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A COMPARISON BETWEEN THE EFFECTS OF EDROPHONIUM AND CHOLINE IN THE SKELETAL MUSCLES OF THE CAT

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The effects of edrophonium and choline have been compared with those of the depolarizing substances acetylcholine, decamethonium, and suxamethonium, in both innervated and chronically denervated tibialis anterior muscles of cats under chloralose anaesthesia. Both edrophonium and choline were more potent antagonists to paralysis by tubocurarine than could be accounted for by their ability to stimulate the motor end-plates directly. It appeared likely that direct depolarization of the end-plate played no part in the anti-curare action of edrophonium and only some part in the anti-curare action of choline. A paralysis produced by the neuromuscular blocking agent, benzoquinonium, was more readily antagonized by a tetanus or by acetylcholine, suxamethonium, and decamethonium than a similar paralysis produced by tubocurarine. The tetraethyl ammonium ion was also slightly more effective against a paralysis by benzoquinonium. On the other hand, edrophonium was about 300 times and choline about five times less potent as an antagonist to benzoquinonium than to tubocurarine. Furthermore, the previous administration of benzoquinonium abolished the antagonistic action to tubocurarine of normally effective doses of edrophonium and reduced that of choline. These results were similar to those previously obtained with neostigmine, physostigmine and ethyl pyrophosphate and suggested that there was some similarity in the mechanism of action of all of these substances. Benzoquinonium, therefore, showed promise as a useful pharmacological tool for distinguishing compounds with this particular type of action. These anti-curare compounds did not appear to act by cholinesterase inhibition, nor by an increase in the sensitivity of the motor end-plates. In common with other workers, we suggest that there is a pre-synaptic mechanism of action.

The mode of action of the anti-curare substance edrophonium has not been fully established, but several mechanisms have been suggested, namely inhibition of cholinesterase (Hobbiger, 1952; Smith, Cohen, Pelikan, and Unna, 1952; Nastuk and Alexander, 1954; Katz and Thesleff, 1957), a direct acetylcholine-like action at the motor end-plates (Wescoe and Riker, 1951; Riker, 1953), and finally a direct action on the nerve endings (Riker, Roberts, Standaert, and Fujimori, 1957; Riker, Werner, Roberts, and Kuperman, 1959). According to Riker *et al.* (1957, 1959), edrophonium reacts with membrane receptors in the nerve endings causing the motor nerve to fire repetitively and thus increasing the transmitter action. A somewhat similar pre-synaptic mechanism of action had previously been proposed by Hutter (1952a) to account for part of the action of choline at the neuromuscular junction.

It was therefore decided to re-examine and compare the properties of edrophonium and choline in the hope that further light might be shed upon their mechanism of action.

METHODS

Cats, anaesthetized with chloralose (80 mg./kg. body weight) injected into the subcutaneous vein of the forelimb, were used throughout the experiments. For the experiments on innervated muscles, shielded silver electrodes were placed on the sciatic nerve of one hind-limb and the nerve was ligated centrally to the electrodes. The limb was set up in a horizontal position on a Brown-Schuster myograph stand, and the tendon of the tibialis anterior muscle was attached to a flat steel spring myograph. Twitches and tetani, which were recorded on smoked paper, were excited by rectangular pulses of 0.2 msec. duration and of twice the strength required to evoke a maximal twitch. For experiments on denervated muscle, the sciatic nerve was divided under sodium pento-

barbitone anaesthesia and degeneration was allowed to proceed for from 17 to 19 days.

Drugs were injected intravenously or close-arterially. For close-arterial injections the preparation described by Brown (1938) was used; all such injections were 0.2 ml. in volume. The drugs used were edrophonium chloride, choline chloride, acetylcholine chloride, tetraethylammonium chloride, suxamethonium chloride, and decamethonium iodide. The solutions were made in 0.9% saline. The doses quoted in the text refer to the quantity of the drug calculated as base. At the beginning of all experiments, atropine sulphate (1 mg./kg.) was administered intravenously.

Results

Stimulant Action

In the unstimulated innervated muscle, close-arterial injection of edrophonium (15 to 100 μg.), choline (0.3 to 1 mg.), decamethonium (0.3 to 1 μg.), and suxamethonium (0.5 to 1.5 μg.) caused, after a short latent period, marked fasciculations of the muscle. Larger amounts of choline (2 to 3 mg.), decamethonium (2 to 5 μg.), and suxamethonium (3 to 5 μg.) caused a quick contraction followed by an immediate relaxation. An increase of the same order in the dose of edrophonium (0.15 to 0.2 mg.) caused a quick contraction of the muscle, but this was followed by only partial relaxation after which powerful fasciculations occurred. With still larger doses (0.3 mg. and above) edrophonium caused a quick contraction which now was followed by a complete relaxation. With acetylcholine (1 μg. or more) a quick contraction followed by immediate relaxation was the only effect seen. Fig. 1 illustrates some of these results. Table I shows a comparison of the doses of all five substances necessary to produce a quick contraction of the muscle approximately equivalent to 50% of the maximal twitch tension.

TABLE I

RELATIVE POTENCIES WITH REFERENCE TO DECAMETHONIUM

The estimates are based on: (a) the doses required to produce an immediate contraction equivalent to about half the maximal twitch tension; (b) the doses required to produce contractures equivalent to about half the maximum; and (c) the smallest doses necessary to produce a distinct decurarization.

	Deca-methonium	Acetyl-choline	Suxa-methonium	Choline	Edro-phonium
(a) Innervated muscles ..	1	0·8–1	1·6	900–1,200	28–40
(b) Chronically denervated muscles ..	1	0·03–0·07	1·7	250–300	15–24
(c) Antagonism to tubocurarine	1	150–200	1·5–2	140–180	1·25–1·8

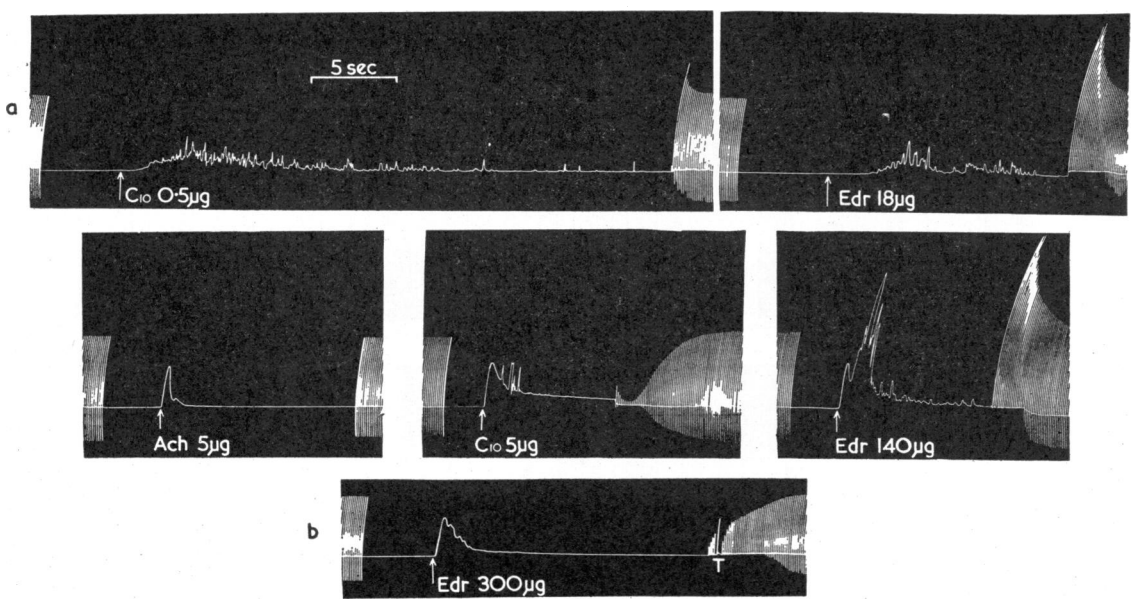

FIG. 1.—a, Cat 3.8 kg.; b, cat 3.1 kg. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. Shortly before each injection, electrical stimulation was stopped and the kymograph speed increased. At C_{10}, decamethonium, at Edr, edrophonium, and at Ach, acetylcholine were injected close-arterially. At T, the sciatic nerve was stimulated at a frequency of 50/sec. for 15 sec.

In the chronically denervated muscle, choline and edrophonium, like decamethonium, suxamethonium and acetylcholine, caused a contracture on close-arterial injection. This effect of edrophonium was demonstrated by Randall (1950). A comparison of the potencies of all five substances is illustrated in Fig. 2. Table Ib shows the combined results of five experiments in which the doses required to produce contractures equivalent to approximately 50% of the maximum were determined.

It is well known that the sensitivity of skeletal muscles to many quaternary ammonium compounds, particularly to acetylcholine, is increased after chronic denervation. In the present experiments, the denervated tibialis anterior muscle became more sensitive to all four stable compounds but to different degrees. The increase in sensitivity was equal for decamethonium and suxamethonium but much greater for edrophonium and choline.

Effects on Maximal Twitch

Small doses of edrophonium cause potentiation of the maximal twitch, while large doses cause neuromuscular block (Randall, 1950). These results were confirmed in the present experiments. There was a striking difference, however, between the effects of edrophonium on the one hand and those of decamethonium, suxamethonium, and choline on the other. Edrophonium caused potentiation of the maximal twitch in doses as low as 2 to 3 μg. administered close-arterially, such doses being five to 10 times smaller than those necessary to cause fasciculations of the resting muscle. Furthermore, potentiation of the twitch tension, without any secondary depression, occurred over a wide range of doses (from 2 to 200 μg.), and only very large doses caused neuromuscular block (Fig. 1). Choline caused potentiation of the maximal twitch in doses approximately half the size of those required to cause fasciculations. The doses of decamethonium and suxamethonium necessary to produce potentiation of the maximal twitch were of the same order as those which caused fasciculations in the resting muscles. In contrast to edrophonium, the range of doses over which choline, decamethonium, and suxamethonium produced only potentiation was narrow, the onset of neuromuscular block occurring with doses only three to four times greater than the smallest necessary to cause potentiation.

Although all four substances caused neuromuscular block, its characteristics were not the same. During partial paralysis produced by edrophonium, tetanic tension was not sustained, and after the tetanus the paralysis was temporarily deepened (Fig. 1b). On the other hand, as Hutter (1952a) showed, paralysis produced by choline resembled that produced by decamethonium and suxamethonium; during the block, tetanic tension was well maintained, and after the tetanus transmission was neither restored nor further depressed.

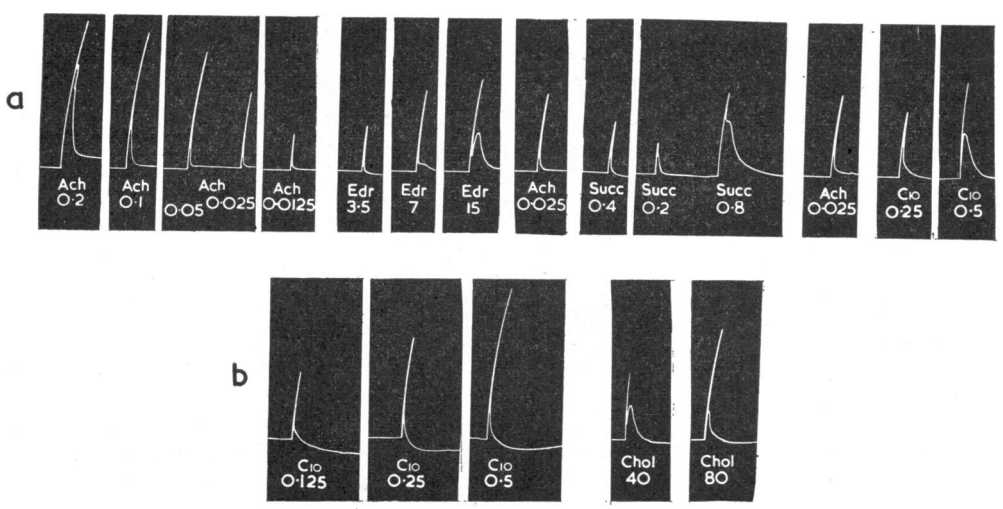

FIG. 2.—a, Cat 3.6 kg.; b, cat 2.8 kg. Chronically denervated tibialis anterior muscle (18 days). At Ach, acetylcholine; at Edr, edrophonium; at Succ, suxamethonium; at C_{10}, decamethonium; at Chol, choline. All injections were made close-arterially. The numerals denote the doses of the drugs in μg.

Anti-curare Action

Sufficient tubocurarine was administered intravenously to produce an 80 to 90% paralysis of the indirectly excited twitches and, at the peak of the blockade, choline, edrophonium, acetylcholine, suxamethonium, or decamethonium was administered close-arterially. Table Ic gives the ratios of the smallest doses capable of causing a distinct decurarization. There was little difference in the minimal effective anti-curare doses of edrophonium, suxamethonium, and decamethonium (Table I; Figs. 3 and 5). This was in marked contrast to their relative abilities to stimulate the resting innervated muscle, in which edrophonium was about 35 times less potent than decamethonium and suxamethonium.

The same was true for choline which, if compared with decamethonium and suxamethonium, possessed about 1/150 to 1/200 of their potency as anti-curare agents but was only about 1/1,000 as active in its ability to stimulate the resting muscle (Table Ia and c).

The results obtained with acetylcholine confirmed those of Hutter (1952a), who showed that large doses were required to antagonize the action of tubocurarine, and that the substance was, in fact, no more effective than a chemically equivalent amount of choline (Fig. 5).

Interaction with Benzoquinonium

Benzoquinonium is a potent neuromuscular blocking drug, very similar in most of its actions to tubocurarine (Hoppe, 1950, 1951; Bowman, 1958). There is, however, one important difference; in the cat, the blockade produced by benzoquinonium is not antagonized by the usual effective anti-curare doses of edrophonium (Randall, 1951). After the administration of benzoquinonium, the ability of normally effective doses of edrophonium to antagonize tubocurarine is abolished (Bowman, 1958). In the present experiments it was found that very large doses of edrophonium (0.5 to 1 mg. close-arterially) did antagonize the blockade produced by benzoquinonium although the antagonism was always preceded by a slight increase in the depth of paralysis (Fig. 3).

Choline to some extent resembled edrophonium in its interactions with benzoquinonium. The substance was more effective in antagonizing paralysis produced by tubocurarine than that due to benzoquinonium. Furthermore, the antagonism by choline of paralysis due to tubocurarine was reduced by the previous administration of benzoquinonium (Fig. 4).

There were, however, quantitative differences in the actions of edrophonium and choline. Paralysis produced by benzoquinonium was only antagonized by edrophonium in doses 300 times greater than those necessary to produce a similar degree of antagonism to tubocurarine (Fig. 3), and a single intravenous dose of benzoquinonium could completely prevent, for periods up to 2 hr., the anti-curare action of a previously effective dose of edrophonium (Bowman, 1958). Choline, on the other hand, was only about five times less

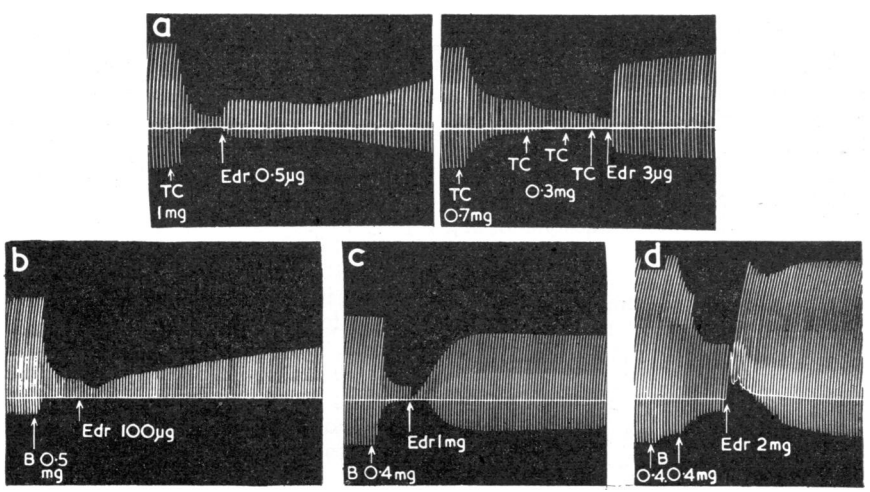

FIG. 3.—a, Cat 3.7 kg.; b, cat 3.2 kg.; c, cat 3.6 kg.; d, cat 4.1 kg. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. At TC, tubocurarine, and at B, benzoquinonium, was injected intravenously. At Edr, edrophonium was injected close-arterially.

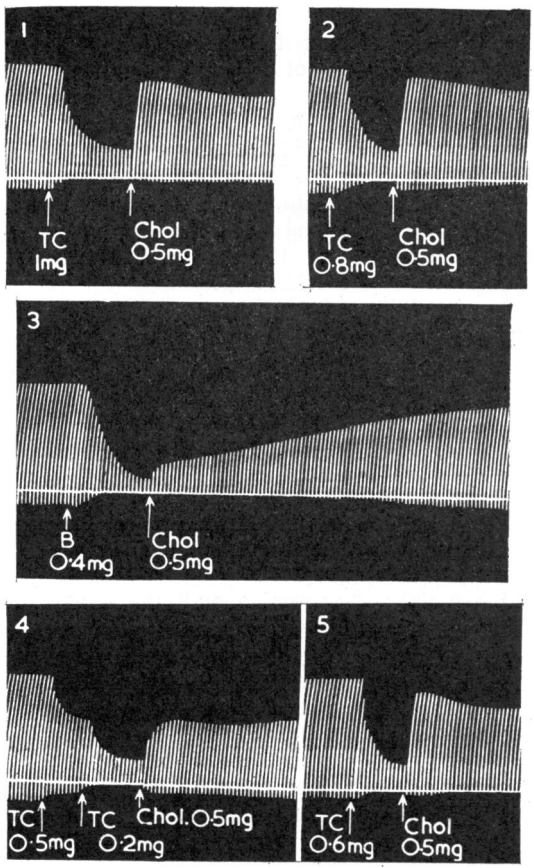

FIG. 4.—Cat 3.4 kg. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. At TC, tubocurarine, and at B, benzoquinonium was injected intravenously. 45 min. elapsed between injections of the blocking agents. At Chol, choline was injected close-arterially.

effective against benzoquinonium than against tubocurarine and its anti-curare effect was never completely abolished after benzoquinonium.

In contrast to edrophonium and choline, tetanic stimulation or the administration of acetylcholine, suxamethonium, or decamethonium was more effective in overcoming paralysis produced by benzoquinonium than by tubocurarine (Fig. 5). These results emphasize again the difference between the actions of choline and edrophonium on the one hand, and those of the purely depolarizing substances on the other. Small doses of acetylcholine were much more effective against benzoquinonium paralysis than against tubocurarine paralysis (Fig. 5), the minimal effective doses being of the order of 15 μg. and 80 μg. respectively. This difference may partly be accounted for by the anticholinesterase action of benzoquinonium (Hoppe, 1951) which, by preventing the hydrolysis of acetylcholine in the blood stream, will allow a greater quantity of that injected to reach the motor end-plates. Large doses of acetylcholine were slightly less effective against benzoquinonium than against tubocurarine (Fig. 5d), possibly because the antagonistic action of large doses of acetylcholine is mainly due to choline itself. Since this latter compound is relatively weak in antagonizing benzoquinonium, this result is not surprising.

Interaction with Injected Acetylcholine

In experiments to study the effects of choline and edrophonium on the response of the muscle to acetylcholine, physostigmine was used for comparison. Maximal twitches were elicited by motor nerve stimulation once every 10 sec. At intervals electrical stimulation was temporarily stopped, and a constant amount of acetylcholine, sufficient to cause a submaximal contraction, was administered close-arterially. After three or four control responses, edrophonium, choline, or physostigmine, in doses known to be sufficient to cause an increase in twitch tension, was administered intravenously. A typical experiment with edrophonium and physostigmine is illustrated in Fig. 6a. While physostigmine always potentiated the response to acetylcholine, edrophonium and choline did not affect it or even slightly depressed it. After physostigmine, the increase in twitch tension produced by edrophonium and choline was always greater, but in spite of this the acetylcholine response was still unaffected.

In a further series of experiments, a similar procedure was carried out except that a partial paralysis of the indirectly excited maximal twitches was maintained throughout by a continuous intravenous infusion of tubocurarine. In the experiment illustrated by Fig. 6b, the amount of acetylcholine had to be increased more than ten-fold during the tubocurarine infusion in order to obtain a response. Edrophonium, injected intravenously, caused an almost complete, though temporary, return of the twitch tension to normal. It did not, however, increase the response to injected acetylcholine. Physostigmine, on the other hand, more than doubled the acetylcholine response. Choline gave similar results to edrophonium.

Synergism with Decamethonium

In addition to having an anticholinesterase action, physostigmine and neostigmine have been

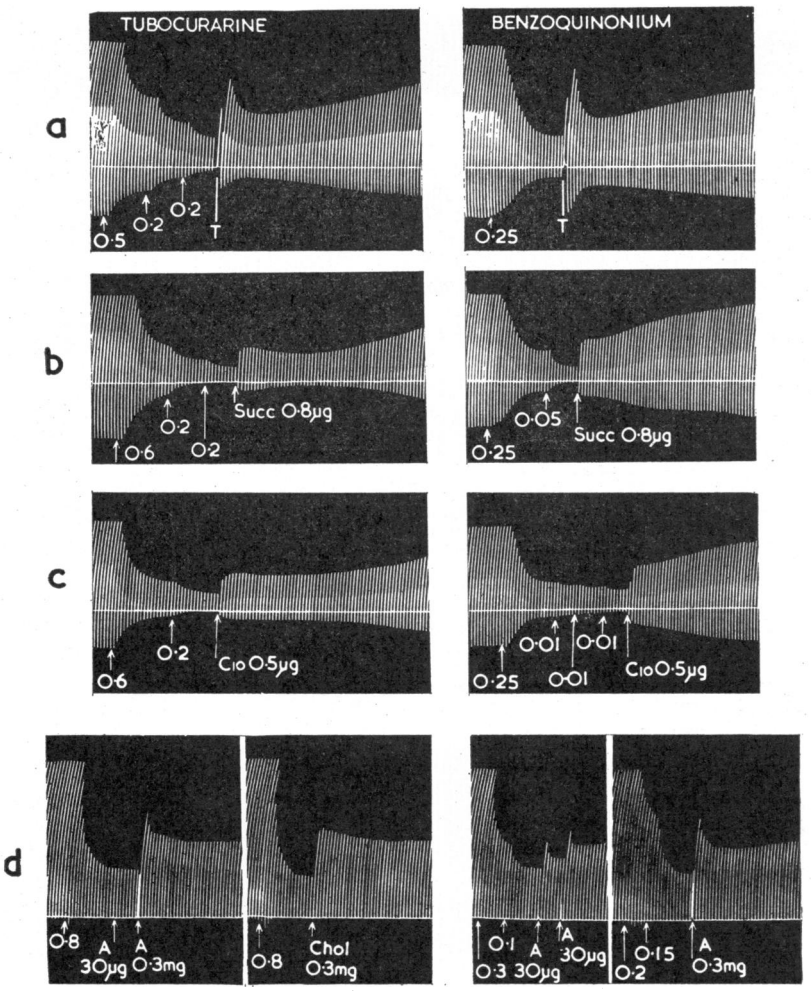

FIG.5.—a, Cat 3.8 kg.; b, cat 3.1 kg.; c, cat 3.9 kg.; d, cat 4.3 kg. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. Records on the left are of paralysis produced by tubocurarine and on the right by benzoquinonium. The unlabelled numerals denote the doses of the blocking agents in mg. administered intravenously. At T, the sciatic nerve was stimulated at a frequency of 50/sec. for 4 sec. At Succ, suxamethonium, at C_{10}, decamethonium, at A, acetylcholine, and at Chol, choline were administered close-arterially.

shown to potentiate the effects of stable choline esters and other quaternary ammonium bases, apparently by increasing non-specifically the excitability of the effector cells (Brown and Harvey, 1941; Zaimis, 1951; Cohen and Posthumus, 1955). In the present study the doses of edrophonium and choline used did not, as already described, potentiate the response to injected acetylcholine. These results provide evidence that the effects of edrophonium and choline cannot be due to an increase in the excitability of the motor end-plates. Nevertheless, some synergism was shown to exist between choline and edrophonium on the one hand, and decamethonium on the other, for the previous administration of a subthreshold amount of either of the former compounds increased the stimulant action of decamethonium. This synergism, however, need not be a consequence of a sensitization of the motor end-plates by edrophonium and choline; it might simply be an additive effect either between decamethonium and acetylcholine, accumulating in the presence of edrophonium, or between choline and

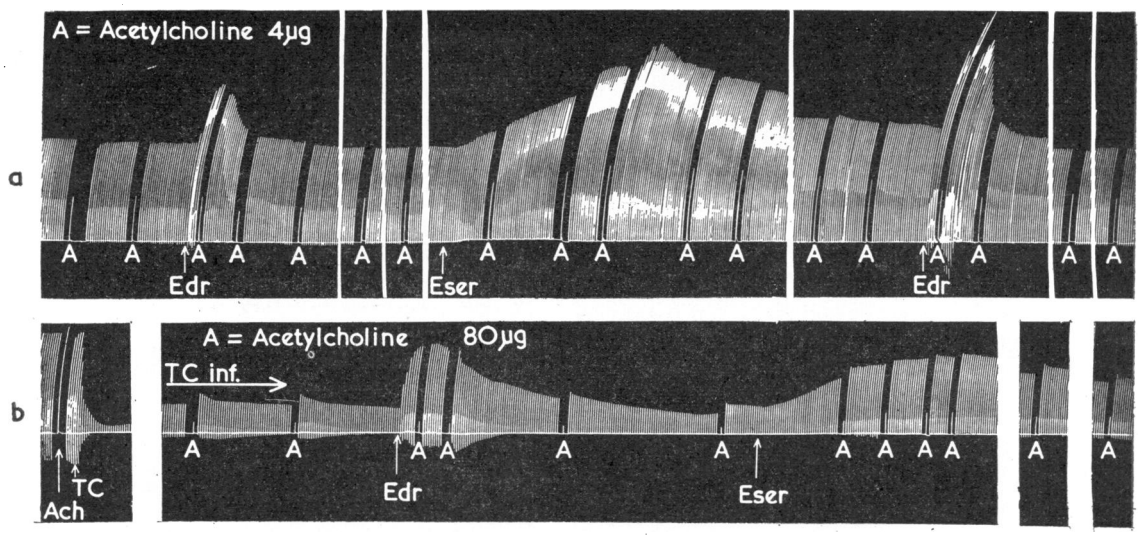

FIG. 6.—a, Cat 3.9 kg.; b, cat 3.5 kg. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. At Edr, 0.5 mg. of edrophonium, at Eser, 1 mg. of physostigmine, at TC, 1 mg. of tubocurarine, and at Ach, 8 μg. acetylcholine were given. The injections were made intravenously, except those of acetylcholine, which were administered close-arterially. In b, during the recovery from the effect of the single injection of tubocurarine, a continuous intravenous infusion of tubocurarine (1.7 mg./hr.) was started and maintained throughout the rest of the experiment.

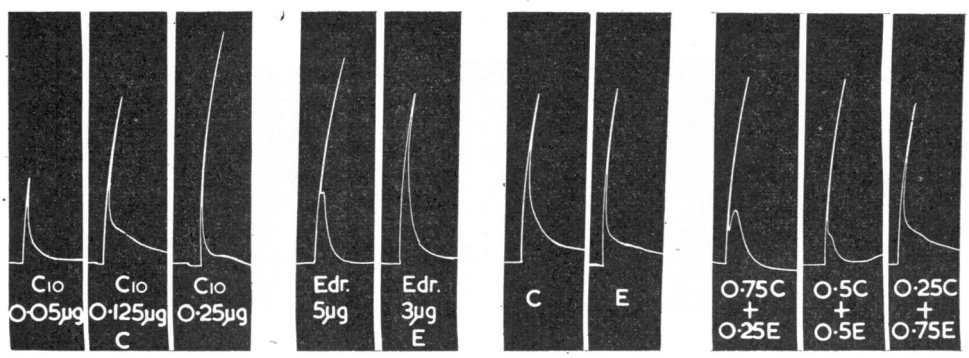

FIG. 7.—Cat 3.7 kg. Chronically denervated tibialis anterior muscle (19 days). At C_{10}, decamethonium, and at Edr, edrophonium were injected close-arterially. C and E were selected as the control doses of decamethonium and edrophonium respectively.

decamethonium, both compounds possessing a direct action on the motor end-plates. Experiments were therefore carried out on chronically denervated muscles from which acetylcholine has disappeared, to determine whether the synergism was due to sensitization of the effector cell. The method was based on that of Gaddum (1959). The quantity of decamethonium necessary to produce a contracture approximately equivalent in tension to 50% of the maximum was determined. The dose, either of edrophonium or of choline, necessary to reproduce this effect was then found, and finally mixtures containing the following proportions of these control doses were prepared: decamethonium:choline or edrophonium; 50%:50%, 25%:75%, 75%:25%. These mixtures were then injected and the responses compared with those elicited by the control doses of each compound alone. Responses to the mixtures similar to those of the control doses would indicate a simple additive effect between the compounds while markedly greater responses would indicate true potentiation.

The responses to mixtures of the compounds containing 50% or more of the control dose of decamethonium showed only a slight increase

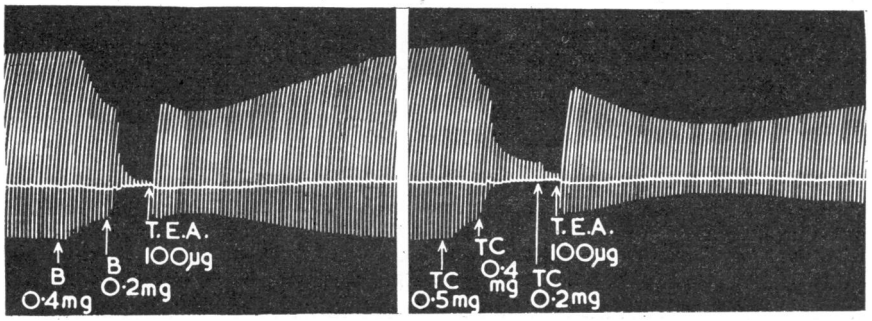

FIG. 8.—Cat 3.9 kg. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. At B, benzoquinonium, and at TC, tubocurarine were injected intravenously. At T.E.A., tetraethylammonium was injected close-arterially.

over the responses elicited by each substance alone, while mixtures containing only 25% often had slightly less action than the control. Fig. 7 illustrates a typical experiment with edrophonium and decamethonium. Synergism between these compounds in the denervated muscle was therefore mainly an additive effect, any true potentiating action being very weak. These results further support the conclusion that an increase in the excitability of the effector cell is unlikely to play a major part in the effects of edrophonium and choline in the skeletal muscles of the cat.

Action of Tetraethylammonium

Stovner (1957a, b, and c; 1958a, b, and c) and Koketsu (1958) have studied the striking anti-curare effect of the tetraethylammonium ion, and concluded that it possesses a pre-synaptic action through which the amount of acetylcholine liberated by each nerve impulse is increased. Because of this, the action of tetraethylammonium on blockade produced by benzoquinonium was studied. Tetraethylammonium was found to be at least as effective in antagonizing paralysis produced by benzoquinonium as that produced by tubocurarine (Fig. 8) showing that its action differs from that of edrophonium and choline. Thus, while all three substances may increase the amount of transmitter liberated by the nerve, there must be some difference in the way in which edrophonium and choline on the one hand, and tetraethylammonium on the other, initiate this effect. Stovner (personal communication) has recently reached the same conclusion.

DISCUSSION

All the effects produced by decamethonium and suxamethonium at the neuromuscular junction are apparently due to a single mechanism of action, namely depolarization of the motor end-plates. For this reason there is little difference between the doses necessary to cause fasciculations of resting muscle, potentiation of the maximal twitch or antagonism to tubocurarine. In the present experiments it was found that the range of doses which produce only potentiation of the maximal twitch, without subsequent blockade, was narrow, and that doses large enough to cause an immediate contraction of the resting muscle were always followed by neuromuscular block.

Edrophonium, on the other hand, caused potentiation of the maximal twitch and antagonism to tubocurarine in doses considerably smaller than those necessary to cause fasciculations of the resting muscle, and the range of doses over which only potentiation of the maximal twitch occurred was extremely wide. Furthermore, even in doses big enough to cause an immediate contraction of the resting muscle, edrophonium did not cause neuromuscular block and the response was usually followed by powerful fasciculations. Extremely large doses were needed to produce neuromuscular block. These results suggest that the direct action of edrophonium on the motor end-plates is very weak and that its effects, except when very large doses are used, must be mediated mainly through acetylcholine. Further evidence that the direct depolarizing action of edrophonium is very weak was obtained from the experiments from benzoquinonium. Acetylcholine, decamethonium, and suxamethonium were effective in antagonizing paralysis produced by benzoquinonium or by tubocurarine, whereas edrophonium antagonized benzoquinonium only in doses at least 300 times greater than those necessary to antagonize tubocurarine. These results agree with those of other workers (Hobbiger, 1952; Smith *et al.*,

1952; Nastuk and Alexander, 1954), who have shown that direct end-plate stimulation plays little, if any, part in the anti-curare action of edrophonium.

The effect of choline appeared to be a combination of an acetylcholine-like action at the motor end-plate and of an edrophonium-like action. It resembled decamethonium and suxamethonium in the normally innervated non-curarized muscle, but its ability to antagonize a tubocurarine paralysis was about five times more powerful than would be expected if the effect were entirely due to a direct action.

Similarity between the actions of edrophonium and choline is apparent also from experiments on chronically denervated muscle. The chronically denervated tibialis anterior muscle gave a contracture-like response to both edrophonium and choline. However, the increase in sensitivity of the denervated muscle to these substances was found to be considerably greater than that to both decamethonium and suxamethonium. This is a puzzling result taking into consideration the weak depolarizing action of edrophonium and choline, but two explanations may be suggested. The response of the denervated muscle to these two substances may not be a true contracture like that produced by acetylcholine and similar drugs (Brown, 1937; Zaimis, 1951). For example, adrenaline has been shown to increase the tension of chronically denervated muscles (Bülbring and Burn, 1936), but, during a study of its effects in various skeletal muscles, it was found that the increase in tension of the denervated muscle due to adrenaline is accompanied by electrical events different from those accompanying the contracture produced by depolarizing drugs (Bowman and Zaimis, unpublished observation). Thus edrophonium and choline may have a different action from acetylcholine in denervated muscle. An alternative explanation might be that degeneration of the motor nerve following denervation results in the destruction of receptor sites to edrophonium and choline. Thus, after denervation, more molecules may be available for an action on the muscle fibre itself.

In the present experiments, choline was shown to be about five times less effective as an antagonist to paralysis produced by benzoquinonium than to that produced by tubocurarine. Furthermore, the previous administration of benzoquinonium was shown to reduce the ability of choline to antagonize a blockade produced by tubocurarine. It was previously shown (Bowman, 1958) that benzoquinonium can completely abolish the anti-curare actions of edrophonium, neostigmine, physostigmine, and ethyl pyrophosphate. These results suggest that a similar mechanism of action may account for the effects of the last four compounds and for part of that of choline.

Masland and Wigton (1940) and Boyd and Martin (1956) have shown that, in addition to its ability to inhibit cholinesterase, neostigmine possesses an action on the motor nerve endings. More recently Riker *et al.* (1957, 1959) concluded that an action on the nerve endings, through which neuromuscular transmission is facilitated, is the main mechanism of action of compounds of the edrophonium type. Hutter (1952a) suggested a similar mechanism of action for part of the effect of choline. An action on the motor nerve endings through which the amount of acetylcholine released by a nerve impulse is increased would explain the present results obtained with edrophonium as well as those previously obtained with neostigmine, physostigmine, and ethyl pyrophosphate. It would also account for part of the action of choline. That the previous administration of benzoquinonium prevented the anti-curare action of edrophonium, neostigmine, physostigmine, and ethyl pyrophosphate, and reduced that of choline, could be explained if, as previously suggested (Bowman, 1958), benzoquinonium prevents the action of these substances on the motor nerve endings. This effect of benzoquinonium must be independent of its curare-like effect on the motor end-plate, firstly because tubocurarine and other curare-like substances do not produce it, and secondly because substances stimulating the motor end-plate directly are still active in the presence of benzoquinonium. Benzoquinonium therefore shows promise as a useful pharmacological tool for distinguishing compounds with an edrophonium-like action. It does not prevent the anti-curare action of substances which act by direct stimulation of the motor end-plates; nor does it affect that of the tetraethylammonium ion.

Both *in vitro* and *in vivo* edrophonium possesses only a very weak anticholinesterase action (Randall and Lehmann, 1950; Randall, 1950; Macfarlane, Pelikan and Unna, 1950; Hobbiger, 1952). Nevertheless, in frog muscle this substance potentiates the effects of acetylcholine (Smith *et al.*, 1952; Hobbiger, 1952; Cohen and Posthumus, 1955; Katz and Thesleff, 1957). Such an action appears unlikely to play a major part in the effects of edrophonium in the cat for, in the present experiments, doses which strongly potentiated the maximal twitch or antagonized tubocurarine did not potentiate the

response to injected acetylcholine. Similarly, choline was shown to have no potentiating action on the response to acetylcholine.

In a previous paper (Bowman, 1958) experiments were described in which an initial dose of tubocurarine, sufficient to produce an 80 to 90% paralysis of the indirectly excited maximal twitches, had been administered intravenously. Four hours later edrophonium, neostigmine, physostigmine, or ethyl pyrophosphate had been administered in a dose large enough to produce a marked increase in twitch tension. During this potentiation, either the same dose of tubocurarine or an equipotent dose of benzoquinonium had been administered. Under these conditions the effect of the second injection of tubocurarine had been much reduced, but that of the benzoquinonium was unaffected. This was so despite the fact that acetylcholine is a more effective antagonist to benzoquinonium than to tubocurarine. Since the anti-curare substance had been administered before the benzoquinonium, such experiments ruled out the possibility that the lack of antagonism to benzoquinonium had been due to its preventing, in some way, the combination between anti-curare substance and cholinesterase. These results, therefore, suggested that the increase in twitch tension and the antagonism to tubocurarine produced by these compounds is not primarily a consequence of an accumulation of acetylcholine, for if this were the explanation the blocking effect of benzoquinonium should also have been much reduced. It is an established fact that neostigmine, physostigmine, and ethyl pyrophosphate inhibit the cholinesterase at the neuromuscular junction, but from the present results it seems that the small amounts of acetylcholine that are released, either spontaneously or as the result of a single nerve impulse delivered every 10 sec., even though protected from destruction, must simply diffuse rapidly away from the end-plate region. It would appear, therefore, that an anticholinesterase action cannot by itself account for the antagonistic action of a substance to paralysis produced by tubocurarine. Once the output of acetylcholine is increased by the effect of the drug on the motor nerve endings, any additional ability of the compound to inhibit cholinesterase may become important in the non-curarized muscle, for, by preserving the acetylcholine beyond the refractory period of the muscle fibre, sufficient may remain in the region of the motor end-plates long enough to facilitate repetitive firing.

The main effect of the anticholinesterase drugs, of edrophonium and part of that of choline at the neuromuscular junction appears, therefore, very similar to that following an indirectly elicited tetanus. Several workers (Hutter, 1952b; Liley and North, 1953; Del Castillo and Katz, 1954; Liley, 1956a and b) have presented evidence that, following high frequency stimulation of the motor nerve, both the spontaneous release of acetylcholine and that liberated by single shocks is increased. The results of Hutter (1952a) with choline led him to suggest (Hutter, 1952b) that the increase in acetylcholine output following a tetanus might be a consequence of the action of choline accumulated at the neuromuscular junction during the repetitive stimulation. Choline, however, is a very inactive compound, and it appears unlikely that the local concentration would ever reach a level sufficient to produce such an effect. Furthermore, the present results showed that, while a tetanus was slightly more effective in antagonizing benzoquinonium than tubocurarine, choline was much less effective against the former blocking agent.

Riker *et al.* (1957) concluded from their results that not only the action of edrophonium but also that of acetylcholine itself is mainly on the motor nerve endings. According to them, on the arrival of an impulse at the nerve ending "acetylcholine is released from a bound state in the motor nerve terminal and reacts with pre-synaptic receptors to initiate the transmitter effect." The present results, however, may be adequately explained on the basis of the classical theory of chemical transmission. Furthermore, the results rule out the possibility that edrophonium and acetylcholine have the same site of action since acetylcholine was very effective in antagonizing benzoquinonium paralysis, while edrophonium was virtually ineffective.

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