STUDIES ON THE MODE OF ACTION OF QUATERNARY AMMONIUM COMPOUNDS WITH MUSCLE RELAXANT AND OTHER PHARMACOLOGICAL ACTIVITIES

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LIST OF PUBLICATIONS

Certain aspects of the work described in this thesis have been published with J.J. Lewis and with D. Edwards, D.E. McPhail and J.B. Stenlake. The publications are as follows:

Neuromuscular Blocking Activity in some NS-Bis-Onium Compounds. J. Pharm. Lond. Suppl. (1959) 11, 91-94T.

Neuromuscular Blocking Agents. Part VII. J. Pharm. Lond. Suppl. (1960) 12, 137-152T.

Neuromuscular Blocking Agents. Part VIII. J. Pharm. Lond. (1961) 13, 543-547.

The following articles have also been published by the author of this thesis conjointly with J.J. Lewis.

The Laboratory Estimation of Curare-Like Activity in Natural and Synthetic Products. Laboratory Practice (1959) 8, No. 10, 333-338; 8, No. 11, 364-368; 8, No. 12, 404-407.

The Laboratory Evaluation of Ganglion Blocking Agents. Laboratory Practice (1960) 9, No. 6, 382-386; 9, No. 10, 712-715; 9, No. 11, 786-789.

Reprints of the above publications are to be found at the end of the thesis.

The conventions used in this thesis are those of the Journal of Physiology. Where journal abbreviations did not appear in the 'Suggestions to Authors' (J. Physiol. (1960), 150, 1-33), resort was made to 'World Medical Periodicals', published by the World Medical Association, New York (1957).

In the case of references to patents, the convention of the Journal of the Chemical Society was used. Figures and Tables appear on the left hand side in the order in which they are referred to in the text. The conventions used in the chemical formulae are also those of the Journal of the Chemical Society.

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CHAPTER I

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CHAPTER II

Studies on the Anti-cholinesterase Activity of a number of Bis- and Polyonium, Aliphatic Compounds Possessing Neuromuscular Blocking Properties.

CHAPTER I

GENERAL INTRODUCTION

The advent of drugs possessing the ability to produce a specific relaxation of skeletal muscle has had far reaching implications in surgery and anaesthetics. Their introduction, approximately twenty years ago, as adjuncts in anaesthesia, has obviated the need for the high doses of the anaesthetic agent otherwise required for the dual purpose of relieving pain and preparing the skeletal muscles for surgical manipulation. Lower doses of the anaesthetic could thus be used solely for the analgesic function, with a consequent gain in the safety margin. Muscle relaxants have also proved valuable in the treatment of tetanus, epilepsy, in the prevention of trauma during electroshock therapy and in several types of orthopaedic procedures.

Each of the three muscle relaxant preparations in current clinical use, namely gallamine, tubocurarine and suxamethonium are capable of producing undesirable side effects. Gallamine may produce tachycardia (Riker & Wescoe, 1951), tubocurarine can cause both histamine release (Sniper, 1952) and ganglion blockade (Foldes, 1960), while the occurrence of muscle pain and the irreversible nature of the paralysis produced

by suxamethonium and other depolarizing drugs are disadvantages to their use (Foldes, 1960). The need for an easily reversible neuromuscular blocking agent, free from side effects and preferably possessing a tubocurarine-like mode of action persists and remains the mandate for continued chemical and pharmacological research in this field.

A very large number of compounds have been prepared since the introduction of the first biologically standardized muscle relaxant preparation, Intocostrin, into clinical anaesthesia (Griffith & Johnson, 1942). The continuing search for better muscle relaxants has necessitated a vigorous laboratory investigation of the precise pharmacological properties of these drugs and present day knowledge stems largely from experiments using laboratory animals. While the correlation of such information with clinical experience is often difficult, only a detailed and accurate experimental assessment of their basic mode of action will permit the safe and rational clinical use of muscle relaxant compounds. The mode of action of Intocostrin was believed to be characteristic of all muscle relaxant compounds but the introduction of decamethonium (Barlow & Ing, 1948a,b; Paton &

Zaimis, 1948a) revealed a second and different mechanism. Present day concepts distinguish at least three basic types of muscle relaxants (Van Rossum, Ariëns & Linssen, 1958). Moreover, the existence of drugs possessing a dual mode of action, first demonstrated by Zaimis (1952), has emphasized the necessity for a careful pharmacological evaluation of these compounds prior to their clinical assessment.

Progress towards an accurate pharmacological investigation of muscle relaxant drugs has been made by the planned preparation of many series of compounds closely related in chemical structure. Among these have been several series of homologous compounds, for example, polymethylene bisquaternary methonium compounds (Barlow & Ing, 1948a,b; Paton & Zaimis, 1948a,b), from the study of which certain correlations between chemical structure and pharmacological activity were derived. The dominant characteristic of the majority of these drugs was the presence of one or more quaternary ammonium groups in the molecule, yet the observed biological response was also affected by other molecular constituents. Among the more interesting chemical features associated with muscle relaxant activity in synthetic compounds, is the ether oxygen function.

While many ether-containing muscle relaxants have been prepared, attempts to evaluate the precise role of the ether link have been vague and inconclusive. On the other hand, its presence in many clinically useful drugs, for example, tubocurarine, tubocurarine dimethyl ether, laudexium, gallamine and oxydipentonium, has underlined the association of this chemical function with the phenomenon of neuromuscular block. One of the principal contributions of pharmacology is to establish the basic mode of action of groups of drugs which appear to act in a similar fashion. By a careful evaluation of the effect of individual structural features on pharmacological behaviour, desirable chemical features may be retained in new drugs and those believed to be responsible for the development of undesirable properties eliminated. Moreover this contribution will be enhanced if the activity of the compounds investigated can be placed in a perspective embracing the entire field of muscle relaxant drugs.

The work undertaken in this thesis represents an attempt to establish the basic mode of action of a number of synthetic muscle relaxant compounds and where possible to correlate their chemical structure

with the pharmacological activity observed. Particular attention was paid to the presence of the ether oxygen function in certain of the compounds studied, and an endeavour was made to assess its contribution to the muscle relaxant potency of these drugs.

The Mechanism of Action of Neuromuscular Blocking Agents.

The classic experiments of Claude Bernard (1856), were not only the first demonstrations that curare exerted its paralyzing action peripherally at the junction of nerve and muscle, leaving both structures unimpaired, but also indicated the specialized nature of this region and stimulated interest in its properties. Subsequent histological and physiological investigations have led to the view that while the nerve impulse traverses the fibres by electrical propagation (Bernstein, 1902), its transmission from neuron to neuron or from neuron to effector cell is mediated by specific chemical substances liberated from the nerve terminals. The existence of chemical mediators or neuro-hormones was first suggested by Elliott (1905), who postulated the existence of sympathin to explain transmission in the sympathetic nervous system. The same concept was subsequently applied to parasympathetic fibres (Le Heux, 1919, 1921; Dale, 1914; Dale & Dudley,

1929; Loewi, 1921; Loewi & Navratil, 1926) and conclusive proof was provided nearly twenty years later by the elegant demonstration by Dale, Feldberg and Vogt (1936) of the existence of acetylcholine in the perfusion fluid from the mammalian neuromuscular junction following stimulation of the motor nerve. The acetylcholine-intensifying action and the anti-curare effect of anticholinesterase drugs (Brown, Dale & Feldberg, 1936; Bacq & Brown, 1937; see also reviews by: Dale, 1937; Brown, 1937a,b; Eccles, 1936, 1937) lent further support to this view, as did the effect of curare-like substances in preventing acetylcholine-induced twitch-like responses of skeletal muscle (Brown et al. 1936).

In order to understand the mechanism of action of muscle relaxant drugs, it is necessary to consider both the structure of the neuromuscular synapse and the normal physiological sequence of events occurring there. Present day concepts are derived from improved recording techniques using microelectrodes by which the intimate mode of action of acetylcholine has been studied intensively (Fatt & Katz, 1951; Fatt, 1954; Del Castillo & Katz, 1956; Eccles, 1957). Recent histological studies of the neuromuscular synapse (Couteaux, 1947,

1955; Robertson, 1956, 1957a,b) have revealed the extensive branching of the motor nerve prior to its termination (Eccles, 1953), and the structural differentiation of the junctional region. The evidence suggests that there is no direct cytoplasmic continuity but merely a close association between the nerve endings and the muscle fibre, the former lying in troughs in the surface of the latter (Eccles, 1957b; Katz, 1959; Couteaux, 1955). Physiological observations have confirmed the apparently specialized role of the motor end plate in neuromuscular transmission (Katz, 1958). The neuromuscular synapse is more readily fatigued in a nerve-muscle preparation than either nerve or muscle fibres (Nachmansohn, 1959a). Furthermore the junctional region possesses not only a high and specific sensitivity to acetylcholine (Buchthal & Lindhard, 1942; Kuffler, 1943, 1945), but also a relatively higher concentration of acetylcholinesterase than other tissues (Couteaux & Nachmansohn, 1940; Couteaux, 1955). Moreover it differs qualitatively and quantitatively from other parts of the muscle and nerve in its response to many drugs, including certain muscle relaxants (Katz, 1959).

Acetylcholine is synthesized by a reaction involving choline and acetylcoenzyme A (Nachmansohn,

1946; Lipmann & Kaplan, 1946) in the presence of choline acetylase which appears to occur in the mitochondria (Hebb & Smallman, 1956). The energy for this reaction is supplied by adenosinetriphosphate (Nachmansohn & Machado, 1943) or from the metabolism of glucose or pyruvate (McIlwain, 1959). In the resting state the acetylcholine is stored in an inactive protein-bound form (Nachmansohn, 1952b) within the motor nerve fibre (Feldberg, 1945, 1950). The presence of vesicles in the terminal portion of motor neurones (De Robertis & Bennett, 1955; Palade, 1954; Palay, 1954) is believed to be intimately associated with this storage of bound acetylcholine.

The release of acetylcholine takes place in response to nerve impulses passing along the motor fibre (Kuffler, 1949). The transmitter may then diffuse across the junctional region to become momentarily attached to a hypothetical specific receptor site on the post synaptic membrane of the motor end plate (Eccles, 1953; Del Castillo & Katz, 1957). There is also evidence to suggest that acetylcholine-receptors exist both in nerve terminals (Masland & Wigton, 1940; Riker, Roberts, Standaert & Fujimori, 1957; Riker, Werner, Roberts & Kuperman, 1959), and in the axon itself

(Rosenberg & Ehrenpreis, 1961). The process of liberation of acetylcholine is neither fully understood nor easily investigated (Del Castillo & Katz, 1956). While its release from the nerve terminal has been frequently studied (Eccles, 1937), this site is not the only source of acetylcholine (Nachmansohn, 1959b). Histochemical (Koelle & Koelle, 1958) and biochemical (Nachmansohn, 1959b,c) evidence has confirmed the presence of acetylcholine in sensory fibres (Chang, Hsieh, Lee, Li & Lim, 1939; Lissak & Pasztor, 1941) and also its release from within both the axon itself (Brecht & Corsten, 1941) and from stimulated denervated muscle (Jarcho, Berman, Lilienthal & Eyzaguirre, 1951; McIntyre, 1958, 1959).

The concentrations of certain cations in the environment, for example sodium, potassium, calcium and magnesium, may influence transmitter release (Nastuk, 1959). By the use of intracellular microelectrode techniques, it has been observed that high concentrations of magnesium ions and low concentrations of calcium ions may reduce the overall amount of acetylcholine liberated (Del Castillo & Engbaek, 1953, 1954). Large concentrations of the latter produce the opposite effect (Del Castillo & Stark, 1952). An intermediate,

mutually antagonistic reaction involving calcium ions and magnesium ions may take place between the arrival of a nerve impulse and the release of acetylcholine (Del Castillo & Katz, 1956; Jenkinson, 1957). High concentrations of both magnesium and calcium cations depress the direct excitability of the muscle membrane (Del Castillo & Engbaek, 1954). Excess potassium ions applied externally to the neuromuscular junction also liberate acetylcholine from nerve endings but the precise role of sodium in this connection has not been clearly established (Del Castillo & Katz, 1956; Nastuk, 1959).

A spontaneous and intermittent release of discrete quanta of acetylcholine occurs at rest at the neuromuscular junction, eliciting minute potential changes in the post junctional membrane which are, however, below the 'threshold' of the adjacent muscle membrane and insufficient to elicit a response (Fatt & Katz, 1952). The synchronous release of a large number of quanta of acetylcholine in response to impulses in the motor nerve (Robertson, 1956; Del Castillo & Katz, 1956; Werner, 1961) produces a depolarization of the end plate of the muscle membrane (Göpfert & Schaefer, 1938; Kuffler, 1942; Fatt & Katz, 1951, 1952; Eccles, Katz

& Kuffler, 1941), which is sufficient to generate a wave of excitation in areas adjacent to the end plate region. This excitatory depolarizing effect, producing the so called 'spike action' potential, propagates in both directions along the membrane of the muscle fibre inducing contraction. Acetylcholine-induced depolarization is associated with rapid alterations in the permeability of the muscle-cell membrane to sodium ions (Hodgkin, 1951), and to potassium and ammonium ions (Nastuk, 1959) and possibly opens up an indiscriminate aqueous channel to all small ions on each side of the membrane (Katz, 1959). Under normal resting conditions, the postsynaptic membrane is moderately permeable to potassium but only sparingly permeable to sodium ions. On depolarization, however, a large, transient increase in the permeability of the end plate membrane to sodium ions takes place (during the rising phase of the action potential), causing a reversal of the potential difference across the cell membrane. This is followed by an increased, more prolonged, permeability to potassium ions. It has been suggested that the energy associated with this ionic transfer may contribute to the propagation of the impulse along the muscle fibre (Hodgkin, 1951). Sites on the muscle cell membrane

which at rest, are occupied by calcium ions, may, on depolarization, be taken up by sodium and potassium ions (Shanes, Freygang, Grundfest & Amatniek, 1959; Adelman & Dalton, 1960). Calcium ions may thus be important in maintaining a selective ionic gradient across the muscle cell membrane (Hodgkin, Huxley & Katz, 1949). The rapid removal of acetylcholine from the site of activity (0.5 to 1 msec), a prerequisite for its essential role in conduction, may be accomplished by several contributing mechanisms. Among these the importance of acetylcholinesterase as a specific (Nachmansohn & Rothenberg, 1944, 1945) hydrolyzing enzyme (Augustinsson & Nachmansohn, 1949) for acetylcholine, has been clearly established. This enzyme, the existence of which was suggested by Dale (1914) to explain the rapid removal of acetylcholine and which was first demonstrated in 1932 (Stedman, Stedman & Easson, 1932), occurs in high concentrations at the neuromuscular synapse (Marnay & Nachmansohn, 1937) probably at the lamellae of the subneural apparatus (Couteaux, 1955). Histochemical evidence for its presence at the motor end plate has also been provided (Couteaux & Nachmansohn, 1940; Couteaux, 1955; Koelle & Friedenwald, 1949). The relatively high rate of hydrolysis of acetylcholine

(Nachmansohn, 1959b) together with the significant prolongation of the transmitter effect by means of acetylcholinesterase inhibitors (Brown et al. 1936; Eccles & MacFarlane, 1949; Fatt & Katz, 1951), have further confirmed the specialized role of acetylcholinesterase in the physiological function of acetylcholine.

Other means of removing acetylcholine by its diffusion into interstices (Brown et al. 1936), its restitution to presynaptic terminals in a way analgous to that demonstrated for choline at nerve endings (Perry, 1953), or by the development of receptors refractory to the transmitter (Thesleff, 1955a,b), have not been experimentally substantiated.

In contrast to the view that transmission was a purely electrical process (Eccles, 1946), the essential role of acetylcholine has now been widely accepted (Eccles, 1949; Katz, 1959; Nachmansohn, 1959b), although chemical transmission at all synapses has not been established and electrical transmission has been demonstrated in the crayfish (Furshpan & Potter, 1957). A difference of opinion, however, persists concerning the precise mechanism of action of acetylcholine at the neuromuscular junction. One school of thought

(Feldberg, 1951; Del Castillo & Katz, 1956) has proposed that acetylcholine is secreted at the terminal nerve membrane and reaches the postsynaptic membrane by diffusion across the subneural space. On the other hand there are proponents of the view that the current generated at the terminal nerve membrane traverses the subneural space liberating acetylcholine at the post-junctional membrane (Nachmansohn, 1952a); i.e. the combination of acetylcholine with the receptor is responsible for the change of conductance and the initiation of ionic movements across the synapse. The existence of protective barriers, surrounding the axon, impermeable to lipid-insoluble quaternary ammonium compounds including acetylcholine (Bullock, Nachmansohn & Rothenberg, 1946; Rothenberg, Sprinson & Nachmansohn, 1948), has proved a serious objection to the latter view. Recent work has, however, revealed that when these are removed, for example by ionic detergents, 'curare' can exert its characteristic blocking action both at the nodes of Ranvier (Walsh & Deal, 1959; Dettbarn, 1960) and in the axon proper (Rosenberg & Ehrenpreis, 1961). Moreover, the liberation of acetylcholine from a nerve fibre has also been confirmed (Brecht & Corsten, 1941). Acceptance of these results supports the view that the

action of acetylcholine at the neuromuscular synapse is merely 'a local intensification of a general process for the propagation of impulses along the nerve fibre' (Dale, 1937, 1954).

It is apparent, despite this controversy, that any drug affecting the synthesis or removal of acetylcholine, or the formation of the acetylcholine-receptor complex, may interrupt the physiological process of neuromuscular transmission. Such drugs can be classified according to their anatomical site of action (neuromuscular blocking agents), their therapeutic effect (muscle relaxants), their pharmacological mechanism of activity, or their chemical or pharmacognostical source of origin (Lewis, 1960a). Consequently, while the term, muscle relaxant, may correctly be given to any drug interfering with any stage in the normal physiological sequence of events, the term, neuromuscular blocking agent, is reserved for those drugs interfering with the actual functioning of the transmitter itself rather than to its presynaptic release or its enzymatic destruction. By definition, both conduction in the motor nerve and the response of the skeletal muscle to direct stimulation remain unimpaired. However, in spite of valid objections (Hunt & Kuffler, 1950;

Paton, 1951), neuromuscular blocking agents are often collectively termed 'curariform' or 'curarizing drugs', irrespective of whether or not their precise mode of action is identical with that of tubocurarine.

From the clinical point of view, neuromuscular blocking agents constitute the most important group of muscle relaxant drugs. Muscle relaxant drugs have been conveniently classified into two main categories, on the basis of their intimate effects on transmitter function:

(a) Non-depolarizing compounds which compete with acetylcholine for receptors on the postsynaptic muscle membrane and interfere with the formation of the acetylcholine-receptor complex. Tubocurarine is generally considered to be the classic example.

(b) Drugs which mimic the action of the transmitter and induce block by a depolarization of the motor end plate, e.g. decamethonium.

In addition, both types of action may be observed in the same molecule, e.g., Tridecamethonium, (Paton & Zaimis, 1952; Zaimis, 1952).

Non-Depolarizing Muscle Relaxant Drugs.

These selectively combine with acetylcholine-receptors in the post-junctional muscle membrane and

so reduce or prevent the depolarizing effect of the transmitter. The combination of acetylcholine and the receptor takes place by a 2 to 3 stage process (Del Castillo & Katz, 1957), but tubocurarine appears only to interfere with the initial site of attachment without being involved in the subsequent changes in the physical properties of the membrane itself. While considerable evidence supports the belief that this antagonism is simply competitive (Fatt & Katz, 1952), this may be an over-simplified view (Del Castillo & Katz, 1957).

Drugs of this type principally affect the end plate potential, which undergoes a progressive reduction in duration, rate of development and amplitude terminating in its complete disappearance (Fatt & Katz, 1951; Thesleff, 1955c). When the amplitude has fallen below the threshold value necessary to 'trigger off' the action potential in the membrane of the muscle fibre, conduction ceases and block is induced (Eccles et al. 1941). Elimination of the drug causes a rapid and complete recovery of transmission (Thesleff, 1955c). A muscle treated with tubocurarine is electrically normal (Paton, 1951), there being no apparent alteration in either the potential or ionic permeability of the

membrane. Although tubocurarine has been observed to cause contractions of denervated muscle when injected intra-arterially (McIntyre, King & Dunn, 1945; Jarcho et al. 1951), the direct excitability of muscle treated with tubocurarine appears indistinguishable from that of the normal fibre (Fatt & Katz, 1951; Paton, 1951; Del Castillo & Katz, 1957). On account of the competitive nature of the block, any local increase in the concentration of acetylcholine, for example, by repetitive stimulation of the motor nerve (Thesleff, 1955c), or the use of anticholinesterase drugs (Riker, 1953), will antagonize the effect of tubocurarine and other members of this group. Moreover, any reduction in the 'threshold' of the muscle fibre will produce similar effects. The action of competitive neuromuscular blocking agents may also be antagonized by increasing the excitability of the muscle membrane to the transmitter, inducing a 'subthreshold' depolarization of the muscle fibre. The anticurare activity of a cathodal current (Burns & Paton, 1951) and the effect of externally applied potassium ions can be explained on this basis. Conversely, agents raising the membrane 'threshold', for example, an anodal current (Paton, 1951), will intensify this type of block.

Depolarizing Muscle Relaxant Drugs.

These achieve their effects primarily by a marked depolarization of the motor end plate in a way analogous to the action of acetylcholine itself. Unlike the tubocurarine-treated muscle, that affected by decamethonium is electrically depolarized at those regions containing motor end plates. This sets up an area of electrical inexcitability at the end plate region preventing the generation of potentials to excite the adjacent muscle membrane. Evidence from experiments describing the development of this effect (Burns, Paton & Dias, 1949; Burns & Paton, 1951; Paton, 1951) in the cat, indicated that the depolarization effect was persistent and long lasting, but more recent observations have opposed this view (Zaimis, 1953; Jenden, Kamijo & Taylor, 1954; Thesleff, 1955a,c; Axelsson & Thesleff, 1958). These have shown that the period of depolarization produced is brief and is followed by a rapid and complete repolarization of the muscle membrane. Neuromuscular block commences during the period of depolarization and the muscle membrane remains insensitive to acetylcholine even in the repolarized condition.

The ability of the muscle membrane to undergo

TABLE I (contd.)

On Cat	Non-Depolarizing Type Drugs	Depolarizing Type Drugs
9. Effect of electric current applied to the end plate region		
Anodal current	Intensifies Block	Lessens Block
Cathodal current	Lessens Block	Intensifies Block
10. Effect on block of reduction in temperature	Reduction in magnitude but not of duration of block	Increase in magnitude and duration of block
11. Nature of Paralysis		
(a) In avian muscle (Chick Test)	Flaccid	Spastic
(b) In hen nerve-skeletal muscle preparation	Flaccid	Contracture super-imposed on block
(c) In frog muscle	Antagonism to contracture caused by depolarizing drugs (Low Intrinsic Activity)	Contracture (High Intrinsic Activity)

TABLE I (contd.)

On Cat	Non-Depolarizing Type Drugs	Depolarizing Type Drugs
5. Effect of administration of different depolarizing muscle relaxants	Antagonistic	Additive
6. Indirect tetanization of the partially blocked muscle	Contraction poorly sustained (Post-tetanic facilitation)	Contraction well sustained
7. Effect of ether anaesthesia	Increased effect (potentiation)	No effect or antagonism
8. Effect of (a) Previous tetanization of the motor nerve	Antagonism	No effect
(b)Potassium	Antagonism	No effect or slight potentiation or antagonism
(c)Edrophonium	Antagonism (of short duration)	No effect or slight potentiation or antagonism
(d)Neostigmine	Antagonism (prolonged)	No effect or slight potentiation or antagonism

TABLE I

A comparison of the pharmacological properties of depolarizing and non-depolarizing muscle relaxants. (After Paton & Zaimis, 1952; Zaimis, 1959; Van Rossum, Ariëns & Linssen, 1958).

On Cat	Non-Depolarizing Type Drugs	Depolarizing Type Drugs
1. Initial excitatory effect on skeletal muscle	None	Transient fasiculations and increased twitch tension
2. Varying order of sensitivity of different muscles (see Pages 74 and 75)	Both 'red' and 'white' fibres are relatively sensitive	Reduced sensitivity of 'red' fibres e.g. soleus. Increased sensitivity of 'white' fibres e.g. anterior tibialis
3. Reversed order of species sensitivity	Rat>Hare, >Monkey>Rabbit >Man>Dog>Cat >Hen	Hen>Cat>Man >Rabbit>Dog, >Monkey>Hare >Rat
4. Effect of administration of different non-depolarizing muscle relaxants	Additive	Antagonistic

repolarization in the presence of a depolarizing agent can be explained, it is suggested, by a reversible change in the end plate receptors, Meyer (1937), causing them to become inert and insensitive to acetylcholine, and producing a 'desensitization block' (Katz & Thesleff, 1957; Axelsson & Thesleff, 1958). In consequence, the high ionic permeability of the end plate to ions cannot be maintained and conduction is impaired. Similar changes in the nature of the drug-receptor complex have been observed using high concentrations of acetylcholine itself (Katz & Thesleff, 1957; Axelsson & Thesleff, 1958). Differences between the two main groups of neuromuscular blocking agents, observed from electrical recording techniques, have explained to a considerable extent their overall pharmacological activities and their differing responses to certain drugs. The major differences in the two main types are given in Table I (Paton & Zaimis, 1952).

Interruption of transmission at the neuro-muscular synapse is not specific to neuromuscular blocking agents but is the common property of a chemically diverse group of drugs, the particular study of which is largely outwith the scope of this thesis. Of these, drugs delaying the enzymatic hydrolysis of

acetylcholine - the anticholinesterases - have received special consideration and been the subject of several reviews (Augustinsson, 1948; Koelle & Gilman, 1949; Whittaker, 1951, Holmstedt, 1959). The effects of botulinum toxin at the neuromuscular synapse (Burgen, Dickens & Zatman, 1949) are believed to be largely due to a fall in the number of units of acetylcholine released by each nerve impulse (Wright, 1955), with a consequent reduction in the frequency of the spontaneous miniature end plate potential. No alteration in the quantal amount of acetylcholine released was, however, observed (Brooks, 1956).

Procaine-induced neuromuscular block has been ascribed to a diminished release of the transmitter substance by each nerve impulse (Harvey, 1939; Foldes, 1959, 1960), while it is also believed to exert a depressant effect on the post junctional end plate membrane (Nicholls & Quilliam, 1956; Del Castillo & Katz, 1957).

The action of these drugs at the neuromuscular synapse cannot be utilised in surgery.

Muscle Relaxants and The Receptor Theory.

It is now generally accepted that pharmacologically active agents can be classified as

specific or non-specific drugs (Beckett, 1956; Beckett et al. 1956; Ing, 1959), i.e. two main types of chemical interference by drugs are envisaged. The term, 'specific', is now generally used to refer to the action of those drugs which, by virtue of their unique chemical structure, possess the ability to react with particular molecular species within cells and form relatively stable combinations with individual cellular constituents. 'Non-specific' drugs, on the other hand, depend for their action upon the modification of the physicochemical characteristics of living cells and so distort the normal sequence of biochemical events proceeding within them. In common with all attempted biological classifications however, no strict line of demarcation separates the two groups. Neuromuscular blocking agents are believed to act in a structurally specific manner, interacting with hypothetical receptors which impose certain limiting spatial and electrical characteristics on drug molecules capable of forming complexes with them.

The concept of receptors was first formally postulated by Ehrlich, who defined the term as 'that combining group of the protoplasmic molecule to which a foreign group, when introduced, attaches itself'

(Ehrlich & Morgenroth, 1910). A similar view was inherent in the lock and key analogy of Fischer (1894) and in the work of Langley (1906) who, in order to explain pharmacological activity, conceived the idea of a 'receptive substance', which could be selectively altered by curare-like agents (Lucas, 1907). Current ideas concerning the nature of receptors, although considerably removed from those of Ehrlich, are still fragmentary and inferential. However, much of the most accurate and fundamental information on drug action, including the work of Clark (1937), and Ing (1936) and in more recent times, the interpretation (Van Rossum et al. 1958) of the mode of action of muscle relaxant drugs by Ariëns and Paton (1961), has depended upon a tacit acceptance of the receptor theory. More recent confirmation of the existence of a receptor has come from studies on the enzymatic hydrolysis of acetylcholine. These have revealed that certain drugs, including acetylcholine and carbaminoylcholine, induce neuromuscular block without impairment of acetylcholinesterase activity (Altamirano, Schleyer, Coates & Nachmansohn, 1955), their effect being attributable to a predilection for a similar cellular entity - the postulated receptor.

Little is known concerning the intimate nature of drug receptors. Early workers envisaged a 'periterminal network' (Boeke, 1929) or a molecular side chain of cellular substance (Langley, 1906), complementary in configuration and polarity to those drugs with which it combined. This view has not been confirmed by recent results obtained from electron microscopy (Estable, 1959) and a narrow but distinct space (150 to 600 Å) between pre- and post synaptic structures has been shown to exist. It has recently been suggested (Cavallito & Gray, 1960) that a more realistic picture of the receptor surface might involve a lattice of anionic groups dispersed in a roughly crystal shaped pattern.

Van Rossum and Ariëns (1957) considered drug-receptor combination to involve a general interaction of complementary electrical fields of force originating in the drug molecule and in the tissue. Electrostatic and Van der Waals' forces were assumed to play the dominant role and certain specific interactions within the general field of force determined the potency of a given drug. The relative unimportance of covalent bonding in the interaction of neuromuscular blocking agents with the receptor is indicated by the

reversible nature of the drug-receptor combination. The receptor has also been regarded as being involved in an enzymatic energy - transport process (Drill, 1958), or as a protein or lipoprotein enzyme with which acetylcholine reacts as a coenzyme to release anions from a substrate in the muscle-cell membrane (Welsh, 1949). The possibility that the receptor may structurally resemble acetylcholinesterase has also been suggested (Roepke, 1937). Although it is well established that many drugs act by interfering with enzymatic processes, it is dangerous to create the generalization that all drugs necessarily do so. Consequently, while certain similarities exist between the receptor and acetylcholinesterase, for example in their common interaction with acetylcholine, the combination of neuromuscular blocking agents with cholinergic receptors in the post synaptic membrane is believed to take place by a non-enzymatic process (Mattocks & Holtan, 1949; Jacobi, Stöesz & McIntyre, 1950; Katz, 1956).

The suggestion has recently been made that the receptor need not be envisaged as a discrete physical unit but rather as a volume in space or sphere of influence, bounded and defined by enzymes, coenzymes

and metallic ions (Martin-Smith & Reid, 1959). On the other hand, another school of thought believe the receptor to be a physical unit, possessing definite spatial and electrical properties. Attempts have been made to predict the molecular shape and electrical characteristics of the receptor by a consideration of similar features found in biologically active molecules, in the belief that the receptor would have properties complementary to those of the most active species (Pfeiffer, 1948; Ing, 1949; Kimura, Unna & Pfeiffer, 1949; Schueler, 1953). Resulting from this approach evidence has accumulated that linear bisquaternary ammonium compounds were more potent muscle relaxants than the corresponding monoquaternary agents (Barlow & Ing, 1948a,b; Paton & Zaimis, 1948a, 1949). This has led to the hypothesis that activity in bisquaternary and other active molecules (Pelikan & Unna, 1952) could be explained by the interaction of optimally spaced onium centres approximately 13 to 15 Å apart, with equidistant co-reactive groups of a biological cholinergic receptor system situated on the motor end plate. The concept of pharmacologically 'bivalent molecules' was introduced (Barlow, 1955) to describe the action of bis-onium salts, for example hexamethonium,

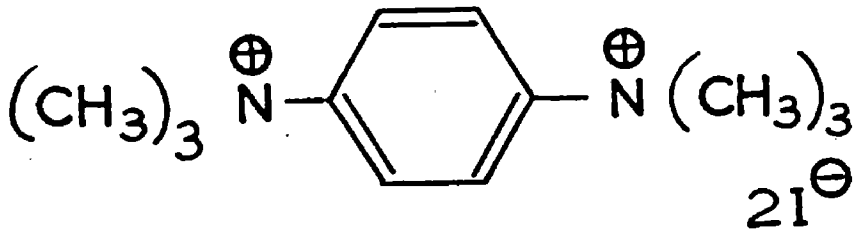
in combining with two sets of receptors at the same time. The complex so formed, it was suggested, would be twice as stable as that formed by a similar 'univalent' molecule for example, tetramethylammonium, with one receptor. One end of the 'bivalent' molecule might act as an anchoring mechanism for the other which would be kept in close proximity to the receptor surface. It has become evident, however, that a one-dimensional approach has over simplified the picture of drug-receptor interaction. The inter-onium distance in non-rigid molecules has no fixed value, on account of conformational isomerism arising from uninhibited rotations about single carbon-carbon bonds in the polymethylene chain (Cavallito, Gray & Spinner, 1954; Macri, 1954). There is therefore no reason to assume that the thermodynamically most stable conformation of the molecule is that actually involved in complex formation with the receptor. The inter-onium distance in tubocurarine, at the time of complex formation with the receptor, formerly taken to be 13 to 15 Å, as it is in the extended conformation of the molecule, is now thought to be less (6 to 12 Å) due to the molecule adopting a folded conformation.

Differences between conductimetric measurements

(Rice, 1956, 1958) and statistically calculated inter-onium distances (Gill, 1959) have underlined the difficulty of assessing the precise molecular conformation participating in any particular drug-receptor combination (Cavallito & Gray, 1960).

The adumbration hypothesis (Loewe & Havery, 1952) emphasized the need for a consideration of the overall molecular tectonic and bonding potentialities, in determining the degree of drug-receptor interaction. It ascribed the muscle relaxant potency of drugs to a spatial competition with acetylcholine, the adumbrating or umbrella-like structure of the molecule hindering access to the receptor site by steric interference. A similar view has been advanced by Wien (1954) and is inherent in Waser's theory concerning the nature of the cholinergic receptor (Waser, 1959, 1960).

The use of rigid molecules appears to offer a more accurate means of assessing the physical characteristics of drug receptors. Acceptance of the view that the characteristics of the receptor may alter in response to the presence of drug molecules, as suggested in the 'induced fit' theory (Koshland, 1958), a premise of the theory of biological relativity (Martin, 1956), has however cast doubts upon the value



I

of this approach. In addition, the view that a limited degree of flexibility is necessary for activity in this type of structure (Gill & Ing, 1958; Gill, 1959) is supported by the lack of ganglion and neuromuscular blocking activity observed in the rigid molecule of NNNN'N'N'-hexamethyl-p-phenylenediamine bisiodide (I) (Wien & Mason, 1953). Quaternary ammonium centres which are separated by a rigid linking structure, appear to be unable to yield sufficiently to permit a close approach to the anionic receptor site. This characteristic appears compatible only with a tubocurarine-like action, which, it is suggested, does not demand a close anionic-cationic combination (Cavallito & Gray, 1960).

Other workers have attempted to interpret pharmacological activity in terms of the 'identity distance' (Long & Schueler, 1954) which is the distance between peptide bonds in a maximally extended peptide and has a value of 3.61 Å or, in terms of the distance between two turns of an α-protein helix which has a value of 5.5 Å (Corey & Pauling, 1953). It is interesting to note that the distance 14.5 Å postulated as the ideal inter-onium distance in bisquaternary ammonium neuromuscular blocking agents (Barlow &

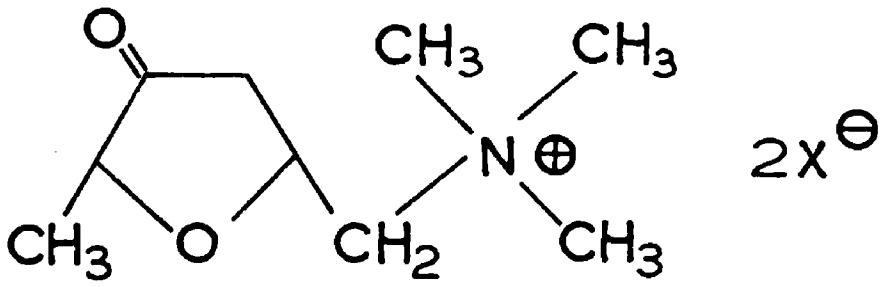
Ing, 1948b; Paton & Zaimis, 1949) is 4 x 3.61 Å and that the distance between the hydrogen bonding groups in oestrogens is approximately 3 x 3.61 Å or 2 x 5.5 Å (Fisher, Keasling & Schueler, 1952).

Experimental evidence, supporting the view that the receptor is a protein, has been provided by Nachmansohn and his associates (Altamirano, Coates, Grundfest & Nachmansohn, 1953). Attempts have been made to isolate and characterize this protein by complex formation with radioactive muscle relaxants using the electric tissue of the electric eel. Initially, experimental evidence indicated strong, but probably unspecific, binding of gallamine to a mucopolysaccharide (Chagas, 1959). Subsequent work using a more refined technique has, however, led to the successful isolation and purification of a protein material (Ehrenpreis, 1959a,b,1960) which showed many characteristics of the *in vivo* receptor substance especially in its ability to bind acetylcholine and muscle relaxant drugs (Schoffeniels & Nachmansohn, 1957).

Present day concepts of the nature of receptors have been considerably clarified by the creation of several pictorial representations based on experimental results. Such representations, although they may be

without reality in much the same way as are the Kekule's structures for benzene, have stressed the value of three-dimensional molecular geometry in the drug molecule, including considerations of the position and electrical nature of the functional groups. Examples of such an approach include the work of Lands (1951) on the muscarinic receptor, of Beckett and his associates (Beckett, 1956; Beckett et al. 1956) on the morphine receptor site and, particularly relevant to this thesis, the experiments of Nachmansohn and Wilson (1951) concerning acetylcholinesterase and of Waser (1959, 1960) on the structure of the cholinergic receptor. Nachmansohn and Wilson (1951) studied the molecular forces associated with the physiological activity of acetylcholine by means of acetylcholinesterase because of the ready availability of this enzyme in a highly purified and stable form (Nachmansohn, 1955). Information from an analysis of the effective molecular forces concerned in the acetylcholinesterase-acetylcholine reaction have been used to promote a better understanding of those forces involved in other transmitter interactions (Nachmansohn, 1959b).

From an examination of the uptake of radioactive



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muscarone (II) by the diaphragm of the mouse, (Waser, 1957, 1959), using autoradiographic techniques, concluded that the cholinergic receptor possessed a three-dimensional pattern. A similar view had been previously advanced (Jenden et al. 1954; Taylor, 1955) in a suggestion that the receptor possessed molecular sieve properties (Barrer, 1947) rather than a planar surface arrangement. From the results of similar experiments using radioactive muscle relaxants, Waser also indicated that the receptor surface was a pore 12-14 Å in diameter in the post synaptic membrane. Many problems, however, concerning the intimate nature and function of the receptor remain unsolved while its postulated existence in sites other than the post-synaptic membrane (Riker et al. 1959; Rosenberg & Ehrenpreis, 1961) remains virtually unexplored.

Muscle Relaxants and the Theory of Biological Antagonism.

A direct consequence of the receptor theory of drug action, and an important factor in the establishment of structure-action relationships in neuromuscular blocking agents, is the principle of biological antagonism. This maintains that a drug competes with a natural substrate, or 'essential metabolite', or perhaps another drug, for the receptor.

A wealth of experimental evidence from different biological disciplines, including bacteriology (Fildes, 1940; Woods, 1940) and pharmacology (Gaddum, 1926; Ing, 1936; Clark, 1937), has confirmed the significance of this principle in a study of the underlying mechanisms of drug action (Work & Work, 1948; Sexton, 1949; Woolley, 1952; Albert, 1960).

Investigations of such mechanisms are best carried out using isolated organs, when the observed effect is mainly attributable to the interaction of the drug under test with the specific receptor system, especially if equilibrium conditions prevail. It is illogical to consider the results from whole animal preparations where complications of distribution, transport, breakdown and excretion may affect to a far greater extent the overall drug response. On the other hand there are some examples where whole animal preparations may best indicate the nature of a pharmacological phenomenon, e.g. Adrenaline reversal. Furthermore, only by means of isolated preparations can the time of exposure of the tissue and the concentration of the drug be controlled to a reasonable extent.

The concept of biological antagonism has been

placed on a quantitative basis, by relating the measured biological response either to the amount of drug absorbed by the receptors or to laws governing enzyme kinetics. Experimental observations have been given theoretical expression by the derivation of a number of equations. All mathematical relationships so derived to explain drug action have been developed fundamentally from the law of mass action, an expression governing thermodynamic equilibria in chemical reactions. Biological systems, however, do not exhibit true thermodynamic equilibrium but exist in a steady state, in which the rates of chemical synthesis and breakdown are balanced.

The correlation of pharmacological activity to adsorption laws has been based on the mathematical equations derived by Freündlich (Clark, 1937) and Langmuir (1916, 1917, 1918) on the assumption that the degree of biological effect was a linear function of the fraction of the receptors occupied by the drug. This premise has however been contested. The early assumptions of Clark (1937) and Gaddum (1937) though supported by Rocha e Silva (1957), have been questioned by Stephenson (1956), Nickerson (1956), Ariëns et al. (1957) and Schild (1957), who for different reasons

have indicated possible exceptions to its overall applicability.

The Freundlich isotherm is unsuitable as a theoretical basis for the explanation of drug action, since it lacks thermodynamic validity and assumes an infinite degree of adsorption, incompatible with observed biological saturation phenomena. Although the Langmuir isotherm (Langmuir, 1916, 1917, 1918) has thermodynamic validity, practical difficulties arise in determining the concentration in the biophase and resort must be made to accepting the concentration in the external phase as a near approximation. This may invalidate the results obtained, Furthermore, in its original form, it is applicable only to bimolecular reactions and to unimolecular layers of adsorption. Therefore it is perhaps more realistic to employ the extended equation of Brunauer and his associates (1938), especially as evidence now exists that certain drug-receptor interactions may be trimolecular (Cavanaugh & Hearon, 1954).

Alternatively, the underlying mechanisms of drug action have been correlated to laws governing enzyme kinetics. In enzymology the principle of biological antagonism has been based on the

Michaelis-Menten equation (1913), and on more recent extensions of this (Lineweaver & Burke, 1934; Haldane, 1930; Kirschner & Stone, 1951), which were derived to explain the interaction of enzymes, their substrates and inhibitors. Michaelis and Menten assumed that enzyme and substrate combined to form an intermediate complex and by applying the law of mass action to this reversible reaction, a relationship was obtained which provided an explanation for many experimental observations and several theoretical-experimental discrepancies (Work & Work, 1948). Since the Michaelis-Menten equation dealt specifically with enzyme systems, its applicability to non-enzymatic mechanisms is not readily apparent. However, the relationship established between the biological effect and the velocity of an enzyme reaction (Michaelis & Menten, 1913), or the law of adsorption (Langmuir, 1916, 1917, 1918), can be expressed mathematically in identical forms, although the former is a kinetically-derived relationship.

Thus equations derived from a consideration of chemical reactions, physical adsorption laws, or enzyme kinetics, may legitimately be applied to drug-receptor interaction. Their identical mathematical

form, however, denies any means of distinguishing which of the three possibilities is responsible for the ultimate mechanism of the drug action.

In more recent years, the concept of biological antagonism has been extended to include instances where the substrate analogue was itself capable of eliciting a response (Chen & Russell, 1950; Furchgott, 1955). Different degrees of antagonism consequently exist (Gaddum, 1957), and several mathematical expressions have been derived to explain them (Gaddum, 1937, 1943; Schild, 1947, 1954; Van Maanen, 1950; Ariëns *et al.* 1957).

For the purpose of this thesis, the explanation of the various types of pharmacological interaction developed by Ariëns and his associates (Ariëns *et al.* 1957), has been chosen for study and application for the following reasons:

(a) The general applicability of the concepts involved to different classes of pharmacological agents (Van Rossum & Ariëns, 1959*a*,*b*,*c*).

(b) The particular reference made to muscle relaxant drugs (Van Rossum *et al.* 1958; Van Rossum & Ariëns, 1959*a*), offering a basis for interpreting the intimate mode of action of these drugs and the possibility of

establishing the effect of certain molecular entities, particularly the ether oxygen function, on muscle relaxant activity.

(c) The procedure developed by Ariëns and his associates, made use of a well known, suitable, laboratory preparation - the frog rectus abdominis muscle, - for the study of the properties of muscle relaxant drugs.

Ariëns had been led to a reconsideration of the accepted views of biological antagonism by the existence of apparent discrepancies encountered during a study of certain drugs (Ariëns, 1954). In many homologous series, certain members apparently possessed a dual mode of action, while presumably acting on the same receptor. The action of certain metabolite-promotor — metabolite-inhibitor derivatives of p-amino benzoic acid (Ariens & Simonis, 1954), the mechanism of action of muscle relaxant compounds which both depolarized and inhibited depolarization (Raventos, 1937; Ginzel, Klupp & Werner, 1951a,b,c), and the role of certain sympathicomimetics which possessed hypertensive and hypotensive properties (Marsh, 1948) seemed paradoxical and inexplicable on the basis of accepted views. Ariëns suggested an explanation of this dual

behaviour by relating drug-receptor interaction to the two basic constants of affinity and intrinsic activity.

The affinity of a drug may be defined as the ability to enter into complex formation with a given receptor. It is directly related to the dissociation constant of the drug-receptor complex (Ariëns, 1954), and this constant can therefore be used to assign to the affinity a numerical value. As defined by Ariëns (1954), the affinity of a drug is the reciprocal of the dissociation constant of the complex formed by the drug and the receptor. In more general terms, affinity may be conceived as a product of a general interaction of electrical fields of force, originating in the drug molecule and the receptor itself, among which contributions from electrostatic and Van der Waal's forces are prominent (Van Rossum & Ariëns, 1957).

The intrinsic activity, on the other hand, is a measure of the power of the drug-receptor complex to evoke a positive biological response. As originally defined (Ariëns, 1954), it is measured by the proportionality constant α, relating the observed biological response to the proportion of receptors

occupied when equilibrium between the drug and the receptor has been achieved. The stimulus to cellular activity may result, however, from changes in the electrical fields of force accompanying formation of the drug-receptor complex, rather than from simple static occupancy of the receptors (Paton, 1961). In consequence, the Ariëns proportionality constant may be split into two components, one being a rate constituent for the speed of dissociation of the drug-receptor complex as stressed by Paton (1961), and indicated by Ariëns, and the other being a constant indicating the effectiveness of the interaction (Ariëns & Simonis, 1960).

In this way, the intrinsic activity of a drug may be due to specific fluxes in the electrical fields of force responsible for the formation of the drug receptor complex.

Both intrinsic activity and affinity vary with the chemical structure, molecular configuration and physio-chemical properties of the drug. In a homologous series, therefore, different degrees of intrinsic activity and affinity may be observed. This approach has offered a means of explaining the mode of action of those compounds, hitherto

unconsidered, whose activity fell somewhere between those evoking a response, high intrinsic activity, and those which induce receptor blockade, low intrinsic activity. Varying degrees of drug-receptor combination were thus believed to control a wide spectrum of pharmacological activity. This view permits new compounds to be logically studied on the basis of their fundamental mode of action.

All the mathematical expressions represent idealized theoretical situations, because of the simplifying assumptions made in deriving them (Ariëns et al. 1957; Nickerson, 1957). In practice, perfect agreement between experimental and theoretical dose-response curves is rarely attained and discrepancies both in the slope (Ariëns et al. 1956a,b; Cavanaugh & Hearon, 1954; Clark & Raventos, 1937; Gaddum, Hameed, Hathway & Stephen, 1955) and symmetry (Ariëns et al. 1957) have been observed. On the other hand, theoretical imperfections have been interpreted in several ways, without invalidating the basic assumptions. For example, a higher order of reaction than bimolecular (Cavanaugh & Hearon, 1954), or a difference in the drug concentration in the bath from that of the biophase (Furchgott, 1955; Ariëns et al.

1957), has been suggested to explain the difference between the slope of theoretical and experimental dose-response curves.

Consequently, while the nature of the receptor system with which drugs react at the neuro-muscular synapse remains hypothetical even today, the interaction of drug molecules with it is believed to obey certain definite mathematical laws, developed on an extended concept of the principle of biological antagonism.

The Classification of Muscle Relaxant Drugs.

It is now apparent that not all compounds possessing muscle relaxant properties act by identical mechanisms (Zaimis, 1959), although it was former practice to group all such drugs together, regardless of their site or mechanism of action. Thus quaternary ammonium bases, pyridine, quinoline, aconitine, muscarine and veratrine, were classified together as 'curarizing' drugs though the manner in which this effect was achieved varied greatly (Santesson quoted by Bovet, 1951). This practice has now been superseded by several attempts to subdivide muscle relaxants into logical divisions on

the basis of their common chemical or pharmacological properties.

The first of these distinguished two main categories the 'pachycurares' and the 'leptocurares' (Bovet, 1951). The 'pachycurares' consisted of bulky molecules which were considered to have a tubocurarine-like mode of action, while the long, thin, 'leptocurares' were assumed to act in a manner resembling acetylcholine. This stereochemical classification has proved unsatisfactory because of the lack of demarcation between the two types and because of two quite different modes of action. Thus several compounds which chemically resemble one class, show pharmacological properties befitting the other. For example, nicotine, which has a thick molecule and might be described as a 'pachycurare' causes neuromuscular block by depolarization in the cat (Paton, 1951), while tridecamethonium, a long thin molecular structure, has a tubocurarine-like action in the chicken (Zaimis, 1952).

The method of classification proposed by Paton and Zaimis (Paton, 1951, 1953; Zaimis, 1951, 1952; Paton & Zaimis, 1952), in which neuromuscular blocking agents were divided into depolarizing and

non-depolarizing drugs, has already been described. This classification has been complicated by variation in the response of different species (Buttle & Zaimis, 1949; Zaimis, 1952, 1953; Bigland, Goetzee, MacLagan & Zaimis, 1958), and of different muscles within the same species, to a particular muscle relaxant drug (Paton & Zaimis, 1952; Jewell & Zaimis, 1954). The effective antagonism of anticholinesterases to depolarization-block in certain species has stressed these difficulties (Bovet, Bovet-Nitti, Guarino, Longo & Fusco, 1951; Su, Kao & Karp, 1951). Furthermore, the two-phase nature of the block induced by certain depolarizing compounds in laboratory animals, (Jenden et al. 1951, 1954; Jenden, 1955) in an isolated human preparation (Sabawala & Dillon, 1959), and in man (Brennan, 1956; Hodges & Foldes, 1956), together with the observation that their depolarizing effect may not account entirely for the block produced (Thesleff, 1955a,b,c), appear outwith the scope of this classification.

By a consideration of both the chemical structure of the drug and the protein nature of the receptor system involved, Foldes (1954) attempted to overcome these difficulties and suggested the division

of quaternary ammonium muscle relaxants into two main groups:

(a) Anti-depolarizing agents - e.g. tubocurarine.

(b) Depolarizing agents - e.g. decamethonium.

While those of the first group produced a similar type of block in all species, that caused by depolarizing agents depended on the nature of the receptor protein of the species involved. In birds, amphibians and certain mammals (e.g. cat) the configuration of the receptor-protein could be altered relatively easily and depolarizing agents caused a depolarization block. On the other hand, where the receptor protein was less prone to configurational changes (e.g. monkey), decamethonium caused an anti-depolarization block. This classification fitted most experimental observations but once more appears too rigid to accommodate drugs possessing characteristics of either group (Zaimis, 1953), or those entirely different from both (Gesler & Hoppe, 1956a,b; Winter & Lehman, 1950). As a corollary to their theoretical treatment of drug-receptor interaction in which mathematical constants are translated into concepts of affinity and intrinsic activity

(Ariëns et al. 1956), Ariëns and his colleagues have utilized the nature of the dose-response curve obtained from experiments using the frog rectus abdominis muscle as a basis for the classification of muscle relaxant drugs (Van Rossum et al. 1958). This classification, confirmed by experiments using whole animal preparations (Ariëns, 1954; Ariëns et al. 1959a,b), eliminates certain difficulties inherent in that of Bovet (Bovet, 1951), while it extends those of Paton and Zaimis (Paton & Zaimis, 1952; Zaimis, 1952) and Foldes (1954). In this classification, muscle relaxant drugs are considered to belong to three principal groups, the depolarizers or cholinomimetics having a high intrinsic activity, for example, decamethonium, the competitors or cholinolytics having a low intrinsic activity, for example tubocurarine, and the non-competitors, for example, prodeconium, which have an affinity for receptors other than those occupied by acetylcholine. The last named group may be further subdivided (Linssen, 1961) into compounds which are believed to block the synthesis of acetylcholine at the neuromuscular synapse, for example, the hemicholiniums (Schueler, 1955;

Reitzel & Long, 1959; Kase & Borison, 1958), the inhibitors of acetylcholine release, for example, botulinum toxin (Ambache & Lessin, 1955) and magnesium ions (Engbaek, 1952), and the anticholinesterases, for example, neostigmine (Holmstedt, 1959). This classification is not rigid and is based on both the molecular properties of the drug and on the inherent characteristics of the neuromuscular synapse. It recognises that variations in the dose level or molecular configuration of a drug may cause gradual changes in intrinsic activity and affinity, with consequent alterations from one category to another.

Chemical Structure and Muscle Relaxant Activity in Synthetic Compounds.

Interest in synthetic muscle relaxants has been maintained over the past twenty years and a large number of drugs have been synthesized and tested. The number of new substances reported is so considerable that it makes a complete bibliography impracticable and the diversity of chemical structures so wide as to render difficult the consideration of each individual chemical type. While compounds other than quaternary ammonium derivatives (Folkers & Major, 1937; Berger & Bradley, 1947), may possess muscle relaxing properties,

the vast majority of compounds exhibiting this type of activity, including virtually all of those employed in anaesthesia, are quaternary ammonium salts. In consequence, a classification based on the quaternary ammonium group would appear to offer a logical means of discussing synthetic muscle relaxants.

Muscle relaxant activity represents an integrated product of many contributory biological, physical and chemical factors including accessibility of the drug to the site of action, the effective concentration which it attains in the biophase and the bonding characteristics of the drug-receptor complex (Cavallito, 1959). Virtually all quaternary ammonium salts of pharmacological interest are believed to exert their principal effects by mimicking or antagonizing the physiological actions of acetylcholine. Many of these compounds exhibit different pharmacological properties depending upon the concentration, the duration of effect and the specific site of action involved. Because they ionize completely, quaternary ammonium salts have an intense affinity for anionic receptors.

The apparent absence of a membrane barrier surrounding the neuromuscular junction (Couteaux, 1947) facilitates access to this site of both ionized simple quaternary ammonium compounds and other more bulky molecular species as evidenced by the rapid onset of action of muscle relaxant drugs. Consequently, variation in chemical structure may have relatively little influence on the potential ability of a quaternary ammonium compound to reach the anatomical site of action.

The muscle relaxant properties of onium salts, first recognized by Crum Brown and Fraser in 1869, have been the subject of several reviews, and reference may be made to the work of Trendelenburg, 1923; Alles, 1934; Ing, 1932, 1936; Craig, 1948; Bovet & Bovet-Nitti, 1948a,b, and references cited therein. The association of rather weak muscle relaxant properties with more potent cholinergic effects in many monoquaternary compounds has virtually eliminated the possibility of their clinical use (Huguenard & Martin, 1950). The isolation of tubocurarine and the determination of the structure of its molecule (King, 1935; Wintersteiner & Dutcher,

1943) revealed the presence of two quaternary ammonium groups. Together with the successful development of techniques for the clinical use of this compound (Bennett, 1941; Griffith & Johnson, 1942; Cullen, 1943), a stimulus was provided for the preparation and study of drugs whose molecule possessed two quaternary ammonium functions. This led to the pharmacological investigation of a large number of aromatic and aliphatic derivatives containing two onium groups, among which were included some of the most active and clinically most useful muscle relaxants (Bovet & Bovet-Nitti, 1948a,b, Bovet, 1951; Barlow & Ing, 1948a,b; Paton & Zaimis, 1948a,b; 1949; Collier & Taylor, 1949). For detailed discussions of the chemical and pharmacological properties of many of the compounds involved, reference may be made to the extensive reports available on the subject, a few of the more recent of which are cited here (Bovet, 1951; Paton & Zaimis, 1952; Schneider, 1953; Cheymol, 1954; Papper & De Beer, Symposium 1955; Bovet, Bovet-Nitti & Marini-Bettolo, 1959; Nachmansohn, Symposium 1959d; Lewis, 1960b). Bisquaternary compounds possess a more intense and prolonged neuromuscular blocking action than the

related monoquaternary derivatives. This may be due to the former possessing a greater ability to form bonds, i.e. a greater number of points of potential receptor attachment, producing an increase in the potential bonding energy of the drug-receptor complex. The design and investigation of polyquaternary derivatives containing three or four nitrogen functions was thus undertaken in the belief that a high degree of activity would be conferred by virtue of their being reactants with a polymeric hypothetical receptor system (Kensler, Langemann & Zirkle, 1954a; Kensler, Zirkle, Matallana & Conduris, 1954b; Edwards, Lewis, Stenlake & Zoha, 1957, 1958a,b; Carey, Edwards, Lewis & Stenlake, 1959; Edwards, Stenlake, Lewis & Stothers, 1959, 1961). In many of the most active polyquaternaries, the ratio of lipophilic to hydrophilic groupings is comparable to that of many active bisquaternary derivatives (Cavallito & Gray, 1960). This suggests that the enhanced potency of many bisquaternary muscle relaxant compounds may be due to these molecules having attained an optimum compromise between lipophilic and hydrophilic molecular bonding functions.

Introduction of hetero-atoms into the onium

substituents may cause changes in the overall size and configuration of the molecule. This may also alter the cationic charge distribution, the secondary bonding characteristics of the compound and its ability to take up molecules of water. Among the most interesting molecular constituents associated with the quaternary ammonium moiety in many muscle relaxant compounds, is the ether oxygen function. Interest in this, originating from the muscle relaxant properties of diethyl ether itself (Auer & Meltzer,1914), has been maintained by the presence of the ether link in tubocurarine (King, 1935) and other clinically useful,similarily acting drugs (Bovet, Depierre & De Lestrange, 1947; Collier, Paris & Woolf, 1948; Collier, 1952; Levis, Preat & Dauby, 1953). Ether anaesthesia has also been observed to potentiate the muscle relaxant activity of non-depolarizing drugs (Foldes, 1959, 1960, and references cited therein). The ether link provides a valency angle (110° in diphenyl ether) approximately similar to that of the carbon-carbon link ($109^{\circ}28'$), while replacement of a -C-C-C-chain by a -C-O-C-chain reduces chain length only slightly, (0.13 Å) when extended conformations are compared. An oxygen atom may thus be interposed in

an alkane chain without major stereochemical disturbance (Sexton, 1949). On the other hand the lone electron pairs on the ether oxygen atom confer polar properties and such molecules are capable of orientation at lipid-aqueous interfaces, for example, cellular membranes. Polar characteristics may also significantly affect the molecular lipophilic-hydrophilic ratio, altering the biological effect by influencing the ability of the drug to reach the hypothetical receptor site. Furthermore, a compound which is predominantly hydrophilic is also often hydrated and although quaternary ammonium functions themselves are believed to be non-hydrated, the presence of polar substituents may confer this property to the overall molecule. Polar groupings may thus be principally concerned with the distribution of the compound between 'sites of activity' and 'sites of loss' and numerous examples indicate the reduced muscle relaxant potency of predominantly hydrophilic molecules (Barlow & Ing, 1948a,b; Hohmann & Jones, 1954; Thesleff & Unna, 1954).

The ability of the molecule to form Van der Waal's bonds may also be influenced by the polar-

conferring properties of the ether oxygen function. These short, weak, secondary bonding structures contribute appreciably towards maintaining the stability of the drug-receptor complex. Moreover, hydrogen bonding between the drug molecule and certain hydrogen atoms, which may be located near the site of drug activity, may be made possible by the presence of the ether link. Formation of hydrogen bonds has often been shown to have a deleterious influence on muscle relaxant activity (Phillips, 1952, 1955; Karczmar & Howard, 1955).

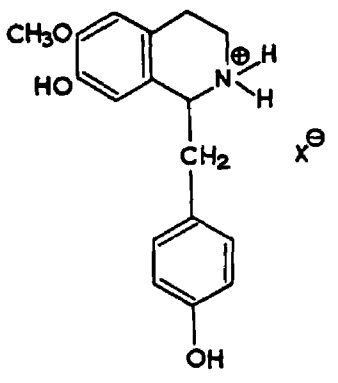
The ether oxygen link may also modify the distribution of the electrostatic charge on the onium centre itself (Taylor, 1951). Thus the inclusion of an oxygen atom to form a morpholine ring may reduce the electrical charge concentration on the onium group and reduce the potency of the compounds compared with that of their piperidine analogues (Cavallito, Soria & Hoppe, 1950; Hazard, Cheymol, Chabrier, Corteggiani, Muller & Gay, 1953; Hazard, Cheymol, Chabrier, Corteggiani, Muller & Bourillet, 1954). Concentration of electrical charge on the onium group is generally favourable to activity in both depolarizing

(Riker, Roberts, Reilly & Roy, 1954) and non-depolarizing drugs (Taylor, 1951).

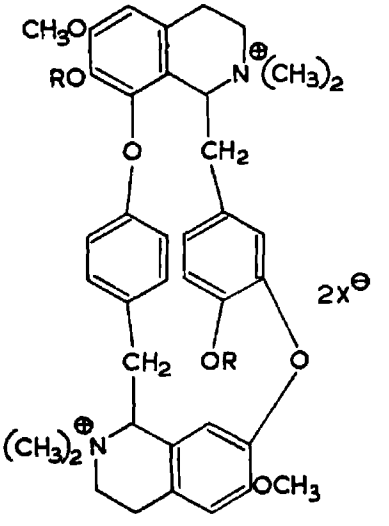
From these considerations it might be expected that the presence of the ether oxygen function would unfavourably modify muscle relaxant potency. Increased polarity, alterations in the charge distribution and bonding propensity of the molecule may, however, be offset by increasing the lipophilic bulk of the onium centres themselves or by increasing the length of the inter-onium chain. In consequence, changes in the intensity of activity induced by the presence of the ether oxygen are often subtle rather than dramatic. Detailed consideration of several of these changes occurring in synthetic muscle relaxants will now be given.

Monoquaternary Derivatives.

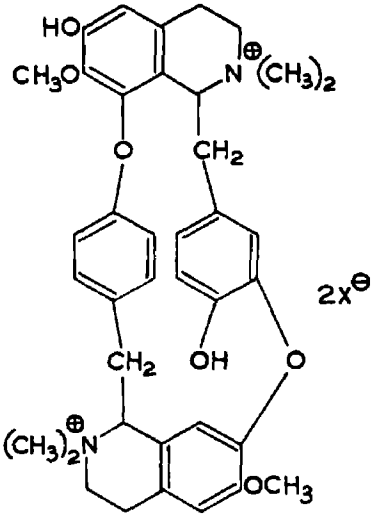
Few monoquaternary ether-containing derivatives possess muscle relaxant properties although simple alkoxytrimethylammonium salts (Rogers, Bovet, Longo & Marini-Bettolo, 1953) and several phenolic ether derivatives of choline (Rosnati, Angelini-Kothny, 1958; Winter & Lehman, 1950; Bovet, Depierre, Courvoisier & de Lestrange, 1949) are reported to


CH3O
HO
H
N
H
CH2
X⊖
OH

III


CH3O
RO
N
(CH3)2
O
CH2
2X⊖
CH2
OR
O
(CH3)2N
OCH3

IV


HO
CH3O
N
(CH3)2
O
CH2
2X⊖
CH2
OH
O
(CH3)2N
OCH3

V

possess this characteristic. The predominance of cholinergic properties (Hey, 1952; Buchel, Guyonneau & Levy, 1957) in monoquaternary compounds is in keeping with similar pharmacological features generally observed in analogous non-ether derivatives. It is interesting to note that (-)-coclaurine (III), which closely resembles half the molecule of tubocurarine (IV, R = H), possesses no neuromuscular blocking activity in dogs, in doses up to 1 mg (Finkelstein, 1951).

Bisquaternary Derivatives.

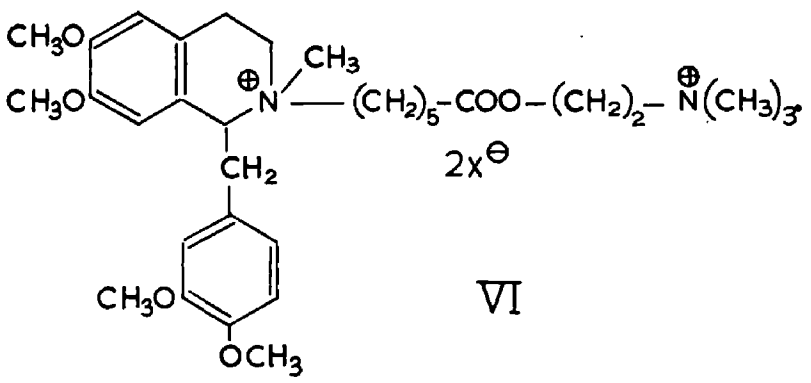
Of those naturally occurring compounds which have now been chemically synthesised, tubocurarine itself, (IV, R = H) possessing two ether linkages, is the most outstanding (King, 1935, 1948; Wintersteiner & Dutcher, 1943).

The (-)-isomer is virtually inactive on the rat (King, 1946). The isomeric (+)-chondocurarine (V) which differs from tubocurarine solely in the position of the methoxyl and hydroxyl substituents is approximately three times more potent on the rabbit than the latter (Wintersteiner, 1959). Tubocurarine dimethyl ether (IV, R = CH_3) (Collier et al. 1948; Collier & Hall, 1950) in which the phenolic groups of

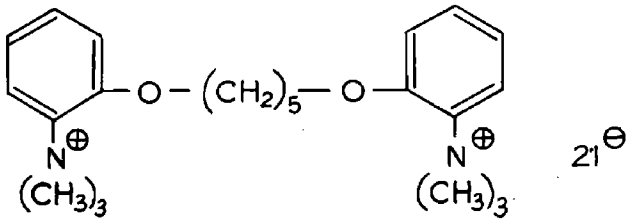
tubocurarine are converted to the methyl ethers, possesses similar qualitative properties to the parent compound. Quantitatively, potency varies considerably with the animal species employed. Tubocurarine dimethyl ether is reported to be more potent than tubocurarine itself on the monkey, rabbit, rat and in the human being but only equipotent on the mouse (Wintersteiner, 1959; Swanson, Henderson & Chen, 1949). On the frog rectus abdominis muscle preparation, under conditions which reduce the ionization of the phenolic groups (low pH), tubocurarine is more potent than its dimethyl ether (Kalow, 1954). Increase in the size of the ether-containing alkyl radical generally reduces potency. Only the diethyl ether is more potent than tubocurarine while the di-n-butyl and the dibenzyl derivatives show a marked reduction in potency. The synthesis and investigation of molecules, the relaxant potency of which was comparable to that of natural products, initially involved aromatic nuclei containing ether functions either in the nucleus itself or in the interconnecting alkane chain. The preparation of these and other related compounds was inspired by the occurrence of similar

molecular features in tubocurarine (Bovet & Bovet-Nitti, 1948a,b; Bovet, 1951). Many of these compounds were structurally complex, containing bulky ring systems with several substituents. Increasing molecular complexity rendered the correlation of changes in the biological response with specific physicochemical variables more difficult. Consequently, it is difficult to establish the precise role of the ether oxygen function in compounds of this type.

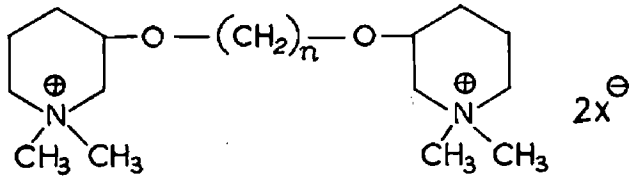
Among the most active products from this series of investigations were the decamethylene bis-tetrahydroisoquinolinium derivatives (Collier & Taylor, 1949; Taylor & Collier, 1951, 1952; Taylor, 1952). Of these, the N-methyltetrahydro derivatives were appreciably more active than the isoquinolinium analogues, indicating the favourable influence of concentration of electrostatic charge on the onium nitrogen atoms for high muscle relaxant activity. 6,7-di- and 6,7,8-tri-methoxy substituents on the benzene ring markedly enhanced the activity of the tetrahydroisoquinolinium derivatives, possibly by enhancing the lipophilic characteristics of the molecule. Of these compounds, salts of decamethylene-α,w-bis-



VI

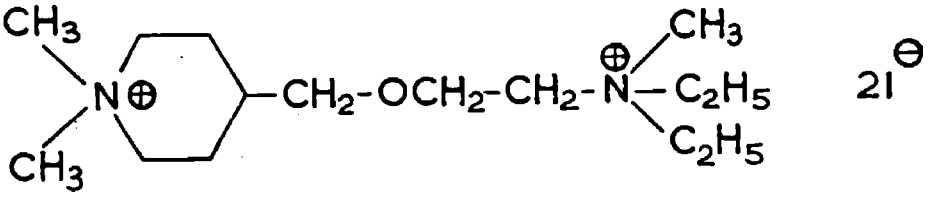


VII

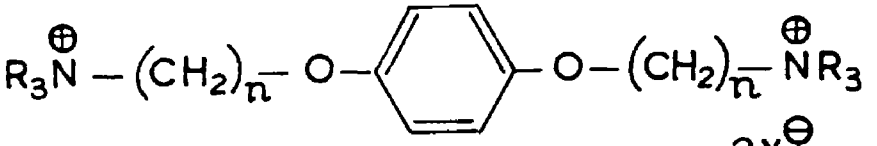


VIII

[1-(3',4'-dimethoxybenzyl)-1,2,3,4 - tetrahydro - 6,7 - dimethoxy -2- methylisoquinoline], (laudexium) were among the most important. This drug is a clinically useful (Bodman, 1952), long acting, muscle relaxant (Collier & Macauley, 1952) which has predominantly non-depolarizing properties. Another related, non-depolarizing compound with a prolonged duration of action is reported to be seven times more potent than suxamethonium (VI) (Collier, Gladych, Macauley & Taylor, 1958). Although investigations were initially concerned with the design of compounds possessing features characteristic of tubocurarine, neuromuscular blocking activity is not confined to complex quinoline or isoquinoline structures but is also the property of simpler molecules. Among ether-containing drugs of this type, the bisquaternary aromatic amines described by Bovet and his associates, for example bis-(2,2'-trimethylaminophenoxy)-1,5-pentane diiodide (VII), were as potent as tubocurarine itself (Bovet, Courvoisier, Ducrot & Horclois, 1946, 1947). Inclusion of the onium moiety as part of a piperidine ring system has also been reported to produce active



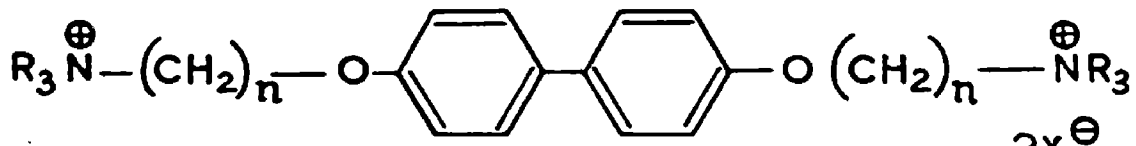
IX



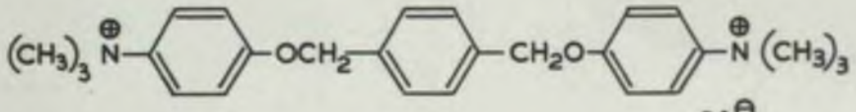
X

compounds (VIII; Fisher & Keasling, 1956, 1958) showing characteristics of both depolarizing ($n \# 12$) and non-depolarizing ($n > 12$) muscle relaxants depending on the length of the inter-onium chain. Other unsymmetric piperidine, bisquaternary, ether-containing compounds for example (IX) possess low muscle relaxant activity (Elpern, 1954). That the presence of the ether oxygen link may increase the potency of such compounds has been suggested by Pradhan and his associates who investigated several closely related di-n-butyl and dibenzyl piperidinium derivatives (Pradhan, Ray, Varadan & De, 1954).

A number of hydroquinone di-ethers have also been prepared and tested for muscle relaxant properties (X). A bibliographic summary and table including structural variations in these compounds have been provided by Bovet (1959). While no attempt has been made to evaluate the contribution of the ether oxygen function in these molecules, there is no evidence to suggest that muscle relaxant potency is enhanced by its presence. Maximum activity was observed when the inter-connecting chain length was 12-15 atoms (Bovet, 1958). Among lower analogues ($n = 2$; $R = CH_3$),



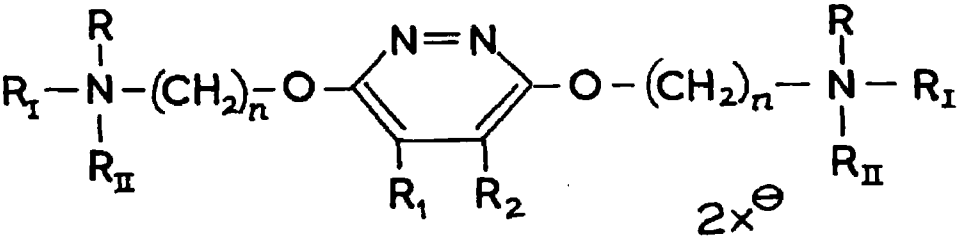
XI



XII

potency was low but replacement of a methyl by an ethyl group on each quaternary nitrogen group enhanced activity (Winter & Lehman, 1950). Modifications in the activity of these compounds produced by alteration in chemical structure is influenced by species variation, which minimises the value of conclusions drawn from different literature sources concerning related compounds. The ortho and meta compounds ($n = 2$; $R = C_2H_5$) are more active than the corresponding para derivatives (Bovet, 1951) while the corresponding bis-dimethylethyl ortho derivatives Diethamine ($n = 2$) and Dipropamine ($n = 3$) have also potent muscle relaxant properties (Winter & Lehman, 1950) and have been clinically investigated (Hunter, 1953a). Variations in the basic hydroquinone structure have produced compounds possessing different degrees of activity. The insertion of a p,p'-biphenyl (XI) centre moiety in which $R = CH_3$ and $n = 2$ yielded a compound with slightly increased activity compared with the reference compound, (Bovet et al. 1947; Levis et al. 1953) while extended ether structures (Funke, Engeler, Jacobs & Depierre, 1949), for example p-xylylene-bis-(p-trimethylammonium phenyl ether) (XII), also possess

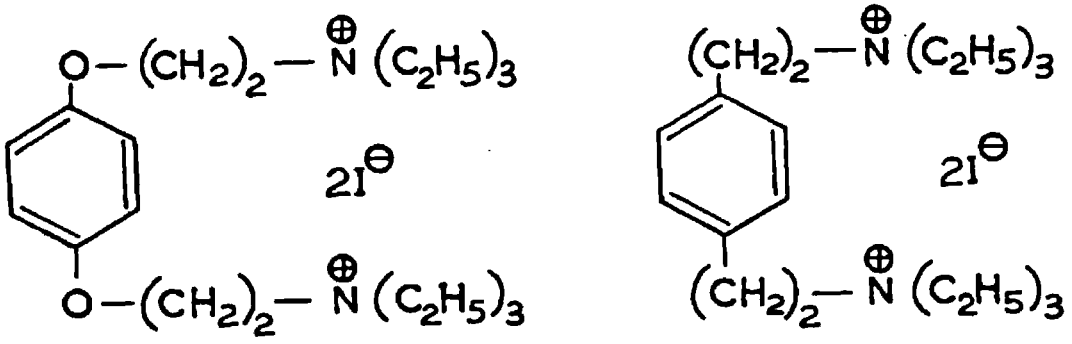
appreciable activity (Funke, Krucker & Depierre, 1950). Ether-containing bisquaternary compounds have contributed to the development of the supporting moiety theory (Cavallini, Costa, Ferrari & Massarani, 1954; Cavallini & Massarani, 1959). This contends that pharmacologically active molecules consist of a radical moiety, which controls the type of biological activity exhibited, together with a supporting moiety which confers affinity for the site of activity. It is interesting to recall the analogy of the 'haptophore-toxiphile' relationship conceived for the process of conferring immunity by Ehrlich. The value of the anchoring 'haptophore' group in enabling the active 'toxiphile' to be effective bears an obvious resemblance to the present day concept of a supporting moiety, as a contributory factor in explaining drug action. Experimental support for the supporting moiety theory has been provided by the investigation of activity in certain steroidal quaternary derivatives (Cavallini & Massarani, 1951) and in certain ether-containing quaternary derivatives of stilboestrol, hexoestrol and diphenylethane (Cavallini *et al.* 1954). The combination, in the latter compounds, of the



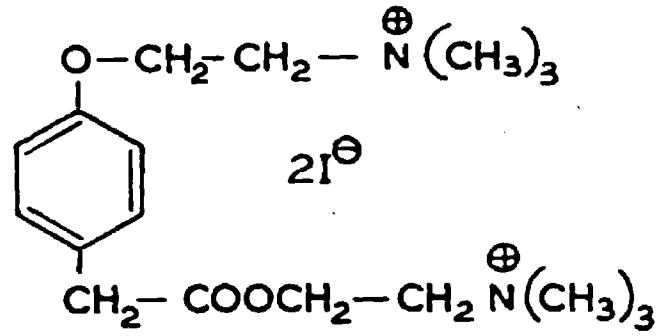
XIII

'stripped down' (Gero & Reese, 1956; Gero & Withrow, 1957) drug molecule or radical moiety, the quaternary ammonium group, and the supporting stilboestrol, hexoestrol or diphenylethane ether moiety produced drugs with high muscle relaxant activity.

Other structurally related ether-containing compounds investigated for neuromuscular blocking activity have included derivatives of biphenyl (Bovet et al. 1947; Levis et al. 1953) and dienoestrol (Hazard et al. 1954). Although muscle relaxant activity is a common feature of these drugs, the precise contribution of the ether oxygen function has not been evaluated. Interest has also been aroused in certain pyridazine ether-containing compounds (Steck, Brundage & Fletcher, 1954; Gesler & Hoppe, 1956) (XIII; n = 2 or 3) reported to exert their muscle relaxant effect by a presynaptic blocking action (Gesler, Lasher, Hoppe & Steck, 1959). This effect is markedly influenced by the frequency of stimulation employed. The role of the ether-oxygen function in influencing muscle relaxant activity in certain phenolic ethers has been more fully investigated by Bovet (1951), who observed a similar curarizing



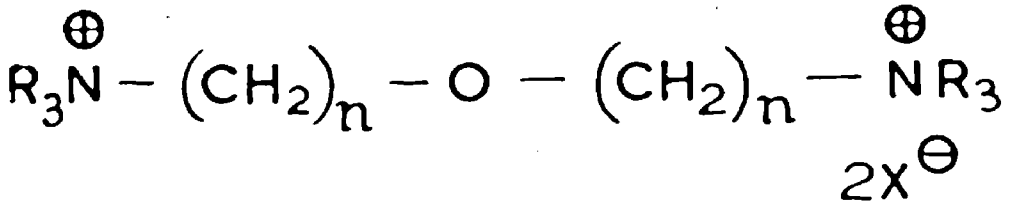
XIV



XV

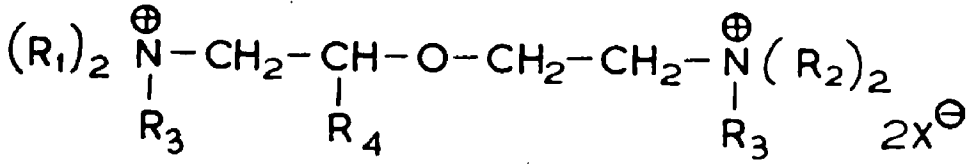
potency on the rabbit for certain ether and non-ether containing compounds (XIV). In derivatives of this type, therefore, the ether oxygen function is relatively unimportant. Pursuit of these studies led this school to investigate the effect of combining both ether and ester functions in the same molecule, (Rosnati & Puschner, 1957) but no obvious advantage was achieved (XV) (Rosnati, Angelini-Kothny & Bovet, 1958).

The difficulty of assessing the effect of the ether oxygen function in aromatic compounds is increased by the complexity of such structures and by the numerous influences on overall biological activity contributed by resonance, steric hindrance and other factors previously discussed in this thesis. In view of this, a study of aliphatic ether-containing muscle relaxants appeared to offer a more useful means of further investigation of this problem. The structure of many of these compounds has been based on that of decamethonium and consists of the insertion of one or more ether linkages into the polymethylene chain linking two quaternary ammonium groups. The quantity, and the diversity of structure of the molecules investigated is considerable and a biblographic summary

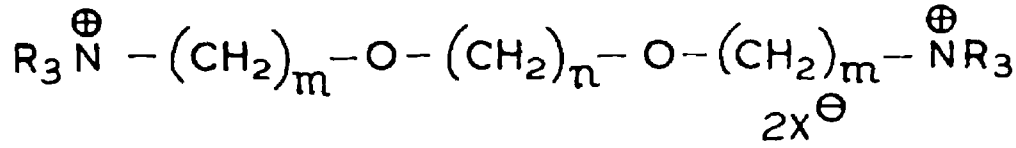


XVI

and structural table of many of the variants involved has been provided by Bovet (1959). Maximum muscle relaxant activity is observed when the interconnecting chain length comprises 11-16 atoms. This is longer than observed in the non-ether analogues (Paton & Zaimis, 1949; Barlow & Ing, 1948b) and reflects the reduction in the effective chain length and the polar characteristics associated with the presence of the ether oxygen function. The former consideration alone (0.13 Å) can hardly account for the marked reduction in potency observed by the introduction of one ether oxygen function in compounds of this type (Ray, Kohli & De, 1957). Among an extensive series of monoethers studied by Levis and her associates (XVI) (1953) and others (G.P.847000/1952; G.P.921,267/1954), appreciable short-acting muscle relaxant properties were observed, which increased with increase in the length of the polymethlene chain (n). Replacement of methyl ($R = CH_3$) by ethyl ($R = C_2H_5$) radicals did not significantly affect potency but increase in the size of the onium substituents beyond the ethyl derivative was claimed to increase the activity of these compounds (Pradhan,



XVII

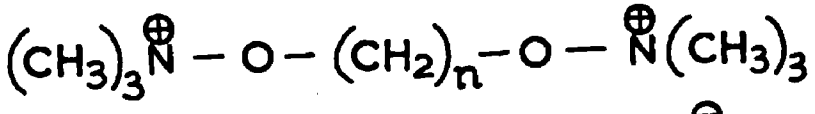


XVIII

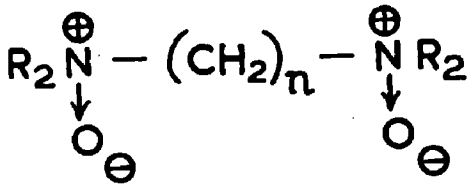
et al. 1954). The most active member, oxydipentonium (n = 5), has been successfully used in certain surgical procedures (Levis et al. 1952: Mundaleer, 1952; Montemartini, 1956).

Ganglion blocking activity associated with non-depolarizing muscle relaxant activity has also been observed in a series of bisquaternary, branched alkyl ethers (XVII) (Lenke, 1957). It is apparent from these considerations that the introduction of one ether oxygen function into straight chain, bis-onium, polymethylene derivatives reduces muscle relaxant potency. Introduction of a second ether function appears to reduce potency further and the alteration in total chain length is again disproportionate to the observed fall in potency (Thesleff & Unna, 1954). In compounds of the general type shown (XVIII), neuromuscular blocking potency (Girod & Hafliger, 1952) is not primarily related to the overall inter-onium chain length but to the middle part (n) and to a lesser extent to the onium substituents (R). While R is ethyl and n is 10, potency is not significantly influenced by the value of m (2,3 or 4), a simultaneous increase in the value of m and a decrease in the value

of n reduces potency to a marked degree. The position of the ether oxygen in the chain may thus appreciably influence the activity of compounds of this type. Weak muscle relaxant properties have also been observed with lower, ethyl homologues (m =2, n = 2,3,4) (Vanecek & Votava, 1955). Hazard and his colleagues (Hazard et al. 1953), investigating analogous compounds in which m and n were 2, found the order of potency, in relationship to the cationic head substituents, to be least favourably influenced by the ether-containing morpholine group. The derivative in which m = 2 and n = 4, possessed potent cholinergic properties (Marsh & Herring, 1951). Muscle relaxant activity has also been reported in related quaternized ether-containing triamines (G.P.848,825/1952) and in certain quaternary ammonium polymers derived from alkyl vinyl ethers (G.P.1,005,273/1957). The combination of an ester and an ether function in the one molecule has produced an active muscle relaxant of short duration, which has been used clinically (Frey, 1955; Hunter, 1959). In this compound, prodeconium (n = 10, m = 2), one methyl group on each nitrogen atom has been replaced by a propyl acetate moiety. It



XIX



XX

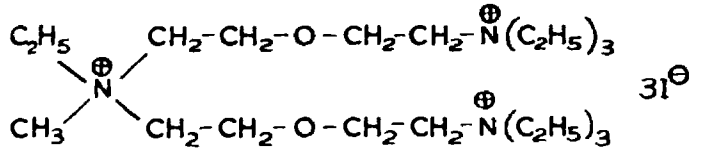
produces a 'mixed' type of block more closely related to tubocurarine than to decamethonium (Rendell-Baker, & Foldes, 1957; Barbieri, Lodi & Malaguti, 1958).

Polymethylene bis-(oxytrimethylammonium) derivatives (XIX n = 5,6 and 10) show anticholinesterase activity only (Palazzo, Rogers & Marini-Bettolo, 1954) while the polymethylene bis-(N-oxides) (XX n = 2-12) are devoid of muscle relaxant properties (Jerchel & Jung, 1952). 'Decamethonium dioxide' is similarly inactive (Hano, Wilimowski & Gieldanowski, 1958).

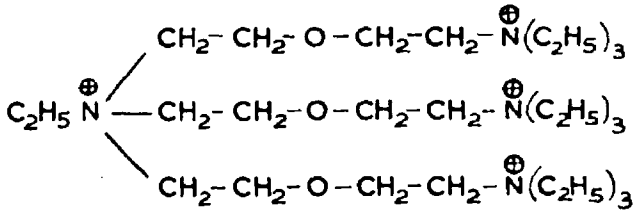
Polyquaternary Derivatives

From a series of β-hydroxyethyltriethylammonium ethers synthesized and investigated by Bovet and his associates (XXI) (Bovet et al. 1947, 1949), gallamine, 1,2,3, tris-(β-triethylammoniumethoxy) benzene triiodide, was observed to possess the most potent tubocurarine-like muscle relaxant properties (Jacob & Depierre, 1950; Bovet & Bovet-Nitti, 1948a,b; Riker & Wescoe, 1951; Wien, 1948) and has proved a clinically valuable drug (Doughty & Wylie, 1946; Mushin, Wien, Mason & Langston, 1949).

The presence of a third quaternary ammonium function in the molecule, it was suggested (Riker &



XXII



XXIII

Wescoe, 1951), might modify the freedom of rotation of the other two side chains attached to the aromatic ring. In this way, the two terminal nitrogen groups may be kept at an optimal interatomic distance for muscle relaxant properties (Cavallito, Schlieper & O'Dell, 1954; Hohmann & Jones, 1954). Few aliphatic, polyquaternary ammonium,ether-containing compounds have however been prepared. Vanecek and Votava (1955) prepared and investigated for muscle relaxant activity one tris-ammonium (XXII) and one tetra-ammonium (XXIII) ether-containing compound. Both were reported to possess weak muscle relaxant properties, the former being slightly more potent than the latter. In neither case was the corresponding non-ether analogue investigated nor was any attempt made to evaluate the contribution of the ether oxygen function to the biological activity of the compound.

It is apparent from the literature cited, that many synthetic aromatic and aliphatic ether-containing derivatives possess muscle relaxant properties to a greater or less degree. It is equally apparent, however, that few attempts have been made to evaluate the contribution of the ether link to the observed

biological effect and a study of the pertinent literature has underlined the existence of this problem rather than contributed to its solution. A precise assessment of the part played by the ether oxygen link is made even more difficult, since different workers have employed different preparations and species, while analogous non ethers have seldom been tested under similar conditions. The compounds in a series of aliphatic, bis-choline ethers possessing ganglion blocking properties, which were synthesized and tested by Fakstorp and his associates (1953, 1954a,b,c, 1955, 1956, 1957a,b,c), were reported to act by means of the attachment of one quaternary ammonium group to the receptor. It was deemed worthwhile to investigate the possibility of applying this hypothesis to neuromuscular blocking activity in certain of these compounds. While onium salts other than those of nitrogen have been of relatively little pharmacological interest, several investigations have been concerned with sulphur-containing derivatives. Early investigations described the reduced nicotinic activity of methylated onium salts of sulphur (Hunt & Renshaw, 1925) their weaker paralyzing effects being related to the larger radius

of the sulphur atom (Ing & Wright, 1933) and to a reduction in the concentration of the cationic charge. The sulphonium analogue of acetylcholine is much weaker than the parent compound (Prelog, Juhasz, Rezek & Stern, 1942) while the insertion of two sulphur atoms into the molecule of suxamethonium (Della Bella, Villiani & Zuanazzi, 1956) markedly reduced the potency but not the type of muscle relaxant activity. Similarly, the disulphonium analogue of decamethonium was less active than the parent compound while the ammonium-sulphonium derivative was intermediate in potency (Walker, 1950). 5,6-Dithiadecamethylene-bistrimethylammonium diiodide is reported to poasess properties qualitatively and quantitatively similar to those of decamethonium (Hunter, 1953b). Replacement of nitrogen by sulphur in several bis-onium ether compounds did not alter muscle relaxant potency (Vanecek & Votava, 1955). The evidence suggests, therefore, that the replacement of nitrogen by sulphur in bis-onium muscle relaxants has no advantageous effect on potency and seldom alters their qualitative pharmacological behaviour.

Purpose of Research

The work described in this thesis was undertaken to investigate the basic pharmacological mode of action of several new synthetic muscle relaxant drugs with a view to investigating, where possible, structure-action relationships among them. In this respect, particular attention was paid to the contribution to muscle relaxant activity, of the ether oxygen function, the effect of altering the length of the polymethylene chain, the number and nature of the onium centres present in the molecule and the nature of the alkyl substituents on the onium centres. Consequently, a number of straight chain, polymethylene, polyquaternary ammonium compounds several of which contained one or more ether functions was investigated for muscle relaxant activity on both intact animals and isolated tissue preparations. Several corresponding non-ether containing compounds were similarly tested. In addition, three sulphonium analogues of decamethonium were examined for muscle relaxant activity. The effects of these compounds on the cardiovascular system and on ganglionic transmission were also observed.

Pharmacological investigations in the field

of muscle relaxants have been increasingly concerned over the past decade with placing the basic mode of drug action on a fundamental mathematical basis. The work of Paton (1961) and of Ariëns and his associates (1957) has already been referred to and the systems of classification of muscle relaxants devised by the latter workers described (Van Rossum et al. 1958). This procedure could, it was believed, logically be applied to investigate the contribution of the ether oxygen function and the effect of other molecular features on the activity of the compounds concerned based on the concept of drug-receptor interaction. Furthermore, this approach appeared to provide a simple method of distinguishing muscle relaxants of potential clinical value from those with little or no clinical value thus eliminating the considerable sacrifice of life entailed in the standard screening procedures adopted for drugs of this class. Consequently, an additional examination of the compounds described and others was made on the basis of their affinity and/or intrinsic activity values, using the frog rectus abdominis muscle preparation described by Ariëns (1954) and a correlation made of these results with those obtained using more conventional methods.

CHAPTER I

EXPERIMENTAL METHODS

Experiments Using Cats.

In all these experiments, cats of either sex weighing between 2.0 and 5.0 kg were used. Anaesthesia was induced by intraperitoneal injection of sodium pentobarbitone solution (50-60 mg/kg). To facilitate the application, when necessary, of artificial respiration, the trachea was cannulated, the amount of air entering or leaving the cannula being controlled by an adjustable sleeve. The external jugular vein on one side was exposed, freed from connective tissue and a heparinised polythene cannula inserted with its pointed end towards the heart. The cannula was then connected by means of rubber tubing to a 50 ml burette containing normal saline solution. Drugs under investigation, dissolved in normal saline solution, were administered by means of a 1 ml graduated tuberculin syringe, injection being made into the rubber tubing connecting the burette and the jugular vein cannula. Each dose of drug was washed in by a suitable volume of saline (3 ml).

The Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

Cat skeletal muscle is characterized by a

marked colour differential into two main groups of fibres and thence into three main types of muscle (red, white and mixed):

(a) Red or slowly contracting fibres, e.g. soleus muscle. These are generally very resistant to depolarizing drugs (Paton & Zaimis, 1951; Jewell & Zaimis, 1954).

(b) White or more rapidly contracting fibres, e.g. anterior tibialis. These are relatively very sensitive to depolarizing drugs (Paton & Zaimis, 1951). Both types of fibres are sensitive to non-depolarizing drugs.

The gastrocnemius muscle consists predominantly of white fibres though a minority of red fibres are also present (Denny-Brown, 1928-29). It was chosen for these experiments since it combined the characteristics of both types of muscle fibres and thus appeared to offer the most accurate means of assessing the overall biological effect of muscle relaxant drugs on mammalian skeletal muscle. Furthermore, use of this muscle would, it was believed, be advantageous if the compounds under review were observed to possess clinical potentialities. The

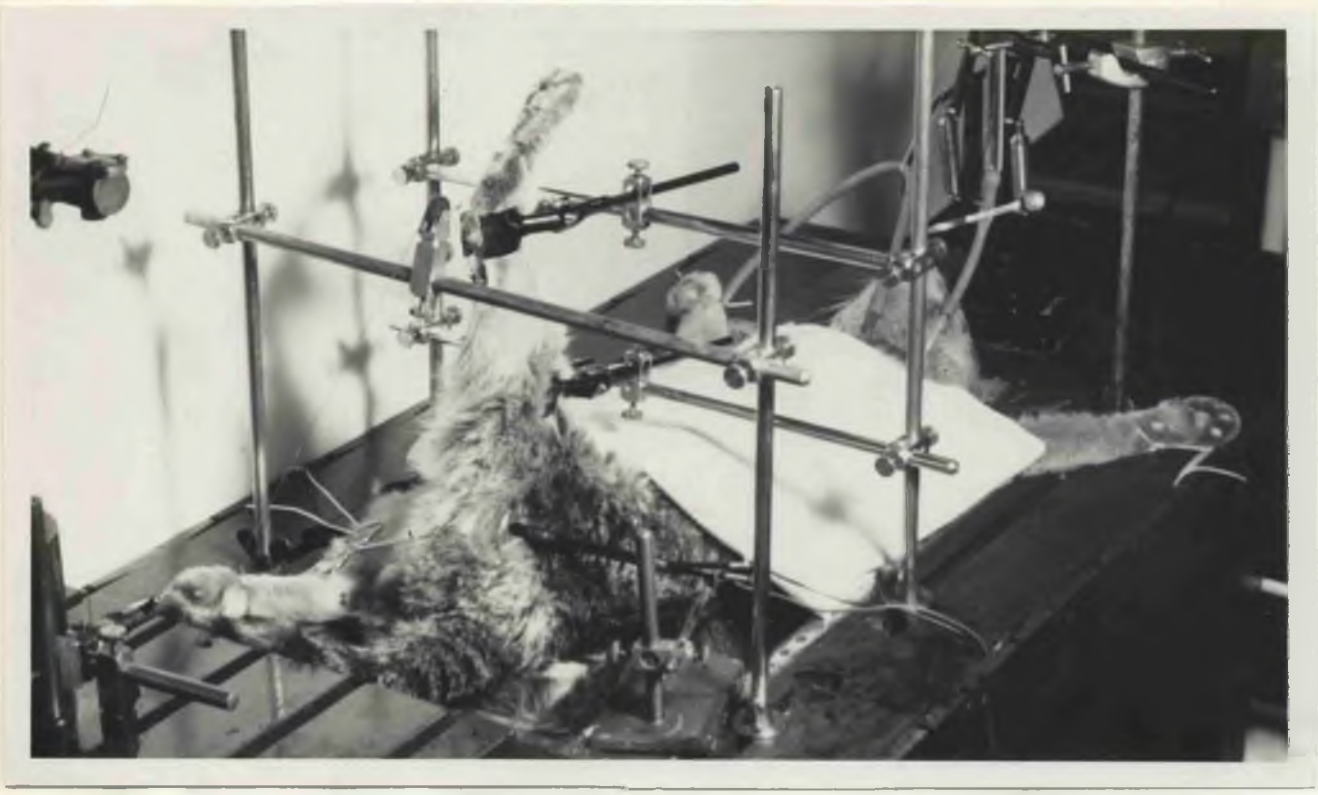


Fig. 1. The cat gastrocnemius muscle-sciatic nerve preparation.

preparation, similar to that described by Bülbring and Burn (1942), may be used to investigate both the qualitative effects of neuromuscular blocking agents and semi-quantitatively to compare the potency of new drugs with that of tubocurarine or decamethonium.

The animal having been prepared as described, one leg was arranged for indirect stimulation of the gastrocnemius muscle via the sciatic nerve. The leg was held with its long axis perpendicular to the operating table and rigidly fixed by means of two clamps, one at the knee joint, and the other at the ankle as shown (Fig. 1). The gastrocnemius muscle was partially freed from surrounding tissues and the Achilles tendon severed at a point near to its insertion in the calcaneus. A strong linen thread was then sewn through the central end of the tendon and the free end of the thread led over pulleys and attached to a Brown-Schuster myograph lever, the writing point of which was adjusted to record the muscle contractions on a moving smoked surface. By means of an incision made through the skin covering the lateral aspect of the thigh, the sciatic nerve was exposed between the hamstring muscles and a pair of shielded platinum

electrodes placed around it. The nerve was then stimulated supramaximally, using a Dobbie-McInnes square-wave stimulator, at a frequency of 6-8/min, at 10 to 20 V and using a pulse width of 2.0 to 3.0 msec. The stimulation and the tension on the gastrocnemius muscle (usually 0.2 - 0.3 kg) remained constant during any one experiment.

Recording The Blood Pressure Of The Anaesthetized Cat.

In these experiments, one of the common carotid arteries, on the opposite side of the neck from the cannulated jugular vein, was freed from the surrounding tissues and ligated as near the cephalad end as possible. A'bull-dog' clip was placed around the artery, distal to the ligature and a heparin-filled glass artery cannula inserted into the artery with its pointed end towards the heart. The cannula was then connected to a mercury manometer by rubber tubing which was filled with heparinised normal saline solution as an anticoagulant. The pressure in the mercury manometer was set at approximately 120 mm of mercury to maintain equilibrium against the blood pressure of the animal. The 'bull-dog' clip was then released and the blood pressure recorded on a smoked surface.

The Nictitating Membrane Preparation.

The jaws of the animal were tied firmly over a brass rod which was clamped to two uprights fixed to either side of the operating table. This maintained the head in a rigid position throughout the recording of the contractions of the nictitating membrane. The animal was then prepared as previously described. By means of a fine needle, a linen thread was passed through the midpoint of the margin of the nictitating membrane of the right eye and tied firmly into place. The thread was then pulled forward and to one side making an angle of about 30° with the long axis of the cat. It was led around pulleys and attached to a frontal-point writing lever and the contractions of the nictitating membrane recorded on a moving smoked surface.

The right cervical sympathetic chain was now dissected free from the combined vago-sympathetic trunk and ligated at as low a point as possible in the neck. The cervical sympathetic chain was severed just above the ligature, placed on a pair of platinum electrodes and kept moist with normal saline solution. Contractions of the nictitating membrane could now be

elicited by stimulation of the cervical sympathetic nerve for short fixed periods of time (15 seconds each minute), at a frequency of 800 to 1200/min, at 8 to 15 V, the pulse width being 0.5 msec to 1.0 msec. A Dobbie-McInnes square wave stimulator was used. These values remained constant during any one experiment. Having obtained standard, reproducible responses from the nictitating membrane by stimulating the nerve trunk, the drugs under investigation were injected into the external jugular vein, one minute prior to the next period of stimulation.

Stimulation of the cervical sympathetic nerve chain reaches the nictitating membrane across the synapses of ganglion cells in the superior cervical ganglion. The nictitating membrane has no vagal (parasympathetic) innervation and can be used for the detection and estimation of sympathetic ganglion block. Therefore, if the drug under test possessed sympathetic ganglion blocking activity, a reduction in the height or elimination of the contraction of the nictitating membrane would be expected.

<u>To record the effect of drugs on the respiration of the anaesthetized cat and rabbit</u>.

Respiratory movements were recorded in the cat

by means of a thread sewn into the skin of the epigastrium at a point along the midline. This thread was then led over pulleys and attached to a frontal-point writing lever. The common carotid artery on one side was used to record the blood pressure of the animal. After a record of the normal respiratory movements had been obtained, the drug under test, dissolved in normal saline solution, was infused (0.75 ml/min) into an external jugular or a femoral vein, by means of a needle cannula attached to a Palmer's slow infusion apparatus.

In order to establish a more accurate picture of the effects of neuromuscular blocking agents on the volume of air exchanged by the lungs, rather than on movements of the respiratory musculature, experiments were carried out using the method devised by Gaddum (1941). To effect a correlation between the respiratory and the neuromuscular paralyzing potency of new drugs, the rabbit was chosen as the test species. Thus the head drop dose could be compared to the dose producing respiratory paralysis. Rabbits, weighing between 1.5 and 4.5 kg, were anaesthetized by administration of urethane solution 25 g/100 ml (approximately 4.5 mg/kg) into a marginal ear vein. Cannulation of the trachea,

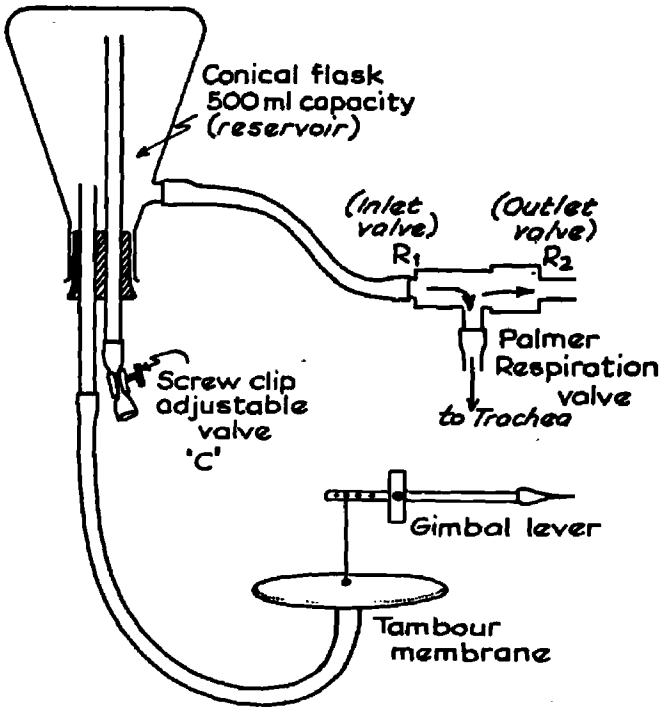


Fig. 2. Diagram of the apparatus used to record respiration, modified from that of Gaddum (1941).

Approximate scale 1 : 5

an external jugular vein on one side and the common carotid artery, on the other side of the neck from the cannulated vein, was then completed.

Experiments on the Respiration of the Anaesthetized Rabbit using the method of Gaddum.

The central limb of a Palmer's 'T' shaped respiratory valve was connected by means of rubber tubing to the trachea cannula. The valve was then connected to a conical flask, acting as a reservoir, and leading to a tambour which recorded the respiratory movements of the animal. The apparatus used is shown in the diagram (Fig. 2). The air pressure in the reservoir is adjusted by the screw-clip valve (C) to maintain the recording lever in a horizontal position.

When the rabbit inspired, air was drawn from the reservoir faster than it entered through C and the consequent drop in pressure in the system was recorded by a depression in the tambour membrane and a rise in the lever. During expiration, the valve R_1 closed, air entered the reservoir at C and normal pressure conditions were restored. The lever fell to its normal resting horizontal position. The expired air from the animal escaped by the outlet valve R_2. Alterations in

the rate and depth of respiration of the animal could then be translated into changes in the frequency and extent of the lever movements. A recording of normal respiration was obtained for several minutes prior to administration of the drug into the external jugular vein. The infusion was carried out at a constant rate of 0.75 ml/min, using a Palmer's slow infusion apparatus. Drugs (0.2 mg/ml)dissolved in normal saline solution were administered via the external jugular vein and,when spontaneous respiration had ceased, infusion of the drug was stopped and the animal placed on artificial respiration. The rate and time taken for infusion of the drug were used to calculate the respiratory paralyzing dose of the drug which was expressed in mg/kg.

Experiments using Avian Species.

Non-depolarizing neuromuscular blocking agents produce a flaccid paralysis in birds whereas depolarizing agents cause a typical spastic paralysis (Paton & Zaimis, 1952; Zaimis, 1954). These effects may be readily demonstrated and the phenomenon can be adopted as a means of distinguishing between these two classes of compounds. Neuromuscular blocking agents of the

intermediate type can be readily distinguished because they produce a response embracing characteristics of both the other types. Depending on the nature of the compound used, an initial spastic paralysis can be shown to change to a flaccid paralysis in the course of the animal's response to the injected drugs.

Work on avian species has been extended to include nerve-muscle preparations (Ginzel et al. 1951a, b,c; Pelikan, Smith & Unna, 1954; Thesleff & Unna, 1954; Crema, Scognamiglio & Bovet, 1959). These studies have primarily involved the use of the gastrocnemius muscle-sciatic nerve preparation of the chicken and have indicated that while non-depolarizing muscle relaxants cause only neuromuscular paralysis, depolarizing activity involves, in addition, a contracture i.e. a shortening of the muscle, independent of the degree of electrical stimulation applied. In the latter case both the shortening effect and the neuromuscular paralysis commenced simultaneously, but the maximum contractural effect is attained before the full depression of the twitch height is observed (Crema et al. 1959).

Experiments on Chicks.

Four to eight day old chicks were used in these experiments. Drugs dissolved in normal saline solution

were injected intraperitoneally using groups of six chicks for each compound and observations made of the nature and mode of onset of the paralysis induced.

The Chicken Gastrocnemius Muscle-Sciatic Nerve Preparation.

The method described was essentially that used by Pelikan and his associates (Pelikan et al. 1954). Leghorn chickens of either sex weighing between 1.0 and 3.0 kg were anaesthetized by slow intravenous injection of sodium pentobarbitone solution (25-35 mg/kg) into a wing vein. In order to maintain anaesthesia, it was found necessary to inject sodium pentobarbitone solution frequently throughout the experiment (5-10 mg/kg at approximately 20 minute intervals). The sodium pentobarbitone solution was diluted with an equal volume of normal saline to facilitate the control of anaesthesia.

The trachea and the external jugular vein on one side were cannulated as previously described for experiments on the cat gastrocnemius muscle-sciatic nerve preparation. Likewise, the tendon of the gastrocnemius muscle of one leg was carefully isolated, cut and attached over pulleys to the lever of a Brown-Schuster myograph by means of a strong linen thread sewn through the cut end. The feathers and skin

covering the lateral aspect of the thigh were removed and the sciatic nerve exposed. Alternatively, access to the sciatic nerve could be gained by blunt dissection of the hamstring muscles on the dorsal aspect of the leg. The distal end of the severed sciatic nerve was then stimulated supramaximally using a Dobbie-McInnes square wave stimulator at a frequency of 6/min, at 12-25 V, the pulse width being 6 msec. The contractions so elicited were recorded on a smoked surface. Drugs under investigation were added via the external jugular vein followed by a fixed volume (3 ml) of normal saline solution.

The Rabbit Head Drop Method.

The Estimation of The Head Drop Dose (H.D.D.).

The basis of this method was outlined by Bennett (1941). The modification described by Varney, Linegar and Holaday (1949) was used. This method of assay is best suited to compounds having a fairly rapid onset of action and a duration of effect comparable to that of tubocurarine.

Rabbits of either sex, weighing from 1.5 to 3.0 kg were used for these experiments. Groups consisting of nine animals were used for each drug under

Fig. 3a. Rabbit 'Bleeding' Box.

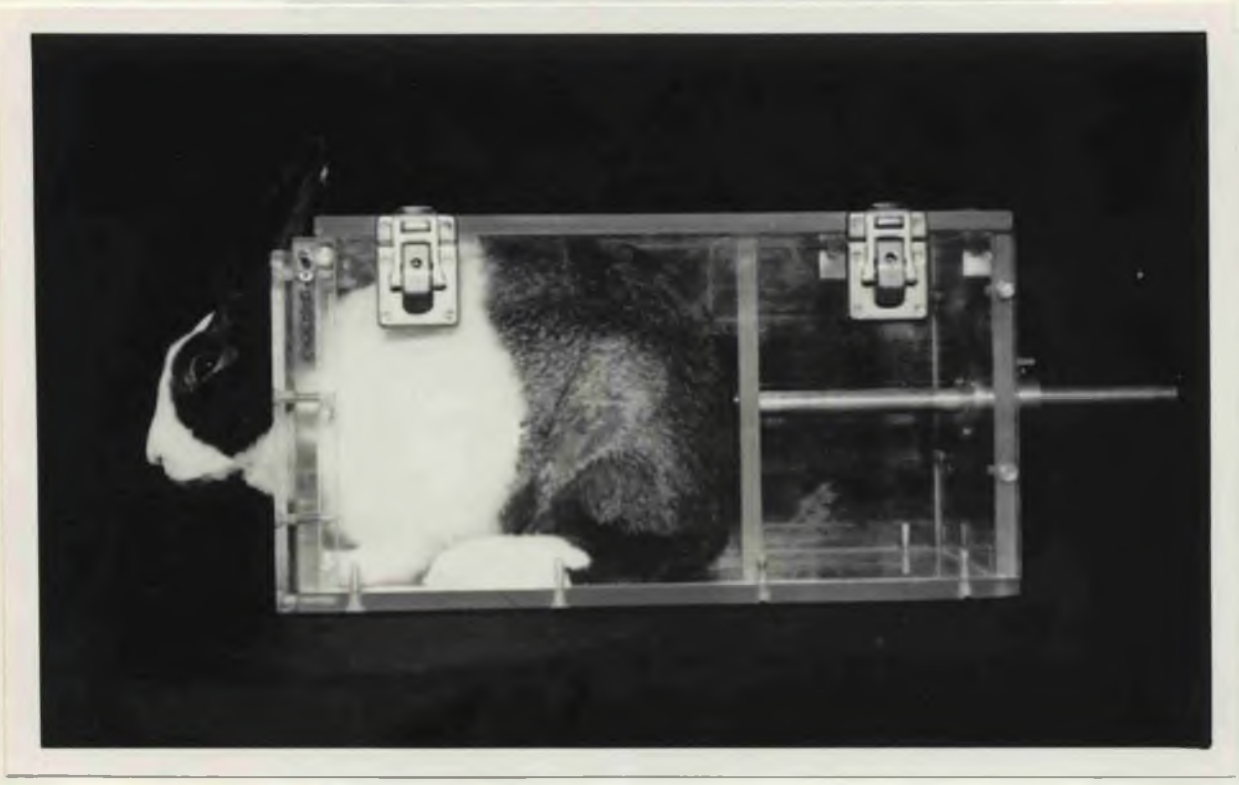


Fig. 3b. Rabbit 'Bleeding' Box with animal prior to commencement of an experiment.

investigation to permit statistical analysis of the results. The rabbits were placed in individual bleeding boxes preferably constructed of perspex, with the head of the animal protruding through the opening at the front (Fig. 3). Drugs under investigation were diluted in normal saline to give a final concentration of 0.01 mg/ml and were administered through a marginal ear vein by means of a Palmer's slow infusion apparatus. This enabled drugs to be given at a constant rate (0.75 ml/min) from an all glass syringe fixed in the apparatus. The animal usually remained quiet until just prior to the end-point of the experiment, when there was a brief period of restlessness. Intravenous infusion was discontinued only when the muscles of the neck were fully relaxed and toneless and a light tap on the muzzle produced no raising of the animal's head. At this point, the reading on the timing device of the slow injection apparatus was noted and the difference between this and the original reading used to calculate the quantity of drug administered. When expressed in mg/ml of body weight this indicated the head drop dose (H.D.D.).

The experiment could also be adapted to

indicate the type of muscle relaxant activity involved. Consequently, the effect of neostigmine (0.1 mg/kg), injected subcutaneously into the rabbit 15 minutes prior to commencement of the experiment, was investigated. The ratio of the head drop dose for the neostigmine-treated animals, to the head drop dose for the untreated animals, was determined. A ratio greater than unity indicated a tubocurarine-like paralysis, while a ratio less than unity was taken to indicate a depolarizing effect.

Experiments On Mice.

Estimation of the Approximate Median Paralysing Dose (P.D.50) and the Approximate Median Lethal Dose (L.D.50).

The method described is based upon that originally used by Thomson (1946) for the assay of insulin. Solutions of drugs, in normal saline, were intraperitoneally injected at different dose levels into groups of ten mice of either sex weighing between 20 and 30 g. The mice were then placed on a fine-mesh wire screen inclined at an angle of 50^{o} to the horizontal. Those mice which developed a typical skeletal muscle paralysis and abruptly slid off the screen within a period of thirty minutes after injection of the drug, were considered to show a positive reaction. The dose at

which 5 out of 10 injected mice slid off the screen, was considered to be the approximate median paralysing dose (P.D.50) and was expressed in mg/kg of body weight. In order to determine the approximate median lethal dose (L.D.50), the dose at which five out of ten mice, in a particular group, died within half an hour, was taken and expressed in terms of mg/kg of the body weight.

Experiments on The Isolated Perfused Rabbit Heart.

The experiment was carried out according to the method of Langendorff (1895) and involved a perfusion of the coronary vessels of the heart through the aorta. The recorded outflow of the heart is not necessarily an accurate assessment of the state of tonus of the coronary vessels, since the former is directly dependant upon the competence of the aortic valves. As a result of aortic incompetence, perfusion fluid may leak into the left ventricle and so to the left atrium and thence to the exterior. Moreover, the volume of coronary perfusate may be increased by a purely mechanical massaging effect which cardiac muscle, stimulated by a cardiotonic drug, may exert upon the coronary vessels. In consequence, an increase in the recorded outflow may falsely indicate a coronary dilatation (Wegria, 1951).

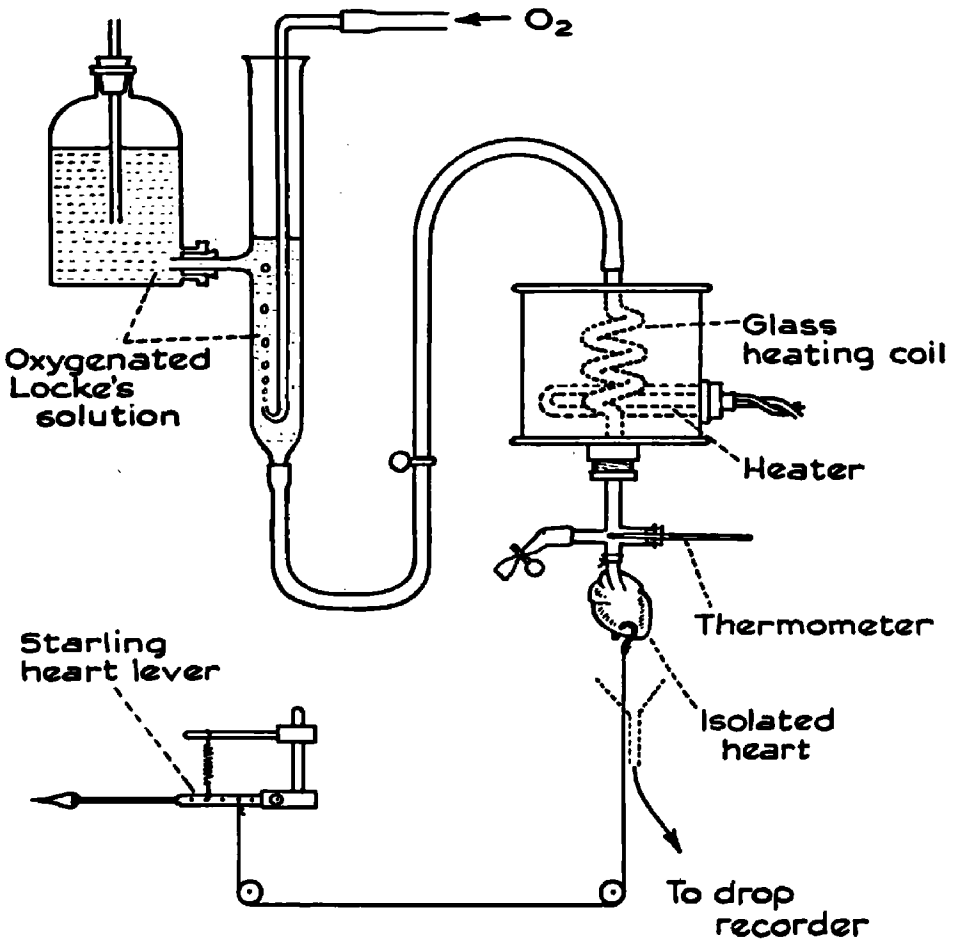


Fig. 4. Diagram of the apparatus used to record the effects of drugs on the isolated rabbit heart. Modified from Langendorff (1895).
Approximate scale 1 : 5

The fluid draining from the heart is thus described as the 'cardiac outflow'. By careful observation of the heart rate, the amplitude of the contractions and the cardiac outflow, an estimate of drug-induced alterations in the cardiac function and in the tonus of the coronary vessels could be obtained.

Rabbits, weighing between 1.0 and 2.0 kg, were killed and exsanguinated. The thoracic cavity was exposed, the lungs removed and a thread tied loosely around the aortic arch near to the origin of the innominate artery. The venae cavae and the aorta were then severed, the pericardium removed and the heart dissected out and placed in warm heparinised Locke's solution to prevent clotting. A stream of Locke's solution was then run through the superior vena cava from a pipette and prior to the insertion of a cannula into the aorta, the heart was gently massaged. Care was taken to ensure that the tip of the cannula was distal to the ostia of the coronary artery.

The preparation was then set up by connecting the cannula to the perfusion apparatus as shown (Fig. 4). Perfusion of the heart with warm (37°C), oxygenated Locke's solution containing double the normal concentration of glucose, to prolong the activity of

the preparation, was commenced at a constant rate and at a pressure of approximately 35mm of mercury, measured by means of a manometer attached to the screw clip but not shown in the sketch, care being taken that no air bubbles entered the aorta. The temperature drop between the thermostatically controlled water bath and the cannula did not exceed 0.2°C. When the heart beat had become regular, which was usually after about fifteen minutes, a supporting thread was tied by means of a fine needle through the tip of the left ventricle. A bent entomological pin inserted into the wall of the right ventricle and connected to a Starling heart lever recorded the contractions of the heart on a smoked drum surface.

Drugs, dissolved in Locke's solution, were injected into the rubber tubing attached to the aortic cannula by a 1 ml graduated tuberculin syringe. The heart rate was counted by inspection and the cardiac outflow recorded by a Thorp Drop-recorder.

Experiments Carried Out Using The Isolated Guinea Pig Ileum.

Inhibition of the Peristaltic Reflex.

Three types of muscular movement are shown by mammalian small intestine:

(a) Pendular motion, the frequency of which is dependent on the size of the animal.

(b) Changes in tone, which are superimposed on the first type of movement.

(c) Peristalsis.

The peristaltic reflex is elicited by an increase in intraluminal pressure from within the intestine and has the specific function of propelling the intestinal contents along the gut. It consists of two phases. Firstly, there is a preparatory phase which is associated with contractions of the longitudinal muscle of the intestine. These contractions are an inherent property of the muscle fibres themselves and remain unaffected by drugs interfering with nervous structures in the intestinal wall. The second phase is an emptying phase, in which contractions of the circular muscle of the intestine take place and this is controlled by the autonomic nervous system, and is susceptible to drugs (Trendelenberg, 1917; Feldberg & Lin, 1949).

The appearance of the peristaltic reflex in response to an increase in intraluminal pressure is due to stimulation of pressure-sensitive receptors in the

intestinal mucosa. The nervous mechanism is controlled from within the wall of the intestine itself and consequently the cutting of extrinsic nerve fibres does not influence the reflex. It is, however, affected by any interference with the sensory nerve fibres in the mucosa.

As the ganglia involved in the peristaltic reflex are probably parasympathetic in origin (Feldberg & Lin, 1949), inhibition of the reflex can be used to estimate the potency of drugs inducing parasympathetic ganglion blockade. The most commonly employed method of studying peristalsis is that of Trendelenburg (1917), who chose isolated guinea pig intestine on account of its having smaller pendular movements and less variability in tone, than that of other mammalian species. Guinea pigs of either sex weighing between 0.3 and 0.5 kg were killed and exsanguinated and a piece of ileum (approximately 7-10 cm) removed from the region proximal to the ileocaecal junction. A piece of thread was tied around the oral end of the segment and the thread attached to a writing lever. The caudal end was connected to a glass 'U' tube connected via a valve to a Mariotte bottle containing Tyrode solution which

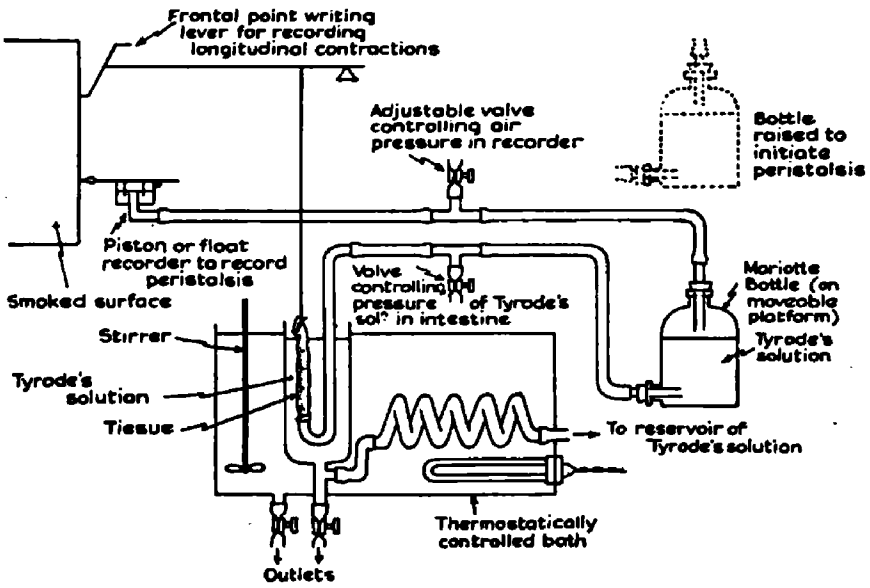


Fig. 5. Diagram of the apparatus used to record the longitudinal and peristaltic movements of the isolated guinea pig ileum. Modified from Trendelenburg (1917).

was placed on a moveable platform (Fig. 5). The bottle could be raised or lowered, as required, to subject the intestine to a regulated, intraluminal hydrostatic pressure. The level of the liquid in the bottle was similar to that in the organ bath which contained the intestine. All air bubbles were removed from the system and the neck of the bottle connected by rubber and glass tubing to a float volume recorder which was connected to a frontal-point lever writing on a smoked surface. The pendular movements of the intestine were recorded, at the same time, by means of a lever attached to the oral end of the preparation. The temperature was maintained thermostatically at 30°C throughout each experiment. In order to prevent fatigue of the preparation, peristalsis was induced for only a short period (15 sec) each minute, a three minute time cycle being used. Drugs under test were added thirty seconds prior to the inducement of peristalsis and the effects on the peristaltic reflex and on the longitudinal contractions were recorded.

Experiments Using The Isolated Frog Rectus Abdominis Muscle.

These experiments were based largely on the method of Chang & Gaddum (1933) modified for muscle

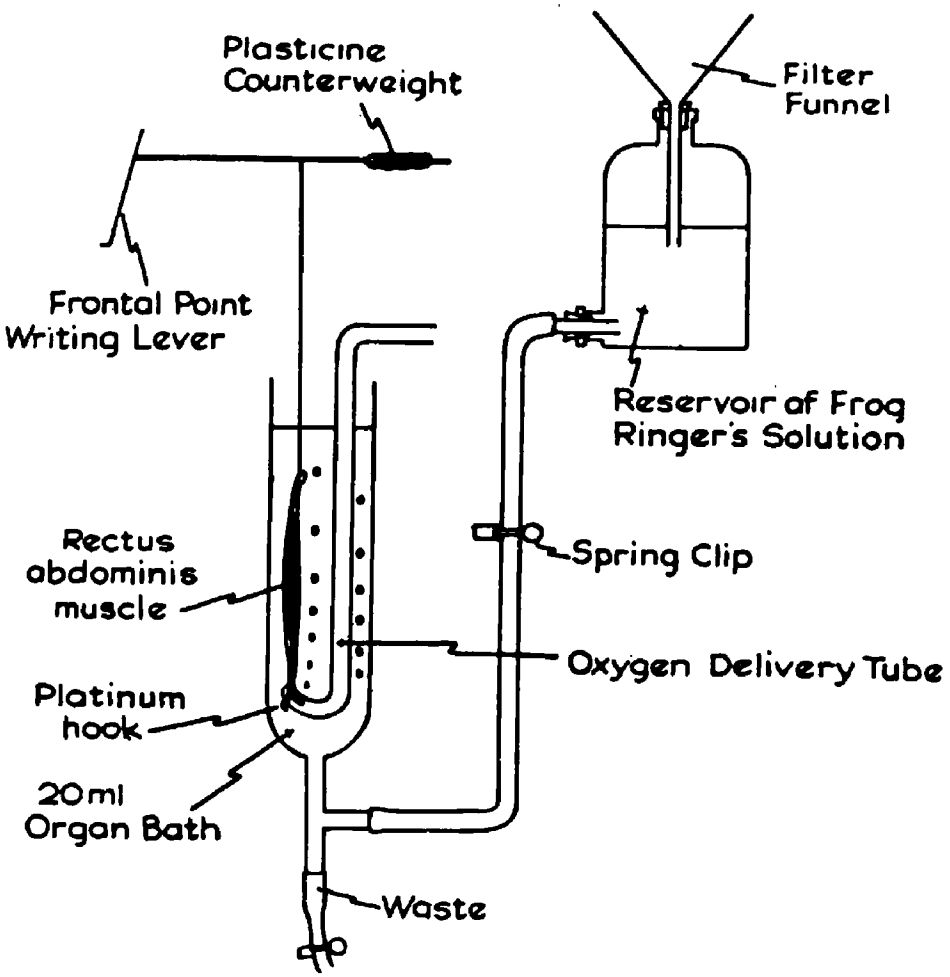


Fig. 6. Diagram of the apparatus used to record the effect of drugs on the isolated frog rectus abdominis muscle.

relaxant drugs by Garcia de Jalon (1947). An adult frog was killed, decapitated and pithed. The skin of the abdomen was removed, the rectus muscle exposed and dissected from its insertion in the pelvic girdle to the point of insertion into the cartilage of the pectoral girdle. One half of this muscle, obtained by longitudinal dissection at the midline, was used in each experiment. A loop of thread was sewn through the xiphisternum and a longer thread tied around the pubic end. The muscle was then fixed, by means of the loop, to the base of a 10 ml organ bath filled with oxygenated Ringer's solution at room temperature. The long thread was then attached to a modified frontal-point writing lever. The apparatus used for these experiments is shown in Fig. 6.

Drugs under investigation, dissolved in normal saline solution, were added to the bath by means of a 1 ml graduated tuberculin syringe. These drugs were first examined for their ability to induce a contracture of the rectus muscle. Accordingly, non-contracture inducing drugs (non-depolarizing) were quantitatively compared with tubocurarine as inhibitors of acetylcholine, while contracture-inducing synthetic compounds were

quantitatively compared with decamethonium. Conditions, once established, remained constant throughout each experiment.

In all experiments, at least two uniform submaximal contractions to the same dose of either acetylcholine or decamethonium were obtained, prior to the investigation of other drugs. A suitable time interval between each dose of acetylcholine was found to be approximately four or five minutes and the resulting contractions were recorded for periods of thirty to ninety seconds. In the case of decamethonium, sufficient time, not generally exceeding twenty minutes, was allowed for the muscle to regain its original length after the contraction. The recording of the contractions themselves took approximately two to three minutes. Acetylcholine-inhibiting drugs were added from thirty to sixty seconds prior to the addition of acetylcholine. The muscle was washed a fixed number of times with fresh frog Ringer's solution at the completion of each time cycle. Several additions of acetylcholine or decamethonium were needed for complete recovery, the length of time taken depending on both the nature and the quantity of the drug added to the bath. The concentrations

of agonists used to induce contractions of the muscle were of the order of 1.0 to 10.0 µg/ml of decamethonium.

Experiments Using Frog Rectus Abdominis Muscle Based On The Technique Of Ariëns And His Associates. (Van Rossum & Ariens, 1959a).

These experiments were performed using the isolated rectus abdominis muscle of the frog Rana temporaria. The dissection of the muscle and the setting up of the preparation were similar to those previously described. The contractures, induced by the action of agonistic drugs, were recorded on a smoked drum surface. In all cases the agonist chosen was decamethonium iodide (2 µmoles/ml) and cumulative dose-response curves were obtained by the stepwise addition of this drug in the following sequence a;a;2a;4a;8a; and so on (a = 0,01 ml added by an automatic-constriction pipette). Each dose of drug was left in contact with the muscle for 15 minutes to permit equilibration, then the concentration of drug was doubled by adding the next dose according to the sequence given above. When the maximum effect had been recorded, the muscle was washed thoroughly for one or one and one-half hours which was the time necessary for

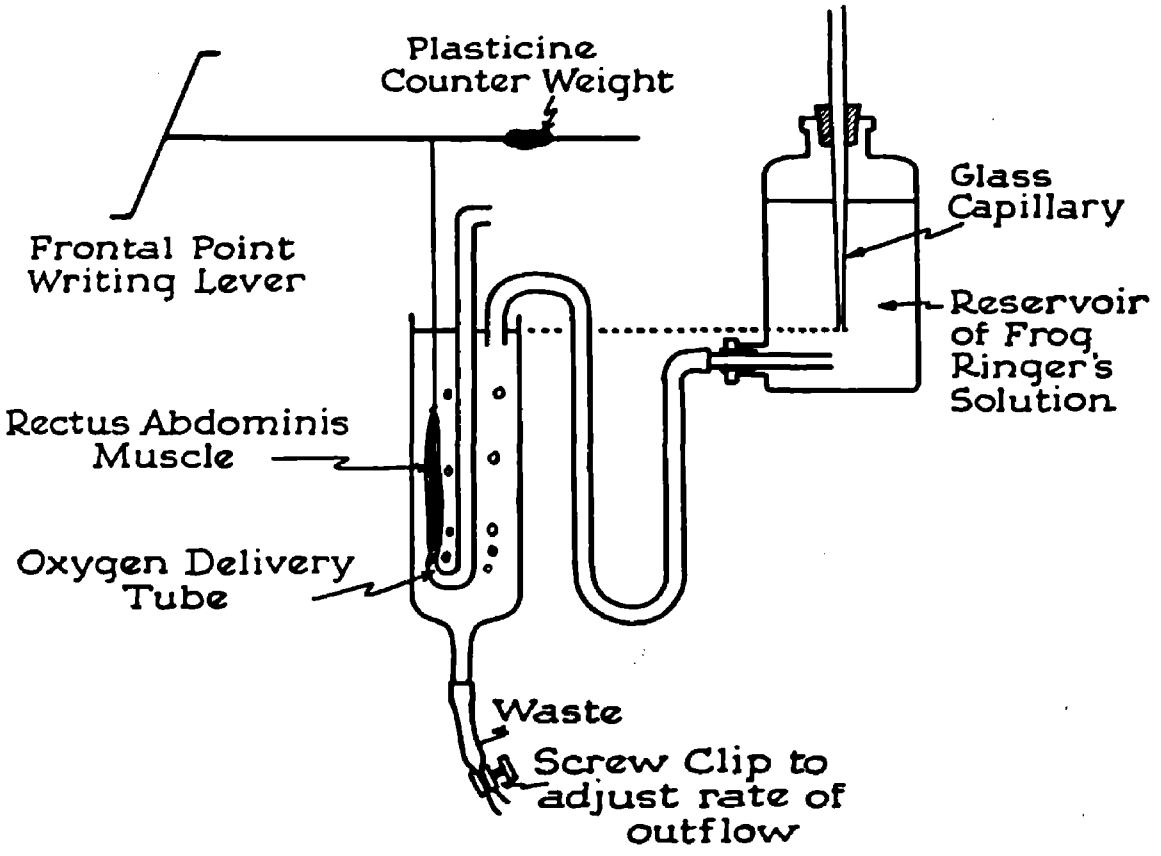


Fig. 7. Diagram of the apparatus used for the continuous washing of the frog rectus abdominis muscle during experiments employing the Ariëns' procedure.

complete relaxation to be attained. This could be carried out in the normal way by continuously emptying and filling the organ bath from the reservoir bottle. Alternatively, the washing could be accomplished automatically by inserting a fine glass capillary in the reservoir, equating the level of the Ringer's solution in the reservoir with that in the bath and permitting the reservoir to empty its contents into the bath at a slow, predetermined rate (Fig. 7).

When washing was complete the investigation of other drugs affecting skeletal muscle could be commenced.

Thus the potency of those drugs which possessed a qualitatively similar agonistic action to decamethonium could be measured by noting the height of the dose response curve elicited by their addition to the bath, as described. Compounds possessing other types of muscle relaxant activity could be investigated by their addition to the bath 15 minutes prior to the addition of decamethonium and their effect on the subsequent dose response curve recorded.

Log-dose response curves were constructed by expressing the effect of each concentration of the drug,

as a percentage of the maximum effect recorded.

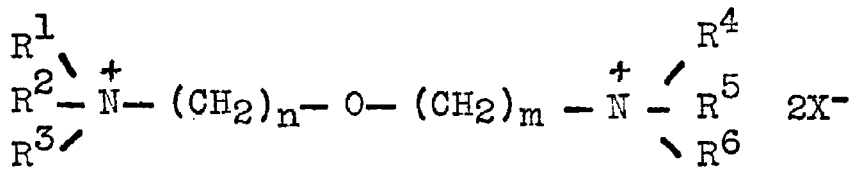
Experiments with each drug under investigation were repeated ten or twenty times and the results analyzed statistically. Experimental and theoretical dose response curves differ in slope but Ariëns has clearly stated that this difference in no way invalidates the use of the experimentally derived curves to compare the affinity and intrinsic activity values of the compounds investigated (Van Rossum & Ariëns, 1959a; Ariëns & Simonis, 1960).

CHAPTER I

Page

TABLE II (contd.)

The chemical structure and code number of compounds in Group 1c and 1d.

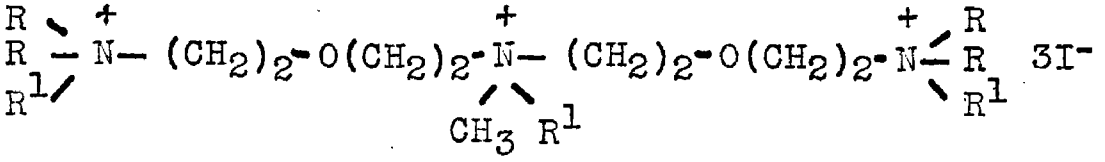


Group 1d

Code No.	Chemical Structure				
	$R^1R^2R^3$	n	m	$R^4R^5R^6$	X
F.&P.16575	$(CH_3)_3$	2	3	$(CH_3)_3$	I
F.& P.16677	$CH_3(C_2H_5)_2$	2	3	$(CH_3)_3$	I
F.& P.8302	$CH_3(C_2H_5)_2$	2	3	$CH_3(C_2H_5)_2$	I
F.& P.16678	$(C_2H_5)_3$	2	3	$(CH_3)_2C_2H_5$	Br
F.& P.8303	$(C_2H_5)_3$	2	3	$(C_2H_5)_3$	Br
F.& P.16701	$(CH_3)_3$	3	3	$(CH_3)_3$	I
F.& P.17843	$(CH_3)_2C_2H_5$	3	3	$(C_2H_5)_3$	Br
F.& P.8212	$(C_2H_5)_3$	3	3	$(C_2H_5)_3$	Br

TABLE II (contd.)

The chemical structure and code number of compounds in Group 1c and Group 1d.

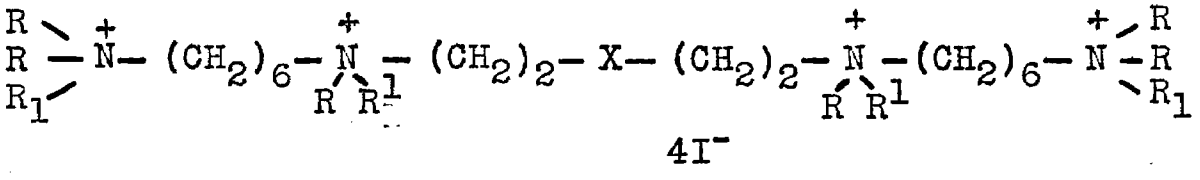


Group 1c

Code No.	R	R^1
E98	C_2H_5	C_2H_5
E99	C_2H_5	CH_3
E100	CH_3	CH_3
E101	CH_3	C_2H_5
E102	CH_3	$n\text{-}C_3H_7$
E103	C_2H_5	$n\text{-}C_3H_7$

TABLE II

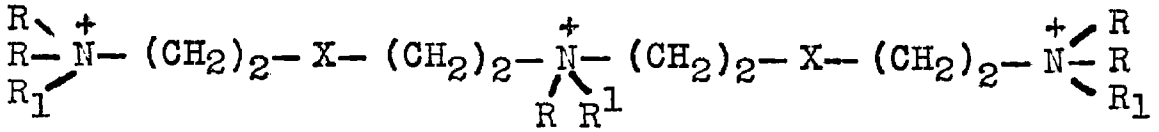
The chemical structure and code number of compounds in Group 1a and Group 1b.



$4I^-$

Group 1a

Code No.	R	R_1	X
E77	C_2H_5	C_2H_5	O
E78	C_2H_5	CH_3	O
E79	C_2H_5	C_2H_5	CH_2
E80	C_2H_5	CH_3	CH_2
E96	C_2H_5	$n\text{-}C_3H_7$	O
E97	C_2H_5	$n\text{-}C_3H_7$	CH_2



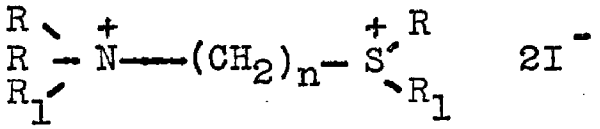
$3I^-$

Group 1b

E90	C_2H_5	C_2H_5	O
E91	C_2H_5	CH_3	O
E93	C_2H_5	CH_3	CH_2
E94	C_2H_5	C_2H_5	CH_2
E95	C_2H_5	$n\text{-}C_3H_7$	CH_2

TABLE III

The chemical structure and code number of compounds in Group 2.



Code Number	R	R_1	n
I	CH_3	C_2H_5	8
II	CH_3	CH_3	10
III	CH_3	CH_3	8

RESULTS

On the basis of their chemical and pharmacological characteristics, the compounds investigated have been divided into two main groups (1 and 2). Group 1 was further divided into 1a, 1b, 1c and 1d. The chemical structure of each compound, together with a reference number, are shown in Tables II and III. In certain cases, the scope of the pharmacological testing was limited by the amount of material available. Compounds in Group 1d were tested for neuromuscular blocking activity on the cat only; other pharmacological properties possessed by these drugs have been reported elsewhere (Fakstorp and his associates, 1954, 1955, 1956, 1957a,b,c). The results are presented under three main headings:

(a) Tests designed to investigate neuromuscular blocking activity and which use conventional techniques.

(b) Investigation of other effects including those at autonomic ganglia, on the heart and on the muscles controlling respiration.

(c) Investigation of the mode of action of certain muscle relaxants on the frog rectus abdominis muscle using the Ariëns' technique.

Experiments to investigate neuromuscular blocking potency were designed to classify the compounds according to the criteria (Paton & Zaimis, 1952) for depolarizing and non-depolarizing drugs of this type.

These criteria are as follows:

(1) Depolarizing drugs produce muscular fasiculation and briefly increase the tension of the muscle twitch prior to its depression.

(2) The ability of edrophonium and neostigmine to constantly antagonize the block produced by non-depolarizing drugs and to potentiate, or only slightly antagonize, the block produced by depolarizing agents.

(3) The reciprocal antagonism of tubocurarine and decamethonium to the block produced by depolarizing and non-depolarizing agents respectively.

(4) The ability of the muscle to maintain a tetanus during partial neuromuscular block by depolarizing drugs as confirmed by several workers including Burns and Paton (1951), Paton and Zaimis (1951). On the other hand the tension in indirectly tetanized muscle, partially blocked by non-depolarizing drugs, wanes rapidly (Paton & Zaimis, 1951; Hutter, 1952).

(5) The induction of a spastic paralysis, in avian

muscle, by depolarizing drugs as opposed to the flaccid paralysis produced by non-depolarizing muscle relaxants.

(6) The ability of depolarizing compounds to induce a contractural response on the isolated frog rectus abdominis muscle and of non-depolarizing drugs to antagonize the contracture.

(7) The ability of neostigmine to effectively increase the head drop dose of non-depolarizing drugs in the rabbit.

Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

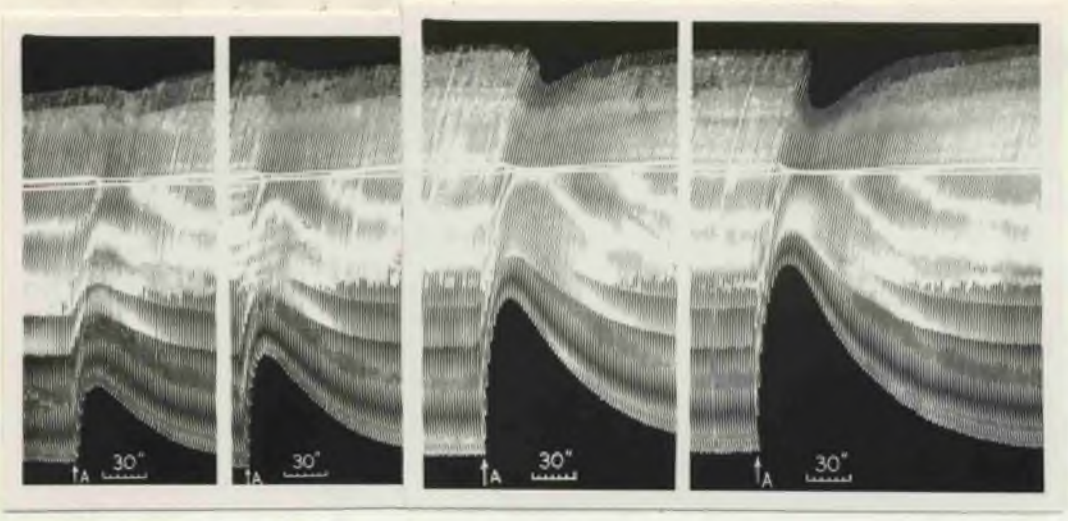
All the compounds, in the doses employed, with the exception of E91 and E100, induced complete or incomplete, reversible block in the cat gastrocnemius muscle-sciatic nerve preparation. The potency of the compounds varied considerably.

The N,N,N,N-tetra-onium compounds (Group 1a), in doses of from 0.05 to 0.10 mg/kg, produced an approximately 50% reduction in the amplitude of the muscle twitch and, with the exception of E78, all were appreciably more potent than tubocurarine. The N,N,N-tris-onium compounds (Groups 1b and 1c) possessed only weak muscle relaxant activity. In all cases,

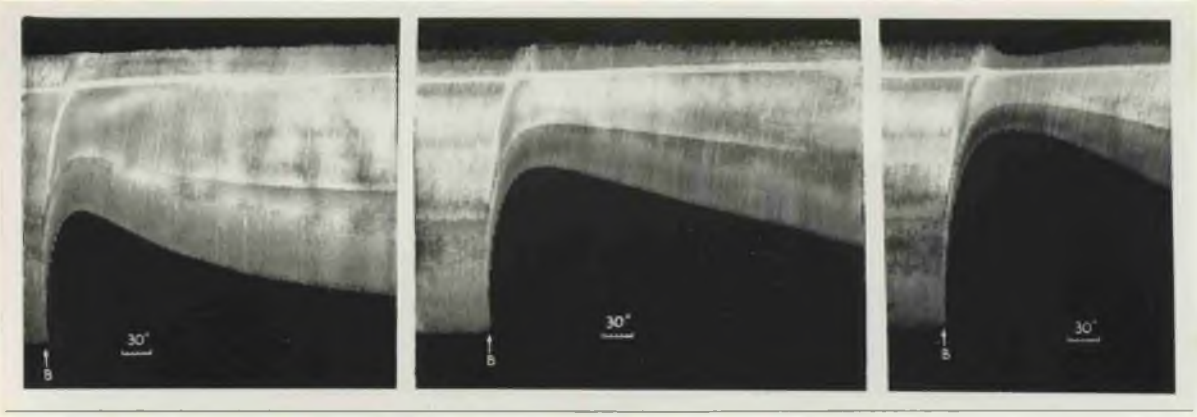
maximum activity was associated with the presence of N - ethyl substituents and, although the ether containing derivatives appeared to be less potent than the corresponding non-ether compounds, there were no qualitative differences between them. The N,N-bis-onium ethers (Group 1d) also possessed weak muscle relaxant activity comparable to that of compounds in Groups 1b and 1c.

All the compounds in Groups 1a, 1b, 1c and 1d appeared to be free from initial stimulant effects and there was no evidence of muscular twitching or fibrillation.

The duration and degree of neuromuscular block induced by all the compounds in Group 1 depended both upon the magnitude of the dose employed and the number of doses administered. Neuromuscular block was more prolonged following the second injection of the same dose than following the first. Block caused by a third and similar dose was more prolonged than that following the second. The effect of a fourth injection of a similar dose often did not significantly differ from that of the third dose. Typical examples of these effects, which were also observed using tubocurarine,



(i)



(ii)

Fig. 8. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contractions downwards. Indirect stimulation via the sciatic nerve. Drugs administered intravenously.

(i) At A, tubocurarine 0.10 mg/kg. There was an interval of 18, 24 and 26 minutes respectively between successive doses.

(ii) At B, E78 0.15 mg/kg. There was an interval of 60 and 90 minutes respectively between successive doses.

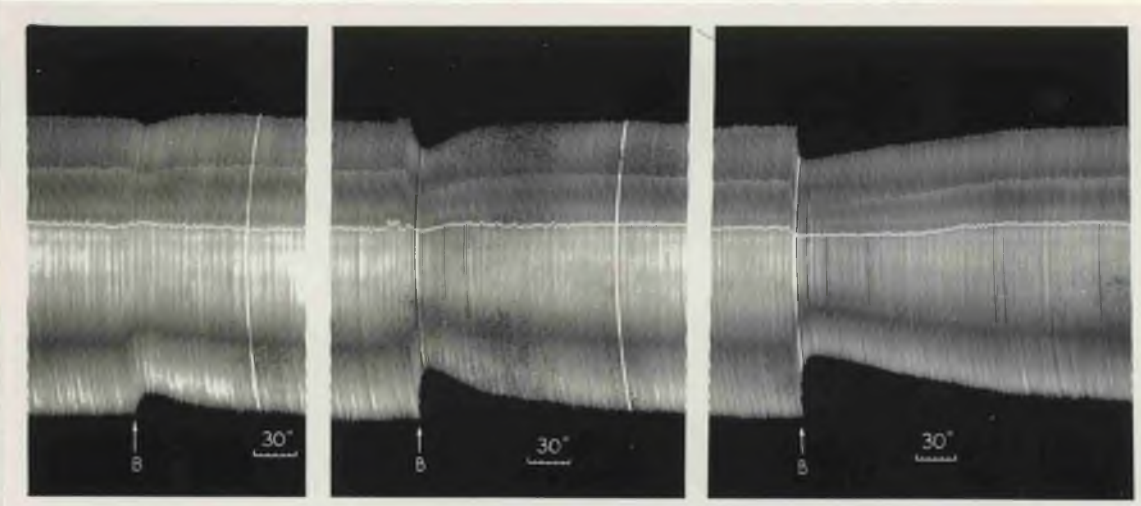


Fig. 9. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Indirect stimulation via the sciatic nerve. Drugs administered intravenously.
At B, compound F. & P.8303 10 mg/kg.
There was an interval of 23 and 35 minutes respectively between successive doses.

are shown in Figs. 8 and 9.

All the active compounds in Group 1 induced a fairly prolonged neuromuscular block and approximately 30-60 min were required for recovery after approximately 50% reduction in twitch amplitude. The onset of paralysis and the time taken for the attainment of the maximum effect of each drug in this group were rapid.

Compounds in Group 2 possessed potent muscle relaxant properties resembling decamethonium rather than tubocurarine. Compounds I (0.1 - 0.5 mg/kg), II (0.05 - 0.1 mg/kg) and III (0.1 - 0.5 mg/kg) reduced twitch amplitude while II (0.25 mg/kg) caused an initial increase in twitch height without a subsequent reduction in amplitude. Generalized, intermittent, fasiculatory movements of the skeletal musculature were produced by compounds II and III. None of the compounds in this group was more potent than decamethonium.

The compounds in Group 2 further resembled decamethonium in that the onset of block was more gradual, the attainment of the maximum effect more prolonged and the duration of effect greater than with tubocurarine and the compounds of Group 1. Thus a dose selected to produce an approximately 20% block

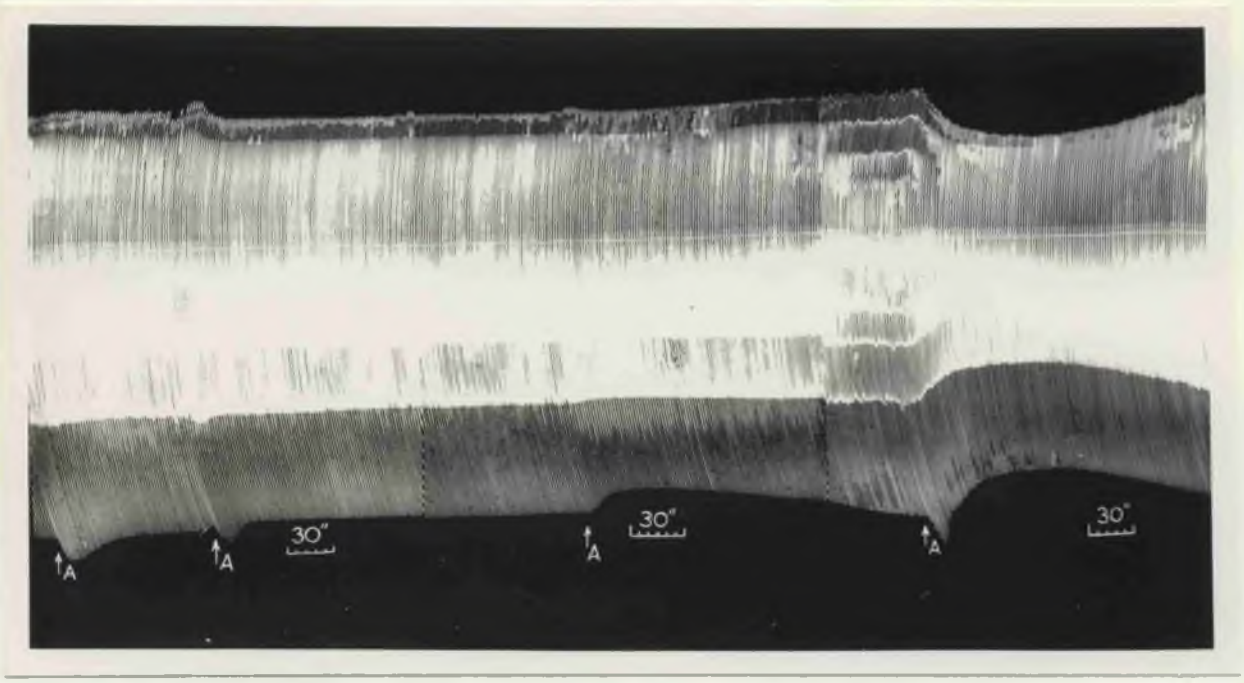


Fig. 10. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Indirect stimulation via the sciatic nerve. Drugs administered intravenously. At A, compound II 0.05 mg/kg.

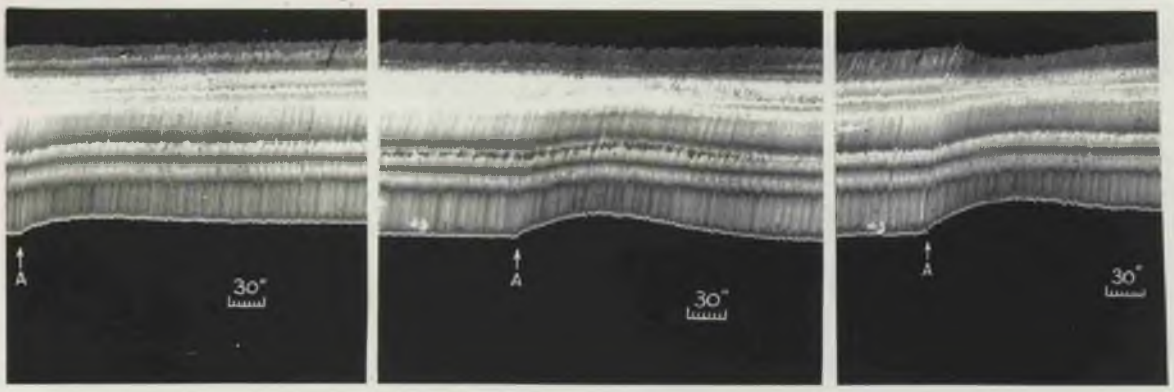


Fig. 11. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia.
Contraction downwards. Indirect stimulation via the sciatic nerve.
At A, compound III 0.10 mg/kg.
There was an interval of 38 and 45 minutes respectively between sucessive doses.

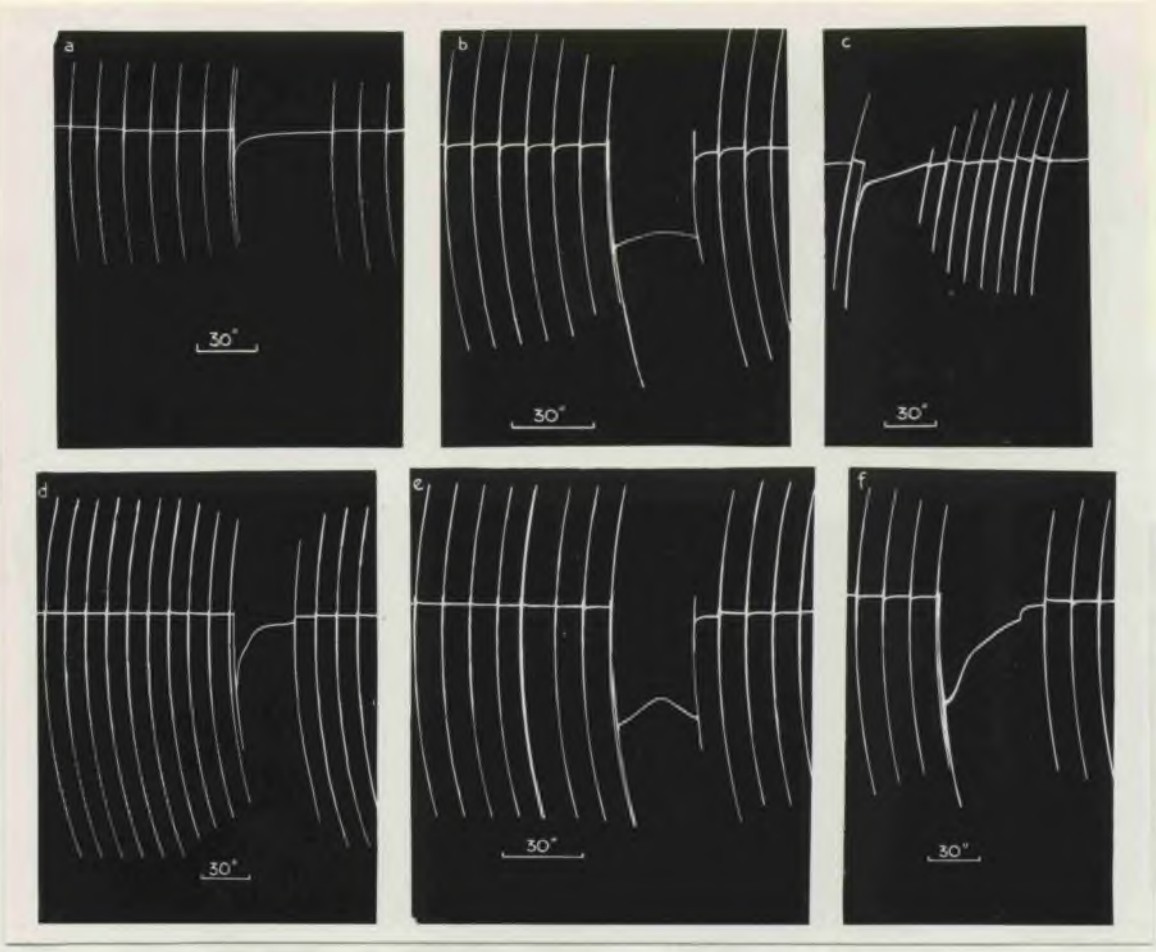


Fig. 12. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect tetanization via the sciatic nerve (1400 impulses/min) during partial block by:

a, Compound E78 0.10 mg/kg,

b, Decamethonium 0.09 mg/kg,

c, Tubocurarine 0.20 mg/kg,

d, Compound E95 0.40 mg/kg,

f, Compound E94 0.30 mg/kg,

e, untreated muscle.

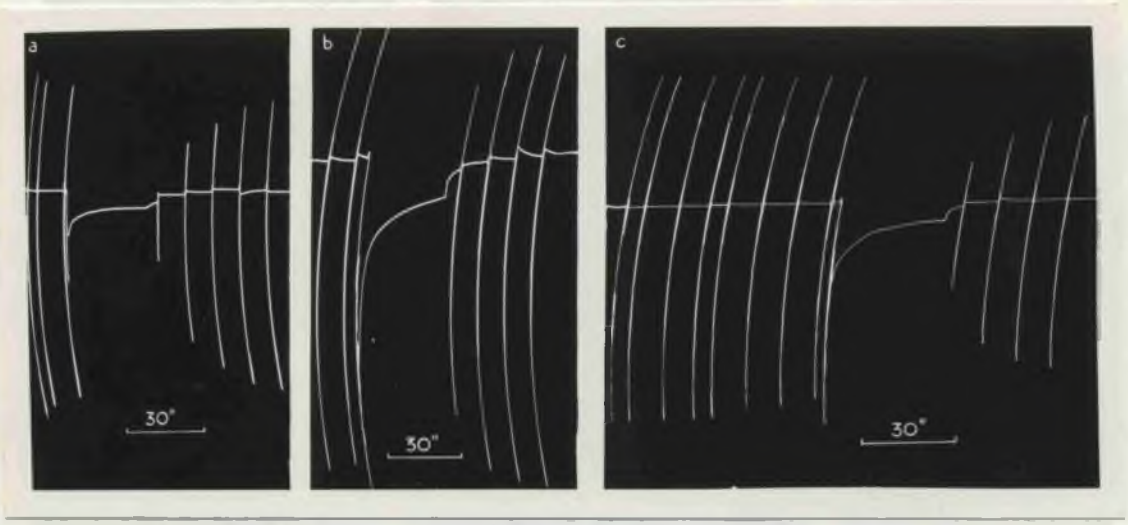


Fig. 13. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect tetanization via the sciatic nerve (1400 impulses/min) during partial block by:

a, Compound F. & P.8303 20 mg/kg,

b, Compound E98 1 mg/kg,

c, Compound E101 20 mg/kg.

required approximately 45 min for complete recovery. Following a subsequent and similar dose of the same drug, given after complete recovery, the duration of the block produced by these compounds was more prolonged and more intense. The phenomenon was less marked, however, than with the compounds in Group 1. This can be explained by the more prolonged period of recovery from the effects of drugs in Group 2 during which a considerable degree of drug elimination may have occurred. These effects are shown in Figs. 10 and 11.

Effect of Indirect Tetanization of the Partially Blocked Muscle.

The results can be divided into two distinct categories. The compounds in Group 1 behaved similarly to tubocurarine and to each other and typical effects are shown in Figs. 12 and 13. The poorly sustained responses of the partially blocked, indirectly tetanized muscle to the compounds E95 (Fig. 12d), E94 (Fig. 12f), E78 (Fig. 12a), F. & P. 8303 (Fig. 13a), E10 (Fig. 13c) and E98 (Fig. 13b) were qualitatively comparable to those produced by tubocurarine (Fig. 12c) and different from the well-sustained tension of the

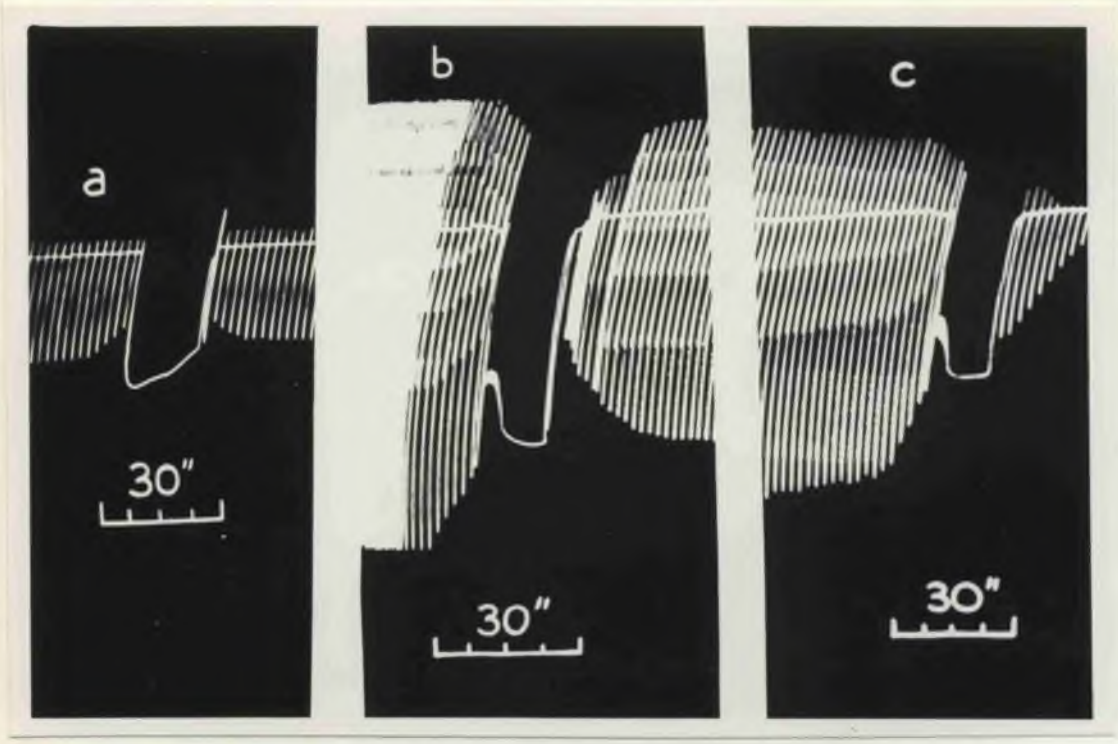


Fig. 14. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contractions downwards. Drugs administered intravenously. Indirect tetanization via the sciatic nerve (1400 impulses/min) during partial block by:

a, compound I 0.3 mg/kg,

b, compound II 0.05 mg/kg,

c, decamethonium 0.02 mg/kg.

tetanized muscle partially blocked by decamethonium (Fig. 12b) which resembled that of the untreated muscle (Fig. 12e).

Fig. 14 shows the well-sustained tension of the tetanized muscle partially blocked by compounds II and III. It is obvious that their behaviour is similar to that of decamethonium.

It is interesting to note that the responses of the muscle, partially blocked by the ether (Fig. 12a) and non-ether (Figs. 12d and 12f) containing compounds, were qualitatively similar.

The Effect of Tubocurarine and Decamethonium on the Block Produced by Compounds in Groups 1 and 2.

When muscle relaxants with similar types of action are given successively, their effects are additive. Dissimilarly acting drugs may show antagonism (Hutter & Pascoe, 1951; Riker & Wescoe, 1951; Paton & Zaimis, 1952). The importance of these phenomena, in confirming the mode of action of the compounds under study, prompted an investigation of the effects of tubocurarine and decamethonium on the block induced by the compounds in Groups 1 and 2.

Investigations were carried out using a dose of

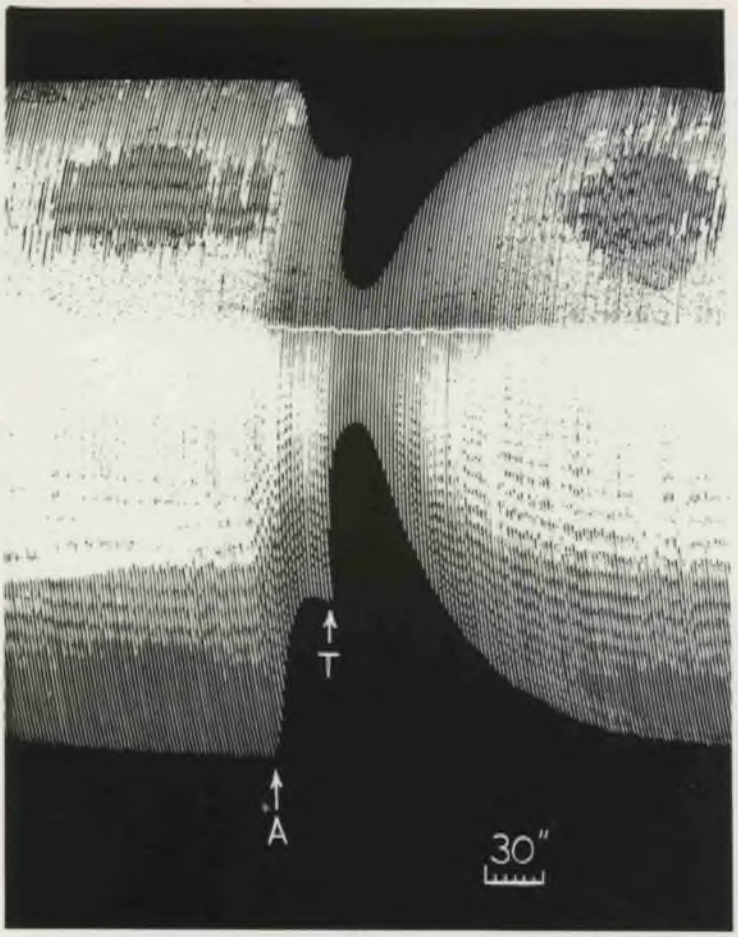


Fig. 15. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E96 0,08 mg/kg.

At T, tubocurarine 0.025 mg/kg.

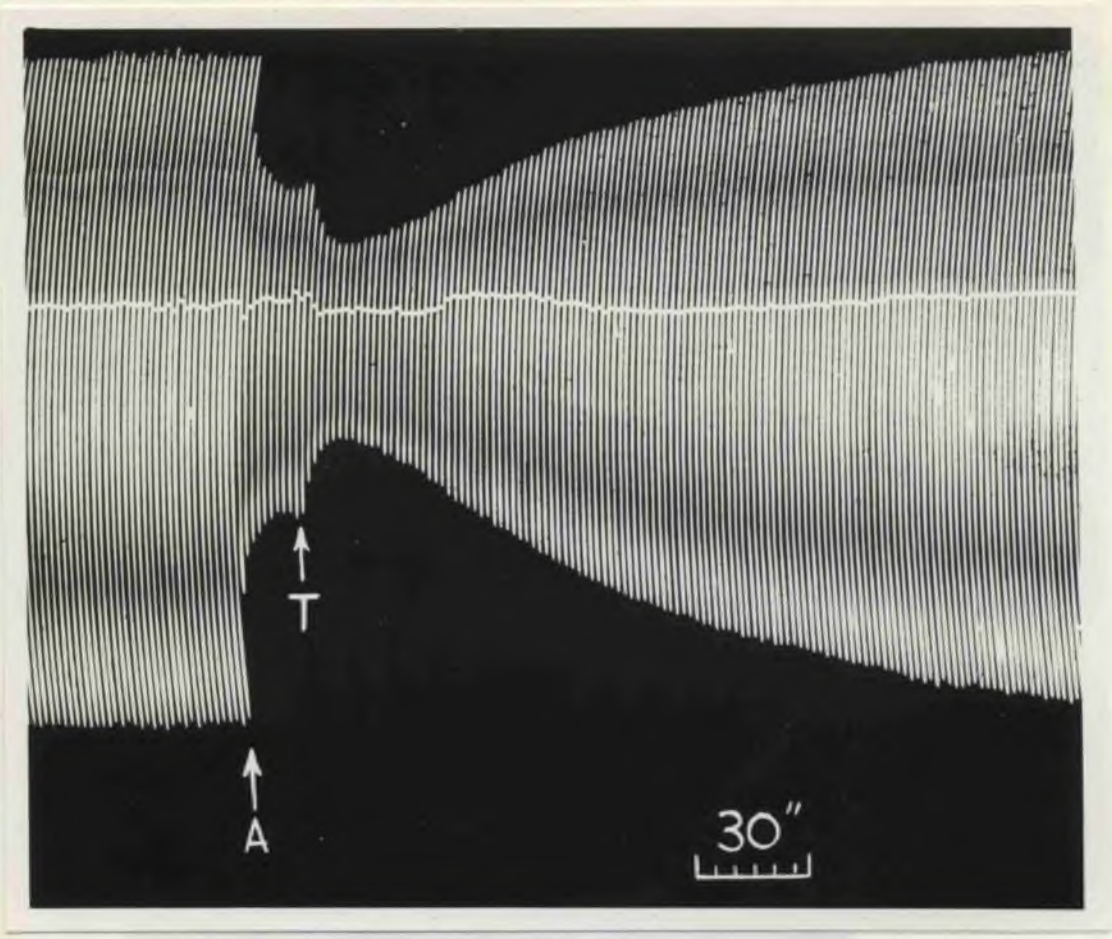


Fig. 16. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound F. & P.8303 10 mg/kg.

At T, tubocurarine 0.10 mg/kg.

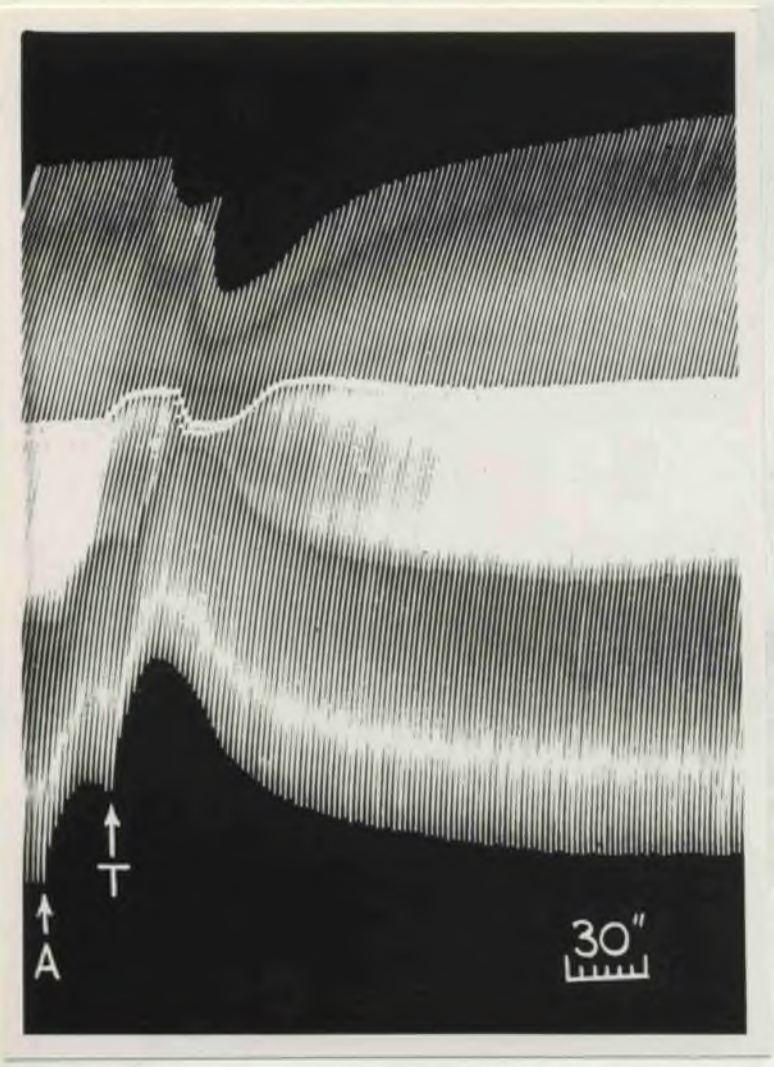


Fig. 17. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E102 7.5 mg/kg.

At T, tubocurarine 0.1 mg/kg.

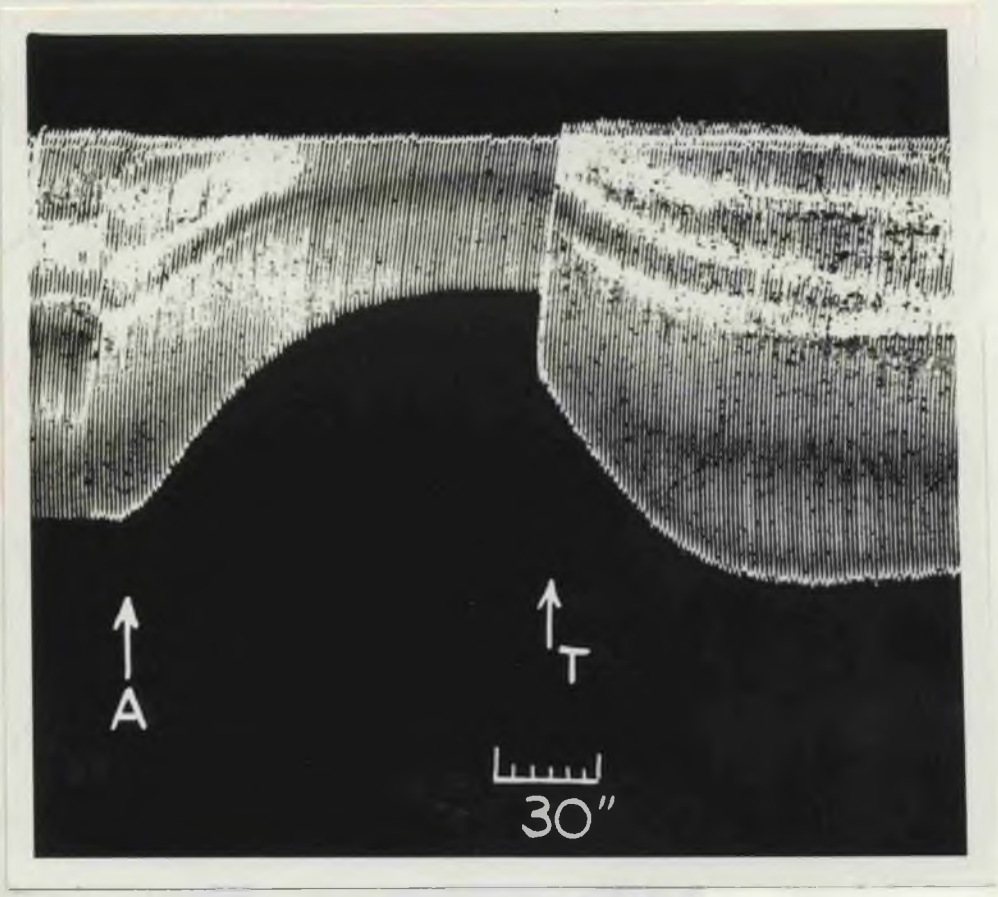


Fig. 18. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound I 0.5 mg/kg.

At T, tubocurarine 0.1 mg/kg.

a new compound and a dose of either tubocurarine or decamethonium selected to produce, when given alone, a measurable but not intense depression of the twitch height.

Tubocurarine.

When a dose of any one of the compounds in Group 1 was followed by a dose of tubocurarine given at the point of maximal depression of twitch amplitude, the intensity of the neuromuscular block was enhanced (Figs. 15, 16 and 17). Thus, in Fig. 15 the dose of E96 (0.08 mg/kg) and the dose of tubocurarine (0.025 mg/kg) were each selected to produce a neuromuscular block of about 40%. When the dose of E96 was followed by a dose of tubocurarine at the point of maximal depression, the degree of neuromuscular block was increased to approximately 80%. In this respect, both the ether and non-ether compounds in Group 1 behaved in a qualitatively identical manner to tubocurarine and to each other. On the other hand, the effect of tubocurarine on the neuromuscular blocking activity of the sulphur-containing decamethonium analogues was quite different (Fig. 18). A dose of compound I was selected to produce an incomplete but

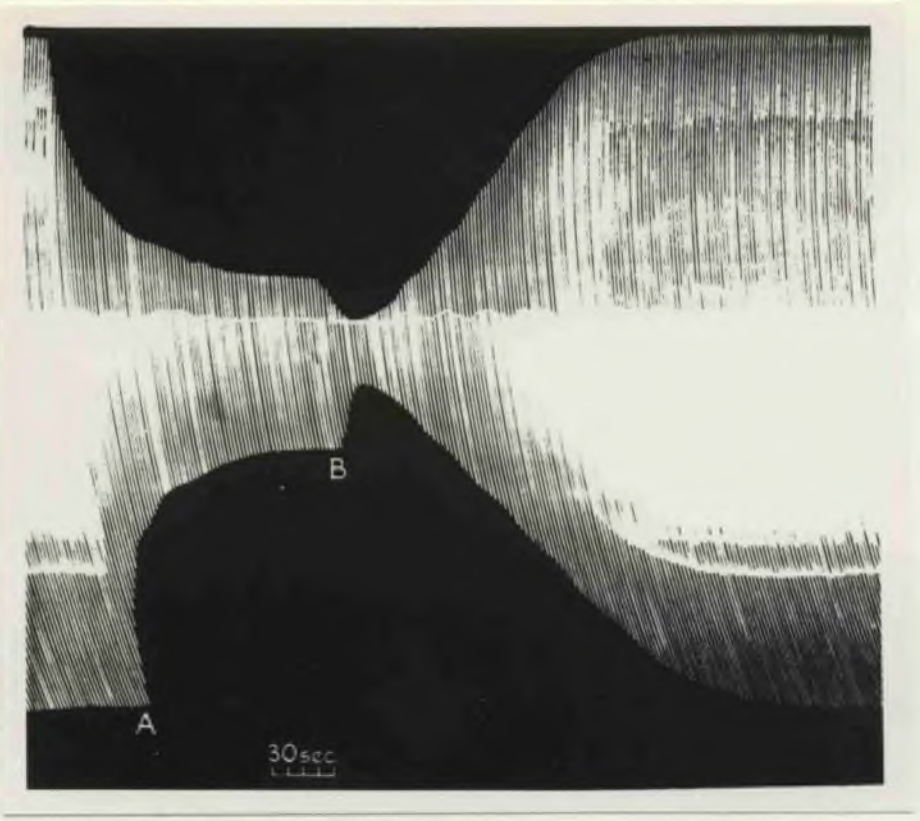


Fig. 19. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound III 0.15 mg/kg.

At B, tubocurarine 0.20 mg/kg.

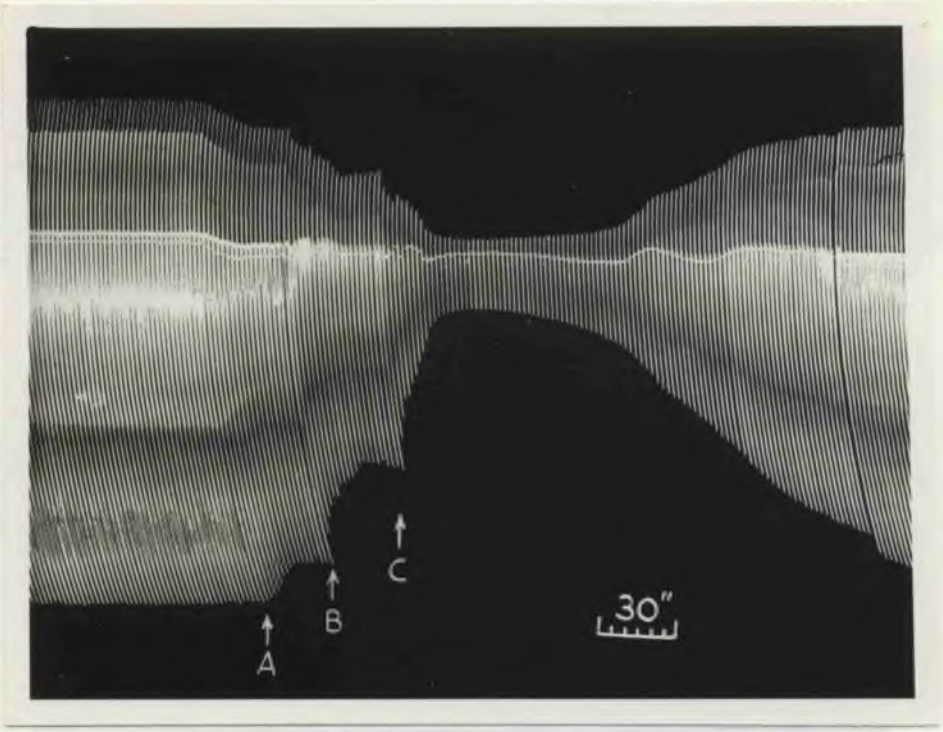


Fig. 20. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound II 0.025 mg/kg.

At B, compound III 0.100 mg/kg.

At C, compound I 0.100 mg/kg.

measurable paralysis and the injection of tubocurarine (0.10 mg/kg), at the point of maximal depression, increased the twitch height. In the case of compound III, the addition of tubocurarine to the partially blocked muscle did not produce the same antagonism as was seen with compounds I and II and a slight reduction in the amplitude of the twitch height was initially produced. This was thought to indicate the presence of mixed blocking properties in this compound but subsequent observations, on other species, did not confirm this view. Consequently, although the effect was reproducible, the enhancing action, demonstrated in Fig. 19, may have been due to the administration of a very large dose of tubocurarine. All three compounds in this group showed additive effects (Fig. 20).

Decamethonium.

The mutually antagonistic effects of depolarizing and non-depolarizing substances (Dellemagne & Philippot, 1952; Hutter & Pascoe, 1951; Paton & Zaimis, 1952) were further investigated by injecting decamethonium (0.02 - 0.05 mg/kg) at the point of maximal depression of the muscle twitch.

Fig. 21. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E103 30 mg/kg.

At B, decamethonium 0.80 mg/kg.

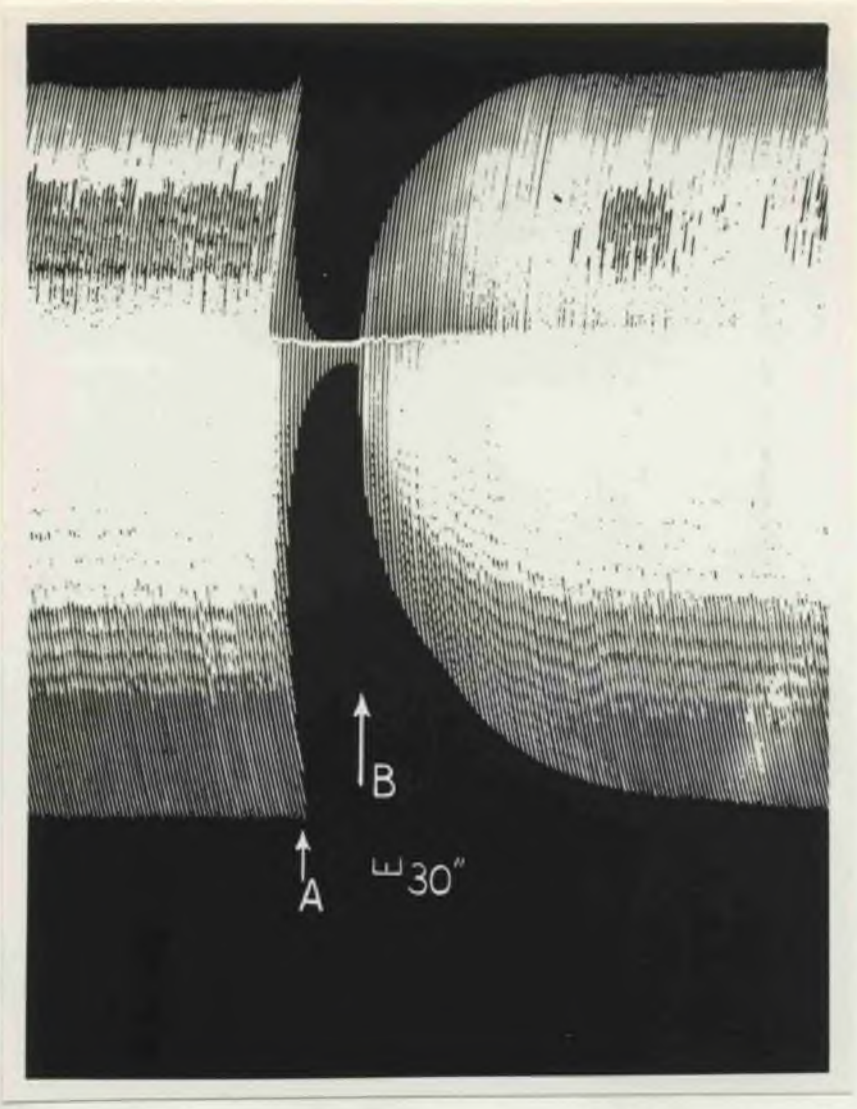


Fig. 22. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E96 0.12 mg/kg.

At B, decamethonium 0.80 mg/kg.

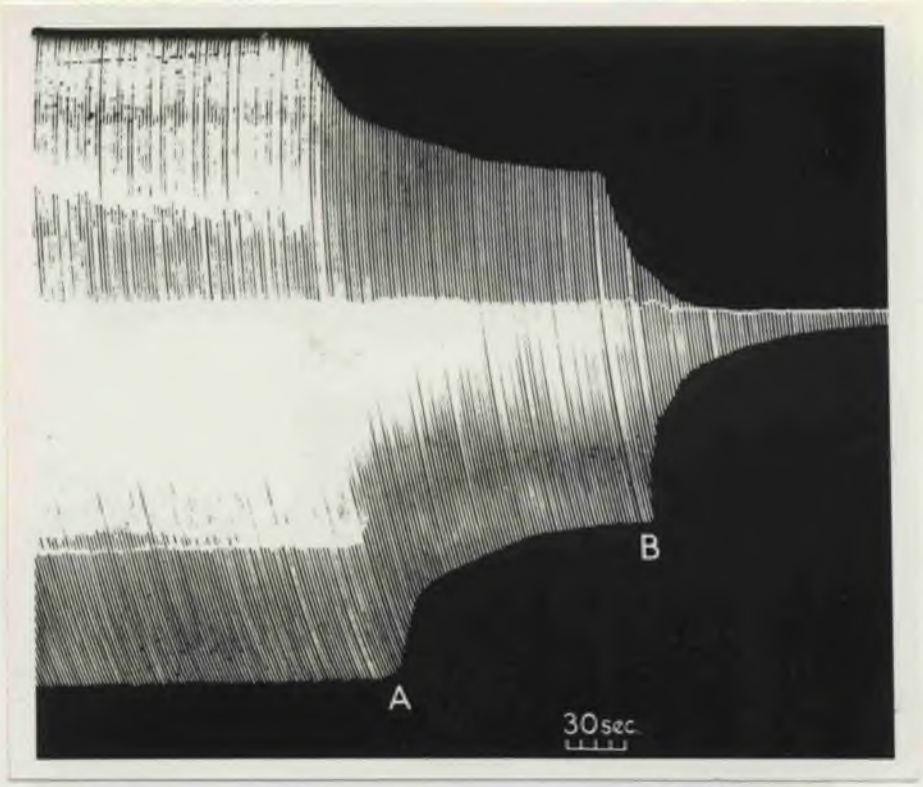


Fig. 23. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound III 0.15 mg/kg.

At B, decamethonium 0.02 mg/kg.

Immediately after injection of decamethonium, the height of the muscle twitch, partially blocked by compounds in Group 1, increased in amplitude and, within approximately five minutes (Figs. 21 and 22), a complete reversal of the block was achieved. On the other hand, decamethonium produced an additive increase in the intensity of the block produced by the compounds in Group 2 (Fig. 23).

The investigations carried out using decamethonium and tubocurarine confirmed the classification of the drugs into depolarizing and non-depolarizing agents. There was again no evidence of qualitative differences between the ether and non-ether compounds in Group 1.

Effect of Ether Anaesthesia.

Ether anaesthesia has been reported to increase the effects of non-depolarizing neuromuscular blocking agents (Burns & Paton, 1951; Foldes, 1959, 1960), whereas the activity of depolarizing drugs appears to be only slightly increased or reduced by this agent (Paton, 1953). Other straight chain aliphatic ethers produce a similar effect. This effect was investigated using compounds in Groups 1 and 2. A dose of drug was selected to produce a consistent, measurable degree of

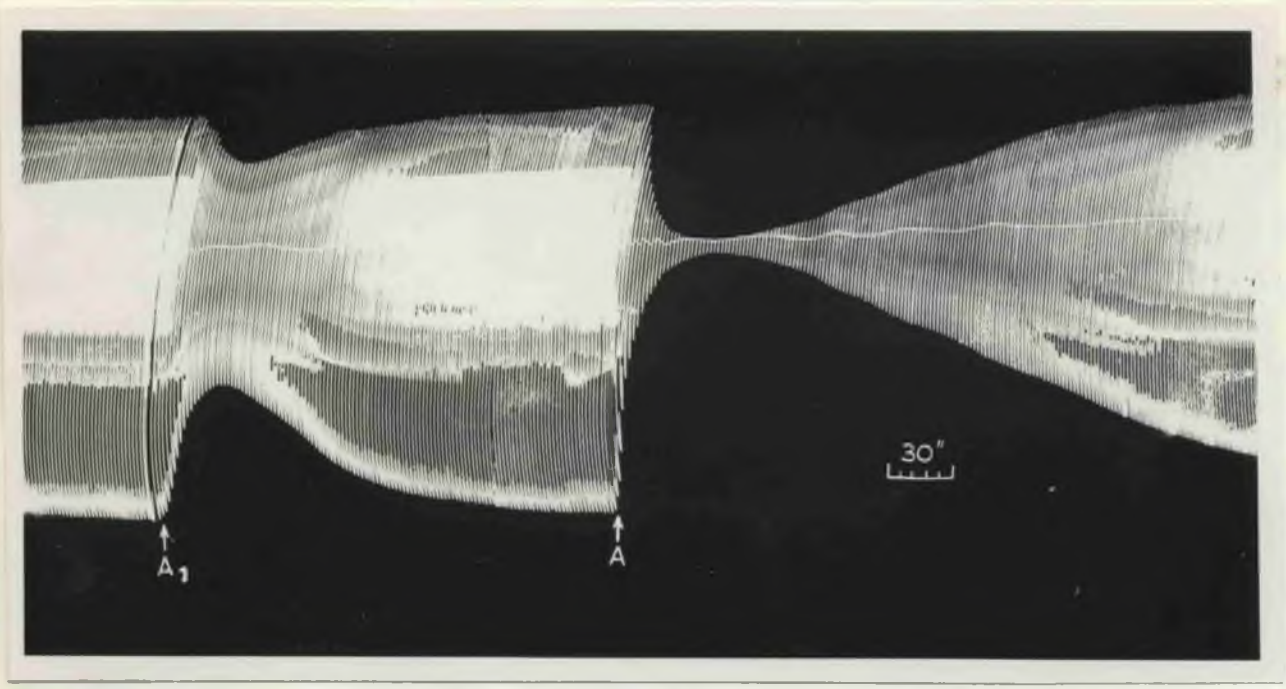


Fig. 24. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A_1, compound E77 0.05 mg/kg.

At A, compound E77 0.05 mg/kg during ether inhalation.

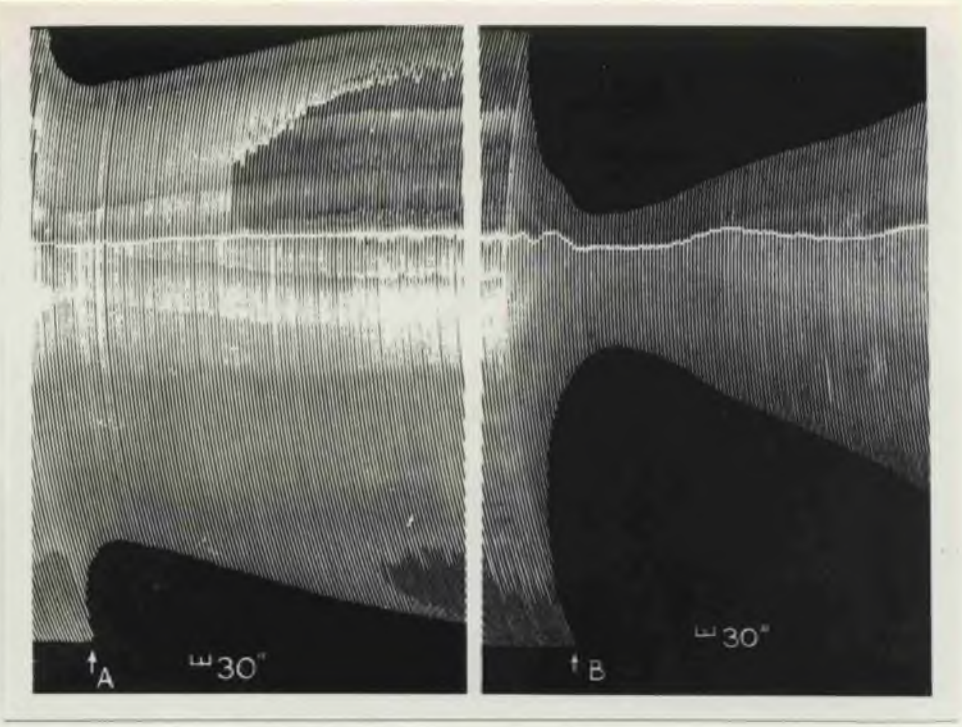


Fig. 25. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E78 0.050 mg/kg.

At B, compound E78 0.050 mg/kg during ether inhalation.

There was an interval of 38 minutes between A and B.

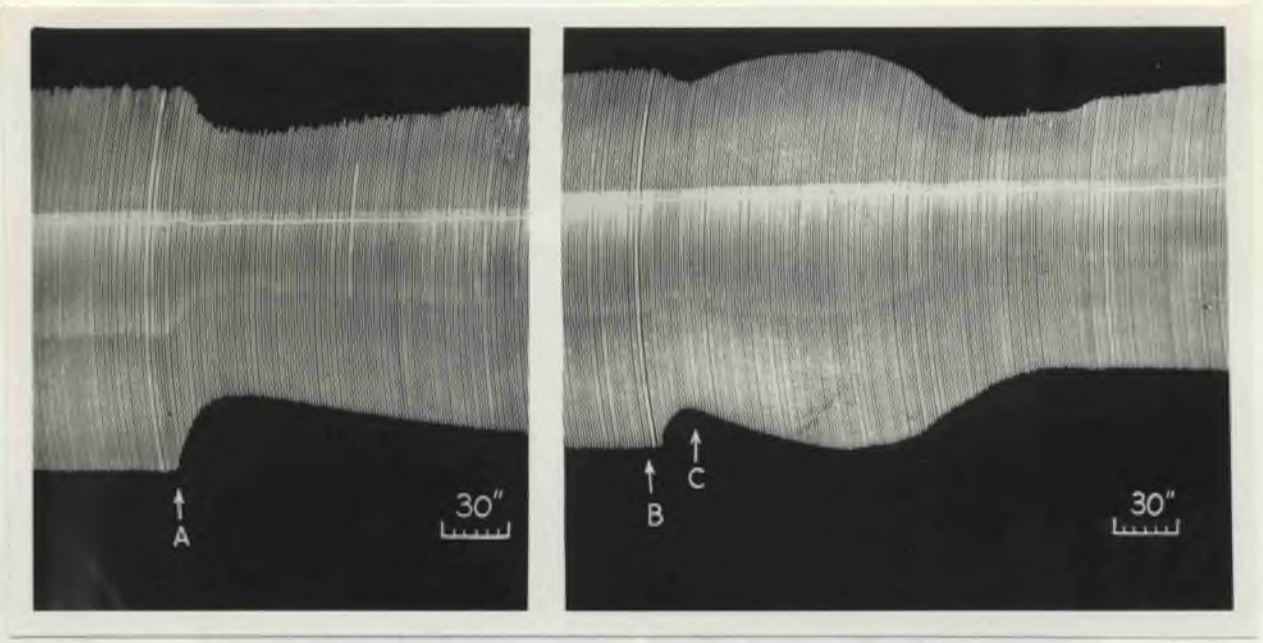


Fig. 26. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound II 0.10 mg/kg.

At B, compound II 0.10 mg/kg during ether inhalation.

At C, ether withdrawn.

neuromuscular block (approximately 20 - 50%). The same dose was repeated, after full recovery had been attained, in the presence of ether vapour administered from a bottle connected to the artificial respiration pump. The administration of the anaesthetic was continued for a period of five minutes, and was begun immediately after the injection of the drug. The neuromuscular blocking action of each of the compounds in Group 1 was intensified by ether anaesthesia but the activity of those in Group 2 was influenced very little. Some typical tracings from these experiments are shown in Figs. 24, 25 and 26. No differences between the ether and non-ether compounds in Group 1 could be observed from these experiments.

Studies On Drug Antagonism.

The activity of muscle relaxant drugs is potentiated, prolonged or terminated by a variety of other agents each of which, in addition, possesses its own characteristic pharmacological actions at sites other than the neuromuscular synapse (Paton & Zaimis, 1952; Foldes, 1959). These may be employed in the laboratory as a means of investigating the nature of the block produced by new muscle relaxants. Thus

anticholinesterases, by virtue of their ability to intensify the action of acetylcholine, antagonize the block produced by non-depolarizing agents (Chase, Schmidt & BhattaCharya, 1947; Bülbring & Chou, 1947; Blaschko, Bülbring & Chou, 1949), but the block produced by depolarizing agents may be potentiated or only slightly antagonized (Hoppe, 1950; Paton, 1951). While the action of those anticholinesterases containing quaternary ammonium groups is generally believed to be due to the prolongation and intensification of the activity of acetylcholine (Hobbiger, 1952; Nastuk & Alexander, 1954; Katz & Thesleff, 1957), both neostigmine (Riker & Wescoe, 1950) and edrophonium (Wescoe & Riker, 1951; Hougs & Johansen, 1958) exert a depolarizing effect at the neuromuscular junction. The anticholinesterases, edrophonium and neostigmine, together with adrenaline, were selected for studies on the stability of the block produced by compounds in Groups 1 and 2. Each was injected into the cat at the point of maximal neuromuscular block or just as recovery commenced.

Edrophonium.

Partial or complete neuromuscular block, produced by the compounds in Group 1, was rapidly and

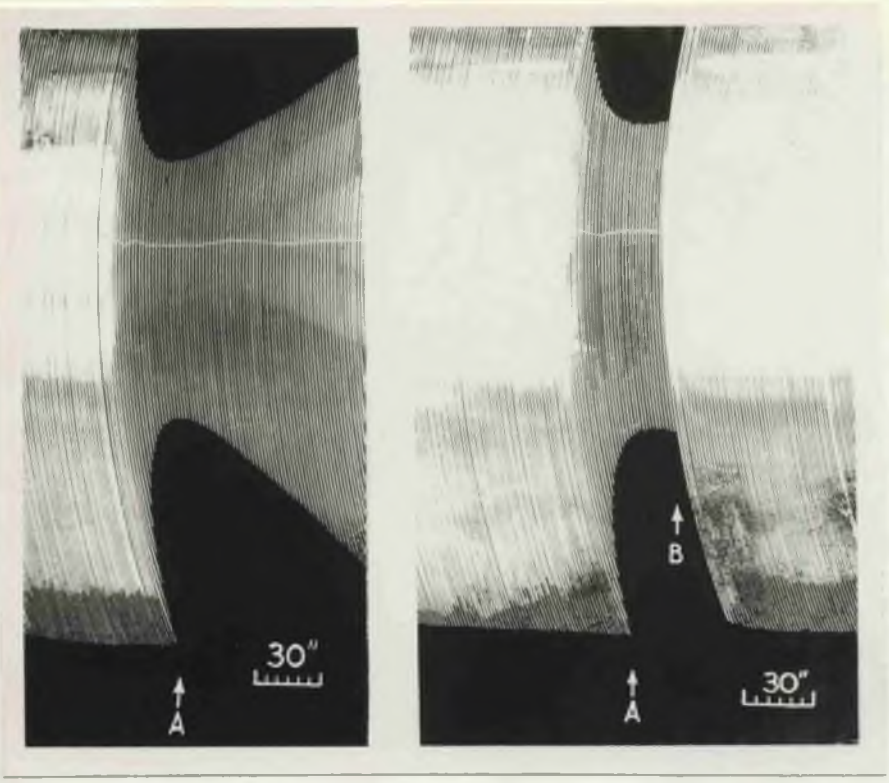


Fig. 27. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E79 0.03 mg/kg.

At B, edrophonium 0.50 mg/kg.

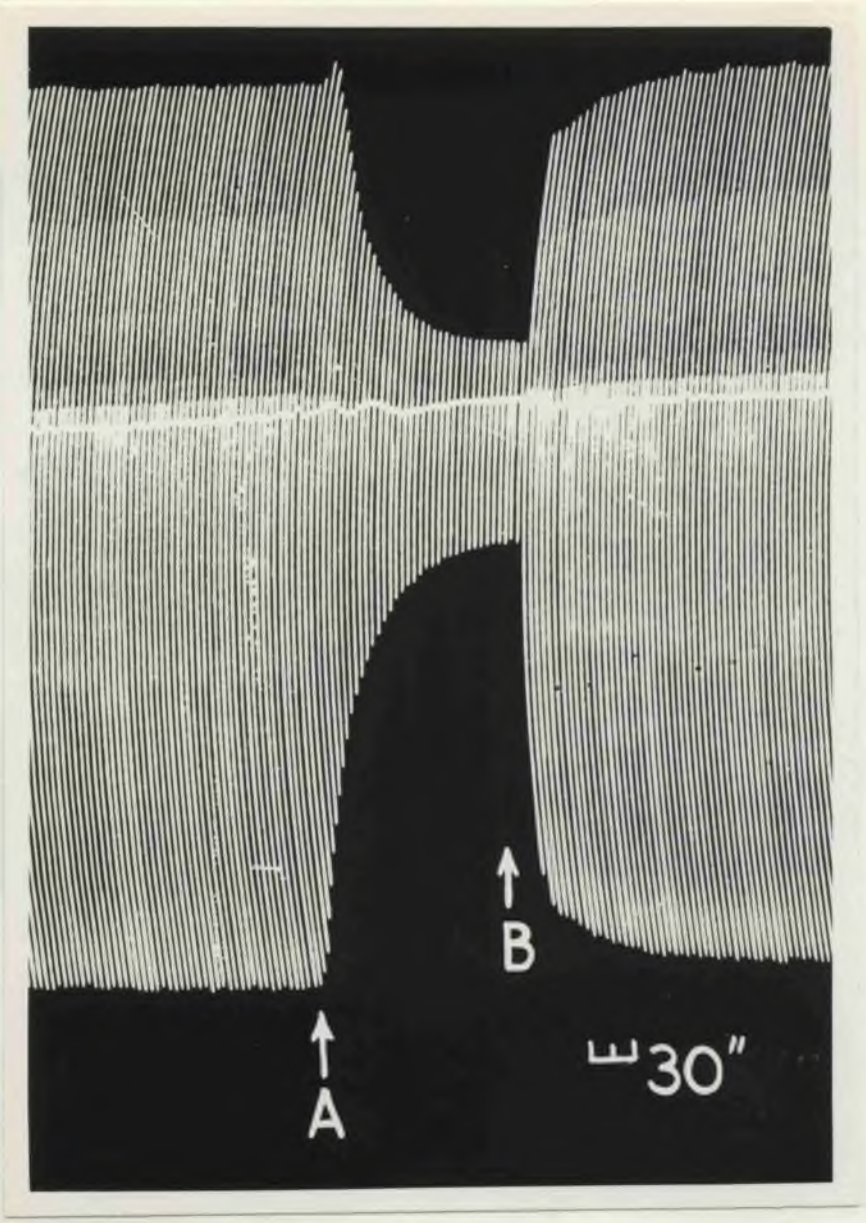


Fig. 28. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E96 0.04 mg/kg.

At B, edrophonium 0.50 mg/kg.

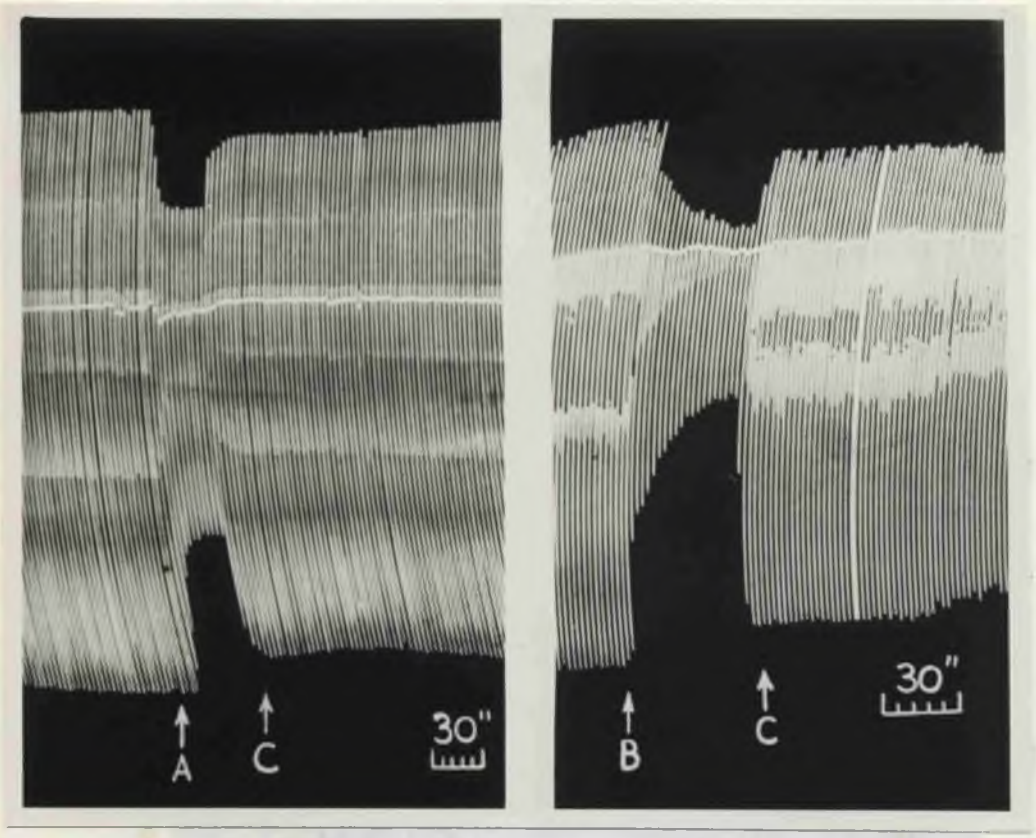


Fig. 29. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound F. & P. 16677 20 mg/kg.

At B, compound F. & P. 8302 10 mg/kg.

At C, edrophonium 0.5 mg/kg.

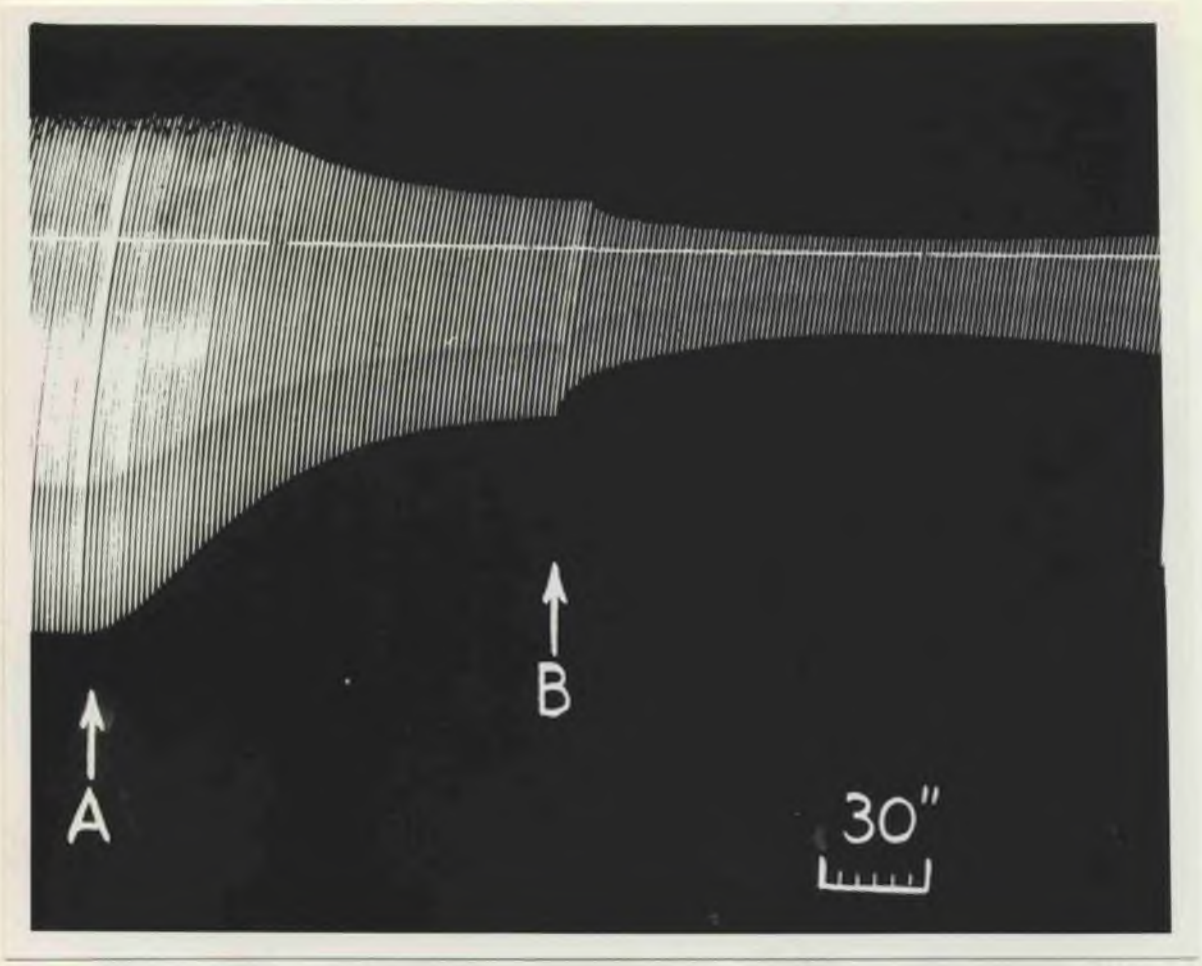


Fig. 30. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound II 0.05 mg/kg.

At B, edrophonium 0.50 mg/kg.

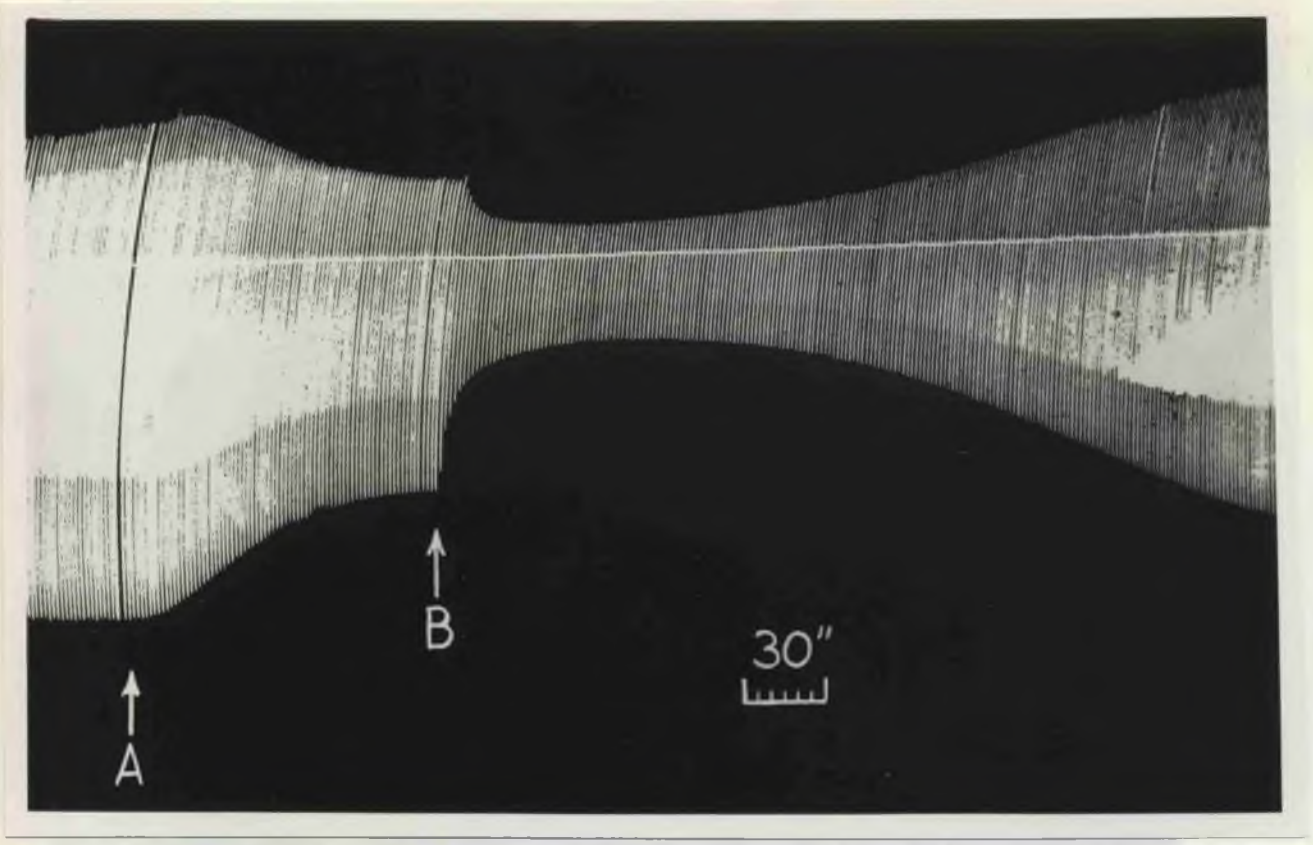


Fig. 31. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, decamethonium 0.05 mg/kg.

At B, edrophonium 0.50 mg/kg.

completely reversed by the intravenous injection of edrophonium (0.5 - 1 mg/kg) at the point of maximum depression of the twitch height. The effect of edrophonium was however short-lived. After complete recovery of the twitch height following edrophonium, a second and similar dose of the drug produced a slight reduction in the degree of neuromuscular block. Typical experiments are shown in Figs. 27, 28 and 29.

The effect of edrophonium on the block produced by the sulphur-containing decamethonium-like compounds in Group 2 was not so striking as that observed using compounds in Group 1, and doses of 0.5 mg/kg caused either a slight intensification or a slight reduction in the intensity of the block. All the compounds in this group behaved similarly to decamethonium and to each other. Typical effects are shown in Figs. 30 and 31.

Neostigmine.

The results obtained using neostigmine and edrophonium were qualitatively similar, although the effect of neostigmine was much more prolonged. Thus a second dose of drug given 30 minutes after the first had been reversed by neostigmine, produced little effect. In doses ranging from 0.02 to 0.10 mg/kg, neostigmine

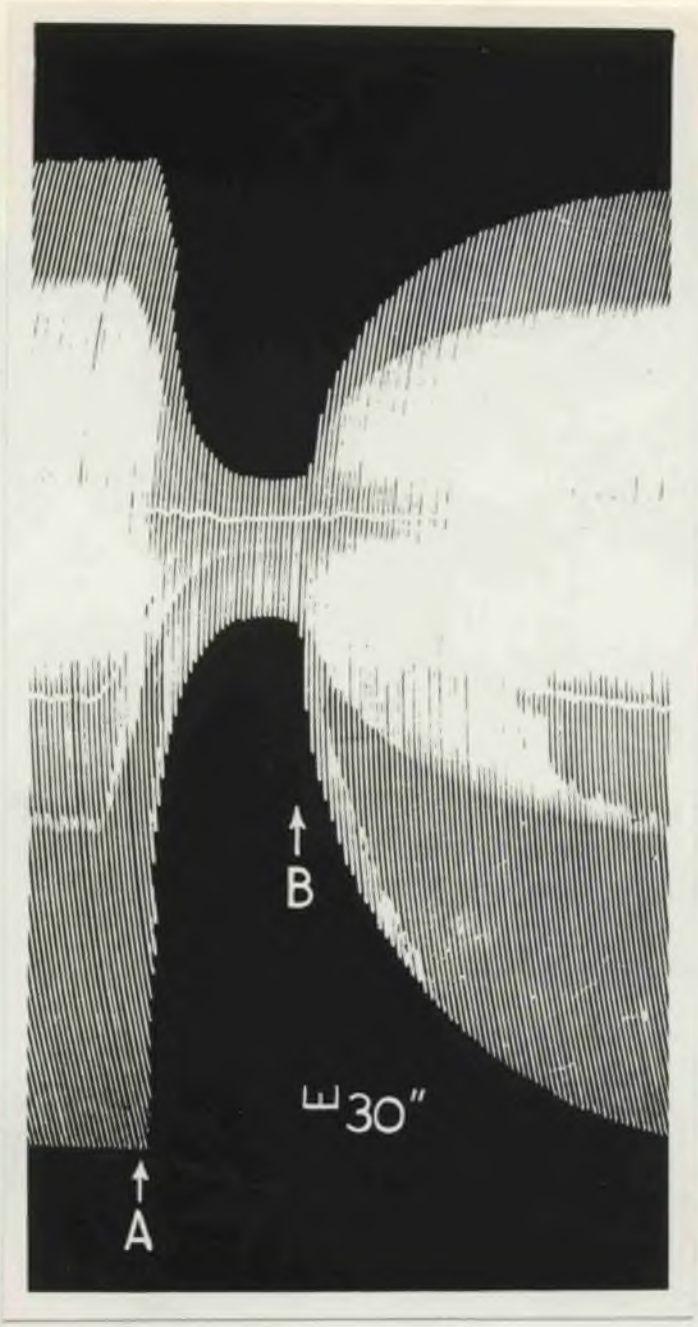


Fig. 32. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E80 0.12 mg/kg.

At B, neostigmine 0.02 mg/kg.

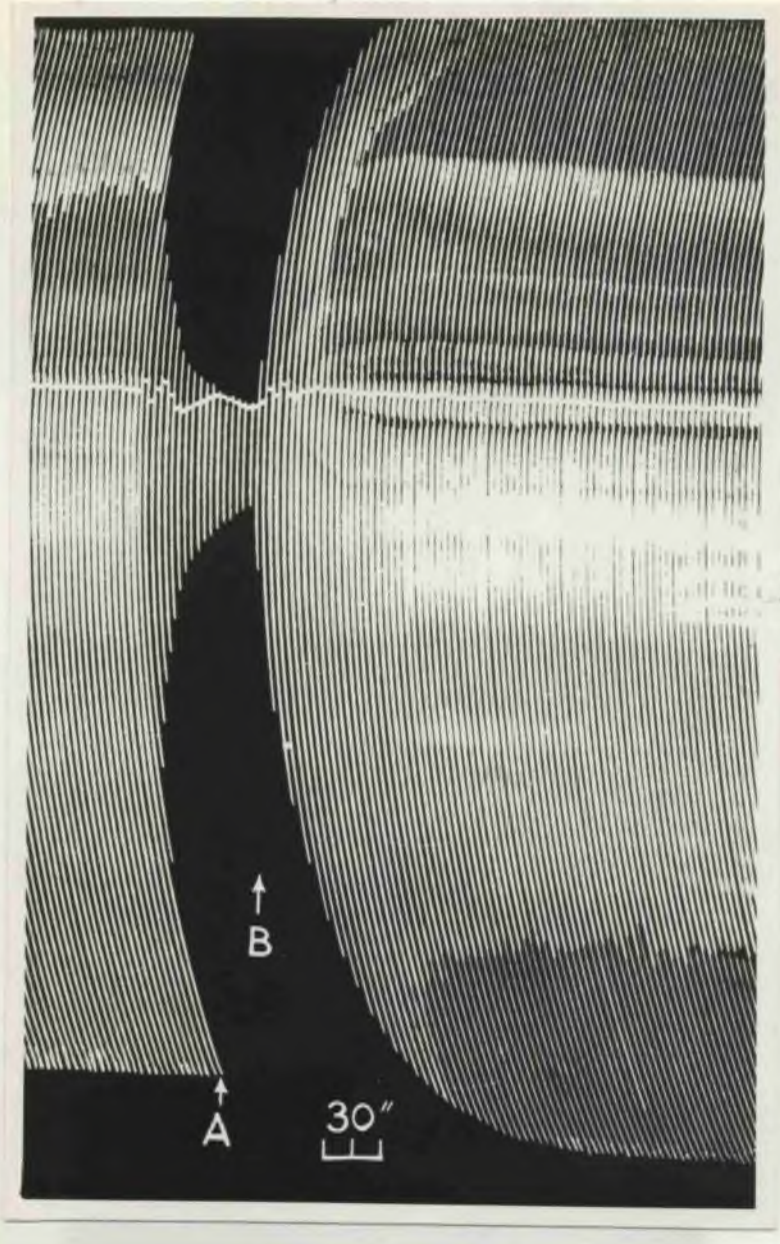


Fig. 33. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E78 0.15 mg/kg.

At B, neostigmine 0.05 mg/kg.

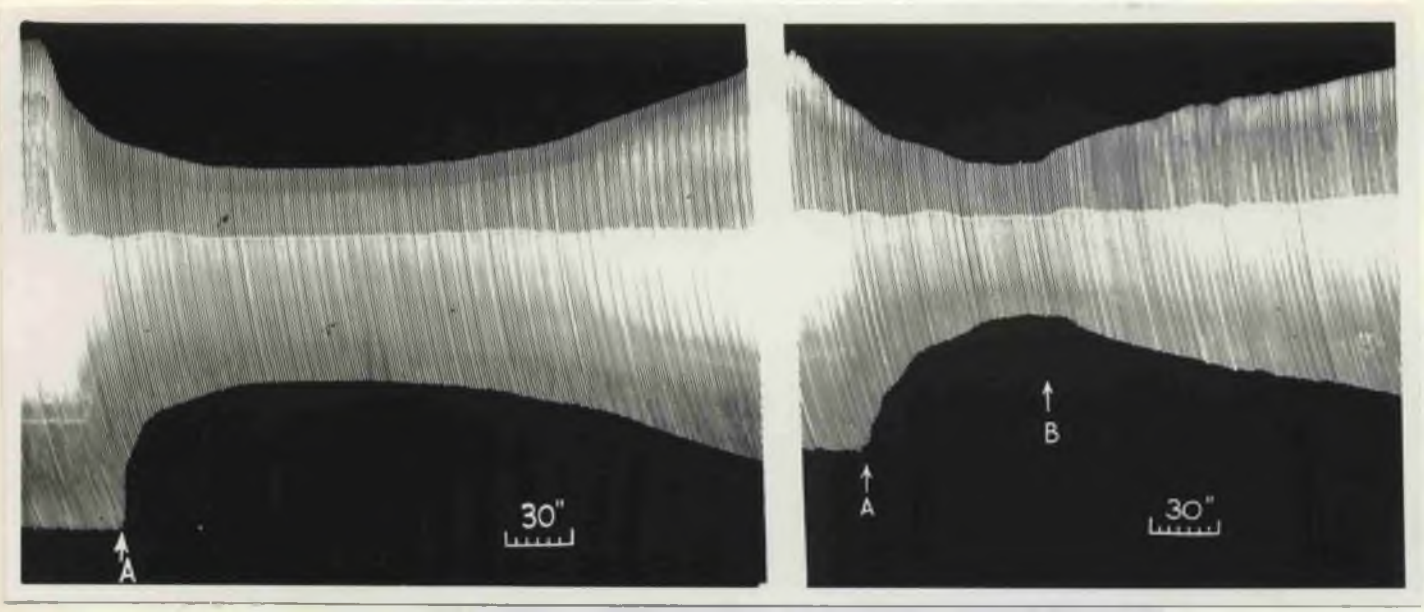


Fig. 34. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Indirect stimulation via the sciatic nerve. Contraction downwards. Drugs administered intravenously.

At A, compound III 0.15 mg/kg.

At B, neostigmine, 0.15 mg/kg.

effectively antagonized those compounds possessing non-depolarizing properties (Group 1) but had little intensifying or antagonizing effect on the predominantly depolarizing agents of Group 2. Characteristic tracings are shown in Figs. 32, 33 and 34.

In these experiments, the ether and non-ether containing compounds produced identical qualitative effects.

Adrenaline.

The mode of action of adrenaline at the neuromuscular synapse is not fully understood (Hunt & Kuffler, 1950 and references cited therein). Adrenaline is believed to exert a dual action on the motor end plate, increasing the quantity of acetylcholine released by indirect stimulation and stabilizing and decreasing the electrical excitability of the muscle fibre (Krnjevic & Miledi, 1958). One or other effect may predominate and consequently antagonism or potentiation may be observed. These effects cannot, it has been suggested (Linssen, 1961), be explained by its hyperpolarizing (Bülbring, 1956; Huidobro, 1960) or hyperglycaemic effects nor by an increase in the blood flow to the site of activity (Hunt & Kuffler, 1950).

The antagonism of adrenaline to the effects of

tubocurarine and other non-depolarizing drugs has been amply confirmed (Gruber, 1914; Rosenblenth, Lindsley & Morison, 1936; Hutter & Loewenstein, 1955; Brown, Bülbring & Burns, 1948; West & Zaimis, 1949). On the other hand, adrenaline, in low concentrations, potentiated the action of tubocurarine and gallamine (Beckett & Ellis, 1955) in the rat (Montagu, 1955). This effect was attributed to a diminished release of acetylcholine. A similar explanation was advanced to explain the inhibition, by adrenaline, of the early depolarizing phase and the polentiation of the late, non-depolarizing phase of acetylcholine, suxamethonium and decamethonium block (Beckett & Ellis, 1955). On the other hand, the stabilizing effect of low concentrations of adrenaline on the end plate might have been responsible (Foldes, 1959). The view that adrenaline may influence neuromuscular transmission by an anticholinesterase effect, has also been suggested (Foldes, 1959).

A dose of each of the compounds under study was selected to produce approximately 30-50% of neuromuscular block and adrenaline was injected at the point of maximum depression of the muscle twitch.

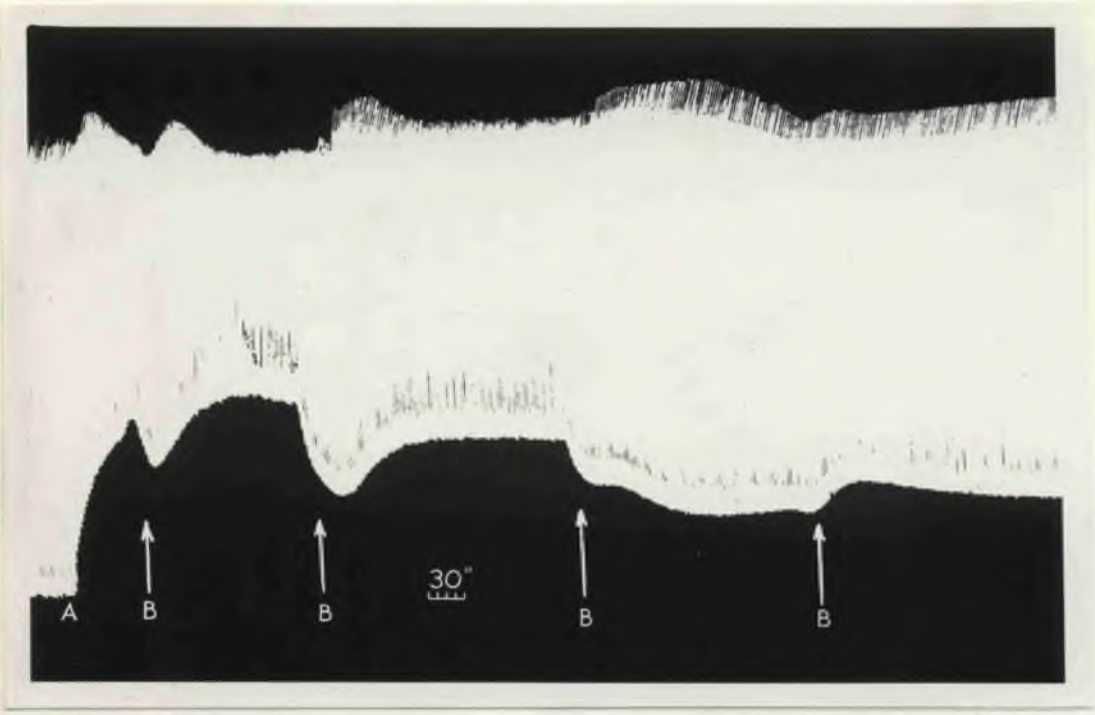


Fig. 35. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E77 0.04 mg/kg.

At B, adrenaline 0.04 mg/kg.

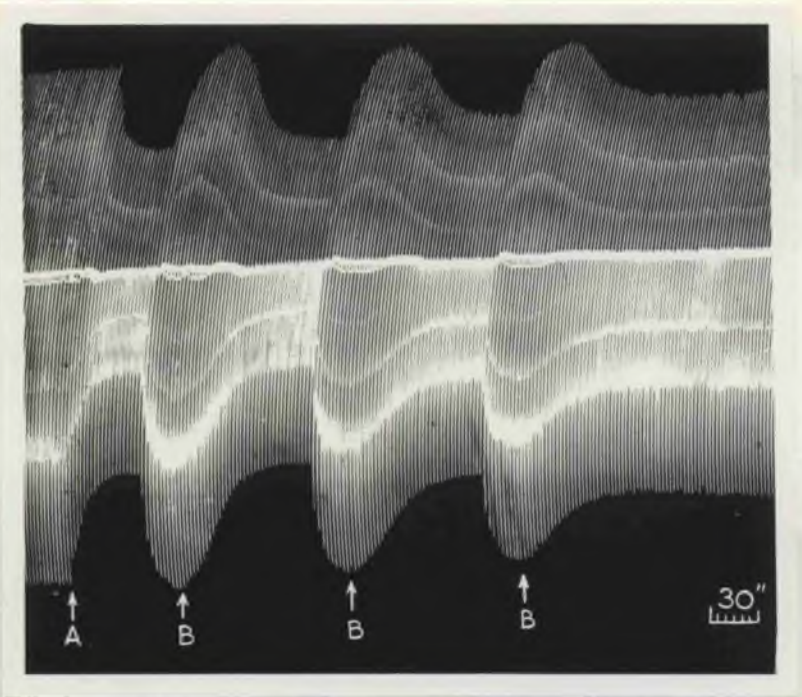


Fig. 36. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E98 0.75 mg/kg.

At B, adrenaline 0.02 mg/kg.

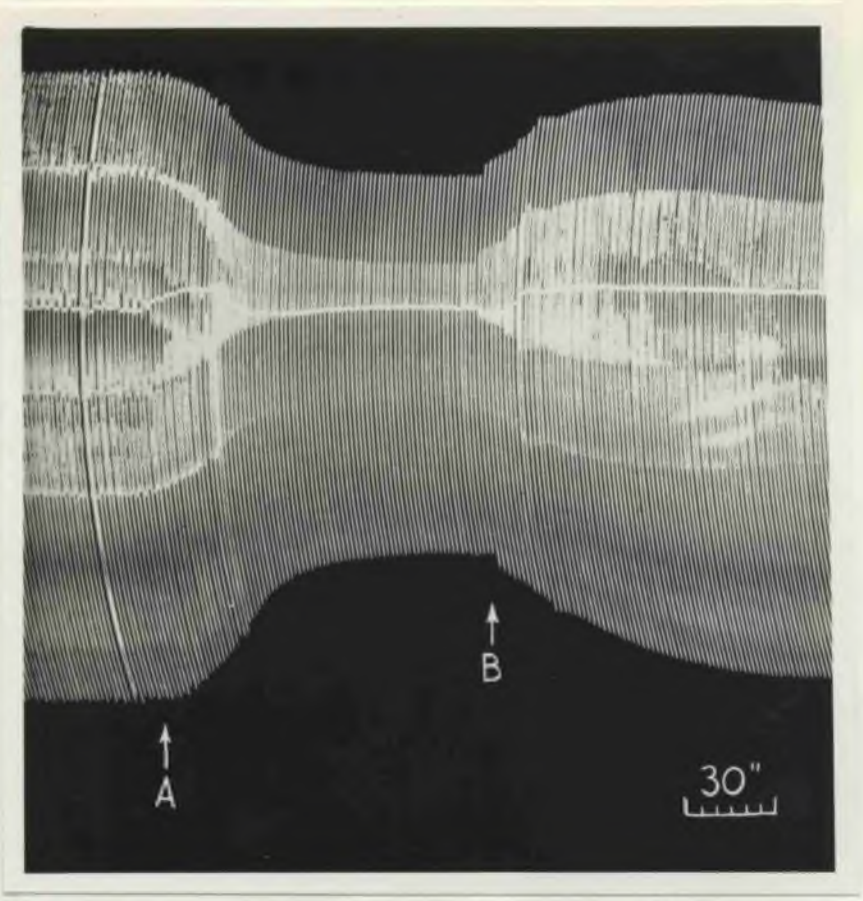


Fig. 37. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, decamethonium 0.06 mg/kg.

At B, adrenaline 0.04 mg/kg.

TABLE V

A tabular comparison of the properties of compounds F. & P.17843, F. & P. 8212, Tubocurarine, I, II, III and Decamethonium on the Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

Compound	Neuromuscular Blocking Activity			Preliminary Excitation of skeletal muscle	Effect of Tubocurarine on Block produced 0.025-0.1 (mg/kg)	Effect of Decamethonium on Block produced 0.02-0.05 (mg/kg)	Effect, on the Block produced of:				Response of partially Blocked muscle to Indirect Tetanus
	Time of onset of maximum paralysis (min)	Duration of Paralysis (50% Block approx.) (min)	Approx. Molar Potency (Tubocurarine = 100)				Edrophonium (0.5-1mg/kg)	Neostigmine (0.05-0.1mg/kg)	Adrenaline (0.02-0.05mg/kg)	Inhalation of Ether Vapour	
F.& P. 17843	1 - 2	25 - 30	0.2	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 8212	1 - 3	20 - 25	2.0	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
Tubocurarine	1 - 6	25 - 30	100	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
I	3 - 5	45 - 50	20 *	Sometimes observed	Antagonistic	Additive	None or slightly Additive	No effect or slightly Additive	Antagonistic	Slightly Additive	Well sustained
II	2 - 4	30 - 40	81 *	Observed	Antagonistic	Additive	No effect or slightly Additive	No effect or slightly Additive	Antagonistic	Slightly Additive	Well sustained
III	1 - 3	55 - 60	28 *	Sometimes observed	Slightly Additive then Antagonistic	Additive	No effect or slightly Additive	No effect or slightly Additive	Antagonistic	Slightly Additive	Well sustained
Decamethonium	1 - 4	35 - 40	100 *	Observed	Antagonistic	Additive	No effect or slightly Additive	No effect or slightly Additive	Antagonistic	Slight Potentiation	Well sustained

* Potency compared to Decamethonium (= 100)

TABLE IV (continued)

Compound	Neuromuscular Blocking Activity			Preliminary Excitation of skeletal muscle	Effect of Tubocurarine on Block produced 0.025-0.1 (mg/kg)	Effect of Decamethonium on Block produced 0.02-0.05 (mg/kg)	Effect, on the Block Produced, of:				Response of partially blocked muscle to indirect Tetanus
	Time of onset of maximum paralysis (min)	Duration of paralysis (50% block approx.) (min)	Approx. Molar Potency (Tubocurarine = 100)				Edrophonium (0.5-1mg/kg)	Neostigmine (0.05-0.1mg/kg)	Adrenaline (0.02-0.05mg/kg)	Inhalation of Ether Vapour	
E103	1 - 3	25 - 30	14.5	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 16575	1 - 3	25 - 30	0.08	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 16677	1 - 2	25 - 30	0.16	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 8302	1 - 3	20 - 25	0.13	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 16678	1 - 2	25 - 30	0.30	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 8303	1 - 3	20 - 25	0.45	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
F.& P. 16701	Approximately 6% Block at 25/mg/kg. Insufficient material to permit further tests										

TABLE IV (continued)

Compound	Neuromuscular Blocking Activity			Preliminary Excitation of skeletal muscle	Effect of Tubocurarine on Block produced 0.025-0.1 (mg/kg)	Effect of Decamethonium on Block produced 0.02-0.05 (mg/kg)	Effect, on the Block Produced, of:				Response of partially blocked muscle to indirect Tetanus
	Time of onset of maximum paralysis (min)	Duration of paralysis (50% block approx.) (min)	Approx. Molar Potency (Tubocurarine = 100)				Edrophonium (0.5-1mg/kg)	Neostigmine (0.05-0.1mg/kg)	Adrenaline (0.02-0.05mg/kg)	Inhalation of Ether Vapour	
E95	1 - 3	35 - 40	2	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E96	1 - 3	30 - 35	234	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E97	1 - 3	40 - 60	263	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E98	1 - 3	25 - 30	3.0	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E99	1 - 3	40 - 45	0.7	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E100			No paralysis with 30mg/kg	None	Inusfficient material to permit further tests						
E101	1 - 3	25 - 30	0.4	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E102	1 - 3	30 - 35	1.0	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained

TABLE IV

A Tabular comparison of the properties of compounds E77, E78, E79, E80, E90, E91, E93, E94, E95, E96, E97, E98, E99, E100, E101, E102, E103, F. & P. 16575, F. & P. 16677, F. & P. 8302, F. & P. 8303 and F. & P.16701, on the Cat gastrocnemius muscle-sciatic nerve preparation.

Compound	Neuromuscular Blocking Activity			Preliminary Excitation of skeletal muscle	Effect of Tubocurarine on Block produced 0.025-0.1 (mg/kg)	Effect of Decamethonium on Block produced 0.02-0.05 (mg/kg)	Effect, on the Block Produced, of:				Response of partially blocked muscle to indirect Tetanus
	Time of onset of maximum paralysis (min)	Duration of paralysis (50% block approx.) (min)	Approx. Molar Potency (Tubocurarine = 100)				Edrophonium (0.5-1mg/kg)	Neostigmine (0.05-0.1mg/kg)	Adrenaline (0.02-0.05mg/kg)	Inhalation of Ether Vapour	
E77	1 - 3	15 - 20	278	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E78	1 - 3	20 - 25	87	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E79	1 - 6	20 - 25	417	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E80	1 - 2	30 - 40	110	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E90	1 - 3	25 - 30	5	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E91	-		No paralysis with 2mg/kg	Insufficient material to permit further tests							
E93	1 - 3	30 - 35	6	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained
E94	1 - 3	40 - 60	50	None	Additive	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Potentiation	Poorly sustained

In all cases, a rapid but brief reversal of the block was obtained. In some cases, the twitch amplitude returned to control levels within a few minutes, but soon declined again almost to the level prior to the injection of adrenaline. In such cases adrenaline was again given successively, until complete recovery was obtained. Characteristic effects are shown in Figs. 35 and 36. Adrenaline (0.04 mg/kg) also exerted a slight temporary antagonism on the block produced by decamethonium and the compounds in Group 2 although the effect was less pronounced than with the compounds in Group 1 (Fig. 37). A summary of the qualitative pharmacological properties of the compounds in Groups 1 and 2, as determined using the cat gastrocnemius muscle-sciatic nerve preparation, is given in Tables IV and V. When the preparation was giving a consistent response, for example 50% inhibition of the twitch amplitude to a fixed dose of any of the drugs in either group, a dose of tubocurarine or decamethonium was selected which produced a similar quantitative effect. The approximate molar potencies, also included in Tables IV and V, were then calculated with reference to tubocurarine for drugs in Group 1 and to decamethonium

TABLE VI (continued)

Compound	Head Drop Doses (H.D.D.)		No.Died / No. Injected	Molar Potency (tubocurarine = 100)
	Individual (mg/kg)	Mean ± S.E.M. (mg/kg)		
E90	3.4, 3.4, 3.9.	3.57 ± 0.15	1/3	5
E91	8 mg/kg produced no head drop.	-	-	-
E98	2.31, 2.6, 2.4, 2.34, 2.8, 2.4. 2.50, 2.5, 2.3 2.8,	2.50 ± 0.20	0/10	0.366
Tubocurarine	0.13, 0.17, 0.15, 0.23, 0.17, 0.23, 0.24, 0.13. 0.16,	0.18 ± 0.02	4/9	100

TABLE VI

Individual and Mean Head Drop Doses of compounds E77, E78, E79, E80, E96, E97, E90, E91, E98 and tubocurarine in the rabbit.

Compound	Head Drop Doses (H.D.D.) Individual (mg/kg)	Mean ± S.E.M. (mg/kg)	No.Died / No. Injected	Molar Potency (tubocurarine = 100)
E77	0.21, 0.21. 0.23, 0.28, 0.23,	0.23 ± 0.01	2/5	108
E79	0.21, 0.19, 0.16, 0.20, 0.29, 0.20, 0.21, 0.20, 0.17.	0.20 ± 0.01	4/9	125
E78	0.30, 0.37, 0.35, 0.36, 0.34, 0.37, 0.35, 0.33, 0.45.	0.36 ± 0.01	0/9	66
E80	0.25, 0.33, 0.27, 0.30, 0.31, 0.29, 0.29, 0.29. 0.26,	0.29 ± 0.01	4/9	82
E96	0.29, 0.28, 0.30, 0.21, 0.26, 0.22, 0.30, 0.30. 0.25,	0.27 ± 0.01	6/9	96
E97	0.25, 0.23, 0.25, 0.20, 0.23, 0.28, 0.24, 0.23, 0.23.	0.24 ± 0.01	6/9	110

TABLE VII

Individual and Mean Head Drop Doses of compounds I, II, II, Oxydipentonium and Decamethonium in the rabbit.

Compound	Head Drop Doses (H.D.D.)		No.Died / No. Injected	Molar Potency (Decamethonium = 100)
	Individual (mg/kg)	Mean ± S.E.M. (mg/kg)		
* I	1.8, 1.3 2.1, 1.6. 1.6,	1.68 ± 0.01	1/5	12
II	0.42, 0.51, 0.49, 0.42, 0.53, 0.47, 0.51, 0.47. 0.44,	0.47 ± 0.01	2/9	41
III	1.5, 1.0 0.9, 1.1, 1.1, 1.1, 1.4, 1.6, 1.9.	1.29 ± 0.33	3/9	14
Oxydipentonium	0.55, 0.55, 0.68, 0.55, 0.54, 0.54, 0.51, 0.56. 0.56,	0.56 ± 0.01	4/9	23
Decamethonium	0.17, 0.13, 0.15, 0.16, 0.21, 0.19, 0.17, 0.22, 0.28.	0.19 ± 0.02	3/9	100

* Insufficient material to permit further observations

for those in Group 2 according to the formula

$$\text{Approximate Molar Potency of New Compound} = \frac{\text{Molecular Weight of New Compound}}{\text{Molecular Weight of Reference Compound}} \times \frac{\text{Dose of Reference Compound producing a certain effect}}{\text{Dose of New Compound producing the same effect}} \times 100$$

The reference compounds, tubocurarine and decamethonium, were arbritarily assigned the value 100.

The Rabbit Head Drop Test.

On account of the limited amount of material available only the potency of compounds in Groups 1a, 2 and compound E98 could be estimated. In addition, the head drop doses of oxydipentonium, decamethonium and tubocurarine were estimated and the results shown in Tables VI and VII.

Whenever possible, groups of nine animals were used to permit statistical analysis. The duration of effect of the compounds in Group 1a varied between 5 and 10 min and complete recovery took approximately 20 min. In some cases, the animals were revived by strapping to an automatic resuscitator, which consisted of a mounted platform tilting through an

angle of 90° to the horizontal. This was not successful in every case and E96 and E97 appeared particularly toxic. Compounds II and III were much shorter-acting than those in Group 1 and apparently complete recovery was observed after approximately 5 min. In keeping with similar observations on the cat, the ether-containing compounds were less potent on the rabbit than the corresponding non-ethers. The sulphur-containing decamethonium analogues were qualitatively similar to, but quantitatively weaker than decamethonium.

The Effect of Neostigmine Upon The Magnitude of The Head Drop Dose.

The head drop dose of the compounds in Group 1a and tubocurarine were found to be appreciably increased by subcutaneous injection of neostigmine (0.1 mg/kg) 15 min prior to injection of the drug under study. This is in keeping with the well known antagonistic effect of neostigmine on tubocurarine-like compounds on other preparations and species as already discussed and is in contrast to the slightly intensifying effect exerted by the anticholinesterases on compounds of Group 2 as indicated by a reduction in the head drop dose.

TABLE VIII

Head Drop Doses (H.D.D.) of compounds E77, E78, E79, E80, E96, E97 and Tubocurarine, in the rabbit, before and after treatment with Neostigmine and the ratio H.D.D.(neostigmine treated)/H.D.D. control.

Compound	Mean Head Drop Dose (H.D.D.) ± S.E.M. (mg/kg)		Ratio: Neostigmine treated / Control
	Control	Neostigmine (0.10 mg/kg), subcutaneously treated	
E77	0.23 ± 0.01 (Five rabbits used)	0.33 ± 0.01	1.43 P 0.001
E79	0.20 ± 0.01	0.29 ± 0.02	1.45 P 0.001
E78	0.36 ± 0.01	0.53 ± 0.02	1.47 P 0.001
E80	0.29 ± 0.01	0.50 ± 0.01	1.72 P 0.001
E96	0.27 ± 0.01	0.37 ± 0.01	1.37 P 0.001
E97	0.24 ± 0.01	0.40 ± 0.01	1.67 P 0.001
Tubocurarine	0.18 ± 0.02	0.30 ± 0.02	1.67 P 0.001

TABLE IX

Individual and Mean Head Drop Dose (H.D.D.) of compounds I, II and III and decamethonium in the rabbit before and after treatment with neostigmine and the ratio H.D.D. (Neostigmine treated)/H.D.D. control.

Name or Code No. of Compound	Mean Head Drop Dose (H.D.D.) ± S.E.M. (mg/kg)		Ratio $\frac{\text{Neostigmine treated}}{\text{Control}}$
	Control	Neostigmine (0.10 mg/kg) subcutaneously	
I	1.68 ± 0.02	*1.60 ± 0.13	0.95
II	0.47 ± 0.01	0.34 ± 0.03	0.72
III	1.29 ± 0.33	1.17 ± 0.33	0.91
Decamethonium	0.19 ± 0.05	0.16 ± 0.01	0.84

* Three observations only due to lack of material.

TABLE X

The qualitative and semi-quantitative properties of compounds E77, E79, E78, E80, E96, E97, E90, E94, E91, E93, E95 and Tubocurarine in the chick.

Compound	No. of Injections required to induce paralysis (1mg/kg /min)	Type of paralysis observed	Approximate Molar potency (Tubocurarine = 100)
E77	5	Flaccid	139
E79	3	Flaccid	230
E78	6	Flaccid	110
E80	4	Flaccid	158
E96	3	Flaccid	242
E97	3	Flaccid	242
E90	No paralysis after three injections 5 mg/kg	-	-
E94	5	Flaccid	101
E91*	6	Flaccid	16
E93	9	Flaccid	52
E95	6	Flaccid	88
Tubocurarine	5	Flaccid	100
Oxydipentonium	2	Spastic	250

*5 mg/kg /min

TABLE XI

The qualitative and semi-quantitative properties of compounds I, II, III and decamethonium on the chick.

Compound	No. of Injections required to induce paralysis (1.0mg/kg/30 sec)	Type of paralysis observed	Approximate Molar potency (Decamethonium = 100)
I	6	Spastic Flaccid	33
II	3	Spastic	66
III	4	Spastic Flaccid	48
Decamethonium	2	Spastic	100

The head drop doses before and after treatment with neostigmine and the ratio of these effects are shown in Tables VIII and IX.

Experiments on Avian Muscle.

Chick paralysis test.

Following intraperitoneal injection of compounds in Groups 1a and 1b (1 to 5 mg/kg every minute until death ensued), a typical tubocurarine-like flaccid paralysis was observed. The sulphonium analogues of decamethonium (Group 2) produced different effects. Compounds I and II (1.0 mg/kg every 30 seconds until death ensued) produced an initial spastic paralysis which became flaccid prior to death. Compound II (1.0 mg/kg every 30 seconds until death ensued) and oxydipentonium (1.0 mg/kg every minute) produced a typical decamethonium-like, spastic paralysis.

In all of these tests, groups of six chickens were used, the results indicating the number of injections required to produce paralysis are shown in Tables X and XI.

Hen Gastrocnemius Muscle-Sciatic Nerve Preparation.

This preparation is primarily of value as a means of differentiating between depolarizing and non-depolarizing muscle relaxants. It may also be used, semi-quantitatively, to compare the approximate

TABLE XII

The molar potency and the type of neuromuscular blocking activity produced on the hen gastrocnemius muscle-sciatic nerve preparation by Group 1c compounds, E99, E100, E101, E102 and E103.

Name or Code Number of Compound	Approx. Molar Potency (Tubocurarine = 100)	Type of Neuro-muscular Block Produced
E98	20.0	Non-Depolarizing
E99	15.0	Non-Depolarizing
E100	*	Non-Depolarizing
E101	5.0	Non-Depolarizing
E102	5.2	Non-Depolarizing
E103	46.0	Non-Depolarizing

* Insufficient material to permit quantitative estimation.

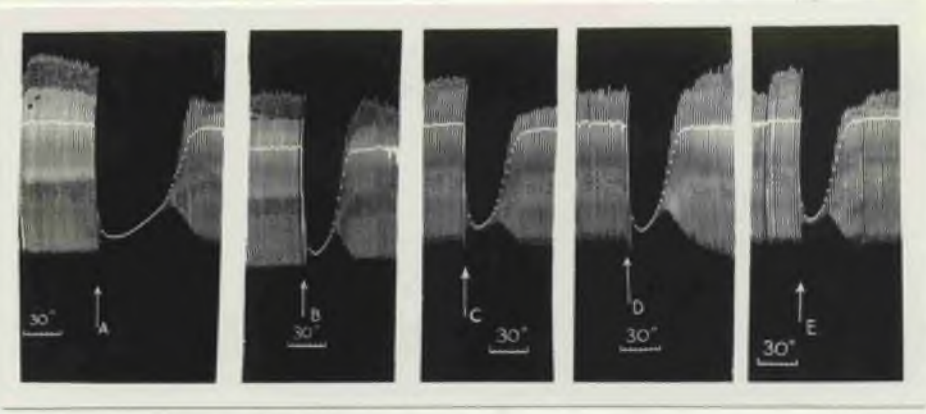


Fig. 38. Hen gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, oxydipentonium 0.10 mg/kg.

At B, compound II 0.02 mg/kg.

At C, compound III 0.05 mg/kg.

At D, decamethonium 0.02 mg/kg.

At E, compound I 0.50 mg/kg.

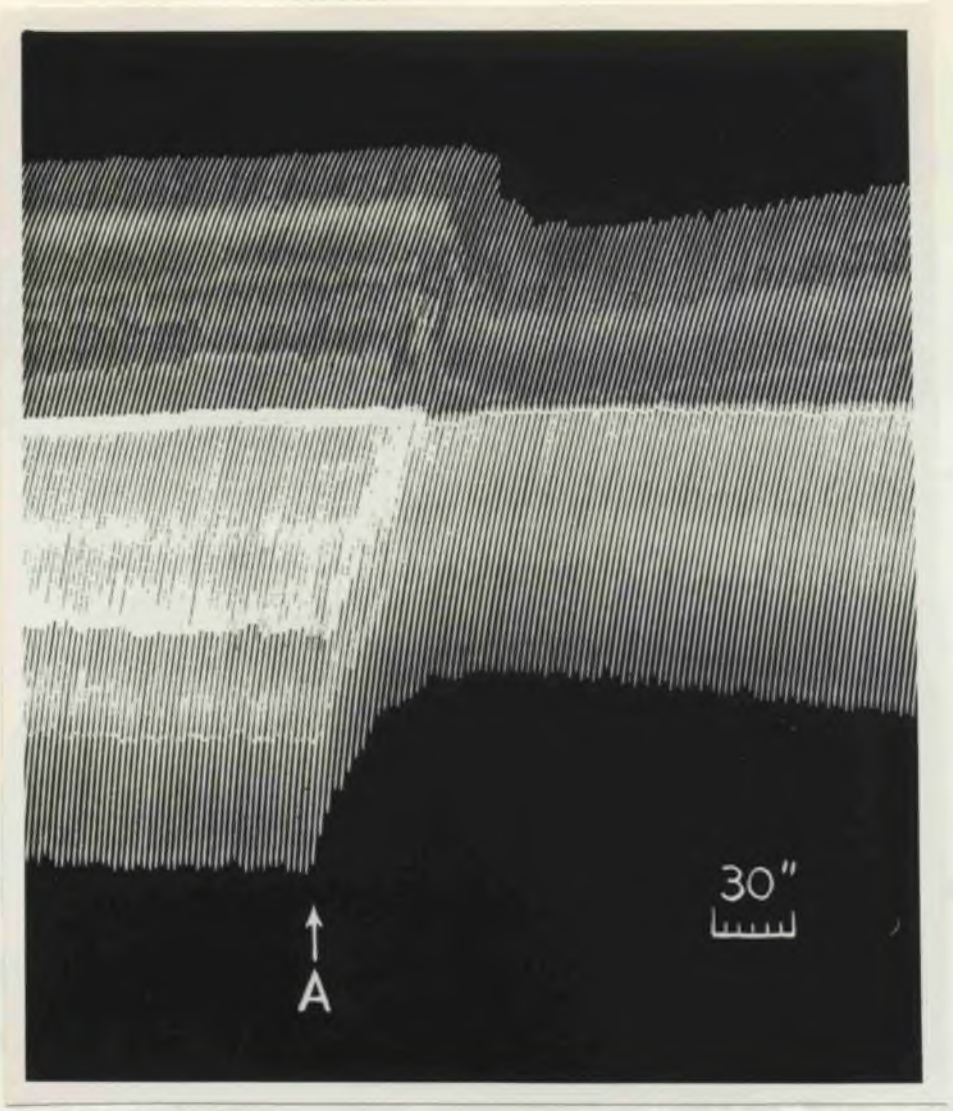


Fig. 39. Hen gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.
At A, compound E100 20 mg/kg.

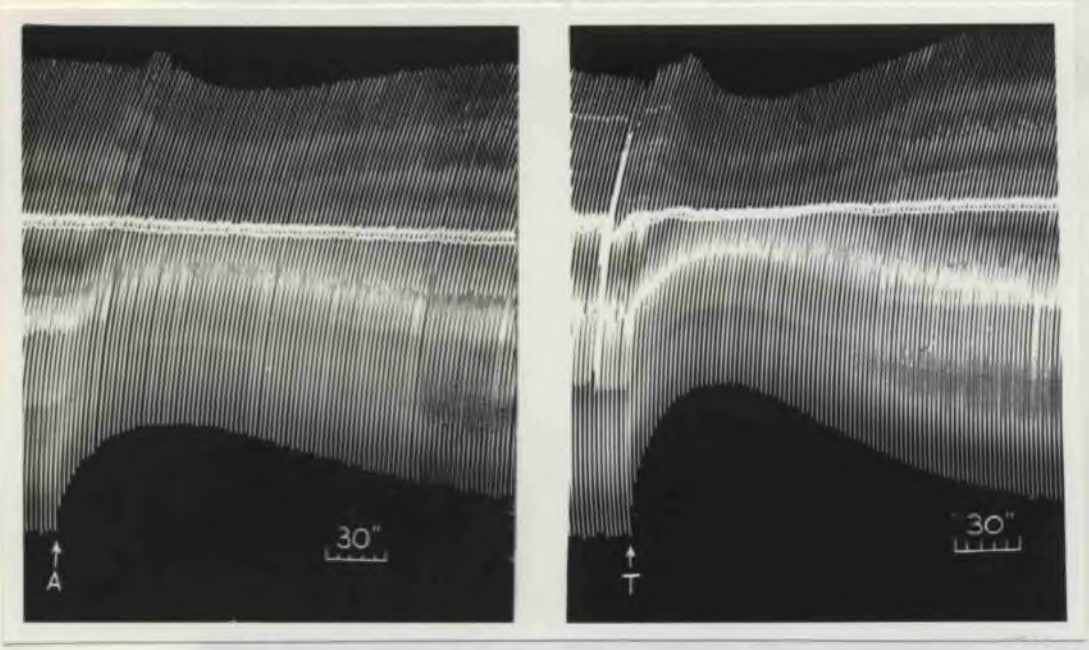


Fig. 40. Hen gastrocnemius muscle-sciatic nerve preparations. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. Indirect stimulation via the sciatic nerve.

At A, compound E101 20 mg/kg.

At T, tubocurarine 0.1 mg/kg.

potencies of new compounds with those of decamethonium and tubocurarine (Table XII).

The compounds tested on this preparation included those of Groups 1a, 1c and Group 2. All the compounds in Group 2, together with oxydipentonium, produced both a contracture and a contraction of the gastrocnemius muscle confirming their depolarizing activity. Compounds I and III were much less potent than II which was approximately equipotent with decamethonium. Typical tracings are shown in Fig. 38. The onset of paralysis was rapid and the duration of block brief. The maximum contracture-producing activity was observed prior to the attainment of the maximum inhibition of twitch height.

On the other hand, compounds in Groups 1a and 1c were devoid of contracture-inducing properties and behaved qualitatively like tubocurarine. The duration of action was found to be more prolonged than when these compounds were tested on a similar preparation in the cat. It should be noted, that compound E100, in which the onium substituents were entirely methyl radicals exhibited no depolarizing properties. Characteristic tracings are shown in Figs. 39 and 40.

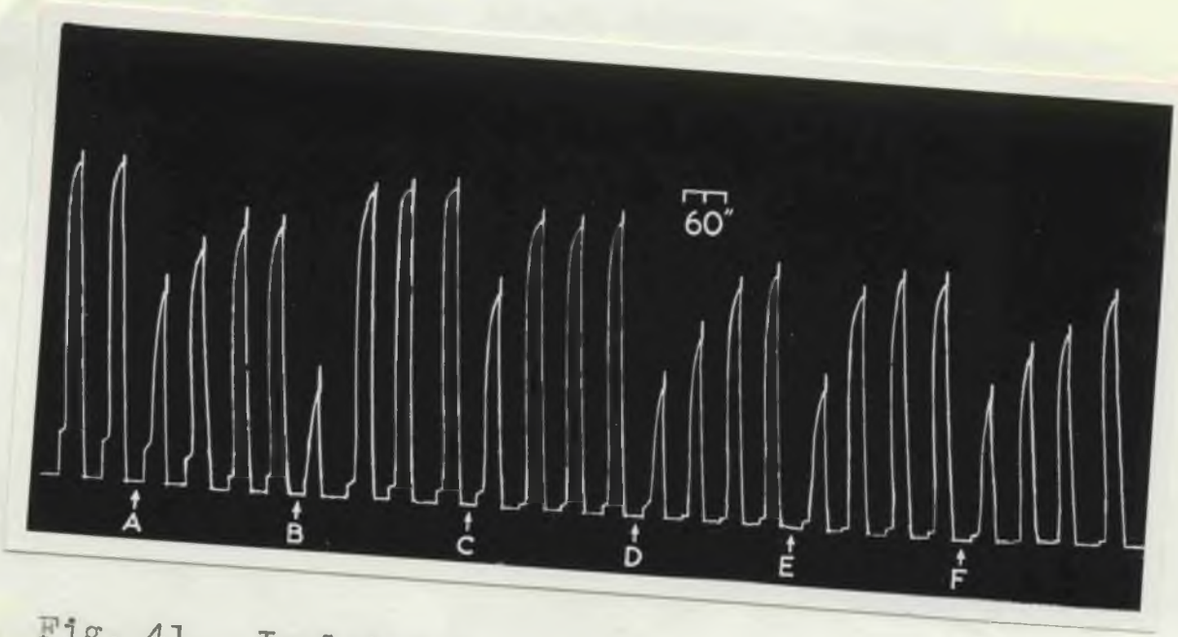


Fig. 41. Isolated frog rectus abdominis muscle.
All contractions were due to 0.01 mg/kg acetylcholine acting for 45 sec.
At A, C and E, compound E80 2, 4 and 6 µg/ml respectively
At B, D and F, tubocurarine 2, 1.5 and 1.0 µg/ml respectively.

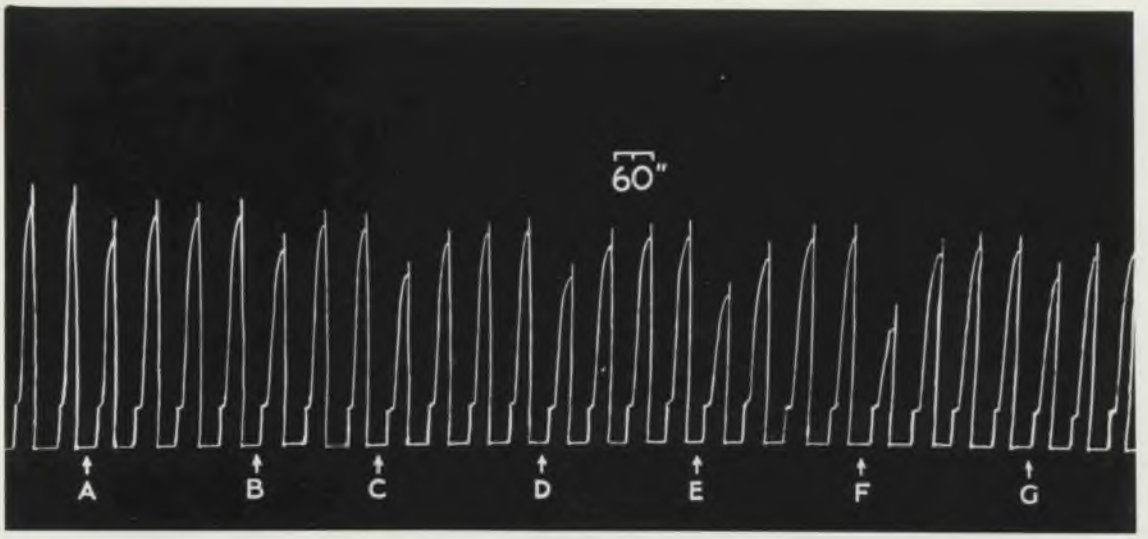


Fig. 42. Isolated frog rectus abdominis muscle.
All contractions were due to 0.01 mg/ml acetylcholine acting for 45 sec.
At A, C, E and G, tubocurarine 0.5, 1, 1.5 and 0.5 µg/ml respectively.
At B, D and F, compound E97 1, 2 and 3 µg/ml respectively.

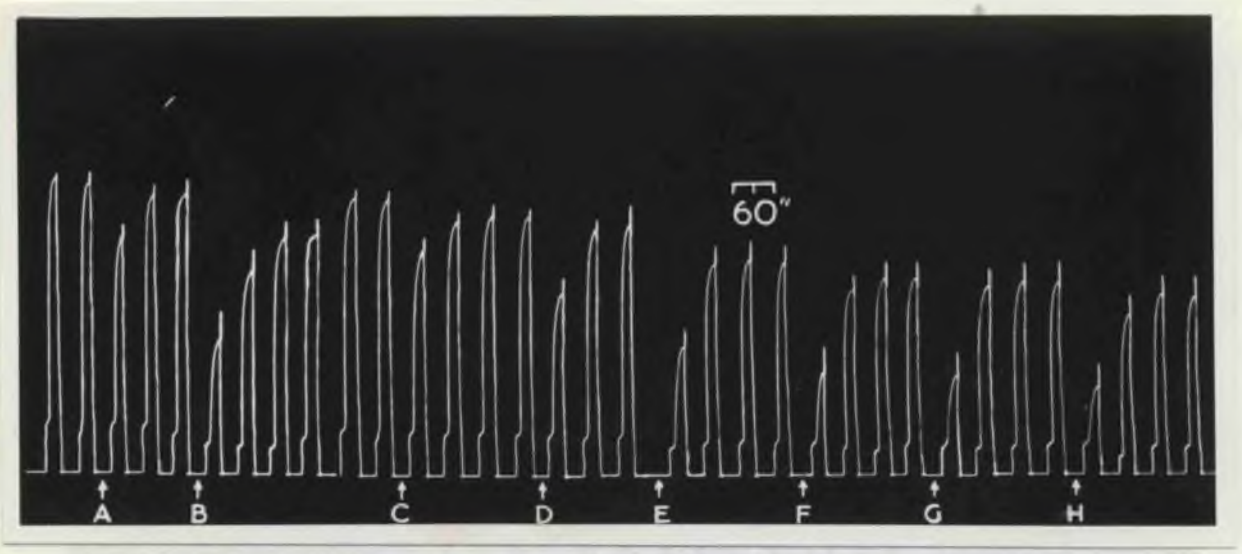


Fig. 43. Isolated frog rectus abdominis muscle.
All contractions were due to 0.025 mg/ml acetylcholine acting for 45 sec.
At A, D, E and G, compound E79 1, 1.5, 2.0 and 2.5 µg/ml respectively.
At B, C, F and H, tubocurarine 1.5, 0.5, 2.0 and 2.5 µg/ml respectively.

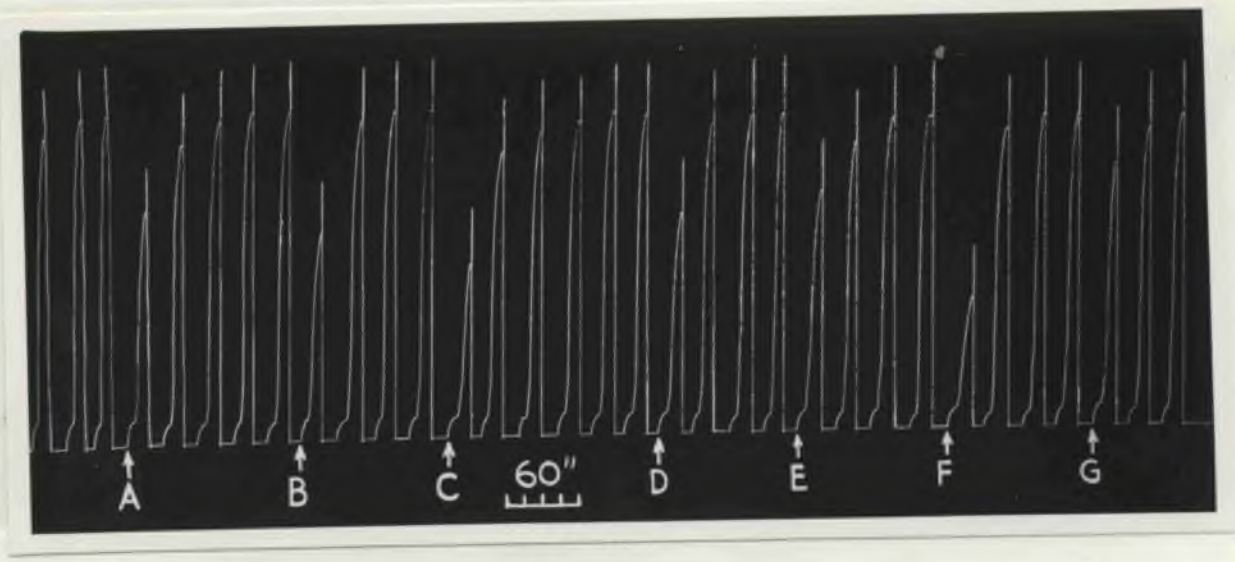


Fig. 44. Isolated frog rectus abdominis muscle.
All contractions were due to 0.01 mg/ml acetylcholine acting for 30 sec.
At A, C, E and G, tubocurarine 3.0, 4.0, 2.0 and 1.0 µg/ml respectively.
At B, D and F, compound E103 20.0, 10.0 and 30.0 µg/ml respectively.

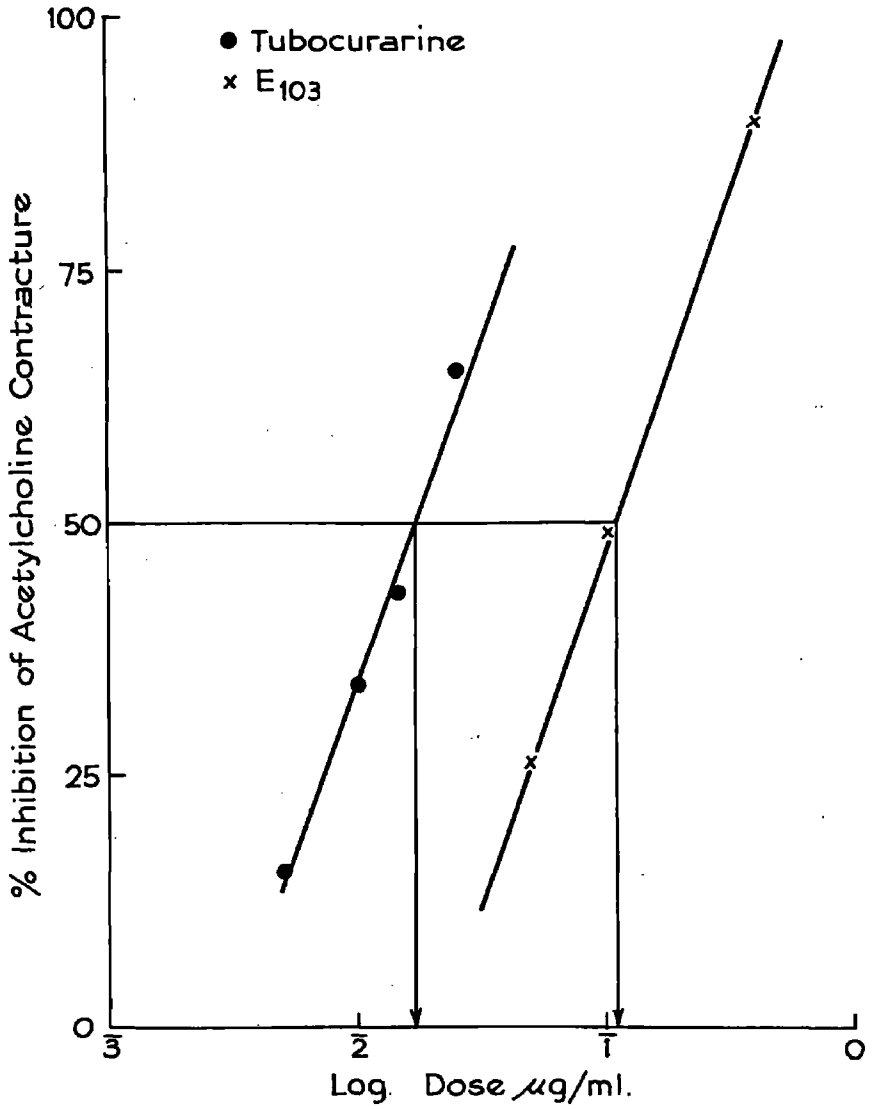


Fig. 45. Frog rectus abdominis muscle. The relative potencies of tubocurarine and compound E103 in inhibiting the contracture induced by acetylcholine.

Fig. 46. Isolated frog rectus abdominis muscle.
All contractions were induced by drugs acting for
2 minutes.
At A, C, E, G, I, K and M, decamethonium,
2.5, 1.0, 2.0, 6.0, 3.5, 3.0 and 1.5 µg/ml respectively.
At B, D, F, H, J, L and N, compound III,
8, 5, 10, 50, 75, 100 and 25 µg/ml respectively.

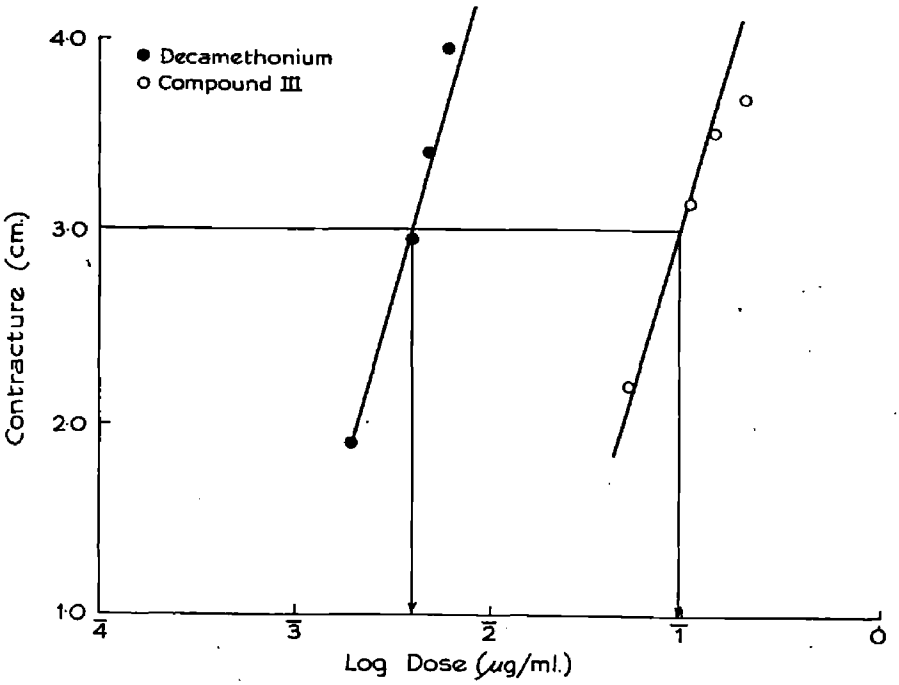


Fig. 47. Frog rectus abdominis muscle. Comparative estimation of the contracture-inducing properties of decamethonium and compound III.

TABLE XIII

A comparison of the potency of the compounds E77, E78, E79, E80, E96, E97, E98, E99, E100, E101, E102 and E103 with tubocurarine and of compounds I, II and III and decamethonium on the frog rectus abdominis muscle.

Compound Code Number	Approximate Molar Potency Tubocurarine = 100
E77	375
E78	77
E79	208
E80	26
E96	63
E97	72
E98	7
E99	4
E100	1
E101	2
E102	2.5
E103	10.5
	Approximate Molar Potency Decamethonium = 100
I	*
II	50
III	7.0

* Could not be estimated. Please see Fig. 48 opposite page 120.

Frog Rectus Abdominis Muscle.

Results obtained using this preparation confirmed the division of the compounds studied into depolarizing (Group 2) and non-depolarizing (Groups 1a, and 1c) agents.

None of the compounds tested in Groups 1a and 1c caused any direct stimulant effect. Each produced graded inhibitory effects on acetylcholine-induced (1 - 10 μg/ml) contractions, acting similarly to tubocurarine (Figs. 41, 42, 43 and 44). On this basis, a comparison of these drugs with tubocurarine was made. The potency of these compounds, as measured by the degree of inhibition of acetylcholine-induced contractions is shown in Table XIII. The potency of each compound was determined graphically as shown in the example (Fig. 45).

Compounds I, II and III (1.0, 0.2 and 1.0 mg/kg respectively) produced a contracture and augmented the effects of acetylcholine on this preparation. Fig. 46 shows typical examples of the contracture-inducing effects of these compounds. All were, however, weaker than decamethonium, the order of potency being decamethonium $>$ II $>$ III $>$ I (Fig. 47 and Table XIII). Compound I possessed very weak stimulant properties.

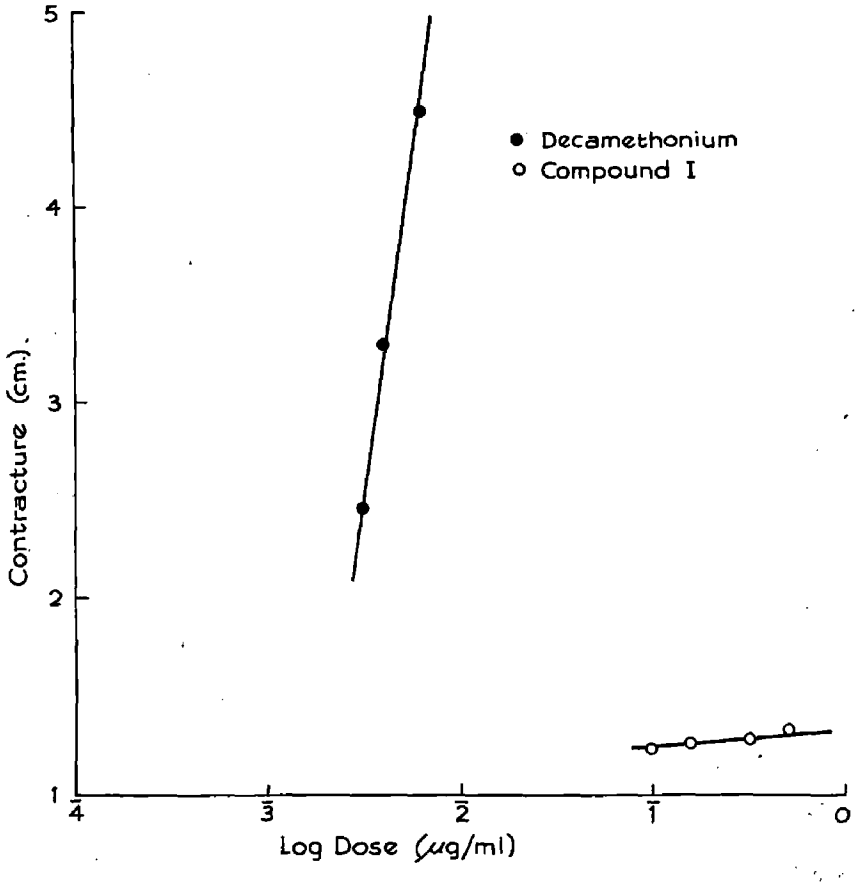


Fig. 48. Frog rectus abdominis muscle. Comparison of the contracture-inducing properties of decamethonium and compound I. It is obviously impossible to compare quantitatively the effects of each drug on this preparation.

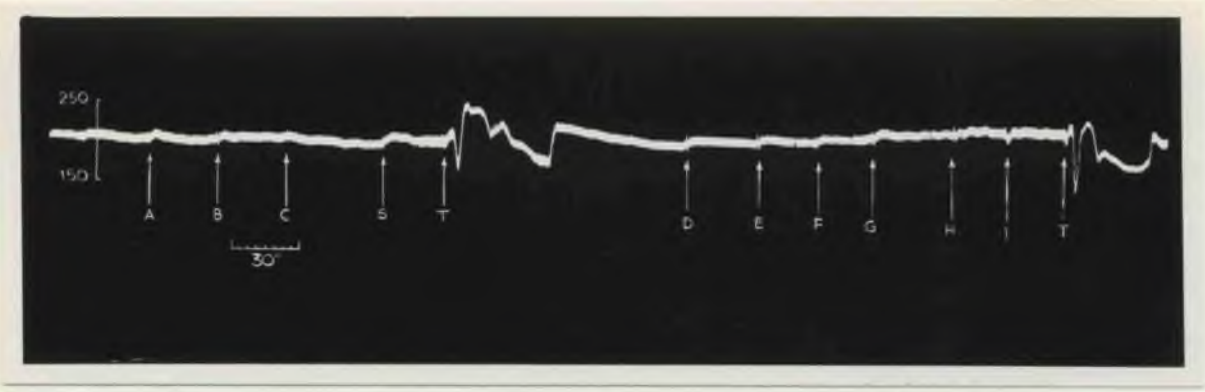


Fig. 49. Cat. Pentobarbitone anaesthesia.
Blood pressure record from common carotid artery.
Drugs administered intravenously. Vertical scale on left hand side indicates the blood pressure in mm of mercury.
At A, B and C, compound E91 0.5 mg/kg, 0.75 mg/kg and 1 mg/kg respectively.
At S, normal saline (0.9%), 4 ml.
At T, tubocurarine 1 mg/kg.
At D and E, compound E79 0.05 mg/kg and 0.1 mg/kg.
At F and G, compound E80 0.05 mg/kg and 0.1 mg/kg.
At H and I, compound E90 0.05 mg/kg and 0.1 mg/kg.

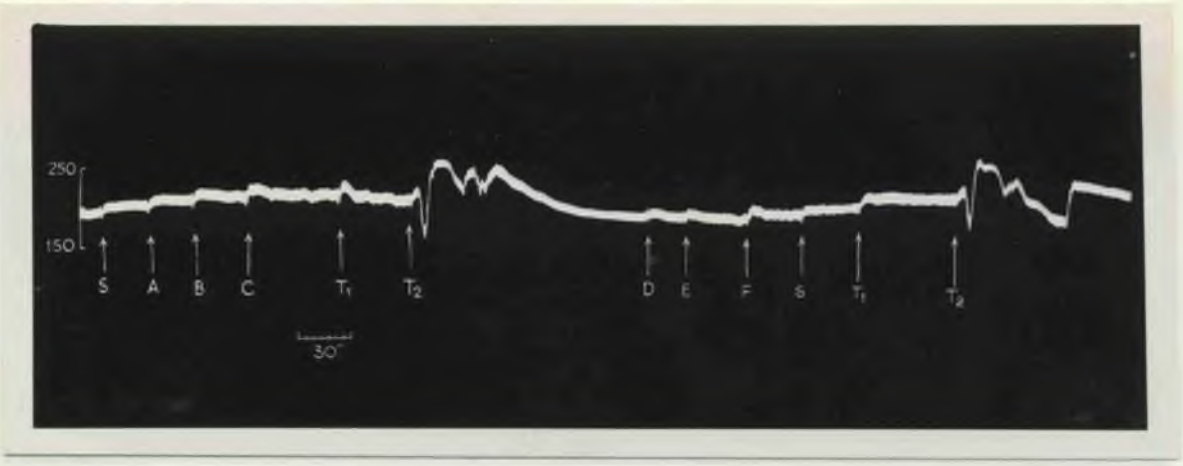


Fig. 50. Cat. Pentobarbitone anaesthesia. Blood pressure record from common carotid artery. Drugs administered intravenously. Vertical scale on left hand side indicates the blood pressure in mm of mercury.

At A, compound E97 0.1 mg/kg.

At B and C, compound E96 0.2 mg/kg and 1.0 mg/kg. respectively.

At T_1 and T_2, tubocurarine 0.5 mg/kg and 1.0 mg/kg respectively.

At D, E and F, compound E78 0.05 mg/kg, 0.15 mg/kg and 0.20 mg/kg respectively.

At S, normal saline (0.9%), 3 ml.

Inspection of the slopes of the log dose-response curves (Fig. 48), showed that a quantitative comparison of compound I and decamethonium was impossible. These results prompted a further investigation of the basic mode of action using the Ariëns' technique.

Results of Tests Other Than Those Designed to Investigate Neuromuscular Blocking Potency.

Effect on the Blood Pressure of the Anaesthetized Cat.

None of the compounds in Groups 1a, 1b and 1c produced any significant rise or fall in the blood pressure of the cat even when administered in doses in excess of those required to induce complete neuromuscular block (Figs. 49 and 50). Tubocurarine (0.5 - 1.0 mg/kg), on the other hand, produced a significant fall in blood pressure when tested on the same preparation, attributable, it has been suggested, to a block of transmission of sympathetic vasoconstrictor impulses at the ganglion synapse (Landmesser, 1947) and to the liberation of histamine (Paton, 1951). The more potent muscle relaxant compounds in Group 1d, which were reported to possess comparatively weak ganglion blocking properties, produced no significant changes in blood pressure levels and in larger doses produced a slightly hypertensive effect which might have been associated with anoxia.

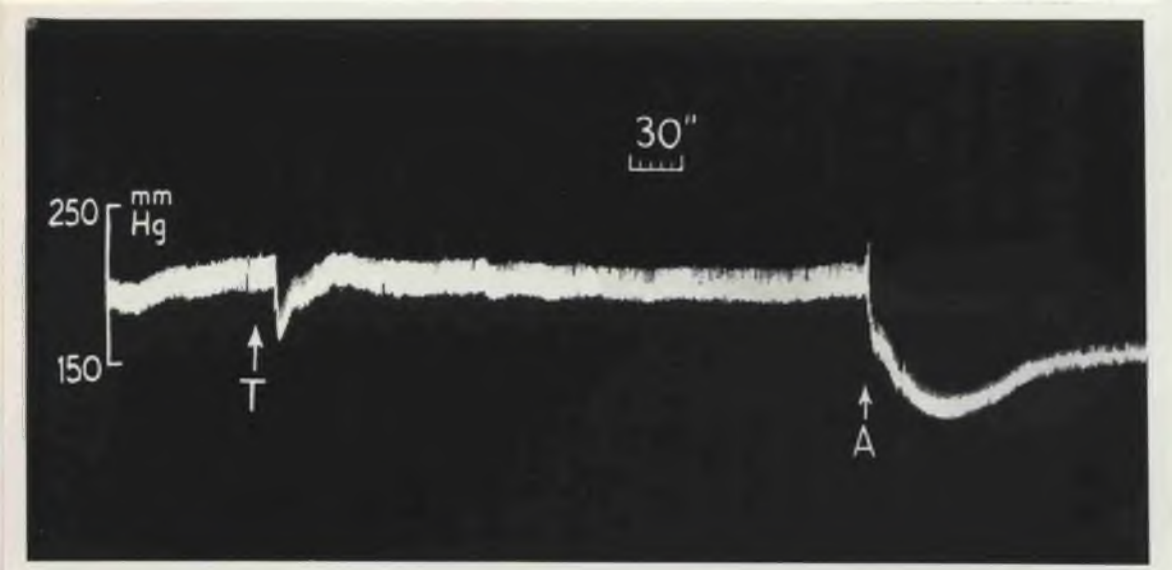


Fig. 51. Cat. Pentobarbitone anaesthesia. Blood pressure record from common carotid artery. Drugs administered intravenously. Vertical scale, on left hand side, indicates the blood pressure in mm of mercury.

At T, tubocurarine 0.10 mg/kg.

At A, compound F. & P. 16677 20 mg/kg.

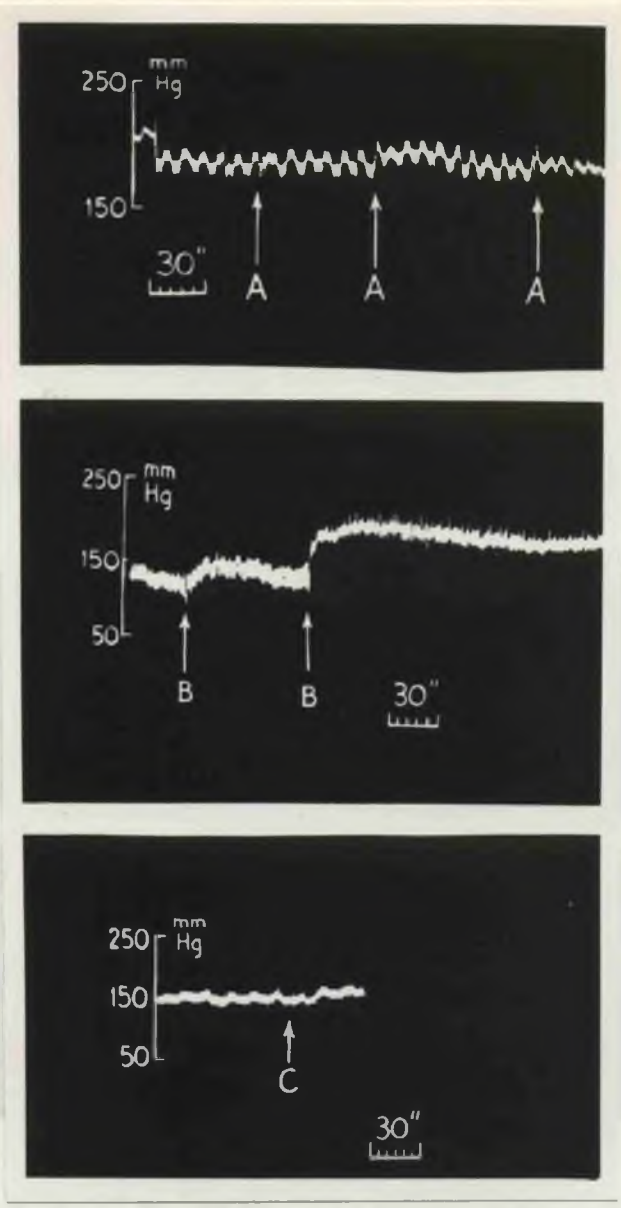


Fig. 52. Cat. Pentobarbitone anaesthesia. Blood pressure record from common carotid artery. Drugs administered intravenously. Vertical scale, on left hand side, indicates the blood pressure in mm of mercury. Recordings from three different animals.
At A, (from left to right) compound I 0.1, 0.5 and 0.6 mg/kg respectively.
At B, (from left to right) compound II 0.05 and 0.25 respectively.
At C, compound III 0.1 mg/kg.

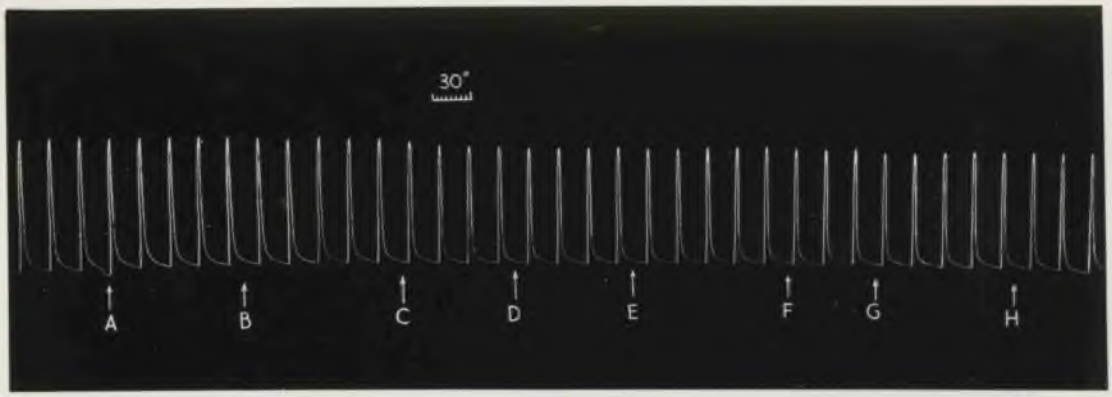


Fig. 53. Cat. Pentobarbitone anaesthesia.
Contractions of the nictitating membrane elicited at 3 minute intervals by preganglionic stimulation of the superior cervical nerve trunk at a frequency of 1200 impulses/min, 10 V and 1.0 msec for 15 sec.
Drugs administered intravenously 30 sec before stimulation.
At A, B and C, compound E94 0.5, 1.0 and 2.0 mg/kg respectively.
At D, E, F and G, compound E95 0.5, 1.0, 1.5 and 2.0 mg/kg respectively.
At H, compound E96 0.5 mg/kg.

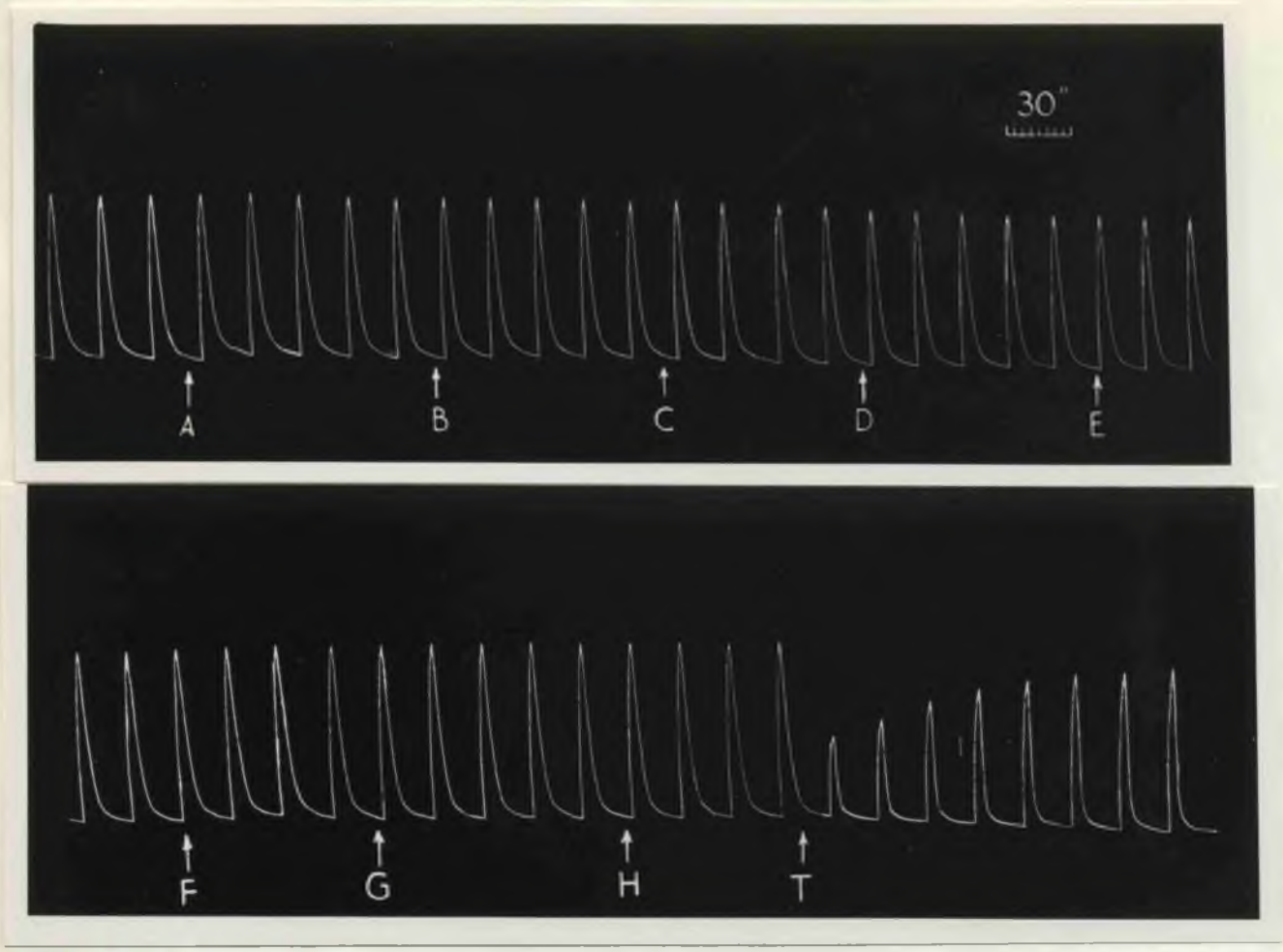


Fig. 54. Cat. Pentobarbitone anaesthesia. Contractions of the nictitating membrane elicited at 3 minute intervals by preganglionic stimulation of the superior cervical nerve trunk at a frequency of 1000 impulses/min 10 V and 1.0 msec for 15 sec. Drugs administered intravenously 30 sec before stimulation.

At A and B, compound E77 0.5 mg/kg and 1 mg/kg respectively.
At C and D, compound E78 0.5 mg/kg and 1 mg/kg respectively.
At E, compound E79 0.5 mg/kg.
At F, compound E80 0.5 mg/kg.
At G, compound E96 0.5 mg/kg.
At H, compound E97 1 mg/kg.
At T, tubocurarine 0.5 mg/kg.

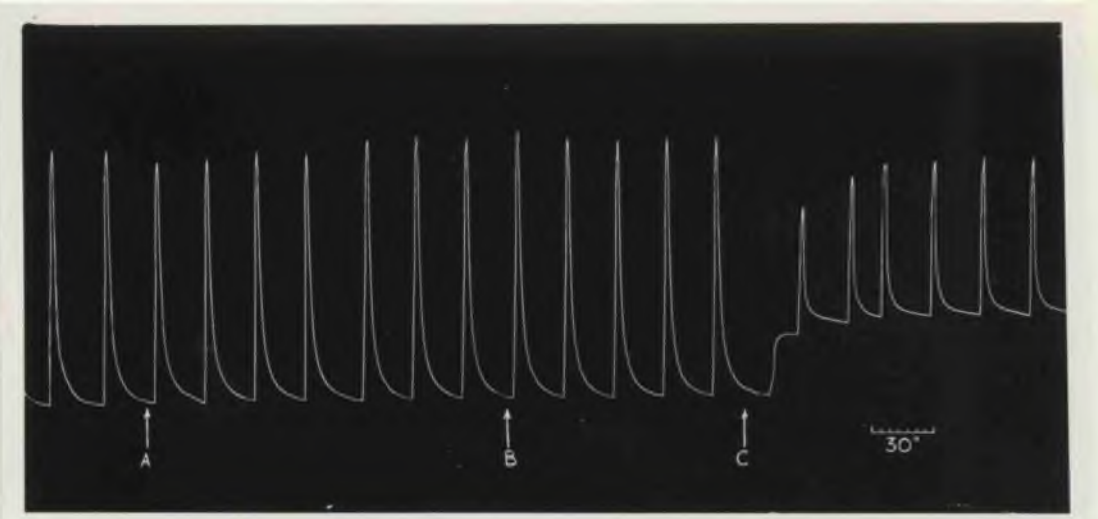


Fig. 55. Cat. Pentobarbitone anaesthesia. Contractions of the nictitating membrane elicited at 3 minute intervals by preganglionic stimulation of the superior cervical nerve trunk at a frequency of 800 impulses/min, 10 V and 1.0 msec for 15 sec. Drugs administered intravenously 30 sec before stimulation.

At A, compound I 0.5 mg/kg.

At B, compound III 0.3 mg/kg.

At C, compound II 0.5 mg/kg.

On the other hand, those compounds possessing potent ganglion blocking properties, for example compounds F. & P. 16575, F. & P. 16677 and F. & P. 16678, produced, as expected, a significant hypotensive action on this preparation (Fig. 51).

The depolarizing compounds of Group 2, in doses sufficient to produce partial neuromuscular block (0.05 - 0.5 mg/kg) exerted no significant effect upon the blood pressure, but in larger doses (0.2 - 0.5 mg/kg) compound II produced a prolonged rise (Fig. 52).

Estimation of Ganglion Blocking Activity.

Cat Nictitating Membrane Preparation. Sympathetic Ganglion Blockade.

Following injection of compound E94 (1 mg/kg), a slight reduction in the height of the contraction of the nictitating membrane was observed (Fig. 53) but none of the other compounds in Groups 1a, 1b, 1c or 2, produced any sympathetic ganglion blocking effect (Fig. 54). Compound II (0.5 mg/kg) produced a prolonged contraction of the nictitating membrane (Fig. 55). In contrast to these effects, tubocurarine (0.5 mg/kg) always produced a marked reduction in the height of contraction of the nictitating membrane (Fig. 54).

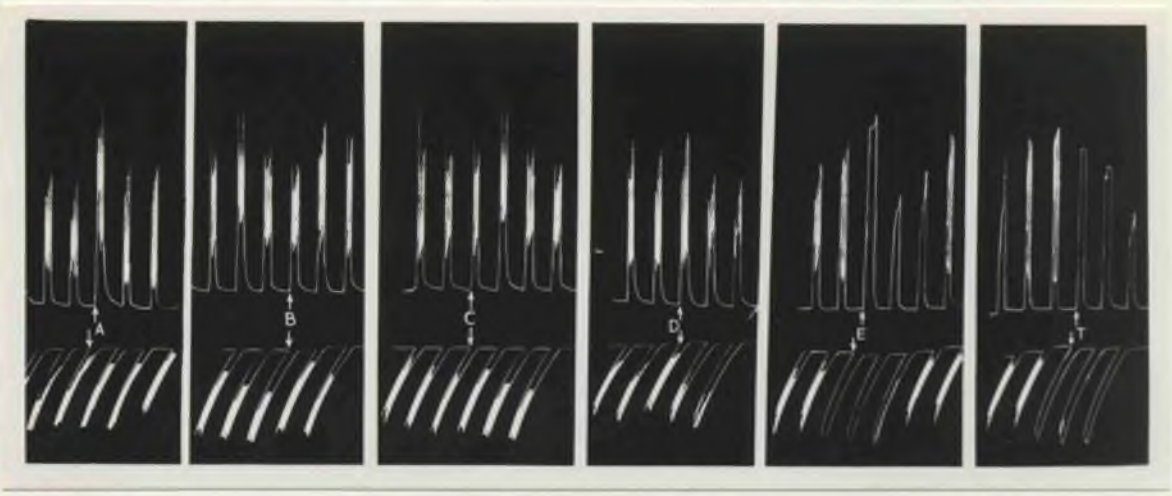


Fig. 56. Guinea Pig ileum. Composite picture. The effect of drugs on contractions of the longitudinal muscle layers (upper trace) and on peristalsis (lower trace) recorded by Trendelenberg's method (1917). Volume of bath used was 50 ml.

At A, compound I 0.5 mg.

At B, compound II 0.1 mg.

At C, compound III 0.5 mg.

At D, decamethonium 0.1 mg.

At E, hexamethonium 0.2 mg.

At T, tubocurarine 0.5 mg.

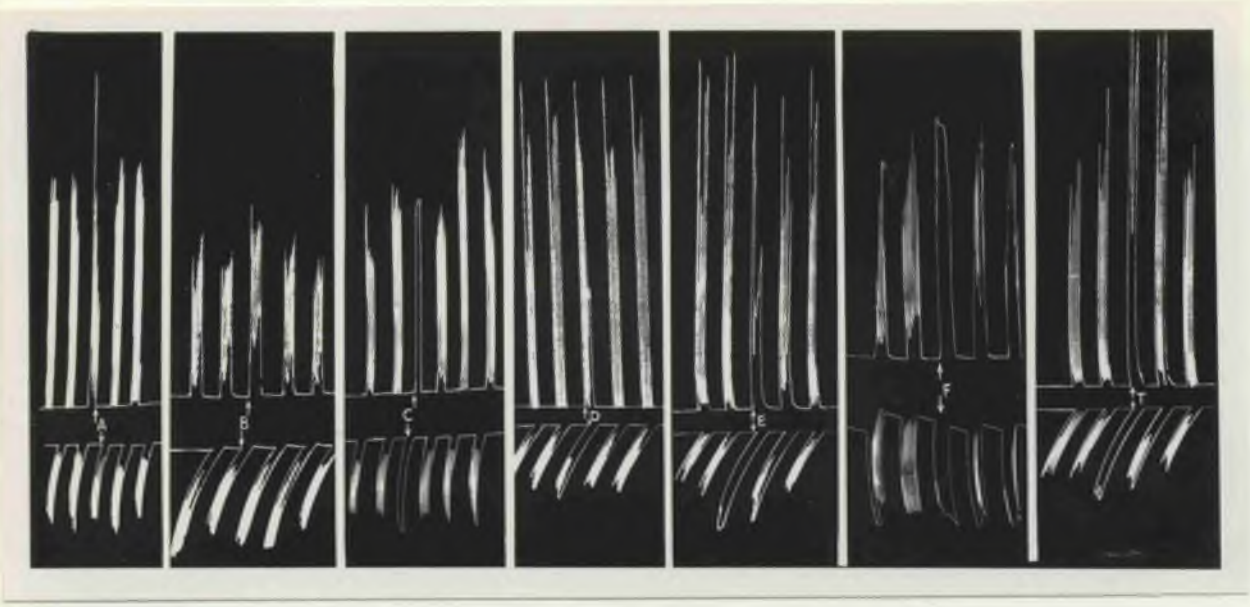


Fig. 57. Guinea Pig ileum. Composite picture. The effect of drugs on contractions of the longitudinal muscle layers (upper trace) and on peristalsis (lower trace) recorded by Trendelenberg's method (1917). Volume of bath used was 50 ml.

At A, compound E77 1 mg.

At B, compound E79 2 mg.

At C, compound E91 1 mg.

At D, compound E95 2 mg.

At E, hexamethonium 1 mg.

At F, compound E98 20 mg.

At T, tubocurarine 1 mg.

Compounds in Group 1d have all been reported to possess ganglion blocking properties when tested on a similar preparation. A full account of their properties has been provided by Fakstorp and his colleagues (1954, 1955, 1956, 1957a,b,c).

Guinea Pig Ileum - Parasympathetic Ganglion Blocking Activity.

None of the compounds in Group 1a (1 - 3 mg) or Group 2 (0.1 - 0.5 mg) (Fig. 56) exerted any ganglion blocking activity on this preparation. Compounds E77 (1 mg), E78 (3 mg) and I (0.5 mg) slightly enhanced the longitudinal contractions of the gut without interfering with peristalsis. Among the compounds in Group 1b, the tris-onium derivatives E91 (1 mg) and E95 (2 mg) blocked peristaltic movements (Fig. 57). This action was especially marked in the former but none of the other compounds in this group exerted any ganglion blocking action. In Group 1c, compound E98, in very high doses (10 - 20 mg) showed a ganglion blocking action but none of the other compounds in this group were active on this preparation.

Experiments Upon Mice.

Estimation Of The Approximate Median Paralyzing Dose (PD50).

Compounds in Groups 1a, 1b and 2 were tested on

TABLE XIV

A comparison of the Potency and Toxicity of compounds E77, E78, E80, E96, E97, E91, E93, E94 and E95 and Tubocurarine, in mice.

Name or Code No. of Compound	Approx. Mean Median Paralyzing Dose (P.D.50) mg/kg ± S.E.M.	Approx. Mean Lethal Paralyzing Dose (L.D.50) mg/kg ± S.E.M.	Therapeutic Index $\frac{\text{(L.D.50)}}{\text{(P.D.50)}}$	Comparative Molar Potency (Tubocurarine = 100)
E77 (5)	0.21 ± 0.004	0.61 ± 0.04	2.91	165
E78 (5)	0.36 ± 0.02	1.2 ± 0.10	3.33	132
E79 (4)	0.20 ± 0.02	0.66 ± 0.06	3.30	174
E80 (4)	0.20 ± 0.02	0.76 ± 0.11	3.80	165
E96 (5)	0.25 ± 0.01	0.52 ± 0.02	2.08	146
E97 (4)	0.24 ± 0.01	0.74 ± 0.05	3.08	151
E91 (6)	No Paralysis with 20 mg/kg	-	-	-
E93 (6)	2.15 ± 0.35	14.6 ± 1.52	6.79	11
E94 (7)	0.90 ± 0.06	3.1 ± 0.22	3.44	22
E95 (8)	2.0 ± 0.16	8.0 ± 0.70	4.0	13
Tubocurarine (7)	0.25 ± 0.01	0.52 ± 0.40	2.08	100

The number, in brackets, in the first column indicates the number of groups of animals tested.

this species. All the compounds tested induced restlessness and increased movement after approximately five to ten minutes. A period of quiet then ensued, prior to the loss of the animals' ability to retain their positions on the inclined plane and the onset of paralysis. Recovery from paralysis was normally complete within twenty minutes. The time of onset of action and the period required for recovery from paralysis were greater for the compounds in Group 2 than for those in Group 1.

Estimation of The Approximate Median Lethal Dose (LD50).

The median lethal dose was determined in a similar way. Following an intraperitoneal injection of a minimal lethal dose of the compounds in Groups 1a and 1b, there was a rapid development of a typical flaccid paralysis followed by failure of respiration. Respiratory collapse, in almost all cases, preceded cardiac arrest.

With compounds in Group 2, death was preceded by clonic convulsions and an extensor spasm of the hindlegs. The therapeutic ratio $(\frac{LD50}{PD50})$, as shown in Table XIV, was highest for compound III and lowest for decamethonium.

The median lethal paralyzing dose for compounds

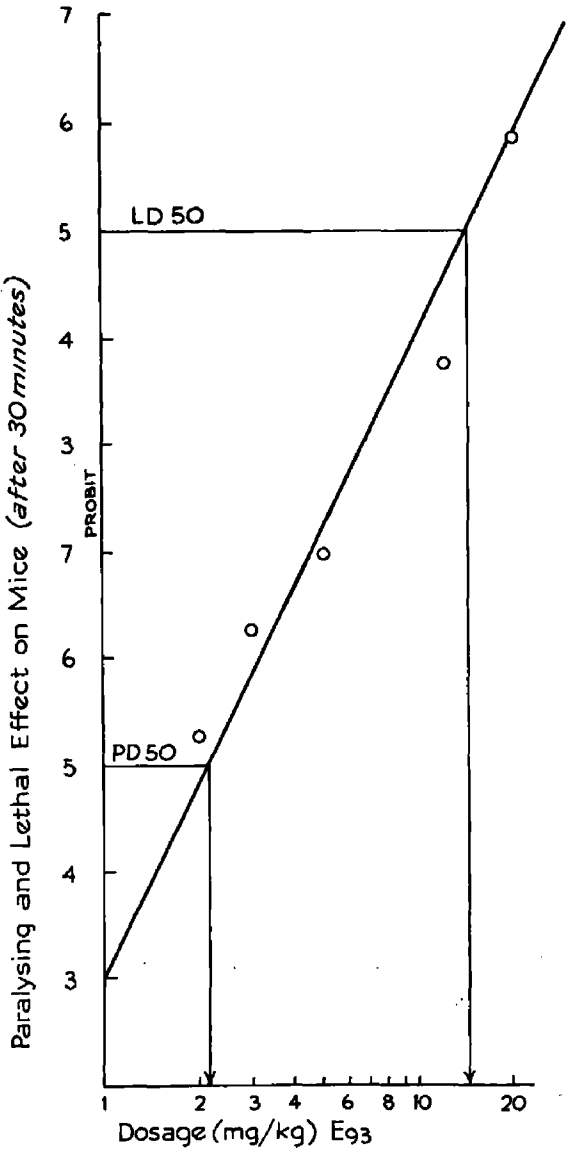


Fig. 58. Diagram of the graphical estimation of the PD50 and the LD50 for compound E93 (Miller & Tainter, 1944). From original graph PD50 = 2.15 $\pm$ 0.27; LD50 = 14.6 $\pm$ 1.52. (Please see accompanying text).

TABLE XV

A comparison of the Potency and Toxicity of compounds I, II and III and Decamethonium in mice.

Compound and no. of Groups tested	Approx. Mean Median Paralyzing Dose (P.D.50) mg/kg ± S.E.M.	Approx. Mean Lethal Paralyzing Dose (L.D.50) mg/kg ± S.E.M.	Therapeutic Index $\frac{(L.D.50)}{(P.D.50)}$	Comparative Molar Potency (Decamethonium = 100)
I (5)	4.1 ± 0.40	11.1 ± 0.60	2.7	44
II (4)	4.1 ± 0.43	9.3 ± 0.60	2.3	44
III (6)	3.1 ± 0.58	24.3 ± 2.8	7.8	55
Decamethonium (8)	1.9 ± 0.11	3.8 ± 0.18	2.1	100

TABLE XVI

Method of calculating the mean P.D.50, L.D.50 and their standard errors (S.E.M.) in mice for compound E95 (Miller & Tainter, 1944).

Group No.	Dose (mg/kg)	Number Paralyzed (%)	Number Killed (%)	Probit Value
1	1	40		4.75
2	2	90		6.28
3	5		10	3.72
4	6		20	4.16
5	7		40	4.75
6	8		60	5.25

From original graph, P.D.50 = 2.0, L.D.50 = 8.0.

Standard Error (P.D.50)

Probit 4 = 1.0

Probit 6 = 2.0

2S = 1.0

No. treated (N) = 20

$$S.E.M. = \frac{2S}{\sqrt{2N}} = \frac{1.0}{6.324} = 0.16$$

Standard Error (L.D.50)

Probit 4 = 5.6

Probit 6 = 11.0

2S = 5.4

No. treated (N) = 80

$$S.E.M. = \frac{2S}{\sqrt{2N}} = \frac{5.4}{8.944} = 0.60$$

$$P.D.50 = 2.0 \pm 0.16$$

$$L.D.50 = 8.0 \pm 0.70$$

in Group 1d, has been estimated by Fakstorp and Pedersen (1957b). The results of compounds in Groups 1a, 1b and 2 were calculated by the graphical method described by Miller and Tainter (1944) and are shown in Table XV. The percentage paralyzed or killed in each group, at each dose level, was plotted on a logarithmic scale, as a probit value, against the dose (mg/kg) of drug producing the effect. The estimated PD50 was that dose corresponding to 50% (probit 5.0) and could be read directly from the graph in mg/kg. The median lethal dose (LD50) was determined in a similar fashion.

In order to estimate the standard error of the PD50 and the LD50, two additional values were read from the graph (Fig. 58 and Table XVI). These corresponded to the dose producing 16% and 84% (probits 4.0 and 6.0) of the effect, the difference between these values being the estimated increment necessary to increase the effects by two probits in this dose range (2S). The approximate average standard error was determined using the following formula

Approximate standard error of the mean (S.E.M.) $= \frac{2S}{\sqrt{2N}}$

The value N indicated the total number of animals in the groups which would be expected to show

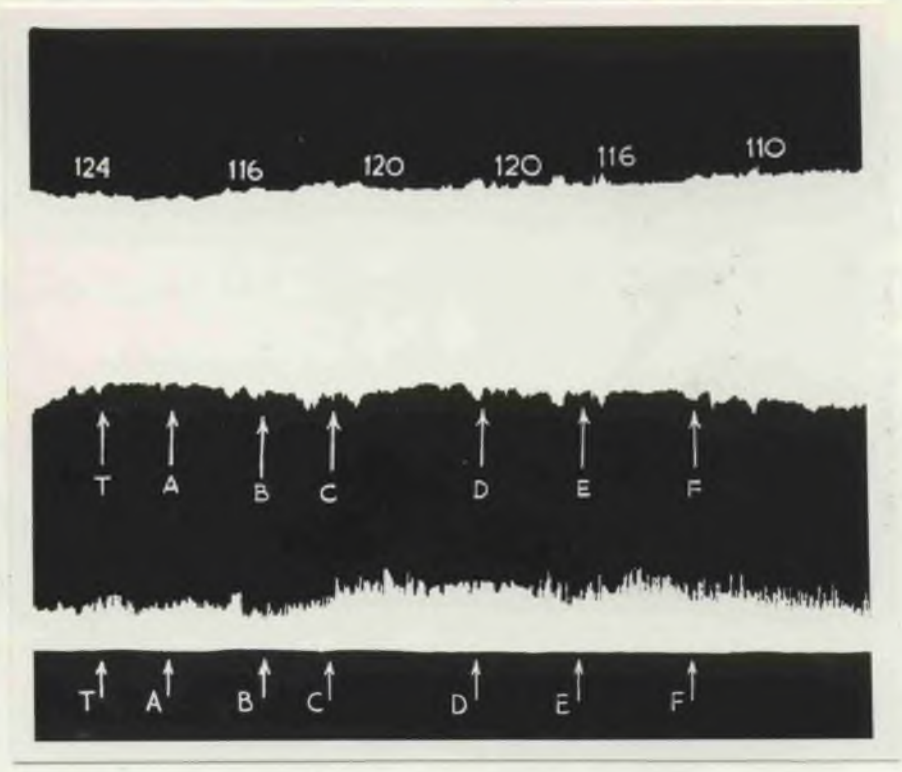


Fig. 59. Isolated rabbit heart. Upper tracing shows heart rate, lower tracing, the cardiac outflow. Numbers represent the heart rate in beats/min.

At T, tubocurarine 1 mg.

At A and D, compound E96 1 mg.

At B and C, compound E97 1 mg.

At E, compound E77 1 mg.

At F, compound E80 1 mg.

results between 6.7% and 93.3% (probits 3.50 and 6.50) as described by Miller and Tainter (1944).

Experiments on the Isolated Perfused Rabbit Heart.

In view of the qualitative and quantitative aspects of the block produced by compounds in Groups 1 and 2, only drugs in Group 1a were believed to possess clinical potentialities. Accordingly, their effects on the isolated rabbit heart preparation were investigated. None of these compounds, in doses from 1.0 to 3.0 mg, produced any significant depressant or accelerating effect on the rate or amplitude of the heart. An example of a characteristic tracing is given in Fig. 59.

From the tests carried out, no toxic effects on the heart muscle of the rabbit could be detected. While it is impossible to forecast, from these experiments, the effect of these drugs on human cardiac muscle, the presence of toxic effects on this preparation would eliminate the possibility of their being clinically investigated.

Experiments on the Respiration of the Anaesthetized Rabbit and Cat.

The clinical importance of the action of muscle relaxants on the muscles controlling respiration, prompted an investigation of the effect of the more

potent compounds upon respiration in experimental animals. However, on account of the lack of material, only the compounds in Group 1a and Group 2 were investigated, the former on the rabbit and the latter on the cat.

Irrespective of the species employed, the continuous intravenous infusion of the compounds under test (E77, E79, E80, E96, E97 and tubocurarine, 0.2 mg/ml; I, 1 mg/ml; II, III and decamethonium, 0.1 mg/ml) always resulted in respiratory paralysis. Spontaneous respiratory movements only returned following the use of artificial respiration although complete recovery was attained in every case.

Each drug was tested several times on different animals. The respiratory paralyzing dose was that administered during the first experiment on each animal. Subsequent doses, less than the first, were not included. The respiratory paralyzing doses of the compounds in Group 1a were similar to their head drop doses (H.D.D.) and all were more potent than tubocurarine in this respect. On the other hand, the doses of compounds I, II and III, required to induce complete respiratory paralysis in the cat, were less than those required to paralyze respiration in the same species.

TABLE XVII

A comparison of the individual and mean respiratory paralyzing doses of compounds E77, E79, E80, E96 and E97 and tubocurarine on the urethane-anaesthetized rabbit and of compounds I, II and III and decamethonium on the pentobarbitone-anaesthetized cat.

Name or Code No. of Compound	Respiratory Paralyzing Dose		Potency (Tubocurarine = 100)
	Individual (mg/kg)	Average (mg/kg)	
E77	0.22, 0.24, 0.21	0.22	139
E79	0.10, 0.16, 0.14	0.13	235
E80	0.21, 0.26, 0.33	0.27	107
E96	0.26, 0.25, 0.30	0.27	119
E97	0.27, 0.24, 0.30	0.27	119
Tubocurarine	0.16, 0.26, 0.24	0.22	100
			Potency (Decamethonium = 100)
I	1.5, 1.6, 1.1	1.4	8
II	0.36, 0.36, 0.36	0.36	31
III	0.42, 0.35, 0.45	0.41	24
Decamethonium	0.09, 0.11, 0.12	0.11	100

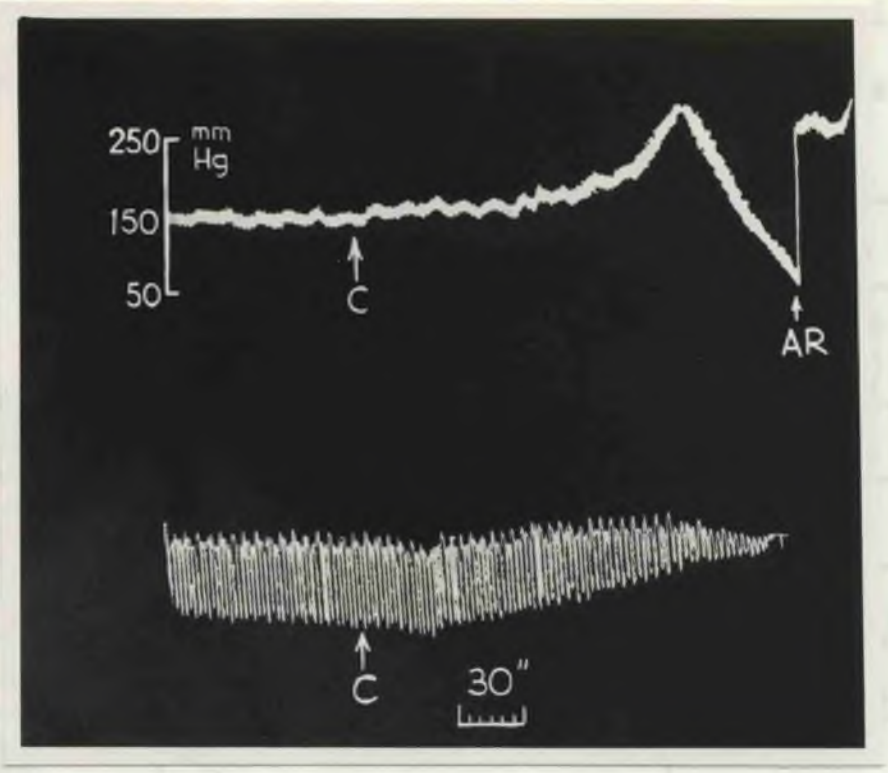


Fig. 60. Cat. Pentobarbitone anaesthesia.
Record of respiratory movements from the epigastrium.
Vertical scale indicates the blood pressure in mm of mercury.
At C, intravenous infusion of compound III 0.20 mg/ml.
At AR, artificial respiration commenced.
Rate of infusion of drug was 0.75 ml/mm.
Rate of respiration of animal at commencement of experiment was 18/min.

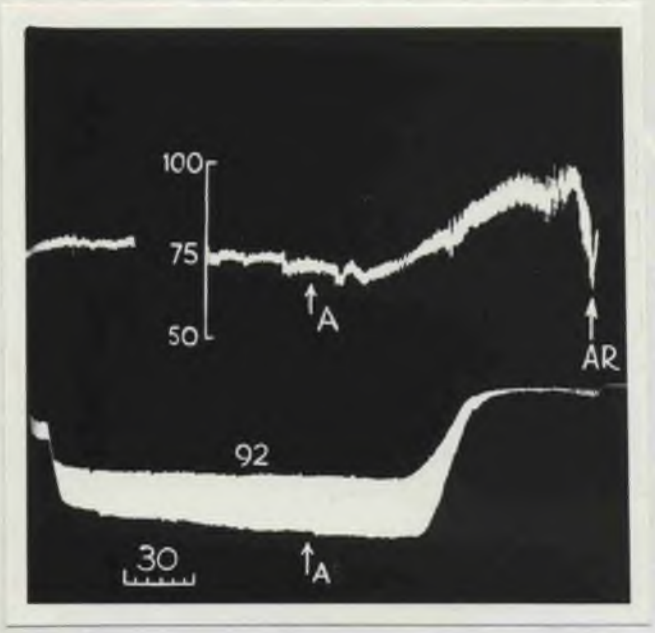


Fig. 61. Rabbit. Urethane anaesthesia.
Record of respiratory movements using the method of Gaddum (1941). Vertical scale indicates the blood pressure in mm of mercury.
At A, intravenous infusion of compound E79 0.20mg/ml.
At AR, artificial respiration commenced.
Rate of infusion of drug was 0.75 ml/min.
Rate of respiration of animal at commencement of infusion was 92/min.

TABLE XVIII

Compounds investigated using the Ariëns' procedure. The compounds investigated using this technique include tubocurarine dimethyl ether and tubocurarine (formulae opposite page 56) compounds E77, E78, E79, E80, E98, E99, E100, E101, E102, E103, I, II, III and decamethonium (formulae in Tables II and III opposite page 99) and oxydipentonium and compound E151 (formulae given below).

Name or Code No. of Compound	Basic Chemical Structure	R	n	An-ion	Type of Block
Oxydi-pentonium	$R_3\overset{+}{N}-(CH_2)_n-O-(CH_2)_n-\overset{+}{N}R_3$	CH_3	5	Cl^-	Depolar-izing
E151	$R_3\overset{+}{N}-(CH_2)n-\underset{R\ R}{\overset{+}{N}}-(CH_2)_n-\overset{+}{N}R_3$	CH_3	6	I^-	Non-De-polar-izing

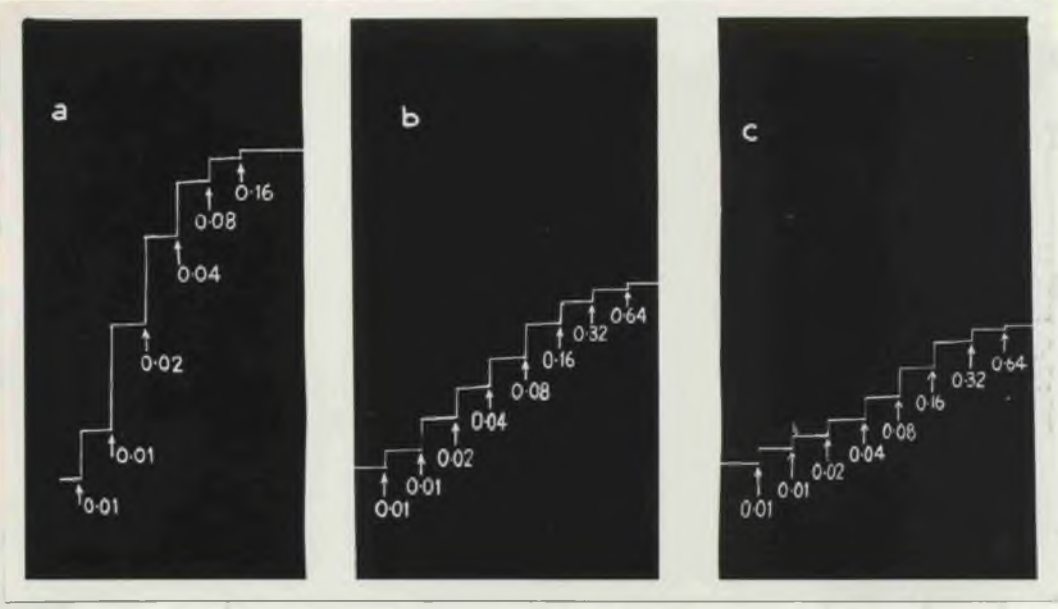


Fig. 62. Frog rectus abdominis muscle preparation. Ariens' procedure. Experimental recordings of contractures from three different pieces of tissue induced by;

At a, decamethonium (2 μmoles/ml)

At b, compound II (2 μmoles/ml)

At c, compound III (2 μmoles/ml)

Volume of bath = 10 ml.

Numbers indicate the volume of drug solution added.

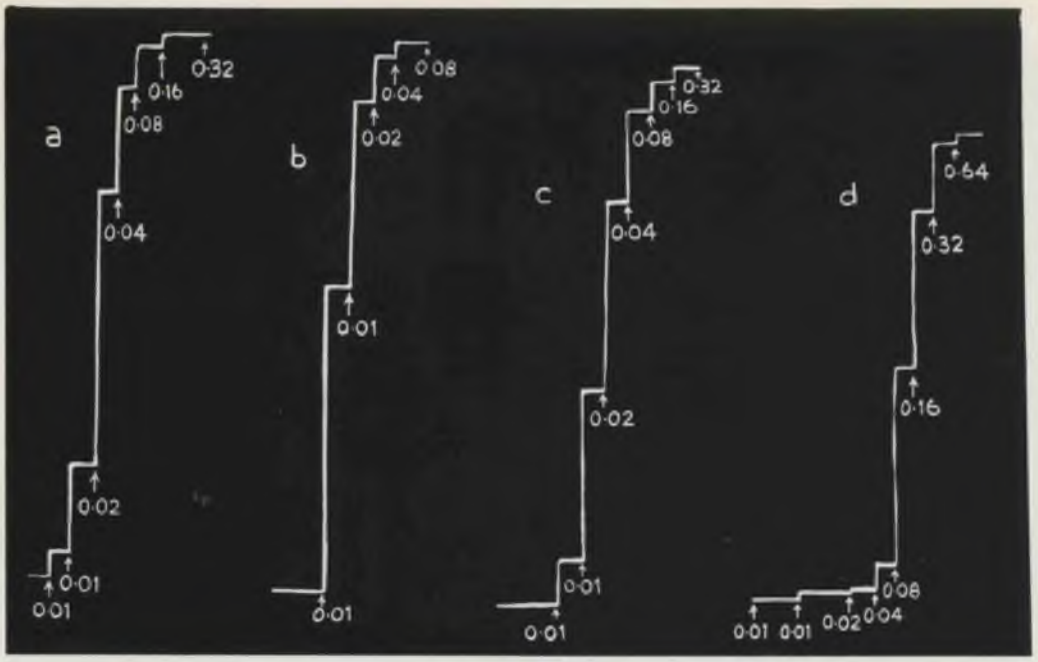


Fig. 63. Frog rectus abdominis muscle preparation. Ariëns' procedure. Experimental recordings of contractures induced by;

At a, decamethonium alone (2 μmoles/ml)

At b, decamethonium (10 μmoles/ml) in the presence of compound E101 (100 μmoles/l)

At c, decamethonium (10 μmoles/ml) in the presence of compound E101 (600 μmoles/l)

At d, decamethonium (10 μmoles/ml) in the presence of compound E101 (1000 μmoles/l).

Numbers indicate the volume of decamethonium solution added.

All the compounds of Group 2 were less potent than decamethonium. The results of these experiments are shown in Table XVII and characteristic tracings shown in Figs. 60 and 61.

Experiments on the Isolated Frog Rectus Abdominis Muscle Using Ariëns' Technique.

The chemical structures of the compounds whose mode of action was investigated using the experimental procedure first described by Ariëns (Ariëns & de Groot, 1954) are shown in Table XVIII. Cumulative log dose-response curves were constructed for agonists alone and in the presence of constant doses of non-depolarizing muscle relaxants. The results, which can be most conveniently expressed graphically, are shown in Figs. 64 to 71.

On the basis of these graphs, a classification of the drugs under investigation was made according to the original scheme devised by Ariëns and his associates (Van Rossum et al. 1958). Typical experimental recordings are shown in Figs. 62 and 63.

The interaction of a substance A with a hypothetical receptor system (γ_o) containing R receptors, is represented by the equation

$$E_A = \frac{\gamma_{oa}}{\frac{K_A}{[A]} + 1}$$

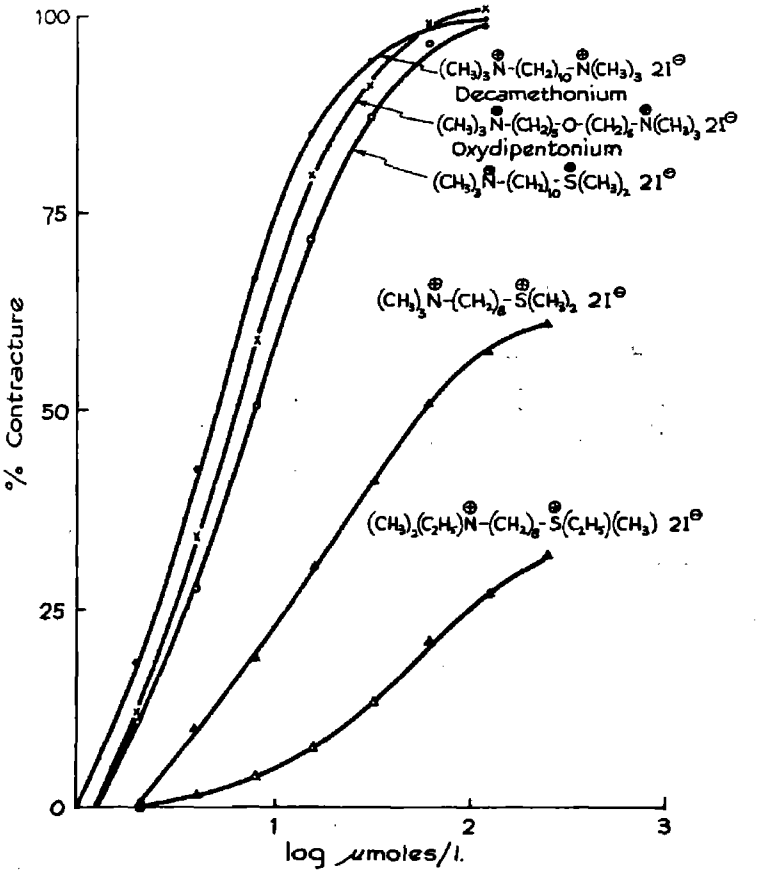


Fig. 64. Cumulative log dose response curves for decamethonium, oxydipentonium and compounds I, II and III performed on the isolated frog rectus abdominis muscle. The variation in the maximum height produced and the displacement of the curves along the abscissa indicate the differences in the intrinsic activity and the affinity values of these compounds.

where E_A is the effect of substance A, K_A the dissociation constant of the complex R A, and α the intrinsic activity of drug A whose concentration is represented by $[\ ]$.

The maximum effect of a compound (E maximum), is proportional to its intrinsic activity. The dose of a compound producing a constant fraction of the maximum response, for example 50%, is proportional to the value of the dissociation constant of the drug-receptor complex,

Fig. 64 represents a comparison of the agonistic, contracture-inducing properties of decamethonium whose intrinsic activity and affinity value was arbritarily assigned unity, oxydipentonium and compounds I, II and III. The order of addition of the drug and decamethonium to the bath was determined from a table of random numbers assigning equal chances of coming first or second to either decamethonium or the drug with which it was being compared. Oxydipentonium and compound II were each compared with decamethonium in twenty experiments while compounds I and III were similarly investigated ten times. Using Student's t test (Snedecor, 1956) no significant difference in the intrinsic activity value of decamethonium, oxydipentonium and compound II

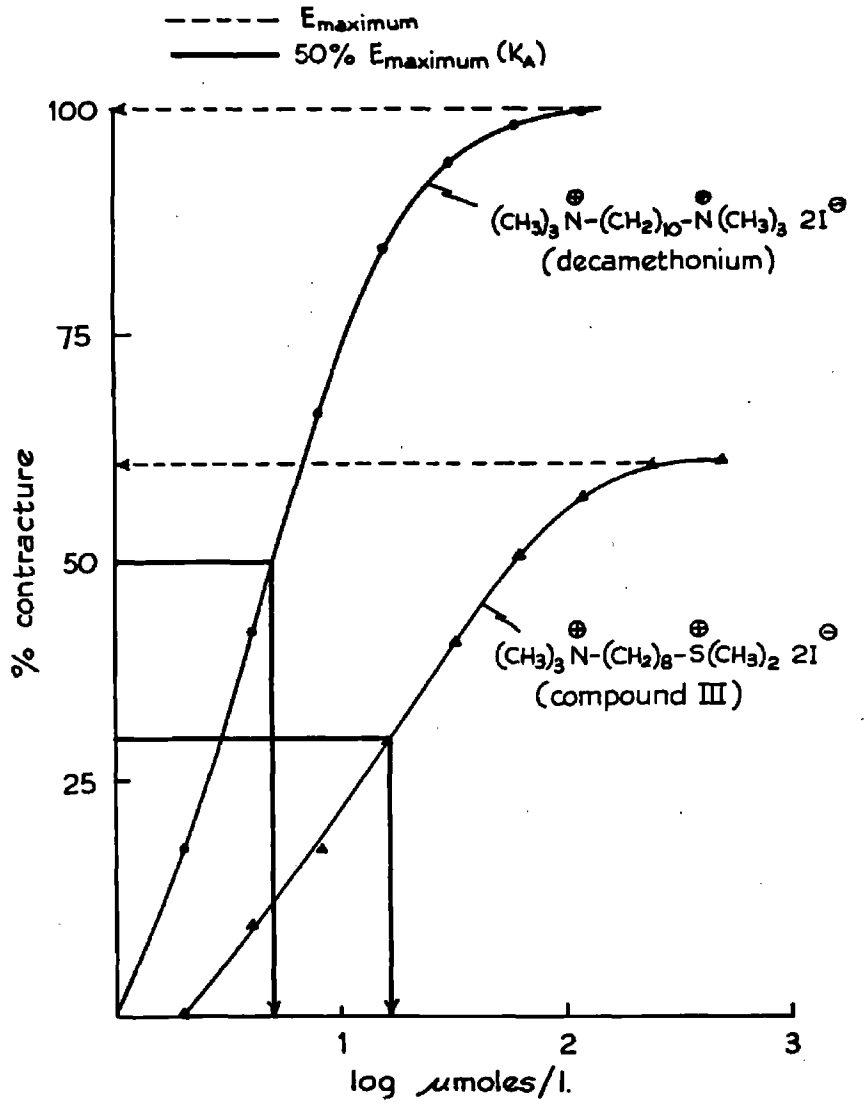


Fig. 65. Frog rectus abdominis muscle. Method of comparing the intrinsic activity and affinity values of decamethonium and compound III.

From original graph,

Emaximum (decamethonium) = 1.0 (arbritarily assigned)

Emaximum (compound III) = 0.6.

50% Emaximum, K_A (decamethonium) = antilog 0.72 = 5.25

50% Emaximum, K_A (compound III) = antilog 1.21 = 16.22

Affinity = $\frac{1}{K_A}$; Intrinsic activity is proportional to Emaximum (Ariëns & de Groot, 1954).

($0.2 > P > 0.1$) was revealed. There were, however, significant differences between the intrinsic activity values of decamethonium and compound I ($P < 0.001$) and between decamethonium and compound III ($P < 0.001$) as indicated by the reduction in the maximum contractural height produced.

The value of the dissociation constant (K_A) for each drug was also calculated by determining that dose which produced 50% of the maximum observed biological response (Ariëns & de Groot, 1954). The reciprocal of this value represented the affinity of the compound. Fig. 65 shows the calculation of intrinsic activity and affinity values for decamethonium and compound III. These values, for all five compounds, appear in Table XIX and are discussed on page 150.

The comparatively low intrinsic activity of compound I, confirmed by observations using the conventional frog rectus assay procedure, prompted a further investigation into the basic mode of action of this drug. Accordingly, a series of log dose-response curves were constructed for different molar doses of this compound and the response of each dose level expressed as a percentage of that observed using a solution of 1 mole/ml. The shape of the curves obtained,

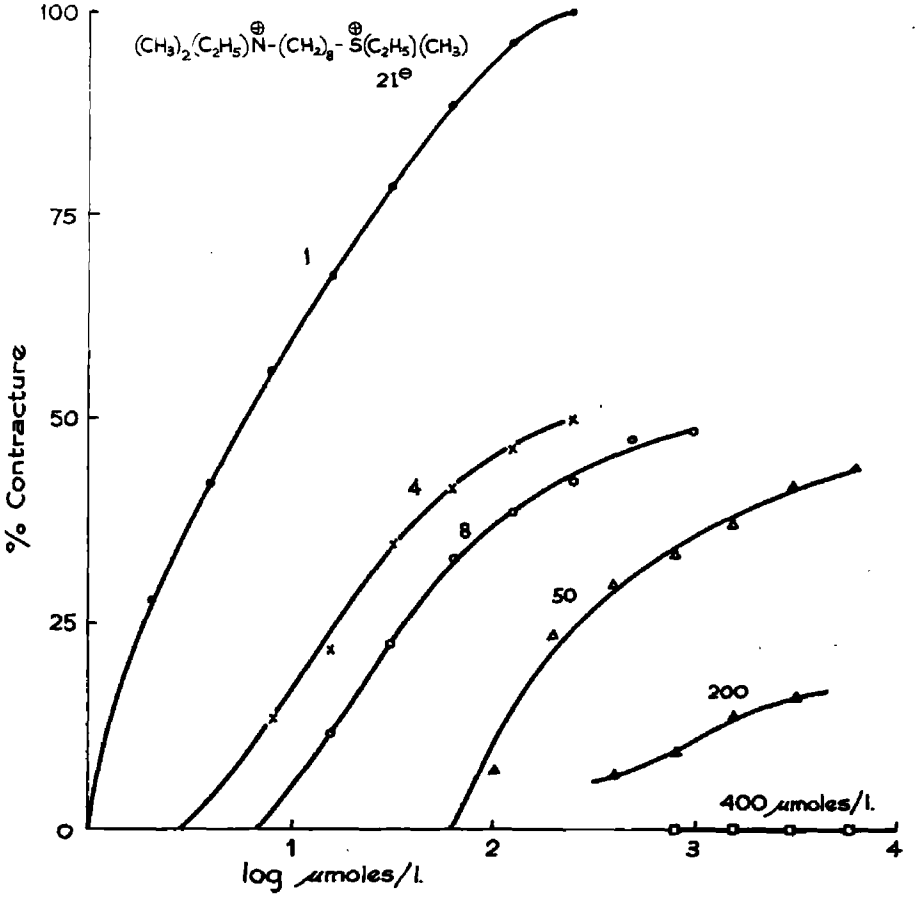


Fig. 66. Cumulative log dose-response curves for various concentrations of compound I performed on the isolated frog rectus abdominis muscle. The agonistic and auto-inhibiting effects of this drug are observed as a gradual decrease in the maximum height and a displacement of the curves along the abscissa.

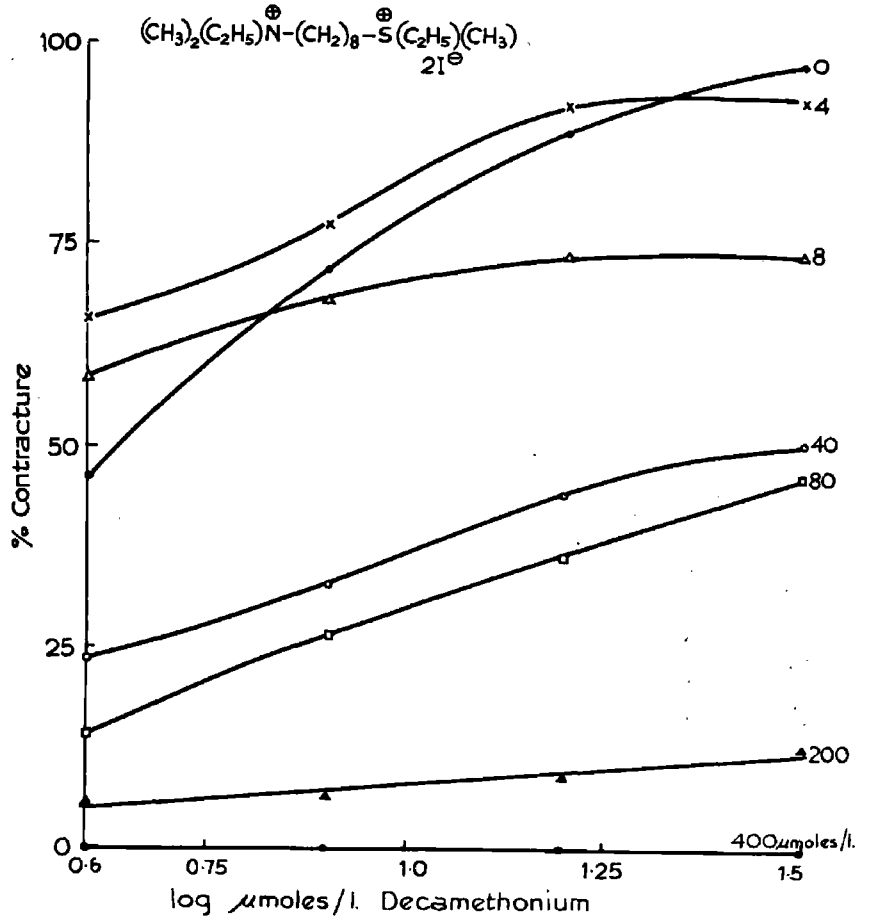


Fig. 67. Cumulative log dose-response curves for decamethonium performed on the isolated frog rectus abdominis muscle alone (0 µmoles/l) and in the prescence of constant concentrations (4, 8, 40, 80, 200 and 400 µmoles/l) of compound I. The agonistic and non-competitively antagonistic properties of compound I appear as a contracture and a decline of the maximum height of the curves.

shown in Fig. 66, indicated that this drug possessed both agonistic and non-competitively antagonistic blocking properties. At higher dose levels, the auto-antagonistic action of the drug was apparent. Investigation of these properties, by constructing a series of log dose-response curves for decamethonium in the presence of different doses of compound I (Fig. 67) confirmed these properties. The dose of compound I employed in any one experiment remained constant throughout. Due to insufficient material, it was impossible to construct hyperbolic log dose-response curves for compound I alone, in order to confirm, quantitatively, the nature of the auto-interaction produced. The preponderance of experimental evidence obtained, however, is not incompatible with the view that compound III possessed auto-inhibitory, non-competitive properties (Ariëns et al. 1957).

According to the Ariëns' classification (Van Rossum et al. 1958), all the remaining compounds possessed Type 2, non-depolarizing properties. The dose of each drug chosen for initial investigation was related to its molar potency compared to tubocurarine, as based on experiments carried out using the conventional

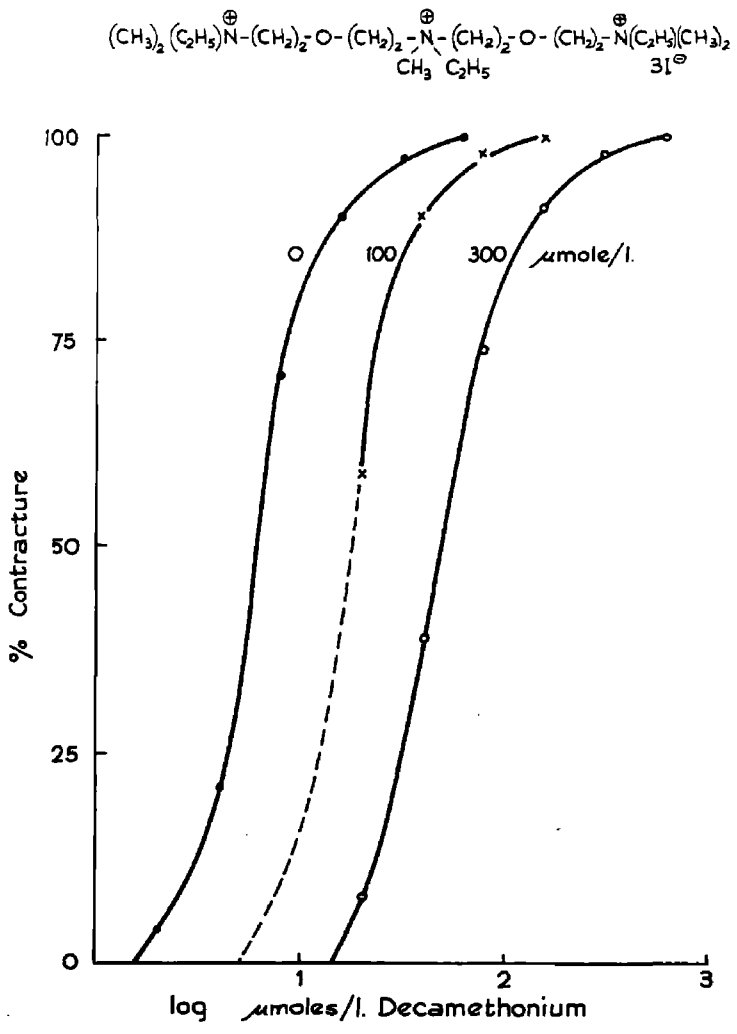


Fig. 68. Cumulative log dose-response curves for decamethonium performed on the isolated frog rectus abdominis muscle, alone (0 µmoles/l) and in the presence of constant concentrations (100 and 300 µmoles/l) of compound E101. The competitively antagonistic properties of compound E101 appear as a displacement of the curves along the abscissa unaccompanied by any reduction in the maximum height.

frog rectus assay method. The dose of tubocurarine initially used (1 μmole/l) was that employed by Ariëns (Van Rossum et al. 1958). Subsequently whole multiples of this dose of tubocurarine and of the other compounds were investigated.

The compounds tested included tris- and tetra-onium compounds containing one or two ether oxygen functions together with several corresponding non-ether homologues. Of these, none possessed any contracture-producing properties on the frog rectus abdominis muscle preparation but when placed in the bath prior to the addition of decamethonium, all displaced the log dose-response curve along the abscissa without affecting either the slope or the maximum observed contractural response. Thus, in the presence of constant doses of these compounds, the decamethonium log dose-response curve suffered a paralled displacement along the abscissa.

A typical example of these effects is shown in Fig. 68.

The interaction of depolarizing and non-depolarizing compounds has been discussed by Ariëns and de Groot (1954). The mathematical relationship

derived to represent this interaction has been expressed as $\frac{E\ max}{E_{AB}} = \frac{K_A}{K_B} \times \frac{[B]}{[A]} + 1$ ($E\ max$ = maximum effect, E_{AB} = combined effect of A and B) where K_A and K_B are the dissociation constants of the drug-receptor complex produced by the depolarizing agent A, $[A]$, and the non-depolarizing compounds B, $[B]$, respectively. From this equation, if parallel log dose-response curves are obtained experimentally for an agonist alone, and in the presence of constant doses of an antagonistic drug, the term $\frac{[B]}{[A]}$ must remain constant. That is, the observed parallelism is related to alteration in the rates of dissociation of the respective drug-receptor complexes involved. The ratio $\frac{[B]}{[A]}$, determined for doses producing 50% of the maximum response, has been termed the 'inhibition index' (Ariëns & de Groot, 1954) and is constant when a truly competitive antagonism between two drugs prevails. In consequence, for the interaction of such compounds, the inhibition indices may be used as a measure of the dissociation constants, or the affinities, of the compounds concerned. When several non-depolarizing compounds are investigated using the same depolarizing compound, for example decamethonium, since K_A for the latter is

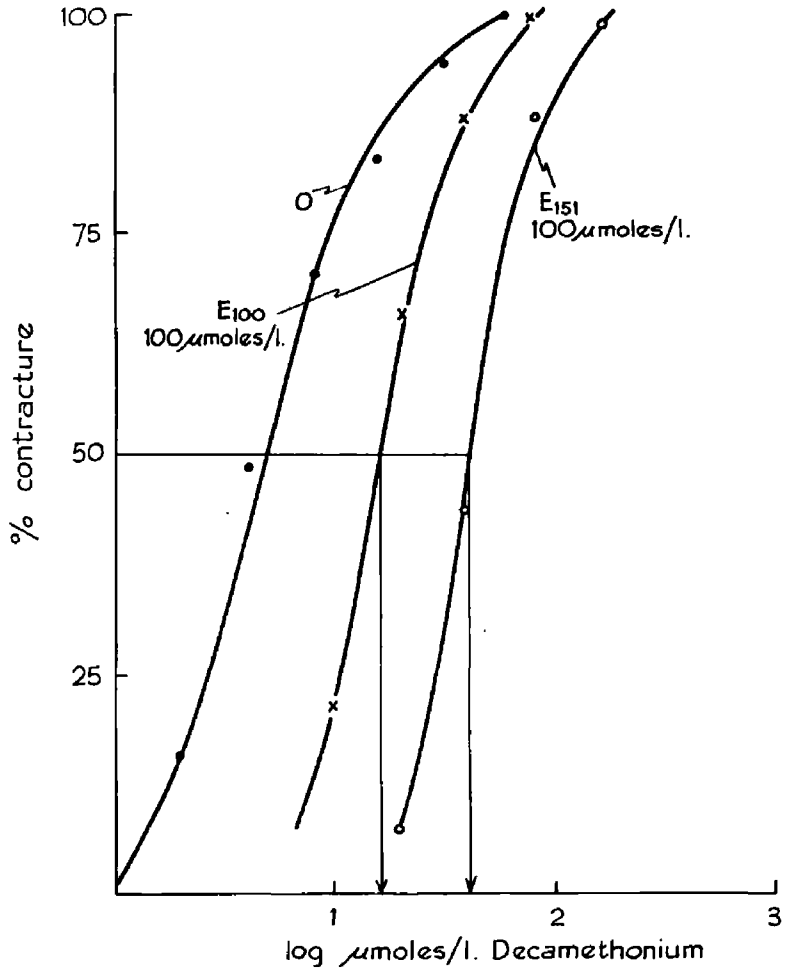


Fig. 69. Frog rectus abdominis muscle.

Method of comparing the 'inhibition indices' of compounds E100 and E151.

From original graph,

$$\text{Inhibition Index (E100)} = \frac{100}{\text{Antilog } 1.18} = \frac{100}{15.14} = 6.6$$

$$\text{Inhibition Index (E151)} = \frac{100}{\text{Antilog } 1.63} = \frac{100}{42.66} = 2.3$$

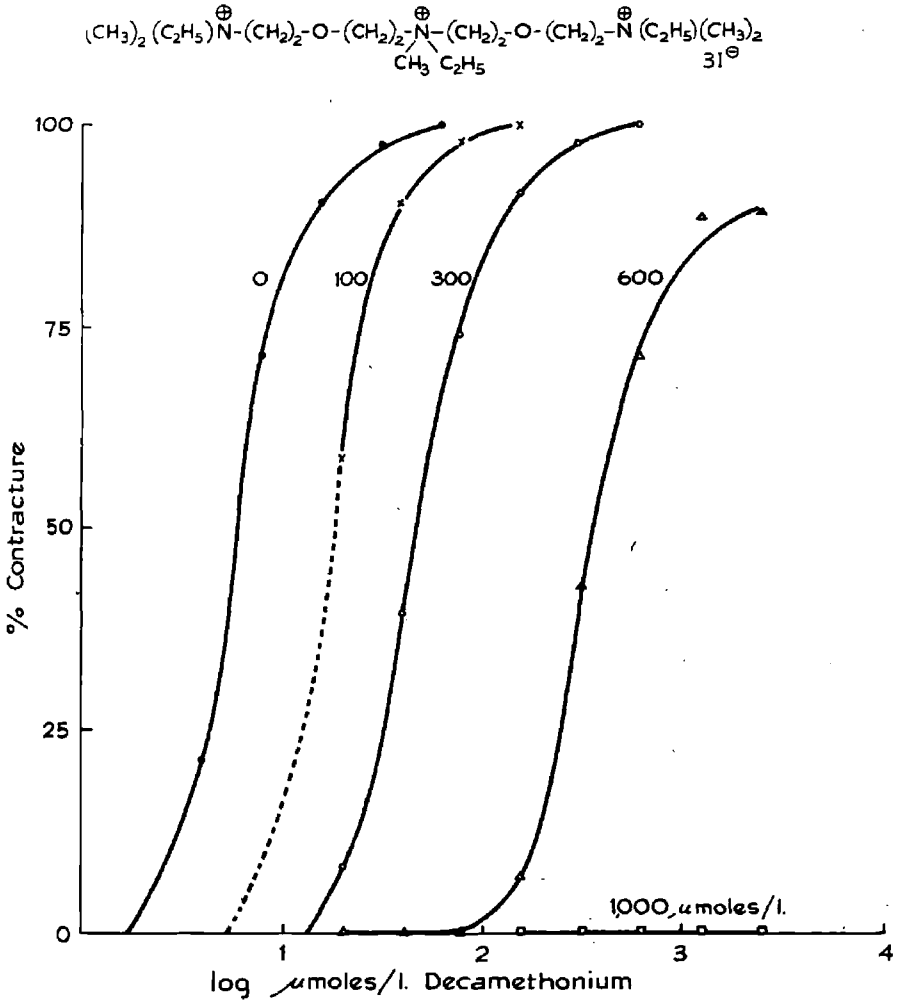


Fig. 70. Cumulative log dose-response curves for decamethonium, performed on the isolated frog rectus abdominis muscle, alone (0 µmoles/l) and in the presence of constant concentrations (100, 300, 600 and 1000 µmoles/l) of compound E.101. The competitively antagonistic properties of compound E101 in lower concentrations (100 and 300 µmoles/l) gives way to non-competitively antagonistic effects at higher concentrations (600 and 1000 µmoles/l) as shown by the decline in the curves and the decrease in the maximum height.

constant, the 'inhibition indices' are inversely proportional to the affinities between the receptor and the non-depolarizing compounds. Accordingly, 'inhibition indices' for these compounds in Group I, tested by the Ariëns procedure, were calculated on this basis and the mean of at least three individual results taken in each case. An example of the method of calculating the 'inhibition indices' for compounds E100 and E151 is given in Fig. 69. The results are shown in Table XX and discussed on page *150*.

When doses of these drugs were used, in excess of those normally required to completely inhibit acetylcholine-induced contractural responses, differences in the shape and height of the decamethonium log dose-response curves were observed. In addition to a non-parallel shift of the curves along the ordinate, a decline in the maximum height of the contractural response was also observed. The shape of these curves was characteristic of a non-competitive antagonism on the basis of the Ariëns' classification (Van Rossum et al. 1958). An example of the transition from a competitively antagonistic to a non-competitively antagonistic drug is shown in Fig. 70.

The role of the ether oxygen function was

investigated by comparing the degree of displacement of the decamethonium log dose-response curve produced by equimolar doses of the ether-containing and the related non-ether compounds. In addition to oxydipentonium and decamethonium, four pairs of compounds were selected for this investigation. These were compounds E77 and E79, E78 and E80, E100 and E151, together with tubocurarine dimethyl ether and tubocurarine. All the compounds were employed in doses at which they antagonized decamethonium competitively. A similar qualitative effect was thus produced by all eight compounds, but quantitative variations in the degree of displacement of the decamethonium log dose-response curve were observed between the ether and the non-ether derivatives. The tetra-onium monoether-containing compounds E77 and E78 respectively had a greater affinity for the decamethonium receptor site than the corresponding non-ether derivatives E79 and E80. On the other hand, the insertion of a second ether oxygen function, which had already been observed to exert a marked deleterious effect on potency, was associated with a reduction in the affinity value of compound E100 below that of E151. Similarly tubocurarine dimethyl ether appeared to possess a lower affinity for the cholinergic receptor site in

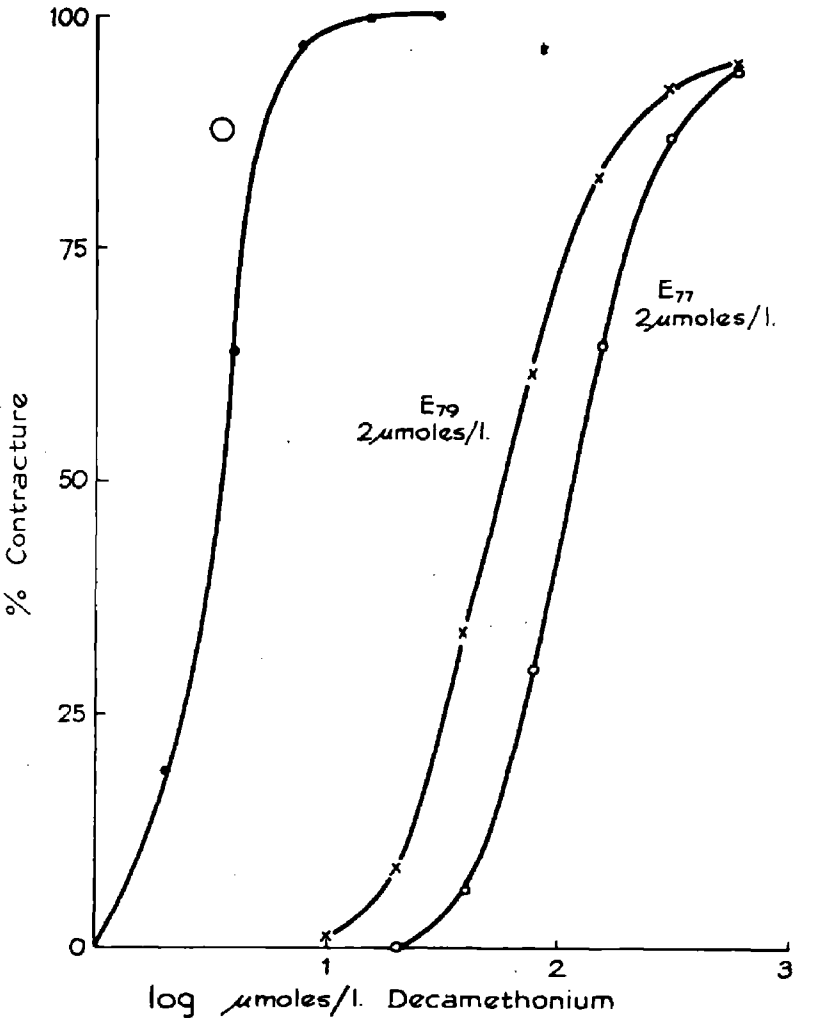


Fig. 71. Cumulative log dose-response curves for decamethonium performed on the isolated frog rectus abdominis muscle alone (0 µmoles/l) and in the presence of equal concentrations of compound E77 and compound E79. The difference in the displacement of the curves along the abscissa represents a difference in the affinity of the two compounds. Both compounds E77 and E79 show competitively antagonistic properties.

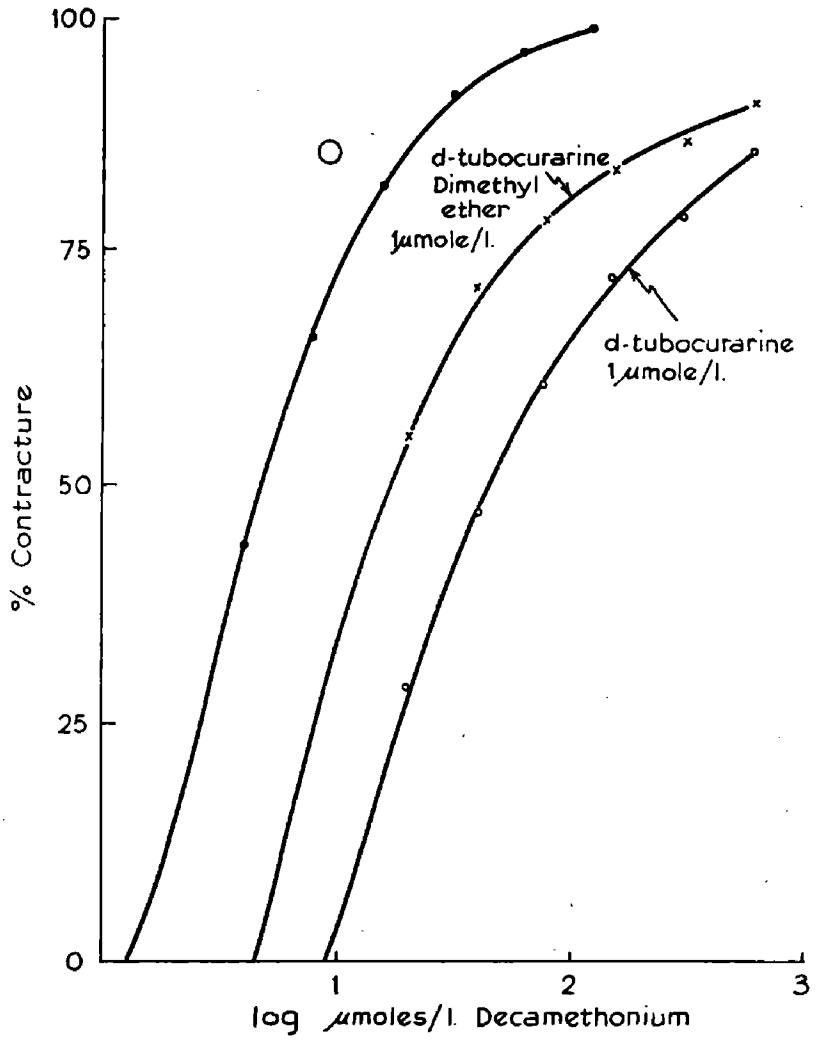


Fig. 72. Cumulative log dose-response curves for decamethonium performed on the isolated frog rectus abdominis muscle, alone (0 μmoles/l) and in the presence of equal concentrations of tubocurarine and tubocurarine dimethyl ether. The difference in the displacement of the curves along the abscissa represents a difference in the affinity of the ether and non-ether compound. Both tubocurarine and tubocurarine dimethyl ether show competitively antagonistic properties.

the frog than tubocurarine itself. Typical examples of these effects are shown in Figs. 71 and 72.

The insertion of an ether oxygen function at the expense of a methylene group into the molecule of decamethonium produced a slight reduction in the affinity value of the compound (oxydipentonium Fig. 64). Since no intrinsic activity differences could be detected, the effect of the ether oxygen function was concerned with the degree of drug-receptor complex formation achieved (affinity) rather than with the power of this complex to evoke a positive biological response.

CHAPTER I

DISCUSSION

The compounds investigated in this thesis all produce a relaxation of skeletal muscle but not by identical peripheral mechanisms. Application of the accepted criteria for the qualitative differentiation of muscle relaxants (Paton & Zaimis, 1952) showed that the compounds of Groups 1a, 1b, 1c and 1d were non-depolarizing neuromuscular blocking agents, while those of Group 2 possessed depolarizing activity.

A study of these compounds failed to establish any clear correlation between chemical structure and pharmacological activity. Apart from the fact that the compounds did not form a regular chemical series, there also exist the general difficulties besetting the derivation of any structure - action relationships, as stressed by Clark (1937) and Ing (1959) and summarized by Reid (1960). The original concept of a two-point attachment of a bisquaternary salt to the receptor (Paton & Zaimis, 1949; Barlow, 1955) has recently been subject to considerable criticism. In addition to the adumbration hypothesis of Loewe and Harvey (1952), already discussed (page 28), the theory of a one-point receptor attachment put forward to

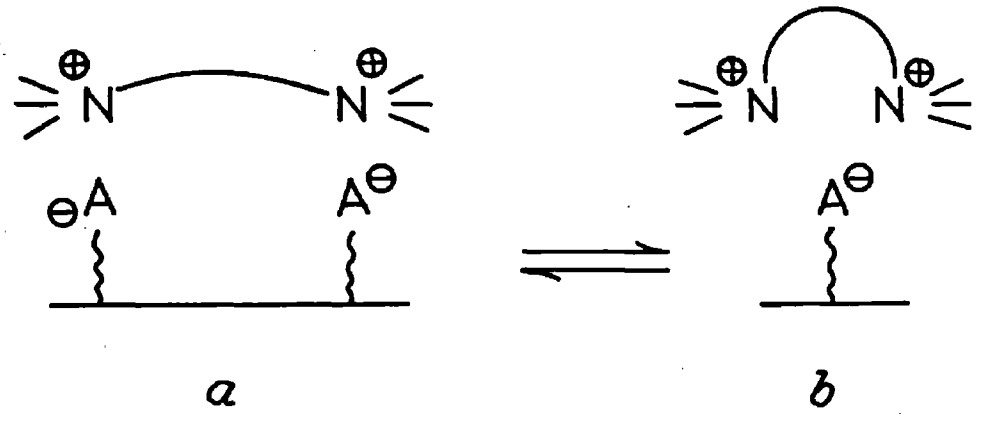


Fig. 74. A diagrammatic representation of the maximally extended form (a) and the ion-pair complex formation (b) in which bis-quaternary salts can exist. In the former, the di-cation is involved with two anionic sites but in the latter an ion-pair, involving only one anionic site, is concerned. (After Cavallito & Gray, 1960).

account for the ganglion blocking potency of a series of bis-choline ether salts (Fakstorp & Pedersen, 1957a,b) might also apply to neuromuscular blocking agents. Furthermore, the use of conductimetric techniques (Brody & Fuoss, 1956) has clearly established that simple polymethylene bisquaternary compounds possess certain properties which are qualitatively and quantitatively different from those of monoquaternary derivatives. Even in dilute aqueous solution, these polymethylene, bisquaternary salts show a marked tendency to associate with other ions (Rice, 1956, 1958). In particular, the combination of a polymethylene di-cation with a single anion has been shown to be extremely stable. This raises the possibility that in drug-receptor complex formation, the two cationic groups do not necessarily adopt a maximally extended conformation covering two anionic sites (Fig. 74a) but form an ion-pair complex with a single anionic site (Fig. 74b) (Cavallito & Gray, 1960). Moreover, it has been suggested that for a two point attachment, the more likely conformation would be a compromise between the maximally extended form (Fig. 74a), favoured on account of its thermodynamic stability, and a less fully

extended form favoured by considerations of entropy. It is thus virtually impossible to consider the receptor site purely in terms of the interquaternary distance in the drug molecule.

Despite the difficulties just enumerated, several attempts have nevertheless been made to derive relationships between chemical structure and muscle relaxant activity. Among aliphatic compounds, attention has been focussed on the nature of the alkyl substituents (Paton & Zaimis, 1952; Barlow, Roberts & Reid, 1953; Thesleff & Unna, 1954; Edwards et al. 1959, Van Rossum & Ariëns, 1959a), on the number and character of the onium groups in the molecule (Vanecek & Votava, 1955; Edwards et al. 1958a,b, 1959, 1961) and on the nature and length of the inter-onium chain (Barlow & Ing, 1948a,b; Paton & Zaimis, 1949; Levis et al. 1953). Although no overall structure-action relationship emerged, it was nevertheless felt worthwhile to examine the series of compounds discussed in this thesis inasmuch as they related to these three variables.

The Nature Of The Alkyl Substituents On The Onium Centres.

In the compounds investigated in this thesis, the alkyl groups attached to the onium centres were

limited to the methyl, ethyl and propyl radicals. With the polymethylene bis-onium compounds I, II and III, which were predominantly decamethonium-like, the replacement of a methyl by an ethyl group was accompanied by a reduction in potency in all the animal species studied. Although conventional experimental techniques revealed no marked qualitative differences between compounds I and III, the replacement of a methyl by an ethyl group was shown, by the Ariëns' procedure, (vide infra), to modify the nature of the block produced from depolarizing to non-competitively agonistic.

In the bis-choline ether series (Group 1d), maximum neuromuscular blocking potency in the cat was observed with the bis-triethylammonium derivatives F. & P. 8212 and F. & P. 8303. At the same time, the potency of these compounds differed significantly from each other, indicating that the nature of the alkyl onium substituents was not the sole determinant of the muscle relaxant activity and emphasizing the role of other factors, especially the interonium distance. The reduction in activity associated with the replacement of ethyl by methyl groups confirms

the findings of Thesleff and Unna (1954) for certain bisquaternary compounds and those of Edwards and his associates (1957, 1958a,b) for certain tris-onium derivatives. The fully methylated derivatives (F. & P. 16575 and F. & P. 16701) possessed very weak muscle relaxant properties but no depolarizing characteristics were observed. The introduction of an n-propyl substituent into bisquaternary compounds of this type (Pradhan et al. 1954) does not appear to reduce potency or cause qualitative changes in the pharmacological activity.

Similar changes have been observed using the tris- and tetra-onium ether and non-ether derivatives described in this thesis. Maximum activity among compounds in Groups 1a, 1b and 1c was associated with the presence of ethyl groupings on the terminal quaternary ammonium centres. For example, among the tetra-onium ether and non-ether derivatives, E77 and E79 were the most potent on the cat, rabbit, mouse and frog. Muscle relaxant potency was slightly reduced by the introduction of propyl substituents (E96 and E97) or more dramatically by substitution of methyl for ethyl groups as in compounds E78 and E80.

There was no evidence of any depolarizing properties in these compounds. Compound E94, in which all the quaternary ammonium functions carried ethyl substituents, was the most potent tris-onium derivative in Groups 1b and 1c. The compounds in Group 1c, in which the central nitrogen atom always possessed at least one methyl substituent, had generally slightly weaker muscle relaxant properties than those compounds in Group 1b in which the central nitrogen atom carried at least one, and the terminal groups two, ethyl radicals. For example, compound E98 was less potent than E90 on the cat and rabbit. The predominance of ethyl radicals has been consistently associated with non-depolarizing activity in compounds of this type. The complete absence of depolarizing characteristics in the purely methyl substituted analogue, E100, confirmed by experiments on the cat, the hen and the frog, and in compound F. & P. 16575 on the cat, is in keeping with similar observations by other workers investigating the type of neuromuscular blocking activity of hexamethonium (Thesleff & Unna, 1954) and the methyl analogue of gallamine (Riker & Wescoe, 1951). It is thus seen that no generally-applicable principle has

emerged relating the nature of the onium substituents to the type or degree of muscle relaxant activity and while early workers associated the presence of methyl substituents with depolarizing activity (Burns & Dale, 1915), there is now ample evidence to refute this generalisation. Moreover, tubocurarine, which is a bis-methonium compound is the classic example of a non-depolarizing agent.

The number and character of the onium groups.

There seems little point in attempting to formulate any relationship linking the type or degree of muscle relaxant activity with the number of the onium centres in the molecule. Decamethonium, suxamethonium and tubocurarine, all containing two quaternary nitrogen centres, are more potent than gallamine which has three. On the other hand, several tris-, tetra-, penta- and hexa-onium compounds show greater muscle relaxant potency than tubocurarine (Edwards et al. 1958a,b, 1959, 1961). Clearly other factors are involved in determining the potency of these compounds.

In the compounds described in this thesis, it is equally impossible to effect a proper comparison

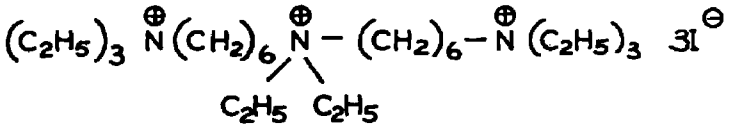
of the compounds in Groups 1 and 2, in which molecular shape and length and steric factors vary so considerably. Nevertheless, it is evident that in Group 1, the general order of potency of the tetra-onium compounds is much greater than that observed in the tris- and bis-onium derivatives. While it cannot be claimed that this is due solely to the extra onium functions involved, this factor, accompanied by an increase in the length of the chain between the terminal nitrogen groups, must be largely responsible. Moreover, the bis-onium compounds (Group 1d) are slightly less potent than the corresponding tris-onium derivatives (Groups 1c and 1b). Thus compound E77, which contains four quaternary centres, is much more potent in the cat, rabbit and chick than compound E90 which contains three. Moreover, compound E90 is more potent in the cat than compound F. & P. 8212 which contains only two quaternary centres. The only difference between these three compounds is in the number of onium centres in the molecule.

There is little evidence, however, to suggest that the number of onium centres exerts a controlling influence on the type of pharmacological activity

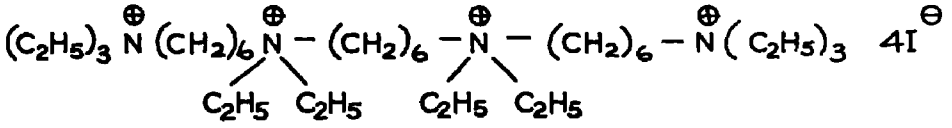
produced. All the compounds in Group 1 show qualitatively similar, muscle relaxant properties, a fact which strengthens the view that the number of onium centres in the molecule influences potency rather than the type of pharmacological activity produced (Edwards et al. 1960)

The fall in muscle relaxant potency, previously noted on replacement of nitrogen by sulphur in bis- and polyonium compounds (see pages 70 and 71 of this thesis), has been confirmed. Compound II possessed weaker muscle relaxant properties than decamethonium on the cat, rabbit, mouse, chick and frog although the type of activity remained unaltered. When compared with the results of Barlow and Ing (1948a), the potency of compound III on the rabbit was less than that of the octamethylene bis-1,8-trimethylammonium salt (Barlow & Ing, 1948a).

When the compounds in Group 1d were tested for muscle relaxant activity on the cat, the results obtained failed to support the concept of a one-point receptor attachment. Compounds possessing similar inter-onium distances (F. & P.8212, F. & P.17843 and F. & P.16701) and (F. & P.16575, F. & P.16677,



(a)



(b)

XXIV

F. & P.8302, F. & P.16678 and F. & P.8303) differed in potency (Table IV). Furthermore, maximum muscle relaxant activity was observed when the N-substituents on both centres were ethyl radicals and successive substitution by larger or smaller alkyl groups, on one or both onium centres, reduced potency.

At the same time there is no evidence to support the view (Paton & Zaimis, 1949) that muscle relaxant activity in bis-onium compounds is produced by a two point attachment.

The Nature and Length of the Inter-Onium Chain.

Relevant comparisons may be drawn between some of the compounds described by Edwards and his associates (1957, 1958a,b) and certain of those discussed in this thesis. The tris-onium compound dihexaazonium (XXIVa) and the corresponding tetra-onium derivative trishexatetrazonium (XXIVb) were respectively more potent than the corresponding compounds E94 and E80 which differed only in having one methylene link in the inter-onium chain. All these compounds possessed the same type of activity. On the other hand, compounds of Group 1d, in which the inter-onium distances are greater than those in Group 1b were, in general, less potent in the cat. In the ether

derivatives of Group 1b, the inter-onium distances were identical to those of compounds in Group 1c but the potencies on various species differed, emphasizing the difficulty of trying to assess the individual contribution of the length of the inter-onium chain to muscle relaxant potency. Moreover, there was no evidence to suggest that, in the compounds studied, alteration in the inter-onium distance modified the type of muscle relaxant activity. It is interesting to note that in other aliphatic polyquaternary compounds the inter-onium distance was believed to be an important determinant of the type of muscle relaxant potency observed (Edwards et al. 1961).

In addition to the influence of variation in the length of the inter-onium chain, its nature may also significantly influence the pharmacological behaviour of synthetic muscle relaxants. From the results obtained, it is obvious that the replacement of a methylene group by an ether oxygen function has reduced potency in the cat, rabbit, chicken and mouse. Thus compounds E77, E78, E96, E90 and E91 are respectively less potent on both mammalian species and on the hen than the corresponding non-ether compounds E79, E80, E97, E94 and E93. Similarly

E99, E100 and E101 are respectively very much weaker in the cat than the related non-ether compounds E74, E151 and E152, the activity of which was reported by Edwards and his colleagues (1959). Similarly, the insertion of an ether oxygen function into the polymethylene chain of hexamethonium (compound F. & P. 16701) or decamethonium (oxydipentonium) reduced muscle relaxant potency (Levis et al. 1953).

Compared to their relative potencies on mammalian species, compounds of Group 1a showed a different order of activity when tested on the frog rectus abdominis muscle preparation. On the frog, the ether containing tetra-onium compounds were more potent than the analogous non-ether derivatives.

Although no single factor can be held responsible for the difference in potency between the ether and non-ether containing compounds, several alternative or contributory explanations may be offered. None of the compounds can be assumed a priori to adopt a maximally extended conformation at the receptor site but even if they did, the small reduction in chain length (0.13Å) due to the insertion of an ether oxygen link seems inadequate to explain the observed fall in

potency. In view of the high water solubility of the corresponding polymethylene non-ether derivatives, it seems unlikely that the introduction of one or two ether oxygen functions would greatly influence the absolute solubility of a compound. It may, however, alter the partition coefficient of the drug by changing the relative water and lipid solubilities. Ether oxygen functions could also enter into hydrogen bonding in the tissues to a greater extent than the corresponding non-ether containing derivatives.

It is evident that, in the compounds studied, the role of the ether oxygen function cannot be explained by the results obtained from the conventional experiments described. In consequence, an attempt was made to investigate this problem by an approach based on the interaction of these drugs with the cholinergic receptor system.

In view of the difficulties which surround attempts to derive structure-action relationships and to place new drugs in their proper perspective, there is a need for a method by which compounds, whose chemical structure is markedly dissimilar, as well as those more closely related in homologous series, can

be classified quantitatively and qualitatively on a common pharmacological basis. A method, which offers some hope of achieving this to a limited extent, has been described by Ariëns and his associates. These workers, following the earlier investigations of Clark, Gaddum and Michaelis and Menten (vide infra) treated drug-receptor interaction mathematically and suggested that drug interaction could be considered in terms of two constants namely the affinity and the intrinsic activity, the nature of which has already been discussed. It must be stressed that this approach does not afford an explanation of the precise mechanism of action of the drugs investigated, but is a method of comparing the effects produced by a series of drugs on a quantitative basis provided that they do not act upon dissimilar receptors. The nature or structure of the receptor involved, in the application of this hypothesis to neuromuscular blocking agents, is not however implied. Consequently, acceptance of the Ariëns' hypothesis accommodates any of the suggested representations of the cholinergic receptor as previously described in the introduction to this thesis.

The affinity and intrinsic activity values of

TABLE XIX

The mean intrinsic activity and affinity values for compounds I, II, III, oxydipentonium and decamethonium estimated by the Ariëns' procedure using the frog rectus abdominis muscle.

Compound and No. of Experiments	Intrinsic Activity	Affinity	Relative Affinity (Decamethonium = 1)
(20) Decamethonium	1.0	0.19	1
(10) Oxydipentonium	1.0	0.16	0.84
(10) Compound I	0.32	0.025	0.13
(20) Compound II	1.0	0.13	0.68
(10) Compound III	0.62	0.062	0.33

TABLE XX

The mean 'inhibition indices' and affinity values (3 observations) of a number of aliphatic polyonium neuromuscular blocking agents, tubocurarine dimethyl ether and tubocurarine.

Code Number	'Inhibition Index'	Affinity	Relative Affinity (Tubocurarine = 1)
E77	0.0163	61.35	2.95
E79	0.034	29.41	1.41
E78	0.243	4.12	0.20
E80	0.307	3.26	0.16
E98	2.10	0.48	0.023
E99	2.60	0.39	0.019
E100	7.15	0.14	0.007
E101	6.33	0.16	0.008
E102	5.23	0.19	0.009
E103	1.96	0.51	0.025
E151	2.43	0.41	0.020
Tubocurarine dimethyl ether	0.168	5.95	0.27
Tubocurarine	0.048	20.83	1.00

the compounds investigated are shown in Tables XIX and XX. If a pictorial representation of the cholinergic receptor site is accepted, a number of suggestions may be put forward to explain the differences observed.

Replacement of a nitrogen atom in decamethonium by a sulphur atom (compound II) has not, by itself, significantly altered pharmacological behaviour but, together with a reduction in chain length, has reduced both the affinity and intrinsic values observed (compounds I and III). The reduction in the number of Van der Waal's forces associated with the tertiary sulphonium atom compared to the quaternary ammonium group and the increased size of the former may reduce the stability and the amount of drug-receptor complex formed. The combined effect of altering the inter-onium chain length and the nature of the centres themselves may also reduce the overall intensity of the cationic charge of the molecule and so impair the electrostatic bond mainly responsible for the production of muscle relaxant activity. Alternatively, the reduction in the chain length from ten (compound II) to eight methylene groups (compounds I and III) might also

reduce the ability of the latter to form an ion-pair complex with one anionic receptor site. The adoption of such a conformation would enhance the charge density of the molecule by reducing the surface area of the charge. The reduction in intrinsic activity could therefore be explained by a reduction in the intensity of the onium cationic charges. Moreover, the reduced ability of compounds I and III to enter into ion-pair complex formation with one anionic receptor would also reduce the stabilising effect of Van der Waal's and other secondary bonding forces. This would reduce the affinity of the compounds concerned.

The replacement of a methyl by an ethyl group on each onium centre involves other steric factors in addition to those already discussed. These may be less marked in a maximally extended molecular conformation than in an ion-pair complex (Fig. 74b, page 137). In the former (Fig. 74a) the ethyl group on each onium centre may sterically hinder the approach of the cationic charge to the anionic receptor berth and so reduce the intensity of drug receptor interaction. Moreover, a less readily definable steric effect may be due to the ethyl group diminishing the ability of the compound to

alter its own conformation to fit that of the receptor site. By this means, the strength of the receptor-bonding effects of secondary forces, for example Van der Waal's bonds, would be reduced. On the other hand, if an ion-pair complex formed between the two onium centres of the molecule and one anionic receptor has reality, then the ethyl groups present may also reduce the concentration of the 'twin' cationic-onium charges by sterically hindering their approach to each other as well as their combined approach to the anionic receptor. It should be stressed however, that since compound I exhibits the ability to combine with an additional receptor system, the above structure-action relationships may only justifiably be applied to its interaction with acetylcholine-like receptors.

Table XX shows the comparative affinity values for a number of non-depolarizing (Type II) muscle relaxants. Since there is no physicochemical evidence to indicate the conformation adopted by these compounds at the receptor site, conclusions concerning the shape of the molecule are even more hazardous than those drawn concerning bis-onium salts. The non-depolarizing properties exhibited by these compounds compared to the

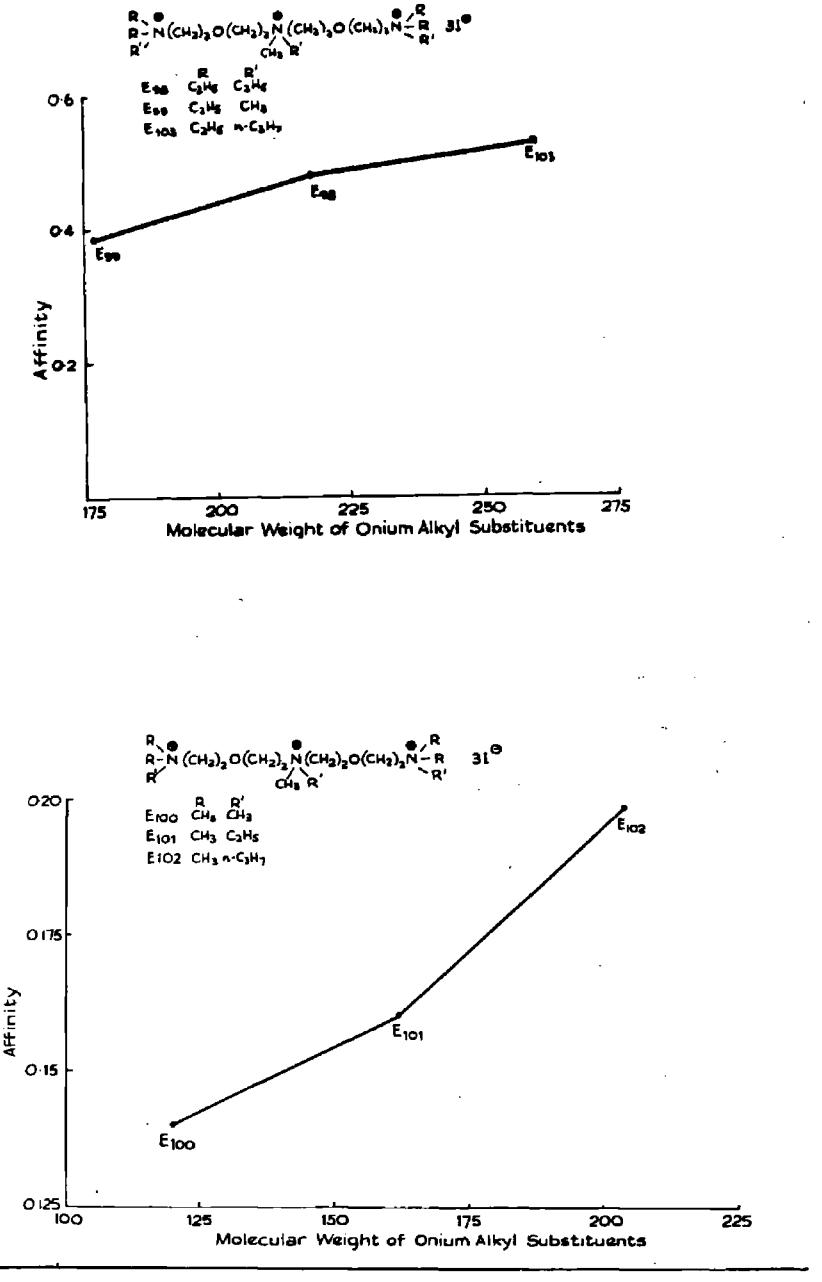


Fig. 73. Frog rectus abdominis muscle. Ariëns procedure. A graphical illustration of the increase in affinity brought about by changes in the molecular weight of the onium alkyl substituents in the homologous series of compounds E98, E99 and E103 (upper graph) and compounds E100, E101 and E102 (lower graph).

predominantly depolarizing effects of bis-methonium salts cannot be accounted for on the basis of the experiments described in this thesis. It has been suggested (Van Rossum & Ariëns, 1959a) that the receptors for depolarizing compounds may differ from those occupied by non-depolarizing agents and that the molecular properties required for a high degree of affinity may vary in either case.

From the results, increased affinity values can be related directly to an increase in the bulk of the onium substituents in the two homologous series of compounds E98, E99 and E103 and E100, E101 and E102 (Fig. 73). No other structural differences exist between the compounds in each series. Similarly, the marked difference in potency between compounds E77 and E78 and E79 and E80 can be directly related to the increased size of the N - substituents. The beneficial effect of fairly bulky alkyl substituents (ethyl and propyl) may be due to their shielding effect on each quaternary function. In addition, a close drug-receptor approach would be sterically hindered by such radicals favouring a tubocurarine-like effect (Cavallito & Gray, 1960). Alternatively, assuming the existence

of non-ionic satellite receptors (Meier, Tripod & Brüni, 1955; Edwards et al. 1959) bulkier onium substituents might be preferentially held there and so help to induce neuromuscular block by preventing the access of acetylcholine to the anionic receptor pore.

The role of the ether oxygen function was investigated by an examination of five pairs of ether and related non-ether compounds under similar conditions. These compounds were tubocurarine and tubocurarine dimethyl ether, decamethonium and oxydipentonium, compounds E79 and E77, compounds E80 and E78, compounds E151 and E100.

Tubocurarine dimethyl ether (IV, R = CH_3) (opposite page 56 of this thesis) contains two methoxyl groups instead of the two hydroxyl groups found in tubocurarine. Otherwise, the compounds are structurally identical and their modes of action also appear similar (Kirschner & Stone, 1951). Due to the difference in structure, tubocurarine dimethyl ether cannot carry any anionic charge while tubocurarine itself contains two phenolic hydroxyl groups capable of undergoing dissociation (Swann, 1951; Kalow, 1953).

The pK_A value of each hydroxyl group is however different, that in the ortho position to the methoxyl groups being the less acidic. Consequently, it has been suggested that tubocurarine can exist in more than one ionized form (Kalow, 1954). Maximum pharmacological activity was observed at pH 6.7 when the hydroxyl groups were virtually unionized. The existence of a partial zwitter ion at high pH values would, it was suggested, reduce the net cationic charge of the entire molecule by partial neutralisation of the cationic charge on both quaternary groups. At the anionic receptor site, therefore, the resulting partial repulsion of the tubocurarine molecule would prevent a close drug-receptor combination and might explain the lower activity of this molecule compared to the dimethyl ether (Kalow, 1954; Cavallito, 1959). This explanation, however, fails to account for the very low potency of the di-n-butyl and dibenzyl ethers of tubocurarine (Wintersteiner, 1959). Moreover, the view that the number and the position of the methoxy substituents in molecules of this type contribute directly to the activity of the molecule as a whole is supported by the marked increase in potency

observed in a series of N,N-dimethyl-1,10-decamethylenebis-tetrahydroquinolinium and tetrahydroisoquinolinium derivatives with increasing substitution of methoxy groups. The increased activity of tubocurarine, compared with that of its dimethyl ether, reported in this thesis, could be due to steric factors residing in the methyl substituents. Alternatively, the hydrogen atom of the hydroxyl group in tubocurarine may conceivably form a hydrogen bond with the receptor, a situation not possible with tubocurarine dimethyl ether. At certain pH values, however, slightly above the physiological range, this phenomenon will be annulled by the opposite effect of anionic dissociation. At this point, both tubocurarine and the corresponding dimethyl ether may be equiactive, (Swanson, Henderson & Chen, 1949; Swanson, Gibson & Powell, 1952).

In mammalian skeletal muscle, dimethyl tubocurarine is claimed to be much more potent than tubocurarine itself (Unna & Pelikan, 1951; Foldes, Machaj, Hunt, McNall & Carberry, 1952). In contrast to its action on the rat (Holmes, Jenden & Taylor, 1951), the effect of tubocurarine on the frog rectus appears

to be uninfluenced by temperature variations (Van Maanen, 1950) and in consequence the species differences observed may be attributable to the specialized response of each neuromuscular synapse to methoxyl and hydroxyl substituents. There is, moreover, no reason to assume that frog and mammalian neuromuscular functions are similar. On the basis of the Ariens' hypothesis, the particular function of these groups can be related to differences in the affinities between them. Kalow (1954) believes that the nucleophilic oxygen, in each hydroxyl group of the tubocurarine molecule, might be attracted to an electrophilic group in the mammalian muscle receptor. Since it is well established that the methyl group has a greater inductive effect than a hydrogen atom, there is a greater electronic density on the oxygen atoms available for bonding to the receptor in the dimethyl ether than in the unionized form of tubocurarine itself. Thus the dimethyl ether would appear the stronger drug. However the most pronounced electrical charge in drugs of this kind is found on the onium centre, while the presence of other substituents, including hydroxyl or methoxyl groups, can only modify

this. Differences in the distribution of tubocurarine and its dimethyl ether *in vivo* (Collier *et al.* 1948; Marsh, 1952) have indicated that the relative potency of these two compounds may also be influenced by differences in the permeability of the drug, the biophase concentration attained and the availability of each drug at the receptor site. The experiments reported in this thesis, carried out at pH 7.5 - 7.8, only indicate that the relative potencies of the compounds were the result of differences in the amount of drug-receptor complex formed. Their non-depolarizing competitive effects were also confirmed. However, it is also necessary to bear in mind that other differences, not indicated by the techniques employed, may also contribute to the potency of these drugs. Thus, while the dimethyl ether has a slightly higher lipid solubility than tubocurarine itself, it is no more effective in blocking conduction at the node of Ranvier, in a single nerve fibre preparation (Dettbarn, 1960). Consequently, although a high degree of membrane permeability is an important factor in determining the activity of these compounds (Rosenberg & Ehrenpreis, 1961), it appears unlikely that this feature is

responsible for the higher potency of tubocurarine dimethyl ether observed in certain preparations. Furthermore, studies on the binding of tubocurarine and its dimethyl ether with the acetylcholine receptor-protein of the electric tissue of the electric eel (Electrophorus electricus) (Ehrenpreis, 1960), has again stressed the influence of the pH of the medium on activity in these molecules. While the dimethyl ether has less affinity than tubocurarine for the receptor protein at pH 7.5, the reverse order applies at pH 9.5. This phenomenon is presumably due to the ionization of the phenolic hydroxyl groups in tubocurarine at the higher pH and supports the view of Kalow (1954) already described and the results of the present investigation.

Oxydipentonium differs structurally from decamethonium in having one ether oxygen function inserted into the polymethylene chain separating the two quaternary ammonium groups. This would produce an increase in the length of the polymethylene chain suggesting a decrease in the effectiveness with which the onium centres might combine with the receptor field. However, no significant difference in

intrinsic activity from that of decamethonium was observed. On the other hand, a slight difference in affinity between the two compounds was apparent and this has been confirmed by other investigators (Van Rossum, 1960). Two main reasons may be suggested to explain this effect. Either or both may operate in determining the potency of oxydipentonium.
(a) Mere increase in chain length, which could, for example, also explain why undecamethonium is less potent than decamethonium (Paton & Zaimis, 1949).
(b) Particular physicochemical characteristics of the ether oxygen atom which might enhance an increased bonding at 'sites of loss' and a disturbance of the lipophilic-hydrophilic balance of the molecule.

The increased electron density on the oxygen atom could lead to an anti-bonding effect causing a reduction in the affinity of the ether-containing compound, as evidenced by the lower activity of oxydipentonium as compared to decamethonium. On the other hand, in the series of bis-choline ethers examined for ganglion-blocking activity by Fakstorp and his colleagues (1957a,b), it was suggested that the proximity of the ether oxygen to the quaternary nitrogen might cause a drift of electrons from the

oxygen atom which could then assume a partial positive charge. The ganglion-blocking potency of these compounds, which was in certain cases greater than that of hexamethonium, was suggested to be due to their stable attachment to the negatively charged esteratic site in the pictorial representation of the acetylcholine receptor suggested by Barlow (1955). The drift of electrons through a carbon chain is known only to be significant however across one or two carbon atoms, but there remains the possibility of electron movement induced across space. Electron drift through the chain, moreover, will not occur where the ether link is symmetrically placed with respect to two onium groups.

While there is no direct evidence to suggest which of these mechanisms is operative, the reduced affinity of the ether-containing derivative oxydipentonium compared to decamethonium supports the view that, in this compound, the ether oxygen link is producing an anti-bonding effect. This view has been supported by the observation that the presence of two ether links in compounds chemically similar (Van Rossum & Ariens, 1959a) was associated with a reduction in

both affinity and intrinsic activity, when compared to decamethonium. Since the alteration in chain length produced by the replacement of two methylene groups by ether links is small, the anti-bonding effect of the oxygen functions may be largely responsible for the reduction in muscle relaxant potency produced. On the other hand, the observed effects may be the resultant of two opposite, rather than two synersistic mechanisms and the suggestions offered must be regarded only as possibilities.

Similarly, when compounds E151 and E100 are compared several factors may contribute to the potency differences observed. Using the Ariëns' procedure however, these variations can be directly related to differences in the amount of the drug-receptor complex formed by each compound although no differentiation between the contribution of the ether oxygen and its effect on the inter-onium chain length can be made.

On the other hand, the role of the ether oxygen function in the mono-ether tetra-onium compounds E77 and E78 can be more precisely defined. In both cases introduction of an ether link increased

the affinity of the compounds compared to that of E79 and E80 in which the ether function was replaced by a methylene ($-CH_2$) unit. In these cases, alteration in chain length and the change in the bond angles produced by the ether function is very small and seems incapable of causing the potency differences observed. It is therefore more likely that the physicochemical properties of the ether oxygen itself, rather than the mere alteration in chain length is responsible. Consequently, the increased affinity might be due to an intensification of the secondary bonding characteristics of the drug-receptor complex which increased the degree of general electrical field interaction attained.

In addition to the chemical and physicochemical considerations discussed, species variation could also account for the differences in the order of potency observed between ether and non ether compounds. In the cat, chick, rabbit and mouse, the ether derivatives are the more potent, while on the frog rectus abdominis muscle, the reverse is true. Although variation in the response of different species to muscle relaxant drugs is well established (Paton & Zaimis, 1952;

Hoppe, 1955), the interesting reversal in the order of potency caused by the replacement of one ether by one methylene link raises the possibility that additional contributory factors exist. Perhaps in whole animal experiments, the ether-containing compounds might undergo increased bonding at 'sites of loss' or attain a reduced concentration in the biophase. Such considerations are obviously more relevant in 'whole animal' experiments than in those involving isolated tissue preparations. Alternatively, the physiological differences in structure between the synapse of mammals and frogs might be responsible, and investigations using tubocurarine dimethyl ether (Kalow, 1954) support this view. At the same time, the effect of variation in the pH values of the bath fluid (Kalow, 1954) on the potency of tubocurarine and its dimethyl ether, in the frog, indicates the difficulty of applying results obtained with certain kinds of chemical structures using an artificial environment.

It is apparent that the ether oxygen function plays no dramatic role in the muscle relaxant activity of the compounds studied and in neither tubocurarine,

its dimethyl ether nor the aliphatic structures examined is there evidence of its ability to qualitatively modify pharmacological behaviour. This view confirms that of Bovet (1951). It may, however, influence the ability of these compounds to combine with the cholinergic receptor by interfering with the general fields of force which contribute to the formation and stability of the drug-receptor complex. As a result, the ether oxygen function may quantitatively alter the pharmacological activity of drugs of this type.

It is extremely difficult to compare the results reported in this thesis with those obtained by other workers. It is to be noted, however, that an increase in the muscle relaxant potency of several aliphatic compounds in the cat was attributed to the influence of an additional oxygen atom in the inter-onium polymethylene chain (Pradhan et al. 1954). No explanation of these effects were given but the slight alteration in chain length appears unlikely to be wholly responsible for the marked potency differences reported. The presumably low intrinsic activity of these compounds (all were tubocurarine-like) makes it

tempting to suggest that the extra ether oxygen function might have increased potency by increasing the affinity of the compounds concerned.

The use of preparations from different species has further complicated the correlation of the results of different workers. Furthermore, the value of some of these preparations in the evaluation of muscle relaxants remains open to question. Vanecek and Votava have, for example, advocated the use of the masseter muscle preparation of the rat (Eicholtz, 1949) as a superior means of pharmacologically investigating drugs of this type. Since this involves a direct stimulation of the muscle itself rather than an indirect stimulation via the motor nerve, conclusions concerning compounds whose pharmacological activity is believed to be primarily effected at the neuro-muscular junction cannot be unreservedly accepted.

Many of these difficulties appear capable of resolution using the Ariëns' procedure which enables all compounds of this type to be classified qualitatively and their potency estimated. The results reported in this thesis from whole animal experiments have confirmed the value of the classification of muscle

relaxant compounds made by Ariëns and his associates (Van Rossum *et al.* 1958). It is interesting to observe a parallel between the affinity values of the compounds tested and their molar potency, compared to tubocurarine, obtained by using the conventional frog rectus assay method. Consequently adoption of the concepts of affinity and intrinsic activity renders the procedure particularly valuable as a means of minimising the difficulty of assessing the muscle relaxant properties of drugs evaluated by different workers.

From the practical point of view, the Ariëns' procedure can be used to screen rapidly large numbers of new compounds. It is useful in the elimination of compounds possessing potentially undesirable properties without the sacrifice of large numbers of experimental animals. In this way the cost of the initial screening process can be considerably reduced and unnecessary waste of animal life avoided. The use of the method facilitates the study of the contribution of individual structural characteristics, in members of a homologous series, thus aiding the design of new compounds.

Possible Clinical Application of the Compounds Studied.

From the clinical view point, the ideal muscle relaxant drug must exert a highly specific action at the neuromuscular synapse without producing adverse side effects. Many synthetic drugs, including those in current clinical use, lack complete specificity by virtue of their capacity to block autonomic ganglia, (tubocurarine) to liberate histamine (tubocurarine) or by the possession of anticholinesterase properties (hexafluorenium). Lack of specificity is more prevalent in non-depolarizing than in depolarizing agents.

For a drug to be considered worthy of clinical investigation, it must possess characteristics superior to those of drugs in current clinical use. Compounds in Group 1d block synaptic transmission in both sympathetic and parasympathetic ganglia (Fakstorp and his associates, 1954, 1957a,b,c, 1958). This property, combined with their weak muscle relaxant properties renders the possibility of their clinical application extremely unlikely.

Among the tris- and tetra-onium compounds studied there was no evidence of any marked ganglion

blocking action while the lack of a hypotensive effect in the cat suggested the absence of histamine-liberating properties. The absence of serious non-specific characteristics was not however accompanied by evidence of any superiority over gallamine or tubocurarine. Indeed while several tetra-onium derivatives were more potent than either of these drugs, this was offset by a slightly more discriminating effect on the respiratory muscles. Moreover the duration of the block produced appeared unlikely to fulfill the present demand for a short acting non-depolarizing muscle relaxant compound.

In spite of the reappraisal of decamethonium in recent years (Lawson, 1958) there appears to be no need for the introduction of further depolarizing muscle relaxants. Consequently since none of the sulphonium analogues possessed any advantages over decamethonium itself, there appears to be little point in offering them for clinical investigation. Furthermore, the appearance of a non-competitive component of action in compound I in addition to the general depolarizing effect of all the drugs in Group 2 is clinically undesirable on account of the lack of a suitable antidote for compounds of this type. The necessity of maintaining neuromuscular block by a single uniform

mode of action raises the question of the apparent non-competitive block produced by very high doses of tubocurarine-like drugs. It is interesting to note that Everett (1948) regarded neostigmine as a 'limited antagonist' to tubocurarine in the rabbit. While neostigmine was effective against 'just paralytic doses' it was claimed to be ineffective against larger amounts of tubocurarine. There is no evidence to indicate whether this effect was caused by the anticholinesterase action of neostigmine or to deny the existence of a non-competitive component in the action of tubocurarine. While the phenomenon of a non-competitive component of action has not apparently affected the clinical value of tubocurarine since the doses used are presumably too small, clinical trials using dihexaazonium, a similarly acting muscle relaxant, suggest that it may have been responsible for the neostigmine-resistant block, which in some patients ensued with the use of this drug (Levy, 1959). On the other hand, the use of very high doses of the antagonist may have invalidated certain of the basic assumptions involved in the theory of drug antagonism, as discussed by Gaddum (1957) and Schild (1957).

One of the major obstacles in assessing the

potential clinical value of a synthetic compound lies in the considerable variation in the response of different species to muscle relaxant compounds. The differences in the response of the frog compared to that of mammals has confirmed the futility of relying on one animal preparation to predict accurately the potency of new drugs (Hoppe, 1955). Consequently, while the Ariëns' procedure appears to be an invaluable screening technique, results from these experiments cannot by themselves warrant the immediate clinical trial of a synthetic muscle relaxant. When the investigation of a particular compound, by this means, has revealed suitable pharmacological properties, a rigorous assessment on other species must also be made. In the final analysis, the potency of muscle relaxant compounds in man may only be obtained by their careful evaluation in man himself.

SUMMARY OF CHAPTER I

Chapter I is divided into four sections; the introduction, experimental methods, experimental results and the discussion.

The introductory section is devoted to a survey of certain aspects of the literature pertaining to neuromuscular blocking agents with particular reference to the study of their pharmacological and electro-pharmacological effects at the motor end plate, their interaction with the hypothetical receptors at this site and the derivation of mathematical equations to explain these effects. The various systems of classification adopted to subdivide muscle relaxants are discussed and a review given of synthetic compounds with neuromuscular blocking activity. Attention was paid, in particular, to ether-containing compounds and to the possible contribution of this function to neuromuscular blocking activity.

A study has been made of the pharmacological action of a number of synthetic bis and polyonium compounds containing one and two ether oxygen functions together with their corresponding or related non-ethers. In addition, three sulphur-containing bis-onium compounds were examined for neuromuscular blocking activity.

Attempts were made to draw structure-action relationships among these drugs and to assess, where possible, the role of the ether oxygen function in the pharmacological activity observed.

The methods used consisted of the conventional techniques commonly employed to evaluate neuromuscular blocking activity using the cat, rabbit, hen, chick, mouse and frog together with the method described by Ariëns, based on drug-receptor interaction, and studied by means of the frog rectus abdominis muscle. The effects of a group of new compounds on the cardio-vascular system, on autonomic ganglia, on respiration and on the isolated rabbit heart were also investigated.

On the basis of their pharmacological activity, the compounds investigated were divided into non-depolarizing and depolarizing drugs. In the former group, many compounds were more potent than tubocurarine while none of the depolarizing compounds investigated was more potent than decamethonium. With the exception of the frog, where the reverse order of potency was true, the ether-containing polyonium compounds were less potent than the corresponding non-ether analogues. By means of the Ariëns' technique, the qualitative and quantitative properties of those compounds investigated

were confirmed and extended and their affinities and/or intrinsic activity values calculated.

From the results obtained using more conventional methods, attempts were made to derive structure-action relationships among the compounds investigated. These were based on considerations of the nature of the alkyl substituents on the onium centres, the number and character of the onium groups, and the nature and length of the inter-onium chain. On account of the chemical dissimilarities among the compounds themselves, the relationships so derived were empirical and resort was made to the Ariëns' interpretation of drug receptor interaction. Accordingly, the activity of all the compounds investigated was placed on the common pharmacological bases of intrinsic activity and affinity and the role of the ether oxygen function in influencing the affinity of neuromuscular blocking compounds established.

In the course of this investigation, the Ariëns' technique has been fully evaluated as a preliminary method in the pharmacological testing of synthetic neuromuscular blocking compounds. None of the compounds investigated appeared to possess properties which might lead to their clinical use.

CHAPTER I

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CHAPTER II

STUDIES ON THE ANTI-CHOLINESTERASE ACTIVITY OF A NUMBER OF BIS- AND POLYONIUM, ALIPHATIC COMPOUNDS POSSESSING NEUROMUSCULAR BLOCKING PROPERTIES.

The term 'choline-esterase' was first suggested to describe the acetylcholine-splitting activity of horse serum (Stedman, Stedman & Easson, 1932) and referred to a group rather than to one particular enzyme. The specificities of these enzymes for their substrates (Richter & Croft, 1942) rendered the original term 'choline-esterase' too vague and necessitated a classification of the enzyme concerned. The term 'true-cholinesterase' was first proposed to include those enzymes which hydrolyzed choline esters present in the erythrocytes and in nervous tissues including acetyl-β-methylcholine but not benzoylcholine (Mendel & Rudney, 1943). The name 'pseudo-cholinesterase' was reserved for those enzymes obtained from serum and other tissues which were capable of hydrolyzing acetylcholine, benzoylcholine and several non-choline esters, but not acetyl-β-methylcholine.

Critics of this method of classification (Glick, 1945; De Laubenfels, 1943) applied the term 'acetylcholine-esterase' or 'specific-cholinesterase'

(Nachmansohn & Rothenberg, 1945, 1946) to those enzymes, present in nervous tissue and erythrocytes, which preferentially catalysed the hydrolysis of acetylcholine.

The general term 'cholinesterase' was then proposed to include both 'acetylcholine-esterase' and the various other enzymes of the serum and tissues, which hydrolyzed other choline esters, including benzoylcholine and butyrlcholine.

At the neuromuscular synapse, 'specific-cholinesterase' or 'acetylcholine-esterase' exclusively and rapidly hydrolyzes acetylcholine liberated endogenously from the end plate receptors. Anti-cholinesterases, by interfering with this process, may induce neuromuscular block in a manner resembling depolarizing muscle relaxants by permitting the accumulation of the transmitter (Briscoe, 1936; Rosenbleuth, Lindsley & Morison, 1936). In addition to their enzyme-inhibiting activity, anti-cholinesterases exert other independent effects at the neuromuscular synapse. In conditions where cholinesterase activity has been completely inhibited, both neostigmine (Riker & Wescoe, 1946) and edrophonium (Randall, 1950; Randall

& Jampolsky, 1953) have been observed to exert a direct excitatory effect on mammalian skeletal muscle-nerve preparations and on the frog rectus abdominis muscle (Miquel, 1946). The action of many quaternary ammonium compounds, not hydrolyzed by cholinesterase, is often potentiated by anti-cholinesterases e.g. decamethonium (Zaimis, 1951) and edrophonium (Cohen & Posthumus, 1955). Certain other bis-quaternary esters including suxamethonium (Hansson, 1958) are so slowly hydrolyzed by cholinesterases that potentiation of their effects by anti-cholinesterases, acting as such, cannot explain the increased and prolonged pharmacological effects observed. This evidence would appear contrary to the view that enzymatic hydrolysis was responsible for the brief neuromuscular block produced by this drug (Castillo & De Beer, 1950).

The well established anti-acetylcholinesterase activity of many quaternary ammonium compounds (Cohen, Warringa & Indorf, 1955; Riker, 1953; Todrick, 1954), many of which possess muscle relaxant activity, led to the view that the active centre of acetylcholine-esterase resembled the anionic site of the end plate

receptor on the postsynaptic muscle membrane. This hypothesis, first suggested by Roepke (1937), has been elaborated in more recent years to include a postulate of the receptor pattern itself, based on the activity of cholinesterase inhibitors (Cohen & Posthumus, 1955, 1957; Cohen et al. 1955; Van Der Meer & Meeter, 1956). That anti-cholinesterases may exert more than one effect at the neuromuscular synapse is accomodated by this view but the resemblance claimed to exist between the cholinergic receptor and cholinesterase leaves unexplained the finding that β,β-dimethylbutyl acetate, a compound closely related to acetylcholine and a satisfactory substrate for cholinesterase, is devoid of acetylcholine-like activity (Banister & Whittaker, 1951).

The activity of muscle relaxant compounds as cholinesterase inhibitors appears to be more marked against 'true' rather than against 'pseudo-cholinesterase' (Cohen et al. 1955; Paton & Zaimis, 1949). This is in keeping with the view that 'true' cholinesterase is responsible for the destruction of acetylcholine at the site of its physiological action (Hawkins & Mendel, 1947) and the weak anti-cholinesterase activity of

non-depolarizing drugs (Paton & Zaimis, 1949). Anti-cholinesterase activity in polymethylene bis-onium compounds was most marked among the higher members of the series which, when compared with eserine possessed considerable activity (Paton & Zaimis, 1949). Decamethylene bis-quinolinium salts, for example, are almost as effective inhibitors of true cholinesterase as eserine itself (Blaschko & Holtan, 1949).

It is apparent that anti-cholinesterase activity may be a contributory factor in the observed muscle relaxant potency of new drugs. The investigation of this effect, especially in compounds chemically similar to those known to possess anti-cholinesterase properties, would, it was believed contribute to a better understanding of the mode of action of new drugs and confirm their qualitative separation on the basis of existing classifications (Paton & Zaimis, 1951; Van Rossum, Ariëns & Linssen, 1958). Accordingly, a number of bis- and polyonium, aliphatic, polymethylene compounds, whose other pharmacological properties have been previously reported (Carey, Edwards, Lewis & Stenlake, 1959; Edwards and his colleagues, 1957, 1958a,b, 1959, 1961; Levis, Preat & Dauby, 1953; Muir & Lewis, 1959), have been investigated for anti-acetylcholinesterase activity.

Method

A cholinesterase-containing preparation from rat brain was made by the method of Fenwick, Barron and Watson (1957) and anti-acetylcholinesterase activity estimated, using the Warburg technique, by a modification of Ammon's method (1933). Acetylcholine chloride was used instead of the bromide described by Fenwick and his associates. In each estimation, a control flask containing no inhibitor and an additional enzyme flask containing distilled water in the side arm were used. The respiration of the brain tissue itself, unaffected by drugs, could thus be measured.

As a result of the hydrolysis of acetylcholine to acetic acid by the enzyme activity present in the rat brain, carbon dioxide was released from the Krebs' bicarbonate solution contained in each test flask. The inhibition of this process by drugs delaying the destruction of acetylcholine was used as a measure of their anti-cholinesterase effect. Different molar concentrations of each drug were employed and the mean of three to six results, expressed as a percentage reduction of the carbon dioxide evolved from the control flask and, allowing for the normal respiration of the

tissue alone, used as a measure of anti-cholinesterase activity. This was then plotted against the logarithm of the molar concentration of the inhibitor used. The negative logarithm of the molar concentration of the drug producing an inhibition of 50%, the pI 50, was then calculated according to the method of Blaschko, Bulbring and Chou (1949).

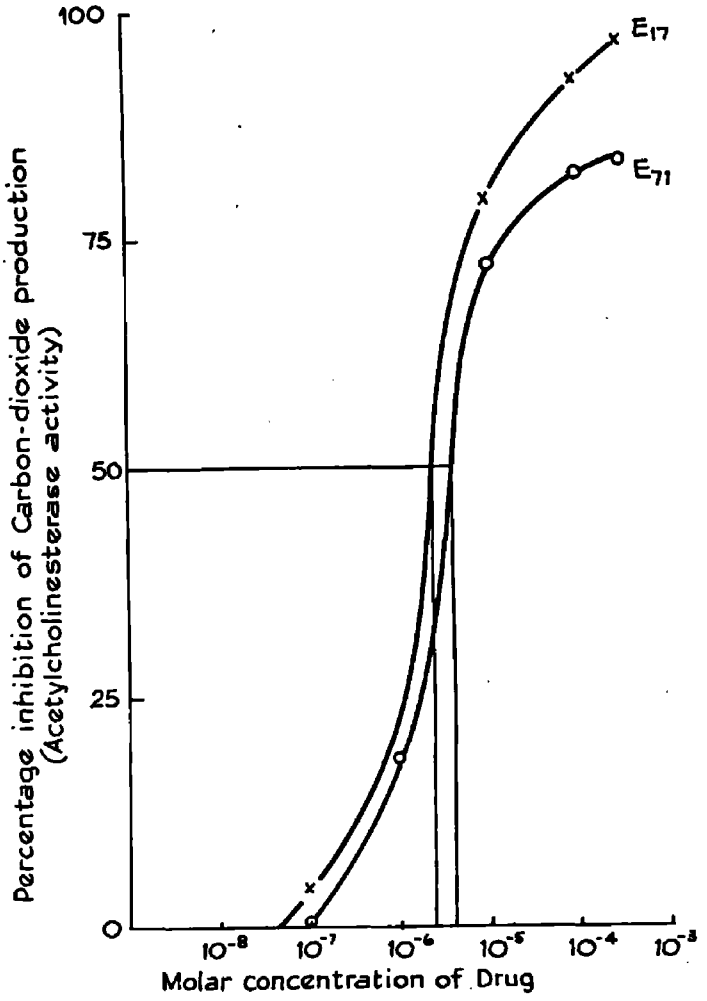


Fig. 75. Method of comparing the anti-acetylcholinesterase activity of compounds E17 and E71 expressed as the PI50 values (Blaschko, Bülbring & Chou, 1949). The PI50 is the negative logarithm of the molar concentration of the drug producing a 50% inhibition of carbon dioxide production.

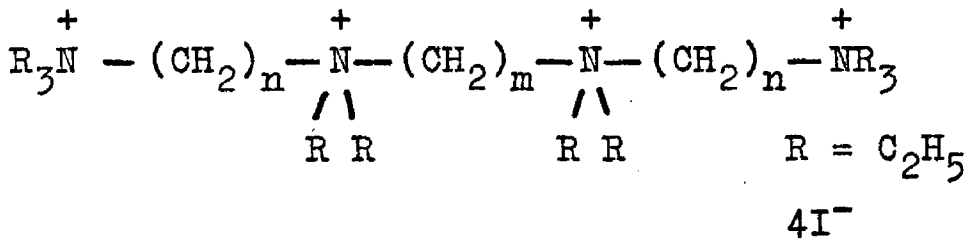
From the original graph,

PI50 (compound E17) = 5.6

PI50 (compound E71) = 5.4

TABLE XXI

The influence of inter-onium distance on anti-acetylcholinesterase activity, indicated by the PI50 value, of a series of tetra-ethonium aliphatic neuromuscular blocking compounds.



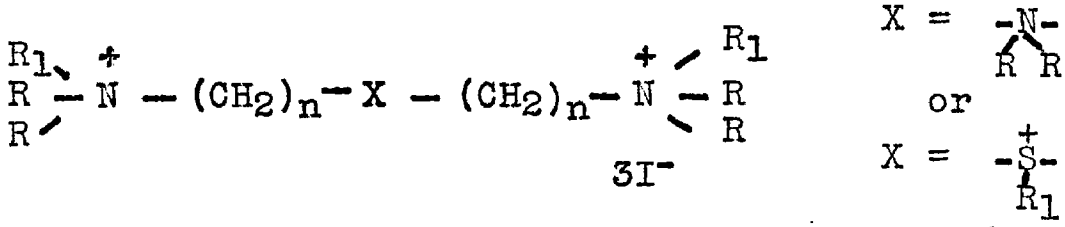
Code Number	n	m	Type of Neuro-muscular Block Produced	PI50
E36	6	6	Non-Depolarizing	3.5
E70	6	8	Non-Depolarizing	2.3
E71	8	6	Non-Depolarizing	5.4
E62	6	10	Mainly Non-Depolarizing but with Depolarizing Features also	4.1
E72	8	8	Mixed, but mainly Non-Depolarizing	5.3
E63	10	6	Mainly Depolarizing	6.2

TABLE XXII (contd.)

Code No.	R	R_1	n	X	Type of Block Produced	PI50
E18	CH_3	CH_3	6	S	Non-De-polarizing	3.5
E61	CH_3	C_2H_5	6	S	"	3.4
E60	C_2H_5	CH_3	6	S	"	3.4
E64	CH_3	n-C_4H_9	6	S	"	4.2
E65	C_2H_5	n-C_3H_7	6	S	"	4.2

TABLE XXII

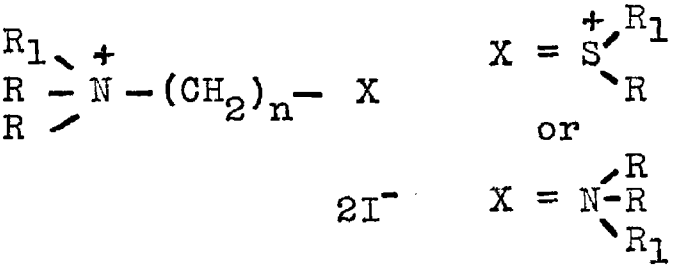
The comparative PI50 values of a series of tris-onium aliphatic neuromuscular blocking compounds showing the effect of alteration in the onium centres, inter-onium chain length and the alkyl substituents on anti-acetylcholinesterase activity.



Code No.	R	R_1	n	X	Type of Block Produced	PI50
E24	C_2H_5	C_2H_5	6	N	Non-De-polarizing	2.3
E41	C_2H_5	C_2H_5	8	N	"	6.0
E31	C_2H_5	C_2H_5	10	N	De-polarizing	5.5
E74	C_2H_5	CH_3	6	N	Non-De-polarizing	3.5
E75	C_2H_5	$n\text{-}C_3H_7$	6	N	"	3.5
E76	C_2H_5	$n\text{-}C_4H_9$	6	N	"	3.6
E82	$n\text{-}C_3H_7$	$n\text{-}C_3H_7$	6	N	"	3.5
E83	$n\text{-}C_3H_7$	C_2H_5	6	N	"	3.5
E84	$n\text{-}C_3H_7$	CH_3	6	N	"	3.7
E93	C_2H_5	CH_3	5	N	"	Nil (at m/200 conc.)
E94	C_2H_5	C_2H_5	5	N	"	"
E27	C_2H_5	C_2H_5	6	S	"	3.3
E40	C_2H_5	C_2H_5	8	S	"	5.7
E30	C_2H_5	C_2H_5	10	S	Mixed	5.9

TABLE XXIII

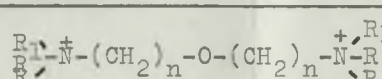
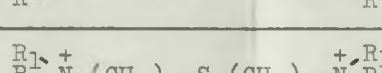
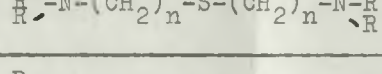
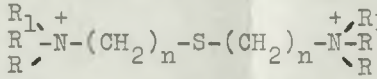
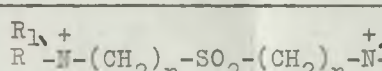
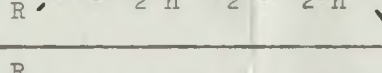
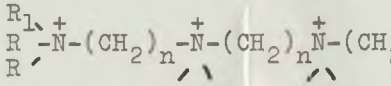
The comparative PI50 values of a series of bis-onium aliphatic neuromuscular blocking agents, showing the effect of alteration of the inter-onium chain length, the alkyl onium substituents and the onium centres on anti-acetylcholinesterase activity.



Code No.	R	R_1	n	X	Type of Neuro-muscular block produced	PI50
C6	CH_3	CH_3	6	$N(CH_3)_3$	Non-depolarizing	3.0
C10	CH_3	CH_3	10	$N(CH_3)_3$	Depolarizing	5.1
C10E	C_2H_5	C_2H_5	10	$N(C_2H_5)_3$	Non-depolarizing	5.1
C11	CH_3	CH_3	11	$N(CH_3)_3$	Mainly depolarizing	5.4
C12	CH_3	CH_3	12	$N(CH_3)_3$	Mixed depolarizing and non-depolarizing	5.5
C13	CH_3	CH_3	13	$N(CH_3)_3$		5.7
I	CH_3	C_2H_5	8	$S(CH_3C_2H_5)$	Mainly depolarizing	2.9
II	CH_3	CH_3	10	$S(CH_3)_2$	Depolarizing	4.9
III	CH_3	CH_3	8	$S(CH_3)_2$	Depolarizing	4.0

TABLE XXIV

The comparative anti-acetylcholinesterase activity, measured as the PI50 values, of a number of bis- and polyonium aliphatic neuromuscular blocking agents.

Name or Code Number	Basic Chemical Structure	R_1	R	n	An-ion	Type of Neuro-muscular Block	PI50
Oxydi-pentonium		CH_3	CH_3	5	2Cl	Depolarizing	5.4
E17 *		CH_3	CH_3	6	$2I^-$	Non-depolarizing	5.6
Ex *		CH_3	CH_3	10	$2I^-$	Mainly Non-depolarizing	5.9
E35 *		C_2H_5	C_2H_5	6	$2I^-$	Non-depolarizing	5.8
E36		C_2H_5	C_2H_5	6	$4I^-$	Non-depolarizing	3.5
E73		C_2H_5	C_2H_5	6	$6I^-$	Non-depolarizing	3.5
E58		C_2H_5	C_2H_5	6	$5I^-$	Non-depolarizing	3.3
Neostig-mine							7.1
Eserine							7.0

* Sulphur atom carries no positive charge

Results and Discussion

The pI50 values for the compounds investigated are shown in Tables XXI - XXIV together with the qualitative nature of the neuromuscular block produced. An example of the calculation of the pI50 values for compounds E17 and E71 is shown in Fig. 75. From the opposite page it can be observed that certain of the compounds investigated (compounds E30, E40, E41, E63, E17, E35, Ex) possessed anti-cholinesterase activity only slightly less than that of eserine and neostigmine. On the other hand, many compounds possessed negligible activity in concentrations not exceeding M/200. It is obvious that anti-cholinesterase activity varied with alteration in chemical structure and the results of the investigation can best be discussed on this basis.

Effect of Change in the Interonium Distance on Anti-cholinesterase Activity.

Interonium distance and especially the distance between the first or last onium centres in the polyonium compounds, is clearly an important factor in the anti-cholinesterase potency of the compounds tested. Increase in the number of methylene groups from five

(compounds E93 and E94) to ten (compound E31) increased potency. A very sharp rise in potency was observed when the chain length increased from six to eight methylene groups (compounds E24 and E41; compounds E27 and E40; compounds E36 and E72). An increase in the effective chain length beyond ten methylene groups (compounds E35, E17 and Ex) did not however, enhance potency. Similar results were observed using bis-onium derivatives (compounds C6, C10, C11, C12, C13 and the fully ethylated analogue of decamethonium) and are in agreement with those of Paton & Zaimis (1949) who reported an increase in anti-cholinesterase activity in a series of polymethylene bis-methonium compounds until the compound containing twelve methylene groups was reached. Potency was reduced however as the interonium chain length reached eighteen methylene groups.

This effect appeared to be dependent only on interonium distance and was unaffected by the size of the onium substituents or the nature of the onium centres themselves.

Effect of Increase in the Number of Onium Centres on Anti-cholinesterase Activity.

Providing that the interonium distance remains

constant, increase in the number of onium centres does not decrease anti-cholinesterase activity. The slight increase in anti-cholinesterase activity with increase in the number of onium centres sometimes observed (for example, compounds E24, E36 and E73; decamethonium and compound E31), is not comparable to the marked influence on potency produced by altering interonium chain length, and cannot be regarded as a major factor in determining the anti-cholinesterase potency of these drugs.

Effect of the Presence of one Sulphonium Atom.

In the N, N, N (compounds E24, E41 and E31) and N, S, N-ethonium series, (compounds E27, E40 and E30), it is clear that replacement of a quaternary nitrogen atom with a tertiary sulphur atom produced no dramatic alteration in anti-cholinesterase activity. Similar effects were observed in the bis-onium compounds II and decamethonium. These results parallel observations on the comparative muscle relaxant potency of these compounds. The compounds E17 and Ex in which the sulphur atom of each is uncharged, were as expected, more potent than the corresponding N,S,N-sulphonium derivatives, indicating the importance of interonium

distance in determining the anti-cholinesterase activity of these compounds.

Effect of Increasing the Size of the Alkyl Substituents on the Onium Centres.

Increase in the alkyl group size is less important than increase in the interonium distance but in both the N, N, N-(compounds E74, E24, E75, E76, E82 and E83) and N, S, N-(compounds E18, E27, E60, E61, E64 and E65) tris-onium series the presence of more bulky substituents was generally associated with a slight increase in potency. In each series, the ethonium derivatives E24 and E27 respectively were the least potent.

Many compounds capable of inducing muscle relaxant properties have an affinity for acetylcholine-receptors both in the post synaptic membrane and on acetylcholine-esterase itself. Consequently, although a number are primarily neuromuscular blocking agents, they also possess anticholine-esterase activity especially at higher concentrations. Moreover, certain drugs may induce muscle relaxation by virtue of their anticholine-esterase properties as indicated in the Ariëns' classification (see page 46 of this

thesis). For example, benzoquinonium is reported to have approximately one-quarter the anti-cholinesterase activity of neostigmine (Hoppe, 1951).

The contribution of the anti-cholinesterase activity to the muscle relaxant potency of the compounds structurally similar to those investigated in this thesis, can only be approximately estimated from these results. Although the anti-cholinesterase action of decamethonium, when compared to eserine, is appreciable, this effect is not believed to account substantially for the muscular fasiculations and initial stimulatory effects observed with this drug (Paton & Zaimis, 1949). Consequently, in those compounds in which anti-cholinesterase potency is less than that of decamethonium, it is unlikely that the accumulation of acetylcholine at the end plate is significantly contributing to the paralysis observed. On the other hand, in those compounds which possess a greater anti-cholinesterase activity than decamethonium (compounds E63, E41, E40, E31 and E30) this possibility cannot be so readily dismissed. It is interesting to observe that in each of these compounds, the distance between the charged onium centres (eight or ten methylene groups) is considerable. However, while it appears that anti-

cholinesterase activity is associated with a fairly large interonium distance, one cannot be certain of the effective distance involved since, as has been already indicated in this thesis (page 137), bisquaternary salts, at least, readily undergo ion pair complex formation. Conclusions concerning the distance between anionic sites on the acetylcholine-esterase enzyme itself cannot therefore be drawn from a consideration of the chemical structure of maximally effective compounds.

That anti-cholinesterase activity may not be contributing to the muscle relaxant potency of compounds of this type has been emphasized by the observations that anti-enzymic effects are not always associated with depolarizing properties. Thus compounds E40, E41, E72 possess a relatively high degree of acetylcholine-esterase activity, in comparison with the other compounds tested, yet are predominantly non-depolarizing muscle relaxants. Furthermore, the presence of depolarizing features in the neuromuscular blocking effects of certain polyonium derivatives, for example compounds E63, E31 and E30, may be independant of the anti-cholinesterase activity observed. In particular the comparable anti-

cholinesterase activity of decamethonium and its fully ethylated analogue, decaethonium, which is a non-depolarizing drug, suggest that the structure of acetylcholine-esterase and the cholinergic receptor may not possess identical features. On the other hand, it has been implied that the receptor for both acetylcholine-like effects and cholinesterase activity may be a single, though differentiated entity (Cohen & Posthumus, 1955; Cohen et al. 1955). The existence of independant 'A', 'B' and 'C' groups on the receptor might, it has been suggested, be responsible for combination with complementary centres of a reactant drug molecule. The 'A' groups are concerned with the interaction of specific cationic-effector drugs responsible for depolarization, the 'C' groups with non-specific cationic drugs which block the receptor without inducing depolarization and the 'B' groups which react with the esterase-active centre of the molecule. Cohen and his colleagues further suggested that the inhibition of true cholinesterase was a non-competitive effect, irreversible by high substrate concentrations and brought about by steric hindrance of the active site. Consequently, the union of a drug and the anionic component of the postulated receptor

could proceed without significantly affecting the inhibition of true cholinesterase. Differences in the anti-cholinesterase potency of non-depolarizing and depolarizing drugs can be explained on this basis. Conversely, the similarity in anti-cholinesterase potency and the qualitatively different neuromuscular blocking effects between decamethonium and decaethonium could be due to differences in affinity for the 'A' or 'C' groups of the postulated receptor site.

The results of the present investigation support the view that acetylcholine-esterase activity and depolarizing muscle relaxant properties are not brought about by drug interaction with identical receptors. The depolarizing activity of some of the compounds examined is thus believed to be independant of their anti-cholinesterase properties. Moreover, these results are not incompatible with the differentiation of a common receptor, as suggested by Cohen and his associates, into centres capable of combining with anti-cholinesterases and into specific and non-specific anionic sites.

CHAPTER II - SUMMARY

In the introduction to this chapter, the differentiation and classification of cholinesterase enzymes has been described and their role at the neuromuscular synapse discussed. The emergence of anti-cholinesterase activity in many quaternary ammonium compounds possessing neuromuscular blocking activity has been correlated with their qualitative effects at the neuromuscular synapse. The possibility that anti-cholinesterase effects contributed, in whole or in part, to depolarizing activity prompted the present investigation of a number of bis- and polyonium neuromuscular blocking agents.

Anti-acetylcholinesterase activity was estimated manometrically using a cholinesterase preparation from rat brain and a wide range of activity, calculated as the pI50 value, observed. None of the compounds tested was more potent than eserine or neostigmine.

The greater anti-cholinesterase-inhibitory action of depolarizing compared to non-depolarizing, agents has been confirmed but a direct correlation between depolarizing effects and anti-acetylcholine-esterase activity could not be established. These

results are not incompatible with the view that the cholinergic and acetylcholine-esterase receptors are probably not identical. The correlation of anti-acetylcholinesterase activity with the chemical structure of the compounds investigated has also been attempted.

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TABLE XXV

Formulae of Physiological Saline Solutions.

Salts g/l.	Frog Ringer's Solution	Tyrode's Solution	Locke's Solution
Sodium Chloride	6.5	8.00	9.00
Potassium Chloride	0.14	0.20	0.42
Calcium Chloride (Anhydrous)	0.12	0.20	0.24
Sodium Dihydrogen Phosphate	0.01	0.05	-
Sodium Bicarbonate	0.20	1.00	0.50
Magnesium Chloride	-	0.10	-
Glucose	-	1.00	1.00

APPENDIX I

In Table XXV, opposite this page, are given the formulae of the physiological saline solutions used in the experimental work described in this thesis. The frog Ringer's solution used in the Ariëns' procedure contained, in addition, glucose (1g/l.). All the chemicals used were of 'Analar' quality and glass distilled water only was employed. Aqueous stock solutions of certain salts were prepared to facilitate the preparation of a saline solution. These stock solutions could be used for at least two weeks after their preparation. Sodium bicarbonate solution was however freshly prepared. Glucose was added in the solid form to each batch of saline solution.

APPENDIX II

Commercially Available Materials

Throughout this thesis, the names of certain commercially available drugs have been abbreviated for convenience. The list of drugs used, together with their shortened names is as follows:

(1) Acetylcholine chloride is described as acetylcholine.

(2) (+)-Tubocurarine chloride is described as tubocurarine.

(3) (-)-Adrenaline hydrogen tartarate is described as adrenaline.

(4) Hexamethonium iodide is described as Hexamethonium.

(5) Decamethonium iodide is described as decamethonium.

(6) Neostigmine methyl sulphate is described as neostigmine.

(7) Edrophonium chloride is described as edrophonium.

(8) Sodium pentobarbitone is described as pentobarbitone.

(9) Ether anaesthetic is described as ether.

(10) Oxydipentonium di-chloride is described as oxydipentonium.

(11) (+)-O,O-Dimethyl tubocurarine di-iodide is described as tubocurarine dimethyl ether.

(12) Eserine salicylate is described as eserine.

The chemical nomenclature of the compounds investigated in this thesis are shown below. Their structural formulae together with the general code number are shown in Tables II and III (opposite page 99), and Tables XXI - XXIV opposite page 223.

Compounds described in Chapter I.

1. 7,7,13,13-Tetraethyl-7,13-diazonia-10-oxanonadecylenebis-(triethyl-ammonium)tetra-iodide is described as compound E77.
2. 7,13-Diethyl-7,13-dimethyl-7,13-diazonia-10-oxanonadecylenebis-(diethyl methylammonium) tetra-iodide is described as compound E78.
3. 7,7,13,13-Tetra-ethyl-7,13-diazonianonadecylenebis-(triethylammonium) tetra-iodide is described as compound E79.
4. 7,13-Diethyl-7,13-dimethyl-7,13-diazonianonadecylenebis-(diethylmethyl ammonium)tetra-iodide is described as compound 80.
5. 7,13-Diethyl-7,13-di-n-propyl-7,13-diazonia-10-oxanonadecylenebis-(diethyl-n-propylammonium) tetra-iodide is described as compound E96.
6. 7,13-Diethyl-7,13-di-n-propyl-7,13-diazonianona-decylenebis-(diethyl-n-propylammonium)tetra-iodide is described as compound E97.

7. 6,6-Diethyl-3,9-dioxa-6-azoniaundecylenebis (triethylammonium) tri-iodide is described as compound E90.

8. 6-Ethyl-6-methyl-3,9-dioxa-6-diazoniaundecylenebis (diethylmethyl-ammonium) tri-iodide is described as compound E91.

9. 6-Ethyl-6-methyl-6-azoniaundecylenebis-(diethylmethylammonium) tri-iodide is described as compound E93.

10. 6,6-Diethyl-6-azoniaundecylenebis-(triethylammonium) tri-iodide is described as compound E94.

11. 6-Ethyl-6-n-propyl-6-azoniaundecylenebis-(diethyl-n-propylammonium) tri-iodide is described as compound E95.

12. 6-Ethyl-6-methyl-3,9-dioxa-6-azoniaundecamethylenebis (triethyl-ammonium) tri-iodide is described as compound E98.

13. 6,6-Dimethyl-3,9-dioxa-6-azoniaundecamethylenebis (diethyl-methyl-ammonium) tri-iodide is described as compound E99.

14. 6,6-Dimethyl-3,9-dioxa-6-azoniaundecamethylenebis (trimethyl-ammonium) tri-iodide is described as compound E100.

15. 6-Ethyl-6-methyl-3,9-dioxa-6-azoniaundecamethylenebis(ethyldimethylammonium)tri-iodide is described as compound E101.

16. 6-Methyl-6-propyl-3,9-dioxa-6-azoniaundecamethylenebis (dimethylpropylammonium)tri-iodide is described as compound E102.

17. 6-Methyl-6-propyl-3,9-dioxa-6-azoniaundecamethylenebis(diethylpropylammonium)tri-iodide is described as compound E103.

18. Octamethylene-1-ethylmethylsulphonium-8-dimethylethylammonium di-iodide is described as compound I.

19. Decamethylene-1-dimethylsulphonium-10-trimethylammonium di-iodide is described as compound II.

20. Octamethylene-1-dimethylsulphonium-8-trimethylammonium di-iodide is described as compound III.

21. 7,7-Dimethyl-7-azoniatridecamethylenebis-(trimethylammonium) tri-iodide is described as compound E151.

22. 7-Methyl-7-Ethyl-7-azoniatridecamethylenebis (dimethylethylammonium) tri-iodide is described as compound E152.

23. 7-Methyl-7-Ethyl-7-azoniatridecamethylenebis (diethylmethylammonium) tri-iodide is described as compound E74.

24. 2-Trimethylaminoethyl-3-trimethylaminopropylether di-iodide is described as compound F. & P. 16575.

25. 2-Diethylmethylaminoethyl-3 trimethylaminopropyl ether di-iodide is described as compound F. & P. 16677.

26. 2-Diethylmethylaminoethyl-3 diethylmethylaminopropyl ether di-iodide is described as compount F. & P. 8302.

27. 2-Triethylaminoethyl-3triethylaminopropyl ether di-bromide is described as compound F. & P. 8303.

28. 2-Triethylaminoethyl-3-dimethylethylaminopropyl ether di-bromide is described as compound F. & P. 16678.

29. 3-3'bis(trimethylamino) di-n-propyl ether di-iodide is described as compound F. & P. 16701.

30. 3-(dimethylthylamino),3'-(triethylamino)-di-n-propyl ether di-iodide is described as compound F. & P. 17843.

31. 3-3'bis(triethylamino)di-n-propyl ether di-iodide is described as compound F. & P. 8212.

Chemical nomenclature of additional commercially non-available compounds investigated in Chapter II.

1. Decamethylene 1,10-bis-(triethylammonium) di-iodide is described as decaethonium.
2. Undecamethylene 1,11-bis-(trimethylammonium) di-iodide is described as compound C11.
3. Tridecamethylene 1,13-bis-(trimethylammonium) di-iodide is described as compound C13.
4. 7,7-Diethyl-7-azoniatridecamethylenebis(triethyl-ammonium) tri-iodide is described as compound E24.
5. 7-Ethyl-7-methyl-7-axoniatridecamethylenebis (diethylmethylammonium) tri-iodide is described as compound E74.
6. 7-Ethyl-7-n-propyl-7 azoniatridecamethylenebis (diethyl-n-propyl-ammonium) tri-iodide is described as compound E75.
7. 7-Ethyl-7-n butyl-7 azoniatridecamethylenebis (diethyl-n-butylammonium) tri-iodide is described as compound E76.
8. 7,7-Di-n-propyl-7 azoniatridecamethylenebis (tri-n-propyl-ammonium) tri-iodide is described as compound E82.

9. 7-Ethyl-7-n-propyl-7 azoniatridecamethylenebis (ethyl-di-n-propylammonium) tri-iodide is described as compound E83.
10. 7-Methyl-7-n-propyl-7 azoniatridecamethylenebis (methyl-di-n-propylammonium) tri-iodide is described as compound E84.
11. 9,9-Diethyl-9-azoniaheptadecamethylenebis (triethylammonium) tri-iodide is described as compound E41.
12. 11,11-Diethyl-11-azoniaheneicosylenebis (triethylammonium) tri-iodide is described as compound E31.
13. 9,9-Diethyl-9-azoniaheptadecamethylenebis (triethylammonium) tri-iodide is described as compound E72.
14. 7,7,14,14-Tetraethyl-7,14-diazoniaeicosylenebis (triethylammonium) tetra-iodide is described as compound E36.
15. 7,7,16,16-tetra-ethyl-7,16-diazoniadocosanebis (triethylammonium) tetra-iodide is described as compound E70.
16. 9,9,16,16-tetra-ethyl-9,16-diazoniatetracosanebis (triethylammonium) tetra-iodide is described as compound E71.

17. 7,7,18,18-tetra-ethyl-7,18-diazoniatetracosane-bis(triethylammonium) tetra-iodide is described as compound E62.

18. 11,11,18,18-tetra-ethyl-11,18-diazonia-octacosane-bis(triethylammonium) tetra-iodide is described as compound E63.

19. 7,7,14,14,21,21,28,28-octa-ethyl-7,14,21,28-tetra-azonia-tetratriacontane bis(triethylammonium) hexa-iodide is described as compound E73.

20. 7-Methyl-7-thioniatridecamethylenebis (trimethyl-ammonium) tri-iodide is described as compound E18.

21. 7-Ethyl-7-thioniatridecamethylenebis(triethyl-ammonium) tri-iodide is described as compound E27.

22. 9-Ethyl-9-thioniaheptadecamethylenebis(triethyl-ammonium) tri-iodide is described as compound E40.

23. 11-Ethyl-11-thioniaheneicosylenebis(triethyl-ammonium) tri-iodide is described as compound E30.

24. 7-Methyl-7-thioniatridecamethylenebis(dimethylethyl-ammonium) tri-iodide is described as compound E61.

25. 7-Methyl-7-thioniatridecamethylenebis(diethylmethyl-ammonium) tri-iodide is described as compound E60.

26. 7-n-butyl-7-thioniatridecamethylenebis(di-methyl-n-butyl-ammonium) tri-iodide is described as compound E64.

27. 7-Ethyl-7-thioniatridecamethylenebis(di-ethyl-n-propyl-ammonium) tri-iodide is described as compound E65.

28. 7-Dioxothioniatridecane-1,13-bis(triethylammonium) di-iodide is described as compound E35.

29. 7-Thiaoniatridecane-1,13-bis(trimethylammonium) di-iodide is described as compound E17.

30. 11-Thiaoniaheneicosylene-bis(trimethylammonium) di-iodide is described as compound Ex.

31. 7,7,14,21,21-Penta-ethyl-7,21 diazonia-14-thionia-heptacosylenebis(triethylammonium)penta-iodide is described as compound E58.

APPENDIX III

A statistical comparison of the intrinsic activities of oxydipentonium and decamethonium. The order of construction of the dose response curves to these drugs (2 μmoles/l) was determined using a table of random numbers and assigning equal chances to either drug of being used first or second on the same piece of tissue.

Expt. No.	1st Drug Added	2nd Drug Added	Height of 1st Contracture	Height of 2nd Contracture	Diff. in Height of 1st and 2nd Contractures
1	Decamethonium	Oxydipentonium	12.8	12.0	0.8
2	"	"	13.8	13.3	0.5
3	Oxydipentonium	Decamethonium	4.1	3.0	1.1
4	Decamethonium	Oxydipentonium	7.7	6.0	1.7
5	Oxydipentonium	Decamethonium	13.3	11.9	1.4
6	Decamethonium	Oxydipentonium	5.8	3.0	2.8
7	Oxydipentonium	Decamethonium	3.6	2.6	1.0
8	"	"	3.3	3.1	0.2
9	Decamethonium	Oxydipentonium	8.4	5.8	2.6
10	Oxydipentonium	Decamethonium	6.0	5.8	0.2

Expt. No.	1st Drug Added	2nd Drug Added	Height of 1st Contracture	Height of 2nd Contracture	Diff. in Height of 1st and 2nd Contractures
11	Decamethonium	Oxydipentonium	4.7	4.6	0.1
12	"	"	8.9	8.1	0.8
13	Oxydipentonium	Decamethonium	2.7	2.2	0.5
14	Decamethonium	Oxydipentonium	5.9	4.6	1.3
15	Oxydipentonium	Decamethonium	4.5	3.1	1.4
16	Decamethonium	Oxydipentonium	5.7	4.6	1.1
17	Oxydipentonium	Decamethonium	7.3	7.1	0.2
18	"	"	3.1	2.4	0.7
19	Decamethonium	Oxydipentonium	11.9	10.1	1.8
20	Oxydipentonium	Decamethonium	13.0	11.1	1.9

The standard Student's '<u>t</u>' test was used to determine the significance of the difference of the means ($\bar{x}_1$ and $\bar{x}_2$) of the contractural responses of the frog rectus abdominis muscle to decamethonium and oxydipentonium.

The variance (s^2) from the mean ($\bar{x}$) for each set of variates was calculated by the equation $s^2 = \frac{Sx^2 - \frac{(Sx)^2}{n}}{n-1}$ where Sx^2 is the sum of squares of x, $(Sx)^2$ is the square of the sum of x and n is the number of variates. The standard deviation of <u>each set</u> of variates (S.D.) is equal to the square root of the variance (s^2). The standard error of each mean (S.E.M.) was calculated from the formula $(\frac{S.D.}{\sqrt{n}})$. The standard deviation from the mean (Sd) for <u>two</u> sets of variates n_1 and n_2 of variance s_1 and s_2 was calculated by the equation $(Sd)^2 = \frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}$. The value of '<u>t</u>' was then obtained by applying the equation, $t = \frac{\bar{x}_1 - \bar{x}_2}{Sd}$ where $\bar{x}_1$ and $\bar{x}_2$ represent the means of the two sets of variates. In tables of '<u>t</u>'-distribution, to obtain the values of P (probability), the degrees of freedom (D.F.)were calculated from the expression, $D.F. = n_1 + n_2 - 2$.

The differences in the heights of the contractural responses in each experiment (shown below) were then compared as follows:-

Decamethonium	Oxydipentonium
0.8, 0.5, 1.7, 2.8, 2.6	1.1, 1.4, 1.0. 0.2, 0.2,
0.1, 0.8, 1.3, 1.1, 1.8.	0.5, 1.4, 0.2, 0.7, 1.9.

Mathematical Symbol	Decamethonium	Oxydipentonium
sx	13.5	8.6
sx^2	25.17	10.6
n	10	10
$\bar{x}$	1.35	0.86
$(sx)^2$	182.25	73.96
$(sx^2)/n$	18.225	7.396
s^2	0.7716	0.356
S.D. $(=\sqrt{s^2})$	0.878	0.597
S.E.M. $(=\frac{S.D.}{\sqrt{n}}$	0.2776	0.1887
Sd	0.3357	
t	1.4596	
P	$>0.1<0.2$	

From these results there is no significant difference in the heights of the contractural responses (intrinsic activities) of the two drugs.