NEUROMUSCULAR PHARMACOLOGY[1,2]

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The acetylcholine released from motor-nerve endings is believed to become adsorbed to receptor substances of the muscle end-plate, following which the permeability of the end-plate for Na^+ and K^+ increases temporarily, the end-plate becomes depolarized, and the end-plate and muscle action potentials and muscle fiber contraction are successively initiated (1, 2). Disturbance of neuromuscular transmission usually results from either deficient or excessive action of acetylcholine on the motor end-plate, or from the action of drugs, changes in ionic concentration, or disease which simulates these effects. Neuromuscular block may occur without any change in the resting potential of the muscle membrane (nondepolarizing block), or following reduction in the resting potential (depolarizing block). It may be characterized descriptively in terms of whether or not it inhibits the depolarizing action of acetylcholine and is reversible by acetylcholine.

NONDEPOLARIZING BLOCKING AGENTS

Neuromuscular block without alteration in resting membrane potential may result from deficient release of acetylcholine from the motor-nerve endings or from inhibition of the depolarizing action of acetylcholine on the motor endplates.

Agents that Inhibit Release of Acetylcholine

Toxins.—The paralysis in botulism (3) and in puffer poisoning (4) is a result of prevention by these toxins of release of acetylcholine from terminal nerve endings. The muscle remains responsive to direct stimulation or to exogenous acetylcholine, but becomes unresponsive to stimulation through the nerve after 30 minutes exposure to the toxin (5). Release of acetylcholine from parasympathetic postganglionic nerve endings is also prevented. The motor paralysis produced by the toxin secreted by salivary glands of the Rocky Mountain wood tick, *Dermacentor andersoni,* has similarly been attributed to deficient release of acetylcholine from nerve endings (6). However, the progression of the ascending paralysis resembles that of

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[2] Abbreviations used in this chapter include: DFP (diisopropyl phosphorofluoridate); DAM (diacetyl monoxime); EA1814, TMB-4 [1,1′-trimethylene-bis-(4-formylpyridinium bromide) dioxime]; EPN [O-ethyl O-(4-nitrophenyl) phenylphosphonothioate]; MINA (monoisonitroso acetone); OMPA (octamethyl pyrophosphortetramide); 2-PAM (pyridine-2-aldoxime methiodide); P2S (pyridine-2-aldoxime methanesulfonate); TEPP (tetraethyl pyrophosphate).

polyneuritis more than botulism (7). This suggests that the tick toxin may block conduction in motor-nerve fibers (8), whereas botulinus toxin more directly impairs release of acetylcholine from the nerve endings. The paralysis produced by diptheria toxin is even more clearly the result of impaired conduction in the motor nerve, as transmission at the neuromuscular junction is not affected. There is some evidence that this toxin may diminish synthesis of acetylcholine within the neurone (9).

Hemicholinium (HC-3).—This compound, which inhibits the synthesis of acetylcholine, blocks transmission at the neuromuscular junction and at the endings of postganglionic parasympathetic nerves. These actions are antagonized by choline, and, to a lesser extent, by analogues and esters of choline (10, 11). Diphenylbutylacetic acid appears to have an effect similar to that of hemicholinium in smooth muscle (12).

Compounds that Inhibit Depolarizing Action of Acetylcholine

Inhibition of the depolarizing action of acetylcholine on the motor end-plate ("acetylcholine-inhibitory" block) occurs in at least three situations:

Block which occurs without change in the resting potential of the muscle membrane, and which is reversible by acetylcholine.—The block produced by *d*-tubocurarine is of this type. Because this drug exerts its effect by competing with acetylcholine for receptor sites at the end-plate, the resulting block has been termed "competitive" (13). This type of block is characterized by inhibition of the depolarizing action of injected acetylcholine and by reversal of the block following the injection of sufficient acetylcholine or anticholinestrease compound. Attempts have been made to identify the receptor substances of the end-plate by their combination with C^{14}-labelled nondepolarizing blocking agents such as *d*-tubocurarine, dimethyl-*d*-tubocurarine, the triethiodide of gallamine (TRIEG), and dimethylisochondodendrine, which complete with acetylcholine for the receptor substances. The substances in eel electric tissue and mammalian skeletal muscle with which these compounds combine have been identified as acid mucopolysaccharides by Chagas (14) and as proteins by Ehrenpreis (15).

Numerous derivatives of *d*-tubocurarine, and related alkaloids, continue to be introduced as muscle-relaxant aids to anesthesia (16). Most are long acting, and apnea caused by excessive administration or unusual reactivity frequently occurs, even though the block can usually be reversed by anticholinesterase compounds, particularly neostigmine (Prostigmin) and its analogues (17). This reversal can sometimes be aided by the administration of potassium salts, epinephrine, or ephedrine (18). Occasionally, however, for reasons that are not clear, irreversible curarization occurs, particularly in elderly, debilitated patients with electrolyte disturbance (19, 20).

The antibiotic, neomycin, has been found to produce neuromuscular block of the nondepolarizing type when administered parenterally in large doses (21, 22). The block is potentiated by ether and antagonized by neostigmine. Streptomycin has a similar but much less marked effect (23).

Block not associated with any change in membrane potential, but not reversible by acetylcholine.—This type of block occurs several minutes after the exposure of frog muscle to acetylcholine or other depolarizing compounds and persists even after recovery of the muscle membrane from the initial depolarization. It is attributable to the development of refractoriness of the motor end-plate to acetylcholine (24). Gamma-aminobutyric acid, glutamine, and some other amino acids inhibit transmission at the crustacean neuromuscular junction and in certain mammalian central synapses (25, 26). The mechanism of these blocks is not known. They are not reversed by acetylcholine, but the gamma-aminobutyric acid block is reversed by picrotoxin. Gamma-aminobutyric acid is present in relatively high concentration in the brain and may be a physiological inhibitor of transmission (27).

Block associated with hyperpolarization or stabilization of the muscle membrane.—The weakness of hypokalemia is probably attributable, in part, to hyperpolarization or stabilization resulting from an increase in the ratio of intracellular to extracellular potassium concentrations (28). The neuromuscular block produced by tetrodotoxin, obtained from the goldfish, has been attributed to stabilization of the muscle membrane by inactivation of the sodium-carrying mechanism (29).

DEPOLARIZING BLOCKING AGENTS

When the end-plate region is in the depolarized state it is inexcitable. This type of block is produced by an excessive concentration of acetylcholine, by anticholinesterase compounds, by choline, and in most animal species by decamethonium and succinylcholine. During the initial stages of neuromuscular block produced by these compounds, there is abnormally prolonged depolarization of the muscle membrane, most marked in the region of the end-plate, and no inhibition of the depolarizing action of injected acetylcholine or anticholinesterase compound; in fact, these compounds intensify the block by their additive effect. However, the neuromuscular block persists after return of normal membrane polarity, because the end-plate develops refractoriness to acetylcholine (30). The properties of this "desensitization" block, which has been ascribed to alteration of the receptor substances from an "effective" to a "refractory" state, vary to some extent with different depolarizing agents.

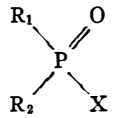
Anticholinesterase Compounds

Organophosphorus compounds.—New agents have been employed as insecticides, chemical warfare agents, and pharmeaceutical agents. Most are of high potency, and may be absorbed through any body surface. Parathion, malathion, O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate (EPN), phosdrin, mipafox, tetraethyl pyrophosphate (TEPP), and octamethyl pyrophosphortetramide (OMPA) (Table I) have been widely used as agricultural insecticides, and their indiscriminate dispersal has resulted in a number

of instances of poisoning, some of them fatal (31, 32). Until recently parathion was the most widely used of the organophosphorus insecticides, and most intoxications, including 6000 in Japan alone, have been caused by this compound. In recent years, there has been increasing use of malathion, which, although less toxic than parathion to mammals, has also produced poisoning after ingestion (33, 34). The organophosphorus chemical warfare agents have been termed nerve gases because of their effect on the central nervous system. Sarin and tabun are among the more important of these, and several instances of intoxication have occurred following exposure to these compounds (35). Diluted solutions of diisopropyl phosphorofluoridate

TABLE I

ORGANOPHOSPHORUS ANTICHOLINESTERASE AGENTS THAT HAVE PRODUCED INTOXICATION IN MAN

General formula: 

Symbol or Common Name	Chemical Name
A. Compounds where X = halogen or CN	
Sarin (GB)	Isopropyl methylphosphonofluoridate
DFP	Diisopropyl phosphorofluoridate
Tabun (GA)	Ethyl-N-dimethyl phosphoramidocyanidate
Mipafox (Isopestox)	N,N′-Diisopropylphosphorodiamidic fluoride
B. Compounds where X = alkyl, alkoxy, or aryloxy	
Phosdrin	Dimethyl 1-methyl-2-carbomethoxyvinyl phosphate
Paraoxon	Diethyl 4-nitrophenyl phosphate
C. Thiol- and thionophosphorus compounds	
EPN	O-Ethyl-O-(4-nitrophenyl) phenylphosphonothioate
Parathion	O,O-Diethyl-O-(4-nitrophenyl)phosphorethioate
Malathion	O,O-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate
D. Derivatives of pyrophosphorus acid	
TEPP	Tetraethyl pyrophosphate
OMPA	Octamethyl pyrophosphortetramide
E. Compound containing a quaternary nitrogen	
Echothiophate (phospholine)	O,O-Diethyl-S-2-trimethylammonium-ethyl phosphonothiolate iodide

(DFP), TEPP, and echothiophate (Phospholine) have been used locally in the management of glaucoma (36). Dilute solutions of DFP, TEPP, hexaethyl tetraphosphate (HETP), OMPA, sarin, and echothiophate have been administered orally or intramuscularly in clinical trials in the management of myasthenia gravis (37).

Quaternary ammonium compounds.—These have been used mainly in the management of myasthenia gravis and, to a lesser extent, of glaucoma and paralytic ileus. Drugs used in the diagnosis and management of myasthenia gravis include neostigmine, pyridostigmine (Mestinon), edrophonium (Tensilon), and ambenonium (Mytelase) (37). Clinical trials have also been carried out with bis-neostigmine (BC-40), bis-pyridostigmine (hexamarium, BC-51, (38a), galanthamine, and lycoramine (38b). Demecarium bromide (BC-48) has recently been used in the management of glaucoma (39).

Anticholinesterase activity.—The inhibitory activity of sarin against the cholinesterase enzymes of the red cells, muscle, and brain is greater than that of the other anticholinesterase compounds, being approximately five times that of tabun, 10 times that of TEPP, 100 times that of DFP or neostigmine, and 4000 times that of parathion (40). Sarin, tabun, and DFP combine with cholinesterase *in vitro* almost irreversibly during the first hour of their reaction and, hence, produce the most prolonged and cumulative effects. The combination of TEPP or parathion with cholinesterase is slightly reversible, whereas the combination of neostigmine with the enzyme is entirely reversible.

Manifestations of anticholinesterase intoxication in man.—All anticholinesterase compounds produce muscarine-like and nicotine-like effects. Organophosphorus compounds of high lipoid solubility, such as sarin, parathion, DFP, malathion, EPN, phosdrin, and tabun, also produce marked central neural effects, while those of low lipoid solubility, such as TEPP, OMPA, and echothiophate (41, 42), and quarternary ammonium compounds, have less effect on the central nervous system. The muscarine-like effects include miosis, increased salivary and bronchial secretion, bronchoconstriction, sweating, and gastrointestinal symptoms, and the central neural effects may include anxiety, headache, convulsions, coma, and respiratory depression (37, 40, 43). The nicotine-like effects on skeletal muscle begin with increased fatiguability and mild generalized weakness which is increased by exertion. This is followed by involuntary muscular twitching, fasciculations, and sometimes cramps. If the exposure has been sufficiently marked, there occurs severe weakness, which is generalized and includes the muscles of respiration. A decrease in tidal volume and respiratory rate and an increase in airway resistance generally occur. Weakness of the muscles of the tongue and pharynx may aggravate upper airway obstruction. The relative importance of central depression of respiration, peripheral neuromuscular block, and bronchoconstriction in causing death varies in different species and with different anticholinesterase compounds and routes of ad-

ministration (44). In man, it is likely that failure of the central drive of respiration is the most important factor, with peripheral neuromuscular block next in importance. Bronchoconstriction appears to be less important in man than in most experimental animals, although airway obstruction resulting from inspissated secretions may occur and may seriously impair ventilatory exchange.

The neuromuscular block produced by anticholinesterase compounds is mainly attributable to the local accumulation of acetylcholine resulting from inhibition of muscle cholinesterase. There occurs an increase in amplitude and frequency of spontaneously occurring miniature end-plate potentials, resulting in the spontaneous appearance of propagated action potentials which cause twitches in individual muscle fibers (fibrillations) and in groups of fibers (fasciculations). When a nerve impulse reaches the myoneural junction, there is increased amplitude and striking prolongation of the end-plate potential, and repetitive firing may take place (45). The muscle-action potential response to a single nerve stimulus is only slightly affected (46), but the responses to subsequent stimuli of a train are progressively reduced, and muscle contraction is not sustained (47). Following neuromuscular block produced by TEPP or DFP, there is recovery from the block before there has been measurable restoration in cholinesterase activity, suggesting that either junctional transmission is not related solely to cholinesterase activity, or is influenced by recovery that is within the limits of experimental error. Some anticholinesterase compounds appear to affect neuromuscular transmission by mechanisms in addition to cholinesterase inhibition. Very large doses of DFP produce further reduction in the muscle response to a single nerve stimulus even after complete inactivation of cholinesterase. Furthermore, this block is accentuated by *d*-tubocurarine, which antagonizes more moderate degrees of anticholinesterase neuromuscular block (47).

Residual effects.—A few instances of persistent paralysis of the extremities with muscle atrophy and loss of tendon reflexes have occurred following exposure to parathion, mipafox, malathion, and EPN (48). Electromyographic studies have shown no evidence of neuromuscular block but have revealed changes similar to those seen in peripheral neuritis. The disorder resembles the peripheral neuritis and demyelinization that occurs more frequently after acute or chronic exposure to triorthocresylphosphate (Ginger Jake paralysis) (49, 50). Demyelinization has been observed in the peripheral nerve and spinal cords in experimental animals following the administration of mipafox, DFP, and other dialkyfluoridates, or triorthocresylphosphate (51). Alkyl organophosphorus compounds containing fluorine appear to be the most neurotoxic, perhaps as a result of liberation of ionic fluorine following rupture of the P-F bond by cholinesterase in the nervous system (52).

Treatment of Anticholinesterase Intoxication

This treatment consists of removal of the toxic agent, administration of

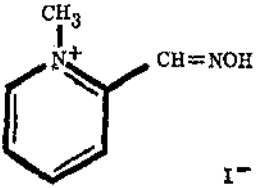
atropine and oxime, removal of oral secretions, maintenance of a patent airway, and mouth-to-mouth artificial respiration if needed (35, 53).

The administration of large doses of atropine ameliorates the muscarine-like effects of anticholinesterase compounds, and to a lesser extent the central neural effects, but has no influence on weakness resulting from neuromuscular block. Until recently, there has been no clinically useful means of accelerating recovery from the neuromuscular block. However, during the past several years, Wilson and others have demonstrated that cholinesterase inhibited by organophosphorus anticholinesterase compounds may be reactivated *in vitro* by derivatives of hydroxamine acid [R – C(= O) — NHOH], and to a greater extent by a number of oximes [R – C(= NOH) – R] (54). Both groups of compounds also react with these inhibitors to inactivate them directly. The action of organophosphorus anticholinesterase agents on smooth, cardiac, and skeletal muscle of experimental animals can be reversed by hydroxamic acid derivatives (54) and oximes (55) and their lethal effects reduced (56), although species differences are wide.

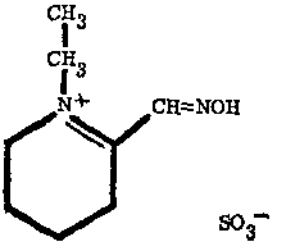
Protection and reactivation by oximes of cholinesterase enzymes inhibited by anticholinesterase compounds in vitro.—The oximes pyridine-2-aldoxime methiodide (2-PAM), pyridine-2-aldoxime methane sulfonate (P2S), 1,1′-trimethylene-bis-(4-formylpyridinium bromide) dioxime (EA 1814, TMB-4), diacetyl monoxime (DAM), and monoisonitroso acetone (MINA) (Fig. 1) cause moderate protection and reactivation of cholinesterase enzymes inhibited by organophosphorus compounds such as sarin (57 to 62). Reactivation by oxime is less after prolonged contact of organophosphorus inhibitor with enzyme. 2-PAM, TMB-4, and to a lesser extent DAM, cause slight protection and very slight reactivation of human cholinesterase enzymes inhibited by quaternary ammonium compounds such as neostigmine and pyridostigmine, but not ambenomium (62) or physostigmine (63). The inhibition of cholinesterase by organophosphorus compounds has been considered to occur by direct phosphorylation of some group at the active center of the enzyme, and reversal of this inhibition by oximes to be caused by displacement of the enzymic group from the phosphorus atom (57). Since the quaternary ammonium anticholinesterase compounds do not contain a phosphorus atom, it is evident that the oximes may reverse cholinesterase inhibition by a more general mechanism than displacement of phosphorus (62).

Reversal by oximes of neuromuscular block produced by anticholinesterase compounds.—The neuromuscular block produced in man by the intraarterial injection of organophosphorus anticholinesterase compounds such as sarin, or quaternary ammonium compounds such as neostigmine, bis-neostigmine, pyridostigmine, bis-pyridostigmine, or ambenomium, is promptly and strikingly reversed in the injected extremity immediately after the intraarterial injection of 2-PAM, DAM, or TMB-4 (62). The effect of each of these oximes on neuromuscular block produced by organophosphorus and quaternary ammonium anticholinesterase compounds is striking and equal, in contrast to lesser ability and marked differences in protection and reactiva-

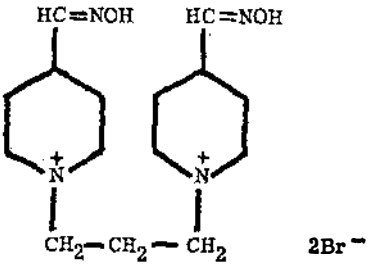
Pyridine aldoxime methiodide (2-PAM)



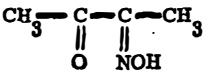
Pyridine aldoxime methane sulfonate (P2S)



1,1'-Trimethylene-bis-(4-formylpyridinium bromide) dioxime (EA 1814, TMB-4)



Diacetyl monoxime (DAM)



Monoisonitroso acetone (MINA)

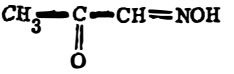


FIG. 1. Formulae of the oximes 2-PAM, P2S, TMB-4, DAM, and MINA.

tion of cholinesterase, including that of muscle, *in vitro*. This suggests that either the enzyme in intact muscle may be particularly accessible to the action of the oximes, or the oximes may restore or protect neuromuscular transmission by another mechanism than restoration or protection of muscle cholinesterase. High concentrations of 2-PAM have been found to produce a reversible block of the action of acetylcholine on isolated frog muscle (64),

while lower concentrations of 2-PAM potentiate the action of acetylcholine, perhaps as a result of cholinesterase inhibition. It seems likely that the reversal by oximes of anticholinesterase neuromuscular block is not only attributable to the reversal of cholinesterase, inhibition, but also, and perhaps mainly, to inhibition of the action of acetylcholine.

The intraarterial injection of high concentrations of 2-PAM or TMB-4 produces a transient neuromuscular block, whereas DAM has no effect (62). The block does not alter the affect of acetylcholine on neuromuscular transmission and is not reversed by acetylcholine or neostigmine, but it is increased by the prior administration of neostigmine. The block may be attributable to a direct action on the muscle fiber (65).

The intravenous administration to man of 500 to 2000 mg. of 2-PAM or DAM, or 150 to 250 mg. of TMB-4, ameliorates to a moderate degree generalized weakness resulting from any of these anticholinesterase compounds (62, 66, 67). Muscular fasciculations are usually reduced to a lesser degree, but may be unchanged. Muscarine-like and central neural symptoms produced by sarin or quaternary ammonium anticholinesterase compounds are not appreciably influenced by oxime administration (62), but those produced by parathion have been reported to be ameliorated (66, 68). It is advisable to administer large doses of atropine intravenously, in addition to oxime, in the management of serious anticholinesterase intoxication.

Distribution and effects of oximes.—The intravenous administration of 1000 mg. (15 mg./kg.) of 2-PAM or DAM produces a plasma concentration of approximately 2 mg./100 ml. (69). 2-PAM is removed primarily by renal excretion and has a half life of 0.9 hour. The renal clearance of 2-PAM is almost three times that of creatinine, with 80 per cent of injected material appearing in the urine as an altered derivative within six hours and most within the first half hour. In contrast, DAM is removed more slowly, primarily by means other than renal excretion and has a half life of 7.2 hours. The renal clearance of DAM is only 6 per cent that of creatinine, and less than 10 per cent of injected material appears in the urine in six hours. Observations in experimental animals indicate that the liver is the main site of destruction of DAM (70). Both 2-PAM and DAM appear to be widely dispersed in total body water, except that 2-PAM, like most quaternary ammonium compounds, does not enter the spinal fluid. DAM, a tertiary amine, does enter the spinal fluid. Rapid intravenous injection of 2-PAM may result in transient mild weakness, diplopia, blurred vision, dizziness, impairment of accommodation, and occasionally headache, nausea, and tachycardia (69). DAM may produce a burning sensation at the site of injection radiating up the injected vein, followed by moderate giddiness, drowsiness, a sensation of warmth and tingling in the abdomen and chest, tachycardia, slight increase or decrease in blood pressure, and mild postural hypotension (62). Rapid injection of large doses may cause bitter taste, paresthesias and decreased position sense in the extremities, decreased sweating, transient loss of consciousness, clonic movements of

the head, and decreased amplitude of the electroencephalogram and of the T-wave of the electrocardiogram (69).

Depolarizing Muscle Relaxants

Biphasic neuromuscular block.—The characteristics of the neuromuscular block produced by most depolarizing agents, including choline, succinylcholine, and decamethonium, depend on the degree and duration of the block. In most instances, signs of stimulation, such as muscular fasciculations occur first. This is followed by the usual manifestations of depolarizing block: equal depression of the muscle-action potentials evoked by successive nerve stimuli, no inhibition of the depolarizing action of acetylcholine, and enhancement of the block by acetylcholine or anticholinesterase compounds. When the block is of severe degree or prolonged duration, these characteristics may change to those of the nondepolarizing, competitive type: progressive depression of successive evoked potentials, inhibition of the depolarizing action of acetylcholine, and reversal of the block by acetylcholine or anticholinesterase compounds (47, 71, 72). In addition, there may be gradually decreasing reactivity (tachyphylaxis) to the effect of repeated doses of depolarizing relaxant (73). The characteristics of this biphasic block have been studied on isolated human intercostal nerve-muscle preparation (74, 75, 76). The second phase of the block may be similar in mechanism to the "desensitization" block produced by acetylcholine, although the latter is not reversible by acetylcholine or anticholinesterase compounds (24, 77). In both instances prolonged or intensive exposure of the end-plate receptors to the depolarizing agent appears to result in altered sensitivity of these receptors to the physiologic transmitter. There is some evidence that the excitation mechanism of the entire muscle membrane may be interfered with, and that the membrane-contractile link may be blocked (78).

Succinylcholine.—This compound has relatively short duration of action and is widely used for brief muscular relaxation, or, in fractional doses or infusion, for prolonged relaxation (79). It is hydrolyzed by plasma cholinesterase to choline and succinylmonocholine, which is in turn hydrolyzed by plasma cholinesterase and by an enzyme in the liver to choline and succinic acid. The hydrolysis of succinylcholine is delayed and its neuromuscular blocking action prolonged when plasma cholinesterase activity is reduced (80). This may occur as a result of hepatic insufficiency or prior administration of an anticholinesterase compound, or, rarely, in otherwise normal individuals as an inherited familial trait. Qualitative differences in plasma cholinesterase may also result in delayed hydrolysis of the drug (81, 82, 83). A selective inhibitor of plasma cholinesterase, hexafluorenium, has been employed to increase the duration and intensity of muscle-relaxing effect of succinylcholine in both anesthetized and unanesthetized subjects (84). The action of the latter drug may be slightly shortened by the intravenous

administration of concentrated human plasma cholinesterase (Cholase) (85), but not enough to be of practical value. Unusual reactivity to the neuromuscular blocking action of succinylcholine may result not only from reduced plasma cholinesterase activity, but also from dehydration, hypokalemia, and possibly hyponatremia, hypocalcemia, and hypermagnesemia (81). It may also occur without apparent cause, perhaps as a result of abnormal fixation of the drug to the end-plate receptors (80, 86). Careful administration of edrophonium or neostigmine sometimes reverses intense succinylcholine block (72), and occasionally administration of potassium chloride may reverse the block, or make it reversible by anticholinesterase compounds (87). This action of potassium is similar to its effect on the nondepolarizing block caused by *d*-tubocurarine, and it is possible that it may occur when the succinylcholine block has become of the same type. Or, it may be due to the replacement of potassium ions which are lost from muscle under the influence of depolarizing agents, as reflected by increased plasma concentration and urinary excretion of this ion (88).

The distribution, metabolism, and elimination of muscle relaxants has been reviewed by Kalow (89), and fetal transmission by Baker (90). Unlike most other drugs with molecular weight of less than 1000, succinylcholine, decamethonium, and *d*-tubocurarine do not cross the placental barrier in significant amount (90, 91). There is some evidence that the fetus may have decreased reactivity to depolarizing agents and increased reactivity to nondepolarizing agents (90).

The combined use of depolarizing and nondepolarizing relaxants presents increased problems (92) and is generally not advisable, even though it decreases postanesthetic muscle pain attributable to the initial stimulating effect of the former (93). Succinylcholine may be used to induce endotracheal intubation and may then be followed by a nondepolarizing drug for maintenance of relaxation if the latter is delayed until the effect of the former has worn off (92). It has been suggested that caution be exercised in the use of procaine in patients receiving succinylcholine, since procaine inhibits plasma cholinesterase (85). Procaine may also affect neuromuscular transmission by inhibiting the release of acetylcholine from motor-nerve endings, by competing with it for end-plate receptors, and by a stabilizing effect on the postjunctional membrane. Oxytocin has also been found to prolong the action of succinylcholine, but the mechanism is not known (94). There is some evidence that ganglion-blocking agents may intensify the neuromuscular effects of both depolarizing and nondepolarizing relaxants (81).

Newer depolarizing blocking agents.—These include hexamethylene-1, 6-biscarbaminoyline bromide (Imbretil), a new muscle relaxant (95), a series of lipoid-soluble long chain derivatives of tetramethylammonium (96), a group of benzyltrimethylammonium iodides (97), a series of bis-onium compounds derived from substituted tropane nuclei (98), and a family of plant pyrrolizidine alkaloids (99).

Tissue extracts.—Gamma-butyrobetaine, which has been extracted from muscle and thymic tissue (100), and other quaternary ammonium compounds which have been extracted from thymic tissue (101), are capable of producing neuromuscular block of the depolarizing type in experimental animals. Maltoxin, an amine from brew malt rootlet, exerts a similar effect (102).

OTHER CHEMICAL AND PHYSICAL AGENTS

Alterations in Electrolyte Concentration

Alterations in concentration of potassium, calcium, sodium, or magnesium in the extracellular fluid may result in derangement of excitation, not only of the motor end-plate, but also of the muscle fiber, nerve, and cardiac and smooth muscle.

Potassium.—The normal resting potential of the muscle membrane is attributed mainly to the concentration gradients of potassium and chloride ions across the membrane; in experimental animals the resting potential varies with these gradients (103). Excitation of muscle is associated with entry of sodium ions and a somewhat smaller loss of potassium ions (104). In normal subjects, rest or administration of glucose, epinephrine, or potassium chloride causes movement of potassium into muscle from the extracellular fluid (28, 105). Exercise, administration of large doses of insulin, deficient intake of potassium, excessive loss of potassium in vomitus, stool, or urine, or alkalosis causes movement of potassium out of muscle. The effect of insulin *in vivo* is probably caused by net movement of potassium into the liver, since, *in vitro,* insulin causes potassium to move into muscle. The latter has been attributed to hyperpolarization of the muscle membrane by the insulin (106).

In patients with periodic paralysis, rest, glucose, insulin, or epinephrine causes an exaggerated movement of potassium into muscle (28). Induced by the prior ingestion of carbohydrate and by rest, the initial event during an attack of periodic paralysis is an abnormal uptake of potassium by muscle. This is followed by a decrease in reactivity of the muscle to the depolarizing action of acetylcholine. This finding is suggestive of an abnormal increase in muscle-membrane potential (hyperpolarization of the muscle membrane) attributable to an increase in the concentration gradient of potassium, but confirmation awaits direct measurement of membrane potential. There is a decrease in muscle responsiveness to nerve stimulation, in propagation of excitation and in contractility, resulting in paralysis. Following the administration of potassium chloride, there is first a return of the extracellular concentration of potassium toward normal. This results in a return toward normal of reactivity to acetylcholine and probably of the muscle membrane potential, which results from a return toward normal of the concentration gradient of potassium. There is a partial return of muscle responsiveness, propagation of excitation, and contractility which results in partial return of strength and increased muscle activity. The latter causes an exaggerated

extrusion of potassium from muscle, which is in turn followed by accelerated improvement in muscle function and strength. Conn (107) has reported that attacks of periodic paralysis are preceded by increased urinary excretion of aldosterone, decreased excretion of sodium, and movement of sodium into muscle. However, these changes do not occur in all patients, and their importance is not clear.

Movement of potassium out of cells and into the extracellular fluid may take place as a result of intravascular hemolysis, tissue damage as in crush injury, or acute acidosis; hyperkalemia may follow, particularly if there is also reduced renal blood flow or other cause of renal insufficiency. Movement of potassium out of muscle into the extracellular fluid also occurs in a rare heritable disease, adynamia episodica hereditaria, described by Gamstorp (108). This is in many ways the opposite of familial periodic paralysis. Attacks of weakness occur during the day rather than at night and at about one hour after exercise. There is a slight to moderate elevation of serum potassium and increased reactivity to acetylcholine. Attacks of weakness may be precipitated by oral potassium administration, prevented by the prior administration of glucose, and relieved by the intravenous administration of calcium.

Weakness resulting from hyperkalemia clinically resembles that resulting from hypokalemia, but the electromyographic and electrocardiographic alterations are different. Since the administration of potassium to normal subjects causes an increase in reactivity of muscle to the depolarizing action of acetylcholine, the weakness of hyperkalemia may be attributable, at least in part, to a decrease in muscle-membrane potential (depolarization), which, in turn, is attributable to a decrease in the concentration gradient of potassium (28).

Calcium.—There is evidence that calcium is necessary for development of the "active state" of contraction of skeletal, cardiac, and smooth muscle (109). The ion enters the muscle fiber during or following depolarization (110), leaves during contraction (111), and may serve as the link between the two processes. Nevertheless, diminution in the concentration of ionized calcium in the plasma below normal results in increased irritability and spontaneous discharge of sensory and motor nerves and of muscle, producing "tetany." Increase in calcium above 12 mg. per cent has an opposite effect and results in hypotonia and weakness, which is attributable to decreased muscle excitability and contractility (112). Hypercalcemia also causes decreased activity of smooth muscle resulting in constipation, anorexia, nausea, and vomiting.

Magnesium.—Reduction in the plasma concentration of magnesium is reported to cause muscle spasm and convulsions resembling tetany, probably because of increased excitability of nerve and muscle. Increase in plasma magnesium concentration following administration of large doses of magnesium salts produces muscular weakness resulting from decreased

muscle excitability and contractility (112), reduction in blood pressure resulting from vasodilatation, and depression of the central nervous system.

Adrenal Hormones

Epinephrine.—This compound reduces muscle fatigue, apparently by increasing the output of acetylcholine from nerve endings (113). Epinephrine, norepinephrine and isoprenaline are also reported to increase twitch tension in slow-contacting muscle such as the cat soleus (114).

Adrenal steroids.—Muscular weakness is a common finding in hyperadrenocorticism. Severe weakness and wasting of the muscles of the pelvis and lower extremities may also take place after prolonged administration of large doses of 17-hydroxycorticosteroids (115). There is increased creatinuria and minimal histologic change. Potassium administration does not affect the weakness or wasting, but discontinuation of steroid is followed by return to normal. The myopathy is believed to be due to altered protein metabolism, but the mechanism is not clear.

Fatigue

Following repetitive nerve stimulation, fatigue of neuromuscular transmission occurs as a result of decreased sensitivity of the endplate to the acetylcholine, increased threshold of the muscle fiber for excitation, and decreased output of acetylcholine from the motor-nerve endings (116). There is difference of opinion concerning the relative rate of fatigue of the contractile mechanism. The nerve trunk is practically indefatigable.

Alteration in Temperature

The optimal muscle temperature for maintaining sustained contraction is reported to be 25 to 29°C. in man (117, 118). In the rat, the maximum twitch tension is attained at 20° in innervated muscle and at 39° in denervated muscle (119). Lowered temperature increases the magnitude and duration of the neuromuscular blocking action of depolarizing agents such as decamethonium, and decreases the blocking action of *d*-tubocurarine (120). It also increases the contractural response to acetylcholine and decreases that to potassium (119).

DISEASE STATES

Myasthenia Gravis

Defect in neuromuscular transmission.—This has many of the characteristics of a nondepolarizing and competitive (acetylcholine-inhibitory) block, similar to that produced by *d*-tubocurarine in normal subjects. In myasthenic patients the block appears to be produced by acetylcholine released in a normal manner during neuromuscular transmission, or by choline or a closely related compound formed following hydrolysis of the transmitter. The intraarterial administration of either acetylcholine or

choline produces in most myasthenic patients a neuromuscular block that inhibits the depolarizing action of acetylcholine and is reversed by acetylcholine or anticholinesterase compounds (47). This competitive and presumably nondepolarizing block is in contrast to the predominantly noncompetitive block produced by these compounds in normal subjects, in whom the impairment is intensified by acetylcholine or anticholinesterase compounds (47). The latter block would appear to be of the depolarizing type, but confirmation of such a mechanism requires measurement of membrane potential, which has only recently been made in normal man (121) and has not yet been applied to the study of disease or drug action. The relationship of these functional changes to abnormalities which have been described in the distal nerve endings and motor end-plates of myasthenic muscle (122, 123, 124) is not clear. A contrasting view of the defect in myasthenia is that it is caused by impaired release of acetylcholine from motor-nerve endings, as suggested by the resemblance between the posttetanic exhaustion of myasthenic muscle and of normal muscle treated with hemicholinium (125).

Different types of neuromuscular block in myasthenia gravis: the acetylcholine-insensitive state.—In some myasthenic patients the block produced by the intraarterial injection of acetylcholine or choline inhibits the depolarizing effect of acetylcholine, but is not reversed by this compound or anticholinesterase compounds (126). Such a block, which resembles one stage of the effect of acetylcholine in the frog (27, 30), may be termed acetylcholine-insensitive. Patients who manifest this type of block respond poorly or not at all to anticholesterase medication; they are clinically acetylcholine- and neostigmine-insensitive. Patients in whom acetylcholine produces the more usual acetylcholine-reversible block may become acetylcholine-insensitive during an exacerbation of their disease, or they may be rendered acetylcholine-insensitive by the repeated intraarterial administration of this compound or choline, or by prolonged repetitive nerve stimulation (126), which probably causes the local accumulation of endogenous acetylcholine or choline. It is this insensitive state that constitutes the main problem in management of the disease and often leads to the administration of large doses of anticholinesterase medication and to confusion as to whether the patient is overdosed (in "cholinergic crisis") or underdosed (in "myasthenic crisis"). Many patients who are thought to be overdosed are really in an insensitive state, and large doses of anticholinesterase compound often render the patient more insensitive (37).

While it is possible that the neuromuscular blocks occurring in myasthenic patients may be a result of abnormal products of acetylcholine or choline capable of producing a competitive or acetylcholine-insensitive block in normal subjects, it seems more likely that they are due to abnormal responses of the motor end-plates to substances normally released from the motor-nerve endings (126). Myasthenic patients react abnormally not only

to acetylcholine and choline, but also to many other quaternary ammonium compounds, including decamethonium (47, 127, 128) and succinylcholine (129). In normal subjects, decamethonium block of moderate degree does not inhibit the depolarizing action of acetylcholine and is not reversed by it or anticholinesterase compounds, whereas decamethonium block of marked degree does inhibit the action of acetylcholine and is reversed by acetylcholine or anticholinesterase compounds. In myasthenic patients, all levels of decamethonium block are reversed by these compounds.

Anticholinesterase compounds used in the management of myasthenia gravis.—The most useful of these are the quaternary ammonium compounds, neostigmine, pyridostigmine (Mestinon), and ambenomium (Mytelase). Bis-neostigmine (BC-40) (38) and bis-pyridostigmine (BC-51, hexamarium) (130) are longer acting quaternary ammonium anticholinesterase compounds which require less frequent administration, but are less suitable for general use because of the danger of cumulation and overdose. Several organophosphorus anticholinesterase compounds have also had clinical trials in the management of myasthenia gravis. These have included DFP, TEPP, HETP, OMPA (131), echothiophate (42), and sarin (37). While these compounds are more prolonged in their action than the quaternary ammonium compounds and produce a more even and sustained increase in strength following the administration of only one or two doses a day, the danger of cumulation and overdose has precluded their general clinical use except under carefully controlled circumstances. The maximal strength obtained after optimal doses of any of these quaternary ammonium or organophosphorus anticholinesterase compounds is approximately the same (37). The compounds differ mainly in their duration of action (organophosphorus compounds > bis-neostigmine and bis-pyridostigmine > pyridostigmine and ambenomium > neostigmine) and in the severity of their parasympathomimetic side effects (organophosphorus compounds > bis-neostigmine and bis-pyridostigmine > neostigmine > pyridostigmine and ambenomium). Recently anticholinesterase compounds have also been administered by local instillation in patients with ocular myasthenia (132).

Management of overdose of anticholinesterase compound.—In the management of myasthenia gravis, graded doses of an anticholinesterase compound are administered until a maximal level of strength is attained in the affected muscles. Unfortunately, in patients with severe myasthenia the maximal strength attained may be far below normal. Increasing doses may result in no further increase in strength, and excessive drug may produce generalized weakness, attributable to the accumulation of an excessive concentration of acetylcholine at the motor end-plates and prolonged depolarization of this region, followed by refractoriness of the end-plates to acetylcholine (126). If overdose is suspected, the simplest procedure is to withhold medication for several hours and carefully observe whether the

strength increases or decreases. It is frequently helpful to observe the effect on the weakness of intravenous administration of the short-acting anticholinesterase compound, edrophonium, or of an oxime. The oximes 2-PAM, DAM, and TMB-4 are capable of reversing neuromuscular block produced by anticholinesterase compounds in myasthenic patients, as in normal subjects (62). The effect of this reversal depends on the prior action of the anticholinesterase compound, since neuromuscular function is restored toward its initial state. Whereas in normal subjects neuromuscular function and strength are returned toward normal, in myasthenic patients the effect depends on the status of the patient at the time of oxime administration. If sufficient anticholinesterase compound was administered to depress function, this is restored to a more optimal level, but if function was optimal at the time of oxime administration, it is restored toward the basal level present prior to the administration of anticholinesterase compound; i.e., it is depressed. It is therefore recommended that myasthenic patients suffering from anticholinesterase intoxication be titrated with successive doses of oxime until strength is restored to the maximal level attained following the administration of optimal doses of anticholinesterase compound.

Unfortunately, many patients with severe myasthenia who are responding poorly to anticholinesterase medication fail to improve following either expectant management or administration of oxime or edrophonium, and they may become worse after any of these measures. The main difficulty in these patients is neither insufficient nor excessive anticholinesterase medication, but a refractory or "insensitive" state of the motor end-plates to the normal depolarizing action of acetylcholine (126). The only therapeutic measure available for this condition is to maintain respiration and reduce the dosage of anticholinesterase medication until response to this medication improves (37). In one patient, prolonged administration of *d*-tubocurarine, with artificial respiration, was followed by improvement in clinical state and in responsiveness to anticholinesterase medication, presumably as a result of the complete rest afforded the end-plates (133). This observation is of interest, but the procedure is too hazardous for general use.

Myasthenic Syndrome With Carcinoma

Some patients with neoplasm, particularly small cell bronchogenic carcinoma, have muscular weakness and fatigue, most marked in the extremities (134). The syndrome resembles myasthenia gravis in symptomatology and, usually, in increased reactivity to *d*-tubocurarine and abnormal reactivity to decamethonium and succinylcholine. It differs from myasthenia gravis in that only a minority of patients respond to neostigmine (135). Furthermore, the action potential and twitch evoked by a maximal nerve stimulus is much lower in relation to the strength of voluntary contraction than in myasthenia gravis, and the degree of facilitation during or after repetitive nerve stimulation is much greater. In some patients, weakness

has appeared many months before the tumor was noted, while in others it has followed the tumor. In some patients evidence of peripheral neuropathy has occurred in the presence of neoplastic disease.

Denervation

The motor nerve appears to exert a direct restraining influence on the chemo-sensitivity of muscle, and removal of this influence is followed by supersensitivity to acetylcholine. Denervation is followed by an increase in the chemo-sensitive area beyond the end-plate region, with no increase in the sensitivity of the receptors themselves (136). A similar increase in the chemo-sensitive area occurs following exposure of muscle to botulinus toxin (137). Since this toxin prevents transmitter release from motor-nerve endings, it appears that this release determines the size of the acetylcholine-sensitive area and that the effect of denervation is caused by interruption of transmitter release. Supersensitivity to acetylcholine has also been reported in mice with hereditary myopathy (138) and in rabbits with vitamin-E-deficient myopathy (139), and has been attributed to local functional denervation.

Spastic States

Unless facilities for endotracheal intubation and artificial respiration are available, neuromuscular blocking agents such as *d*-tubocurarine are too hazardous for use in the relief of muscle rigidity or spasm. Centrally acting agents are used for this purpose, in spite of their relatively low efficacy. In parkinsonism, up to 25 per cent improvement in muscle rigidity and tremor can be achieved by the use of anticholinergic drugs such as belladonna alkaloids, trihexyphenidyl (Artane), cycrimine hydrochloride (Pagitane), procyclidine hydrochloride (Kemadrin), benztropine methanesulfonate (Cogentin), or orphenadrine (Disipal) (140). Diphenhydramine (Benadryl) may have some value as an adjunct to these drugs. Chlorpromazine (Thorazine) and Rauwolfia alkaloids are of no practical value, and may occasionally produce a parkinsonian-like state. Muscle spasticity, whether central or peripheral in origin, may be temporarily alleviated by the intravenous administration of mephanesin (Myanesin) or its congener methocarbamol (Robaxin), but is usually little affected by the oral administration of these drugs, or meprobamate (Equanil, Miltown) or zoxazolamine (Flexin).

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