Brit. J. Pharmacol. (1961), **17**, 176–195.

ACTIONS OF TRIETHYLCHOLINE ON NEUROMUSCULAR TRANSMISSION

BY

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(*Received June* 6, 1961)

The effects of the triethyl analogue of choline (triethyl 2-hydroxyethyl ammonium) on muscular activity have been studied in conscious rabbits, chicks, dogs and a cat. The contractions of the tibialis anticus and soleus muscles of cats under chloralose anaesthesia, and of the tibialis anticus muscle of rabbits under urethane anaesthesia and the isolated diaphragm preparation of the rat were also used. In conscious animals, triethylcholine caused a slowly developing muscular weakness which was more severe after exercise and which resembled the symptoms of myasthenia gravis. In nerve-muscle preparations triethylcholine had a selective action in reducing the contractions of muscles elicited by a high rate of nerve stimulation while leaving unaffected the contractions caused by slower rates of stimulation. During the paralysis of the tibialis muscle of the cat produced by triethylcholine, action potentials recorded from the motor nerve were unaffected and the muscle responded normally to injected acetylcholine and to direct electrical stimulation. The failure of neuromuscular transmission produced by triethylcholine was reversed by injection of choline, but anticholinesterases were ineffective. Choline reduced the toxicity of triethylcholine in mice. It is concluded that triethylcholine produces transmission failure at the neuromuscular junction by interfering with the ability of the nerve endings to synthesize acetylcholine. The possibility that triethylcholine is itself acetylated by the nerve endings and released as an inactive neurohormone is discussed. It was shown that triethylcholine was devoid of depolarizing action and curare-like blocking action. It possesses a transient ganglion blocking action of the tetraethylammonium-type as shown in experiments in which it caused a fall in blood pressure and blocked the response of the nictitating membrane to pre- but not to post-ganglionic stimulation of the cervical sympathetic nerve.

This paper deals with observations on the pharmacological actions of triethylcholine (triethyl 2-hydroxyethyl ammonium) with particular reference to its actions at the neuromuscular junction. Triethylcholine produces muscular weakness after exercise and selectively depresses the contractions of muscles caused by high rates of nerve stimulation. These findings led to the suggestion that a substance with this type of action might be of value in the control of neurogenic spastic states (Bowman & Rand, 1961). Triethylcholine has been shown to be effective in relieving the spasm of experimental tetanus in rabbits (Laurence & Webster, 1961). Most of the previous work on triethylcholine has been concerned with its ability to replace choline in nutrition ; the substance has been shown to be capable of replacing choline in some vitaminic actions but not in others (Channon & Smith, 1936 ; Channon,

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Platt & Smith, 1937; Channon, Platt, Loach & Smith, 1937; Moyer & du Vigneaud, 1942; Jukes & Welch, 1942; Horowitz, Bonner & Houlahan, 1945; McArthur, Lucas & Best, 1947; Stekol & Weiss, 1950; Welch, 1950; Sebrell & Harris, 1954). The first pharmacological investigations were carried out by Hunt & Taveau (1909), who stated that triethylcholine lacked both the muscarinic and nicotinic actions of acetylcholine. According to Keston & Wortis (1946), acetylcholine-induced contractures of the frog rectus abdominis muscle are unaffected by the presence of triethylcholine. These authors also showed that the toxicity of triethylcholine in mice is antagonized by choline.

METHODS

Toxicity. Determinations of acute toxicity were made on male mice (A.R.C. strain) of about 20 g body weight, and in rabbits. The effects of repeated daily injections of triethylcholine were observed in 20 mice, 10 rats, 3 rabbits, 1 cat and 3 dogs.

Observations in conscious animals. In these experiments animals were exercised, restrained or observed without interference other than the injection. Rabbits and young chicks were exercised by turning them on to their backs, which procedure led to their making continual attempts to regain their normal position; they were restrained by placing them inside a wire framework which only allowed small movements. A cat was exercised by continually moving it from a preferred position, which it attempted to regain; it was not necessary to restrain the cat, since, when left alone, it moved infrequently. Dogs were exercised by making them follow their keeper and by playing, and they were restrained by leashing.

Observations on anaesthetized animals. Cats were anaesthetized by intravenous injection of chloralose (8 ml./kg of a 1% solution) to which pentobarbitone sodium (6 mg/kg) was added. Rabbits were anaesthetized by intravenous injection of urethane (6 ml./kg of a 25% solution).

The sciatic nerves in both legs were divided between ligatures, and bipolar platinum electrodes were placed on the peripheral portion of each nerve. The hind limbs were fixed in a horizontal position by means of steel drills through the knee and ankle joints. The tendons of both the tibialis anticus muscles and, in some experiments in cats, the tendon of a soleus muscle were detached at their insertions and tied to flat steel spring myographs. Muscle contractions were recorded on smoked paper. The nerves were stimulated by rectangular pulses usually of 10 to 100 μsec duration and of at least twice the strength required to evoke a maximal twitch. The stimulus to the nerve of one of the legs was passed through a 1:1 isolation transformer so that there was no common connexion between the electrodes to the two nerves. When required, muscles were stimulated directly with rectangular pulses of supramaximal strength and of 1 msec duration applied between the tendon and the drill in the femur. Acetylcholine was given by close-arterial injection to one tibialis muscle as described by Brown (1938). Other drugs were injected intravenously. In experiments on the knee jerk, contractions of a quadriceps femoris muscle were elicited at a rate of 1/min by tapping the patellar tendon with a Palmer automatic hammer.

In some experiments action potentials in response to nerve stimulation were recorded simultaneously from the right tibialis muscle and from the left common peroneal nerve. The cat was laid face downwards and the left sciatic nerve was fully exposed in the popliteal space and covered with liquid paraffin. Stimulating electrodes were placed as far centrally as possible, at the level of the trochanter. Nerve action potentials were recorded by means of bipolar platinum electrodes placed on the left common peroneal nerve, which was crushed peripherally to prevent the muscle action potential from complicating the recordings. Muscle action potentials in response to stimulation of the right sciatic nerve were led off from the tibialis anticus muscle by glass-mounted platinum wires inserted through the belly and tendon, or by concentric needle electrodes. Each pair of recording electrodes was connected to a differential amplifier (Tektronix type 122 battery-driven pre-amplifier) and the action potentials

were displayed on a double-beam oscilloscope (Tektronix type 502) and photographed on 35 mm film.

Respiratory movements were recorded by Gaddum's method (Gaddum, 1941). Contractions of both nictitating membranes were recorded simultaneously in cats. The pre-ganglionic cervical sympathetic nerve on one side was stimulated through bipolar platinum electrodes. The post-ganglionic fibres on the other side were stimulated by exposing the superior cervical ganglion and placing bipolar platinum electrodes so that one electrode was in contact with the ganglion and the other with the post-ganglionic fibres.

Blood pressure was recorded with a mercury manometer from a carotid artery in experiments on muscle tension and on respiratory activity, and from a femoral artery in experiments on the nictitating membrane.

Rat diaphragm. The isolated hemidiaphragm of the rat with the phrenic nerve attached was set up as described by Bülbring (1946) and suspended in McEwen's (1956) solution at 32° C in a 100 ml. bath. The nerve was stimulated by pulses of supramaximal strength and of 0.2 msec duration.

RESULTS

Acute toxicity

Mouse. The LD50's of triethylcholine chloride and of triethylcholine iodide administered intravenously, subcutaneously and orally to mice are given in Table 1. The LD50 and the 95% confidence limits were determined by the method of Litchfield & Wilcoxon (1949). Lethal doses of triethylcholine produced a slowing of respiratory movements, gasping and cyanosis, and in some of the mice there were

TABLE 1
TOXICITY OF TRIETHYLCHOLINE IN MICE
LD50 and 95% confidence limits in parentheses (mg/kg)

Route	Triethylcholine salt	
	Iodide	Chloride
Intravenous	79 (62–101)	69 (53–90)
Subcutaneous	100 (88–113)	88 (71–109)
Oral	3,900 (3,277–4,641)	—

terminal convulsions which were probably asphyxial in origin. The observations of Keston & Wortis (1946) that the toxicity of triethylcholine in mice is antagonized by choline were confirmed. Fig. 1 shows the LD50 of triethylcholine in the presence of 1/2, 1/4, 1/8 and 1/16 of the LD50 of choline chloride. The optimum protective dose of choline chloride was 100 mg/kg, which increased the LD50 of subcutaneous injections of triethylcholine chloride approximately 7-fold. We were unable to demonstrate an antagonistic action of choline on the lethal dose of triethylcholine when the compounds were given intravenously.

Rabbit. Ten rabbits were each injected with 100 mg/kg triethylcholine iodide intravenously. Five of them were continuously exercised immediately after the injection and all of these died between 6 and 17 min later. The other five were restrained ; four of these survived and one died 35 min after the injection. Death was caused by respiratory failure ; heart beats continued for 0.5 to 2 min after the last breath. The surviving rabbits developed pronounced muscular weakness which

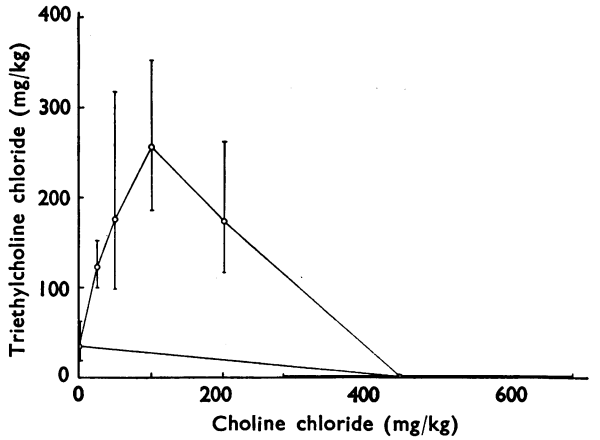

Fig. 1. The effect of choline in reducing the toxicity of triethylcholine. The circles indicate the LD50 of triethylcholine alone, of triethylcholine in combination with 4 different doses of choline, and of choline alone. The 95% confidence limits of the LD50 of triethylcholine alone and in combination with choline are indicated by the vertical lines, and of choline alone by the horizontal line. The straight line joining the LD50 points for triethylcholine alone and choline chloride alone is that to be expected if the toxicities were additive (Gaddum, 1959). All the data for this figure were obtained from one batch of mice.

was at its peak 25 to 35 min after injection ; 1.5 to 2 hr after injection full recovery had taken place, and no effect of triethylcholine was detectable even after exercise.

Chronic toxicity

The effect of daily injections of triethylcholine iodide on the growth of mice and rats is shown in Table 2. The high dose in mice, approximately 25 mg/kg, killed

TABLE 2

EFFECT OF 14 DAILY DOSES OF TRIETHYLCHOLINE IODIDE ON GROWTH IN MICE AND RATS

Mice were dosed by intravenous injection, rats by intraperitoneal injection. Asterisk denotes value significantly greater than control, $P<0.05$

Species	Daily dose/ animal	No. in group	Deaths	Initial mean wt. (g)	Mean wt. after 14 days	Mean % increase in wt.
Mouse	Control	10	0	25·8	28·0	7·6
	0·375 mg	10	1	23·9	26·3	11·0
	0·625 mg	10	5	23·0	28·6	20·2*
Rat	Control	5	1	118	213	81
	2·5 mg	5	0	116	211	82
	4·0	5	0	112	231	105*

5/10 of the group in the course of the 14 days during which the injections were given. This dose is less than half of the lower 95% confidence limit of the LD50 of single injections, which suggests that there has been a cumulative effect with injections repeated daily. The surviving mice in the high dose group and the mice and rats in the other groups were indistinguishable from the control animals in

general appearance. The mean body weight of both mice and rats in the high dose groups showed a significantly greater increase than their controls.

Three rabbits each weighing about 1.5 kg at the start of the experiment were injected with 37.5 mg of triethylcholine iodide daily for 28 days. There was no apparent deleterious effect of these injections and their final weights were each about 2.25 kg. One dog (18 kg) injected with 20 mg/kg daily for 8 days and two dogs (6 kg and 7 kg) each injected with 50 mg/kg daily for 15 and 14 days respectively showed no abnormal signs after the immediate effect of each injection had passed off. Most of the injections in dogs were made intravenously, but on some days they were given by the subcutaneous or by the intramuscular route.

The effect of triethylcholine in conscious animals

Chick. Intravenous injection of 20 mg/kg triethylcholine iodide did not cause spastic paralysis and so differed from depolarizing substances (Buttle & Zaimis, 1949). There was no immediate effect on the chick, but if it was repeatedly placed on its back it lost the ability to right itself after 15 to 20 trials; a control chick was not fatigued by these manœuvres. After resting for 1.5 to 2 min the chick recovered the ability to stand. This sequence of fatigue and recovery could be repeated several times during the 30 min following a single injection of triethylcholine.

Rabbit. Intravenous injection of 10 to 25 mg/kg triethylcholine iodide had no apparent effect on restrained rabbits. However, if the rabbits were repeatedly exercised, muscular weakness appeared about 10 min after the injection and reached its peak after 30 min ; 90 min after injection exercise no longer produced weakness. The signs of muscular weakness were demonstrated in several ways. When a rabbit was exercised by turning it on to its back it made vigorous attempts to right itself, but after an injection of triethylcholine its movements became less vigorous until the ability to right itself was lost. If the rabbit was then allowed to rest for 1 or 2 min it recovered the ability to regain its normal posture. The weakness was confined to those muscles which were exercised. For example, if loss of righting ability was produced by continually turning a rabbit on to its left side, then a single trial by turning it on to its right side showed no impairment. In other experiments one leg was repeatedly pulled out until the rabbit lost the ability to withdraw that leg ; at this time the other legs were withdrawn normally. Withdrawal of the fatigued leg occurred after 30 to 60 sec rest. The production of muscular weakness by exercise could be repeated several times after a single injection of triethylcholine ; in the rest intervals between periods of exercise rabbits appeared normal.

Intravenous injection of larger doses of triethylcholine iodide (50 mg/kg) had more marked effects. Muscular weakness appeared after spontaneous movement on the part of the rabbit, so that the gait became incoordinated. A slight degree of head drop was present and the stance was affected. Continuous exercise caused prostration, but after resting for 2 to 5 min there was considerable recovery. Respiratory movement continued throughout. The time course of the effects was

similar to that occurring after 25 mg/kg. The effects of intramuscular injection were greater than those of the same dose intravenously.

Choline chloride (4 mg/kg) injected intravenously at the height of the response to triethylcholine caused rapid recovery to normal muscular activity. Neostigmine methylsulphate (0.15 mg/kg) did not reverse the muscular weakness.

Cat. Intravenous injections of 50 mg/kg of triethylcholine iodide (3 observations) produced an immediate relaxation of the nictitating membrane and a dilatation of the pupil. The nictitating membrane returned to normal in 10 min, but the pupillary dilatation persisted for about 1 hr. Signs of muscular weakness and disturbance in walking movements appeared 10 to 15 min after injection. Complete exhaustion after walking for a few feet was observed 25 to 35 min after injection. However, after a rest of 1 to 2 min another period of walking was possible. Small movements, such as withdrawal of the leg from a forced extension, were possible even when prostrated by exercise. Respiratory movements were always adequate. The ability to make more sustained movements returned in 35 to 40 min and full recovery in 80 to 100 min after injection.

Intraperitoneal injections (2 observations) produced the same general pattern of events, except that the weakness was more pronounced and persisted longer, so that full recovery was delayed to 150 min after injection. The pupillary dilatation and relaxation of the nictitating membrane were less marked and only occurred at the height of the signs of muscular weakness.

Dog. Intravenous injection of 20 mg/kg triethylcholine iodide had no immediate effect in dogs, but 50 mg/kg produced a relaxation of the nictitating membrane with return to normal by 10 min. When dogs were continuously exercised signs of muscular weakness appeared 6 to 10 min after the injection of 20 to 50 mg/kg, and reached a maximum after 20 to 30 min. This effect of the injection was not detectable after about 1 hr. The first sign of muscular weakness was lack of co-ordination in walking ; the head was not held erect, the lower jaw hung open and the eyelids drooped. After strenuous exercise, such as running, dogs were unable to support their own weight, although they were not paralysed since small movements were still made and respiratory movements continued. If the dogs were then allowed 2 to 3 min of rest they were again capable of a short period of strenuous exercise. Even when only lightly exercised, so that muscular weakness was not produced, triethylcholine had an effect on the behaviour of the dogs ; they frequently adopted relaxed resting positions and seemed to be disinclined to obey their keeper's orders. In this condition they alerted to stimuli such as noise and movement, but they did not stand or walk except after repeated commands from their keeper.

An intramuscular injection of triethylcholine caused a more pronounced and longer-lasting effect than an intravenous injection of the same dose.

Observations on nerve-muscle preparations

Cat. In the cat under chloralose anaesthesia, the intravenous injection of triethylcholine caused a slowly developing decrease in the size of the contractions of the tibialis and soleus muscles when the frequency of nerve stimulation was high, but

at low rates of stimulation the contractions were not affected. The higher the frequency of nerve stimulation, the greater the effect of triethylcholine in depressing muscle contractions, and the more rapid the onset of its effect. In order to study these effects, it was necessary to use a frequency of stimulation which itself did not cause fatigue of the preparation. In control experiments twitches of a tibialis muscle in response to nerve stimulation at frequencies greater than 2/sec gradually decreased in size, often to below 20% of their original level, after 2 hr of continuous stimulation. When the frequency of stimulation was 1/sec the tension of the twitches increased during the first 2 to 3 min and then remained constant. Therefore, in the majority of the experiments on the anaesthetized cat, the highest frequency of stimulation used was 1/sec.

Figs. 2, 3 and 4 show that intravenous injections of triethylcholine decreased the tension of indirectly excited maximal twitches of the tibialis anterior muscle stimu-

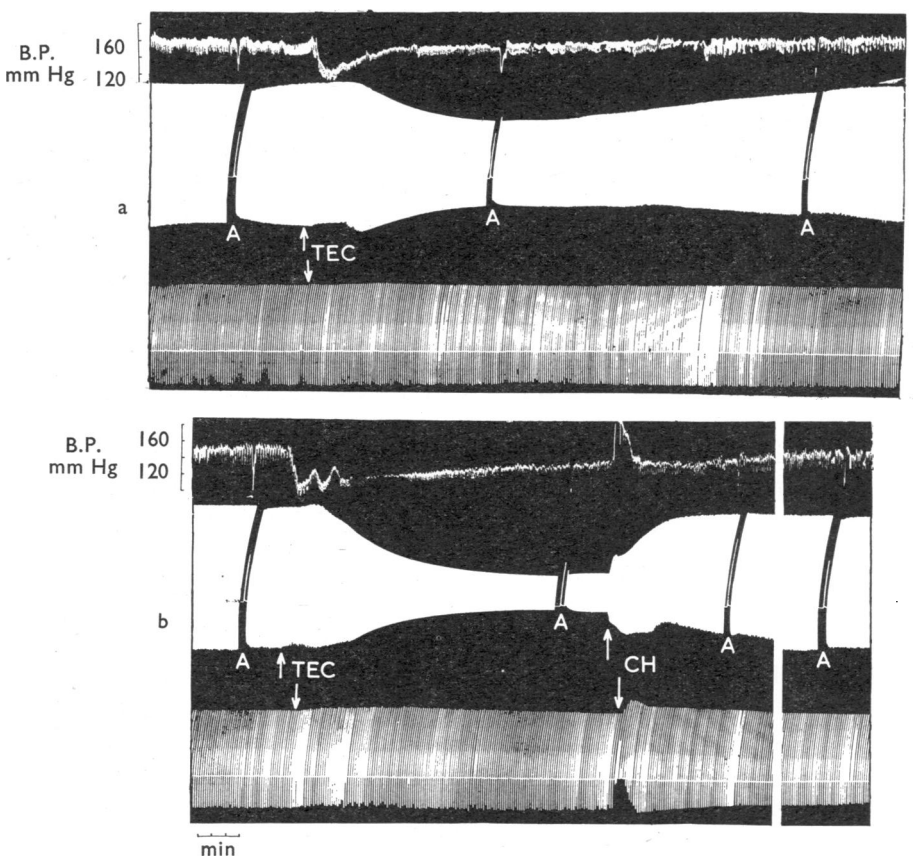

Fig. 2. Cat, 4.0 kg. Upper record, blood pressure, middle and lower records, maximal twitches of right and left tibialis anticus muscles elicited indirectly 1/sec and 1/10 sec respectively. At A, 10 μg acetylcholine was injected close-arterially to the right tibialis anticus muscle, electrical stimulation being temporarily stopped during the injection. At TEC in a, 10 mg/kg, and at TEC in b, 30 mg/kg of triethylcholine chloride were injected intravenously. At CH, 5 mg/kg choline chloride was injected intravenously.

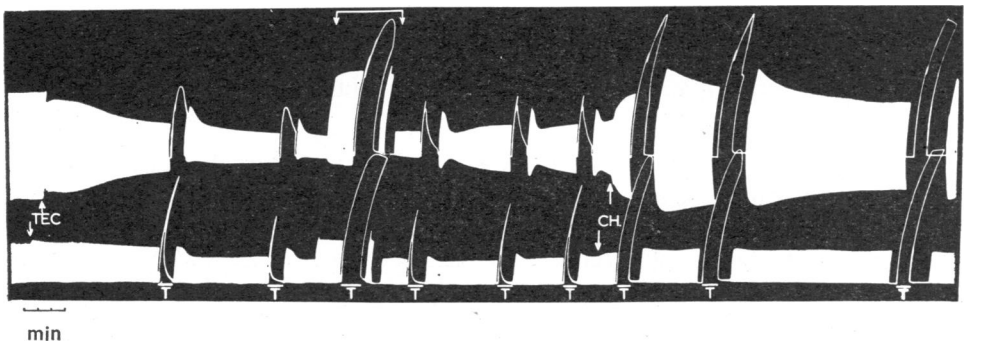

Fig. 3. Cat, 5.5 kg. Maximal twitches of the tibialis anticus (upper record) and soleus muscles (lower record) elicited indirectly once every second. At TEC, 35 mg/kg of triethylcholine iodide, and at CH, 5 mg/kg of choline chloride were injected intravenously. At T, tetani were elicited at a frequency of 50/sec for approximately 6 sec. During the tetani the kymograph speed was increased. During the period marked ↓‾‾↓, the muscles were stimulated directly. The animal had previously recovered from an initial injection of 35 mg/kg of TEC.

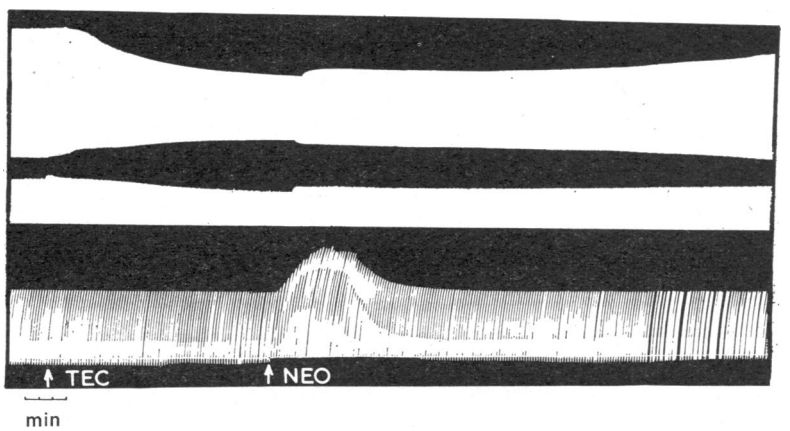

Fig. 4. Cat, 3.2 kg. Upper and middle records: maximal twitches of the right tibialis anticus and soleus muscles respectively, elicited indirectly once every sec. Lower record: maximal twitches of the left tibialis anticus muscle elicited indirectly once every 10 sec. At TEC, 30 mg/kg of triethylcholine iodide, and at NEO, 0.1 mg/kg neostigmine methylsulphate injected intravenously.

lated at a frequency of 1/sec, but the twitches of the contra-lateral tibialis, which was stimulated once every 10 sec (Figs. 2 and 4), were not affected. The time of onset of the effect of triethylcholine on muscle contractions, while dependent on the frequency of stimulation, was but little affected by increasing the doses of triethylcholine iodide from 20 mg/kg up to 50 mg/kg, and occurred 6 to 12 minutes after injection when the frequency of stimulation was 1/sec. The time from injection to the maximum reduction of contractions, and the duration of the effect, depended on the dose. After an initial injection of 40 mg/kg triethylcholine iodide the maximum degree of block occurred about 20 min after injection and the twitches

regained their normal tension 60 to 80 min later. With the second and subsequent injections of triethylcholine, some cumulative effect was seen. For example, Fig. 5 shows that a second injection of triethylcholine produced a greater degree of block with a more rapid onset.

In some experiments the first effect of an injection of triethylcholine was to produce a small increase in twitch tension, which can be seen in Fig. 2 in the record from tibialis muscle and in Figs. 3 and 4 in the records from soleus muscle. With the second and subsequent injections the initial increase was sometimes replaced by an abrupt transient depression of the twitches, which was then followed by the usual more slowly developing and longer-lasting paralysis. This is evident in Fig. 3, which illustrates the response to a second injection of triethylcholine.

With equal frequencies of nerve stimulation the maximal twitches of the tibialis muscle were always blocked to a greater extent than those of the soleus muscle. This difference is illustrated in Figs. 3 and 4. At the height of the effect of triethylcholine in another experiment the twitches of the tibialis muscle were depressed by 66.7% while those of the soleus were depressed by 27.5%.

During a partial paralysis of the maximal twitches, the tension of an indirectly elicited tetanus was markedly reduced and waned during the period of stimulation (Fig. 3). This inability of the muscle to sustain tetanic tension was more pronounced in the soleus than in the tibialis muscle, possibly because the frequencies of tetanic stimulation used (50 to 100/sec) were well above the physiological range in the case of the soleus muscle. After the tetanus the maximal twitches of both muscles were at first slightly increased in tension, but subsequently declined to a level below that before the tetanus. Fig. 5 is the record of an experiment in which contractions

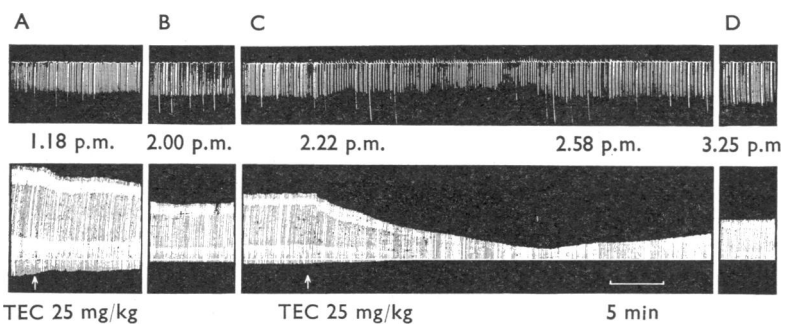

Fig. 5. Effect of triethylcholine on respiratory movements (upper) and contractions of tibialis anticus muscle (lower) in cat. Inspirations are downwards and tibialis contractions upwards. The sciatic nerve was stimulated at 100/sec for 0.2 sec every 5 sec. The doses of triethylcholine (TEC) refer to the iodide.

of a tibialis muscle were recorded in response to nerve stimulation at 100/sec for 0.2 sec every 5 sec. In the original record it can be seen that initially the tetanic contractions were well maintained, but after triethylcholine the contractions resembled twitches.

We have never observed a complete neuromuscular paralysis with doses of triethylcholine iodide up to 50 mg/kg, and the greatest decrease recorded in the twitch tension of the tibialis muscle was of the order of 80 to 90%.

At the height of a partial paralysis produced by triethylcholine, direct stimulation of the muscle produced normal contractions. Fig. 3 shows maximal twitches and a tetanus produced by direct stimulation at the time of maximum block of the indirectly excited contractions of both the tibialis and the soleus muscles. Throughout the blocking action contractions of the tibialis muscle produced by close-arterial injections of acetylcholine remained equal in tension to those produced before triethylcholine (Fig. 2).

Simultaneous recordings of muscle and nerve action potentials before and after triethylcholine are shown in Fig. 6. Triethylcholine markedly reduced the amplitude

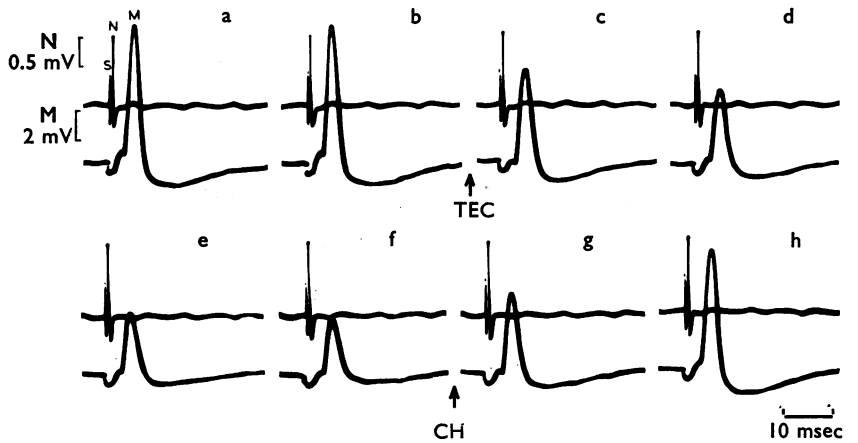

Fig. 6. Cat, 2.1 kg. Maximum action potentials recorded from the peripheral end of the left common peroneal nerve and from the right tibialis anticus muscle (concentric needle electrodes) in response to stimulation of the nerve once every second. S, Stimulus artifact; N, nerve action potential; M, muscle action potential; a and b were recorded before injection with an interval of 10 min between them; c, d, e and f were recorded 12, 14, 16 and 20 min respectively after the intravenous injection of 75 mg/kg of triethylcholine iodide; g and h were recorded 1 and 3 min after the intravenous injection of 5 mg/kg choline chloride.

of the muscle action potential, but was without effect on the shape and size of the nerve action potential. Triethylcholine iodide injected close-arterially in doses up to 5 mg did not cause contraction of the tibialis muscle.

Rabbit. In the rabbit under urethane anaesthesia, triethylcholine produced effects on the contractions of the tibialis muscle similar to those described in the cat, except that higher frequencies of stimulation were required to demonstrate them. Thus in the rabbit, short bursts of high frequency stimulation were found more suitable than single pulses. In the majority of experiments the muscle of one leg was excited every 5 sec by stimulating the nerve at a frequency of 100 or 250/sec for a period of 0.2 sec. With this type of stimulation, the contractions decreased in tension over the first 2 to 3 min and then remained constant at approximately

80% of the initial tension for several hr. The muscle of the other leg was simultaneously excited by single supramaximal shocks applied to the nerve once every 5 sec.

In the experiment illustrated in Fig. 7, triethylcholine iodide (40 mg/kg) caused an initial transient decrease in the tetanic contractions, followed by a more slowly developing block of the contractions which was more rapid in onset than that seen in the cat. The duration of the effect was similar in the two species. The slowly stimulated muscle was completely unaffected by triethylcholine.

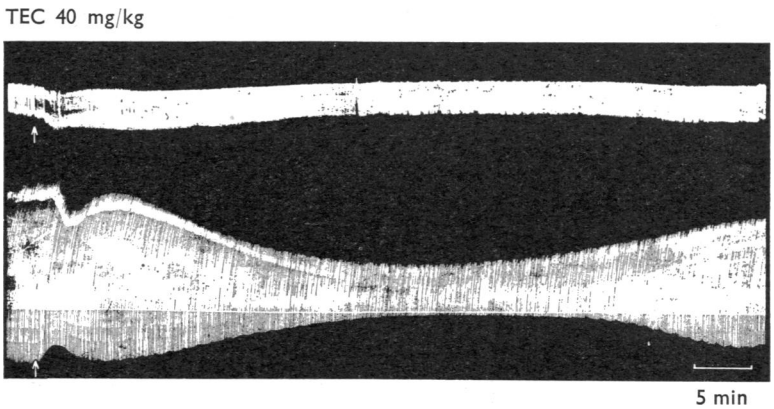

Fig. 7. Effect of triethylcholine on respiratory movements (upper trace) and contractions of tibialis anticus muscle (lower trace) of rabbit. Inspirations are downwards and muscle contractions upwards. The sciatic nerve was stimulated at 100/sec for 0.2 sec every 5 sec. The tetanic contractions of the tibialis muscle were depressed, but respiration was not impaired after the intravenous injection of 40 mg/kg triethylcholine iodide.

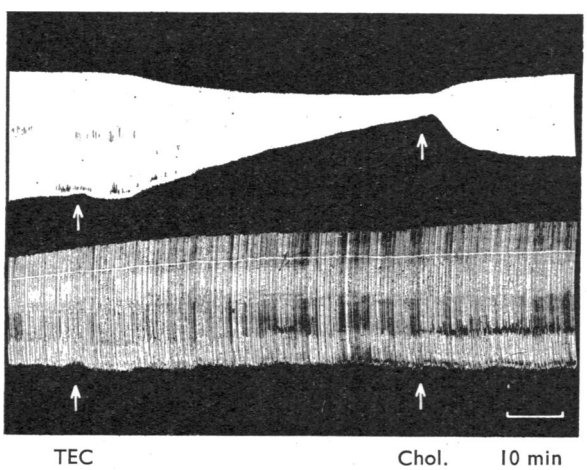

Fig. 8. Maximal twitches of 2 hemidiaphragms from one rat mounted in the same 100 ml. organ bath. Contractions are downwards. In the upper tracing the phrenic nerve was stimulated at 1/sec and in the lower at 1/10 sec. At TEC 40 mg of triethylcholine iodide and 1 hr later at Chol. 10 mg of choline chloride were added to the bath.

Rat diaphragm. The contractions of the isolated rat diaphragm to stimulation of the phrenic nerve were gradually reduced after the addition of triethylcholine to the bath when the rate of nerve stimulation was high, but were not affected by low rates of stimulation. The experiments of Fig. 8 show the contractions of both hemidiaphragms from one rat mounted in the same bath. The upper tracing, which is of a diaphragm driven at 1/sec, shows a marked reduction in contractions 1 hr after 0.4 mg/ml. triethylcholine iodide; the lower tracing shows that triethylcholine did not reduce the contractions of a diaphragm driven at 1/10 sec. The maximum rate of nerve stimulation which did not result in a reduction of the contractions of the diaphragm in the presence of 0.4 mg/ml. triethylcholine iodide was about 1/3 sec. With frequencies of stimulation higher than 1/sec the rate of onset of failure was more rapid. The initial effect of triethylcholine on the diaphragm was to produce a slight increase in the contractions, which was followed by the slowly developing block when stimulation was rapid.

The effects of gallamine and of tubocurarine were tested on two hemidiaphragms stimulated at 1/sec and 1/10 sec in the same bath. With these neuromuscular blocking drugs the greatest effect was on muscle with a high rate of nerve stimulation, but some degree of block was detectable at slow rates. Triethylcholine, on the other hand, even in concentrations up to 5 mg/ml., which was the maximum administered, did not reduce the contractions of the diaphragm produced by slow rates of stimulation.

Effect of neostigmine and edrophonium. Neostigmine and edrophonium, in doses of 100 μg/kg and 200 μg/kg respectively, caused only a small increase in the tension of the triethylcholine blocked twitches in the cat. These doses were twice as great as those necessary completely to antagonize a similar degree of paralysis produced by tubocurarine. Fig. 4 illustrates the slight effect of neostigmine at the height of a partial paralysis produced by triethylcholine in the tibialis and soleus muscles of the cat. The small increase in twitch tension produced by neostigmine may well have been a consequence of repetitive firing of some of the unblocked fibres, rather than to an increase in the number of fibres contributing to the gross tension. Evidence in support of this was provided by the experiments on the rabbit in which tetanic contractions were recorded in place of twitches. In these circumstances repetitive firing cannot be produced and neostigmine and edrophonium were completely without effect on the partially blocked contractions.

Effect of choline. Choline antagonized the effect of triethylcholine in reducing the contractions of indirectly stimulated muscles. In both cat and rabbit the intravenous injection of choline chloride in doses of 1 to 5 mg/kg caused a striking and rapid reversal of the effect of triethylcholine (Figs. 2, 3 and 6). A similar reversal was obtained in the isolated diaphragm preparation when choline was added to the bath in a concentration of 50 to 200 μg/ml. (Figs. 8 and 9). The same amounts of choline given first prevented or reduced the development of the block produced by triethylcholine; this effect in the rat diaphragm is illustrated in Fig. 9. With the larger doses of choline chloride (5 mg/kg) in the cat, the antagonistic action took place in two stages; an initial abrupt but short-lasting effect was followed by a more slowly developing sustained and complete antagonism (Fig. 2). The initial

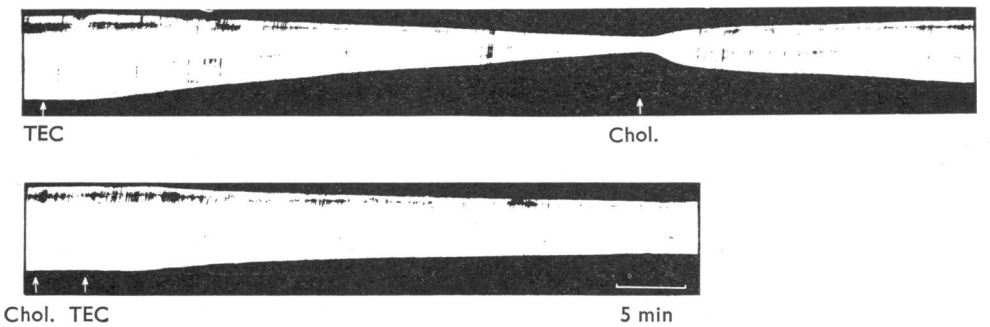

Fig. 9. Maximal twitches of isolated rat diaphragm (contractions downwards) elicited by stimulation of the phrenic nerve at a frequency of 1/sec. At TEC 200 μg/ml. of triethylcholine chloride and at Chol. 50 μg/ml. of choline chloride were added to the bath. In the upper tracing choline reversed the action of triethylcholine. In the lower tracing, which was obtained from the same preparation, choline added first reduced the action of triethylcholine.

rapid antagonism was probably caused by the direct end-plate depolarizing action of choline, since other depolarizing drugs (decamethonium and suxamethonium) produced a similar effect. With the latter drugs, however, the more sustained secondary phase of antagonism was absent. Doses of choline greater than 5 mg/kg caused an initial sharp increase in the partially blocked twitches which was often followed by a pronounced but short-lasting increase in the depth of block. The twitch tension then rapidly recovered and complete antagonism of the triethylcholine block occurred. Triethylcholine did not therefore appear to prevent the block by depolarization which large doses of choline are known to produce (Hutter, 1952). The slowly stimulated muscle on these occasions was also temporarily paralysed by choline.

During the antagonistic action of choline in the cat, contractions of the muscle produced by injected acetylcholine were regularly increased. Fig. 2 illustrates this effect which is probably a consequence of the weak anticholinesterase action of large amounts of choline (Augustinsson, 1948).

In control experiments on the cat and on the rat diaphragm, in which the nerve was stimulated at a sufficiently high frequency to cause fatigue of the contractions, choline was without any beneficial effect and, in fact, often hastened the decline of the twitch tension.

Knee-jerk. In a few experiments, knee-jerks elicited once every minute were recorded from one leg, simultaneously with maximal twitches of the tibialis muscle of the opposite leg elicited indirectly once every sec. Triethylcholine, in doses of 40 mg/kg injected intravenously, depressed the maximal twitches but was without effect on the reflexly induced contractions of the quadriceps muscle.

Comparison with hemicholinium

In the cat, hemicholinium (Schueler, 1955) was effective in smaller doses than triethylcholine, but its effects differed from those of triethylcholine in certain respects. In doses of 1 to 2 mg/kg hemicholinium dibromide caused an immediate decrease in

the contractions produced both by nerve stimulation and by the close-arterial injection of acetylcholine. This was followed by a more slowly developing decrease in twitch tension during which the response to acetylcholine returned to, and was maintained at, the control level. Choline, in doses which completely antagonized the paralysis produced by triethylcholine, caused an abrupt but only short-lasting antagonism to hemicholinium. As with triethylcholine, the soleus muscle was more resistant to the blocking effect of hemicholinium than the tibialis muscle and, like the paralysis produced by triethylcholine, that produced by hemicholinium wore off more rapidly when the frequency of stimulation was reduced. Fig. 10 illustrates these effects

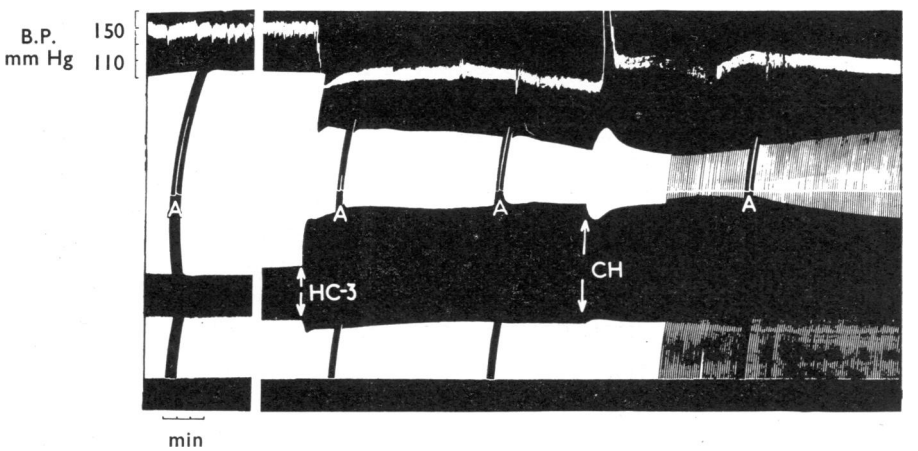

Fig. 10. Cat, 3.4 kg. Upper record: blood pressure; middle and lower records: maximal twitches of the tibialis anticus and soleus muscles respectively elicited indirectly once every second and, towards the end of the record, once every 10 sec. At A, 8 μg acetylcholine injected close-arterially to the tibialis anticus muscle. Electrical stimulation was temporarily stopped while acetylcholine injections were made. At HC-3, 2 mg/kg of hemicholinium dibromide, and at CH, 5 mg/kg choline chloride were injected intravenously.

of hemicholinium. The initial abrupt decrease in twitch tension, during which the response of the muscle to acetylcholine was depressed, was probably a consequence of the weak curare-like action of hemicholinium (Reitzel & Long, 1959a ; Schueler, 1960).

Comparisons of the relative potency of hemicholinium and triethylcholine, taking the doses of hemicholinium used by other workers (Reitzel & Long, 1959a and b ; Wilson & Long, 1959), is difficult since the effect obtained depends on the parameters of the stimulation. For example, Reitzel & Long (1959a) used stimuli of 3 msec duration, but in the majority of our experiments the pulse width was 50 μsec. On a few occasions, the pulse width was increased to 3 msec during the experiment and this caused a marked increase in the response of the muscle to a single shock applied to the nerve. Recordings of the gross muscle action potential with belly-tendon electrodes showed that the response had become markedly repetitive and under these conditions the block produced by both triethylcholine and

hemicholinium was increased. When compared under the same conditions, hemicholinium was shown to be about 20 times more potent than triethylcholine on a weight basis. However, the doses of choline required to reverse the effects were larger in the case of hemicholinium.

A further comparison between triethylcholine and hemicholinium was made on the frog rectus abdominis muscle. Hemicholinium dibromide in a concentration of 150 μg/ml. caused a 50% reduction in the response to acetylcholine, but triethylcholine iodide even in concentrations up to 5 mg/ml. did not reduce the acetylcholine responses. Triethylcholine did not itself produce contraction of the rectus abdominis muscle.

Respiratory movements

In the experiments to determine the toxicity of triethylcholine in mice and rabbits death was apparently due to respiratory failure, and in the isolated diaphragm preparation of the rat, contractions elicited by phrenic nerve stimulation were depressed by triethylcholine. Therefore experiments were carried out in which respiratory movements were recorded.

The effect of the first injection of triethylcholine in the rabbit was a slight hyperpnoea, which was probably reflexly induced by the fall in blood pressure (Fig. 7). After large doses of triethylcholine iodide (100 mg/kg) the rate and depth of respiratory movements were decreased and became erratic. These changes did not occur immediately after the injection, but developed slowly. After the injection of choline the rate and depth of respiratory movements gradually increased and became more regular.

Our previous experiments showed that the rate of failure of responses to nerve stimulation depended on the frequency of stimulation. Therefore it was important to establish the relationship between failure of a muscle contracting in response to stimulation of its nerve and the failure of contraction of the respiratory muscles in response to the respiratory drive. The experiment of Fig. 7 shows that a single injection of triethylcholine iodide (40 mg/kg) into a rabbit was without effect on respiratory movements, but markedly decreased the response of the tibialis muscle to stimulation of the sciatic nerve. A similar experiment on a cat is shown in Fig. 5. The injection of triethylcholine iodide (25 mg/kg) produced, after 40 min, a 40% decrease in the tension developed by the tibialis muscle in response to brief tetanic shocks to the nerve. At this time the only effect on respiratory movements was a slight slowing. A second injection of 25 mg/kg triethylcholine led to an 85% decrease of response of the tibialis muscle and the respiratory movements were further slowed and became erratic and smaller in amplitude. However, they were clearly sufficient to maintain life. In other experiments in which 40 mg/kg triethylcholine iodide was given there was a pronounced reduction in the contractions of the tibialis muscle, but spontaneous respiration persisted.

The depressor action of triethylcholine

The intravenous injection of triethylcholine produced a transient fall in blood pressure in rabbit, cat (Figs. 2 and 11) and dog. In the experiments on the conscious cat and dog we observed a relaxation of the nictitating membrane immediately after

the injection of 50 mg/kg of triethylcholine. Therefore it seemed likely that the depressor action of triethylcholine may have been due to ganglionic blockade, especially since triethylcholine is closely related to the ganglion blocking drug tetraethylammonium. The experiment of Fig. 11 shows that the reduction

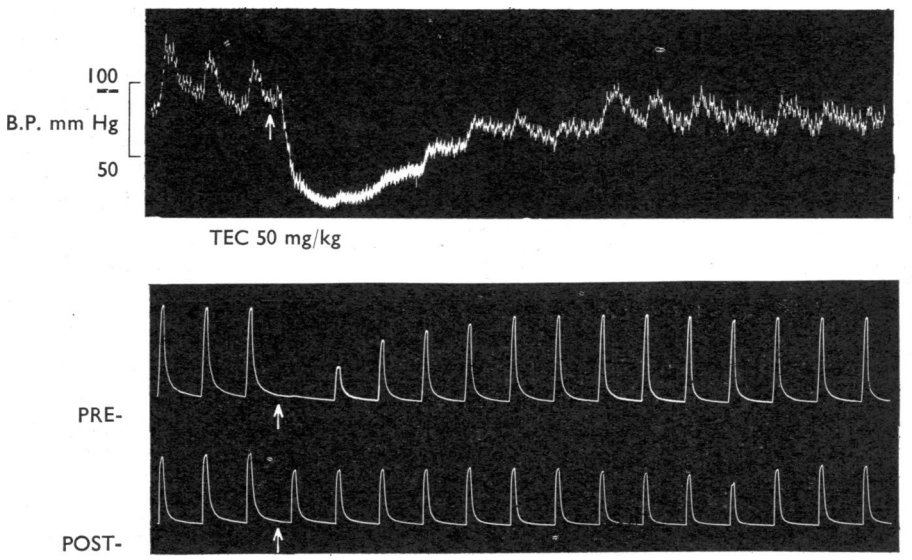

Fig. 11. Cat, 2.8 kg. Effect of triethylcholine iodide on blood pressure (upper tracing), and on the contractions of nictitating membranes in response to sympathetic nerve stimulation with 0.5 msec pulses at 20/sec for 12 sec every 2 min. The contractions of the left nictitating membrane (middle trace) are in response to preganglionic nerve stimulation and the contraction of the right nictitating membrane (lower trace) are to postganglionic nerve stimulation.

in response of the nictitating membrane to pre-ganglionic nerve stimulation was completely blocked at a time corresponding to the maximum fall in blood pressure and the recovery of response of the nictitating membrane was approximately parallel with the recovery of the blood pressure.

In the dog the pressor effect of 0.15 mg/kg of tetramethylammonium and the fall in blood pressure caused by stimulation of the peripheral end of the cervical vagus nerve were transiently blocked by 30 mg/kg of triethylcholine iodide. Intravenous injections of 10 to 30 mg/kg triethylcholine iodide produced a fall in blood pressure of 10 to 30 mm Hg. This depressor response was not blocked by atropine in doses up to 1 mg/kg, but was absent after complete ganglion block by hexamethonium bromide (4 mg/kg).

DISCUSSION

The most striking property of triethylcholine is its ability gradually to reduce the size of contraction of a muscle rapidly stimulated through its motor nerve. Thus, in experiments on nerve-muscle preparations *in situ* and in the organ bath, triethylcholine reduced the contractions in response to motor nerve stimuli at a high rate

while producing no effect where the rate of stimulation was low. Similarly in conscious animals injected with triethylcholine, muscular weakness was more pronounced after exercise, unexercised muscles being relatively unaffected. A more selective blocking action on muscles stimulated at a high rate is also seen with curare-like drugs (Preston & Van Maanen, 1953), but with these drugs the difference in effect on rapid and slow stimulation is much less marked than with triethylcholine.

Our experiments allow us to locate the site of action of triethylcholine. When muscle contractions are markedly reduced by triethylcholine, conduction in the motor nerve trunk is unaffected since the nerve action potentials were unchanged in shape and size. The muscle responded normally to direct electrical stimulation and to injected acetylcholine, which shows that the block is not due to an effect on the contractile mechanism itself nor to a decrease in the sensitivity of the motor end-plates to acetylcholine. The chemical similarity of triethylcholine to choline, together with the fact that choline reverses the effects of triethylcholine, make it likely that triethylcholine interferes with the metabolism of choline at the nerve endings, probably by interfering with the synthesis of acetylcholine. The slow onset of the paralysis and its dependence on high activity in the motor nerves support this view, since the pre-formed depots of acetylcholine must be exhausted before transmission fails. Further work is necessary to elucidate the precise mechanism of action by which triethylcholine may interfere with acetylcholine synthesis, but it is of interest to speculate. The substance appears to resemble hemicholinium in its mechanism of action. (For a recent review of the actions of the hemicholiniums, see Schueler, 1960.) Hemicholinium has been found to prevent the synthesis of acetylcholine by the choline-acetylase of organized brain tissue but to be without this effect after disruption of the cell membranes (MacIntosh, Birks & Sastry, 1956; Gardiner, 1957). These results led to the suggestion that hemicholinium competes with choline for a carrier mechanism in the cell membrane and causes transmission failure at cholinergic junctions by rendering the nerve terminals deficient in choline and consequently in acetylcholine (MacIntosh, 1959). Triethylcholine and hemicholinium possess properties in common; both are more effective during high rates of nerve stimulation and both are antagonized by choline. The molecule of triethylcholine is small compared to that of the bisquaternary hemicholinium, and it is possible that, in addition to preventing the combination of choline with the carrier mechanism, triethylcholine may itself be transported to intracellular sites in place of choline. Burgen, Burke & Desbarats-Schonbaum (1956) found from *in vitro* studies that triethylcholine was acetylated by the choline acetylase of brain tissue almost as effectively as choline itself. The acetyl ester of triethylcholine is 5,000 times less active than acetylcholine on the frog rectus abdominis muscle (Holton & Ing, 1949), so that, if triethylcholine were acetylated in the nerve terminals and subsequently released as a "false" transmitter, it would be ineffective in causing muscle contraction.

The idea that triethylcholine might be synthesized into a physiologically inert neurohormone and thus cause transmission failure is not new. Burgen *et al.* (1956) and Stovner (1958) speculated on this possibility. The latter author, however, was unable to demonstrate such an effect in the isolated diaphragm preparation of the rat, probably because he used an insufficiently high rate of nerve stimulation.

Choline antagonizes tubocurarine block (Hutter, 1952), but this antagonism is short lasting in comparison with its reversal of triethylcholine block. Injected choline disappears rapidly from the circulation (Appleton, Levy, Steele & Brodie, 1951). Bligh (1953) found that the plasma choline in dogs returned to its normal level 10 to 20 min after intravenous injection of 5 mg/kg choline chloride. The main loss appeared to be due to destruction by choline oxidase in liver and kidney.

The property of choline to antagonize the actions of triethylcholine at the neuromuscular junction indicates that the level of choline normally present may modify the effects of triethylcholine. Schueler (1960) suggested that the variation in the toxicity of hemicholinium between different batches of mice might depend on the dietary choline intake. Bligh (1952) found that the mean free choline content of cat plasma was 0.7 μg/ml. (range 0.5 to 0.8); rabbit plasma had a higher choline content (mean 2.9 μg/ml., range 1.2 to 5.2). In our experiments we found that it was more difficult to produce neuromuscular block with triethylcholine in the rabbit than in the cat. It is conceivable that this difference is related to the different choline contents. According to Bligh (1952) human plasma contains 1 to 2 μg/ml. of choline.

In the cat, the soleus is a slow-contracting muscle containing a high proportion of red fibres; its function is concerned mainly with the maintenance of posture and as such it is adapted for prolonged activity. The tibialis anticus muscle, on the other hand, is a fast-contracting flexor muscle containing a high proportion of white fibres. Of the two muscles, the soleus was considerably more resistant to the paralysing action of triethylcholine and hemicholinium when maximal twitches of both were elicited at the same frequency. This difference in sensitivity suggests that the nerve terminals in the soleus muscle may contain greater reserves of preformed transmitter and provides a possible explanation of the surprising resistance of the respiratory muscles to the blocking action of triethylcholine. When tetanic contractions of the tibialis and soleus muscles were recorded the difference in sensitivity to triethylcholine was less marked. Denny-Brown (1929) showed that postural tone in the soleus muscle was maintained by rates of firing of the order of 10/sec. In the present experiments, the frequency of tetanic stimulation used was 50 or 100/sec and, therefore, well above the physiological range for this muscle. During such tetanic contractions the acetylcholine reserves are probably rapidly exhausted, and the tension produced will therefore depend upon the amount of acetylcholine which can be freshly synthesized in the presence of triethylcholine or of hemicholinium.

Unlike most drugs, the action of triethylcholine is greater after subcutaneous, intraperitoneal or intramuscular injection compared with intravenous injection. For example, in the conscious cat, rabbit and dog the effect of a dose given intramuscularly or intraperitoneally is about the same as twice the dose given intravenously. The effect of triethylcholine in producing muscular weakness is slow in onset and therefore is favoured by an injection route which results in a longer persistence in the circulation than would follow intravenous injection. Triethylcholine is not attacked by choline oxidase (Wells, 1954), but it is probably excreted rapidly by the kidney.

Desmedt (1958) has demonstrated the similarity between electromyograms of muscles from myasthenic patients and from cats injected with hemicholinium ; those of cats injected with tubocurarine were quite different in his test procedure. He suggested therefore that " the myasthenic condition results from a presynaptic biochemical defect chronically impairing synthesis of acetylcholine." In our experiments on conscious animals triethylcholine produced a state resembling myasthenia gravis, and these results lend some support to Desmedt's conclusions that there is a presynaptic biochemical defect in this disease. However, the defect in myasthenia gravis and that produced by triethylcholine differ in that the weakness of myasthenia is antagonized by anticholinesterases, while the effects of triethylcholine are not influenced. There are two possible explanations for the inability of anticholinesterases to antagonize triethylcholine. Firstly, triethylcholine itself may possess anticholinesterase activity. Secondly, if triethylcholine is acetylated in the nerve ending, the false transmitter released will be preserved by anticholinesterase (acetyl triethylcholine is hydrolysed by cholinesterase, Holton & Ing, 1949 ; Burgen *et al.*, 1956).

We are indebted to Mr H. J. Fearn for carrying out the toxicity experiments, and to Dr E. A. Gill, Department of Pharmacology, Oxford, and Messrs Ward, Blenkinsop & Co. for gifts of triethylcholine.

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