential, suggesting that the speed of muscle conduction is slowed (82). Similar effects follow direct stimulation of curarized and denervated muscle. In contrast, when the nerve is stimulated tetanically, a pronounced decrease of the tension response results particularly at higher frequencies. As the dose of quinine is increased the tension decline occurs at progressively lower frequencies (82, 149). Further, the refractory period of the muscle is lengthened as tested by two closely spaced nerve stimuli (139), and also a decrease in the electrical excitability of the muscle is observed (82). Quinine depresses the response of muscle to ACh and in a partially curarized muscle it causes a further decrease in the response to nerve stimulation. This has been interpreted as indicating that quinine has a "curare-like" action. In the absence of studies of the electrical events at the junction under such conditions, the findings may be explained alternatively by a reduction in the ex-

citability of the muscle fiber. Thus the e.p.p., reduced to a critical level by partial curarization, would be unable to set up impulses because of the muscle's raised threshold. A number of additional effects of quinine warrant discussion. Several actions of eserine are antagonized by quinine, including the potentiation of the tension response to single nerve stimuli and the depression to tetanic stimulation. Quinine can also abolish the repetitive firing of the muscle fiber in a number of cases where curare has no effect. These are found in denervated muscle, myotonia and veratrine-treated muscle, as well as in the repetitive muscle effects of DDT, 2,4-D and metrazol and in muscle with Ca lack (59, 60). A probable explanation of the above actions may be found in the lowered excitability of the muscle fiber after quinine. More satisfactory explanations of the mechanism might be obtained by studies of impedance changes and electrical behavior of the membrane under the influence of this drug. Conversion of quinine into a quaternary salt, as in quinine methochloride, reduces the above actions and produces more striking junctional blocking effects (84). The increase in refractoriness of skeletal muscle produced by quinine may bear a similarity to the effect of quinidine on cardiac muscle (125). In general, quinine appears to slow conduction, increase refractoriness and lower excitability. However, critical experiments on most of these points have not been done with newer methods.

Veratrine: The best known effect of veratrine is the marked long-continued response to single nerve or muscle stimuli. The tension increase is mainly due to propagated repetitive muscle fiber impulses (8, 39, 56, 65, 113); it is not a contracture or local shortening, although the latter may follow on high frequency discharges. The myotonic reaction, occuring as a disease process in man and goats, is similar to the veratrine effects; both are reduced by activity and by quinine (30, 170).

Veratrine first affects the muscle fibers, then the nerve. Its action on both structures, in terms of membrane changes, appears similar (3, 72). First of all, the after-potential is increased and, if it reaches a critical height, it in turn sets up another propagated potential. In isolated muscle fibers, discharge rates of about 300/sec. can be reached. The negative after-potential collapses during each muscle impulse and is built up again, the rate of rise being related to the drug concentration. The whole explosive veratrine effect is initiated somehow during the "breakdown" process of the impulse, since even in strongly veratrinized preparations subthreshold potential changes are unaffected. The veratrine after-potential, however, is a separate process and not merely a delayed recovery of the muscle membrane after the spike (113). While nerve and muscle membrane is thus drastically affected, as becomes evident when propagation occurs it appears remarkable that the e.p.p. itself and the "transmitter" time course are not appreciably changed. It therefore fails to act on the mechanism so exclusively affected by curare. No special use of this property has so far been made in physiological studies. It has been argued, however, that since veratrine changes the current flow resulting from nerve impulses, without changing the e.p.p., the latter is not set up by action currents (115). All the "typical"

veratrine actions can occur at concentrations which do not change the muscle resting potential. Further, the threshold for excitation by ACh, KCl, or electric stimulation is not appreciably changed (113). For a comprehensive review of the pharmacology of veratrum alkaloids, see Krayer and Acheson (106).

Discharges of the veratrine type, reproducing myotonic effects, have been studied in the rat with the use of 2,4-D (2,4-dichlorophenoxyacetate), DDT (2,2-bis-p-chlorophenyl 1,1,1-trichloroethane) and metrazol, and the relationship to other rhythmic phenomena of this type has been discussed (59, 60).

Epinephrine: Since the work of Gruber (76) and of Orbeli (140), it has been known that administration of epinephrine or stimulation of sympathetic nerves produces a considerable increase in the contractile response of striated muscle to nerve stimulation. This increase is seen particularly in fatigued muscle and is considered by several writers (34, 37, 95) to result from an action of epinephrine on n-m transmission, since direct stimulation of the curarized muscle did not yield comparable changes. However, a recent paper of Brown, Bülbring and Burns (24) indicates that the epinephrine effect in increasing twitch tension is not accompanied by a larger muscle action potential. The duration of the muscle action potential appeared to be prolonged, suggesting that the speed of propagation of the muscle impulse was slowed and that this might account for the tension changes. The epinephrine effect, therefore, does not appear to be due to a greater number of fibers contracting. It is not clear what the relationship of fatigue is to this action of epinephrine, and it would be of considerable interest to have more detailed information on the changes in muscle conduction velocity with correlated tension measurements, particularly since similar phenomena have been reported with other agents (see potassium, quinine, etc.). Recently Brown and Goffart (28) have described changes in the demarcation potential of cat muscle produced by stimulation of the sympathetic outflow or administration of epinephrine by the intravenous or intra-arterial route. These changes consisted of a 5–10% increase of the muscle polarization potential, and the time course of this effect paralleled the increase in twitch response. While the present evidence suggests that epinephrine acts on skeletal muscle by some effect on muscle conduction, and that alterations in blood flow can be excluded, the possibility of a direct action on the contractile elements remains. Perhaps some similarity may exist between the action of epinephrine on striated muscle and on cardiac muscle. For additional information about the effects of epinephrine, Burn's review should be consulted (37). While the possibility of sympathetic innervation of skeletal muscle fibers seems excluded (88), the question of the physiologic effect of the sympathetic outflow on muscle remains.

ELECTROLYTES

Effects of inorganic ions on n-m transmission must be interpreted with a view to the general effects on the irritability of excitable tissues. In the case of the n-m junction, effects on nerve fibers, nerve terminals, release of transmitter and post-junctional membrane, as well as on the muscle fiber itself, must be considered. Most of the studies concerning electrolytes and action potentials have

been done on nerve. Muscle appears to share many properties in this respect, such as the reversal of membrane potential during propagated impulses (73, 138).

Potassium: This ion has been studied in particular by Walker and Laporte who followed the effects of the intra-arterial injection of K^+ on the e.p.p. of curarized frog muscle (166). Following the injection of K^+, the e.p.p. was first increased and transmission was partially restored. In spite of this continued increase in e.p.p., transmission failed again, suggesting a raised threshold of the muscle fibers. Later the e.p.p. was reduced, particularly to nerve volleys at short intervals. As would be expected from the progressive depolarizing action of K^+, the whole nerve-muscle system deteriorates in the presence of K^+ excess. Contrary to earlier statements (109), K^+ does not excite the endplates exclusively and, in accordance with its uniform depolarizing action, can set up muscle impulses anywhere along the muscle (112). Small differences in excitability to K^+ along the fibers, however, would not have been distinguished. As one would expect, curarine, acting only on the endplate, does not antagonize the K^+ effects. Also procaine, which prevents propagated responses, is not appreciably effective in preventing local contractions at the site of K^+ application, in concentrations which otherwise would have set up propagated contractions. In this respect the K^+ effect resembles an applied cathodal current (114). In rat muscle, K^+ causes an increase in twitch height and duration to single nerve shocks, accompanied by a diminution in the amplitude of the propagated muscle action potential (165). These changes probably indicate a decreased rate of muscle conduction.

Sodium: Although considerable information is available on the electrolyte balance in relation to Na^+ in muscle, few studies have been made of its specific effect on n-m mechanisms. The recent studies of Nastuk and Hodgkin (138) show that the magnitude of the muscle membrane potential reversal with activity depends on the concentration of external Na^+. These findings, similar to those in the squid axon (89), have been interpreted as indicating a selective permeability of the membrane to Na^+ during activity. It would be of interest to know the effect of changes in Na^+ on junctional potentials. A preliminary report on the depolarizing action of ACh on the endplate regions of frog muscle (61) indicates that this effect is dependent on the concentration of external Na^+.

Calcium: The effect of calcium excess and lack has been studied a great deal in nerve, but much less in muscle. Reduction of calcium produces an instability of the medullated and non-medullated nerve, reduction of accommodation, and accompanying oscillatory membrane fluctuations, eventually resulting in "spontaneous" discharges. Ca^{++} increase, on the whole, reduces "excitability", and slows and blocks conduction.

In the nerve-muscle preparation the first sign of reduced Ca^{++} is a repetitive contraction to a single nerve volley, due to multiple motor-unit discharges. At the same time the endplates themselves become hyperexcitable, since with gradual lowering of a preparation into Ca^{++}-free Ringer solution no excitation results until the innervated portions reach the solution. Then the "spontaneous" discharges in frog muscle consist mainly of single, and only few grouped, mus-

cle fiber responses, indicating relatively little nerve participation at that stage. The particular involvement of the junctional region is strikingly illustrated with single nerve-muscle fiber preparations (110). Discharges from the junction also occur in curarized preparations, where the participation of nerve is excluded (4, 110). Curare, as expected, does block those discharges in calcium lack or tetany which are due to repetitive firing of the nerve itself (see below). Hyperexcitability is followed by block of transmission, which occurs before the well-known spontaneous nerve discharges start. During the "hyperexcitable" stage of Ca^{++} reduction, the ACh sensitivity is greatly increased. This state is also indicated by the rhythmic discharges set up with pressure or stretch (4). In the course of further Ca^{++} reduction, the e.p.p. and "transmitter" duration is first shortened, then reduced until block results. This may possibly be analogous to the events at the superior cervical ganglion where ACh production is reduced with corresponding transmission failure (86). Many similarities in facilitation and depression phenomena exist between the sympathetic ganglia and n-m junctions during Ca^{++} reduction, as studied extensively by Brink, Bronk and Larrabee (19). The block of n-m transmission can be overcome by repeated stimuli, due to e.p.p. summation. Brown and Harvey (31) kept a kid on a calcium-deficient diet and observed facilitation phenomena, with a second and several subsequent nerve stimuli progressively restoring impaired transmission. After that initial facilitation no depression was seen, such as is prominent in the response of curarized muscle to tetanic stimuli. Similarly their chicks on a calcium-deficient diet showed the same phenomena, which is an exaggeration of the normal state in the fowl (29). The analogy to the symptoms of Ca^{++} reduction is particularly striking in cats with thyroids and parathyroids removed and also in frogs without parathyroids (111). While their symptoms were relieved by Ca^{++} addition, the severity could not be directly correlated with the serum calcium level. In humans the most prominent clinical symptoms during tetany, according to Kugelberg (121), result from nerve discharges which may actually originate in different regions along the course of the peripheral axons.

With Ca^{++} lack, a small membrane depolarization is observed, not confined to any special muscle region. If this has any connection with spontaneous discharges, it is surprising why they should start preferentially at junctions. Also the ineffectiveness of curarine in preventing discharges from endplates may be significant, a fact which brings out another similarity to fibrillating denervated muscle. The latter also becomes excessively sensitive to stretch and pressure, particularly at the endplate regions.

The rhythmic discharges in muscle during reduction of Ca^{++} arise from slowly developing potential changes, as described by Adrian and Gelfan (4). There also appears to be a possibility of finding a synchronizing mechanism between groups of endplates, probably of "electrical" current field nature, since grouped discharges can arise in curarized muscles.

Ca^{++} increase blocks n-m transmission, presumably by increasing the threshold of the muscle membrane surrounding the endplate (110). It also increases the size of the e.p.p. in curarized preparations, which accounts in part for its

anti-curare action (52, 66). In agreement with the above, the effect of Ca^{++} increase and decrease was also reported by Coppée, in particular its interaction with other drugs (40).

Magnesium: The striking effects of narcosis and paralysis which are produced in animals by the administration of magnesium has led to a number of more detailed studies of its effects. Magnesium affects the peripheral nerve and the central nervous system in higher concentrations than those causing peripheral paralysis (58). Lorente de Nó has shown that concentrations of Mg^{++} less than 0.02 M produce no narcotic effect on frog nerve (128). The mechanism of the peripheral action of Mg^{++} in causing paralysis is not clear, but in certain concentrations there results a n-m block with the nerve still conducting and the muscle excitable by direct stimulation (15, 20, 21, 90). Associated with this block is a lowered excitability to applied ACh (58, 130). However, Ashkenaz (6) has shown that the electrical threshold of normal or denervated muscle fibers is raised severalfold by Mg^{++} in about 0.01 M concentration. The threshold changes are temporarily antagonized by Ca^{++}.

Fenn and Haege found that with external concentrations of Mg^{++} below 4 mM there was no exchange of Mg^{++} with frog muscle, while 4–8mM Mg^{++} was found to penetrate the muscle against a concentration gradient (67). It is not clear whether the effects of Mg^{++} are correlated with penetration. Mg^{++} and Ca^{++} appear to play an opposing role in the enzymatic breakdown of adenosine triphosphate (74). It is surprising that in spite of the great magnesium content of muscle relatively few studies have been done on its function.

PHARMACOLOGY OF THE SMALL-NERVE SYSTEM

The mechanism of the small-nerve motor system to frog's striated muscles resembles in many respects that found in crustaceans (98, 134). Nerve fibers of about 5 μ emerging through the ventral roots innervate numerous muscles in the body, and when stimulated repetitively, cause local non-propagated muscle shortening around the n-m junction, in striking contrast to the well-known twitch-motor-unit response. Detailed studies of the myoneural processes and of the reflex function of the small-nerve system have also been made, and it appears that this system serves a tonic function, producing graded, relatively slow, contractile effects, suitable for the maintenance of prolonged shortening (117, 120, 160, 161). This is in contrast to the function of the cat's small-nerve system which acts as a muscle proprioceptor regulator (*cf.* above).

It now appears that the small-nerve motor fibers of frog innervate separate muscle fibers from those supplied by the larger group (above 5 μ). The small-nerve innervated muscle fibers seem to be unable to conduct propagated muscle impulses and they also are distinct pharmacologically (116). The sartorius of frog represents a muscle innervated nearly exclusively by the large motor fibers while, for instance, the rectus abdominis or iliofibularis has in addition a dense small-nerve supply. The latter muscle has a distinct portion in which the small-nerve fibers are quite numerous (slow portion) and one portion where they are practically absent (quick or twitch portion). If the "slow" portion of the ilio-

fibularis, or the whole rectus is immersed into a variety of drugs, like KCl or ACh, tension development and muscle shortening follow which are maintained roughly for the duration of the drug application. In a sartorius or in the "quick" portion of the iliofibularis there will result a tension rise which, if comparable concentrations are used, will disappear in several minutes in spite of the continued presence of the drug and the continued depolarization of the muscle (116). The distinct pharmacological behavior of the small-nerve supplied iliofibularis portion was well recognized by Sommerkamp (158) who called it the "tonus-bundle."

It is now clear that the ACh assays, for which the frog rectus is frequently used, have been actually done on the small-nerve innervated muscle fibers. The effect of anti-cholinesterases on the small- and large-nerve innervated muscle systems has also to be distinguished. In the small-nerve innervated muscles potentiation refers to an increase in the contracture caused by ACh, while in the twitch muscles it generally refers to a repetitive muscle firing to a single nerve volley.

The pharmacology of the small-nerve transmission system in frog is practically unknown. Bremer's well-known n-m contractures are particularly sensitive to atropine (17). It seems that many of his studies apply to the small-nerve innervated portions of frog's muscles. The same appears to have been the case with Wachholder and Ledebuhr's (164) investigations on the ACh effect. Since some of the membrane properties of muscle components differ, one may also expect a varied drug effect. *Normally* the sartorius-type muscle tends to give twitch responses while contractures and maintained local shortening are not so readily obtained (*cf.* below).

Differences in the pharmacology of mammalian striated muscles or their components may exist, but this field has not yet been explored.

CONTRACTURE

The most complete review on this topic remains that of Gasser (69). Contracture may be defined as a non-propagated prolonged reversible shortening of a muscle. Many types of "contracture," including that caused by veratrine, have now been found to be set up by a tetanus of propagated impulses (56, 65, 113). Contracture in twitch-producing striated muscle, functioning "normally," seems exceptional. However, during "fatigue," during drug action, after denervation, etc., the muscle membrane tends to lose its ability to conduct impulses. In such a state a local depolarization will produce a non-propagated contractile change in the same region of a muscle fiber (151). Procaine in concentrations which do not change the muscle resting potential will abolish conducted muscle responses and, under these conditions, the relationship between membrane changes and contractile effects, set up electrically or pharmacologically, can be studied conveniently (114). One may assume at present that the muscle membrane depolarization is the first step in the normal process of contractile element activation, the propagated impulse therefore causing the whole muscle fiber to contract, while a local change sets up a corresponding contractile activity. In

twitch-producing muscle fibers, like those of the sartorius, a relatively quick accommodation process sets in between the membrane and contractile elements (see above).

N-m contractures have also been observed in eserinized twitch-muscles by Cowan (44), and by Feng (66) who also recorded potential changes. It now seems clear that those contractures were set up and maintained by the prolonged junctional potentials (53). Feng also showed that n-m contractures may block propagated muscle impulses set up by direct stimulation (64). This may be important in comparing responses from direct and indirect stimulation. Using barium in concentrations of 1:2000, Feng produced eserine-like effects (*cf.* above) on nerve and muscle in addition to contractures which he localized at the region of the nerve terminals (63). These effects were antagonized by calcium. A series of papers by Feng and co-workers quoted in his review (66) still deserve attention for many details on nerve-muscle problems.

DENERVATED MUSCLE

A great volume of work was done on these problems during the war, with interest sharply declining afterwards. The development of electromyography has greatly helped in the diagnostic aspects of denervations. No novel concepts on the pharmacology of denervated muscle seem to have emerged.

The increased sensitivity of denervated muscle to applied or injected drugs can be strikingly shown with ACh, although many other agents act likewise (47). For an extensive treatment of this subject, see Cannon and Rosenblueth (38) and Tower (163), and also the discussion by Loewi (127).

Muscles also acquire a tendency to give non-propagated shortening following denervation. Such contractures interfere with propagated muscle impulses set up by fibrillation (23). There is now good evidence that the fibrillation of denervated mammalian muscle starts at the endplate region (87). Even frog muscle can be brought to the verge of spontaneous fibrillation after long denervation, and single muscle fiber discharges lasting for minutes can be set up around the endplates by light touch, stretch or electric stimulation (109, 150). The differentiation in response to chemical excitation of innervated and nerve-free regions of the muscle fibers still persists after denervation (109). Further, it seems significant that paralytic doses of curare in cats do not interfere appreciably with fibrillation in spite of their otherwise specific action in depressing endplates (153, 157, also unpublished). The effect of curarine, however, in antagonizing depolarization by ACh is still observed in denervated muscle. This seems to imply that although histologically all nerve components have disappeared and other changes in the "endplate" have occured, the behavior of the "receptor substances" where this competitive reaction takes place is not qualitatively changed. Such facts add to the many other indications of specialized properties of the post-junctional region.

Quinine is an effective agent to prevent fibrillation, and it also depresses the ACh sensitivity of muscle fibers (157). Muscles so treated show no appreciable change in the course of atrophy. *d*-Tubocurarine in the dog has also been shown

to depress fibrillation, presumably by a different mechanism, namely, by producing contractures and thus preventing propagated impulses (133). The property of producing contractures is also shared by some quaternary ammonium compounds (see above) which, besides blocking, also have excitatory effects resembling ACh (*cf.* 143). Neostigmine, but not eserine, also causes contracture in denervated muscles (152). This is probably related to its quaternary ammonium structure.

The cause of fibrillation is obscure; it has been suggested that it's due to ACh reaching the junctions through the circulation. This is contraindicated by the absence of a blocking effect by curare, and by the occurrence of a near-fibrillation state in completely isolated preparations of frog muscle after long denervation.

GENERAL COMMENTS

It is clear from the above discussion that differentiation of the functional components of the junctional system should lead to a better understanding of many drug effects. Three physiologically distinct parts of the junction can at present be considered, namely, the nerve fiber and its termination, the endplate, and the muscle fiber itself. In the intact functioning system strict localization is frequently difficult but can be obtained, particularly in isolated structures.

As regards the pharmacology of the nerve fiber and its termination, the chief difficulty is that most drugs have been tested exclusively on the myelinated fiber along its course. As the fiber approaches the endplate, it loses its medullated sheath and subdivides into fine filaments which, according to Couteaux (42), obtain a new surrounding structure, the teloglia. We do not know the rate of excitation spread and other characteristics of the terminal nerve network. Such information would provide much needed data about the nerve events which lead up to the production of the "transmitter" whose time course we now know. Provisionally we may assume that agents which do not alter the latency of the e.p.p. do not interfere with nerve terminal processes, as has been demonstrated for curare. However, for agents which are supposed to act on the nerve terminals, such as botulinus toxin, no such formation is available.

The study of the muscle fiber proper can be done in isolation from nerve, and the endplate can also be excluded by using the endplate-free regions of muscle. This is possible only in a few muscles such as the frog sartorius and even here inspection will reveal that the pelvic end is not always nerve-free (109). The gracilis preparation of the cat, with discrete end plate foci (25), is of promise for such studies. Since it is known that many curare preparations do not affect the muscle excitability, it is possible to obtain, in this way, a reliable test of muscle performance excluding nerve and endplate. However, even here, difficulties arise with "direct" electrical stimulation of large muscles or of those with short non-parallel fibers. Even intense current may fail to excite all elements which are not favorably situated in relation to the lines of current flow. Due to different excitability of nerve and muscle, selection of the proper stimulus duration and electrode size and placement is helpful.

A study of the "endplate" itself raises much greater difficulties. It is not possible to correlate strictly the histological endplate with that delineated by the physiologist, particularly since for all purposes the "endplate" and muscle membranes seem continuous. Thus, potential changes spread freely over both structures; the e.p.p. spreads along the muscle fiber and the muscle impulse, in turn, may completely depolarize the endplate region (107, 108). The clear-cut histochemical demonstration of cholinesterase (104), probably localized in the palisade-like structure of Couteaux and seen in the sole plate nuclei, may help in clarifying the basis for chemical specialization of the endplates (Koelle and Friendenwald, unpublished).

It can be seen that only a few tests are available which give satisfactory information by themselves regarding drug actions on the component structures of the myoneural junction. Present methods, furthermore, need extension since they were not designed primarily for pharmacological studies, which were usually incidental to other physiological investigations.

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