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THE NEUROMUSCULAR BLOCKING ACTION OF BENZOQUINONIUM CHLORIDE IN THE CAT AND IN THE HEN

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Benzoquinonium (Mytolon) has been shown to produce a curare-like rather than a decamethonium-like paralysis of neuromuscular transmission in the tibialis anterior and soleus muscle of cats and the gastrocnemius muscle of hens. In the cat, but not in the hen, benzoquinonium had an additional action in preventing both the twitch potentiating action and the anti-curare action of edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate. Paralysis produced by benzoquinonium was antagonized by injected acetylcholine and by acetylcholine liberated by tetanic stimulation of the motor nerve but, in the cat, anticholinesterases were without effect even when they were first administered in circumstances in which acetylcholine accumulation might be expected to occur. These findings suggested that inhibition of cholinesterase played little part in the skeletal muscle effects of the anticholinesterases studied. Although anticholinesterases did not antagonize benzoquinonium paralysis in the cat, they nevertheless potentiated the antagonistic action of injected acetylcholine.

The muscle-paralysing action and other pharmacological properties of benzoquinonium, *p*-benzoquinone-2:5-bis-N-(3-aminopropyl)-benzyl-diethylammonium chloride, were originally investigated by Hoppe (1950, 1951) and the compound subsequently received clinical trial as a muscle relaxant in conjunction with surgical anaesthesia (Arrowood, 1951; Foldes, 1951; Dundee, Gray, and Rees, 1952; Hunter, 1952; Gordon, 1953).

The mechanism of its blocking action has not been fully established. In the frog, mouse, rabbit, dog (Hoppe, 1951), and man (Arrowood, 1951) it has been shown to produce effects similar to those of tubocurarine which interrupts neuromuscular transmission by raising the threshold of the motor end plates to acetylcholine. In the cat, however, its action is believed to resemble that of the depolarizing substance, decamethonium. This latter belief is based mainly on the observations of Randall (1951), who showed that, in the cat, paralysis produced by benzoquinonium, like that produced by decamethonium, is not antagonized but actually slightly increased by the administration of the anti-curare substance, edrophonium.

From the work of Zaimis and her colleagues (Buttle and Zaimis, 1949; Paton and Zaimis, 1952; Jewell and Zaimis, 1954; Zaimis, 1953, 1954, 1956–7), it is clear that if there is a depolarizing element present in the action of a neuromuscular blocking agent, it will be more evident in the tibialis anterior muscle of the cat and in certain avian muscles than in the muscles of most other species. The cat and the hen were therefore used in the present experiments in which the effects of benzoquinonium chloride were compared more fully with those of tubocurarine chloride and decamethonium iodide in an attempt to throw further light on its mode of action.

METHODS

Cats.—Cats were anaesthetized with chloralose (80 mg./kg.) injected into the subcutaneous vein of the fore-limb or into the internal saphenous vein. A hind limb was set up in a horizontal position on a Brown-Schuster myograph stand. Shielded silver electrodes were placed on the sciatic nerve and the nerve was ligated central to the electrodes. Twitches and tetani of the tibialis anterior and soleus muscles were excited by rectangular pulses of 0.2 msec. duration and of twice the strength required to evoke a maximal twitch. The muscles were attached to flat steel springs and their contractions recorded on a smoked paper. For experiments on denervated muscles, the sciatic nerve on one side was divided

high in the thigh under sodium pentobarbitone anaesthesia. Degeneration was allowed to proceed for from 14 to 21 days after which the animal was anaesthetized with chloralose as described above. Direct stimuli of maximal strength and of 0.5 msec. duration were applied between the tendons of the muscles and the drill in the femur. Electrical contact with the tendons was made through shielded silver wires attached to saline-soaked pads of cotton wool. In order to ensure that the current supplied for direct stimulation was not short-circuited through the other tissues, the muscles were insulated by enclosing their bellies in rubber finger-stalls smeared with liquid paraffin. Drugs were injected intravenously through a cannula in the jugular vein or intra-arterially through a cannula in the cut central end of a branch of the femoral artery. When close-arterial injections to the tibialis anterior muscle were made, the method described by Brown (1938) was used.

Hens.—Hens were anaesthetized by the slow injection of pentobarbitone into a wing vein. When a sufficient depth of anaesthesia was reached (usually after the injection of about 40 mg./kg.) a similar quantity of pentobarbitone was injected intramuscularly. Twitches and tetani of the gastrocnemius muscle were elicited indirectly in the same way as in the experiments on cats. Drugs were injected intravenously through a cannula in the jugular vein.

Solutions of all drugs were made up in 0.9% w/v NaCl solution.

Results

After the administration of several doses of benzoquinonium, a gradual fall in blood pressure occurred and death occasionally ensued due to circulatory collapse. These effects were prevented by previous atropinization, and for this reason atropine (1 to 2 mg./kg.) was administered intravenously at the beginning of many of the experiments. Such treatment did not, however, alter the results to be described. Similar circulatory effects were described in man by Dundee *et al.* (1952).

In the cat, benzoquinonium chloride administered intra-arterially in doses of 30 to 50 μg. or intravenously in doses of 0.25 to 0.3 mg./kg. caused a 90 to 100% paralysis of the indirectly excited maximal twitches of the tibialis anterior and soleus muscles. In all the experiments benzoquinonium was approximately twice as potent by weight as tubocurarine. Like tubocurarine (Paton and Zaimis, 1951), benzoquinonium paralysed the soleus to a slightly greater extent than the tibialis anterior muscle (Fig. 7*a*) and paralysis of the respiratory muscles accompanied the effect on the limb muscles. In contrast, decamethonium had little effect on the soleus and respiratory muscles in doses which produced a 90 to 100% paralysis of the tibialis anterior (see also Paton and Zaimis, 1951). Potentiation of the maximal twitch and muscle fasciculations, such as are observed after injection of decamethonium (Paton and Zaimis, 1949), never followed the administration of benzoquinonium. During complete block of the indirectly excited maximal twitches, the muscles

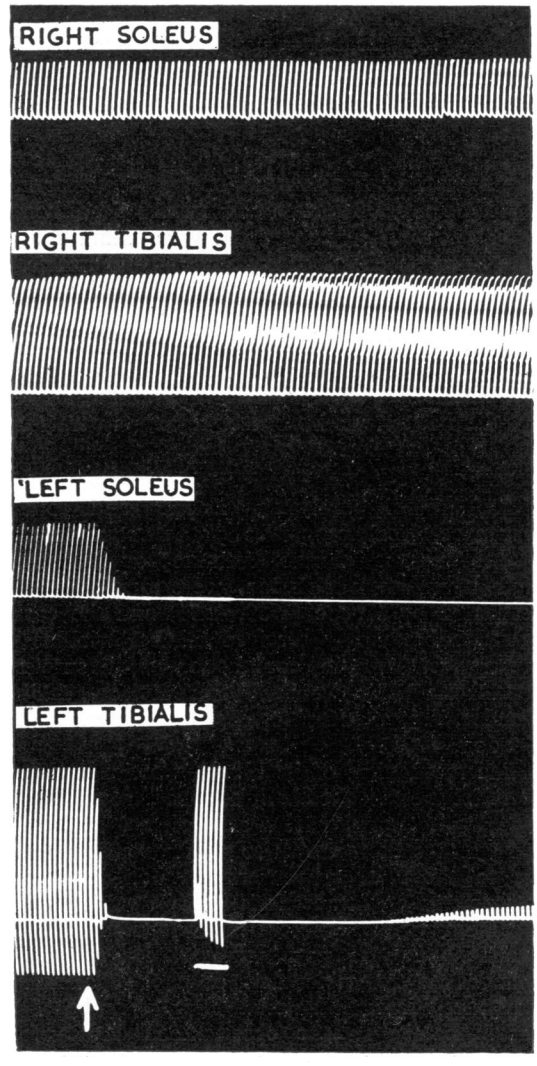

FIG. 1.—Cat 2.8 kg. Simultaneous recording of maximal twitches of both tibialis anterior muscles and both soleus muscles once every 10 sec. The muscles of the right leg were chronically denervated (18 days) and directly stimulated. Those of the left leg were normal and stimulated indirectly. At the arrow, 1 mg. of benzoquinonium was administered intravenously. During period marked by horizontal bar, left tibialis anterior was stimulated directly.

were still able to respond normally to direct stimulation (Fig. 1) and the contractions of the directly stimulated chronically denervated muscles were unaffected by benzoquinonium (Fig. 1) showing that the paralysis is not brought about through an action on the muscle fibre itself. Unlike decamethonium and other depolarizing substances, benzoquinonium even in large doses of up to 1 mg./kg. intravenously did not cause contracture in chronically denervated muscles (Fig. 1).

Benzoquinonium, tubocurarine, and decamethonium blocked the response to close-arterial injection of acetylcholine to a greater extent than the responses to maximal indirect stimuli. This effect with benzoquinonium is illustrated by Fig. 2

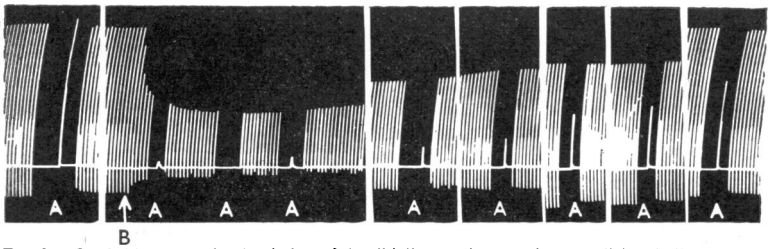

FIG. 2.—Cat 3.4 kg. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. At *A*, electrical stimulation was stopped and 5 μg. of acetylcholine was injected close-arterially. At *B*, 20 μg. of benzoquinonium was injected close-arterially.

and shows that a reduction in the quantity of acetylcholine liberated from nerve terminals cannot play a major part in its blocking action.

In the hen, the intravenous administration of benzoquinonium in doses of 150 to 200 μg./kg. caused a 90 to 100% paralysis of the indirectly excited maximal twitches of the gastrocnemius muscle. A similar degree of paralysis but of longer duration was produced by the intravenous administration of 0.8 to 1 mg./kg. of tubocurarine. The administration of benzoquinonium or tubocurarine reduced or abolished the contracture produced by a subsequent injection of decamethonium but did not alter the character of the response (Fig. 3) as does the previous administration of mecamylamine (Bennett, Tyler, and Zaimis, 1957). These results taken together demonstrate the absence of a depolarizing action of benzoquinonium and indicate that it produces a curare-like rather than a decamethonium-like paralysis of neuromuscular transmission.

In both the cat and the hen, cross cumulative effects occurred between benzoquinonium and tubocurarine when doses of each were administered alternately and, when either substance was administered at the peak of the effect of the other, an increase in the degree of block occurred. The additive effects of benzoquinonium and tubocurarine in the cat are illustrated in Fig 4*a*. On the other hand, benzoquinonium and decamethonium were seen to be mutually and markedly antagonistic (Fig. 4*b*).

Like the effect of tubocurarine and in contrast to that of decamethonium, neuromuscular block produced by benzoquinonium was antagonized by the administration of suxamethonium (20 to 30 μg./kg. intravenously), acetylcholine (30 μg. intra-arterially) or KCl (15 mg. intra-arterially). Paton and Zaimis (1949) used the response to tetanic stimulation of the motor nerve as an indication of the mode of action of neuromuscular blocking substances and the results of such a test are illustrated by Fig. 5. During partial paralysis by decamethonium, tetanic tension was fairly well maintained and after the tetanus there was no antagonism of the block. During partial paralysis by tubocurarine or benzoquinonium, however, tetanic stimulation produced only a brief "twitch-like" response from the muscle and after

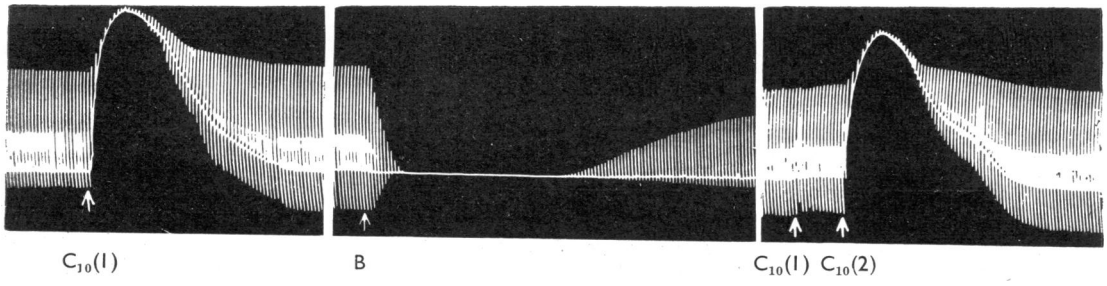

FIG. 3.—Hen 2.7 kg. Maximal twitches of the gastrocnemius muscle were elicited indirectly once every 10 sec. At $C_{10}(1)$, 40 μg. of decamethonium; at $C_{10}(2)$, 100 μg. of decamethonium; and at *B*, 0.4 mg. of benzoquinonium were injected intravenously.

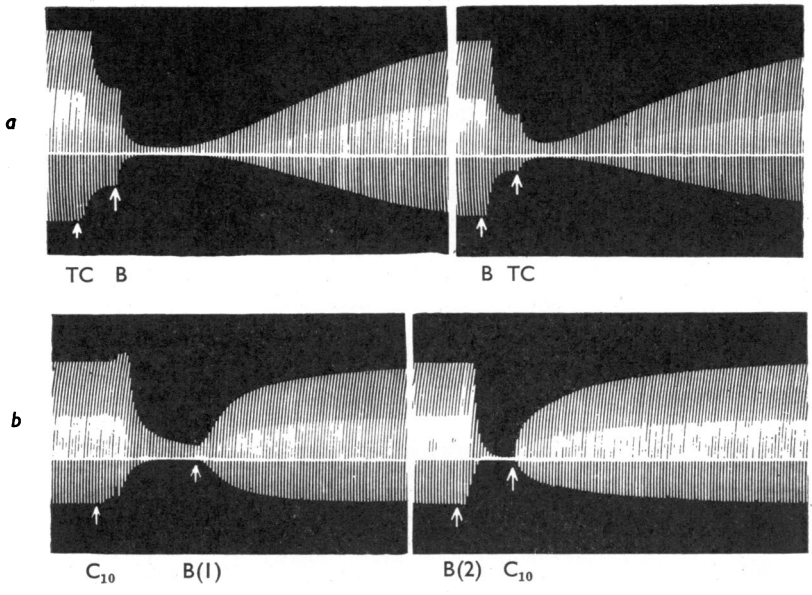

FIG. 4.—*a*, cat 3.8 kg. *b*, cat 2.9 kg. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. At ***TC***, 0.4 mg. of tubocurarine. At ***B***, 0.25 mg., at ***B(1)***, 0.2 mg., and at ***B(2)***, 0.6 mg. of benzoquinonium. At C_{10}, 100 μg. of decamethonium. All injections were made intravenously.

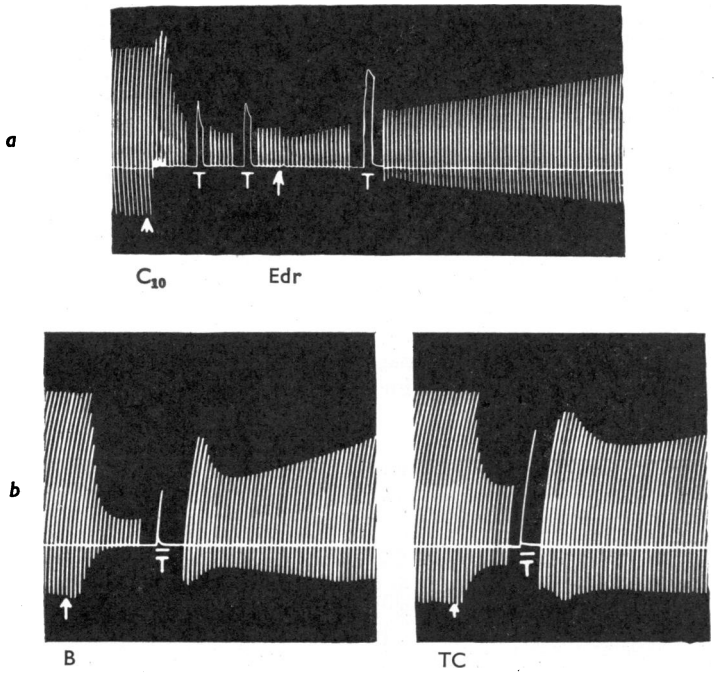

FIG. 5.—*a*, cat 2.7 kg. *b*, cat 3.4 kg. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. At C_{10}, 100 μg. of decamethonium; at ***Edr***, 0.3 mg. of edrophonium; at ***B***, 0.6 mg. of benzoquinonium; at ***TC***, 0.8 mg. of tubocurarine. All injections were made intravenously. ***T*** indicates tetanus of 30 sec. duration and at a frequency of 50/sec.

the tetanus the block was markedly antagonized.

It has long been known that adrenaline antagonizes a partial paralysis produced by tubocurarine when it is administered during the effect (Rosenblueth, Lindsley, and Morison, 1936). No such antagonism occurs, however, when decamethonium is used (West and Zaimis, 1949). On the other hand, when adrenaline is administered first, the blocking action of tubocurarine is potentiated, while that of decamethonium is reduced (Paton and Zaimis, 1950). These results were confirmed in the present experiments on the cat and it was shown that in this respect, also, benzoquinonium resembled tubocurarine (Fig. 6). As with tubocurarine, the antagonistic action of adrenaline to benzoquinonium paralysis was more marked at higher rates of stimulation.

Bennett *et al.* (1957) have shown that the previous administration of mecamylamine potentiates the blocking action of tubocurarine but reduces that of decamethonium, at the same

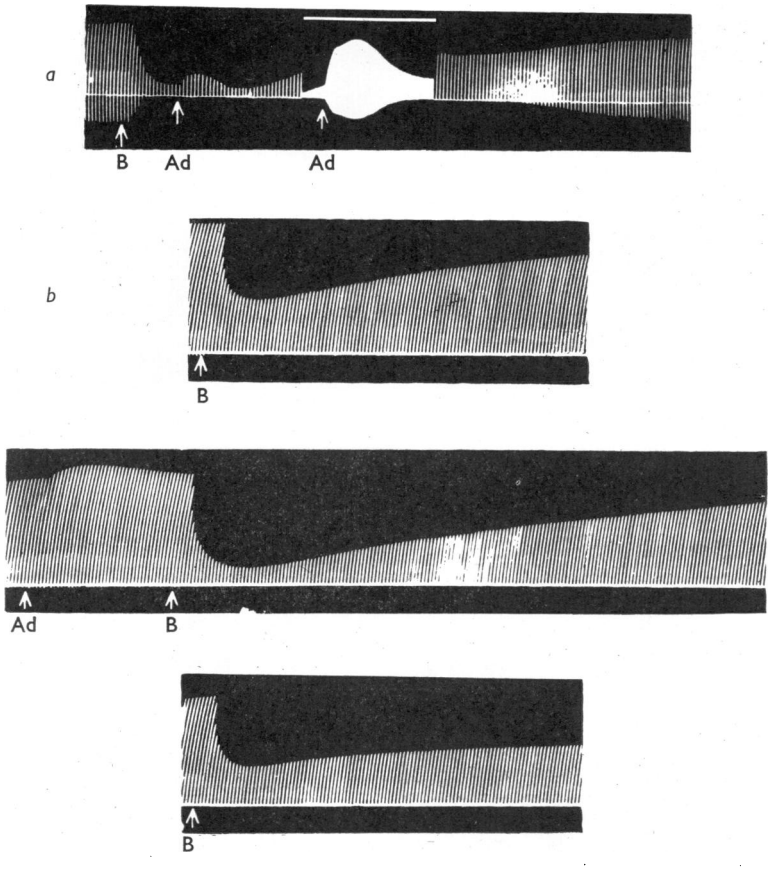

FIG. 6.—*a*, cat 2.4 kg. *b*, cat 3.1 kg. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. At ***B***, 0.5 mg. of benzoquinonium, and at ***Ad***, 50 μg. of adrenaline, were administered intravenously. In *a* during period marked by horizontal bar the frequency of stimulation was increased to 60/min. In *b*, 90 min. elapsed between injections of benzoquinonium.

time altering the character of the response. These results were confirmed and again benzoquinonium was shown to resemble tubocurarine, its effect being potentiated by mecamylamine.

The results so far described show that benzoquinonium produced a curare-like paralysis of neuromuscular transmission, but in spite of this, as Randall (1951) also demonstrated, its effect in the cat is not antagonized by edrophonium.

In addition to edrophonium, neostigmine, eserine and tetraethyl pyrophosphate were studied. In some experiments edrophonium (30 to 300 μg./kg.), neostigmine (30 to 300 μg./kg.), eserine (50 to 300 μg./kg.) or tetraethyl pyrophosphate (0.5 to 1 mg./kg.) was administered intravenously at the peak of the paralysis produced either by tubocurarine or by benzoquinonium. In others a small dose of either benzoquinonium or tubocurarine was administered at a constant time interval, which varied in different experiments from 30 min. to 4 hr., and edrophonium, neostigmine, eserine or tetraethyl pyrophosphate was injected 1 to 20 min. before the third or fourth dose of the blocking agent. All four substances readily antagonized tubocurarine in both types of experiment, but in the cat no antagonism to benzoquinonium could be demonstrated by either method (Fig. 7). In fact, when administered during the paralysis, edrophonium and neostigmine often slightly increased the block produced by benzoquinonium.

During these experiments it was observed that the administration of edrophonium, neostigmine, or eserine during partial block produced by tubocurarine was without effect in cats which had previously received benzoquinonium. Fig. 7*a* and *b* illustrates this effect in the case of edrophonium and neostigmine. This abolition of the anticurare effect lasted approximately 2 hr. after the intravenous administration of 0.2 mg./kg. of benzoquinonium. After the administration of benzoquinonium, edrophonium and neostigmine usually slightly deepened the paralysis produced by tubocurarine. Large doses of tetraethyl pyrophosphate (1 mg./kg.) still produced some antagonism, however, although the effect was reduced (Fig. 7*e*). Owing to the long duration of action of tetraethyl pyrophosphate, its anti-curare action showed marked tachyphylaxis. Accurate conclusions could not, therefore, be drawn from experiments in which its anti-curare action, both before and after the administration of benzoquinonium, was studied in the same animal. However, in an animal which had previously received benzoquinonium, the anti-curare action of the first injection of tetraethyl pyrophosphate was found to be non-existent or weak compared with control responses studied in different animals (Fig. 7*c* and *e*).

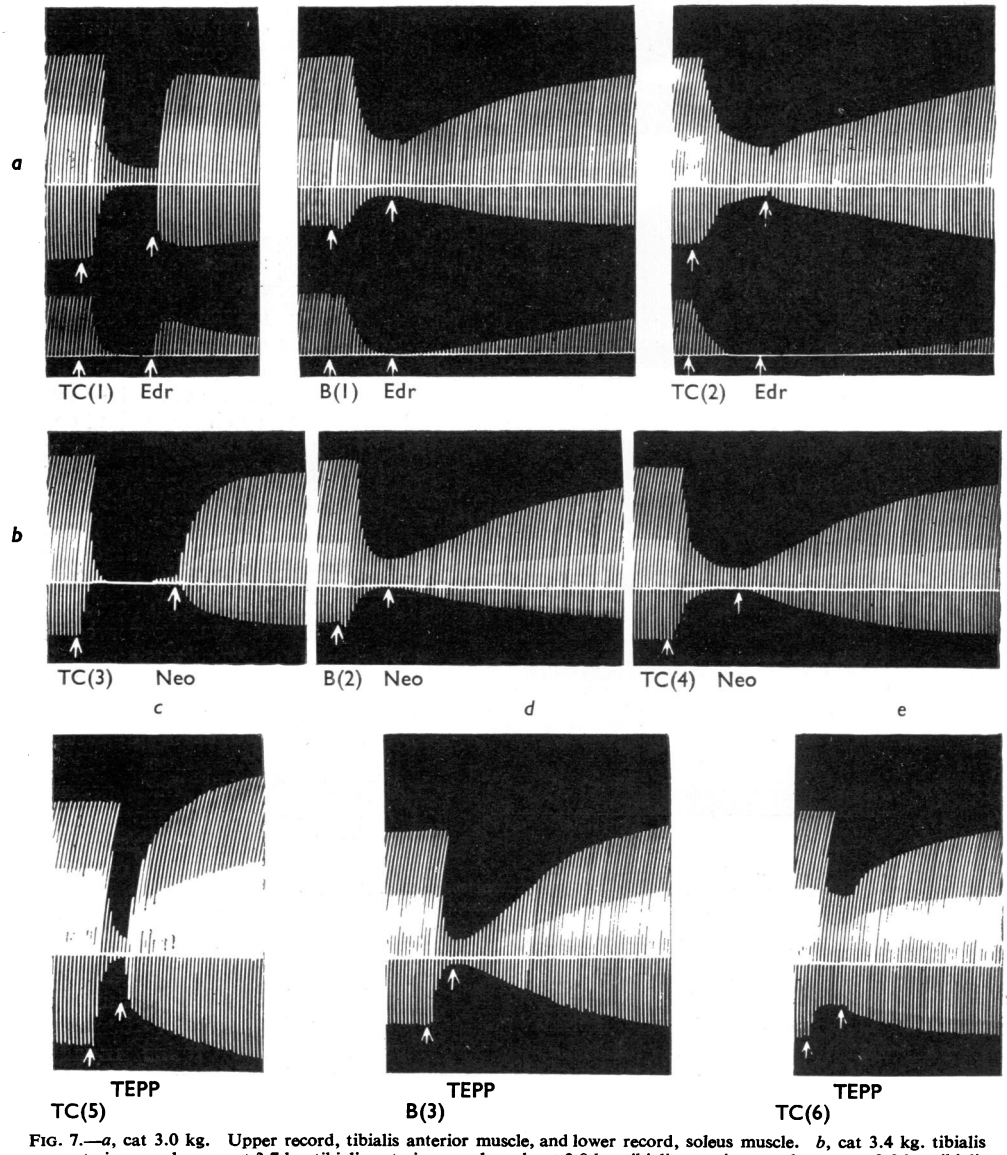

FIG. 7.—*a*, cat 3.0 kg. Upper record, tibialis anterior muscle, and lower record, soleus muscle. *b*, cat 3.4 kg. tibialis anterior muscle. *c*, cat 3.7 kg. tibialis anterior muscle. *d*, cat 2.9 kg. tibialis anterior muscle. *e*, cat 3.2 kg. tibialis anterior muscle. All records are of maximal twitches elicited indirectly once every 10 sec. At ***TC(1)*** 1 mg., at ***TC(2)*** 0.6 mg., at ***TC(3)*** 1.2 mg., at ***TC(4)*** 0.9 mg., at ***TC(5)*** 1.3 mg. and at ***TC(6)*** 0.8 mg. of tubocurarine respectively. At ***Edr***, 0.3 mg. of edrophonium; at ***Neo***, 0.15 mg. of neostigmine; at ***TEPP***, 3 mg. of tetraethyl pyrophosphate. At ***B(1)*** 0.3 mg., at ***B(2)*** 0.5 mg. and at ***B(3)*** 0.6 mg. of benzoquinonium. All injections were given intravenously. In *a* and *b*, 1 hr. elapsed between the doses of the blocking agents. In *e*, 0.5 mg. of benzoquinonium had been administered 45 min. previously.

This prevention of the anti-curare action was studied in another way, namely sufficient tubocurarine to produce a 50 to 70% paralysis of the maximum twitches was injected and, at the peak of the effect, a small dose of benzoquinonium was administered, which was insufficient by itself to produce any decrease in the maximal twitch tension. This was followed by the administration of either edrophonium, neostigmine, eserine, or tetraethyl pyrophosphate, none of which showed any antagonistic action in spite of the fact that under these circumstances the majority of the

muscle fibres must have been blocked by tubocurarine. Although after the administration of benzoquinonium, the anticholinesterases no longer antagonized tubocurarine, other antagonistic substances were still effective. Thus the paralysis was still antagonized by tetanic stimulation of the motor nerve and by the administration of acetylcholine, suxamethonium, decamethonium, KCl, and adrenaline. The lack of antagonism by anticholinesterases is not, therefore, the result of a change in the mechanism of tubocurarine blockade.

Small doses of benzoquinonium also prevented the potentiation of the unblocked maximal twitch produced by the anticholinesterases although with large doses of tetraethyl pyrophosphate some effect was still to be seen. Fig. 8 illustrates this effect with edrophonium.

It was considered possible that benzoquinonium in some way prevents the anticholinesterases from reaching their site of action in the cat. Experiments were therefore designed in which the anticholinesterases were administered to animals which had not previously received benzoquinonium. Under these circumstances acetylcholine accumulation should occur and some antagonism to subsequently administered benzoquinonium might be expected. These experiments were carried out as follows. In order to determine the sensitivity of the preparation to competitive blockade, 0.4 mg./kg. of tubocurarine

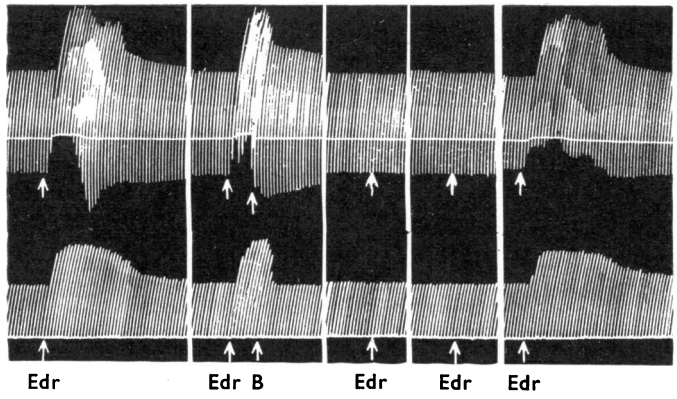

FIG. 8.—Cat 2.7 kg. Simultaneous recording of maximal twitches of the tibialis anterior muscle (upper record) and of the soleus muscle (lower record) elicited indirectly once every 10 sec. At ***Edr***, 0.3 mg. of edrophonium, and at ***B***, 0.15 mg. of benzoquinonium, were administered intravenously. 30 min. elapsed between each injection of edrophonium.

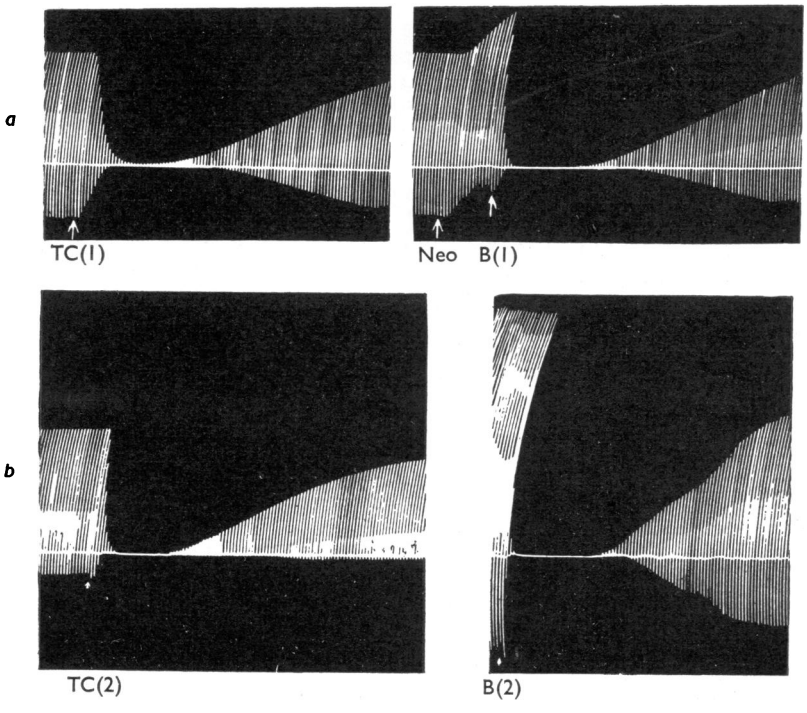

FIG. 9.—*a*, cat 3.4 kg. *b*, cat 3.2 kg. Maximal twitches of the tibialis anterior muscles were elicited indirectly once every 10 sec. At ***TC(1)*** 1.4 mg. and at ***TC(2)*** 1.3 mg. of tubocurarine. At ***B(1)*** 0.7 mg. and at ***B(2)*** 0.65 mg. of benzoquinonium. At ***Neo*** 0.2 mg. of neostigmine. 3¼ mg. of tetraethyl pyrophosphate was administered 15 min. before ***B(2)***. All injections were given intravenously. In both *a* and *b*, 4 hr. elapsed between the injections of the blocking agents.

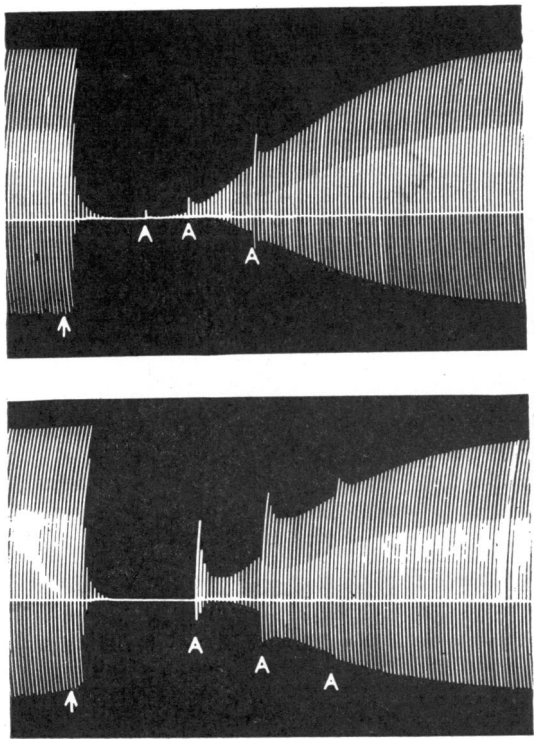

FIG. 10.—Cat 4.1 kg. Maximal twitches of the tibialis anterior muscle were elicited indirectly once every 10 sec. At the arrows, 0.7 mg. of benzoquinonium was administered intravenously. At *A*, 30 μg. of acetylcholine was administered intra-arterially. 10 min. before the second injection of benzoquinonium, 0.4 mg. of eserine was administered intravenously. 90 min. elapsed between the injections of benzoquinonium.

was administered intravenously. Four to five hours later neostigmine (100 μg./kg.), eserine (150 μg./kg.) or tetraethyl pyrophosphate (1 mg./kg.) was administered and this was followed 10 to 20 min. later by 0.2 mg./kg. of benzoquinonium. Since in all experiments benzoquinonium was twice as potent as tubocurarine, this dose would be expected to produce a comparable response. A long interval was allowed to elapse between the doses of the blocking agents in order to reduce cumulative effects to a minimum. Even under these circumstances, no antagonism to benzoquinonium could be demonstrated; in every case the paralysis produced was equal to, or slightly greater than, that produced by the initial injection of tubocurarine. Fig. 9 illustrates typical results with tetraethyl pyrophosphate and neostigmine.

Although in the cat edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate did not antagonize paralysis produced by benzoquinonium, they nevertheless potentiated the antagonistic action of injected acetylcholine. Eserine and tetraethyl pyrophosphate were the most potent in this respect while the effect of edrophonium was weak or non-existent. The upper record of Fig. 10 illustrates the antagonistic action of injected acetylcholine against paralysis by benzoquinonium. Ten minutes before the second injection of benzoquinonium, eserine was administered, but in spite of this the paralysis was not reduced. The antagonistic action of injected acetylcholine, however, was markedly potentiated.

An interesting species difference was demonstrated by experiments on the hen in which edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate readily antagonized paralysis produced both by tubocurarine and by benzoquinonium (Fig. 11).

DISCUSSION

The present experiments demonstrate that in the cat and the hen, as in other species, benzoquinonium caused a reversible paralysis of the neuromuscular junction typical in many respects of that produced by substances such as tubocurarine. That benzoquinonium did not possess a depolarizing action is shown by the lack of any muscle fasciculations or potentiation of the maximal twitch and by the absence of a contracture in avian or chronically denervated mammalian muscle. In fact, benzoquinonium and depolarizing substances such as decamethonium, suxamethonium, and acetylcholine were shown to be mutually antagonistic.

The administration of benzoquinonium, even in doses too small to affect the maximal twitch tension, was shown to reduce or abolish both the potentiating action on the maximal twitch and the anti-curare action of edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate. These results show that, in addition to its neuromuscular blocking action, benzoquinonium prevents the skeletal muscle effects of these anticholinesterases in the cat. The fact that anticholinesterases did not antagonize paralysis produced in the cat by benzoquinonium appears, therefore, not to be a consequence of the mechanism of its blocking action as Randall (1951) believed and as is the case with decamethonium (Paton and Zaimis, 1949), but rather to be the result of this additional action.

Randall (1952) showed that the replacement of the benzyl groups of benzoquinonium with nitrobenzyl or cyano-benzyl radicals resulted in compounds, the blocking actions of which were antagonized by edrophonium in the cat. Ran-

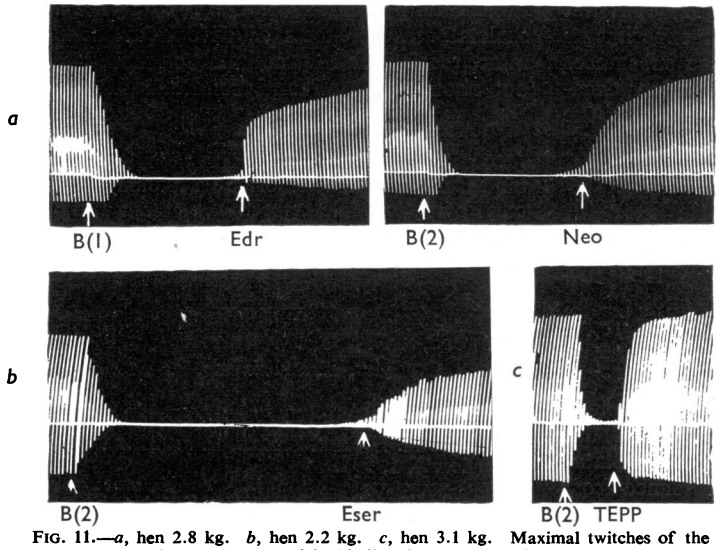

FIG. 11.—*a*, hen 2.8 kg. *b*, hen 2.2 kg. *c*, hen 3.1 kg. Maximal twitches of the gastrocnemius muscle were elicited indirectly once every 10 sec. At ***B(1)*** 0.4 mg. and at ***B(2)*** 0.5 mg. of benzoquinonium. At ***Edr*** 0.3 mg. of edrophonium. At ***Neo*** 0.15 mg. of neostigmine. At ***Eser*** 0.4 mg. of eserine. At ***TEPP*** 3 mg. of tetraethyl pyrophosphate. All injections were made intravenously.

dall interpreted this as a conversion from a decamethonium-like to a curare-like substance, but the present experiments indicate that such substitution probably abolished the "anti-edrophonium" effect without altering the mechanism of the blocking action. Hoppe, Funnell, and Lape (1953, 1955) showed that the replacement of the benzyl groups with ethyl groups also resulted in a compound which was antagonized by edrophonium so that it appears that the presence of unsubstituted benzyl groups on the quaternary nitrogen centres of benzoquinonium is an important factor in the "anti-anticholinesterase" effect in cat skeletal muscle.

Although benzoquinonium has been shown to produce a curare-like paralysis in all the species studied, considerable species difference exists with regard to the effects of anticholinesterases. The present experiments have shown that while edrophonium, neostigmine, eserine, and tetraethylpyrophosphate are completely ineffective in the cat, they are all effective antagonists to benzoquinonium in the hen. Other species appear to lie between these extremes; for example, it has been reported that in the dog (Hoppe, 1951) and the rabbit (Hoppe *et al.*, 1955) neostigmine was slightly effective particularly when it was administered before benzoquinonium. Edrophonium, however, was without effect in the rabbit. According to Dundee *et al.* (1952), neostigmine antagonized paralysis produced by benzoquinonium in man.

Hoppe (1951) has shown from *in vitro* studies that benzoquinonium itself possesses some anticholinesterase activity. A potent inhibitor of the acetylcholinesterase at the neuromuscular junction might, by inhibiting all the enzyme in the region, prevent the effects of other anticholinesterases administered subsequently. The present experiments showed, however, that even when edrophonium, neostigmine, eserine, or tetraethyl pyrophosphate was administered first, the paralysing action of benzoquinonium was not reduced. Furthermore, in some recent experiments it has been shown that the previous administration of the potent anticholinesterases, eserine and neostigmine, does not abolish the antagonism to tubocurarine produced by edrophonium or tetraethyl pyrophosphate (Bowman, unpublished observations). These results therefore supply evidence that the anticholinesterase action of benzoquinonium cannot be the explanation for the failure of edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate to antagonize its blocking action.

From a chemical point of view, it is unlikely that benzoquinonium can reactivate an inhibited enzyme or that it can combine chemically in the blood with all of the different types of anticholinesterase studied; the fact that the anticholinesterases are active antagonists to benzoquinonium in the hen is also evidence against such a possibility.

Neuromuscular block produced by benzoquinonium is antagonized by injected acetylcholine and by acetylcholine liberated from the motor nerve during tetanus, and yet, in the cat, anticholinesterases are without effect even when they are administered first. These facts suggest that inhibition of cholinesterase and consequent acetylcholine accumulation is not the main mechanism responsible for the skeletal muscle effects of edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate. Some workers (Riker, Wescoe, and Brothers, 1949; Wescoe and Riker, 1951; Riker, 1953) have expressed the opinion that the main action of edrophonium, neostigmine and similar compounds in skeletal muscle is a direct acetylcholine-like stimulation of the motor end plate. The work of others (Randall and Lehmann,

1950; Randall, 1950; Hobbiger, 1952) does not support this conclusion and the present experiments also rule out such a possibility since substances which are known to possess such an action (decamethonium, suxamethonium, and acetylcholine itself) readily antagonize benzoquinonium paralysis while edrophonium and neostigmine do not. Riker, Roberts, Standaert, and Fujimori (1957) have recently put forward evidence that the repetitive firing seen in the motor nerves of cats under the influence of compounds of the edrophonium and neostigmine type is not, as is generally believed, entirely the result of retrograde activation of nerve by muscle but rather that it is partly brought about by an action of such substances on the motor nerve terminal. They concluded that, as a consequence of this action, neuromuscular transmission is facilitated and they summarized some of the evidence from other sources which points to a similar action of eserine and dyflos. Douglas and Paton (1954), in their study of the motor end plate depolarization produced by tetraethyl pyrophosphate in the cat, pointed out that they had no evidence to exclude the possibility that tetraethyl pyrophosphate causes a release of acetylcholine from nerve. Such an action would account for the skeletal muscle effects of anticholinesterases and would imply that whatever change occurs, when the nerve impulse reaches the nerve ending, is exaggerated or prolonged so that under the influence of these substances a greater quantity of acetylcholine is released by each stimulus. If such is the case, benzoquinonium, in addition to its action at the motor end plate, might inhibit the skeletal muscle effects of anticholinesterases by competing with them for receptor sites on the motor nerve terminal.

The anticholinesterase action of benzoquinonium which was demonstrated by Hoppe (1951), and which is presumably responsible for the muscarinic effects observed in man (Dundee *et al.*, 1952) and in the cat, may therefore merely be a reflexion of the other action. It is not unlikely that a substance which prevents the action of a second substance at one site may have a similar action to the second substance at another. The present experiments showed that neostigmine, eserine, and tetraethyl pyrophosphate in the presence of benzoquinonium still potentiate the skeletal muscle effects of injected acetylcholine, and experiments now in progress indicate that benzoquinonium does not prevent the muscarinic effects of anticholinesterases. The conclusion is therefore drawn that benzoquinonium produces a curare-like blockade of the neuromuscular junction and in addition, in the cat, it prevents the skeletal muscle effects of edrophonium, neostigmine, eserine, and tetraethyl pyrophosphate without abolishing their anticholinesterase activity.

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