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A COMPARISON OF THE RESPONSES OF THE TENUISSIMUS MUSCLE TO NEUROMUSCULAR BLOCKING DRUGS IN VIVO AND IN VITRO

BY

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In view of the differing responses to decamethonium which have been reported for the isolated tenuissimus muscle on the one hand and the tibialis muscle of the cat on the other hand, the responses of the tenuissimus muscle to neuromuscular blocking drugs were studied both *in vivo* and *in vitro* and compared with those of the tibialis anterior muscle. In both muscles *in vivo*, the block produced by decamethonium had all the well-known characteristics of blockade due to long-lasting depolarization, independent of the number of doses given. However, when the tenuissimus muscle was studied *in vitro* its responses were considerably altered. When decamethonium was left in contact with the isolated muscle, maximum paralysis developed quickly, but then the muscle recovered despite the continued presence of the drug in the bath. This contrasts with the effect obtained *in vivo*, where a steady application of decamethonium produced steady blockade. This spontaneous recovery *in vitro* occurred to a smaller extent with each successive dose. In addition, the usual antagonism between depolarizing and competitive drugs was only seen during the earlier part of an experiment and rarely occurred after several doses. It is suggested that the differences between *in vivo* and *in vitro* responses of the tenuissimus muscle to decamethonium could be due to detrimental changes in ionic concentration gradients resulting from immersion in an artificial fluid.

Decamethonium and suxamethonium are quaternary ammonium compounds which block neuromuscular transmission in man and the cat by mimicking acetylcholine (Zaimis, 1951 ; Bovet & Bovet-Nitti, 1955 ; Cannard & Zaimis, 1959). Burns & Paton (1951) have shown that in the gracilis and tibialis muscles of the cat, *in vivo*, this likeness to acetylcholine is due to the ability of decamethonium to cause a persistent depolarization of the end-plate region. However, Thesleff (1958) has questioned the existence of a block due solely to depolarization. He found that in the tenuissimus muscle of the cat, *in vitro*, during a continuous application of decamethonium or suxamethonium, the motor end-plate was initially depolarized but this depolarization was slowly abolished and replaced by desensitization to the transmitter. Furthermore, during this period of desensitization the well-known antagonism between depolarizing and non-depolarizing drugs did not occur.

In view of this discrepancy it was decided to investigate the effects of neuromuscular blocking drugs on the tenuissimus muscle both *in vivo* and *in vitro* and

to compare the findings with those on the tibialis anterior muscle, the reactions of which, to various neuromuscular blocking drugs, have been extensively studied (Paton & Zaimis, 1952; Zaimis, 1953; Jewell & Zaimis, 1954).

METHODS

Experiments in vivo

Cats were anaesthetized with a mixture of chloralose (80 mg/kg) and pentobarbitone sodium (5 mg/kg) injected intravenously. Contractions of the tenuissimus muscle of one hind limb together with those of the tibialis and soleus muscles of the other hind limb were recorded

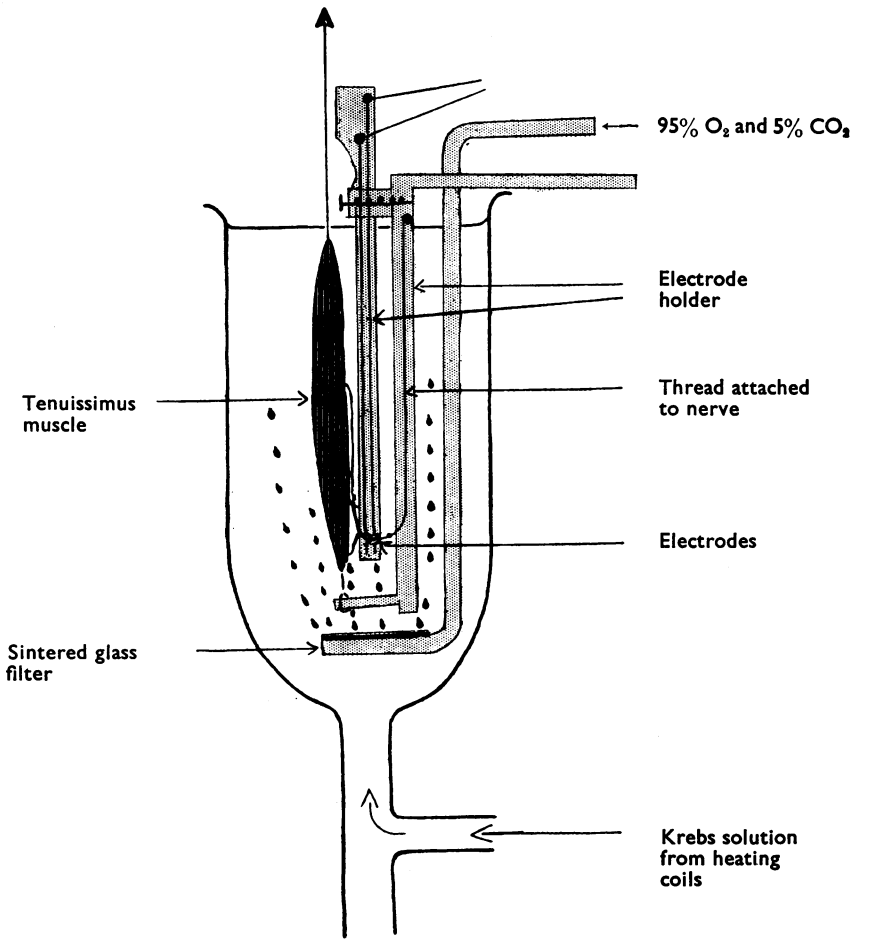

Fig. 1. Diagram of the apparatus used for experiments on the tenuissimus muscle *in vitro*.

simultaneously. The method used to record contractions of the tibialis and soleus muscles was that described by Bigland & Zaimis (1958) and the limb was mounted horizontally.

The tenuissimus muscle is approximately 10 cm long and 0.5 cm wide and is ribbon-like in appearance. It runs from the second caudal vertebra to a diffuse attachment on to the fascia of the biceps femoris muscle (Adrian, 1925). In order to record its contractions the

tendon of the muscle was freed from the biceps femoris muscle and dissection continued for approximately 5 cm. Further separation was not possible because blood vessels enter the muscle every 3 to 4 cm. The limb was mounted vertically. The nerve to the tenuissimus muscle arises from the sciatic nerve high in the thigh and branches into two just before reaching the muscle. As it was not possible to free the entire muscle from neighbouring tissues, it was necessary to minimize stimulus spread. The sciatic nerve was tied above and cut below the point of origin of the tenuissimus nerve and a small pair of shielded electrodes were tied on to the main branch of the tenuissimus nerve.

Throughout the experiments great care was taken to maintain the temperature of the three muscles constant; in the case of the vertically mounted limb, extra radiant heating was needed.

Experiments in vitro

The cats were anaesthetized with chloralose, the tenuissimus muscle carefully exposed in the popliteal space and its nerve ligated and cut close to its origin from the sciatic nerve. Two ligatures were then placed round the muscle, one approximately 3 cm central and the other 5 cm distal to the point of entry of the nerve; the muscle was then cut and quickly transferred to a 200 ml. bath filled with oxygenated Krebs solution of the following composition: Na^+ 145 mM, K^+ 5.9 mM, Ca^{++} 2.5 mM, Mg^{++} 1.2 mM, Cl^- 126 mM, HCO_3^- 25 mM, $H_2PO_4^-$ 1.2 mM, SO_4^{--} 1.2 mM.

A modified rat-phrenic-nerve diaphragm electrode (Barnes & Duff, 1953) was used to suspend the muscle in the bath, as shown in Fig. 1. The bathing fluid was oxygenated with 95% oxygen and 5% carbon dioxide bubbled continuously through a sintered glass filter so that the bubbles impinged on the muscle (Creese, Scholes & Whalen, 1958). Throughout the experiment the temperature was maintained at 37° C.

In order to record isometric contractions from both *in vivo* and *in vitro* preparations, the muscle tendon was attached to a flat steel spring or a transducer valve (Radio Corporation of America). Contractions were elicited by square-wave pulses of 0.2 msec duration and twice the strength required to evoke a maximal twitch delivered to the nerve once every 10 sec via isolation transformers. The maximal twitches were recorded either on a smoked drum or photographed from the cathode ray oscilloscope screen.

RESULTS

Comparison of the tibialis and the tenuissimus muscles in vivo

In the first series of experiments the physiological characteristics of the tenuissimus muscle together with its responses to neuromuscular blocking drugs were compared with those of the tibialis anterior muscle *in vivo*.

Physiological characteristics

In the cat, the tibialis is known to be a "fast" muscle made up of predominantly white fibres (Denny-Brown, 1929), but no information could be found for the twitch speed of the tenuissimus muscle. Therefore, a comparison of the two muscles was made, and the results obtained showed that the twitch speed of the tenuissimus was only slightly slower than that of the tibialis anterior muscle. Maximal twitches of the two muscles were recorded simultaneously using transducer valves. Fig. 2 demonstrates a typical result of an experiment where the temperature of both muscles was 35° C. The tibialis twitch reached its peak in approximately 20 msec and the overall duration was 80 msec. The corresponding values for the tenuissimus muscle were 30 msec to peak and 100 msec duration. A further similarity between the two muscles was shown by their responses to tetanic stimula-

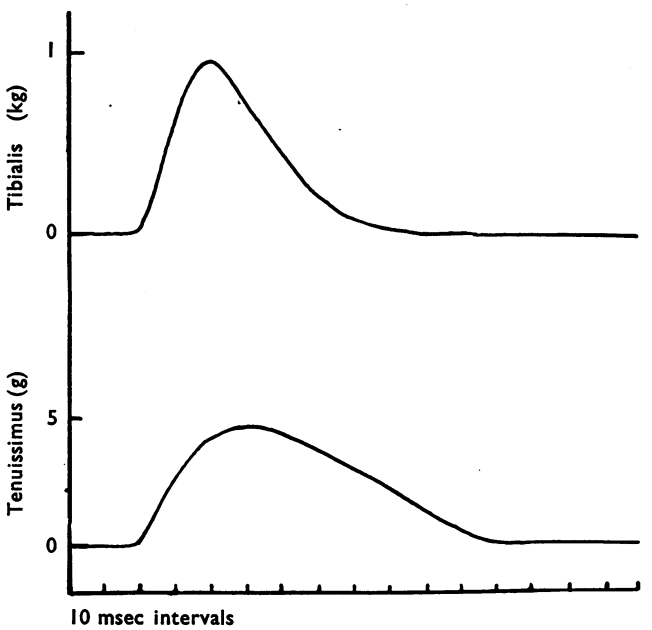

Fig. 2. Cat, 3.5 kg. Chloralose anaesthesia. Simultaneous electrical records of indirectly elicited maximal twitches of the tibialis anterior and tenuissimus muscles. The temperature of both muscles was 35° C.

tion. Both muscles had approximately the same fusion frequencies, and following a tetanus marked potentiation of the maximal twitch occurred, irrespective of the duration of the tetanus or the frequency of stimulation. This effect is shown for the tenuissimus muscle in the first section of Fig. 3.

Responses to neuromuscular blocking drugs

When the responses of the tenuissimus muscle to both competitive and depolarizing neuromuscular blocking drugs were studied, further similarities between this muscle and the tibialis anterior muscle were found. In both muscles the block produced by depolarizing neuromuscular blocking drugs was preceded by potentiation of the maximal twitch, antagonized by tubocurarine and potentiated or unaffected by anticholinesterase drugs. Slight tachyphylaxis occurred in both muscles, but the characteristics of the blockade remained unchanged throughout the experiment, irrespective of the number of doses given or their magnitude. The only noticeable difference between the two muscles was that the tenuissimus was slightly less sensitive to decamethonium than the tibialis muscle (Fig. 4). In contrast, when tubocurarine was used the reverse order of sensitivity was found.

Fig. 4 illustrates the results of an experiment in which a small dose of tubocurarine effectively antagonized the block in both muscles. A similar antagonism is also shown in section *c* of Fig. 3 ; in this experiment tubocurarine was administered

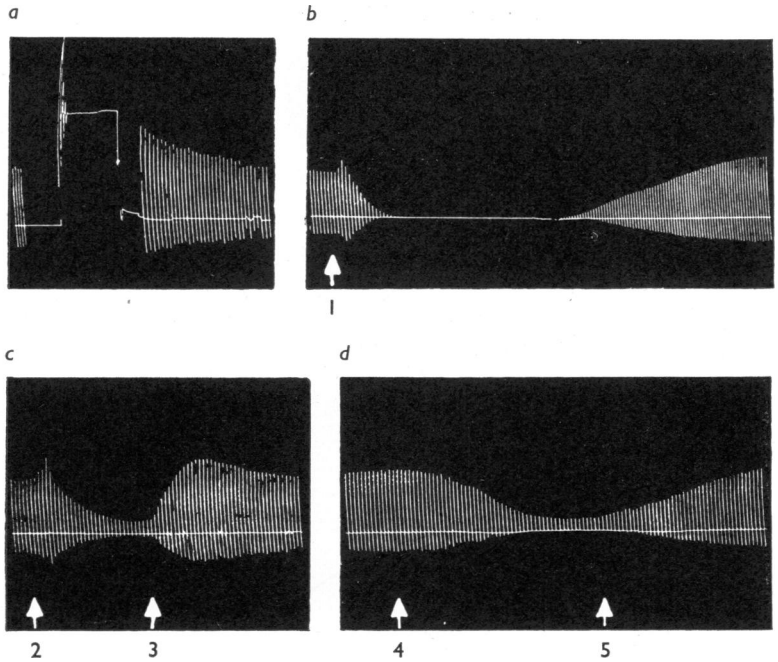

Fig. 3. Cat, 2.1 kg. Chloralose anaesthesia. Record of maximal twitches of the tenuissimus muscle *in vivo*. The first section shows the effect of stimulation at a frequency of 50/sec. This was followed by 5 successive doses of decamethonium of which the last three are shown. At arrow (1) 60 μg, at (2) 50 μg, and at (4) 150 μg of decamethonium were administered. At (3) 200 μg of tubocurarine and at (5) 200 μg of neostigmine were given.

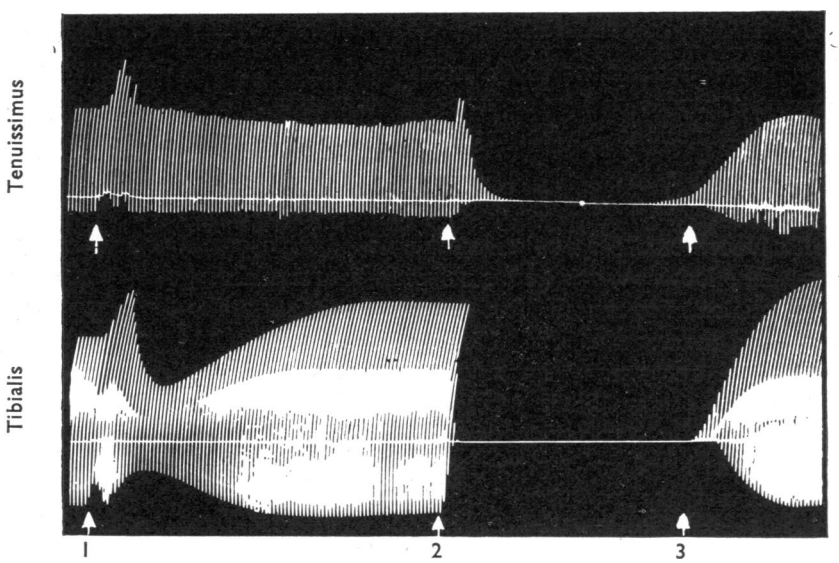

Fig. 4. Cat, 3.4 kg. Chloralose anaesthesia. Simultaneous recording of maximal twitches of the tenuissimus muscle (upper tracing) and tibialis muscle (lower tracing) elicited by indirect stimulation delivered once every 10 sec. At arrow (1) 80 μg and at (2) 110 μg of decamethonium. At (3) 200 μg of tubocurarine.

after the fourth injection of decamethonium. The same figure (last section) also illustrates the characteristic ineffectiveness of neostigmine in antagonizing the decamethonium block.

Experiments on the tenuissimus muscle in vitro

The indirectly elicited maximal twitches of the isolated tenuissimus muscle increased slowly for the first hour after dissection and then remained steady for at least 6 hr. A similar initial increase in twitch height has been reported for the isolated diaphragm preparation of the rat and guinea-pig and attributed to recovery from the trauma and asphyxia of dissection (Creese, 1950 ; Jenden, 1955).

The tenuissimus muscle *in vitro* was found to be very sensitive to decamethonium, concentrations between 0.15 and 0.2 μg/ml. producing approximately 90% block. A comparison with the concentrations needed to produce a comparable degree of paralysis in other isolated preparations (Table 1) shows that the tenuissimus muscle

TABLE 1

COMPARISON OF CONCENTRATIONS OF DECAMETHONIUM AND TUBOCURARINE WHICH CAUSE APPROXIMATELY 90% BLOCK IN DIFFERENT ISOLATED MUSCLES

Muscle	Decamethonium μg/ml.	Tubocurarine μg/ml.	Reference
Cat tenuissimus	0·15	2·4	This paper
Rabbit lumbrical	1·0	—	Jenden, Kamijo & Taylor (1954)
Guinea-pig diaphragm	3·0	1·0	Jenden (1955)
Rat diaphragm	100–200	—	Thesleff (1958);
	—	0·25	Holmes, Jenden & Taylor (1951)

is approximately ten times more sensitive than the rabbit lumbrical preparation, thirty times more sensitive than the guinea-pig diaphragm and one thousand times more sensitive than the rat diaphragm. In contrast, there is only slight variation between the sensitivities of these muscles to tubocurarine.

When decamethonium was added to the bath fluid a steady state of paralysis was reached in 5 to 10 min. If the bath fluid was changed at this stage the block took a similar time interval to recover. Subsequent doses given at regular time intervals produced the same magnitude of blockade. It was found that neostigmine administered during the first or any subsequent blockade was always ineffective. On the other hand, tubocurarine, as a rule, effectively antagonized the first and second doses of decamethonium in any experiment: this is shown in Fig. 5.

Following the administration of tubocurarine it was necessary to change the bath fluid at least six times before the muscle regained its initial sensitivity to decamethonium. This meant that there was necessarily an interval of at least 60 min between doses. Fig. 5 shows the effect of adding a dose of decamethonium when the muscle had only been washed 4 times following the administration of tubocurarine (section C): under these circumstances the block was reduced. This effect

is also known to occur in other isolated tissues (Chou, 1947; Jenden, Kamijo & Taylor, 1954). Because of this prolonged residual effect, in many instances tubocurarine was administered towards the end of the experiment when several doses of decamethonium had already been given. However, this procedure revealed a rather unexpected finding: it was noticed that while tubocurarine effectively antagonized the early doses of decamethonium its effectiveness decreased when

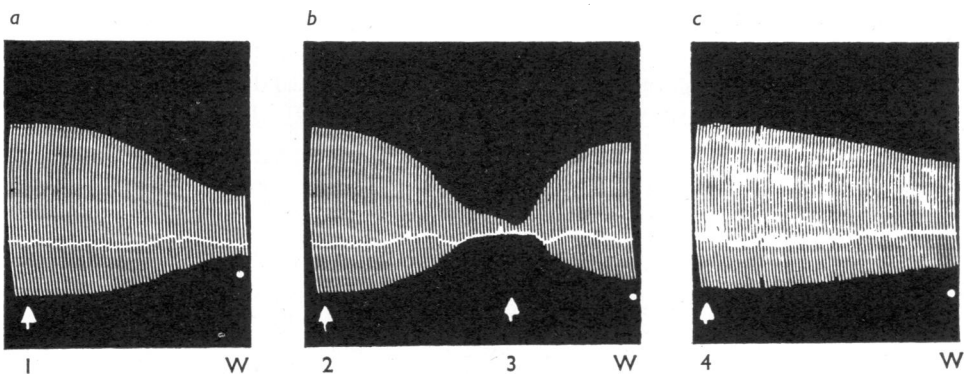

Fig. 5. Record of indirectly elicited maximal twitches of the tenuissimus muscle *in vitro*. Each section shows the effect of adding decamethonium to the bathing fluid; at (W) the solution was changed and the muscle left to recover. At arrows (1), (2) and (4) 30 μg of decamethonium added to bath and at (3) 200 μg of tubocurarine added at point of maximum paralysis. Interval of 60 min between *b* and *c* during which solution was changed 4 times.

administered during subsequent blockades. Thus its effectiveness as an antagonist could be correlated with the number of times that the muscle had been treated with decamethonium. After several doses of the depolarizing drug and at a stage when the muscle appeared to be in good condition, tubocurarine antagonism occurred in only 15 to 20% of the occasions on which it was tested. A further interesting point was that, independent of the effectiveness or ineffectiveness of tubocurarine,

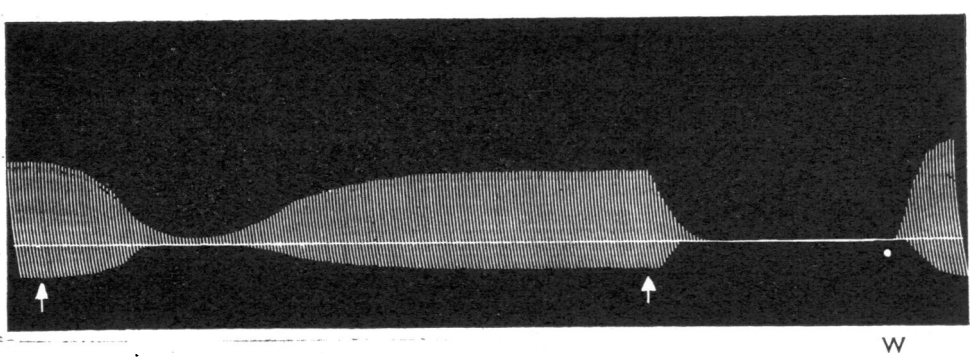

Fig. 6. Record of maximal twitches of tenuissimus muscle *in vitro*. At first arrow, 30 μg of decamethonium was added to bath and left in contact with the muscle for 60 min. At second arrow, further identical dose of decamethonium added. Bath solution changed at (W).

anticholinesterase drugs remained unable to antagonize the decamethonium block. Such a situation is totally different from that recorded *in vivo*.

Thus it appears that the responses of the isolated tenuissimus muscle to decamethonium differ from those obtained from the same muscle *in vivo*. This point is further emphasized by the results of another series of experiments in which decamethonium was left in contact with the muscle for 1 hr. Under these circumstances maximum paralysis developed in about 10 min but then waned, and 30 min later the paralysis had almost completely disappeared. At this stage, however, a

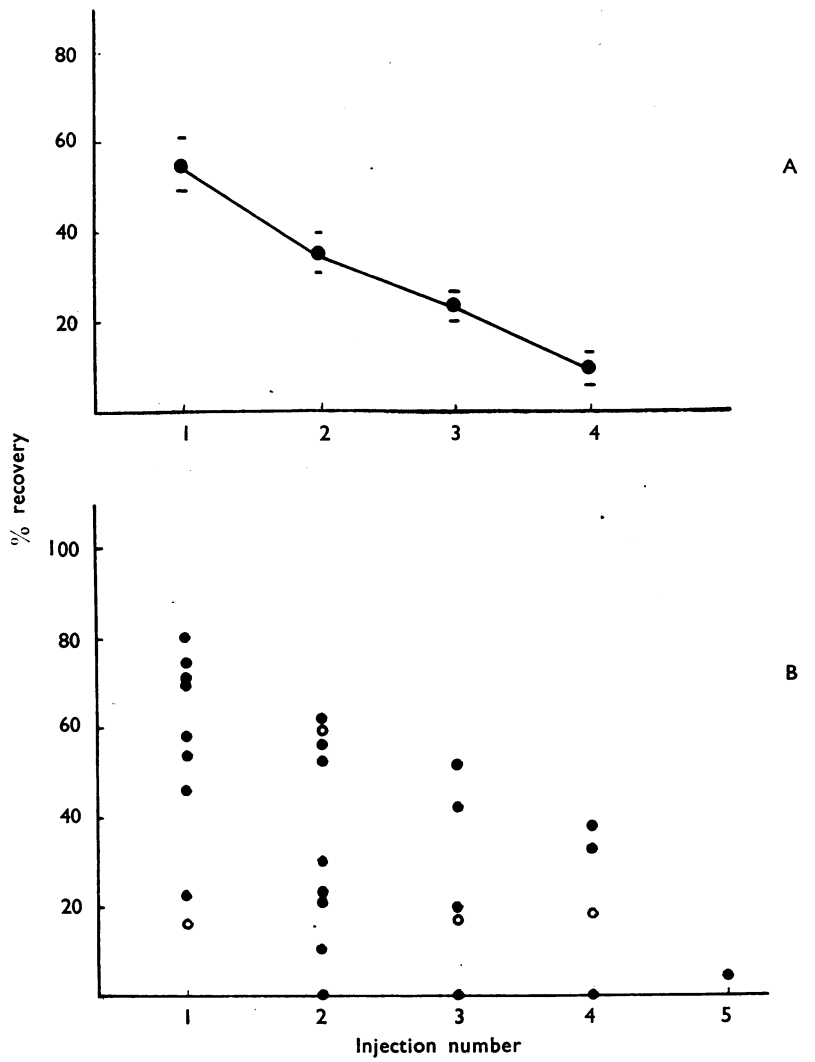

Fig. 7. Percentage spontaneous recovery occurring with successive doses of decamethonium. Section A shows mean values and standard errors of observations from 15 experiments. Individual values from these experiments are shown in section B.

further identical dose of decamethonium restored the paralysis: this result is shown in Fig. 6.

In several experiments an effective dose of the drug was left in contact with the muscle until the block had reached its new steady level, and at this point the entire contents of the bath were removed and preserved at 37° C. When this solution was tested on a fresh muscle it proved fully active and produced a block comparable to that obtained in the first instance.

The results of 15 experiments *in vitro* are shown in the lower half of Fig. 7, in which percentage spontaneous recovery is plotted against the number of doses of decamethonium which had been administered. As the size of the peak block varied from one experiment to another, according to the dose administered, the spontaneous recovery was expressed as a percentage of the peak block. Although this varied widely from one experiment to another, when the mean percentage recovery for each injection number was calculated, the results clearly showed that the spontaneous recovery was greater during the first application of decamethonium than for subsequent doses.

This spontaneous recovery of the isolated muscle while remaining in contact with the drug is in contrast to the behaviour of the tenuissimus muscle *in vivo*,

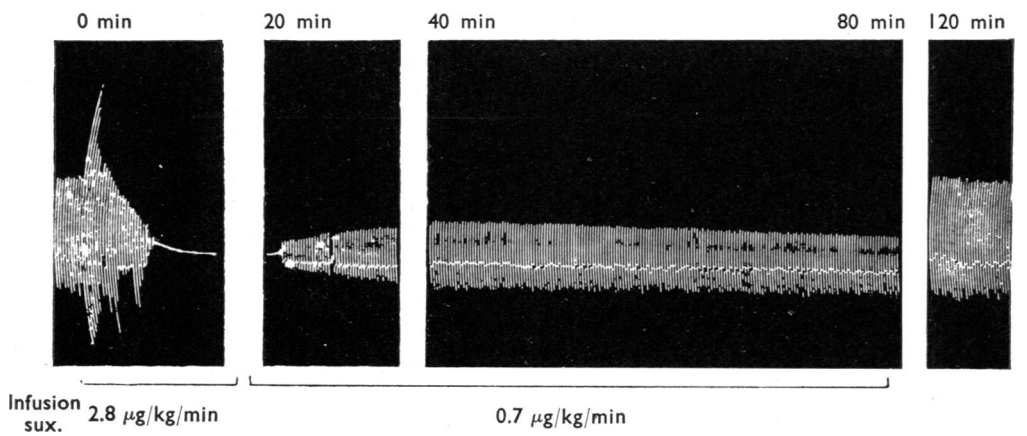

Fig. 8. Record of maximal twitches of the tenuissimus muscle *in vivo*. Suxamethonium infusion started at rate of 2.8 μg/kg/min and later reduced to 0.7 μg/kg/min. The infusion rate was then maintained constant throughout the experiment. Artificial respiration given during the infusion.

where the continuous infusion of a depolarizing neuromuscular blocking drug at a constant rate produces a steady degree of paralysis. Fig. 8 illustrates an experiment in which suxamethonium was infused at a constant rate for more than 40 min, during which time the degree of paralysis remained constant.

A further interesting point was that, despite this waning spontaneous recovery, the peak block for a given dose was constant throughout the experiment irrespective of the number of previous doses of decamethonium. This again contrasts with the results obtained *in vivo*, where slight tachyphylaxis occurred in the tenuissimus muscle.

DISCUSSION

The results just described show that, *in vivo,* the tenuissimus is very similar to the tibialis muscle, both in its physiological characteristics and in its responses to neuromuscular blocking drugs. In both muscles, the time course of the twitch was short and tetanic stimulation was followed by potentiation of the maximal twitch. This latter effect was independent of either the duration of the tetanus or the frequency of stimulation.

The similarity between the tenuissimus and tibialis muscles *in vivo* was further emphasized by their responses to pharmacological tests. In both muscles, the interruption of neuromuscular transmission produced by decamethonium had all the characteristics of a depolarization block (Paton & Zaimis, 1952): the paralysis was preceded by spontaneous fasciculations of the muscles and potentiation of the maximal twitch. Furthermore, the block was antagonized by tubocurarine but unaffected by anticholinesterase drugs. Moreover, after repeated single injections or prolonged infusions of the depolarizing drug the characteristics of the blockade remained unchanged in both muscles. Thus, for the muscle *in situ* the characteristics of the blockade were independent of the length of application of the drug.

This uniformity and predictability of response was lost when the tenuissimus muscle was studied *in vitro.* The most striking change which occurred was the recovery of neuromuscular transmission while decamethonium was still in contact with the muscle. This spontaneous recovery was marked during the first blockade but steadily decreased with each subsequent dose. These effects were quite different from those obtained *in vivo,* where a continuous slow infusion of suxamethonium produced a steady paralysis.

Varying results were also obtained when the antagonism between competitive and depolarizing neuromuscular blocking drugs was studied. While tubocurarine effectively antagonized the first dose of decamethonium, it proved less effective in antagonizing subsequent blockades. This phenomenon was found to be related to the number of previous doses of decamethonium and independent of the number of applications of tubocurarine. The only reaction which remained constant throughout an experiment was the complete ineffectiveness of anticholinesterase drugs to antagonize the action of decamethonium. From these results it appears that there is little similarity between the responses obtained from the tenuissimus muscle *in vitro* and those obtained from either the same muscle or from other well-studied mammalian muscles *in vivo.*

In contrast, comparisons can be drawn between the present results *in vitro* and those reported by Thesleff (1958) for the isolated tenuissimus muscle. He found that "the initial antagonism between non-depolarizing and depolarizing drugs is not maintained in the desensitized state and that, on the contrary, the desensitization produced by depolarizing drugs is additive to that produced by tubocurarine and vice versa."

Furthermore, it is interesting that many similarities can be drawn between the behaviour of the tenuissimus muscle *in vitro* and other isolated muscle preparations; in particular, the rabbit lumbrical muscle (Jenden, 1955); the guinea-pig diaphragm (Jenden, Kamijo & Taylor, 1954); and human intercostal muscle preparation (Dillon

& Sabawala, 1959). Although there are considerable differences in the sensitivity of these muscles to decamethonium, the time course of the blockade and the phenomenon of spontaneous recovery are almost identical.

In all these muscles maximum paralysis develops in approximately 10 min and the block then recovers to reach a new steady state in approximately 20 min. The finding that there is a marked difference between the spontaneous recovery following the first and subsequent applications of decamethonium is not reported by most of these authors, possibly because no such comparison was made. However, Jenden *et al.* (1954) commented that " successive doses produce increasing phase 1 block and slower recovery from it to a lower, earlier point of maximum recovery." Furthermore, if the results shown in the third illustration in their paper are calculated as in the present experiments, it is found that the first dose of decamethonium was followed by 90% spontaneous recovery whereas, by the third dose, recovery had fallen to 50%.

This similarity between the isolated cat, guinea-pig, rabbit and human muscles is very surprising, since *in vivo* the muscles of these species differ in their responses to decamethonium: in the rabbit and guinea-pig decamethonium exhibits a dual mode of action (Zaimis, 1953 ; Hall & Parkes, 1953), whereas in the cat and man the paralysis has all the characteristics of a block due to long-lasting depolarization (Burns & Paton, 1951 ; Cannard & Zaimis, 1959).

In the cat *in vivo* following a single administration or a continuous application of decamethonium or suxamethonium, there is a fairly good parallelism between blockade and depolarization measured either electrically or pharmacologically (Burns & Paton, 1951 ; Zaimis, 1959 ; Paton, 1962). In contrast, *in vitro,* despite the continued presence of the same drugs, the block either " recovers " spontaneously as in the present experiments, or remains steady, while depolarization is replaced by " desensitization " as in Thesleff's experiments. In conclusion these unusual responses of the isolated muscles to depolarizing neuromuscular blocking drugs would appear to be the result of changes occurring in the muscle when isolated from the body and immersed in an artificial fluid, but unrelated to physiological differences between the neuromuscular junctions of these various mammalian muscles.

One interesting point which emerges from these findings is that, at a stage when the responses to depolarizing drugs are profoundly altered, the twitch tension can remain unchanged. Thus " pharmacological tests," as used in the present experiments, appear to provide an early indication of changes in the physiological properties of the isolated muscle.

It has previously been reported by Creese (1954) that, although the contractions of the rat diaphragm preparation remain steady for many hours when stimulated at low frequencies, the ability of the tissue to maintain the normal concentration gradient between cell and environment is seriously impaired. This results in a gradual depolarization of the inner fibres and significant loss of potassium from the muscle. These effects were accelerated by anoxia or an increase in stimulation frequency, and if continued unchecked eventually resulted in a decrease in twitch tension (Creese, Scholes & Whalen, 1958).

The literature of recent years contains many references to changes in the ionic content of mammalian tissues following immersion in artificial electrolyte media. In particular, Krnjevic & Miledi (1958) have shown that in the isolated rat diaphragm muscle at the end of a 5 hr experiment, consisting of alternate periods of stimulation and rest, the intracellular concentration of sodium is practically that of the bathing solution. At this stage the intracellular concentration of potassium has fallen to less than one-third of the control value. These ionic changes were accompanied by a considerable reduction in the indirectly elicited twitch tension. These effects could be minimized by reducing the temperature to 22° C. In contrast, when this experiment was repeated using the same muscle *in situ*, only slight changes in intracellular sodium and potassium concentration occurred. The deterioration of the isolated muscles was attributed, at least in part, to impaired aerobic metabolism. Creese (1960) and Creese & Northover (1961) have followed the changes in intracellular Na^+ and K^+ in the isolated rat diaphragm and have shown that these concentrations can only be maintained at control levels by using solutions with a high K^+ content and fortified with serum or proteins. Even then the muscle is very sensitive to changes in oxygen supply or stimulation frequency.

In conclusion, it appears that there is adequate evidence to support the suggestion that mammalian muscle undergoes a gradual deterioration when isolated from the body and studied *in vitro*. Consequently the transference of results obtained from experiments *in vitro* to the intact animal must be treated with caution.

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