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ACTION OF DECAMETHONIUM ON RAT DIAPHRAGM

BY

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Decamethonium can act as a depolarizing and as an antidepolarizing inhibitor of neuromuscular transmission (Taylor & Nedergaard, 1965). Jenden, Kamijo & Taylor (1954) and Jenden (1955) described a biphasic effect of decamethonium on isolated rabbit lumbrical muscle and guinea-pig diaphragm preparations. On adding decamethonium to a nerve-muscle preparation in an organ bath the response to nerve stimulation was rapidly blocked (phase I), but recovery occurred in spite of the continued presence of decamethonium ; this recovery was followed by a slowly developing depression (phase II) which took several hours to reach a steady state. MacLagan (1962) found in a cat's tenuissimus muscle and nerve preparation *in vitro* decamethonium produced a short-lasting paralysis followed by recovery. However, *in vivo* she found that a continuous infusion of a "depolarizing neuromuscular blocking drug" caused a steady paralysis with no biphasic effect. This difference in response to decamethonium has been further investigated in the present experiments using rat nerve-diaphragm preparations.

METHODS

Laboratory rats weighing 170–210 g were killed by bleeding after light ether anaesthesia. An entire or hemidiaphragm was removed with its phrenic nerves or nerve and set up as described below. The solutions used, unless otherwise stated, contained K^+ 5.9 mM, Ca^{++} 2.1 mM, Na^+ 156 mM, Cl^-138 mM, HCO_3^-24.9 mM, $H_2PO_4^-$1.2 mM, dextrose 11.1 mM, sucrose 13.7 mM and were gassed with a mixture of 95% oxygen and 5% carbon dioxide. The nerves were stimulated with a rectangular pulse of 0.3–0.5 msec at twice the minimal voltage required to produce a maximal single contraction of the muscle. The effects of decamethonium iodide, tubocurarine chloride and suxamethonium chloride were studied. At the end of each experiment the amount of sodium and potassium in the diaphragm was determined by flame photometry after ashing with 10:1 mixture of concentrated nitric and perchloric acid and dissolving in distilled water.

Organ bath experiments

Isolated hemidiaphragms from rats weighing 170–190 g (weighing 80–140 mgm at end of the experiments) were immersed in a bath at 37° C and the contractions recorded by a spring lever on a smoked drum. In some experiments both the right and left hemidiaphragms were set up in the same bath, but their nerves were stimulated separately. The height of contraction became constant after about 15 min and remained constant for up to 9 hr (when the rate of stimulation was slower than once a second).

Perfusion experiments

Before removal from a rat (170–210 g) of the entire diaphragm (weighing 150–400 mgm at end of the experiment) the inferior vena cava was cannulated through the right auricle and the

polythene catheter tied so that its tip was lying just anterior to the diaphragm. The inferior vena cava was ligated in the abdomen just posterior to the diaphragm. The diaphragm was perfused with the solution described *via* the catheter at a rate between 2 and 4 ml./min. The rate of perfusion in any one experiment varied by less than 0.5 ml./min. Drugs could be added to the perfusion fluid by a slow injector (Palmer). The preparation was mounted on a holder (Fig. 1), placed in an empty jacketed organ bath maintained at 37° C and gassed with 95% oxygen and 5% carbon dioxide. The container was open at the base and the effluent from the muscle was collected. The contractions of the muscle were recorded on a smoked drum. In some experiments the muscle was perfused with a solution containing 42K before reverting to a solution without radioactivity. The radioactivity of the effluent, collected in 4 min samples, was measured in a type M6 liquid Geiger Mueller counter and corrections for decay, paralysis time and background were applied. In the perfusion experiments, the muscle was oxygenated by exposure of the surface to 95% oxygen and 5% carbon dioxide as well as by oxygenation of the perfusion fluid.

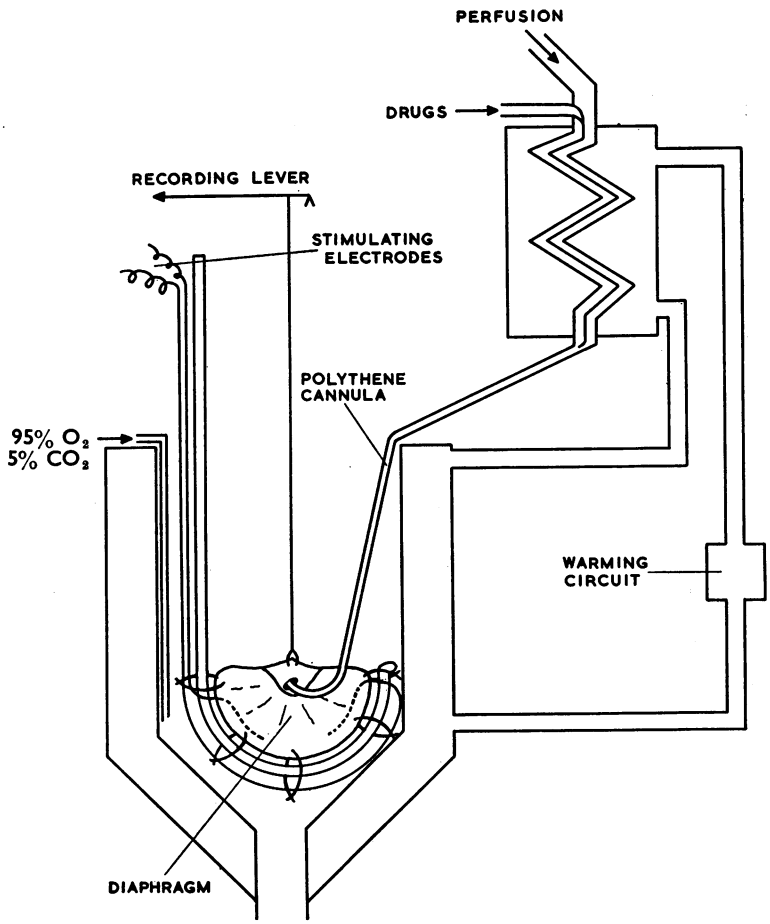

Fig. 1. Rat diaphragm perfusion. An entire diaphragm is cannulated *via* inferior vena cava and suspended in a warm atmosphere of 95% oxygen and 5% carbon dioxide. The muscle or its nerves can be stimulated. Container, which is open at its base, and perfusion fluid are warmed. By means of slow perfusion pump drugs can be added to perfusing fluid.

RESULTS

Immersed non-perfused diaphragm

In 30 preparations two types of response were seen when decamethonium was added to the stimulated nerve-diaphragm preparation. A biphasic effect occurred 13 times (Fig. 2). The first phase of the biphasic response consisted of a rapid initial paralysis, maximal between the 3rd and 18th min and after recovery a second phase of paralysis gradually developed for several hours. A monophasic, slowly developing paralysis occurred in 17 preparations (Fig. 3). This paralysis persisted for several hours, as long as observations continued. Two factors were important in eliciting a biphasic response:

(1) A slow rate of stimulation. When decamethonium 30–70 μg/ml. was added within 90 min of removing the diaphragm from the rat, a biphasic response was always produced (11 diaphragms) if the rate of stimulation was slower than 0.2/sec, but a monophasic

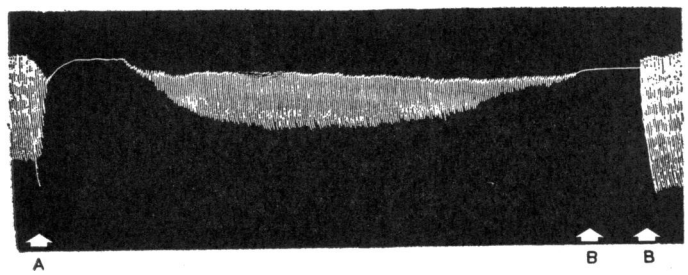

Fig. 2. Immersed rat diaphragm preparation. Biphasic response to decamethonium (0.06 mg/ml.). At A decamethonium added 80 min after removal from rat. Between BB stimulation was stopped and bath fluid changed three times. From A to B was 71 min.

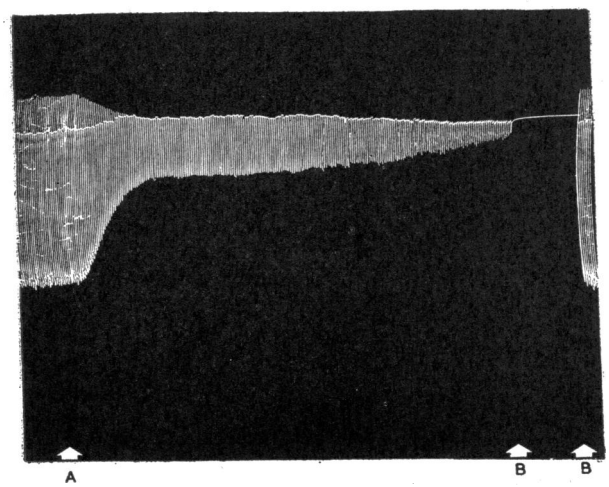

Fig. 3. Immersed rat diaphragm preparation. Monophasic response to decamethonium (0.06 mg/ml.). At A decamethonium added 180 min after removal from rat. Between BB decamethonium was washed out. From A to B was 59 min.

response (eight diaphragms) always occurred with rates raster than 0.5/sec. At rates between 0.2 and 0.5/sec the response was either monophasic (two diaphragms) or biphasic (four diaphragms). In four experiments the two diaphragms from the same rat were stimulated in the same bath at different rates and the more slowly stimulated produced a biphasic response to decamethonium while the more rapidly stimulated had a monophasic block, suggesting that the type of response was not dependent on changes in the bath fluid. In five experiments in which suxamethonium was used instead of decamethonium the same results were obtained.

(2) Freshness of the preparation. A monophasic response occurred in all five experiments in which the decamethonium had been added more than 120 min after the rat had been killed.

The following additional observations were noted in further experiments.

1. If at the end of an experiment in which either a biphasic or a monophasic response had been obtained the bath fluid was added to a second diaphragm preparation a biphasic or monophasic response was obtained independent of the response of the first muscle.

2. If a second dose of decamethonium was added a monophasic response was always obtained.

3. During the first phase, tubocurarine 0.5 μg/ml. produced a slight reversal of the block but during the second phase it rapidly increased the severity of the block.

4. The response of the muscle to direct stimulation was not altered at any stage of the block.

5. Muscles with a monophasic response contained less potassium than those with a biphasic response. In five experiments the mean ($\pm$SE) potassium content of a slowly stimulated muscle during recovery from a phase I block, 30 min after adding decamethonium, was 56.8 ($\pm$2.9) μ-mole/g wet weight. In four experiments the mean potassium level during the monophasic block of a rapidly stimulated muscle 30 min after adding decamethonium was 45.5 ($\pm$3.0) μ-mole/g w.w. In seven experiments the potassium level in the muscle after it had been stimulated in an organ bath without decamethonium for up to 100 min at the rate of 0.2/sec was 74.0 ($\pm$3.5) μ-mole/g w.w.

Perfusion experiments

With rates of stimulation slower than once every 4 sec the contractions remained equal for over 8 hr. The addition of tubocurarine to the perfusing fluid completely abolished the contractions. The flow rate could be varied between 1 and 4 ml./min without altering the amplitude of the contractions. At flow rates of less than 1 ml./min the preparation became visibly dry on the surface and at faster rates than 4 ml./min there was gross distension of the inferior vena cava and later of the vessels in the muscle.

After the addition of decamethonium to the perfusing solution the contractions decreased within 30 sec. Concentrations of decamethonium above 0.1 mg/ml. abolished the contractions within 2 min. When the decamethonium infusion ceased the contractions began to recover within 30 sec. Single rapid injections of 0.5 mg decamethonium into the perfusing fluid to give a concentration of about 0.5 mg/ml. for about 15 sec

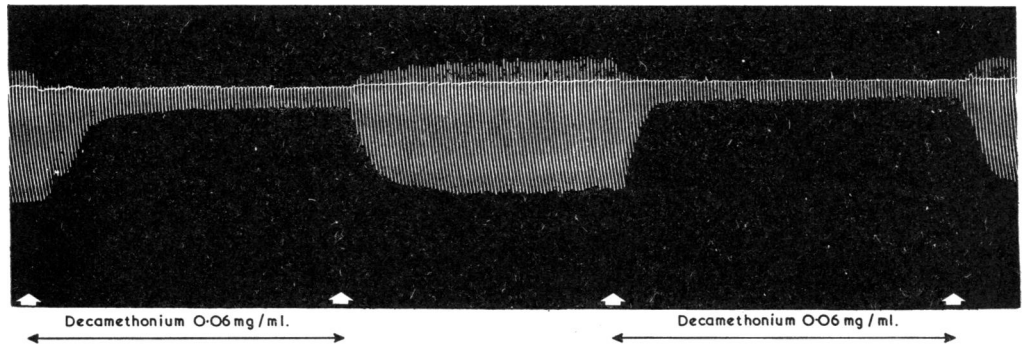

Fig. 4. Perfused rat diaphragm preparation. Decamethonium was added to perfusing fluid to give concentration of 0.06 mg/ml. for two 24 min periods with intervening period without decamethonium.

caused complete paralysis within 35 sec; full recovery occurred within 90 sec. When smaller doses were perfused for long periods (Fig. 4) the block still began rapidly but took up to 10 min to become steady. Recovery was rapid after the drug was stopped. There was no tachyphylaxis to decamethonium (Fig. 4). A biphasic effect was never seen. The addition of tubocurarine to the decamethonium infusion increased the block. Unlike either the monophasic or biphasic block seen in the organ bath the block produced by decamethonium on the perfused diaphragm remained steady for long periods. Decreasing the potassium concentration in the perfusing fluid to 1.18 mM did not alter the type of response, but at the end of the experiment the muscle contained less potassium than when using the normal perfusing fluid (Table 1).

TABLE 1

POTASSIUM CONCENTRATION IN RAT DIAPHRAGM MUSCLE PERFUSED WITH FLUIDS AT VARYING POTASSIUM CONCENTRATIONS

All experiments lasted between 120 and 130 min. The rate of stimulation was never greater than once every 16 sec. Each mean is the result of four experiments

Potassium concn. of perfusing fluid (mM)	Potassium concn. of muscle (μM/g wet weight)	
	Mean	S.E.
5·9	73·3	3·1
3·5	60·7	2·5
2·4	56·4	2·5
1·2	55·2	4·1
0	48·6	3·1

The efflux of 42K, measured at the same time as the muscle contractions, was independent of the rate of stimulation when this was slower than 0.5/sec. The efflux was least when the perfusing fluid contained no potassium and increased with increasing potassium concentrations. The results are given in Fig. 5. The rate constant was calculated for the efflux of 42K from the whole tissue and no correction was made for extracellular diffusion. In a perfusion experiment the distance from the capillaries to the muscle fibres is small compared to the distance for perfusion in an organ bath. When the perfusing fluid contained 5.9 mM potassium, decamethonium did not alter the efflux of 42K. With potassium concentrations of 3.5, 2.4, 1.2 and 0 mM, decamethonium

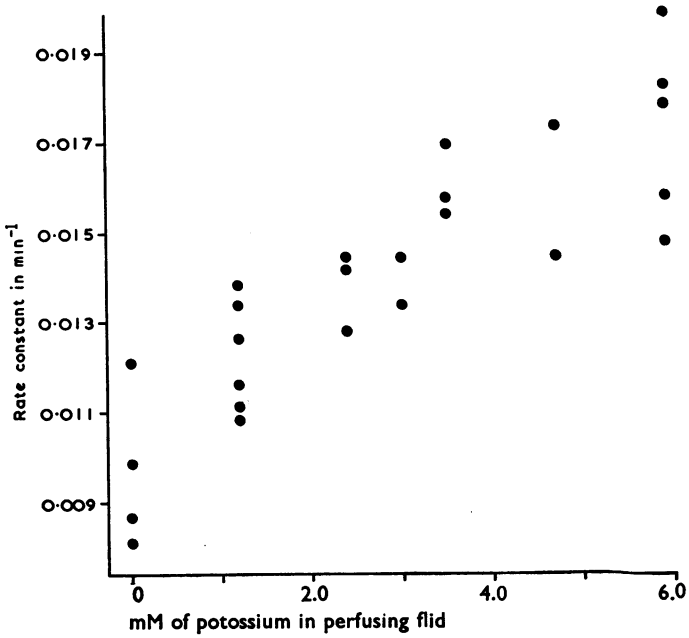

Fig. 5. Efflux of 42K from 25 perfused rat diaphragm preparations. Ordinate represents rate constant in min^{-1}, abscissa the mM of potassium in perfusing fluid.

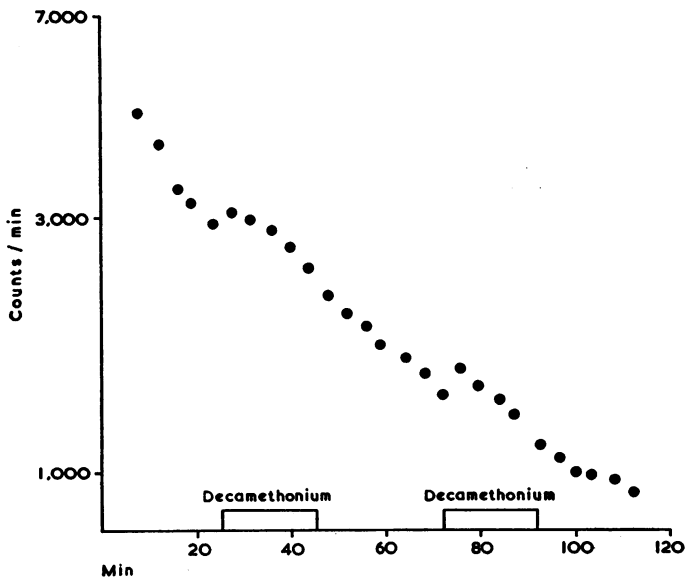

Fig. 6. Perfused rat diaphragm preparation. Ordinate represents corrected count of 42K in effluent. Decamethonium was added tc perfusing fluid for two separate periods to give concentration of 0.06 mg/ml.

TABLE 2

RATE CONSTANTS FOR EFFLUX OF POTASSIUM 42

The rate constants were calculated for the efflux of potassium 42 from the whole tissue before and during the addition of decamethonium (0·06 mg/ml.). Varying perfusion solutions were used

Potassium in perfusing fluid (mM)	Rate constant for 42K (min^{-1})	
	Before decamethonium	During decamethonium
0	0·0122	0·0144
1·18	0·0117	0·0134
2·3	0·0128	0·0136
3·5	0·0169	0·0177
4·8	0·0174	0·0178
5·9	0·0183	0·0180

increased the efflux of potassium, particularly at the lower concentrations (Table 2). A further dose of decamethonium after full recovery from an initial dose produced a second increase in the efflux (Fig. 6).

DISCUSSION

Two types of experiments were performed. In the first the tissue was immersed in a bathing fluid, while in the second the tissue was perfused while suspended in 95% oxygen and 5% carbon dioxide. The perfusion technique had several advantages. The contractions of the muscle were measured during the collection of the effluent. The volume of the effluent was small and all of the fluid had been in close proximity to the muscle fibres. The drug came rapidly into contact with the muscle fibres and could be stopped suddenly. The size and thickness of the muscle was relatively unimportant since oxygen in the perfusing fluid diffuses from the capillaries.

In the experiments with the immersed nonperfused muscle, the rate of stimulation and the duration of the experiment were found to influence the type of block produced. The response to tubocurarine suggests that the first phase block is predominantly due to depolarization, whereas the second stage is due to competitive or "antidepolarizing" block (Taylor & Nedergaard, 1965). This supports the previous work of Jenden *et al.* (1954). It has been shown in the rat diaphragm that the potassium concentration of the muscle is reduced by rapid stimulation (Creese, Hashish & Scholes, 1958) and by anoxia (Creese, 1954). Rapid stimulation and keeping the muscle for a long period in the bath would lead to potassium loss from the muscle, and in the present experiments these same conditions abolished the first phase of the biphasic response, producing a monophasic response.

MacLagan (1962) suggested that as the biphasic response never occurred *in vivo* and only *in vitro* then the biphasic response was due to deterioration. This is only one of the possible explanations. In the present experiments the biphasic response was abolished in preparations subjected to conditions likely to lead to deterioration such as keeping the muscle in the organ bath for long periods and rapid stimulation. Similarly repeated doses of decamethonium led to a decrease in phase I of the biphasic block. The potassium contents of muscles which produced a biphasic block were higher than in those which did not. In those organ bath experiments in which the biphasic response was not produced the response to decamethonium consisted of a gradually increasing block. This monophasic response was similar to the biphasic response minus the initial block and recovery—i.e., the monophasic response was like phase II of the biphasic response.

From the present experiments it can be concluded that the monophasic block is evidence that deterioration has occurred, while the biphasic block occurs in less deteriorated muscles. It is as though deterioration protects the nerve muscle preparation from the phase I block of decamethonium.

In the perfusion experiments the neuromuscular block produced by decamethonium was always steady, though in the immersed non-perfused preparation a steady block was never obtained. MacLagan (1962) found that with a cat muscle *in vitro*, depolarizing drugs did not produce a steady response but that there was always a steady response when the drug was given *in vivo*. The results she obtained *in vivo* were similar to the results of the perfusion experiments described in this paper ; in both there was a steady response and in both the drug entered *via* the capillaries. In an organ bath the drug must diffuse across either the pleural or peritoneal surface of the diaphragm or through a cut edge before it can diffuse within the muscle itself. The drug reaching the muscle *via* the capillaries may act differently. Decamethonium is concentrated at the endplate region of muscle fibres (Waser, 1962).

Taylor, Creese & Scholes (1964) showed that iodocholinium, a compound related to decamethonium, is taken up by two separate processes by the rat diaphragm. Taylor & Nedergaard (1965) have suggested that phase II of the biphasic response to decamethonium is due to entry of decamethonium into muscle fibres, and that before entry it acts as a depolarizing drug and after entry as an anti-depolarizing inhibitor. In an organ bath the drug may be absorbed by the muscle fibres at a site different from where it is absorbed *in vivo* or in a perfused organ. With an immersed muscle the diffusion would be rapid at the cut ends of the muscle where the peritoneal or pleural membrane is cut. The drug would meet a part of the muscle membrane different from the area most plentifully supplied by capillaries. The difference in the response to decamethonium when given by perfusion or in the bath fluid might be due to the difference in the site of action of the decamethonium.

The rate of efflux of 42K from a perfused muscle followed a single exponential course in the range studied. As the potassium concentration of the perfusing fluid was decreased below 5.9 mM the efflux rate decreased, although the net loss, as measured by the total potassium at the end of the experiment, increased. With perfusion fluids containing 5.9 mM potassium, decamethonium had no effect on the efflux of potassium. With potassium concentrations below 4.7 mM the potassium efflux increased when decamethonium was added. The effect on sodium movement was not measured. This increase was not subject to tachyphylaxis and was independent of the total muscle potassium. As the potassium level in the muscle continuously falls throughout the experiments and repeated doses of decamethonium produce a similar change in the potassium efflux, the internal potassium is not responsible for the increased efflux of 42K on adding decamethonium to the muscles perfused with a low potassium concentration. These experiments suggest that the external potassium concentration is an important factor in controlling the efflux of potassium in the presence of decamethonium. However a steady level of paralysis of the perfused rat diaphragm was produced by decamethonium at all concentrations of potassium in the perfusing fluid between 5.9 and 1.2 mM. Therefore there was no correlation between the various changes in the efflux of potassium in the presence of decamethonium and the type of paralysis.

The experiments described show that the action of decamethonium on the rat diaphragm depends on the manner in which the decamethonium reaches the muscle. The biphasic response, seen in simple organ bath experiments, cannot be produced by perfusion of decamethonium. Previously published conclusions on the mechanism of action of decamethonium need to be restated in terms of the way in which the drug reaches the muscle. Explanations of the action of decamethonium which depend on a biphasic response (Taylor & Nedergaard, 1965) seem unlikely to apply to the drug's action *in vivo*. Conditions in the whole animal are probably approached more closely by the perfusion method of testing drugs than by the organ bath experiments.

It is probable that changes in the ionic concentrations of the intracellular fluid, the mode of access of the drugs and the maintenance of normal metabolism affect not only the degree but also the type of response to neuromuscular blocking drugs.

Experimental studies of these variables, separately, may throw new light on the mode of action of the depolarizing drugs.

SUMMARY

1. A biphasic neuromuscular block to decamethonium occurs in a rat phrenic nerve-diaphragm preparation in an organ bath. However, the response is monophasic with rapid stimulation or in an old preparation.

2. When a similar preparation suspended in oxygen is maintained by perfusion *via* the inferior vena cava, decamethonium produces a steady paralysis unlike either the monophasic or biphasic responses. Altering the potassium concentration of the perfusing solution between 1.2 mM and 5.9 mM does not alter the type of response.

3. The efflux of potassium 42 from the perfused rat diaphragm has been studied. With perfusing fluids containing 5.9 mM of potassium decamethonium does not alter the efflux, but with solutions containing less than 4.7 mM potassium efflux is increased. The efflux changes were not related to a change in the type of block.

4. The type of response to decamethonium depends on the method by which the drug reaches the muscle and the state of the muscle at the time.

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