STUDIES ON THE MODE OF ACTION OF PHARMACOLOGICALLY ACTIVE SUBSTANCES FROM NATURAL SOURCES WITH ADDITIONAL STUDIES ON SOME SYNTHETIC NEUROMUSCULAR BLOCKING AGENTS

A Thesis submitted to the University of Glasgow in candidature for the degree of

Doctor of Philosophy

in the

Faculty of Medicine

by

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March, 1958.

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ACKNOWLEDGMENTS

I have pleasure in recording my indebtedness to the many people who have aided me in the preparation of this thesis.

I am grateful to Professor Stanley Alstead, Regius Professor of Materia Medica and Therapeutics at the University of Glasgow, for his encouragement and kindness.

In particular, I wish to offer my sincerest thanks to Mr. J. J. Lewis of this department who suggested to me the problems investigated. Throughout my post-graduate training, he has been a constant source of helpful advice and guidance, friendly criticism and whole-hearted encouragement.

I wish to thank my colleagues in the department for their help in different ways.

I am indebted to Dr. S. D. Silvay of the Mathematics Department of the University for his guidance in the statistical analysis contained in the thesis.

I am very grateful to Mr. R. Callander for drawing all the line diagrams and for lettering the figures, and to /

to Miss I. Moon, Miss S. Grace, Miss J. Hall and Mr. P. Leitch for their invaluable assistance with the photographic work.

I wish also to thank Miss E. J. Newell who typed the thesis, and with whom it has been a pleasure to co-operate.

Lastly, I am grateful to the following persons for their kindness in generously supplying the materials investigated:

(1) Dr. C. D. Falconer of Ciba Laboratories Ltd. and Dr. J. A. Lumley of Riker Laboratories Ltd.

(2) Dr. J. B. Stenlake and Dr. D. Edwards of the Royal College of Science and Technology, Glasgow.

(3) Dr. J. A. D. Jeffreys of the Department of Chemistry, University of Leeds.

(4) Dr. M. F. Grundon of the Department of Chemistry, Queen's University, Belfast.

LIST OF PUBLICATIONS.

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

(1) The Pharmacology of Some Hydroxybenzylisoquinoline Derivatives.

J. Pharm. Pharmacol., (1957), 9, 955.

(2) Some NSN-Tris-quaternary Neuromuscular Blocking Agents.

J. Pharm. Pharmacol., (1957), 9, 1004.

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

The following publications have been submitted or are about to be submitted to the Editor of the Journal of Pharmacy and Pharmacology:

(1) Neuromuscular Blocking Agents - Part II. The Preparation and Properties of a Series of NSN- and NNN-Trisethonium Compounds (with D. Edwards, J. J. Lewis and J. B. Stenlake).

(2) /

(2) Neuromuscular Blocking Agents - Part III. The Preparation and Properties of a Series of NNNN-Tetraethonium Compounds (with D. Edwards, J. J. Lewis and J. B. Stenlake).

(3) A Note on the Pharmacology of Serpentine and Rescinnamine, Two Minor Alkaloids of Rauwolfia serpentina.

In addition, part of this work was communicated, jointly with J. J. Lewis, at the following meetings:

(a) British Pharmacological Society at Edinburgh in July, 1956 - The Properties of some synthetic alkaloids related to curare.

(b) British Pharmacological Society at Oxford in July, 1957, - Some tris quaternary compounds with neuromuscular blocking activity.

(c) British Pharmacological Society in London in January, 1958,- The pharmacology of some tris- and tetra-quaternary salts with neuromuscular blocking activity.

(d) British Pharmaceutical Conference at Bristol in September, 1957, -

(1) /

(d)..continued.

(1) The Pharmacology of Some Hydroxybenzyl-isoquinoline Derivatives.

(2) Some NSH-Tris quaternary Neuromuscular Blocking Agents. (and with D. Edwards and J. B. Stenlake).

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PART I

STUDIES ON THE MODE OF ACTION OF RESCINNAMINE

CHAPTER I

INTRODUCTION

The introduction into clinical medicine and the subsequent wide therapeutic use of various extracts and alkaloids of Rauwolfia serpentina Benth in the treatment of high blood pressure and certain types of mental disorders, has recently aroused much interest among pharmacologists, clinicians and organic chemists. The root of R. serpentina has been used for centuries in Indian folk medicine for the treatment of a wide variety of conditions ranging from snake bite and dysentery to various types of mental disorder including excitement, maniacal behaviour associated with psychoses, epilepsy and anxiety.

The use of this drug in human hypertension has been a recent development. Its beneficial effects in this condition were first reported by Chakravarty and his colleagues[1] in 1951.

Rauwolfia is a paratropical genus of about 130 species[2,3] consisting of shrubs and small trees. Rauwolfia serpentina Benth (family Apocynaceae) is a small erect shrub about three feet high which is indigenous to India, Burma, Malaya, Siam and Java.

Of /

Of the twenty or more alkaloids which have now been isolated from R. serpentina, reserpine has been the most extensively studied from both the pharmacological and clinical aspects. Two recent articles[2,3] review the available literature.

Rescinnamine, another clinically important alkaloid of Rauwolfia serpentina, was isolated in 1954 by Klohs and his colleagues[4,5]. This compound has been characterised as the 3 : 4 : 5 - trimethoxycinnamic acid ester of methyl reserpate, and like reserpine it is a tertiary indolic base related to yohimbine (Fig. 1, p.3).

Clinical trials of rescinnamine by Smirk and McQueen[6] and Hershberger et alia[7] have indicated that there is apparently no important difference between the hypotensive effects of rescinnamine and those of reserpine. They also observed that mental symptoms occurring in patients treated with reserpine such as depression, diurnal somnolence, lassitude, nocturnal dreaming and nightmares may often be relieved by changing the patients to rescinnamine, although the change to rescinnamine does not influence the control of blood pressure. Its administration was observed to be accompanied by much the

same /

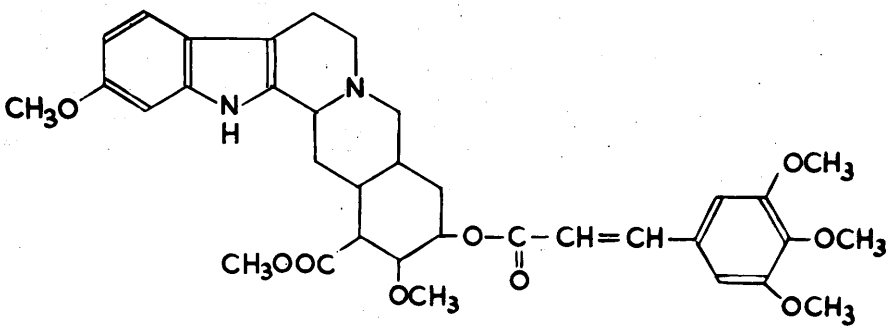

Figure 1. The structural formula of reserpinnamine.

same qualitative side effects as noted after reserpine, but the severity of the side effects was less[7,8]. Lemieux and his colleagues[9], however, observed that orally administered rescinnamine was clinically less potent than reserpine in the treatment of hypertension.

In lower animals rescinnamine has been reported to have hypotensive properties similar to, or even greater than those of reserpine[10] and also to have certain quantitatively different but otherwise similar effects on the central nervous system.

In the perfused, innervated but otherwise isolated hindquarters of the rat, in which the vasomotor tone had been increased by infusion of noradrenaline, pitressin or barium chloride, McQueen and Blackman[11] have shown that both reserpine and rescinnamine exert a direct vasodilator effect. Rescinnamine has also been described by Cronheim et alia[12] as having all of the typical pharmacological properties of reserpine and Alseroxylon (Rauwiloid - Riker) - a concentrate of the pharmacologically active and clinically useful alkaloids of the root of Rauwolfia serpentina. When given orally or by intravenous injection to normotensive dogs, it was shown

to /

to cause bradycardia, hypotension and, at higher dose levels, sedation. The prolongation of pentobarbitone-induced sleep in mice, which has been suggested as an index of the sedative activity of Alseroxylon[13], is also observed with rescinnamine. Rescinnamine has been reported to cause an increase in the pressor response to adrenaline[12], and reversal of the pressor response to hypoxia in dogs[12]. The pressor responses due to bilateral occlusion of the carotid arteries and electrical stimulation of the central end of the cut vagus in dogs have also been reported to be significantly diminished by rescinnamine[12].

While rescinnamine has been reported to possess similar therapeutic properties to reserpine in the treatment of hypertension in man, this drug has not so far been thoroughly investigated either in man or in laboratory animals. It was therefore felt that a more thorough evaluation of this drug was necessary in order to confirm and extend previous work. Very little information is available about the effects of rescinnamine on the cat, or on isolated tissues and organs. It was therefore decided to study the properties of rescinnamine on /

on isolated tissues as well as on the blood pressure of the anaesthetised cat etc.

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C H A P T E R II

A. MATERIALS

Throughout this part of the thesis, the names of certain drugs have been abbreviated. The list of drugs used in this part, together with their shortened names, is as follows:-

(1) Acetylcholine chloride	is	described	as	acetylcholine
(2) Atropine sulphate	"	"	"	atropine
(3) (±)-Tubocurarine chloride	"	"	"	tubocurarine
(4) (-)-Adrenaline hydrochloride	"	"	"	adrenaline
(5) (-)-Noradrenaline bitartrate	"	"	"	noradrenaline
(6) Histamine acid phosphate	"	"	"	histamine
(7) 5-Hydroxytryptamine creatinine sulphate	"	"	"	5-hydroxy-tryptamine
(8) Sodium pentobarbitone	"	"	"	pentobarbitone
(9) Chloralose	"	"	"	chloralose.

The composition and methods of preparation of all physiological saline solutions used in this investigation are to be found in Appendix I (pages 334 and 336).

In Appendix I are detailed also the methods used in preparing /

preparing solutions of rescinnamine.

The conventional abbreviations for volumes and weights of the metric system are used throughout this thesis.

CHAPTER II

B. EXPERIMENTAL

1. Experiments on the Blood Pressure of the Anaesthetised Cat.

METHOD.

Cats of either sex, weighing between 2.0 and 4.0 kg. were used. Anaesthesia was induced by means of an intraperitoneal injection of chloralose. A one per cent solution of chloralose in distilled water was made. When this solution was used, a dose of 8 ml. per kg. (80 mg. per kg.) was usually sufficient to produce surgical anaesthesia in about 30 minutes.

In some cats sodium pentobarbitone was used as the anaesthetic. 60 mg. per kg. injected intraperitoneally was found to be an adequate anaesthetic dose.

The anaesthetised cat was laid on its back upon an operating table; the legs were secured to the table and the head extended by passing a string through the skin at the apex of the lower jaw. The skin covering the neck was cut away from the sternum up to the apex of the mandible. The fascia covering the trachea was divided at the midline and the blunt point of an aneurysm needle was passed between the muscles of the neck and thence around the trachea. A strong /

strong linen thread was passed around the trachea and a transverse incision made in it by means of a scalpel. The cut edge of the partly severed trachea was held in a pair of blunt forceps and a tracheal cannula inserted and tied into place. This was done as a precautionary measure in case it became necessary (due to drug-induced respiratory depression or failure) to keep the cat alive under artificial respiration. The amount of air entering and leaving the cannula could be controlled by means of an adjustable sleeve or side tube.

The external jugular vein, usually of the left side, was then cannulated. To do this the skin of the left anterolateral part of the neck was removed and the left external jugular vein exposed. The fascia covering the vein was carefully removed, a thread was tied around the cephalad end and a bulldog clip was put on the vein on the cardiac aspect. A small transverse cut was made in the dilated vein by means of a pair of sharp pointed iris scissors. A vein cannula, filled with a solution of heparin, was then inserted through the incision with the pointed end toward the heart. The cannula was connected by means of rubber tubing to a 50 ml. burette containing normal /

normal saline. All the air bubbles had been previously removed from the system. The observation that the saline in the burette ran freely into the vein indicated that the cannula had been correctly inserted. Having completed the cannulation of the trachea and of the external jugular vein, an artery cannula was inserted into one of the carotid arteries. The artery was first tied off as near to the head as possible: a bulldog clip was then placed on the artery about three cm. below the ligature and a thread was passed under the vessel midway between the ligature and the bulldog clip. A small transverse cut was made in the artery by means of a pair of sharp pointed iris scissors. An artery cannula, filled with a solution of heparin, was then inserted through the incision with the pointed end towards the heart. The cannula was connected to a mercury manometer, and the space between the mercury and the artery filled with a twenty-five per cent solution of sodium thiosulphate as an anticoagulant. Air having been displaced from the cannula and the pressure in the manometer set at about 120 mm. of mercury, the artery clip was removed. A writing flag on one arm of the mercury manometer recorded the blood pressure on a smoked surface.

Drug solutions were injected into the rubber connection between the vein cannula and the burette. Each injection was followed by the infusion of 4 ml. of saline from the burette. The blood pressure was usually recorded from the cannulated common carotid artery, but when a reflex pressor response was elicited by means of bilateral occlusion of the common carotid arteries, the blood pressure level was recorded from one of the cannulated femoral arteries.

The effects of rescinnamine on various pressor reflexes were studied by comparing the nature and magnitude of the reflex responses observed before and after the injection of rescinnamine. At least three uniform reflex responses were obtained before injection of rescinnamine. Pressor responses were obtained following the elicitation of the following reflexes:-

(1) The Carotid Sinus Pressor Reflex. Bilateral occlusion of the common carotid arteries for a period of twenty to thirty seconds: this reflex was elicited either by the placing of bulldog clips on to the arteries or by pulling upon threads looped loosely around them.

(2) Occlusion of the Abdominal Aorta. The abdominal cavity was opened by a midline incision. The rectus muscles /

muscles and the fasciae were retracted, the viscera pushed to the right and the abdominal aorta carefully dissected free from the fascia at a point a little below the diaphragm. A thread was then passed loosely around the aorta by means of an aneurysm needle at a point just above the origin of the coeliac artery. The abdominal aorta was occluded for ten seconds by pulling upon the thread.

(3) Stimulation of the Cut Central End of the Cervical Vagus. The right vago-sympathetic trunk was carefully dissected free from its fascia and separated from adjacent structures. The vagus was then freed from the cervical sympathetic trunk with which it runs. The vagus was divided by means of scissors at as low a point in the neck as possible. The central end of the cut vagus was placed upon a pair of platinum electrodes which were connected to the output of a stimulator. The nerve was stimulated by means of square wave impulses at a frequency of 800 to 1,000 per minute, at 8 to 12 volts and with a pulse width of 0.5 to 1.0 msec. Stimulation was continued for periods of fifteen to twenty seconds.

(4) Stimulation of the Greater Splanchnic Nerve. This nerve joins the coeliac ganglion which is situated a little

below the coeliac artery near to its origin from the aorta, and distributes fibres to the renal and suprarenal plexuses and to the other abdominal nerve plexuses. The coeliac ganglion was carefully dissected free and the greater splanchnic nerve traced upwards and cleared from fascia. The nerve was divided close to the ganglion and the cut central end was placed upon a pair of platinum electrodes which were held in position by means of a clamp, and the abdomen closed. The nerve was stimulated with fifteen seconds bursts of impulses by means of a Dobbie McInnes square wave stimulator, at a frequency of 800 to 1200 per minute, at 10 volts and with a pulse width of 1.0 to 1.5 msec.

(5) Hypoxia. The cat was allowed to inhale a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide from a Douglas bag for one and half minute periods.

In a number of anaesthetised cats (pentobarbitone or chloralose), the blood pressure level was raised by the continuous infusion of solutions of adrenaline or noradrenaline, and rescinnamine was injected when the elevated blood pressure level had become steady. The concentration of the adrenaline or noradrenaline solutions which were infused /

infused was 0.10 mg. per ml. and the rate of infusion was 1.0 ml. per minute from a Palmer's slow infusion apparatus.

2. Experiments on the Blood Pressure of Spinal Cats.

METHOD.

Cats within the weight range of 2.0 to 3.0 kg. were given atropine (1.0 mg. per kg.) by intraperitoneal injection, about 15 minutes before induction of anaesthesia by ether. The common carotid arteries were dissected free from the accompanying vagosympathetic trunks and tied. The trachea was next freed from adjoining tissues and cannulated. The tracheal cannula was connected by means of rubber tubing to a bottle containing ether. The ether bottle could be joined rapidly and easily by means of rubber tubing to an artificial respiration pump. The cat was then turned over, and the spinal cord exposed in the vicinity of the long spine of the second cervical vertebra. The bony covering of the spinal cord and finally the cord itself were cut by means of bone forceps. As soon as the cord was transected, artificial respiration was commenced. Bleeding was arrested by means of cotton wool swabs soaked in hot normal saline. A probe was inserted through the foramen magnum, pushed up into the brain and the brain destroyed. The cut end of the spinal canal was plugged with /

with plasticine and the area swabbed clear. The skin over the back of the neck was closed with the aid of surgical clips, and the animal again turned on to its back. One of the carotid arteries was cannulated and connected to a conventional pressure recording system filled with a 25 per cent sodium thiosulphate solution. A mercury manometer carrying a writing flag on one arm was incorporated into this system and arranged so as to record the blood pressure on a smoked surface. The external jugular vein was cannulated and connected by rubber tubing to a burette filled with normal saline. Drug solutions were injected into the rubber connection between the vein cannula and the burette. Each injection was followed by the infusion of 4 ml. of saline from the burette.

In all experiments, the preparation was left to settle for at least one hour before any experiment was carried out. No drug was given unless the blood pressure level had remained constant for from 15 to 30 minutes.

3. Experiments on Isolated, Perfused Rabbit and Kitten Hearts.

The isolated hearts of both rabbits and kittens were perfused according to the method of Langendorff[1]. This involves perfusion of the coronary vessels through the aorta. /

aorta. Wegria[2], in his review on the pharmacology of the coronary circulation, quotes several published criticisms of this method. It is pointed out that the recorded outflow will give a true picture of the state of tonus of the coronary vessels only if the aortic valves are competent. This is not always so. In the event of aortic incompetence, some perfusion fluid will leak past the valves into the left ventricle and so into the left atrium, and thence to the exterior. The increased outflow may therefore exceed the true coronary outflow by the amount of fluid which has passed into the left ventricle. The volume of fluid draining into the right atrium via the ventricle is not constant and, in addition, cannot be measured satisfactorily. It is also pointed out that the volume of coronary perfusate may be increased by a purely mechanical massaging effect which cardiac muscle - stimulated by a cardiotonic drug - has upon the coronary vessels. Under these circumstances, an increase in outflow might be taken to indicate a coronary dilatation which in fact was not present. For these reasons it was decided that the fluid draining from the heart should be described simply as the "cardiac outflow". In spite of the objections raised to the use of this method, it was felt /

felt that the Langendorff preparation would still give some useful information about the effects of drugs on cardiac function *in vitro*. By carefully observing the heart rate, the amplitude of the contractions, and at the same time measuring the outflow, an estimate of alterations of cardiac function as well as of the tonus of the coronary vessels can be obtained.

METHOD.

Rabbits and kittens used were within the weight ranges of 1.0 to 2.0 kg. and 0.6 to 1.0 kg. respectively. The animal was killed by a blow on the head. The throat was cut and the blood allowed to drain out. The animal was then placed upon its back on a dissecting board and the thoracic cavity exposed, care being taken not to damage the heart with scissors or other instruments. The lungs were removed and a thread was tied loosely around the aortic arch proximal to the origin of the innominate artery. The venae cavae and aorta were then severed and after removing the pericardium, the heart was lifted out of the thorax and placed in a dish of warm Locke's solution (Appendix I p.336) at a temperature of about 37°C, and containing a little heparin to prevent the blood inside the heart from clotting. A stream of Locke's solution was allowed /

allowed to run through the superior vena cava from a pipette, and the heart was squeezed gently. After washing, a cannula was tied into the aorta taking care that its tip was distal to the coronary ostia. The preparation was then set up by connecting the cannula to the perfusion apparatus. Perfusion of oxygenated Locke's solution, containing double the normal concentration of glucose, was started at a constant rate of flow and at a pressure of 35 mm. of mercury, care being taken that no air bubbles entered the aorta. Any blood remaining in the preparation was rapidly washed away, and as a rule the heart started to beat immediately. After about thirty minutes, when the beat had become regular, a supporting thread was tied by means of a fine needle through the tip of the left ventricle. A bent entomological pin was inserted into the wall of the right ventricle and connected to a Starling's heart lever which recorded the contractions of the heart on the surface of a smoked drum. Doubling the normal concentration of glucose in the perfusion fluid gave a more active preparation, and one which was fatigued less easily. The Locke's solution from the two reservoirs used flowed through heating coils in a water bath maintained thermostatically at 37°C. The two coils were connected

by /

by a glass Y-piece which was joined to the aortic cannula by a short length of rubber tubing. The temperature drop between the thermostatically controlled water bath and the cannula was never more than 0.2°C.

Rescinnamine was dissolved in Locke's solution in one of the reservoirs to give the concentration of 1.0 µg. per ml. Alternatively a measured volume of the control solution was mixed with Locke's solution in the bottle. The other bottle contained Locke's solution. Solutions of other drugs in Locke's solution were injected by means of a one ml. tuberculin syringe into the rubber tubing attached to the aortic cannula. In some experiments, a solution of rescinnamine was also injected in this way. The heart rate was counted by inspection of the tracing or by direct observation, and the outflow measured at five minute intervals by collecting the perfusate for a period of one minute. In some experiments the outflow was measured by means of a Gaddum outflow recorder.

4. Experiments on the Isolated Auricles of the Guinea Pig.

These experiments were carried out in order to study the action of rescinnamine on isolated cardiac muscle.

METHOD. /

METHOD.

Adult guinea pigs of either sex were killed by a blow on the head. The throats were cut and the blood allowed to drain out. The hearts were removed as rapidly as possible and immersed in well-oxygenated Locke's solution. Using a pair of fine scissors, the ventricles were carefully removed and the auricles placed upon a cork mat and moistened frequently with Locke's solution. All extraneous tissue was dissected away until the horseshoe shaped auricles alone remained. These were then suspended in a 50 ml. organ bath by means of two bent entomological pins to which fine cotton threads were tied. One thread was connected to the oxygen delivery tube at the base of the bath, the other to a Starling's heart lever set up so as to record the contractions of the auricles upon a smoked surface. After about twenty to thirty minutes, the beat of the auricles had usually become regular and the experiment was commenced. All drugs were added to the bath as solutions in Locke's solution, and by means of a one ml. tuberculin syringe. Rescinnamine was added in the form of a solution as described in Appendix I, page 334. The effect of each drug was observed for a period of sixty seconds, after which the fluid in the bath was replaced. Sufficient /

Sufficient time was allowed for the auricles to regain a normal regular rhythm and amplitude of beat before the next addition of drug.

5. Experiments using Strips of Horse Artery.

METHOD.

Lengths of carotid artery were removed at the slaughter house from freshly killed horses. A portion of the artery was freed from fascia and a strip about four cm. in length and two mm. wide was made by cutting the artery into a spiral by means of a pair of iris scissors. Threads were tied to both ends of the segment and the strip of artery set up in a forty ml. organ bath containing oxygenated Tyrode's solution (Appendix I, page 336) at 37± 0.5°C. The thread at one end of the artery strip was fixed to the lower end of a glass tube supplying oxygen to the bath; the thread at the other end was attached to a modified frontal point writing lever giving a magnification of about 1 in 10. The strip was stretched for about fifteen minutes by means of a ten gramme weight. Before the experiment was started the additional weight was removed and the lever readjusted. Contractions of the strips of artery were induced by addition to the bath of adrenaline, /

adrenaline (1.0 to 2.0 µg. per ml.), noradrenaline (1.0 to 2.0 µg. per ml.), 5 - hydroxytryptamine (0.10 to 0.50 µg. per ml.), acetylcholine (0.02 to 0.1 µg. per ml.) or histamine (1.0 to 2.0 µg. per ml.). These were added to the bath as solutions in Tyrode's solution by means of a tuberculin syringe. Two types of experiments were performed.

(1) Contractions of the artery strip were elicited by the addition to the bath of the stimulant drug. While the artery strip was still in a stage of contraction, rescinnamine (2.0 to 40.0 µg. per ml.) was added to the bath. In most cases the artery strip relaxed following addition of rescinnamine to the bath. Comparisons were made by repeating the experiment using a control solution.

(2) Stimulant drugs (in the dose range mentioned above) were added to the bath and the effects observed for eight minutes. Before adding rescinnamine, at least two uniform submaximal contractions were obtained to the same dose of the stimulant drugs added to the bath at fifteen to twenty minute intervals. Rescinnamine (1.0 to 10.0 µg.,per ml.) was added to the bath ten minutes before the next addition of the stimulant drug and allowed to remain in contact with the tissue for eighteen minutes. The experiments were repeated using the control solution.

6. Experiments on the Isolated Perfused Rat Hindquarters.

METHOD.

In these experiments the pressure at which the physiological fluid passed through the blood vessels was kept constant, and the alterations in the rate of outflow of the perfusion fluid which were produced by the drug were recorded by means of Gaddum's drop recorder. The vessels were perfused with oxygenated Locke's solution at room temperature.

Rats of either sex, weighing between 200 and 300 g., were killed by a blow on the head. The throats were cut and the blood allowed to drain out. The abdominal cavity was opened by means of a longitudinal incision extending from the sternum to the anus. The rectum, the oesophagus and the inferior and superior mesenteric arteries were divided between ligatures. The abdominal viscera were then removed. This brought into view the abdominal aorta which was cannulated. The body wall and vertebral column were transected above the point of cannulation and the cannula attached to the perfusion system by means of fine rubber tubing. The perfused hind-quarters were set up in the apparatus shown in Fig. 2, p. 27.

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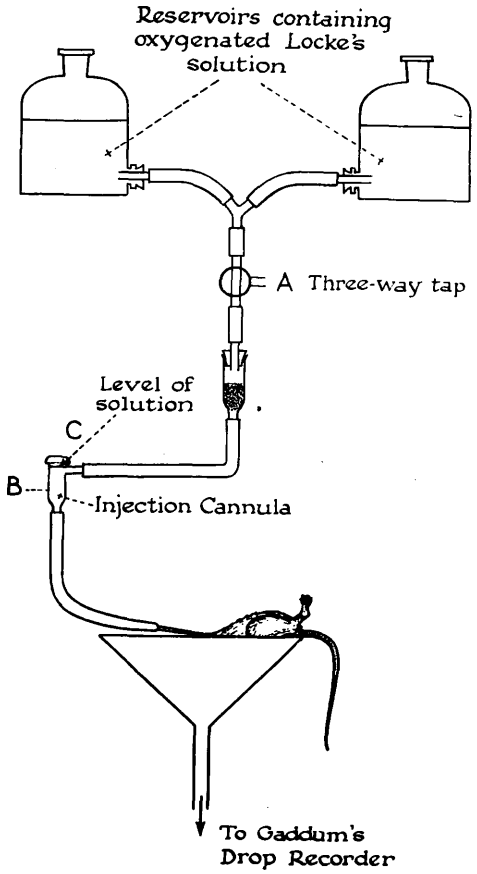

Figure 2. Diagram of the apparatus used for perfusion of the isolated rat's hindquarters.

Two reservoirs are included in this apparatus. These are joined by means of a glass three-way stopcock. The rate of flow of fluid from the bubble trap to the injection cannula is controlled by the tap A in Fig. 2, p.27, and can be adjusted to a suitable rate value at the beginning of each experiment. The injection cannula, (the design of which was based upon that suggested by Gaddum and Kwiatkowski[3]) (Fig. 2,B), allowed the drug solutions to be injected at a constant rate. This was achieved by injecting the solution with a tuberculin syringe, fitted with a fine needle, through the rubber cap at a rate such that the level of fluid in the cannula (Fig. 2,C) was unaltered during the process. The hindquarters preparation was placed on a muslin rest lying in a filter funnel. The outflow was led <u>via</u> the filter funnel to the contacts of a Gaddum drop recording assembly[3].

After setting up the preparation, a uniform outflow record was obtained for at least 15 minutes before drugs were injected. Rescinnamine was dissolved in the Locke's solution in one of the reservoirs to give the desired concentration (0.10 to 10 µg. per ml.). Alternatively, a measured volume of the control solution was mixed with the Locke's solution. In a few experiments, the solution

of /

of rescinnamine was injected into the cannula by means of a tuberculin syringe.

7. Experiments carried out Using the Isolated Guinea Pig Ileum.

METHOD.

Guinea pigs of either sex, weighing between 0.3 and 0.5 kg., were killed by a blow on the head and the throats cut in order to drain out the blood. The abdominal cavity was opened and a piece of ileum about 3 cm. long was removed from the region about 3 cm. proximal to the ileocaecal junction. It was then freed from extraneous tissue and the contents washed out by means of a stream of Tyrode's solution (Appendix I , p.336). Threads were tied to both ends of the segment which was then set up in a 2 ml. organ bath (Fig.3, p.30) containing oxygenated Tyrode's solution. One thread was attached to a modified frontal point writing lever, and the other to a hook fixed into the base of the bath. The fluid in the bath was oxygenated by passing oxygen through a hypodermic needle fixed into the base of the bath. The temperature was maintained thermostatically at 30± 0.5°C. Solutions containing the stimulant drugs were added and washed out automatically using the overflow principle. The inlet

tube /

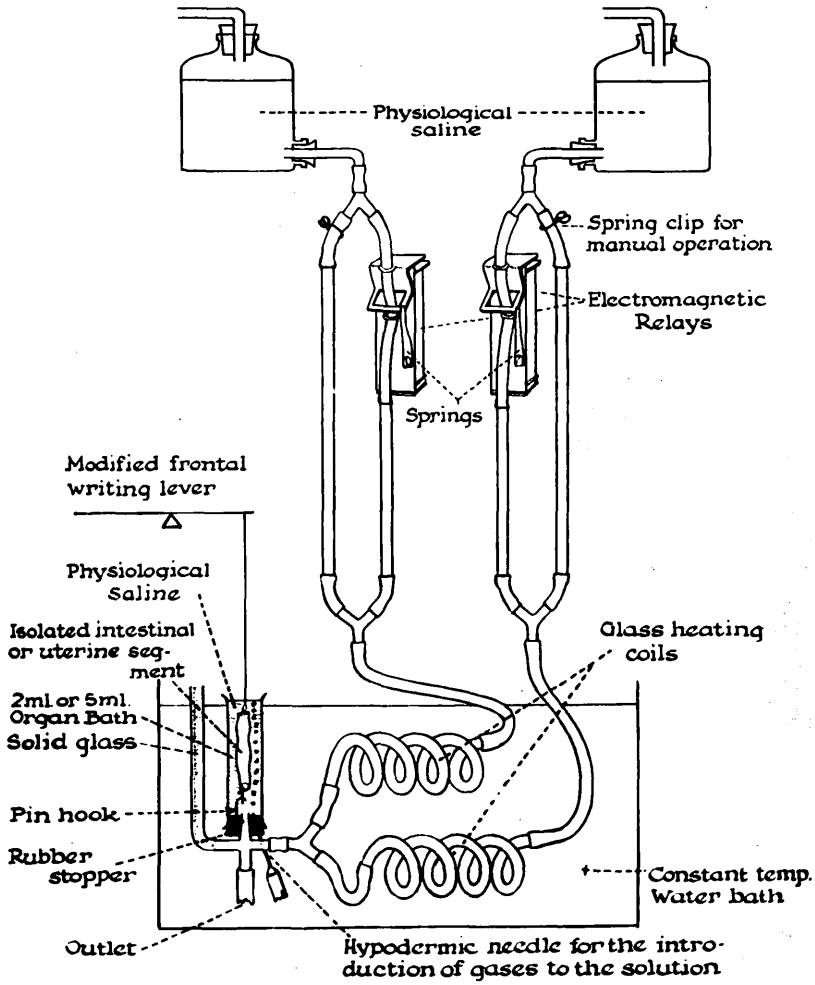

Figure 3. Diagram of the semi-automatic isolated organ bath used for experiments upon isolated strips of guinea pig ileum.

tube at the lower end of the bath was connected via heating coils to two reservoirs, one of which contained the Tyrode's solution, the other a solution of the stimulant drug in the same fluid. The dose of the stimulant drug to be used was determined at the start of each experiment by adding it to the bath by hand. The stimulant drug was then dissolved in Tyrode's solution to give the dilution required, and the automatic apparatus switched on. The electrical controlling equipment (Appendix II, p.337) replaced the saline colution in the bath by a solution containing the stimulant drug, at three minute intervals. Since the solution containing the stimulant drug flowed into the bath for five seconds, there was complete replacement of the solution in the bath. Reproducible submaximal contractions were obtained to acetylcholine or histamine added at three minute intervals and left in contact with the tissue for thirty seconds. At the end of this period the acetylcholine or histamine solution was washed out by the automatic inflow for a period of five seconds of pure Tyrode's solution. The rescinnamine solution was added to the bath by hand one minute before the next inflow of acetylcholine or histamine. This point in the cycle was indicated by a signal /

signal light placed in the circuit (Appendix II, p.337). The contractions were allowed to return to a constant level before the next addition of rescinnamine.

8. Experiments Carried Out Using the Isolated Rabbit Duodenum.

METHOD.

Rabbits of either sex, weighing between 1.5 and 2.5 kg. were killed by a blow on the head. The throat was cut and the blood allowed to drain out. The abdominal cavity was opened and a piece of duodenum about 4 cm. long removed. This was freed from fatty and other tissues. Threads were tied to both ends of the segment, and the piece of duodenum was set up in a 40 ml. organ bath containing oxygenated Locke's solution at 37± 0.5°C. The thread at one end of the duodenum was fixed to the lower end of a glass tube supplying oxygen to the bath, and the thread at the other end was attached to a modified frontal point writing lever giving a magnification of 1 in 8. Adrenaline (0.05 to 0.10 µg. per ml.) and acetylcholine (0.004 to 0.04 µg. per ml.) in solution in Locke's solution were added to the bath by means of a one ml. tuberculin syringe, and the effect was observed for two minutes. At the end of this period the fluid in the bath was replaced several times by running in fresh Locke's solution /

solution. The next addition of adrenaline or acetylcholine was not made until the tissue regained its original length. The solution of rescinnamine (1.0 to 4.0 µg. per ml.) and the same volume of the control solution were added to the bath two minutes before the addition of adrenaline or acetylcholine, and the effects observed.

9. Experiments Using the Isolated Frog Rectus Abdominis Muscle.

METHOD.

The procedure used for preparing the muscle to record the effects of drugs was essentially similar to that described by Burn[4]. An adult frog was stunned by means of a blow on the head, decapitated and pithed. The frog was laid on its back upon a cork covered dissecting board to which it was pinned. The rectus muscle was exposed by cutting away the skin of the abdomen, and then it was dissected from its insertion into the pelvic girdle to its insertion into the cartilage of the pectoral girdle. The rectus muscle was freed from the underlying connective tissues, removed from the frog and then suspended in an organ bath of 20 ml. capacity by means of two threads tied to either end of the muscle. A loop was made in the thread /

thread at one end in order to fix the muscle to the bent wire in the base of the bath, and a long thread left at the other end. The long thread was tied to a modified frontal point writing lever which gave a magnification of 8 to 10 times. The bath (Fig.4, p.35) contained 20 ml. of oxygenated frog Ringer's solution (Appendix I, p.336) at room temperature. Acetylcholine was dissolved in frog Ringer's solution to give the concentration required and added to the bath by means of a one ml. graduated tuberculin syringe. The concentration of acetylcholine used to produce contractions of the muscle was from 0.10 to 0.20 µg. per ml.

Solutions of rescinnamine (5.0 to 50 µg. per ml.). or the control solution (Appendix I, p.334), were added to the bath in the similar way. Uniform submaximal contractions to the same dose of acetylcholine were obtained before the effects of rescinnamine were studied. The time interval between each dose of acetylcholine was six minutes; the resulting contractions were recorded for ninety seconds. The bath was washed out with fresh frog Ringer's solution between each dose of acetylcholine. Rescinnamine in the dose range of from 5 to 50 µg. per ml., or the control /

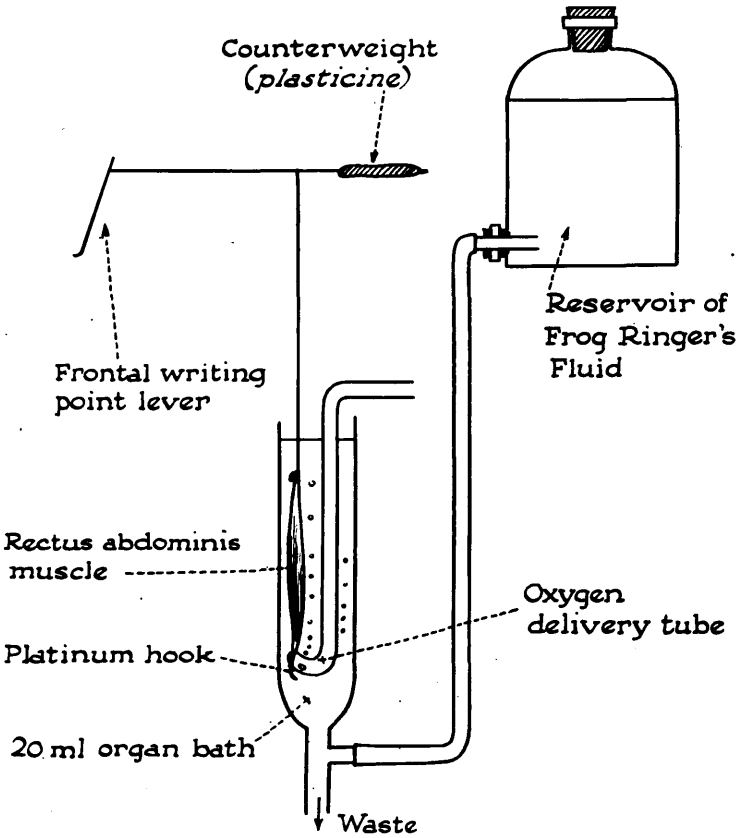

Figure 4. Diagram of the apparatus used for experiments upon the isolated frog rectus abdominis muscle.

control solution, were added one minute before the next addition of acetylcholine. Before the next addition of rescinnamine, sufficient time was allowed for the muscle to regain its original length.

In some experiments the effects of tubocurarine and atropine upon rescinnamine-induced contractions of the rectus muscle were studied. Tubocurarine (5.0 to 10 µg. per ml.) and atropine (1.0 to 10 µg. per ml.) were added to the bath either one minute before the addition of rescinnamine or during the actual rescinnamine contraction. The apparatus used for these experiments is shown in Fig.4, p.35.

10. Experiments on the Nictitating Membrane of the Anaesthetised Cat.

METHOD.

In these experiments cats of either sex, weighing between 2.0 and 3.0 kg., were used. The cat was anaesthetised by means of an intraperitoneal injection of sodium pentobarbitone (see p. 11), and tracheal and vein cannulae inserted as described on pages 11 and 12. The head was rigidly fixed by passing a brass rod between the jaws and then tying the jaws firmly together with string /

string. The ends of the rod were then gripped firmly in clamps, and these were supported on uprights fixed to the side of the operating table. By means of a fine needle, a silk thread was passed through the mid-point of the margin of the nictitating membrane of the right eye, and was tied firmly into place. The thread was then pulled forward and to one side, thus making an angle of about 30° to the long axis of the cat. It was then led around pulleys and attached to a frontal point writing lever. The contractions of the nictitating membrane were recorded on a smoked surface.

The right cervical sympathetic chain was now dissected out and a fine cotton thread tied tightly around it at as low a point as possible in the neck. The chain was severed above the ligature and low in the neck. The cut preganglionic cervical sympathetic chain was then placed upon a pair of platinum electrodes and kept moist with normal saline. Contractions of the nictitating membrane were elicited by stimulation of the cervical sympathetic by means of square impulses at a frequency of 800 to 1,200 per minute, 8 to 15 volts, the pulse width being 0.5 to 1.0 msec. In any one experiment frequency of stimulation, voltage and pulse width were constant.

The /

The nerve was stimulated, at 3 minute intervals, for 15 seconds. Having obtained standard reproducible responses of the nictitating membrane by stimulating the nerve trunk, a solution of rescinnamine (0.50 to 2.0 mg. per kg.) was injected into the external jugular vein one minute before the next period of stimulation.

[illegible], [illegible] M., (1952), Practical Pharmacology, Blackwell Scientific Publications, Oxford, page

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4. Burn, J. H., (1952), Practical Pharmacology, Blackwell Scientific Publications, Oxford, page 2.

CHAPTER III

RESULTS

1. Blood Pressure of the Anaesthetised Cat.

Rescinnamine in doses of 0.50 to 2.0 mg. per kg. produced a sharp fall in blood pressure, the level of which usually returned to its original value (in some cases reaching a higher level) within five to ten minutes (Fig. 5, p.41). After the initial fall no significant change in the blood pressure level was observed for from one to two hours. Three to four hours after the intravenous injection of rescinnamine, a significant drop in the blood pressure level (50 to 80 mm. Hg.) was observed. This latency of action on the blood pressure is similar to that observed by Cronheim et alia[1] in urethane-anaesthetised dogs following the administration of rescinnamine. The control solution had no observable action on the blood pressure level. In anaesthetised cats which had to start with a low blood pressure level (100 mm. Hg. or less), there was no significant fall in blood pressure even after four or five hours. Along with the lowering of blood pressure, a significant reduction in the heart rate was also noticed. Some of the control cats, however, showed hypotension and bradycardia /

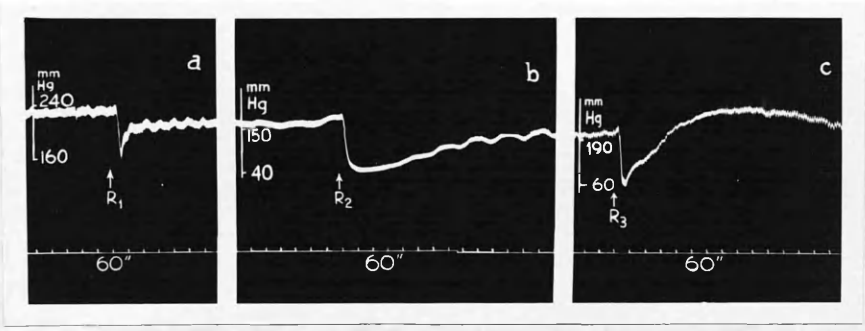


Figure 5. Cat. Pentobarbitone anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously.

At R_1 rescinnamine 0.50 mg. per kg.

At R_2 " 0.10 mg. per kg.

At R_3 " 0.10 mg. per kg.

bradycardia but to a lesser extent.

Rescinnamine in the dose range of 0.5 to 1.0 mg. per kg. caused either no reduction or a slight reduction in the response to both adrenaline, 0.5 to 2 µg. per kg., and noradrenaline, 0.5 to 2 µg. per kg. (Fig. 6, p.43 and also Fig. 10, p.48). Rescinnamine did not modify the characteristic depressor effects of acetylcholine (0.50 to 1.0 µg. per kg.) or histamine (0.50 to 1.0 µg. per kg.) on the blood pressure of the anaesthetised cat. (Fig. 6, p.43).

The pressor response to bilateral occlusion of the carotid arteries was significantly reduced following 0.50 to 1.0 mg. per kg. of rescinnamine but was not abolished (Fig.7, p.44). The maximum reduction was observed about thirty minutes after the injection of rescinnamine.

Following the injection of 0.50 mg. per kg. rescinnamine, pressor responses due to occlusion of the abdominal aorta or stimulation of the central end of the greater splanchnic nerve were reduced by about sixty per cent at the end of about one hour (Fig. 8, p.45).

Some /

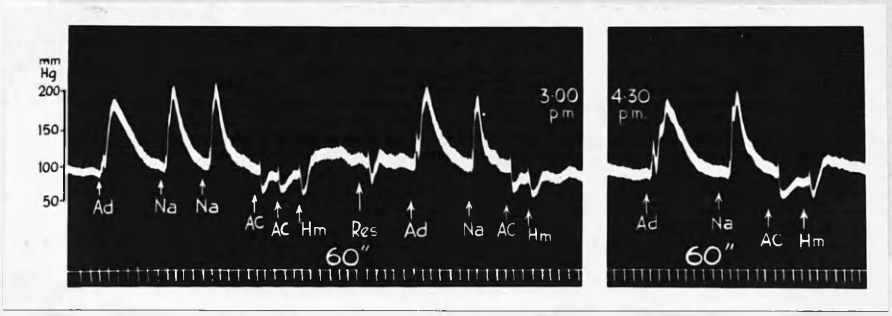


Figure 6. Cat, chloralose anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously.

At Ad, adrenaline 1.0 µg. per kg.

At Na, noradrenaline 1.0 µg. per kg.

At Ac, acetylcholine 1.0 µg. per kg.

At Hm, histamine 1.0 µg. per kg.

At Res, rescinnamine 1.0 mg. per kg.

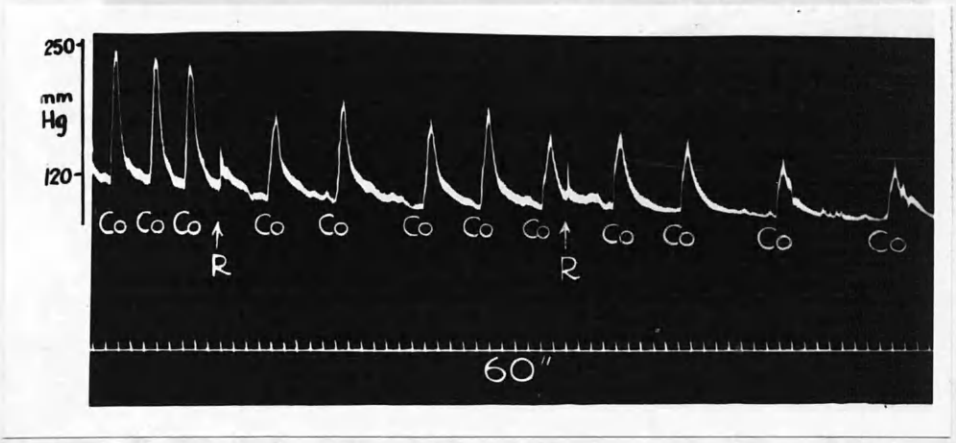


Figure 7. Cat, chloralose anaesthesia. Blood pressure record taken from the femoral artery. Drugs administered intravenously.

At Co, bilateral carotid occlusion for 30 seconds.

At R, rescinnamine 0.75 mg. per kg.

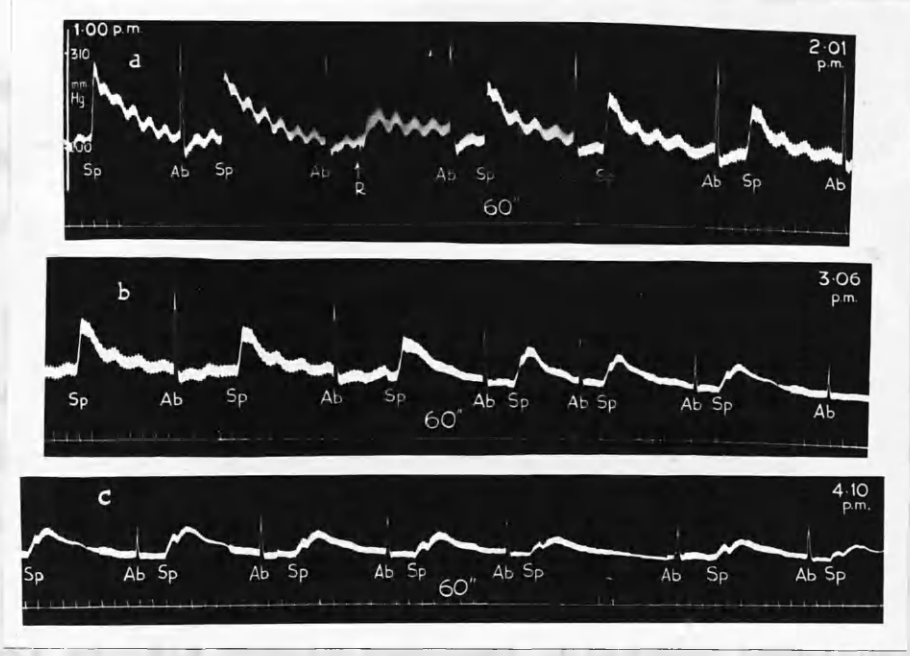


Figure 8. Cat. Pentobarbitone anaesthesia. Blood pressure record taken from the common carotid artery. Drugs administered intravenously.

At Sp, stimulation of the cut central end of the greater splanchnic nerve at 10 volts, 1,000 impulses per minute, pulse width 1.0 msec. for 15 seconds.

At Ab, compression of abdominal aorta for 10 seconds.

At R, rescinnamine 1.0 mg. per kg.

Part b is in continuation of a, and part c is in continuation of b.

Some reduction in the magnitude of these two reflex responses was observed within ten minutes after rescinnamine administration.

Following 0.75 to 1.0 mg. per kg. of rescinnamine, the magnitude of the pressor response caused by electrical stimulation of the central end of the right vagus was reduced within ten minutes, and abolished at the end of about thirty minutes. In some cats there was an actual reversal of this reflex response following rescinnamine (Fig. 9, p. 47).

Following 0.50 mg. per kg. rescinnamine, the pressor response elicited in the cat by hypoxia was found to be completely blocked within about fifteen minutes. Prior to the administration of rescinnamine, hypoxia caused only a pressor response but after rescinnamine had been given the pressor response was changed into a complex depressor-pressor -depressor response (Fig. 10, p. 48). The control solution had no observable effects upon any of these reflexes.

One mg. per kg. of rescinnamine caused a slight reduction in the level of the blood pressure which had been artificially raised by continuous infusion of adrenaline

or /

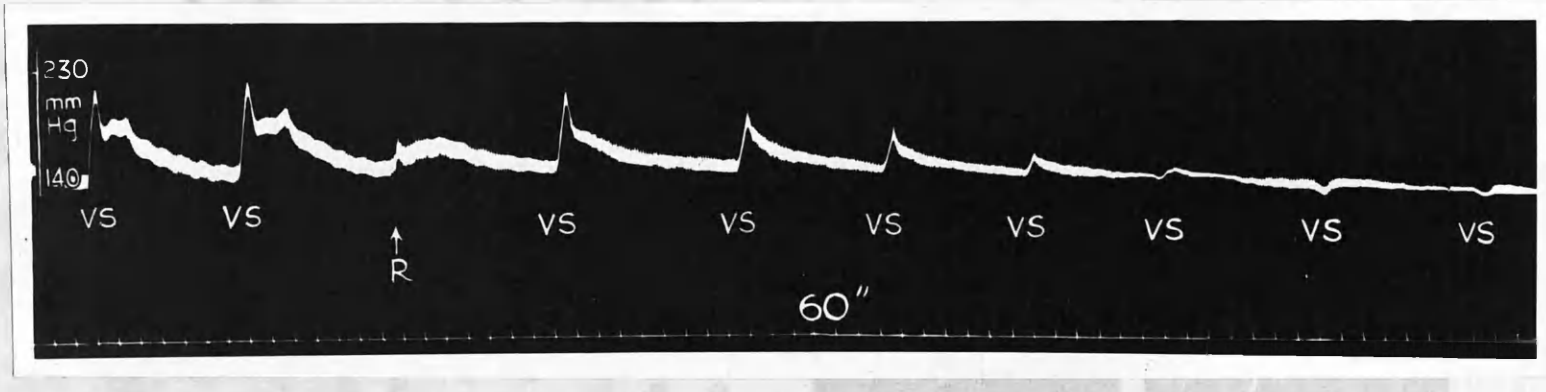


Figure 9. Cat. Pentobarbitone anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously.

At VS, stimulation of cut central end of the right vagus at 15 volts, pulse width 1 msec., 1000 impulses per minute for 30 seconds.

At R, rescinnamine 0.75 mg. per kg.

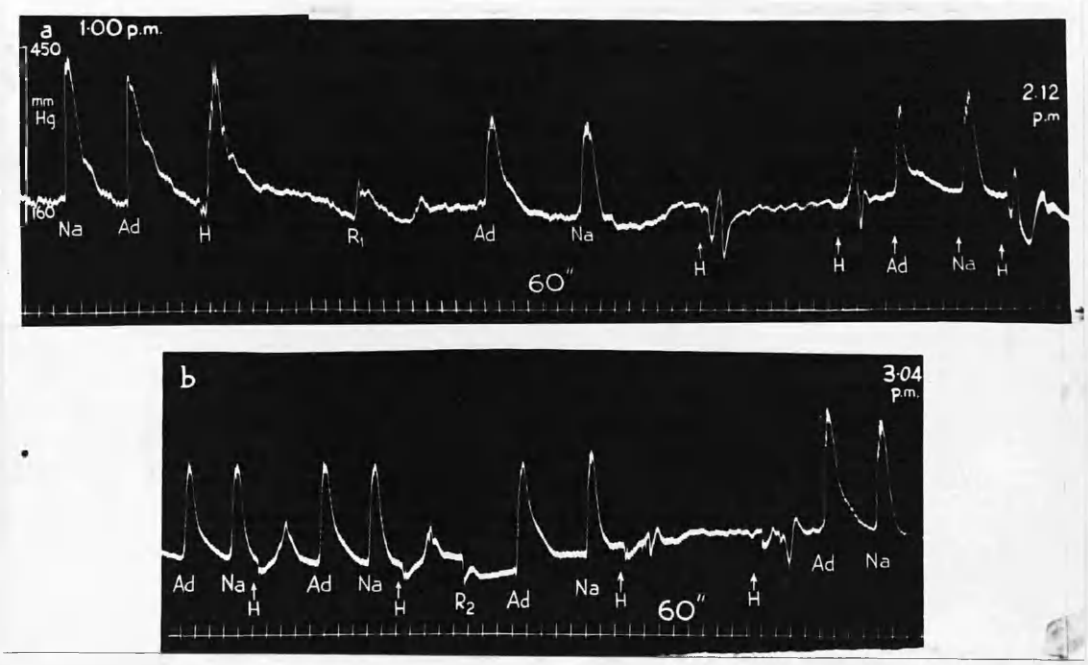


Figure 10. Cat. Chloralose anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously.

At Na, noradrenaline 2.0 µg. per kg. + 4 ml. saline

At Ad, adrenaline 2.0 µg. per kg. + 4 ml. saline

At H, inhalation of mixtures of 95% nitrogen and 5% CO_2 for 1½ minutes.

At R_1, Rescinnamine 0.50 mg. per kg. + 4 ml. saline

At R_2, Rescinnamine 1.0 mg. per kg. + 4 ml. saline

Part b is continuation of part a.

or noradrenaline. In bigger doses (2.0 to 4.0 mg. per kg.), rescinnamine lowered both adrenaline and noradrenaline hypertension to normal levels within a few minutes (Figs.11 and 12, pages 50 and 51). The blood pressure usually remained at the normotensive level although the infusion of adrenaline or noradrenaline was continued. Anaesthetised (pentobarbitone or chloralose) and spinal cats behaved in the same way. The control solution caused no significant effect.

2. Blood Pressure of the Spinal Cat.

In spinal cats, the blood pressure of which was usually about 100 mm. Hg., rescinnamine caused no fall in the blood pressure level even after several hours. The characteristic effects of acetylcholine, histamine, adrenaline and noradrenaline on the blood pressure were not modified by rescinnamine.

3. Isolated Perfused Rabbit and Kitten Hearts.

No differences were noted in the results obtained when the experiments were carried out with either rabbit or kitten hearts. Perfusion of 1.0 µg. per ml. of rescinnamine decreased the rate and amplitude of the contractions /

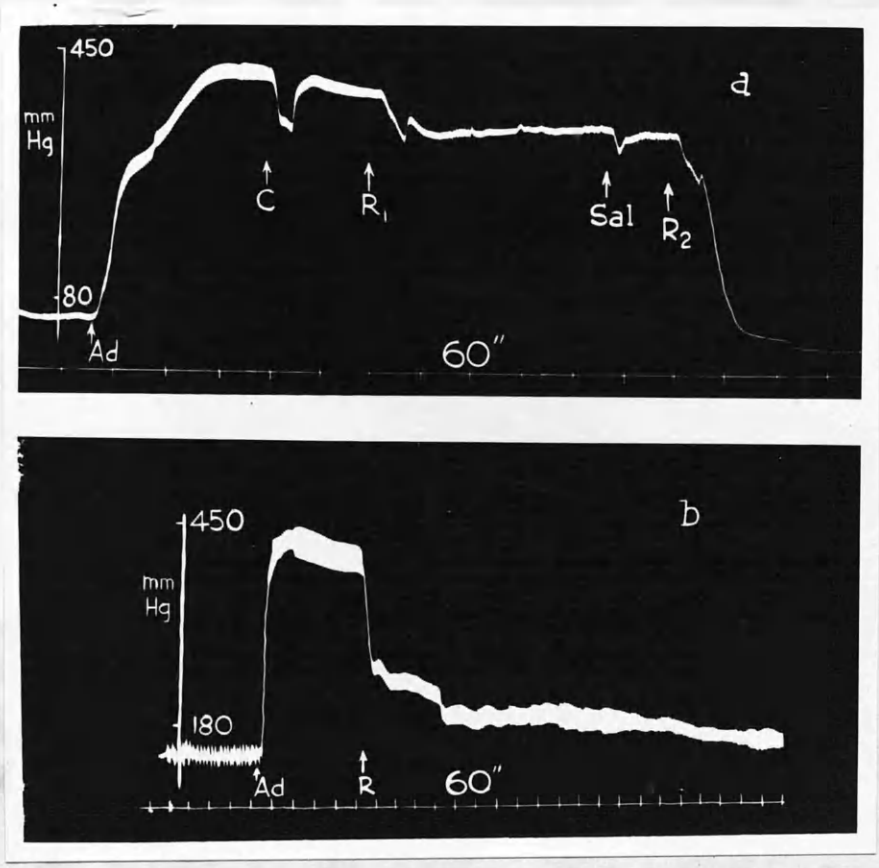


Figure 11. Cat. (a) Pentobarbitone and (b) chloralose anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously.

At Ad, infusion of adrenaline 0.10 mg. per ml. at the rate of 1.0 ml. per minute.

(a) At C, Control solution.

At R_1, Rescinnamine 1.0 mg. per kg.

At R_2, " 4.0 mg. per kg.

At Sal, Saline 4 ml.

(b) At R, Rescinnamine 2.0 mg. per kg.

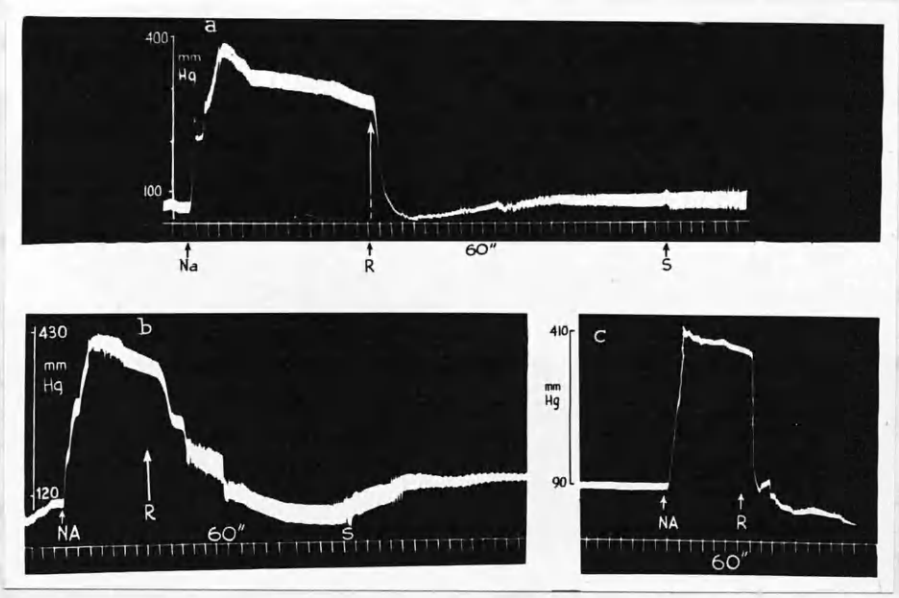


Figure 12. Cat. (a) Chloralose anaesthesia, (b) pentobarbitone anaesthesia and (c) spinal animal. Blood pressure record from the common carotid artery. Drugs administered intravenously.

At Na, infusion of noradrenaline 0.10 mg. per ml. at the rate of 1.0 ml. per minute.

(a) At R, Rescinnamine 2.0 mg. per kg.
At S, infusion of noradrenaline stopped.

(b) At R, Rescinnamine 2.0 mg. per kg.
At S, infusion of noradrenaline stopped.

(c) At R, Rescinnamine 2.0 mg. per kg.

contractions of the heart (Fig. 13, p.53). The decrease in the rate and amplitude was gradual and became evident within a few minutes after the start of the perfusion of the rescinnamine solution. The heart outflow was also decreased. The rescinnamine effect was not reversible, and the decreased rate and amplitude did not increase after withdrawal of the drug (Fig. 13b, p.53). The control solution equivalent to 1.0 µg. per ml. of rescinnamine had qualitatively similar, but quantitatively much weaker effects upon the heart.

The increased rate and amplitude of contraction produced by adrenaline, 0.50 µg., or noradrenaline, 0.50 µg., were not altered by rescinnamine, 25 to 50 µg. (Fig.14a,p.54). The effect of acetylcholine (0.05 µg.) was also unaffected by prior injection of 25 to 50 µg. rescinnamine (Fig.14b,p.54). When rescinnamine was injected at this dose level through the perfusion cannula, there was an immediate decrease in the amplitude which recovered within a few minutes but did not return to the original level. The heart rate and heart outflow were not significantly altered.

4. Isolated Guinea Pig Auricles.

When rescinnamine in concentrations 2.0, 4.0 or 8.0 µg. per ml. was added to the bath, there was an immediate reduction /

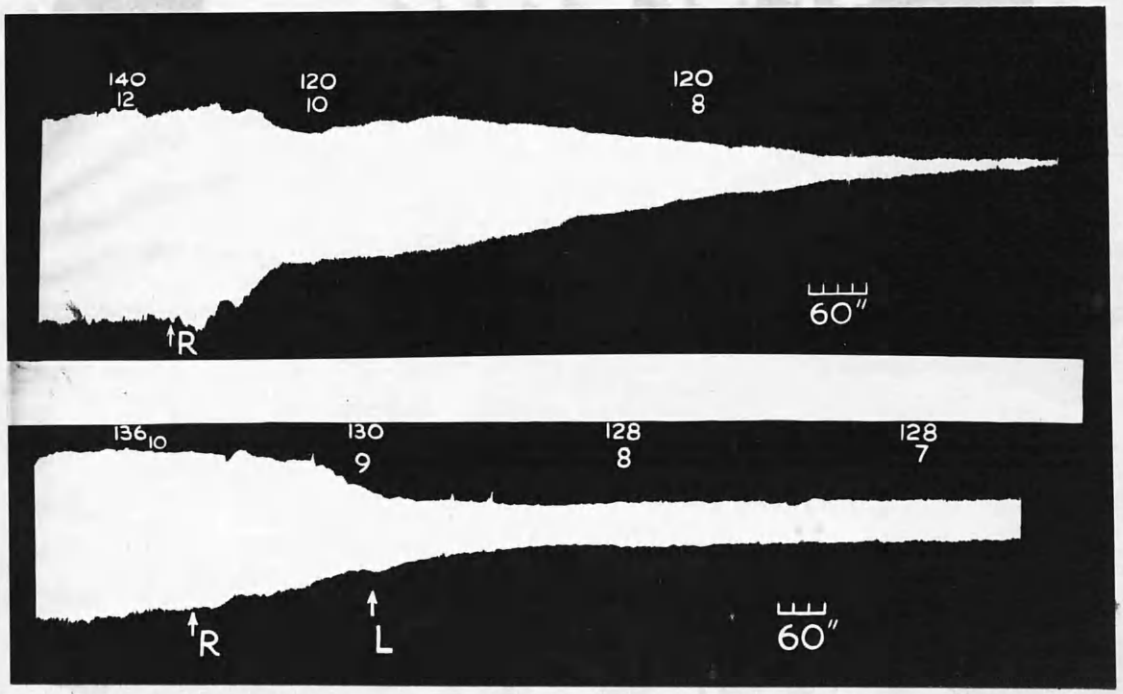
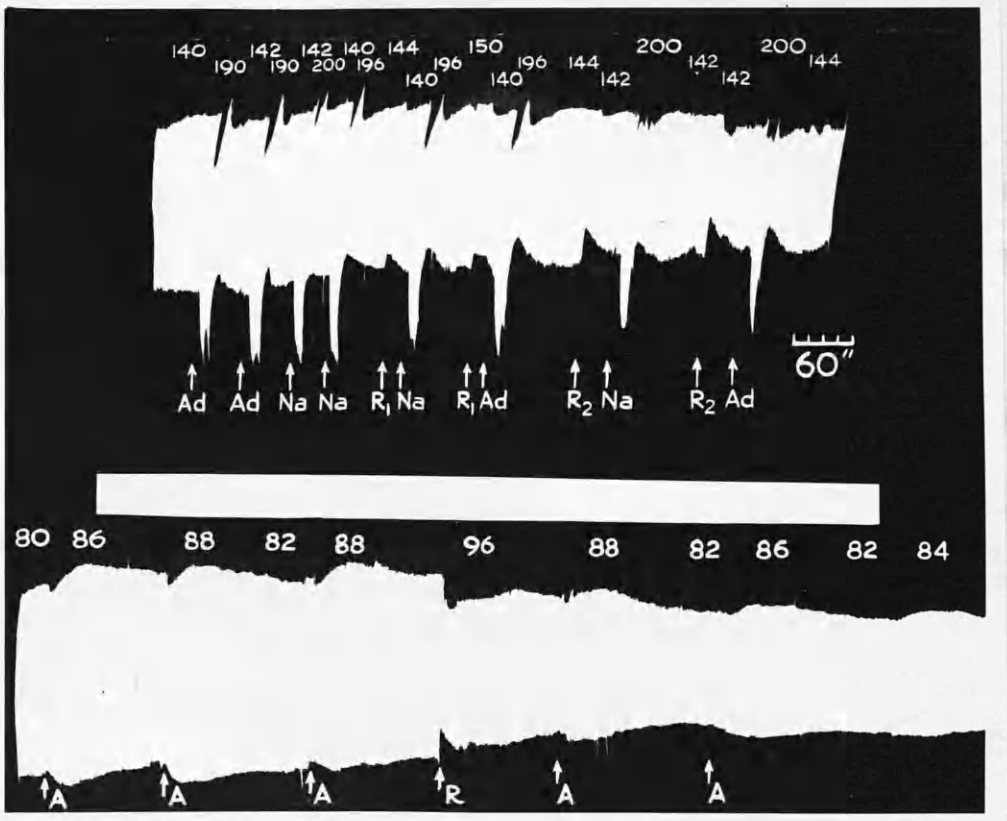


Figure 13. Isolated rabbit heart. Numbers above the recording - upper row heart rate in beats per minute, lower row cardiac outflow in ml. per minute.

(a) At R, perfusion with rescinnamine 1.0 µg. per ml.

(b) At R, perfusion with rescinnamine 1.0 µg. per ml. At L, rescinnamine replaced by Locke's solution.

a



b

Figure 14. Isolated kitten heart.

(a) Numbers above the recording refer to the number of beats per minute; upper row normal beat, middle row after administration of adrenaline and noradrenaline and lower row after administration of rescinnamine.

At Ad, adrenaline 0.5 µg.

At Na, noradrenaline 0.5 µg.

At R_1, rescinnamine 25 µg.

At R_2, rescinnamine 50 µg.

(b) Numbers above the recording refer to the number of beats per minute.

At A, acetylcholine 0.05 µg.

At R, rescinnamine 50 µg.

reduction of the rate and amplitude of the contractions. After washing, the rate and amplitude returned to their original levels (Fig. 15a, p. 56).

Rescinnamine (2.0 to 8.0 µg. per ml.) did not show antagonism or potentiation to the increased rate and amplitude produced by noradrenaline (0.02 µg. per ml.) or adrenaline (0.02 µg. per ml.) (Fig. 15b and c, p. 56). The control solution had no significant effect.

5. Strips of Horse Carotid Arteries.

Rescinnamine in the dose range of 2.0 to 40.0 µg. per ml. relaxed sustained contractions of artery strips induced by adrenaline (1.0 to 2.0 µg. per ml.), noradrenaline (1.0 to 2.0 µg. per ml.), 5 - hydroxytryptamine (0.10 to 0.50 µg. per ml.), acetylcholine (0.02 to 0.1 µg. per ml.) or histamine (1.0 to 2.0 µg. per ml.). The degree of relaxation depended upon the magnitude of the dose of rescinnamine used. The results of some of these experiments are shown in Fig. 16, p. 57. The control solution produced no observable effects. Rescinnamine in doses of from 2.0 to 40.0 µg. per ml. had little or no direct effect upon the artery strip.

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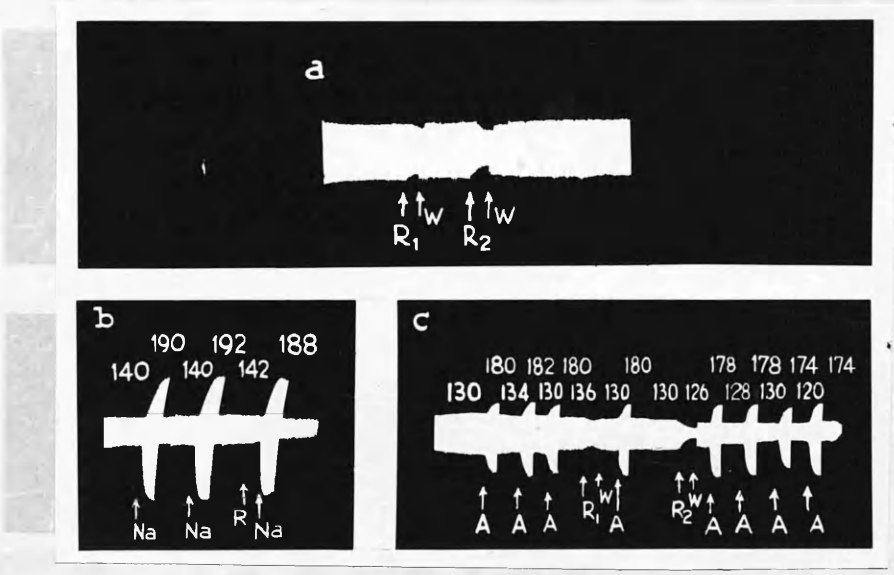

Figure 15. Isolated guinea pig auricles. Numbers above the recordings refer to the number of beats per minute.

(a) At R_1, rescinnamine 2.0 µg. per ml.
At R_2, " 4.0 " " "
At W, wash out.

(b) At Na, noradrenaline 0.04 µg. per ml.
At R, rescinnamine 2.0 " " "

(c) At A, adrenaline 0.04 µg. per ml.
At R_1, rescinnamine 2.0 " " "
At R_2, " 8.0 " " "
At W, wash out.

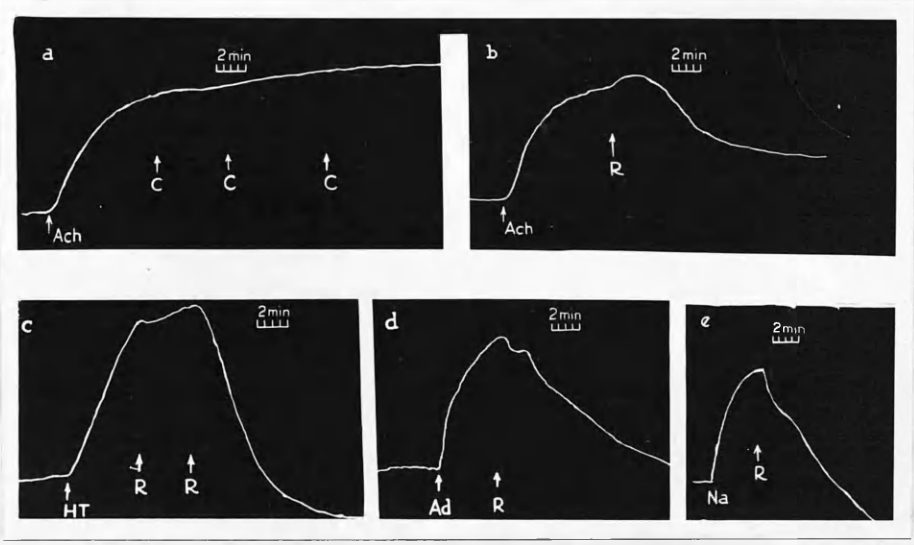

Figure 16. Isolated strips of horse carotid artery.

(a)	At Ach,	acetylcholine	0.02 µg. per ml.
	At C,	control solution	
(b)	At Ach,	acetylcholine	0.02 µg. per ml.
	At R,	rescinnamine	40.0 " " "
(c)	At HT,	5-hydroxy-tryptamine	0.10 µg. per ml.
	At R,	rescinnamine	5.0 " " "
(d)	At Ad,	adrenaline	2.0 µg. per ml.
	At R,	rescinnamine	40.0 " " "
(e)	At Na,	noradrenaline	2.0 µg. per ml.
	At R,	rescinnamine	20.0 " " "

Prior addition of rescinnamine in doses of from 1.0 to 10.0 µg. per ml. reduced the magnitude of the contraction induced by adrenaline (1.0 to 2.0 µg. per ml.), noradrenaline (1.0 to 2.0 µg. per ml.), 5 - hydroxytryptamine (0.005 to 0.05 µg. per ml.), acetylcholine (0.02 to 0.10 µg. per ml.) or histamine (0.10 to 0.50 µg. per ml.) (Fig.17, p. 59). The control solution did not show any significant effect upon the contractions induced by these drugs.

6. Isolated Perfused Rat Hindquarters.

In the perfused hindquarters of the rat, rescinnamine has been shown by McQueen and Blackman[2] to have a direct peripheral vasodilator effect similar to that caused by reserpine.

When perfused in concentrations of 0.10 to 1.0 µg. per ml., solutions of rescinnamine caused no change in the rate of outflow. When the concentration was increased to 10.0 µg. per ml. there was a gradual decrease in the outflow apparently due to constriction of the blood vessels. A marked constrictor effect was also observed when rescinnamine in the dose range of 0.05 to 0.10 mg. was injected through the injection cannula. No antagonism

was /

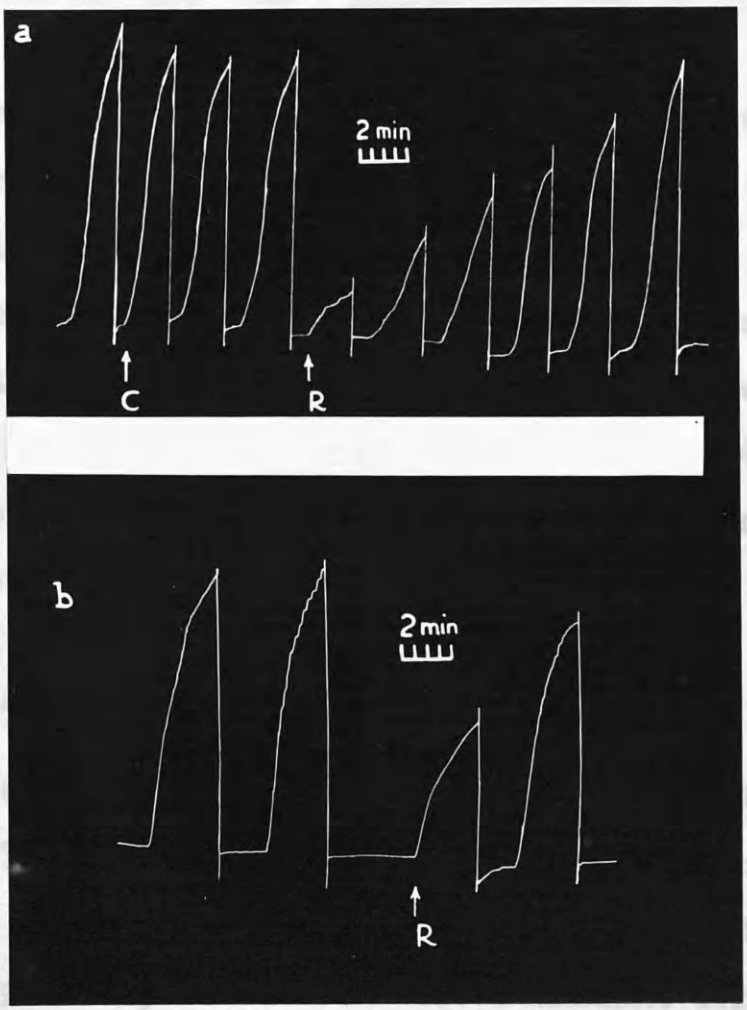

Figure 17. Isolated strips of horse carotid artery.

(a) All contractions produced by acetylcholine 0.05 µg. per ml. Addition of acetylcholine to the bath was preceded 10 minutes earlier by

at C, control solution, and

at R, rescinnamine 10 µg. per ml.

(b) All contractions produced by 5-hydroxytryptamine 0.30 µg. per ml. Addition of 5-hydroxytryptamine was preceded 10 minutes earlier by

at R, rescinnamine 10 µg. per ml.

was shown to the vasoconstriction caused by 0.10 to 0.40 µg. adrenaline following injection (0.10 µg.) or perfusion (1.0 µg. per ml.) of rescinnamine. The control solution produced almost the same constrictor effect as the solution of rescinnamine. In this investigation, it has not therefore been possible to demonstrate that in the isolated perfused rat hindquarters rescinnamine has a direct peripheral vasodilator effect.

7. Isolated Guinea Pig Ileum.

In some preparations rescinnamine, in doses of from 1.5 to 3.0 µg. per ml., showed a direct stimulant effect whereas in others no direct action, even in doses of up to 20 µg. per ml., was seen. The stimulant effect was completely inhibited by 0.1 µg. atropine (Fig.18, p.61). The control solution showed no direct action.

Contractions of the guinea pig ileum following the addition to the bath of acetylcholine (0.04 to 0.08 µg. per ml.) or histamine (0.04 to 0.10 µg. per ml.) were reduced by 2.0 to 20.0 µg. per ml. of rescinnamine (Fig.19, p.62). The extent of the reduction in the amplitude of the contraction appeared to be related to the dose of rescinnamine in the case of acetylcholine, but not in /

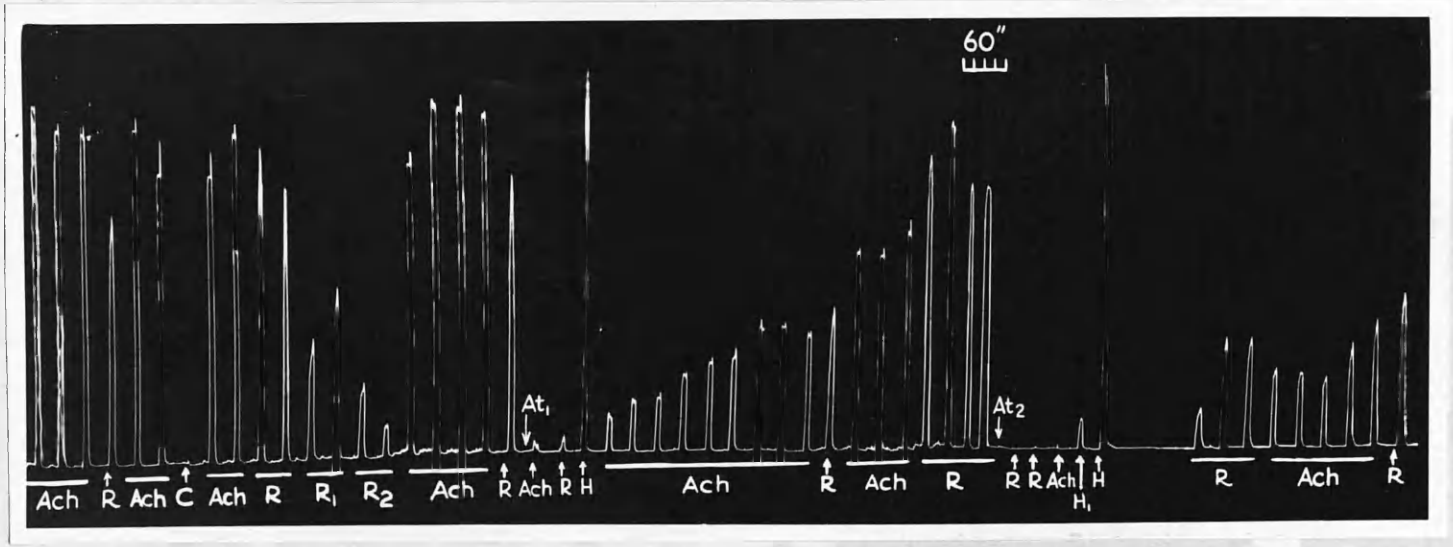

Figure 18. Isolated guinea pig ileum.

At Ach,	contraction caused by	acetylcholine	0.005 µg. per ml.
At R,	" " "	rescinnamine	3.0 " " "
At R_1,	" " "	"	2.0 " " "
At R_2,	" " "	"	1.0 " " "
At C,	control solution.		
At At_1 and At_2,	atropine		0.05 and 0.1 " " " respectively.
At H and H_1,	histamine		0.30 and 0.03 µg. per ml. respectively.

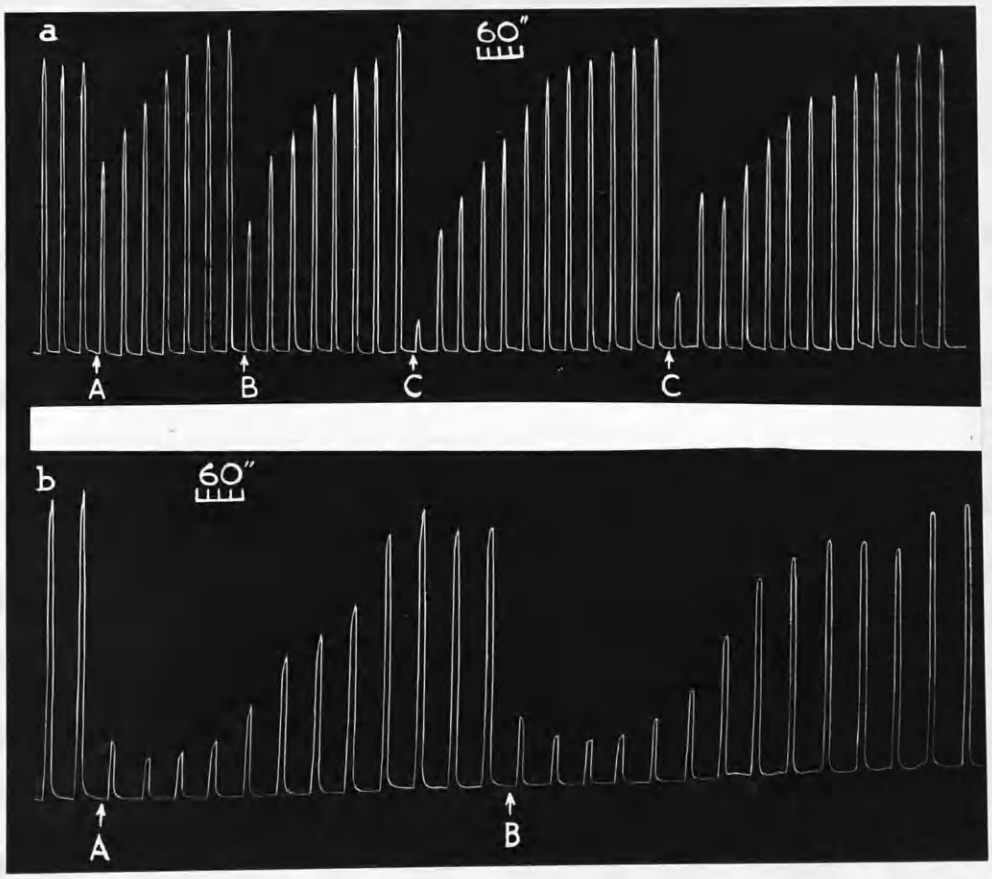

Figure 19. Isolated guinea pig ileum.

(a) All contractions produced by acetylcholine 0.08 µg. per ml. Addition of acetylcholine to the bath was preceded one minute earlier by

at A, rescinnamine 5 µg. per ml.
at B, " 10 µg. per ml.
at C, " 20 µg. per ml.

(b) All contractions produced by histamine 0.10 µg. per ml. Addition of histamine to the bath was preceded one minute earlier by

at A, rescinnamine 8 µg. per ml.
at B, " 4 µg. per ml.

in the case of histamine. Recovery of the contractions to the control height was usually complete, although the time required for the histamine-induced contraction to recover was the more prolonged (Fig.19b, p.62).
In the case of histamine the maximum inhibition was always produced some time after the addition of rescinnamine - generally after two to four additions of histamine (Fig.19b). In the case of acetylcholine, the contraction induced immediately after addition of rescinnamine showed the maximum inhibition (Fig.19a, p.62). The control solution had no inhibitory effect.

8. Isolated Rabbit Duodenum.

Two to four µg. per ml. of rescinnamine had no effect upon the spontaneous activity of the rabbit duodenum, but there was usually a reduction in the normal tone as recorded by a fall in the level of the recording lever (Fig.20b, p.64). The control solution had no effect.

Contractions of the duodenum following addition to the bath of acetylcholine (0.004 to 0.04 µg. per ml.) and the reduction in tone produced by 0.05 to 1.0 µg. per ml. of /

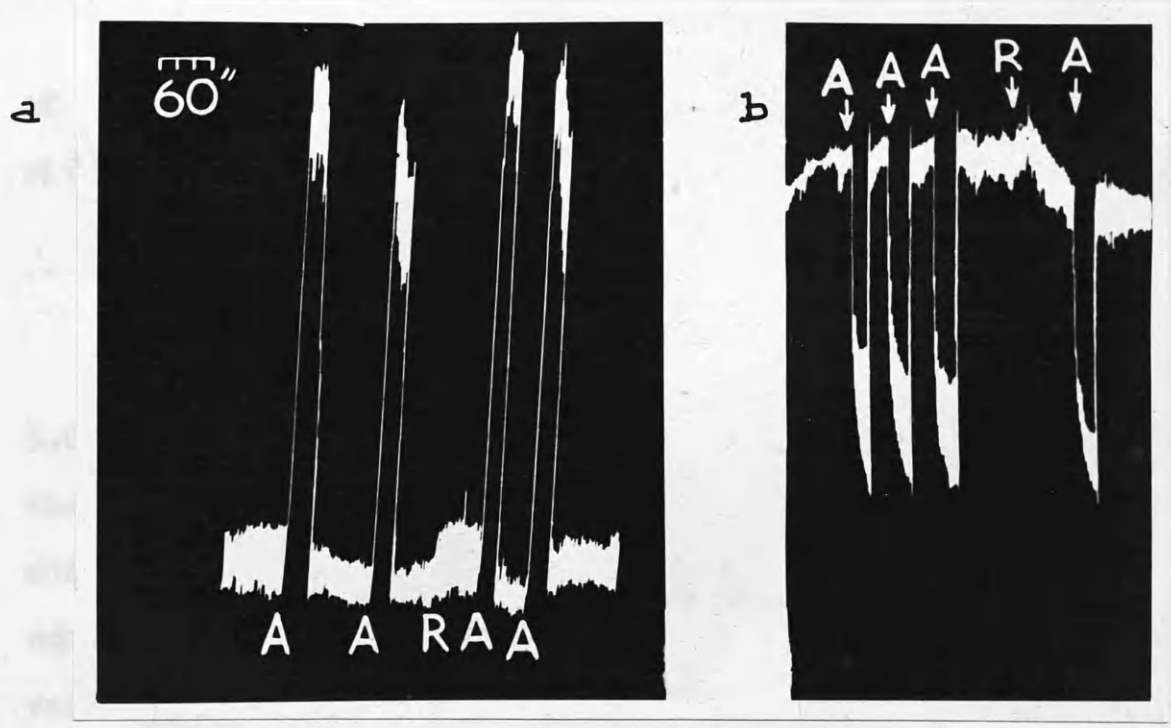

Figure 20. Isolated rabbit duodenum.

(a) At A, acetylcholine 0.004 µg. per ml.
At R, rescinnamine 1.0 µg. per ml.

(b) At A, adrenaline 0.05 µg. per ml.
At R, rescinnamine 2.0 µg. per ml.

of adrenaline were unaffected by doses of rescinnamine within the range of 1.0 to 4.0 µg. per ml. (Fig.20, p.64).

9. Isolated Frog Rectus Abdominis Muscle.

In some experiments rescinnamine in doses of from 5.0 to 50 µg. per ml. had a direct stimulant effect upon the rectus muscle (Fig.21, p.66). Immediately after the addition of rescinnamine to the bath there was a slow contractural response. The magnitude of the contracture varied from test to test, even when the same dose was used. (Fig.21).

The contracture was not reduced by prior addition to the bath of atropine (1.0 to 10.0 µg. per ml.) or tubocurarine (5.0 to 10.0 µg. per ml.). The control solution had no direct stimulant effect.

When rescinnamine did not cause a contracture, it showed antagonism to acetylcholine-induced contractions of the rectus. Following 50 µg. per ml. of rescinnamine, the magnitude of the acetylcholine-induced contractions was reduced by about 30 per cent, but doses of less than 30 µg. per ml. had no significant effect (Fig.22b, p.67). When rescinnamine (10 to 20 µg. per ml.) was allowed to remain /

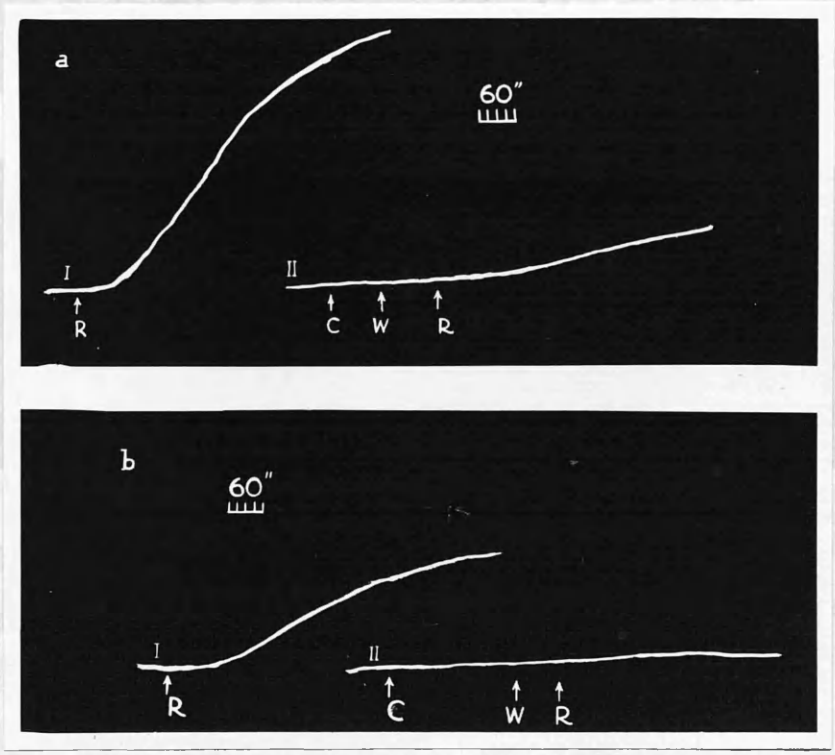

Figure 21. Isolated frog rectus abdominis muscle.

(a) At R, rescinnamine 50 µg. per ml.
At C, control solution
At W, wash out.

(b) At R, rescinnamine 15 µg. per ml.
At C, control solution
At W, wash out.

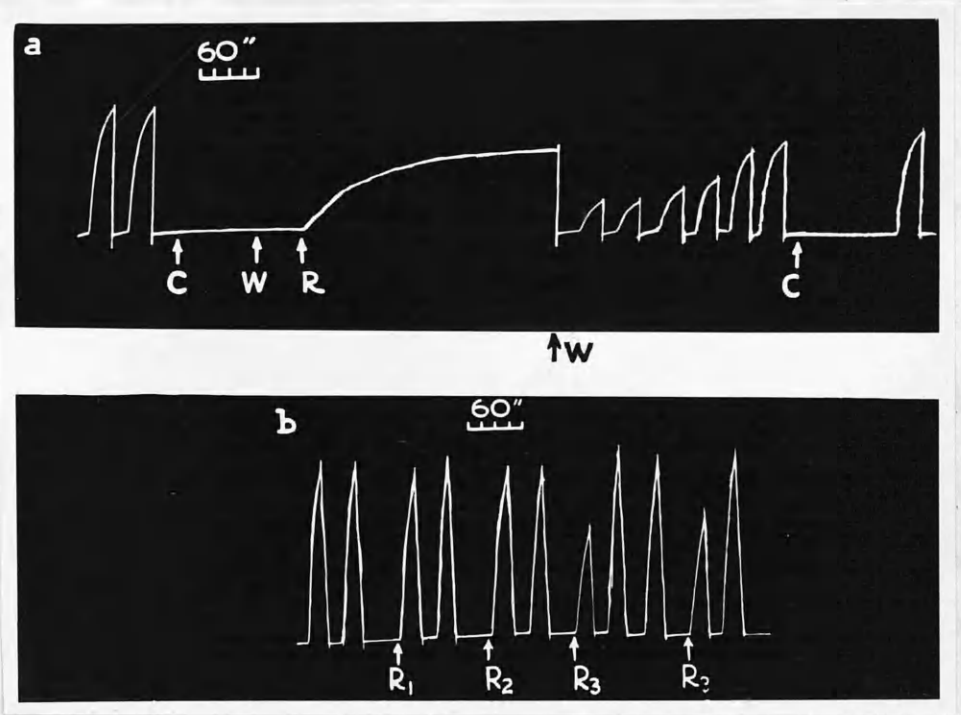

Figure 22. Isolated frog rectus abdominis muscle.

(a) Unlabelled contractions produced by acetylcholine 0.15 µg. per ml.

At C, control solution

At W, wash out

At R, rescinnamine 20 µg. per ml.

(b) All contractions produced by acetylcholine 0.10 µg. per ml.

At R_1, rescinnamine 10 µg. per ml.

At R_2, " 20 µg. per ml.

At R_3, " 50 µg. per ml.

remain in contact with the rectus for longer periods, a marked inhibition of the acetylcholine-induced contraction was observed (Fig.22a, p.67).

10. Nictitating Membrane of the Anaesthetised Cat.

Rescinnamine in doses of from 1.0 to 2.0 mg. per kg. showed no direct effects upon the nictitating membrane of the anaesthetised cat. There was no immediate effect upon the magnitude of contractions due to indirect tetanization of the cervical sympathetic, but about thirty minutes after an injection of 2.0 mg. per kg. a significant reduction of the amplitude was observed. After about three hours had elapsed, the magnitude of the contraction was reduced to about 30 per cent of the original (Fig.23, p.69).

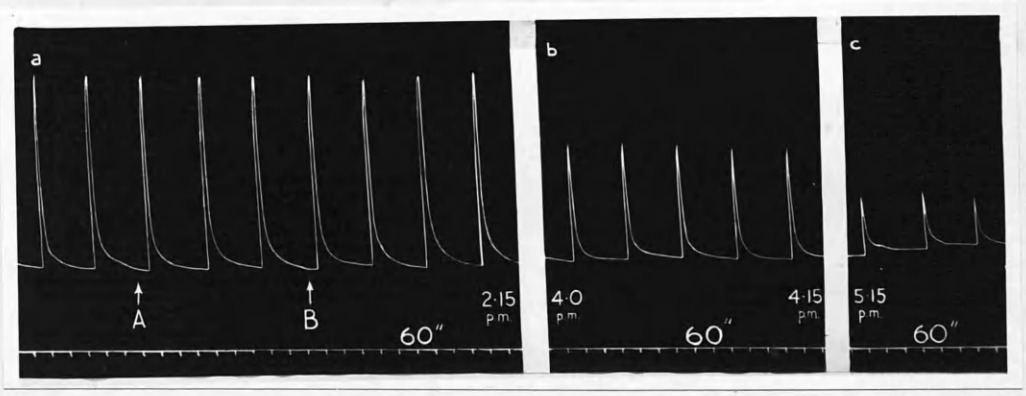

Figure 23. Cat. Pentobarbitone anaesthesia.

Contractions of nictitating membrane elicited at intervals of three minutes by preganglionic stimulation at a frequency of 1,000 impulses per minute, 10 volts, pulse width 1 msec. for 10 seconds.

At A, rescinnamine 0.5 mg. per kg. intravenously.

At B, rescinnamine 2.0 mg. per kg. intravenously.

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CHAPTER IV

DISCUSSION

Rescinnamine causes an immediate but short-lived, sharp fall in the blood pressure level of the anaesthetised cat (Fig.5 ,p.41). There is also some reduction in the level of the blood pressure at the end of three or four hours and this might be considered to indicate a hypotensive action. On the other hand, there is almost always a reduction in the blood pressure level of the anaesthetised cat after three or four hours on the operating table, and control experiments indicate that rescinnamine has apparently very little hypotensive effect upon the normotensive cat. Cronheim et alia[1] have, however, claimed that rescinnamine has a definite hypotensive action on normotensive dogs.

Rescinnamine abolishes or reduces hypertension due to adrenaline or noradrenaline in anaesthetised and spinal cats, but ganglion blocking agents and reserpine increase the hypertension caused by injected adrenaline or noradrenaline[2]. Rescinnamine does not alter or may slightly inhibit the pressor responses due to adrenaline or noradrenaline in the cat, but in the dog it has been reported to potentiate the pressor response to adrenaline[1].

The /

The magnitude of the pressor response which is due to stimulation of the pressoreceptors, and which follows bilateral occlusion of the carotid arteries, is reduced significantly following rescinnamine. It has been concluded by Heymans[3] that drugs which cause the muscular walls of the carotid sinus to contract increase their intrinsic tension and decrease their distensibility, thus causing stimulation of the receptors at the endings of the carotid sinus nerves. This stimulation reflexly induces a fall in the systemic arterial blood pressure and decreases the hypertension normally produced by a decrease of the blood pressure in the carotid sinus. Drugs which relax the arterial walls of the carotid sinus and increase their distensibility induce a reflex rise of systemic arterial blood pressure. Stimulation of chemoreceptors by inhalation of a low-oxygen mixture also produces a reflex rise of blood pressure in cats. These reflex pressor responses, as well as those produced by faradization of the central end of the cut vagus and the splanchnic nerves and by occlusion of the abdominal aorta, may have the same mechanism of action, i.e. liberation of adrenaline and noradrenaline from the nerve endings of the adrenergic nerves[4], the reflex being mediated through /

through higher centres in the brain. The fact that adrenergic blocking agents and ganglion blocking agents depress these reflex pressor responses supports this view. Although the peripheral sympathetic nervous system is considered to represent the final step responsible for the rise of blood pressure which is brought about by these reflexes[2] and noradrenaline is the transmitter set free from the adrenergic nerve endings[4], the differences in the composition of the adrenal medullary secretion after stimulation of different afferent nerves and of presso- and chemoreceptors, are also of importance in causing the blood pressure to rise[5, 6]. In cats it has been shown that when these pressor reflexes are brought into play, about two-thirds of the medullary secretion is normally noradrenaline. As has already been indicated, rescinnamine antagonises the pressor responses elicited by faradization of the splanchnic nerves and by bilateral occlusion of the carotid arteries and abdominal aorta, and it abolishes the pressor responses due to faradization of the cut end of the afferent vagus and hypoxia. Antagonism to these pressor reflexes indicates that rescinnamine may act by interfering with sympathetic activity in the central nervous system, because it /

it shows no antagonism to the peripheral effects of circulating adrenaline or noradrenaline. Rescinnamine may also exert its hypotensive effect by a specific depressant action upon the sympathetic ganglia, and the observation that it markedly reduces the responses of the nictitating membrane to stimulation of the preganglionic sympathetic fibres lends support to this view. In the dog, rescinnamine has been reported to cause reversal of the pressor response to hypoxia, diminution of the pressor response to bilateral carotid occlusion and blockade or reversal of the blood pressure rise elicited by faradization of the afferent vagus[1]. Bein[7], using reserpine, has observed similar effects in cats anaesthetised with dialurethane; but in contrast to rescinnamine, reserpine does not cause inhibition of the pressor response caused by stimulation of the afferent splanchnic nerves. Bein considered that reserpine acted upon the central nervous system and had a direct effect upon the sympathetic autonomic centres in the brain which were responsible for the regulation of the blood pressure.

Depression of the rhythmic activity of preparations of isolated cardiac muscle was observed following the use of /

of rescinnamine. The rate and amplitude of the contractions of the isolated kitten and rabbit heart were decreased and the cardiac outflow was reduced. In these preparations, the site of action of rescinnamine appears to be directly upon the cardiac muscle. On the other hand, rescinnamine does not seem to alter the characteristic actions of cardiotonic drugs such as adrenaline, noradrenaline etc. on the isolated heart. Rescinnamine caused depression of the spontaneous activity of the isolated auricles but did not alter the stimulant effects of adrenaline or noradrenaline.

No vasodilator effect was observed in the isolated rat hindquarters preparation following perfusion of rescinnamine. McQueen and Vleckman [8], however, observed vasodilatation in the perfused rat hindquarters in which vasomotor tone had been increased by the infusion of noradrenaline. In the isolated rat hindquarters preparation, it is perhaps to be expected that little, if any, neurogenic vascular tone should remain and that, in these circumstances, little or no vasodilator effect would be expected.

In experiments performed using isolated strips of horse /

horse carotid artery, rescinnamine showed little direct relaxant effect but caused relaxation of contractures of artery strips elicited by adrenaline, noradrenaline, 5-hydroxytryptamine, histamine or acetylcholine. Prior addition of rescinnamine to the bath also antagonised the effects of these stimulant drugs.

In the isolated guinea pig ileum, rescinnamine depressed the stimulant actions of acetylcholine and histamine. It also caused a slow contraction of the frog rectus abdominis muscle. This effect was followed by a reduction in the magnitude of acetylcholine-induced contraction of the rectus muscle, i.e. not only had rescinnamine a direct stimulant action on the muscle but it antagonised the stimulant action of acetylcholine.

The isolated tissue preparations used in this investigation differ widely in their sources, structure and properties. Certain characteristic features of the action of rescinnamine on isolated muscle preparations can however be demonstrated. These are, its general depressant effects on the muscular activity produced by other stimulant drugs and the non-specific nature of these depressant effects.

It /

It is not possible to suggest one single mechanism of action which can account for all of the effects of rescinnamine observed in the intact animal and in isolated tissue preparations. While rescinnamine undoubtedly has some effects upon the central nervous system, it appears from the study of its effects upon isolated tissue preparations that it may also have definite peripheral sites of action. Many of the peripheral effects of rescinnamine may, however, be explained if it is assumed that rescinnamine acts by depressing the ability of smooth cardiac and skeletal muscle to contract. The site or sites of this action within the cells cannot as yet be defined.

From experiments on intact animals and on isolated tissues, it is difficult to assess the clinical value of a drug. In the anaesthetised normotensive cat rescinnamine causes little or no fall in the blood pressure level except for a short-lived, sharp initial fall. Pharmacological studies with this alkaloid were made by Cronheim et alia[1] who, however, found it to possess all of the typical properties /

properties of reserpine and of the alseroxylon fraction of Rauwolfia serpentina as measured on the normotensive anaesthetised dog. This is in agreement with the clinical findings obtained by Hershberger and his co-workers[9] and by Smirk and McQueen[10] who reported rescinnamine to have similar hypotensive effects to reserpine in man. That rescinnamine markedly reduces adrenaline and noradrenaline hypertension in anaesthetised cats may have some significance in its use as a hypotensive drug in man. Lemieux and his colleagues[11], on the other hand, did not agree with the clinical results obtained by the previous investigators[9,10] following oral administration of rescinnamine, although they observed a significant lowering of blood pressure in man following its intramuscular administration.

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CHAPTER V

SUMMARY.

Rescinnamine has been used in the treatment of hypertension and is claimed to be an effective hypotensive agent in man.

The pharmacological properties of rescinnamine have been studied in experimental animals and there appears to be little or no difference between the properties of rescinnamine and reserpine.

In Chapter II of this section, various experimental procedures using intact animals and isolated tissue preparations in the study of the pharmacological properties of rescinnamine are described.

In Chapter III, the results of these studies are described. In the normotensive anaesthetised cat, rescinnamine caused little or no fall in the level of the blood pressure. It depressed or abolished the pressor responses elicited by (a) faradization of the central end of the cut vagus, (b) faradization of the splanchnic nerves, (c) bilateral occlusion of the carotid arteries, (d) compression of the abdominal aorta and by (e) hypoxia. Hypertension in anaesthetised or spinal cats due to the infusion /

infusion of adrenaline or noradrenaline was reduced by an injection of rescinnamine, but the pressor effects of single injections of adrenaline or noradrenaline were either unaltered or slightly depressed.

Although its action is delayed, rescinnamine has been shown to possess some ganglion blocking activity.

Rescinnamine depressed the activity of preparations of isolated cardiac muscle. In isolated vascular (horse carotid artery) and intestinal (guinea pig ileum) smooth muscle, rescinnamine reduced the magnitude of drug-induced contractions.

In preparations of the isolated frog rectus abdominis muscle, although rescinnamine had some direct stimulant effect, acetylcholine-induced contractions were inhibited.

In Chapter IV, the results obtained in the present study as well as those of other workers are discussed. The possibility of a significant peripheral component in the effects of rescinnamine in human hypertension is also discussed. It is concluded that while the drug has a definite action upon the central nervous system - which probably contributes largely to its antihypertensive effect /

effect in man - peripherally induced relaxation of the vascular smooth muscle may also play a part.

PART II

A. STUDIES ON SOME SYNTHETIC TRIS- AND TETRA-QUATERNARY NEUROMUSCULAR BLOCKING AGENTS.

CHAPTER I

INTRODUCTION

The oldest known neuromuscular blocking agents are those grouped together as the curare alkaloids. The term "Curare" is a wide one and includes many types of South American arrow poison. Curare has a long and romantic history which has been well described in a comprehensive review by McIntyre[1] who has discussed the botanical sources, nomenclature and chemical identification etc. Sir Walter Raleigh[2] in his "Discovery of Guiana", published in 1595, mentions the arrow poisons of the South American Indians, and the first eye witness account of the preparation of curare was given by Humboldt[3] who believed that curare was prepared by the natives of British Guiana from various species of Strychnos.

Prior to the important contribution of Gill[4] in supplying a sufficient amount of the authentic drug which had been prepared from Chondrodendron tomentosum, different specimens of curare were not of uniform chemical constitution or pharmacological activity. The side effects which were produced by any given sample of curare were also very variable. The only consistent observation was that the drug produced a paralysis of skeletal /

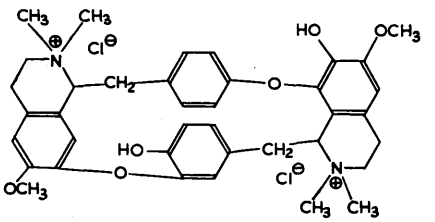
skeletal muscle. Yet another major obstacle was the fact that the sources of the different curares had not been satisfactorily identified. Some semblance of order was created by Boehm[5] who showed that there were three kinds of curare which were commonly available. These could be classified upon the basis of the containers in which they were stored, transported and appeared on the market. These were: tube curare, contained and marketed in bamboo tubes, pot curare, found in earthenware jars and calabash curare, found in gourds. This system of classification has many obvious limitations and is no longer commonly used since purified preparations of constant composition and potency are now available for experimental investigation and clinical use. Alkaloidal extracts are indeed seldom now employed, having been replaced by tubocurarine - a pure crystalline alkaloid.

The main raw material for the preparation of the various curares which were used by the South American Indians was either the bark of one of the many species of the genus Strychnos (most frequently, Strychnos toxifera), or of Chondrodendron tomentosum, but many other plants appear /

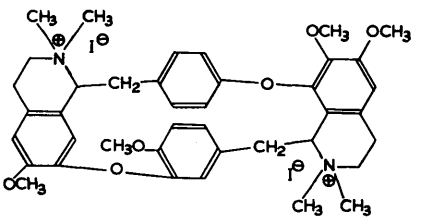
appear to have been used.

Although Boussingault and Roulin[6] prepared an extract from curare in 1827, the first systematic approach to the problem of the isolation of the alkaloids of curare was made by Boehm[5]. Boehm isolated certain quaternary bases called curarines from the varieties of curare which were then available. Boehm named the products obtained from calabash curare, the curarines, those extracted from pot curare, the protocurarines, and the extracts of tube curare, the tubocurarines. He also isolated from pot and tube curare tertiary bases with weak neuromuscular blocking activity which he called curines. The chemistry of tube and pot curare was later investigated by King[7]. In 1938 McIntyre obtained the first clinically useful, standardized curare preparation from Chondrodendron tomentosum. Subsequently crystalline tubocurarine (Fig. 24 , p.87) (previously isolated from tube curare by King[7] in 1935) was prepared from the same plant by Wintersteiner and Dutcher[8]. Dimethyltubocurarine (Fig. 24 ,p.87), a clinically useful derivative of tubocurarine, was also prepared by King[7] and by Wintersteiner and Dutcher[8].

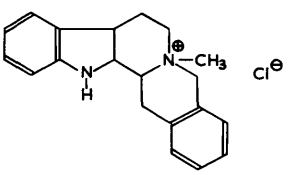
Calabash /



A. Tubocurarine Chloride



B. Dimethyl Tubocurarine Iodide



C. C-Toxiferine-I Chloride

Figure 24. The structural formulae of tubocurarine, dimethyl tubocurarine and C-toxiferine-I.

Calabash curare was studied by Wieland and his associates[9 to 12], by Karrer and Schmid[13], by Schmid and Karrer[14 to 17], by Waser[18,19] and by Marsh.[20] Of the numerous alkaloids isolated from calabash curare, one of the most interesting is C-toxiferine-I (Fig. 24, p. 87). It is thought to be the most potent of all the neuromuscular blocking agents, yet it contains only one quaternary nitrogen atom. C-toxiferine-I was also isolated from the bark of Strychnos toxifera by King[7] and studied by Paton and Perry[21] who found that this compound showed less variation of activity from species to species than any of the neuromuscular blocking agents which had been investigated up to this time.

Another type of neuromuscular blocking agent which occurs naturally is β-erythroidine, an alkaloid which was isolated by Folkers and Major[22] from the seeds of Erythrina americana. Dihydro-β-erythroidine which was prepared from β-erythroidine by Folkers and Major[23] also possesses neuromuscular blocking activity and is about six times as potent as β-erythroidine. In contrast to tubocurarine and the other alkaloids of curare, both of these compounds are tertiary amines and contain no quaternary /

quaternary nitrogen atoms.

Although Humboldt and Bonpland[24] as long ago as 1821 had carried out on the frog nerve-muscle preparation what were probably the first experiments with curare, the earliest scientifically designed experiment to demonstrate the neuromuscular blocking activity of curare was that described and carried out by Claude Bernard[25,26] in 1850. In his classic experiment, Bernard showed that curare paralysed its victims by blocking the transmission of the impulse from nerve to muscle. One leg of a decapitated frog was tightly ligatured and an extract of curare injected intra-peritoneally. This technique ensured that the injected drug did not enter the circulation of the ligatured limb, although the rest of the animal was affected. When the nerve was stimulated above the ligature, the ligatured limb contracted and it was concluded that the drug did not affect the nerve itself. When the opposite limb was stimulated in a similar way it did not contract, but if an electric shock or a small quantity of acid was applied directly to the muscle, then this contracted in a normal fashion. Bernard concluded that the drug had no direct /

direct action on the nerve or muscle and must therefore act upon the neuromuscular junction.

The history of the modern clinical usage of curare may be said to date from the studies of West[27] who, in 1932, employed in the treatment of patients with tetanus and certain spastic disorders a highly purified fraction prepared from crude curare. In 1940, Bennett[28] introduced the drug as an adjuvant to the leptazol shock treatment of psychiatric disorders, and in 1948 Griffith and Johnson[29] reported the results of the first clinical trial of curare as a muscular relaxant in general anaesthesia. In their pioneer studies, Griffith and Johnson[29] administered curare in the form of Intocostrin, a purified standardized fluid extract obtained from Chondrodendron tomentosum.

In addition to the purified natural form of curare, tubocurarine, we possess to-day a large number of synthetic muscle relaxants some of which are used in clinical medicine. These are pure chemicals of known composition and their pharmacological effects can be measured with a high degree of precision. Since their effects are readily predictable, they are widely used in surgery /

surgery to cause muscular relaxation by blocking the transmission of impulses from the motor nerve to the muscle and thereby permitting the use of smaller doses of volatile and other anaesthetics with a very desirable reduction in the associated undesirable toxic side effects and in post-operative morbidity and mortality. The muscle relaxants are especially valuable in abdominal pelvic and thoracic surgery, and in various orthopoedic procedures. They have been used to facilitate laryngoscopy, bronchoscopy and oesophagoscopy. They are also useful in some nervous disorders which cause spastic paralysis, and in controlling various convulsive states including those induced by electro shock therapy.

The isolation and characterisation of tubocurarine from tube curare by King[7] in 1935 gave a renewed impetus to the investigation of the mode of action of neuro-muscular blocking agents. Dale and his co-workers[30] showed that when a motor nerve was stimulated, a nerve impulse was initiated and that when this reached the nerve endings it brought about the release of acetyl-choline at the neuromuscular junction. Cowan[31] demonstrated /

demonstrated that the acetylcholine liberated by the motor nerve impulse in response to nerve stimulation depolarized the end-plate and the potential difference produced by this depolarization (called the end-plate potential by Eccles and his co-workers[32]) was responsible for the initiation of the propagated muscle action potential which produced the muscular contraction. Although certain aspects of the mechanism of neuromuscular transmission are still controversial, it is now generally accepted that both electrical phenomena and the acetylcholine-acetylcholine esterase system play important roles in neuromuscular transmission[33,34]. According to the "chemico-electrical" theory of Feldberg[33], the depolarization of the end-plate is caused by the acetylcholine secreted at the terminal membrane which diffuses through the sub-neural space to reach the post-junctional membrane. In contrast to this, Nachmansohn[35] in his "electro-chemical theory" claims that the current generated at the terminal membrane reaches the post-junctional membrane by jumping the "few microns-wide" sub-neural space and then liberates acetylcholine which is responsible for the depolarization of the end-plate. For convenience, the sequence of events in neuromuscular transmission /

transmission may be summarised as follows: (1) the propagated nerve impulse causes the liberation of acetylcholine at the region of the end-plate, (2) acetylcholine is adsorbed on to cholinergic receptors on the post-junctional membrane, (3) the end-plate is partly or completely depolarized with consequent generation of the end-plate potential, (4) the end-plate potential reaches a critical level and the muscle action potential is initiated, (5) acetylcholine is hydrolysed by acetylcholinesterase to acetic acid and choline (it is possible that end-plate depolarization and enzyme hydrolysis occurs simultaneously), (6) the end-plate becomes repolarized, (7) muscular contraction occurs towards the end of the phase of repolarization, (8) the choline acetylase system forms acetylcholine by a synthetic process from choline and acetate ions. (This is a simplification of what is certainly a complex biochemical process[36].)

Much of the work on the physiology of neuromuscular transmission was done with the help of muscle relaxants. The work of Burns and his co-workers[37], Burns and Paton[38], Paton and Zaimis[39] and others gave a considerable amount of /

information on the mechanism of action of the neuromuscular blocking agents. A neuromuscular blocking agent may be defined as a drug which interferes with the transmission of the nerve impulse through the neuromuscular junction to the muscle fibres without modifying conduction in the nerve, and without affecting twitch tension in response to direct stimulation. Although compounds other than quaternary ammonium derivatives, e.g. tertiary amines[22,23] are also capable of blocking neuromuscular transmission, the clinically useful neuromuscular blocking agents are all quaternary bases which produce neuromuscular block by inhibiting either the depolarization or the repolarization phases of neuromuscular transmission. Attempts have been made by a number of investigators to classify the quaternary neuromuscular blocking agents. The method of classification proposed by Paton and Zaimis[39] distinguishes three types of neuromuscular block: (1) competitive block, (2) depolarization block and (3) mixed or intermediate block. Competitive block is caused by drugs which produce their effects by competing with acetylcholine at the end-plate region. The end-plate potential is progressively reduced to a level below that of /

of the normal threshold value until impulse propagation ceases and muscle contraction is abolished. A large number of synthetic or naturally occurring substances of this type are known. Tubocurarine is generally considered to be the typical representative of this group since it is by far the most thoroughly studied.

Depolarization block may be contrasted with that caused by competition by virtue of the fact that depolarizing drugs produce a persistent depolarisation of the end-plate. This causes an electrical inexcitability at the end-plate region which is sufficient to prevent the generation of end-plate potentials which are of a magnitude adequate to cause excitation of the adjacent muscle membrane. On the basis of its actions on mammalian (cat), avian (chick) and amphibian (frog) muscle, decamethonium may be taken as being the most typical representative of the group of drugs which produce block by depolarization. End-plate depolarization caused by decamethonium and other depolarizing drugs is associated with an initial phase of excitation or increased excitability. It is during this phase that spontaneous fasciculation of the muscle and potentiation /

potentiation of twitch height with repetitive firing to single nerve impulses are seen. As depolarization persists, the phase of increased excitability soon passes over into one of inexcitability.

The properties of drugs producing mixed or intermediate types of neuromuscular block are different from the two types described. When tridecamethonium which is a higher member of the methonium series is injected into chicks, a contractural response is seen but there slowly ensues a mixed type of paralysis in which the legs are contracted and the head and neck are flaccid. Finally there appears a typical generalised flaccid paralysis. In the cat successive injections of tridecamethonium lead to an increasing refractoriness, to paralysis of the tibialis muscle and to an increasing sensitivity of the soleus muscle to neuromuscular block. Thus decamethonium-like activity is replaced by a tubocurarine-like effect. In the alkyl trimethyl ammonium series studied by Dallemagne and Philippot[40,41], the lower members have predominantly decamethonium-like properties and cause depolarization of the end-plate, whereas the higher members have a mixed competitive-depolarizing type /

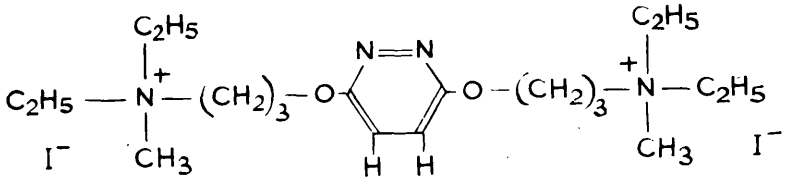
type of effect. Similar results have been obtained with a series of choline esters of adipic acid[42,43] in which the methyl groups on the choline nitrogen atoms were successively replaced by ethyl groups. Adipylcholine itself is a depolarizing agent and this is still true when one methyl group is replaced by an ethyl group. With three ethyl substituents on each nitrogen atom it becomes tubocurarine-like in its properties and antagonises the neuromuscular blocking activities of the parent compound. The diethyl methyl derivative is intermediate in its properties, so that it can, for instance, produce a contracture of avian muscle and also terminate a similar contracture which has been elicited by administration of the parent substance. The block produced by this type of drug is not typical of either competitive block or of the depolarizing block, and is described as a mixed or intermediate block.

The classification suggested by Bovet et alia[44] grouped decamethonium, suxamethonium and related substances under the heading "leptocurares"; compounds related to tubocurarine and gallamine were described as "pachycurares". In proposing the use of this system of /

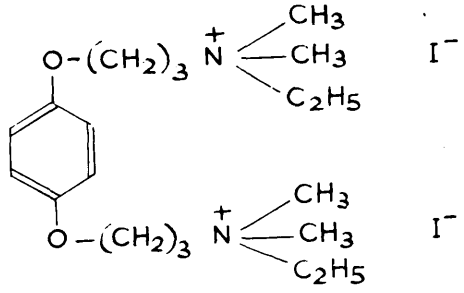
of classification, Bovet and his colleagues suggested that those neuromuscular blocking agents with long, thin molecules should be called leptocurares. The leptocurares all possessed decamethonium-like properties, whereas those drugs with fat or thick molecules (termed pachycurares) resembled tubocurarine. This is not always true. Nicotine which has a thick molecule, and might be described as a pachycurare, causes neuromuscular block in the cat by depolarization of the muscle. Tridecamethonium which has a structure which would lead to its being classified as a lepto-curare behaves pharmacologically, in some respects, like tubocurarine - a pachycurare - in that when injected into chicks it does not cause the typical spastic paralysis obtained with decamethonium, but produces an effect resembling that caused by tubocurarine. Another objection to Bovet's system of classification is that it is a rather exclusive one, and implies that there are two discrete types of compound with two quite different modes of action. As has been shown this is certainly not the case. More recently, Foldes[45] has divided the neuro-muscular blocking agents into (1) non-depolarizing and /

and (2) depolarizing agents. This system of classification possesses some advantages, and it offers a system into which most of the available experimental findings can be fitted, but it is difficult to allot to either group certain substances which have been shown to have some properties in common with decamethonium, others in common with tubocurarine and still others not shared by either tubocurarine or decamethonium. 3, 6 - bis (3-diethyl-aminopropoxy) pyridazine bis - methiodide studied by Gesler and Hoppe[46] possesses these anomalous properties and more closely resembles the compound dipropamine investigated by Winter and Lehman[47]. These two compounds are shown in Figure 25, page 100.

Each classification may possess certain merits of its own but the classification suggested by Paton and Zaimis appears to be more suitable for the purpose of discussing the various types of neuromuscular blocking agents which are quaternary ammonium derivatives. When tubocurarine and other substances having a similar mechanism of action are classed as competitive, and decamethonium and other substances with a similar type of action are described as depolarizing neuromuscular blocking /



A. 3,6-*bis*(3-diethyl-aminopropoxy) pyridazine *bis*-methiodide



B. Dipromamine

Figure 25. The structural formulae of

A. 3,6,-bis(3-diethyl-aminopropoxy) pyridazine bis-methiodide and

B. Dipromamine.

blocking agents, a number of substances which cause neuromuscular block, but in a manner different from that caused by either tubocurarine or decamethonium yet sharing certain properties in common with these compounds, may be conveniently classed as drugs with an intermediate type of action. The competitive and depolarizing types of neuromuscular blocking agent have properties which appear to be characteristic of the members of each group; but the compounds which belong to the intermediate class cannot be rigidly differentiated from the members of either of these two former groups. Compounds which are described as being intermediate in type may have certain properties in common with tubocurarine, others in common with decamethonium: some properties may be unique and have nothing in common with either of these compounds. In the case of both the competitive and the depolarizing type of neuromuscular blocking activity, it may be assumed that the members of both classes compete with acetylcholine for cholinergic receptors at the end-plate and are capable of preventing the access of acetylcholine to these receptors, partly because of their greater stability and partly because of their greater affinity for these receptors. After being

adsorbed /

adsorbed on to the cholinergic receptors the members of the two groups behave in a different manner. This behaviour may be described as follows: After adsorption on to the cholinergic receptors, the activity of the competitivie type of neuromuscular blocking agent is limited to the prevention of the access of acetylcholine to these receptors. The configuration of the receptor remains unchanged; there is no depolarisation, nor is there any change in the resting potential of the end-plate and consequently no muscular contraction can be initiated. This type of neuromuscular blocking agent exhibits a uniform behaviour and causes flaccid muscular paralysis in all of the mammalian, avian and amphibian species investigated.

Neuromuscular blocking agents which act by depolarization cause a typical depolarization block in birds, amphibians and in certain mammals (cats and man)[38]. These agents cause depolarization of the end-plate in a similar manner to acetylcholine but, in contrast to acetylcholine, the depolarization persists, spreads to the parts of the muscle fibre adjacent to the end-plate and makes the muscle fibre insensitive to subsequent stimulation. /

stimulation. In certain other species of mammals however, except perhaps for a brief period of depolarization, the block produced by these agents shows the typical characteristics of competitive block.

The difference in the activity of the two groups of neuromuscular blocking agents can be explained by the hypothesis[48] that the type of block produced by these compounds depends primarily upon the chemical structure of the agent, and secondarily upon the properties of the cholinergic receptors. The chemical structure of the competitive type of neuromuscular blocking agent is such that, under physiological circumstances, they will not change the configuration of the receptors and will produce a competitive block in all species. The block produced by the depolarizing blocking agents depends on the properties of the receptors in the postjunctional membrane of the species involved. In some species where the configuration of the receptors can be changed easily, relatively small doses produce a depolarization block. In other species where the receptors are more resistant to changes of configuration, small doses of depolarizing drug produce /

produce no neuromuscular block at all and large doses cause a competitive type of neuromuscular block.

The major differences in the two types of neuromuscular blocking agents are described in Table 1, page 105.

TABLE I

Comparison of Competitive and Depolarizing Muscle Relaxants

	Competitive type of muscle relaxant	Depolarizing type of muscle relaxant
1. Initial excitatory effect on striated muscle	None	Transient fasciculation i.e. excitation
2. Effects of administering different competitive muscle relaxants	Additive	Antagonistic
3. Effects of administering different depolarizing muscle relaxants	Antagonistic	Additive
4. Indirect tetanization of the partially blocked muscle	Poorly sustained contraction	Well sustained contraction
5. Effect of ether anaesthesia	Increased effect	No effect or antagonism
6. Effect of		
(a) Previous tetanization of the motor nerve	Antagonism	No effect
(b) Potassium	Antagonism	No effect
(c) Anticholinesterases	Antagonism	Little effect; occasionally feeble potentiation or antagonism
(d) Edrophonium	Antagonism	No effect or potentiation
7. Effect of current applied to the end-plate region.		
(a) Anodal Current	Intensifies paralysis	Lessens paralysis
(b) Cathodal Current	Lessens paralysis	Intensifies paralysis.

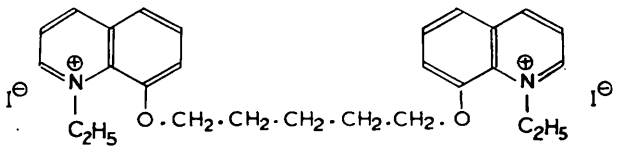
The first attempts at the production of synthetic muscle relaxants were the syntheses by Crum-Brown and Frazer[49] in 1869 of the quaternary derivatives of strychnine and brucine, methylstrychnine and methylbrucine. Crum-Brown and Frazer repeated Bernard's experiments and showed that these quaternary derivatives of strychnine and brucine had certain properties analagous to those of natural curare. It was recognised later that this type of action was a characteristic of many quaternary amines[50], and that some tertiary ammonium compounds such as stilbamidine[51], quinine, nicotine and β-erythroidine had similar properties. Many well known drugs including atropine, quinine, strychnine etc. show a marked increase in neuromuscular blocking potency when their nitrogen atoms are quaternized, while the neuromuscular blocking activity of β-erythroidine and dihydro-β-erythroidine is actually abolished by quaternization of the nitrogen atom[52]. Other atoms may be substituted for nitrogen; thus neuromuscular blocking activity has been reported among sulphonium, phosphonium, arsonium, stibonium and iodinium compounds[52].

Partly because of the relative difficulty in getting supplies of Chondrodendron tomentosum and partly because of the incidence of untoward side effects[53,54] which occasionally accompanied /

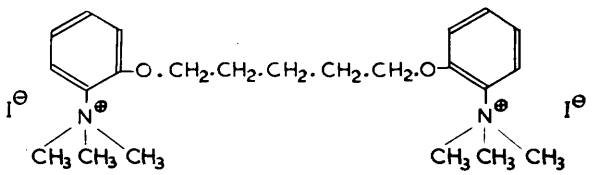
accompanied the use of tubocurarine, a systematic search for synthetic neuromuscular blocking agents was started by several independent groups of investigators. The main object of this was to obtain synthetic compounds with a more selective action on the neuromuscular junction. The early attempts by West[55] to utilize in man the curarizing action of trimethyl hexyl ammonium iodide, and that of trimethyl-octylammonium iodide by Burman[56], were not successful because the actions of these compounds were not sufficiently selective.

Many of the recent advances in the history of synthetic neuromuscular blocking agents are due largely to the work of Bovet and his co-workers who, after a consideration of the chemical structure of tubocurarine, attempted the synthesis of quinoline and isoquinoline derivatives with curarizing action.

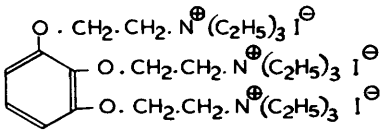
In 1946 Bovet and his colleagues[57,58] described the preparation and neuromuscular blocking activity of 8^{1} - 8^{11} - diquinolyloxy - 1,5 - pentane, di-iodoethylate (3381 R.P.)(Fig.26, p.108). This compound was the first synthetic compound which had significant curarizing activity in mammals and which had a potency and selectivity comparable with /



A. 3381 R.P.



B. 3565 R.P.

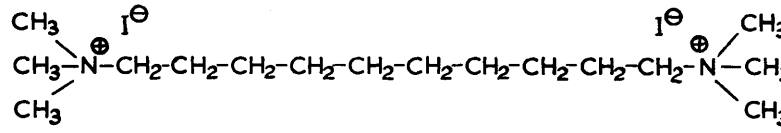


C. Gallamine Triethiodide

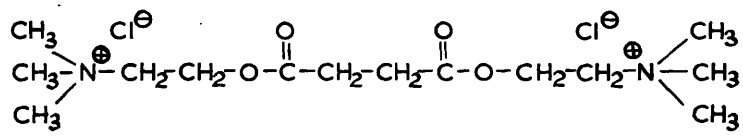
Figure 26. **The structural formulae of 3381 R.P., 3565 R.P. and gallamine, (3697 R.P.).**

with that of the naturally occurring alkaloid. That even simpler compounds which lacked the quinoline or iso-quinoline nucleus could exhibit curarizing activity was shown in 1947 by Bovet and his co-workers[57,59] when they synthesised and tested a series of bis quaternary derivatives in the aromatic amine series; the most interesting member of this series was the bis-(dimethyl amino 2, 2 - phenoxy) - 1, 5 pentane diiodomethylate (3565 R.P.) (Fig.26, p.108). In certain species and in certain preparations, this compound was equal to or even greater in neuromuscular blocking activity than tubocurarine. In the same year, a series of ethers of β-hydroxyethyltriethylammonium were prepared and examined by Bovet et alia[58,60]. Tri-(β-triethylammoniumethoxy) - 1,2,3-benzene triiodide (Gallamine) (Fig.26, p.108), the most potent member of this series, showed both in experimental animals and in clinical trials certain promising features. The undesirable side effects which occasionally follow the use of tubocurarine were absent. When the potency of this compound was estimated by the rabbit head drop test[61], it was shown to have an activity of about one fourth of that of tubocurarine. Its use in anaesthesiology /

anaesthesiology was first reported by Huguenard[62] and later by Mushin and his associates[63]. Gallamine appears to act in the same way as tubocurarine in that the neuromuscular block is antagonised by neostigmine[63] and edrophonium[64,65], and the neuromuscular blocking effect is potentiated by ether anaesthesia[66]. The duration of action of gallamine is significantly shorter than that of equipotent doses of tubocurarine, and cumulative effects result from the repeated administration of this drug. Shortly after the publication by Bovet and his co-workers of the results of these studies on the pharmacology of gallamine, Barlow and Ing[68,69] and Paton and Zaimis[70,71,72] simultaneously but independently reported on the neuromuscular blocking activity of decamethonium, the most active member of the polymethylene bis trimethylammonium series (Fig.27, p.111). It was from the members of this series of compounds that drugs which were appreciably more potent than tubocurarine were obtained[68 to 72]. Decamethonium was found to be about ten times as potent as tubocurarine in causing neuromuscular block in the tibialis muscle-sciatic nerve preparation of the cat. Eserine and neostigmine had no effect on decamethonium block but tubocurarine /



A. Decamethonium Iodide



B. Suxamethonium Chloride

Figure 27. The structural formulae of decamethonium and suxamethonium.

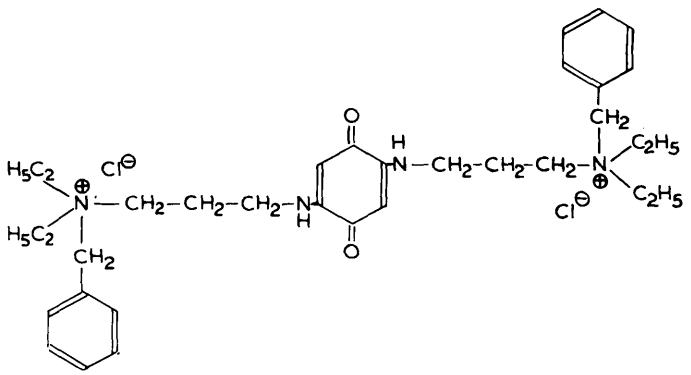
tubocurarine had an antagonistic effect[72]. Decamethonium was first used in clinical anaesthesia by Orgone et alia[73] in 1949. It is a satisfactory agent for the production of muscular relaxation for moderately short surgical operations but has latterly been used very little.

The dimethyl ether of tubocurarine was prepared by King[7] in 1935 and the pharmacological properties of this compound were described by Collier and his co-workers[74] in 1948. In this compound the two hydroxy-groups in the tubocurarine molecule were replaced by methoxy-groups (Fig.24, p.87). Dimethyltubocurarine was found to be about three times as potent as tubocurarine in man[75] and to possess pharmacological properties similar to those of tubocurarine, except that the histamine liberating and ganglion blocking action of this compound were less marked[76,77]. Dimethyltubocurarine was evaluated clinically by Stoelting and his co-workers[78,79] in 1948.

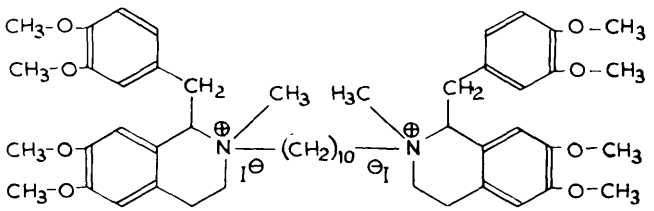
The neuromuscular blocking activity of suxamethonium, which may be looked upon as bis acetylcholine (Fig.27, p.111), was discovered independently in 1949 by Bovet et alia[80] and by Phillips[81]. Its neuromuscular blocking activity was overlooked /

overlooked by Hunt and Taveau[82] (who investigated this compound in 1906), because their experimental technique involved the use of curare to immobilise the experimental animals. Although suxamethonium is a potent neuromuscular blocking agent it has a short duration of action. This was believed to be because the molecule of suxamethonium was almost completely hydrolyzed in the organism by plasma cholinesterase. Hydrolysis first took place in two stages: first, fairly rapidly, to form succinylmonocholine and choline[83 to 85], and secondly, much more slowly, to form succinic acid and choline. The paralysing effect of suxamethonium was found not to be antagonised by neostigmine or edrophonium and was actually intensified and prolonged by these two anticurare agents. The pharmacological properties of suxamethonium were also studied by Castillo and de Beer[86] and by de Beer and his colleagues[87], and the first clinical trials were reported by Brucke *et alia*[88], Thesleff[89] and Mayrhofer and Hassefurther.[90] In relaxant doses, (provided that adequate carbon dioxide removal is ensured) suxamethonium only affects neuromuscular transmission[91].

Another interesting compound, benzoquinonium (Fig.28, p.114), /



A. Benzoquinonium Chloride



B. Laudexium Iodide

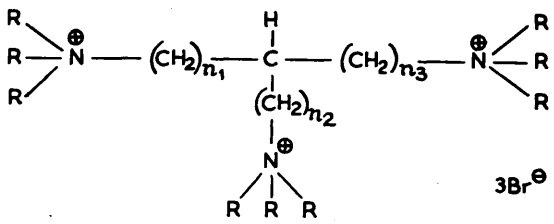
Figure 28. **The structural formulae of benzoquinonium and laudexium.**

p.114), which had a high degree of neuromuscular blocking activity was synthesised by Cavallito et alia[92] but primarily as part of a programme of study of agents with antibacterial activity. It was investigated pharmacologically by Hoppe[93,94] in 1950. This compound was found to be five times more potent than tubocurarine when tested by means of the rabbit head drop method, but in man it was only slightly more potent, while its duration of action was intermediate between that of tubocurarine and decamethonium. Benzoquinonium reduced the effectiveness of a subsequent injection of decamethonium[92], and the muscular relaxation caused by this drug was potentiated by ether anaesthesia[95] and was not preceded by initial stimulation. In these respects it resembled tubocurarine but on the other hand its neuromuscular blocking activity was only partially and irregularly antagonised by neostigmine[96] and was actually intensified by edrophonium. The first clinical trial of this drug as a muscle relaxant in anaesthetised patients was reported by Arrowood[95]. Besides its neuromuscular blocking action, benzoquinonium stimulated the vagus causing a marked increase in salivary and bronchial secretion, and a tendency to bradycardia[96,97].

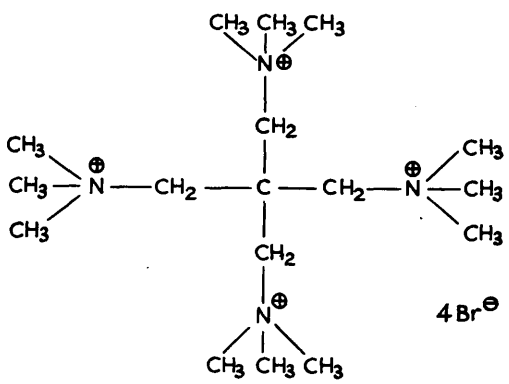
Laudexium /

Laudexium (Fig.28,p.114), another clinically useful neuromuscular blocking agent, was synthesised by Taylor and Collier[98,99] during an investigation of a series of decamethylene bis - 1:2:3:4 - tetrahydro-?-methyl isoquinolinium salts. Its pharmacological properties were studied by Collier and Macauley[100] in 1952. This compound was first used in clinical anaesthesia by Bodman et alia[101] in 1952. In the rabbit and the cat, laudexium was more potent; and in the rat and the mouse it was less potent than tubocurarine. When tested on the sciatic nerve-tibialis muscle preparation of the cat, its duration of action was about 50 per cent longer than that of tubocurarine. Neuromuscular block was antagonised by neostigmine[102] and markedly potentiated by ether anaesthesia[103] and its cumulative effect was greater than that of tubocurarine.

A new series of aliphatic trisquaternary ammonium compounds (Fig.29,p.117), in which the number of carbon atoms between the quaternary nitrogen centres varied from five to nine, were prepared and examined for pharmacological activity by Kensler et alia[104] in 1954. Two types of activity were observed, namely, the production of neuromuscular blockade and the inhibition of vagal slowing of /



A. R = CH_3 or C_2H_5



B. SKF 2091

Figure 29. The structural formulae of

A. 5-(4-diethylaminobutyl)-1,9-bis diethyl amino-nonane triethobromide (R = C_2H_5, $N_1 = N_2 = N_3 = 4$) and

B. Tetrakis-(dimethyl aminomethyl)-methane tetra-methobromide (SKF 2091).

of the heart. The most potent compound among this series was found to be 5 - (4-diethylaminobutyl) - 1,9 - bis diethylaminononane triethobromide (444E, Fig.29,A, p.117) where R = C_2H_5 and $n_1 = n_2 = n_3 = 4$) which had about 40 per cent of the activity of gallamine when tested by the rabbit head drop test. Its mode of neuromuscular blocking action was similar to that of gallamine and tubocurarine in that in the kitten phrenic nerve-diaphragm preparation its paralysing effects were antagonised by edrophonium, and it was found to antagonise the stimulant actions of acetylcholine on the isolated frog rectus abdominis muscle.

When it was found that bis and tris quaternary derivatives possessed potent neuromuscular blocking activity, it was not unnatural to expect a search to be made for compounds containing even more quaternary nitrogen atoms. Tetrakis - (dimethylaminomethyl) - methane tetra methobromide (SKF 2091), the most potent of the series of tetraquaternary ammonium aliphatic methonium compounds, was examined by Kensler et alia.[105] The structural formula of this compound is shown in Fig.29,p.117. In the kitten phrenic nerve-diaphragm preparation this compound showed neuromuscular blocking activity of a type which resembled that of decamethonium rather than that of tubocurarine /

tubocurarine in that (a) the block was not antagonised by neostigmine or edrophonium, (b) it produced contracture of the isolated rectus abdominis muscle of the frog and (c) injection into the chicken produced a spastic not a flaccid paralysis.

Although tubocurarine, gallamine, suxamethonium and a number of other muscle relaxants are being widely used in clinical practice and are in general satisfactory, all of them are at times guilty of causing untoward side effects. Histamine liberation and ganglion blocking activity are shown by tubocurarine, an inhibitory effect upon the cardiac vagus is shown by gallamine and a lack of suitable antidotes is a major disadvantage in the use of decamethonium and suxamethonium. The search for the ideal muscle relaxant can by no means be considered concluded and active investigations are still being carried on in this field. The greatest need is, without doubt, for a short acting relaxant which acts by competition with acetylcholine because, although suxamethonium is usually satisfactory for short surgical procedures, it causes initial stimulation of the skeletal muscles, and occasional reports of prolonged apnoea have appeared in the literature.[106,107]

A /

A series of aliphatic straight chain polymethylene ethonium compounds having three and four quaternary centres (Figs.30,31 and 32, pages 121, 122 and 123), which were synthesised by Edwards and Stenlake[108,109], have been investigated for neuromuscular blocking activity. These compounds have more than two quaternary centres and they are all ethonium derivatives. This work has been carried out for the following reasons. It was hoped to demonstrate in this series not only neuromuscular blocking activity but also ganglion blocking, and in consequence hypotensive, activity. Ganglion block is not caused by any of the compounds which have been investigated, but their neuromuscular blocking properties were so interesting that it was thought well worth while pursuing the study of this aspect. In some of the trisquaternary compounds the nitrogen atom in the centre has been replaced by sulphur or by a non-ionised sulphone group (Fig. 30,(1) and 31). The methonium analogue of compound dihexasulphonium (Fig.31 (1)) was also available.

The work described in this part of the thesis was undertaken to investigate the neuromuscular blocking activity of these compounds in different species in order to correlate the degree and type of muscle relaxant activity /

1. $(C_2H_5)_3\overset{+}{N}(CH_2)_6\,SO_2\,(CH_2)_6\overset{+}{N}(C_2H_5)_3\;.\;2I^-$

7-Dioxothiatridecane - 1 : 13 - *bis* (triethyl ammonium iodide) (dihexone)

2. $(C_2H_5)_3\overset{+}{N}(CH_2)_n\overset{+}{N}(CH_2)_n\overset{+}{N}(C_2H_5)_3\;.\;3I^-$

C_2H_5 C_2H_5

A. $n = 6$, 7:7-Diethyl-7-azoniatridecylene *bis* (triethylammonium) triiodide (dihexaazonium)

B. $n = 8$, 9:9-Diethyl-9-azoniaheptadecylene - *bis* (triethylammonium) triiodide (dioctaazonium)

C. $n = 10$, 11:11-Diethyl-11-azoniaheneicosylene *bis* (triethylammonium) triiodide (didecaazonium)

Figure 30. **The structural formulae of dihexone, dihexaazonium, dioctaazonium and didecaazonium.**

1. $(CH_3)_3 \overset{+}{N} (CH_2)_6 \overset{+}{\underset{\underset{CH_3}{|}}{S}} (CH_2)_6 \overset{+}{N} (CH_3)_3 \cdot 3I^-$

7-Methyl-7-thioniatridecylene *bis* (trimethyl ammonium) triiodide.
(dihexasulphonium trimethiodide)

2. $(C_2H_5)_3 \overset{+}{N} (CH_2)_n \overset{+}{\underset{\underset{C_2H_5}{|}}{S}} (CH_2)_n \overset{+}{N} (C_2H_5)_3 \cdot 3I^-$

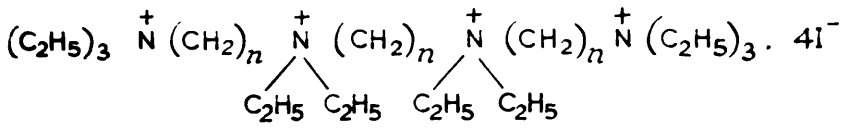
A. n=5, 6-Ethyl-6-thioniaundecylene *bis* (triethylammonium) triiodide.
(dipentasulphonium)

B. n=6, 7-Ethyl-7-thioniatridecylene *bis* (triethylammonium) triiodide.
(dihexasulphonium)

C. n=8, 9-Ethyl-9-thioniaheptadecylene *bis* (triethylammonium) triiodide.
(dioctasulphonium)

D. n=10, 11-Ethyl-11-thioniaheneicosylene *bis* (triethylammonium) triiodide.
(didecasulphonium).

Figure 31. The structural formulae of dihexasulphonium trimethiodide, dipentasulphonium, dihexasulphonium, dioctasulphonium and didecasulphonium.



A. $n=6$, 7:7:14:14-Tetraethyl-7:14-diazoniaeicosylene *bis*(triethylammonium) tetraiodide (*tris*hexatetrazonium)

B. $n=10$, 11:11:22:22-Tetraethyl-11:22-diazoniadotriacontylene *bis*(triethylammonium) tetraiodide (*tris*decatetrazonium)

Figure 32. The structural formulae of

A. **tris**hexatetrazonium and B. **tris**decatetrazonium.

activity with the chemical structure. The relationship of pharmacological activity to chemical structure in the neuromuscular blocking agents is a complex subject. An apparently small alteration in molecular configuration may profoundly change the type of action of a compound and cause a large alteration in potency. Well known examples of this are the changes in the neuromuscular blocking activity brought about by the replacement of methyl by ethyl groups on the quaternary nitrogen atoms of the neuromuscular blocking agents[110 to 116]. The distance between the quaternary centres is also reported to be one of the main factors upon which the potency and the mechanism of action of the neuromuscular blocking agents depend[69,70,72,115]. In this investigation, the relationship of pharmacological activity to chemical structure of some members of the new series of compounds has been studied with reference to (a) the influence of the length of the polymethylene chains separating the quaternary centres, (b) replacement of the central quaternary nitrogen atom by a sulphone (SO_2) group or by a quaternary sulphonium group and (c) the influence of the number of quaternary centres present in the molecules. In addition, the activity of these compounds on the cardio-vascular system, on ganglionic transmission and on respiration of the cat have been studied /

studied in some detail and comparisons with tubocurarine have been made.

The series of compounds investigated have now been protected by letters patent and one compound, dihexasulphonium, is undergoing clinical trial.

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C H A P T E R II

A. MATERIALS

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

(1)	Acetylcholine chloride	is described as		acetylcholine.
(2)	Atropine sulphate	"	"	atropine.
(3)	(+)-Tubocurarine chloride	"	"	tubocurarine.
(4)	(-)-Adrenaline hydrochloride	"	"	adrenaline.
(5)	(-)-Noradrenaline bitartrate	"	"	noradrenaline.
(6)	Histamine acid phosphate	"	"	histamine.
(7)	5-Hydroxytryptamine creatinine sulphate	"	"	5-hydroxy-tryptamine.
(8)	Hexamethonium bromide	"	"	hexamethonium.
(9)	Decamethonium iodide	"	"	decamethonium.
(10)	Neostigmine methyl sulphate	"	"	neostigmine.
(11)	Edrophonium chloride	"	"	edrophonium.
(12)	Eserine salicylate	"	"	eserine.
(13)	Potassium chloride	"	"	potassium.
(14)	Sodium pentobarbitone	"	"	pentobarbitone.

(15) /

(15) Ether anaesthetic is described as ether.

The compounds investigated in this section of the thesis, together with their shortened names, are shown below. Their structural formulae are shown in Figures 30, 31 and 32, pages 121, 122 and 123.

1. 7:7-Diethyl-7-azoniatridecylenebis(triethylammonium) triiodide is described as dihexaazonium. (Fig.30,2A.)

2. 9:9-Diethyl-9-azoniaheptadecylenebis(triethylammonium) triiodide is described as dioctaazonium. (Fig.30,2B.)

3. 11:11-Diethyl-11-azoniaheneicosylenebis(triethylammonium) triiodide is described as didecaazonium. (Fig. 30, 2C.)

4. 7:7:14:14-Tetraethyl-7:14-diazoniaeicosylenebis(triethylammonium) tetraiodide is described as trishexatetrazonium. (Fig. 32, A.)

5. 11:11:22:22-Tetraethyl-11:22-diazoniadotriacontylenebis(triethylammonium) tetraiodide is described as trisdecatetrazonium. (Fig. 32, B.)

6. 7-Methyl-7-thioniatridecylenebis(trimethylammonium) triiodide is described as dihexasulphonium trimethiodide. /

6. /trimethiodide. (Fig. 31, (1).)

7. 6-Ethyl-6-thioniaundecylene<u>bis</u>(triethylammonium) triiodide is described as dipentasulphonium. (Fig. 31, 2A.)

8. 7-Ethyl-7-thioniatridecylene<u>bis</u>(triethylammonium) triiodide is described as dihexasulphonium. (Fig. 31, 2B.)

9. 9-Ethyl-9-thioniaheptadecylene<u>bis</u>(triethylammonium) triiodide is described as dioctasulphonium. (Fig. 31, 2C.)

10. 11-Ethyl-11-thioniaheneicosylene<u>bis</u>(triethylammonium) triiodide is described as didecasulphonium. (Fig. 31, 2D.)

11. 7-Dioxothiatridecane-1:13-<u>bis</u>(triethylammonium) iodide is described as dihexone. (Fig. 30,(1).)

All the compounds mentioned in this section are aliphatic straight chain polymethylene derivatives. <u>Tris</u>-hexatetrazonium and <u>tris</u>decatetrazonium each have four quaternary nitrogen atoms whereas the others (excepting dihexone) each have three quaternary centres. The quaternary /

quaternary centres in each compound are separated by polymethylene chains of varying lengths (Figs. 30, 31 and 32; n = 5, 6, 8 or 10). Dihexone is similar in structure to dihexamonium and dihexasulphonium except that the central $\overset{+}{N}$ or $\overset{+}{S}$ atom has been replaced by a nonquaternary sulphone ($-SO_2$) group. This compound is therefore a bisquaternary derivative. All the compounds described are ethonium derivatives with the exception of dihexasulphonium trimethiodide which is the methyl analogue of dihexasulphonium and is a methonium derivative. All the compounds described are freely soluble in water.

The composition and methods of preparation of all physiological saline solutions used in this investigation are to be found in Appendix I (pages 334 and 336).

The conventional abbreviations of the metric system for volumes and weights are used throughout this thesis.

CHAPTER II

B. EXPERIMENTAL

1. Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

METHOD.

Cats of either sex, weighing between 2.0 and 5.0 kg., were anaesthetised by means of an intraperitoneal injection of sodium pentobarbitone. The commercial solution containing 60 mg. per ml. of sodium pentobarbitone (Nembutal-Abbott) was employed. A dose of 60 mg. per kg. was usually adequate for the production of surgical anaesthesia within 10 to 15 minutes.

Having anaesthetised the cat, the external jugular vein and the trachea were cannulated in a manner similar to that described on pages 11 and 12 of this thesis. Having completed the cannulation of the trachea and the external jugular vein, the left leg was prepared for indirect stimulation of the gastrocnemius muscle via the sciatic nerve. The gastrocnemius muscle was partially dissected free from the surrounding tissues, and the achilles tendon severed at a point near to its insertion into the calcaneus. A strong linen thread was tied around the free end of the tendon. The leg was held with its long axis perpendicular

to /

to the operating table and fixed rigidly by means of two clamps, one at the knee joint and the other at the ankle (Fig.33, p.146). The thread tied to the achilles tendon was led over pulleys and attached to a myograph lever, the writing point of which was adjusted so as to record the contractions of the muscle upon 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. It was then crushed proximally between the jaws of a pair of artery forceps, and a pair of shielded platinum electrodes were placed around the nerve distal to this point. The nerve was stimulated using single shocks by means of a Dobbie McInnes square wave generator at a frequency of 4 to 8 per minute, at 10 to 20 volts, the pulse width being 2.0 to 3.0 msec. In any one experiment frequency, voltage and pulse width were kept constant; but in some experiments the muscle was also tetanized indirectly by using a frequency of 1,500 impulses per minute: and in others the muscle was also stimulated directly at 40 volts after it had become unresponsive to indirect stimulation. The tension placed upon the muscle varied between 0.2 and 0.3 kg., and in any one experiment tension was kept constant. The tension produced /

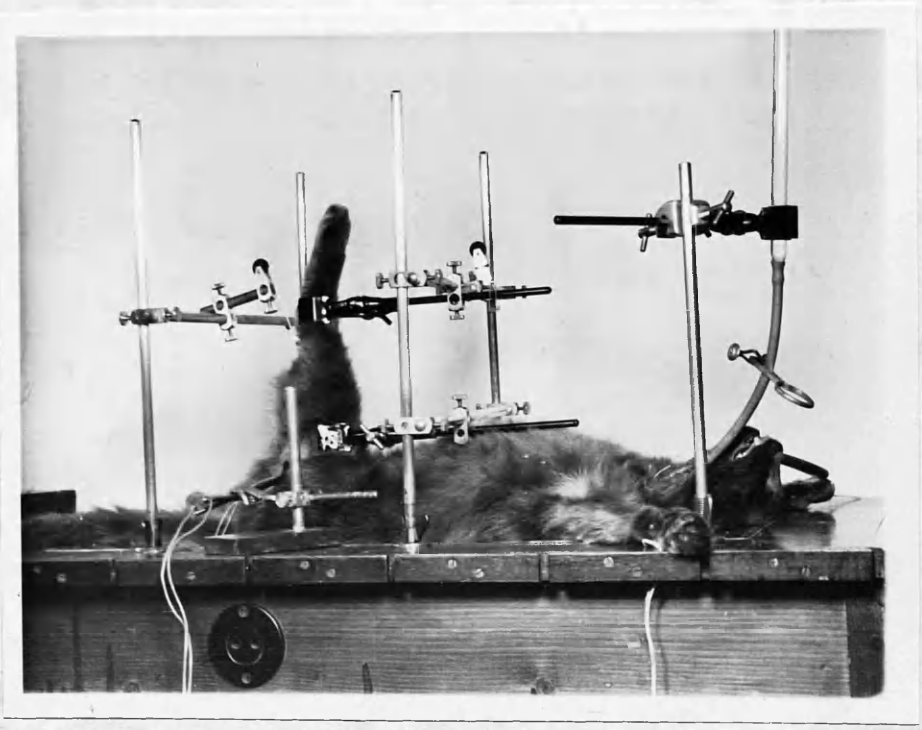

Figure 33. Method of recording contractions of the gastrocnemius muscle of the cat in response to indirect stimulation via the sciatic nerve.

produced by the contraction of the muscle in response to electrical stimulation varied from experiment to experiment but usually remained between 0.6 to 0.8 kg. The drugs used were administered in the form of solutions in normal saline. Injection was made into the rubber connection between the jugular vein cannula and the burette. The solution of the drug was washed by means of 4 ml. of saline.

2. The Rabbit Head Drop Method.

This method was introduced by Holaday[1] in 1941 but the procedure was first described in detail by Varney et alia[2]. The procedure followed in the experiments to be described is a modification of that described by Varney et alia[2].

METHOD.

Rabbits of either sex, weighing between 1.5 and 3.0 kg., were used. Nine rabbits were used for each drug. The rabbits were placed into individual wooden bleeding boxes in such a way that the head protruded through the opening at the front of the box (Fig. 34, p.148). The animals were allowed to sit quietly in the cages for some time before the experiment was started. In all cases rabbits which had /

Figure 34. Rabbit Head Drop Test.

I and II, before injection of drug.

III and IV, after injection of drug.

had not previously been used for experimental purposes were employed. The drugs under test, and tubocurarine and decamethonium, were diluted with normal saline to give solutions with a final drug concentration of 0.05 mg. per ml. This was injected through a marginal ear vein. The injection was made by means of a Palmer's slow infusion apparatus at a constant rate of 1.4 ml. per minute using a 50 ml. all glass syringe connected by means of rubber tubing to a "No.12" Record fitting hypodermic needle. The needle was inserted into one of the marginal ear veins and was held in place by means of a bulldog clip fixed just peripherally to the point of puncture. A strip of adhesive tape was used to fix the rubber tubing firmly to the ear. The piston of the syringe was lubricated by means of liquid paraffin before use, so as to ensure smooth running. After making sure that all the air bubbles had been expelled from the syringe and the rubber tubing, the needle was inserted into the vein, the reading of the syringe was noted and the apparatus immediately switched on. The rabbit remained quiet until a few minutes before the end-point was reached, when there was usually a brief period of restlessness. Soon after this point was reached, the rabbit's head began to fall but the animal was still able to raise it voluntarily.

Infusion /

Infusion was stopped as soon as the muscles of the neck were fully relaxed and toneless, and the animal could no longer raise its head in response to a light tap on the muzzle. At this moment the reading of the syringe was again noted. The difference between the two readings gave the volume of solution injected. The dose required for head drop was calculated and expressed in mg. per kg. of body weight (See Table 3, p.226). The antagonism of neostigmine to the effects of the drugs injected, as well as to the effects of tubocurarine and decamethonium, was also investigated, again using groups of nine rabbits. For this purpose, a dose of 0.1 mg. per kg. of neostigmine was injected subcutaneously into the rabbit 15 minutes before the intravenous infusion was started. The ratio - head drop dose for the neostigmine-treated animals / head drop dose for the untreated animals - was determined. If this value was greater than unity, this was interpreted as evidence of neostigmine antagonism and if less than unity, as evidence of potentiation by neostigmine of the neuromuscular blocking effects of the compounds under test.

3. Experiments upon Mice.

Estimation of the Approximate Median Paralysing Dose (PD50) METHOD. /

METHOD.

Solutions of drugs were injected intraperitoneally into mice of either sex, weighing between 20 and 30 g. Groups of 10 mice were injected at different dose levels, and were placed on a fine-mesh wire screen inclined at an angle of 50° to the horizontal. This method was originally used by Thomson[3] for the assay of insulin in mice. Those mice, which developed a typical skeletal muscle paralysis and abruptly slid off the screen within a half-hour period after drug injection, 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 (PD 50). This was expressed in mg. per kg. of the body weight.

4. Experiments upon Chicks.

Four to eight day old chicks were used in these experiments. Drugs in solution in normal saline were injected intraperitoneally into groups of six chicks for each compound (10 to 40 mg. per kg.), and the nature and mode of onset of the paralysis which developed was observed.

5. Experiments using the Isolated Rat Phrenic Nerve-Diaphragm Preparation.

METHOD.

The /

The procedure adopted was essentially that described by Bülbring[4]. Adult rats of either sex were killed by a blow on the head, the throats cut and the blood allowed to drain out. The rat was then laid on its back upon a cork-covered dissecting board to which it was pinned. The skin over the chest was removed and the thorax opened along the right side of the sternum. The frontal part of the right thoracic wall was removed. The mediastinum behind the sternum was severed, and a cut was made just above the frontal insertion of the diaphragm. Care was taken not to damage the phrenic nerve which is sometimes attached to the ribs. The frontal part of the left thoracic wall was then removed and the phrenic nerve exposed. Both lobes of the left lung were removed, and the left phrenic nerve carefully freed from fat and other tissues: the utmost care was taken not to injure it. The left abdominal muscles were cut along the costal margin. Holding the last rib with a pair of forceps, a segment of diaphragm was then dissected out. Two converging cuts were made through the ribs towards the tendinous part of the diaphragm, and parallel to the direction of its muscle fibres. The cuts were made about 5 mm. to the right and 5 mm. to the left of the point where the phrenic nerve entered the diaphragm. /

diaphragm. The strip of diaphragm was dissected out beyond its tendinous part with about 5 cm. of phrenic nerve attached to it. The final preparation was fan-shaped, being 2 mm. wide at the tendinous end and about 20 mm. wide at the costal margin. The costal margin of the diaphragm segment was fixed to a J-shaped glass rod by means of platinum wires, and a thread was tied around the tendinous end. The preparation was then set up in a 100 ml. organ bath (Fig.35,p.154) containing double glucose Tyrode's solution. The J-piece held the costal margin of the segment in position at the bottom of the organ bath, while the thread tied at the tendinous end was fixed to a light isotonic heart lever writing upon a smoked drum surface. Tyrode's solution,containing double the usual amount of glucose, was supplied to the bath from a reservoir via the heating coils. The temperature of the bath was maintained thermostatically at 29$\pm$ 0.5°C. A sintered glass distribution tube was fixed at the bottom of the bath to provide a vigorous supply of oxygen with which the bath fluid was aerated in a form of fine bubbles. A thread was now tied to the cut end of the phrenic nerve and, by means of a fine needle, about one cm. of the phrenic nerve was drawn into a fluid electrode[5]. The fluid electrode

containing /

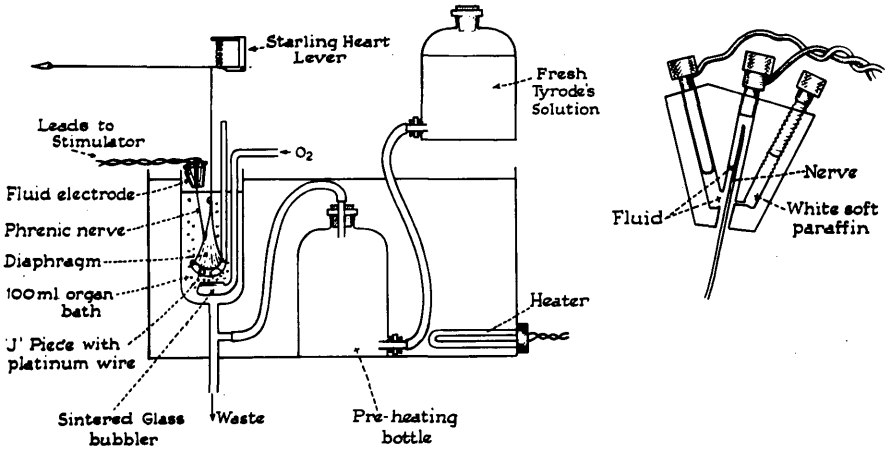

Figure 35. Diagram of the apparatus used for recording contractions of the kitten or rat diaphragm produced by electrical stimulation of the phrenic nerve.

containing the nerve was filled with double glucose Tyrode's solution and the hole at the bottom of the electrode was sealed off with soft paraffin. By this arrangement, the fluid surrounding the nerve at the point of stimulation was not in electrical contact with the fluid surrounding the muscle in the bath. The nerve was left sufficiently slack to allow for any movement caused by the contraction of the diaphragm. Single square wave impulses were applied to the nerve by means of a Dobbie McInnes' square wave generator at a frequency of 6 to 10 per minute, at 8 to 12 volts, the pulse width being 0.5 to 2.0 msecs. In any one experiment frequency, voltage and width were kept constant.

Drugs (in solution in double glucose Tyrode's solution) were added to the bath by means of a 1 ml. tuberculin syringe. The drug was allowed to act for three minutes after which the Tyrode's solution in the bath was changed. Between the addition of one dose of the drug and the addition of the next, there was an interval of about 15 minutes during which the Tyrode's solution was changed several times.

West[6] observed that the preparation gave a constant response /

response of good magnitude for a longer period, if the temperature of the bath was lowered from 37°C to 20°C. He also found that the rate of recovery of the muscle after the addition of a dose of tubocurarine was greatly accelerated by adding potassium chloride to the bath after the tubocurarine had been washed out. In the present series of experiments, it was observed that lowering the bath temperature from 37°C to 29°C without addition of potassium chloride gave a satisfactory recovery, and that the magnitude of the muscle contraction was maintained in a satisfactory manner.

Chou[7], using the phrenic nerve-diaphragm preparation for the assay of curare-like substances, allowed the solution of tubocurarine to act for 3 minutes. In the experiments described in this section, this feature of Chou's technique was adopted because it was thought that if the drug was allowed to act upon the tissue for long enough to produce its maximal effect, then so much time would be needed to wash the drug off the receptors that few comparisons could be made upon one preparation.

6. Experiments carried out on the Kitten Phrenic Nerve-Diaphragm Preparation.

Details of the relative potency of curare-like compounds /

compounds and tubocurarine on the phrenic nerve diaphragm preparation of different species of animals were published by Wien[8] who found that the triethyliodide of tri (diethylaminoethoxy) - 1 - 2 - 3 benzene was equal in potency to tubocurarine on the diaphragm of the rabbit, had one-fifth of the potency of tubocurarine on the diaphragm of the kitten, but possessed less than one-hundredth of the potency of tubocurarine on the diaphragm of the rat. The wide species variations of potency among neuromuscular blocking agents is one of the striking features of this group of compounds.[8] It was therefore decided to observe the effects of these compounds upon similar preparations obtained from different species of animal. With this object in view, it was decided to use the kitten phrenic nerve-diaphragm preparation as well as that taken from the rat.

METHOD.

The dissection and method of assembly of the preparation was similar to that described for the rat phrenic nerve diaphragm preparation (see p.152).

Kittens of either sex, weighing between 200 and 400 g., were used. The muscle was stimulated indirectly via the phrenic nerve by square wave impulses from a Dobbie Cinnes stimulator, /

stimulator (10 volts, 8 per minute, pulse width 1.0 to 3.0 msecs.).

It was found that this preparation was as satisfactory as the rat phrenic nerve diaphragm preparation, although Bülbring[4] observed that even with single shocks given at the slow rate of 5 per minute, the muscle twitches slowly declined in magnitude. It was found, however, in this investigation that muscle twitch height was uniform and the recovery after tubocurarine and other neuromuscular blocking agents was satisfactory for many hours, even when the rate of stimulation was as much as 10 per minute.

7. Experiments using the Isolated Frog Rectus Abdominis Muscle.

METHOD.

The preparation was set up as described in page 33 of this thesis. Acetylcholine and decamethonium were dissolved in frog Ringer's solution to give the concentration required and added to the bath by means of a tuberculin syringe fitted with a suitable record fitting needle.

In all experiments at least two uniform submaximal contractions to the same dose of either acetylcholine or decamethonium were obtained before the effects of any of

the drugs under investigation were studied. The time interval between each dose of acetylcholine was six minutes; the resulting contractions were recorded for ninety seconds. In the case of decamethonium, sufficient time (about thirty minutes) was allowed for the muscle to regain its original length after the contraction, and the contractions were recorded for periods of two to three minutes. In each case the drug being investigated was added one minute before the addition of acetylcholine or decamethonium. The muscle was washed with fresh frog Ringer's solution between each dose of acetylcholine or decamethonium. Before the next addition of the drug under test, sufficient time was allowed for the muscle to regain its original length. Several additions to the bath of acetylcholine or decamethonium were needed for complete recovery. The length of time taken depended upon the size of the dose of the drug added to the bath. The concentrations of stimulant drugs used to produce contractions of the muscle were from 0.10 to 0.20 µg. per ml. of acetylcholine, and from 1.5 to 2.5 µg. per ml. of decamethonium. The apparatus used for these experiments is shown in Figure 4, page 35.

8. Experiments on the Blood Pressure of the Anaesthetised Cat.

METHOD.

Cats of either sex, weighing between 2.0 and 3.5 kg., were used. Induction of anaesthesia, dissection and assembling of the preparation are described on pages 11 to 13 of this thesis. The blood pressure was recorded from the common carotid artery, and drugs were administered via the external jugular vein.

9. Experiments on the Nictitating Membrane of the Anaesthetised Cat.

METHOD.

Cats of either sex, weighing between 2.0 and 3.5 kg., were used. The cat was anaesthetised by means of an intraperitoneal injection of 60 mg. per kg. sodium pentobarbitone (see page 11.). The preparation was then set up in a manner similar to that described on page 36 of this thesis. The contraction of the nictitating membrane was elicited by stimulation of the cervical sympathetic by means of square impulses at a frequency of 800 to 1,200 per minute, 10 to 12 volts, pulse width 0.5 to 1.5 msec. In any one experiment frequency of stimulation, voltage and pulse width were constant. The nerve /

nerve was stimulated at 3 minute intervals for periods of 15 to 20 seconds at a time. Having obtained standard reproducible responses of the nictitating membrane by stimulating the nerve trunk, drugs were injected into the external jugular vein one minute before the next period of stimulation. The next injection of drug was not made until the contractions of the nictitating membrane had regained their original height.

10. Experiments on the Respiration of the Anaesthetised Cat.

METHOD.

In these experiments healthy cats of either sex, weighing from 1.5 to 2.5 kg., were used. Anaesthesia was induced by 60 mg. per kg. of sodium pentobarbitone injected intraperitoneally. The external jugular vein and the trachea were cannulated in a way similar to that described in pages 11 and 12. Respiratory movements were recorded by means of a thread sewn into the skin of the epigastrium. This was led over pulleys and attached to a recording lever having a frontal writing point. After obtaining a standard record of the respiratory movements for a period of about fifteen minutes, the drug solution was infused into the external jugular vein at a constant rate of 0.8 ml. /

ml. per minute from a Palmer's slow infusion apparatus. The drug was in solution in normal saline and in all cases the strength of solution was 0.2 mg. per ml. The infusion was stopped as soon as spontaneous respiration ceased, and the volume of the solution which had been injected noted. The cat was then placed under artificial respiration.

11. Experiments on Isolated Perfused Rabbit and Kitten Hearts.

METHOD.

The preparation was set up in a manner similar to that described on page 20 of this thesis. Drugs dissolved in Locke's solution were injected into the rubber tubing attached to the aortic cannula by means of a one ml. tuberculin syringe fitted with a "number 20" record fitting needle. The heart rate was counted by inspection, and the outflow was measured by collecting the perfusate for a period of one minute, at five minute intervals, after each injection of the drug. In some experiments, the outflow was measured by means of Gaddum's outflow recorder.

12. Experiments carried out Using the Isolated Rabbit Duodenum.

METHOD.

The /

The experimental procedure was similar to that described on page 32 of this thesis. The drug, in solution in Locke's solution, was added to the bath by means of a tuberculin syringe and the effect observed for ninety seconds. At the end of this period the fluid in the bath was replaced several times by running in fresh Locke's solution.

13. Experiments carried out Using the Isolated Guinea Pig Ileum.

METHOD.

The experimental procedure was similar to that described on page 29 of this thesis. The stimulant drug used was acetylcholine which was added at three minute intervals and left in contact with the tissue for thirty seconds. The drug under test was added to the bath by hand one minute before the next automatic inflow of acetylcholine. The contractions were allowed to return to a constant level before the next addition of the drug under investigation.

14. Experiments on the Perfused Rat Hindquarters.

METHOD.

The experimental procedure was similar to that described /

described on page 26 of this thesis. Drugs dissolved in Locke's solution were injected into the injection cannula by means of a tuberculin syringe fitted with a "number 20" record fitting hypodermic needle.

15. Experiments upon Mice.

Determination of the Approximate Median Lethal Dose.

METHOD.

In these experiments drugs were administered by intraperitoneal injection into groups of ten mice, each mouse weighing between 20 and 30 g. The weights of the individual members of each group were approximately equal to a variation of not more than ± 0.5 g. The dose at which five out of ten mice died within half-an-hour was taken as the median lethal dose (LD 50) for that particular group of mice, and was expressed in terms of mg. per kg. of the body weight.

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CHAPTER III

RESULTS.

1. Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

(A) Neuromuscular blocking activity. All of the compounds which were tested reduced the twitch height of the gastrocnemius muscle in response to indirect stimulation via the sciatic nerve. There was incomplete, or complete, neuromuscular block depending upon the dose administered. The block was completely reversible.

The compounds dipentasulphonium, dihexasulphonium, dihexaazonium, didecasulphonium and didecaazonium caused about 50 per cent reduction of the amplitude of the muscle twitch when doses from 0.15 to 0.20 mg. per kg. were injected, whereas 0.30 to 0.35 mg. per kg. was needed to cause complete neuromuscular block. The doses needed to produce these effects were comparable in magnitude with the doses of tubocurarine required to produce similar effects on twitch height.

The compounds dihexasulphonium trimethiodide, dioctasulphonium, dioctaazonium, dihexone and trisdecatetrazonium were less potent than tubocurarine, and in order to produce equivalent effects doses, which were from between two to eight /

eight times greater than those of tubocurarine, were needed. Trishexatetrazonium was about three times more potent than tubocurarine and about 0.10 mg. per kg. was needed to cause complete block.

The duration of neuromuscular block depended upon the magnitude of the dose injected as well as upon the number of doses which had been administered. The bigger the dose, the more prolonged was the duration of effect. Neuromuscular block was more prolonged following the second injection of the same dose than it was following the first dose. Block caused by the third and similar dose was of longer duration than that following the second dose.

When neuromuscular block following administration of any one of the compounds, dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexazonium, dioctasulphonium, dioctazonium and trishexatetrazonium, was just complete the duration of block was about 30 minutes. The maximum degree of neuromuscular block was usually reached within 1 to 2 minutes following the injection. In this respect all of the compounds referred to behaved in a similar way. The type of effect observed resembled that produced by an equipotent dose of tubocurarine. Dihexone showed /

showed similar properties but the duration of effect was a little longer. Figures 36, 37 and 38 (pages 169,170 and 171) illustrated the type of neuromuscular block caused by some of these compounds.

The duration of block caused by didecasulphonium, didecaazonium and trisdecatetrazonium was different from that produced by tubocurarine. If a dose was selected so as to produce an approximately 80 per cent block, about one and a half hours were needed for complete recovery. Following a subsequent and similar dose of the same drug (given after complete recovery), the duration of block was much more prolonged. These three compounds appeared to possess from two to four times the duration of effect of tubocurarine given in an equipotent dose. The onset of neuromuscular block caused by these compounds was gradual and the maximum degree of block was usually not reached until about 10 minutes after the administration of the drug (Fig. 38, b and c).

Potentiation of twitch height was only observed after injection of didecaazonium (Fig. 38,c). Potentiation of twitch height after didecaazonium was accompanied by generalised fasciculatory movements of the skeletal muscles.

Although /

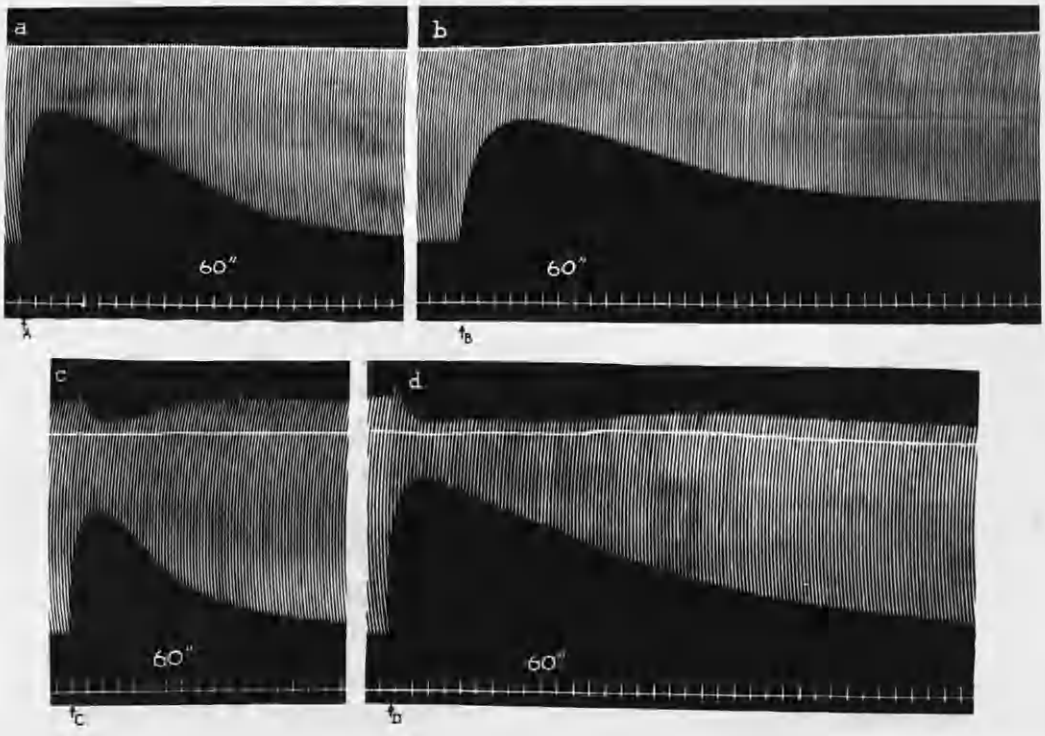

Figure 36. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, trishexatetrazonium 0.05 mg. per kg.

(b) At B, tubocurarine 0.15 mg. per kg.

(c) At C, dioctaazonium 0.30 mg. per kg.

(d) At D, dihexone 1.50 mg. per kg.

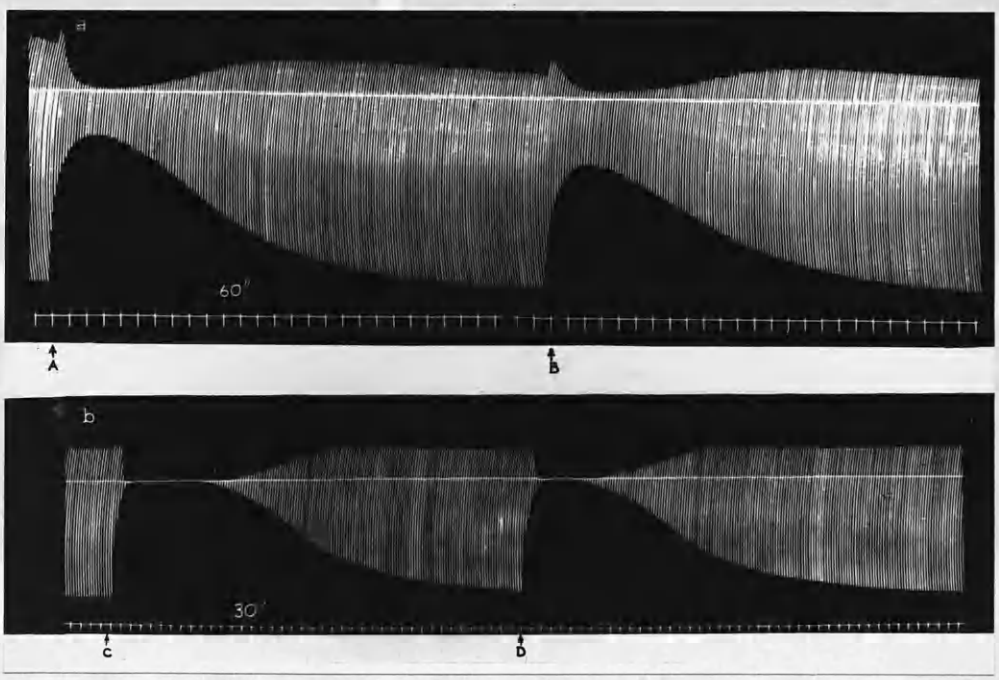

Figure 37. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, dihexasulphonium 0.15 mg. per kg.
At B, dipentasulphonium 0.15 mg. per kg.

(b) At C, dihexasulphonium 0.50 mg. per kg.
At D, dipentasulphonium 0.50 mg. per kg.

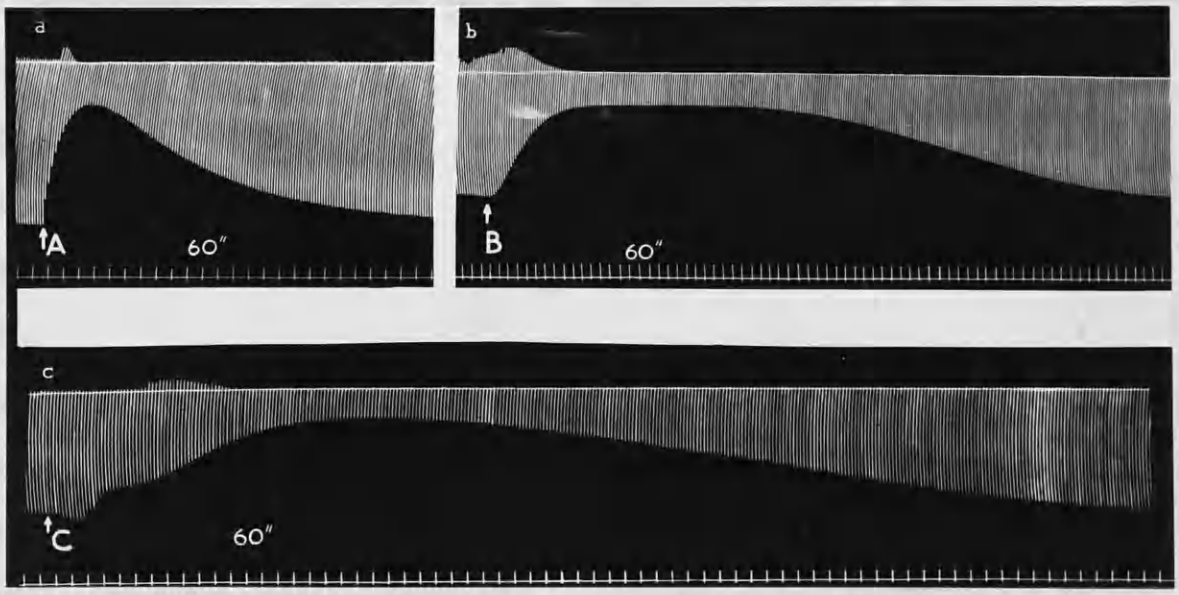

Figure 38. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, tubocurarine 0.25 mg. per kg.

(b) At B, didecasulphonium 0.30 mg. per kg.

(c) At C, didecaazonium 0.25 mg. per kg.

Although there was no increase in twitch height, a similar fasciculatory response was observed following injection of didecasulphonium and trisdecatetrazonium. As a rule, muscular fasciculations were intermittent in nature and appeared in a few groups of muscles at a time. When bigger doses were used, the muscular movements observed were very vigorous. Muscular fasciculation was never observed following administration of any of the other compounds. The gastrocnemius muscle responded to direct electrical stimulation during the period of interruption of nerve-impulse transmission. In each case the amplitude of the muscle contraction following direct stimulation during complete neuromuscular blockade was less than that obtained following direct stimulation before injection of the drug. This indicates some degree of direct depressant action upon the muscle which is also observed when tubocurarine is used. A typical example of this kind of effect is shown in Figure 39, page 173. The amplitude of the muscle contraction on direct stimulation after complete neuromuscular block was from 20 to 40 per cent of the control height following didecasulphonium and trisdecatetrazonium, while in the case of the other compounds the amplitude varied from 50 to 80 per cent of the value obtained before /

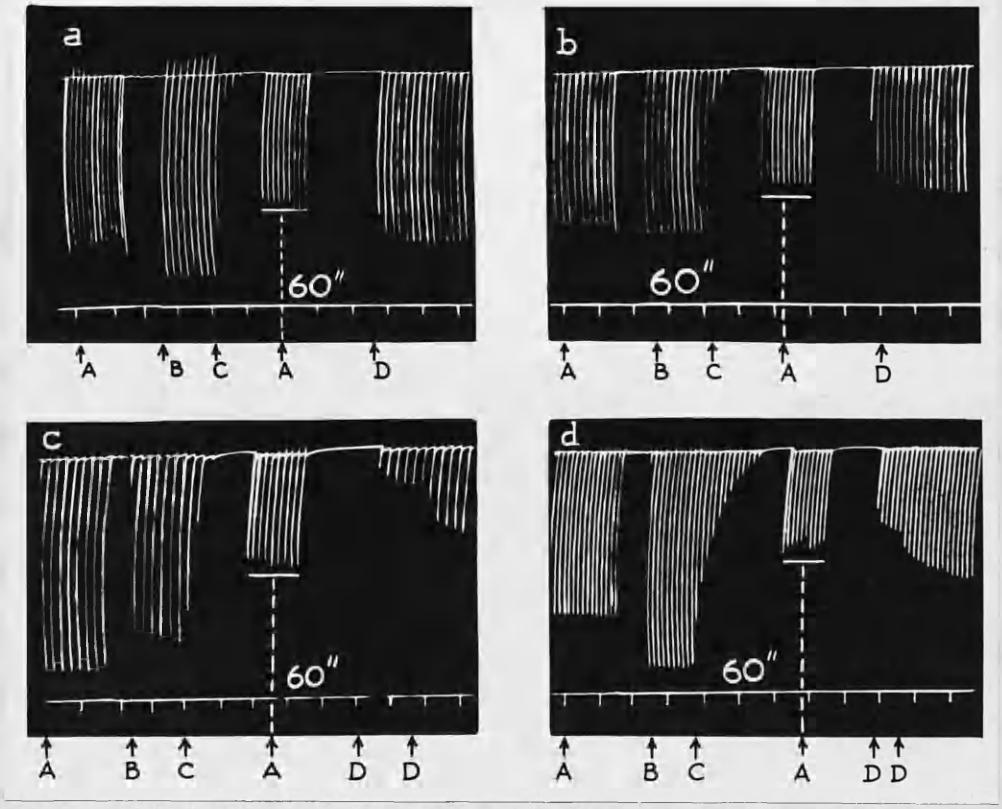

Figure 39. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Direct stimulation at 40 volts. Contraction downwards. Drugs administered intravenously.

	At A,	direct stimulation	
	At B,	indirect stimulation	
(a)	At C,	dipentasulphonium	0.50 mg. per kg.
	At D,	edrophonium	0.50 mg. per kg.
(b)	At C,	dihexasulphonium	0.50 mg. per kg.
	At D,	edrophonium	0.50 mg. per kg.
(c)	At C,	dihexaazonium	0.20 mg. per kg.
	At D,	edrophonium	0.20 mg. per kg.
(d)	At C,	trishexatetrazonium	0.06 mg. per kg.
	At D,	edrophonium	0.50 mg. per kg.

before injection of the drug.

Subsequent doses of the same drug given after complete recovery appeared to produce an increased effect. This effect was demonstrated in the cat gastrocnemius muscle-sciatic nerve preparation, and is shown in Figure 40, page 175 . A similar effect was seen when tubocurarine was used. (Fig.40,a.) In this figure, if part b is considered, it can be seen that the first dose of 0.30 mg. per kg. of dioctaazonium reduced the twitch height by about 15 per cent and the preparation required 8 minutes for recovery; the second and similar dose administered 10 minutes after the first dose resulted in a depression of about 40 per cent and needed 16 minutes for recovery. The effect of third injection of a similar dose of dioctaazonium caused the twitch height to be depressed by about 90 per cent and required about 30 minutes for complete recovery. The effect of the fourth dose can be seen not to differ significantly from that of the third dose. That further cumulation was not taking place suggested that the period of 30 minutes between injections allowed a sufficient amount of the drug to be eliminated to balance the effects of any dose subsequently given. All the compounds behaved in a similar fashion with the exception of didecasulphonium, didecaazonium /

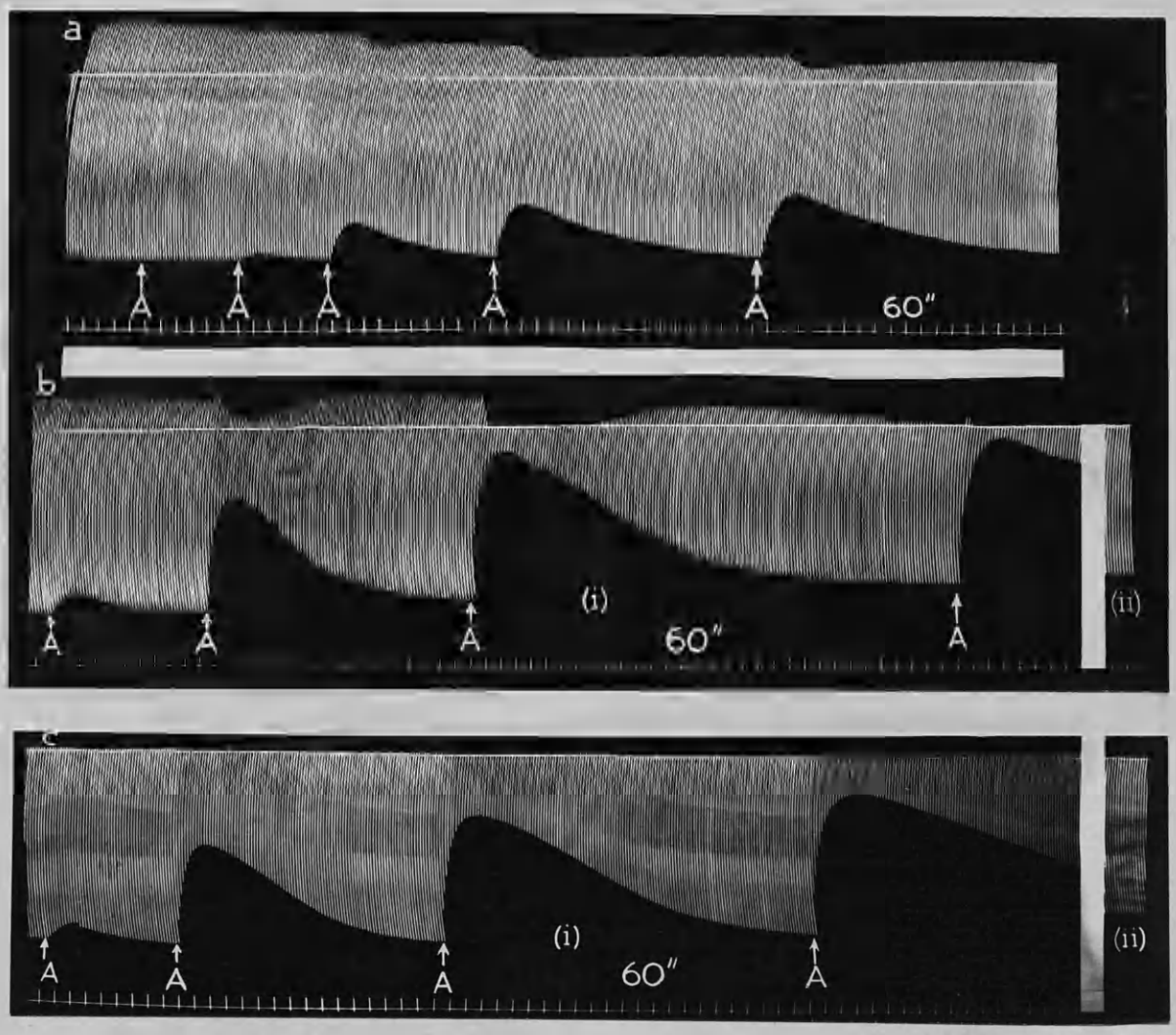

Figure 40. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, tubocurarine 0.075 mg. per kg.

(b) At A, dioctaazonium 0.30 mg. per kg.

There was an interval of 40 minutes between (i) and (ii).

(c) At A, dihexaazonium 0.20 mg. per kg.

There was an interval of 25 minutes between (i) and (ii).

didecaazonium and trisdecatetrazonium, which showed less tendency to produce cumulative effects.

(B) Effect of Indirect Tetanization of the Partially Blocked Muscle.

If the muscles partly blocked by tubocurarine are tetanized indirectly the tension rapidly wanes[1,2]. Previous tetanization decreases the intensity of the neuromuscular block produced by tubocurarine or by other drugs having a similar mechanism of action[1,2]. In contrast to this, tetanization does not affect the intensity of a neuromuscular block caused by decamethonium or other depolarizing agents, and the tetanus is well maintained in cat muscles in which partial neuromuscular block has been produced[1,3,4].

When the compounds under test were used, the effects observed were of two distinct types. Some behaved in a similar way to tubocurarine, while others had properties in common with decamethonium. The compounds dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dehexaazonium, dihexone, dioctaazonium, didecasulphonium and trishexatetrazonium were of the former group and the compounds, dioctasulphonium, didecaazonium and trisdecatetrazonium were of the latter. It was observed that the tetanic /

tetanic response of a normal muscle was well sustained, i.e. the tension did not wane. In Figures 41, 42 and 43, (pages 178, 179 and 180) the results of a typical experiment are shown. Figures 41 and 42 show the poorly sustained responses of the muscle, tetanized indirectly via the sciatic nerve, when partly blocked by dioctaazonium (Fig.41,a), didecasulphonium (Fig. 41,b), dihexaazonium (Fig. 41,c), dihexasulphonium (Fig. 41,e), trishexatetrazonium (Fig.42,b), dipentasulphonium (Fig.42,c) or by dihexone (Fig. 42,d). These effects are compared with those seen in the muscle partly blocked by tubocurarine (Fig. 42,a), decamethonium (Fig. 42,e), and of untreated muscle (Fig. 41,d). Figure 43 shows well sustained tension of the tetanized muscle partly blocked by dioctasulphonium (Fig. 43,a), trisdeca-tetrazonium (Fig. 43,b), didecaazonium (Fig. 43,c) and decamethonium (Fig. 43,e) which are compared with the well sustained tension of normal muscle (Fig. 43,d) during indirect tetanization. Indirect tetanization during neuromuscular block caused by didecaazonium, trisdecatetra-zonium and didecasulphonium, has no influence on the degree of block and is shown in parts b, c, and d of Figure 44 (page 181). In contrast to these observations, the muscle twitch amplitude during neuromuscular block following the use of dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium /

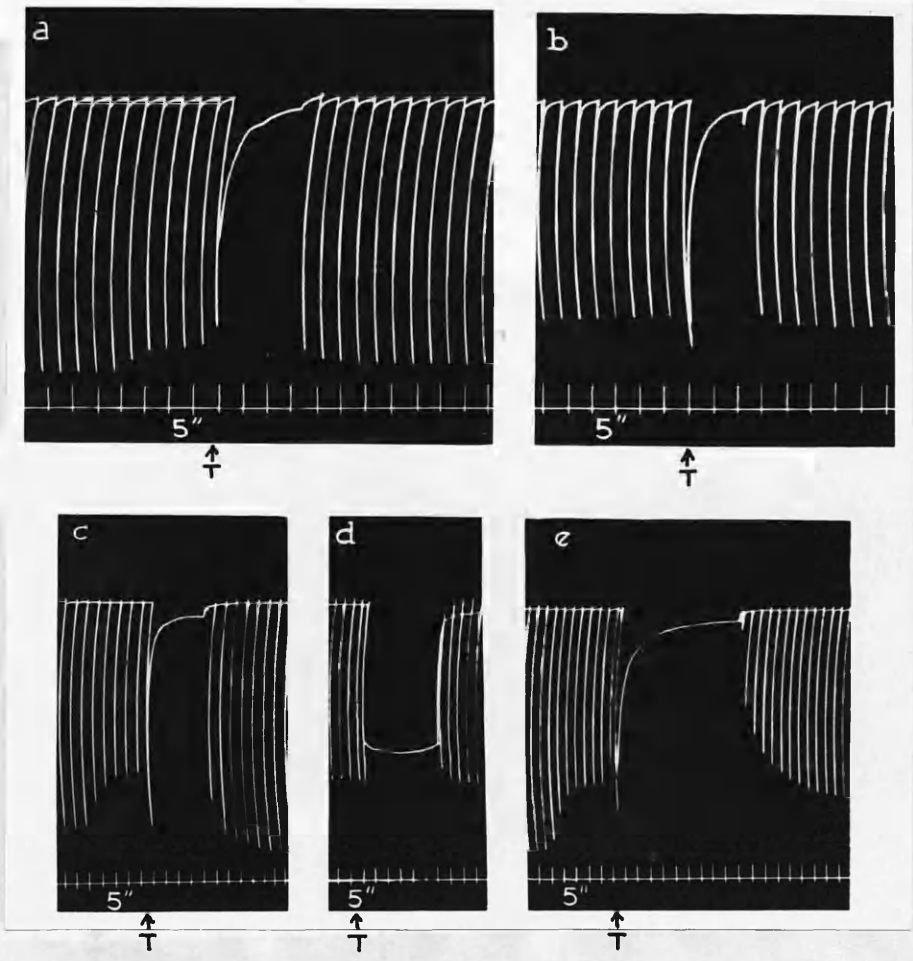

Figure 41. Cat gastrocnemius-sciatic preparation.

Pentobarbitone anaesthesia. Contraction downwards.

Drugs administered intravenously.

At T, indirect tetanization via sciatic nerve, during

(a) partial block following dioctaazonium 0.20 mg. per kg.

(b) " " " didecasulphonium 0.20 mg. per kg.

(c) " " " dihexaazonium 0.06 mg. per kg.

(e) " " " dihexasulphonium 0.10 mg. per kg.

(d) no drug administered.

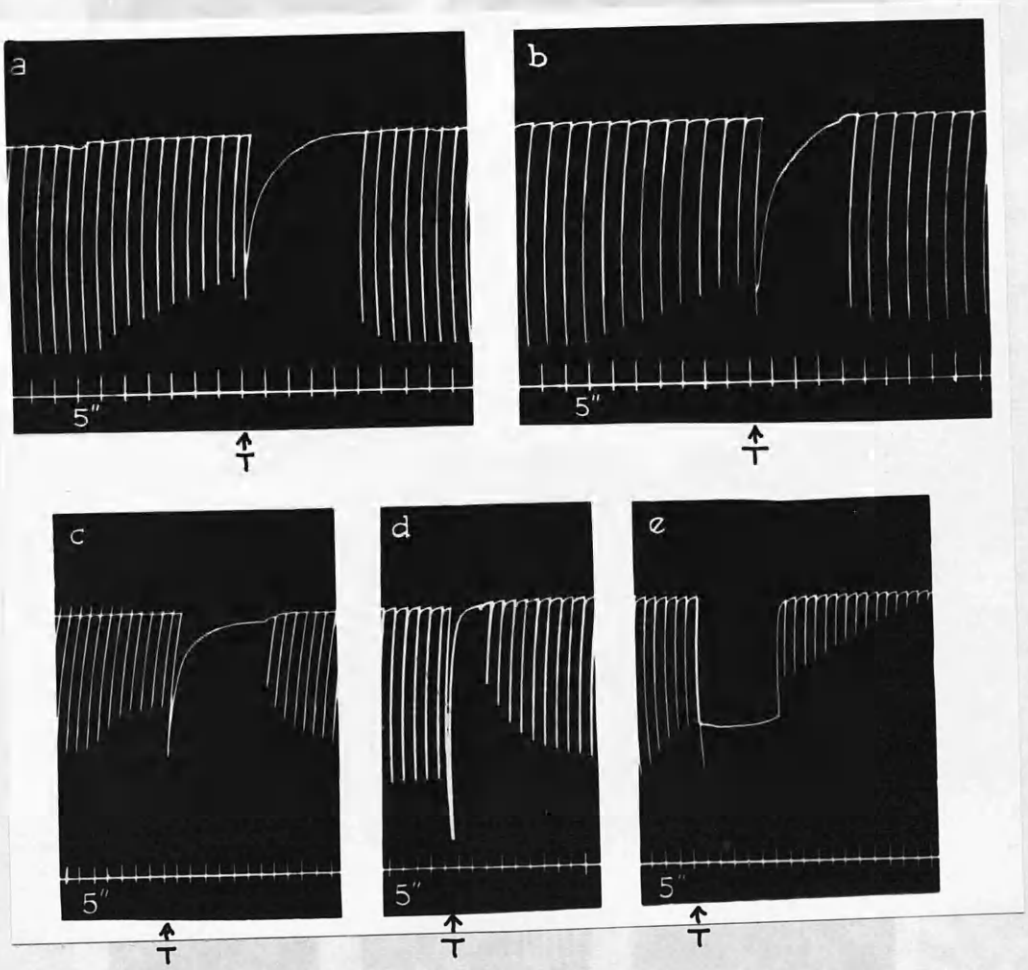

Figure 42. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously.

At T, indirect tetanization of the gastrocnemius (1500 impulses per minute) via the sciatic nerve during partial block by

(a) tubocurarine	0.05 mg. per kg.
(b) trishexatetrazonium	0.02 mg. per kg.
(c) dipentasulphonium	0.05 mg. per kg.
(d) dihexone	1.0 mg. per kg.
(e) decamethonium	0.02 mg. per kg.

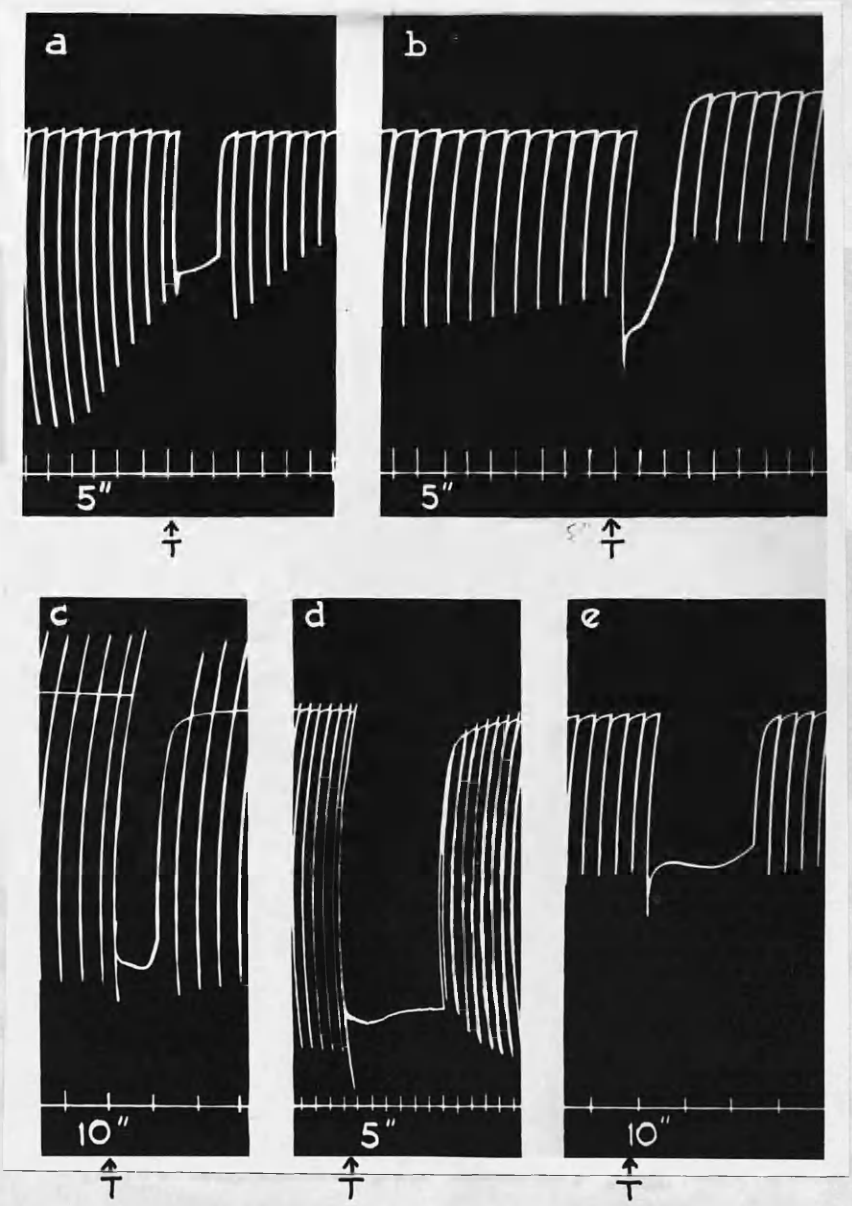

Figure 43. Cat gastrocnemius-sciatic preparation.
Pentobarbitone anaesthesia. Contractions downwards.
Drugs administered intravenously.
At T, indirect tetanization via sciatic nerve (1500 impulses per minute) during partial block by
(a) dioctasulphonium (b) trisdecatetrazonium
(c) didecaazonium (e) decamethonium
and untreated muscle (d).

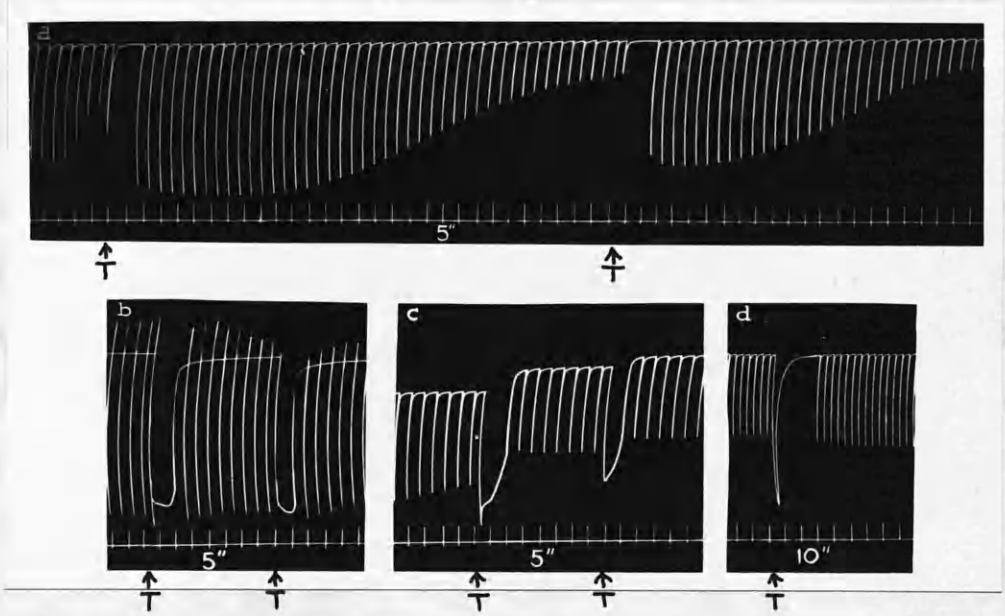

Figure 44. Cat gastrocnemius-sciatic preparation.

Pentobarbitone anaesthesia. Contraction downwards.

Drugs administered intravenously.

At T, indirect tetanization of the gastrocnemius (1500 impulses per minute) via the sciatic nerve during partial block by

(a) dioctaazonium 0.10 mg. per kg.

(b) didecaazonium 0.10 " " "

(c) trisdecatetrazonium 0.10 " " "

(d) didecasulphonium 0.20 " " "

dihexasulphonium, dihexaazonium, dihexone, dioctasulphonium, dioctaazonium and trishexatetrazonium is temporarily increased (decurarization) by indirect tetanization. This is shown in part 'a' of Figure 44 (page 181), which illustrates a typical example of this type of effect. Although the tension of the tetanized muscle is fairly well maintained during block by dioctasulphonium, indirect tetanization produces some degree of temporary decurarization (Fig. 43,(a), p.180).

(C) The Effect of tubocurarine. The effect of the different competitive neuromuscular blocking agents is additive[5] and that of depolarizing agents antagonistic to the block produced by them in the cat muscle-nerve preparation[6,7]. In attempting to elucidate the mechanism of action of new compounds with neuromuscular blocking activity, these facts are very important and it was therefore decided to investigate whether the effects of the drugs being studied were additive with or antagonistic to tubocurarine. If these drugs have the same mechanism of action as tubocurarine, then the neuromuscular blocking effects of any one of them would be expected to summate with that of tubocurarine. To investigate this point, a dose of one of the new compounds and a dose of tubocurarine were selected /

selected to produce, when given alone, a measurable but not intense neuromuscular depression of comparable degree. When the dose of any one of the compounds dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexazonium, dihexone, dioctasulphonium, dioctazonium and trishexatetrazonium was followed by a selected dose of tubocurarine given at the point of maximal effect of the former agents, the extent of neuromuscular block was further increased. The same end result occurred when the order of administration of the two drugs was reversed. Figure 45, (page 184), shows typical effects observed in this particular type of experiment. In this figure, the dose of dihexasulphonium (0.06 mg. per kg.) and the dose of tubocurarine (0.06 mg. per kg.) were selected to produce a neuromuscular block of about 30 per cent (parts I and II of Fig. 45). When the dose of dihexasulphonium was followed by a dose of tubocurarine at the point of maximal depression, the degree of neuromuscular block was increased to about 60 per cent (part III of Fig. 45). Approximately the same end result occurred when the order of administration of dihexasulphonium and tubocurarine was reversed (part IV of Fig. 45). The effect of two consecutive injections of either dihexasulphonium or tubocurarine was in /

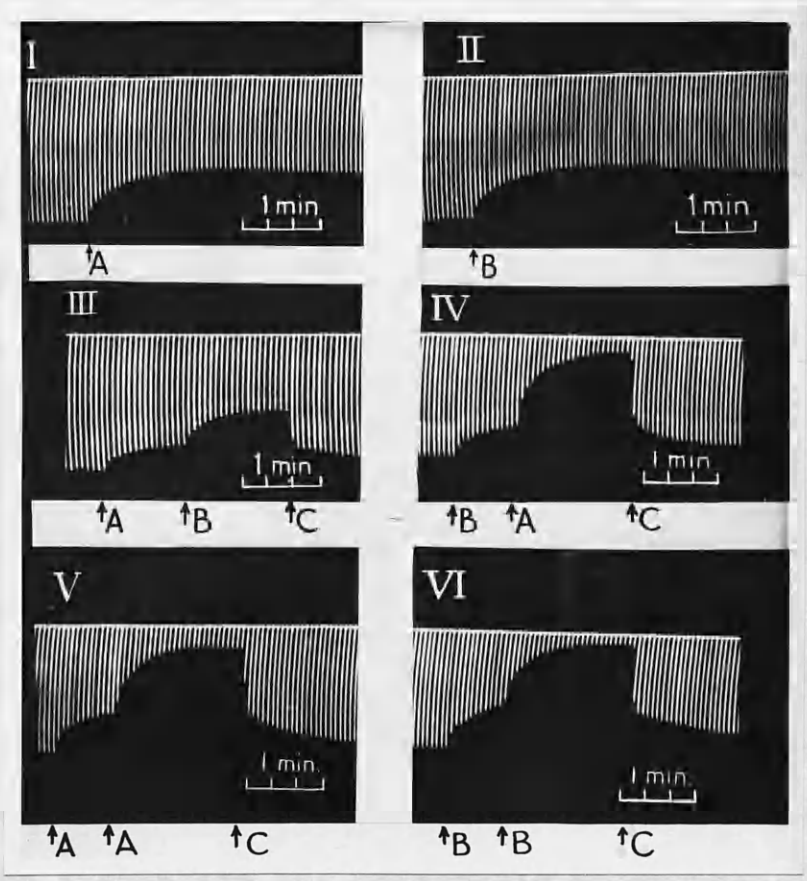

Figure 45. Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

At A, dihexasulphonium 0.06 mg. per kg.

At B, tubocurarine 0.06 mg. per kg.

At C, edrophonium 0.50 mg. per kg.

in no way different from the mixed responses (parts V and VI of Fig. 45). This effect was typical of those observed with dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexazonium, dihexone, dioctasulphonium, dioctazonium and trishexatetrazonium, all of which had additive effects with tubocurarine as well as with one another. The effects of didecasulphonium, didecazonium and trisdecatetrazonium, when given in sequence with tubocurarine were different. This is illustrated in Figures 46 and 47, pages 186 and 187. In part b of Figure 46, a dose of didecasulphonium (0.50 mg. per kg.) and a dose of tubocurarine (0.25 mg. per kg.) were selected to produce incomplete but measurable neuromuscular blocking effects. When the dose of didecasulphonium had produced its maximum depressant effect, it was followed by the dose of tubocurarine and the extent of the neuromuscular block was further increased. On the other hand when the dose of tubocurarine was followed by the dose of didecasulphonium, at the point of maximal depression caused by tubocurarine the twitch height was increased instead of being further depressed (Fig. 47, part a), an effect which would not be expected if both drugs possessed the same mechanism of action. Didecazonium and trisdecatetrazonium behaved in

a /

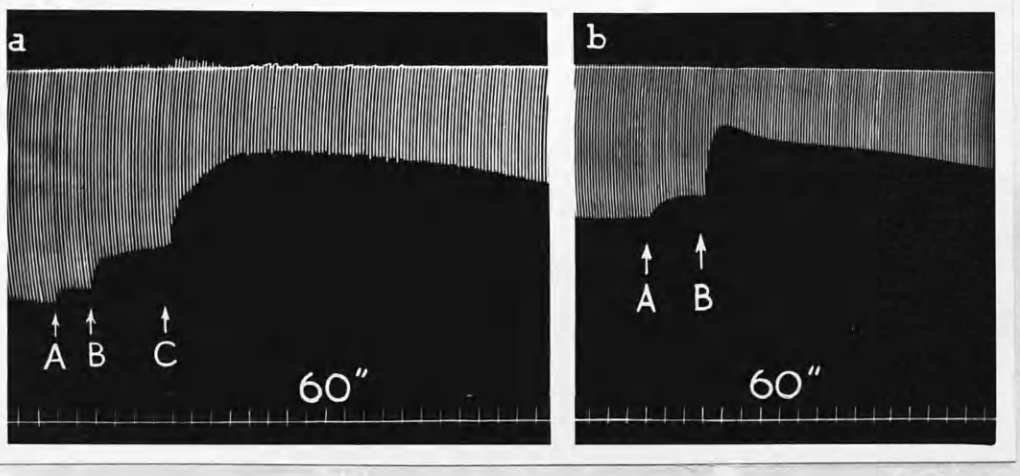

Figure 46. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation *via* sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, *tris*decatetrazonium 0.50 mg. per kg.
at B, *tris*decatetrazonium 0.75 mg. per kg.
At C, tubocurarine 0.20 mg. per kg.

(b) At A, didecasulphonium 0.50 mg. per kg.
At B, tubocurarine 0.25 mg. per kg.

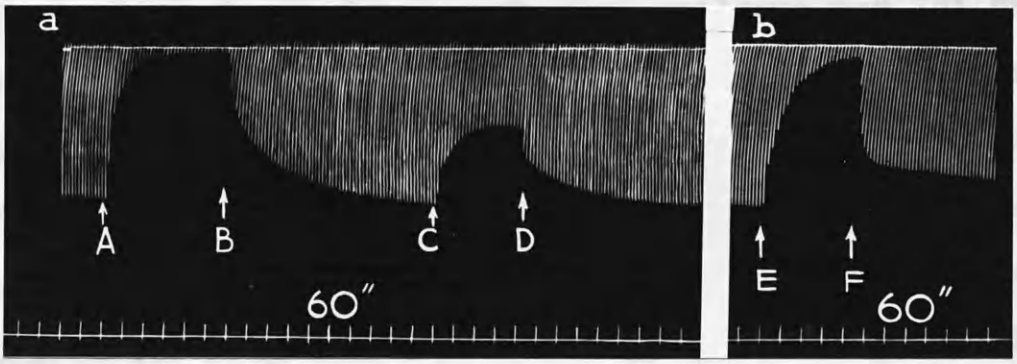

Figure 47. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	tubocurarine	0.15 mg. per kg.
	At B,	didecaazonium	0.30 mg. per kg.
	At C,	tubocurarine	0.10 mg. per kg.
	At D,	didecasulphonium	0.30 mg. per kg.
(b)	At E,	tubocurarine	0.12 mg. per kg.
	At F,	trisdecatetrazonium	0.70 mg. per kg.

a similar way to didecasulphonium (Figs. 46 and 47). When the same experiment was done using didecasulphonium, didecaazonium or trisdecatetrazonium with decamethonium, the effect observed was an additive one irrespective of whether the dose of decamethonium employed was followed, or preceded by the dose of didecasulphonium, didecaazonium or trisdecatetrazonium (Figs. 60 and 62, pages 213 and 217 These drugs had mutually additive effects. This feature of the actions of didecasulphonium, didecaazonium and trisdecatetrazonium is shared with decamethonium rather than with tubocurarine.

(D) Effect of ether anaesthesia. Ether anaesthesia has been reported to increase the effects of competitive neuromuscular blocking agents[3,8], whereas the neuromuscular block caused by depolarizing agents is either unaffected or antagonised by this agent[9]. This point was investigated with respect to each member of the group of compounds listed in pages 141 and 142 . A dose was selected which produced a measurable neuromuscular block (about 20 to 50 per cent). The same dose was repeated in the presence of ether anaesthesia. Immediately after injection of the drug, ether vapour was administered for a period of ten minutes to the pentobarbitone-anaesthetised cat from a bottle /

bottle connected to the artificial respiration pump. The degree of inhibition of the muscle twitch by the same dose of the drug injected before and during ether anaesthesia was compared. The effects of all the compounds, except didecasulphonium, didecasazonium and trisdecatetrazonium, were potentiated and prolonged by ether anaesthesia. The effect of didecasulphonium was slightly potentiated by ether anaesthesia. The effects of ether anaesthesia upon the effects of didecasazonium and trisdecatetrazonium could not be investigated due to the very prolonged effect of these compounds and incomplete recovery. Some typical tracings obtained from these experiments are shown in Figures 48 and 49, pages 190 and 191.

(E) Studies on drug antagonism. The neuromuscular block produced by tubocurarine or other substances having the same mechanism of action is antagonised by depolarising drugs[7,9,10], anticholinesterases[11], edrophonium[12,13], adrenaline[14] and potassium ions[15]. Anticholinesterases increase the neuromuscular blocking activity of depolarising agents[16,17]. Edrophonium has either no effect on or potentiates a depolarization block[10,18]. Neostigmine, eserine, edrophonium, decamethonium, adrenaline and potassium chloride were used for the studies on drug antagonisms /

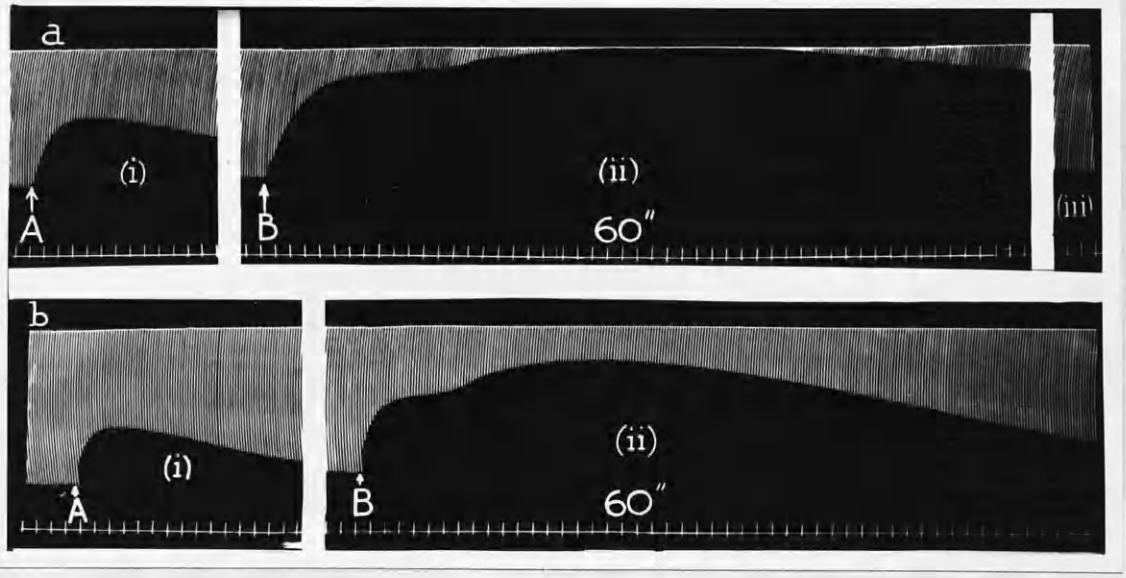

Figure 48. Cat gastrocnemius-sciatic preparation.

Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards.

Drugs administered intravenously.

(a) At A, trishexatetrazonium 0.03 mg. per kg.

At B, trishexatetrazonium 0.03 mg. per kg. with ether anaesthesia.

26 minutes elapsed between (i) and (ii) and 90 minutes between (ii) and (iii).

(b) At A, dihexaazonium 0.06 mg. per kg.

At B, dihexaazonium 0.06 mg. per kg. with ether anaesthesia.

10 minutes elapsed between (i) and (ii).

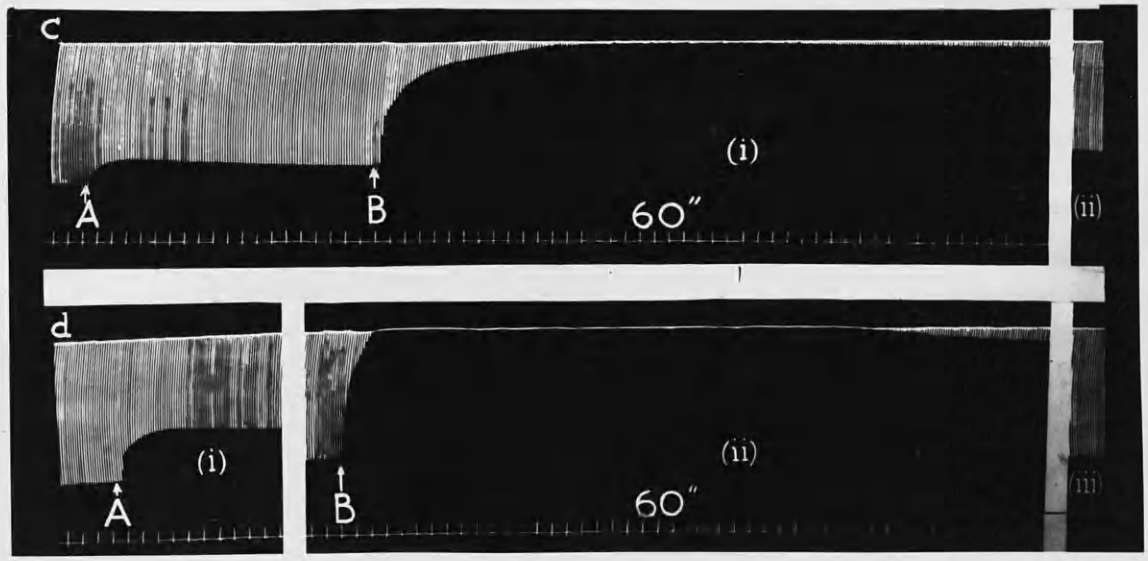

Figure 49. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(c) At A, dioctaazonium 0.07 mg. per kg.

At B, dioctaazonium 0.07 mg. per kg. with ether anaesthesia.

130 minutes elapsed between (i) and (ii).

(d) At A, dioctasulphonium 0.20 mg. per kg.

At B, dioctasulphonium 0.20 mg. per kg. with ether anaesthesia.

30 minutes elapsed between (i) and (ii) and 80 minutes between (ii) and (iii).

antagonisms which are described in this section. The antagonists were injected into the cat at the point of maximal neuromuscular block or at the point where recovery was beginning. The extent of the recovery caused by the antagonist was compared with that of the control. The effects of the antagonists are described separately.

(i) Edrophonium:

Partial or complete neuromuscular block produced by dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexaazonium, dioctaazonium and tris-hexatetrazonium was rapidly and completely reversed by the intravenous injection of edrophonium in a dose range of 0.20 to 1.0 mg. per kg. (Figs. 50 and 51, pages 193 and 194). After a suitable dose of edrophonium, the muscle twitch rapidly gained its original height (sometimes within less than one minute). This effect was similar to that observed when tubocurarine was used (Fig.51,b). The effect of edrophonium appeared to be short-lived because, if after complete recovery of the twitch height following edrophonium a second and similar dose of the drug was given inside a period of about ten minutes, the neuromuscular block was of approximately similar intensity to that obtained before edrophonium was administered. This effect /

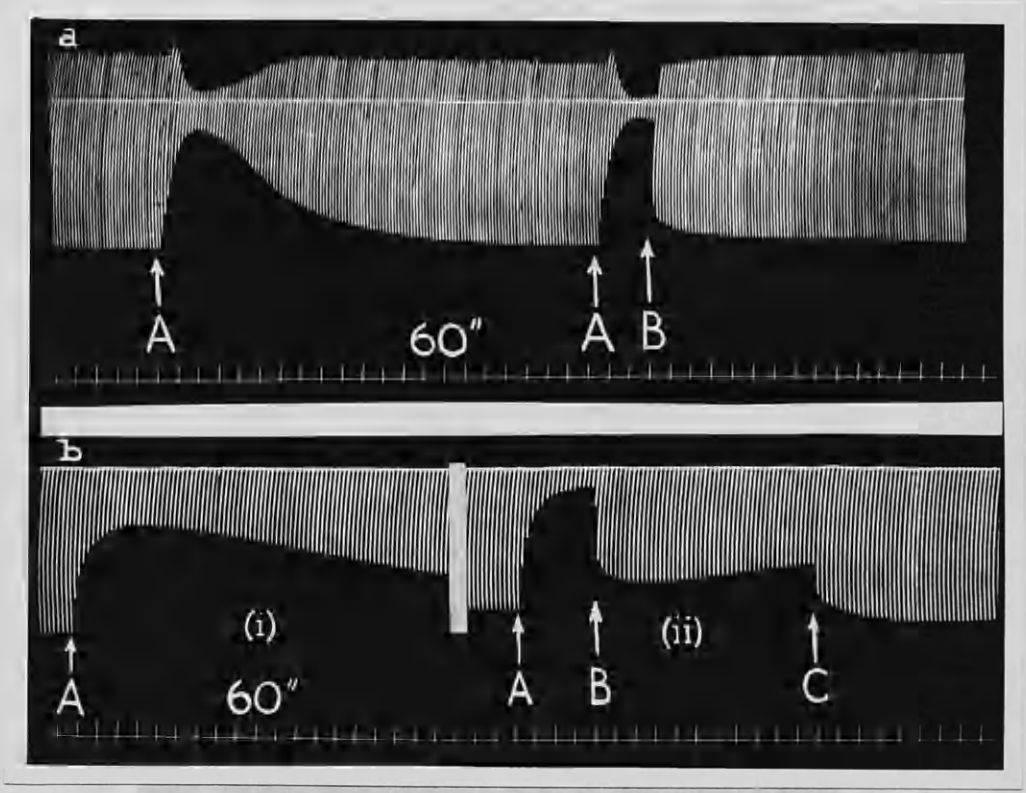

Figure 50. Cat gastrocnemius-sciatic preparation.

Pentobarbitone anaesthesia. Indirect stimulation *via* sciatic nerve. Contraction downwards.

Drugs administered intravenously.

(a)	At A,	dipentasulphonium	0.50 mg. per kg.
	At B,	edrophonium	0.30 mg. per kg.
(b)	At A,	dioctaazonium	0.20 mg. per kg.
	At B,	edrophonium	0.50 mg. per kg.
	At C,	edrophonium	1.0 mg. per kg.

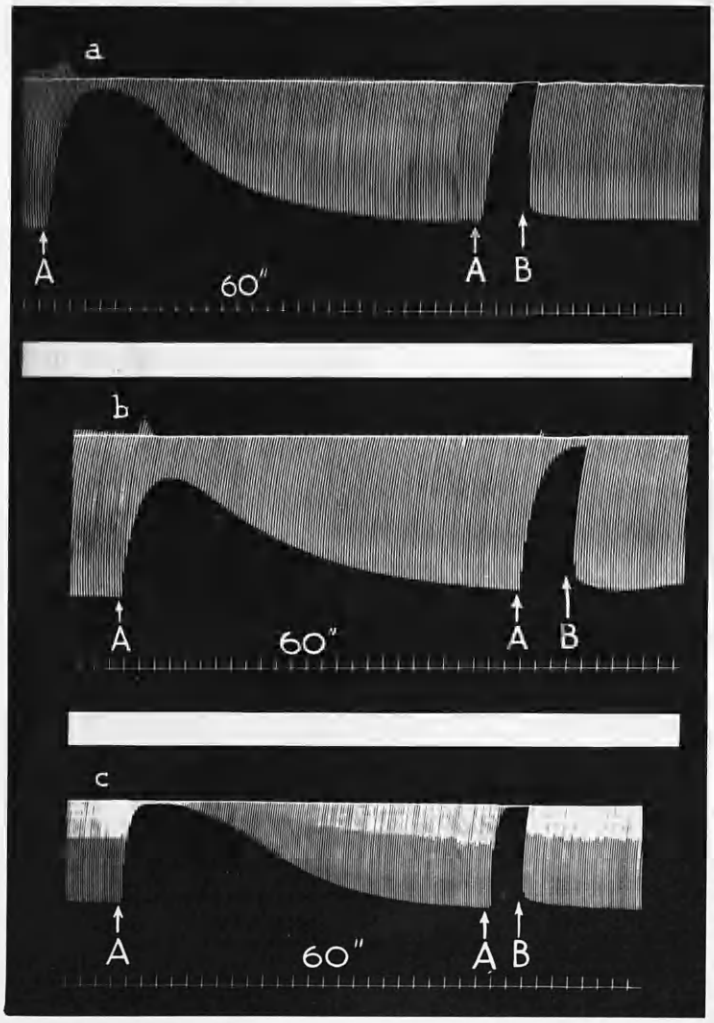

Figure 51. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A, trishexatetrazonium	0.07 mg. per kg.
	At B, edrophonium	0.70 mg. per kg.
(b)	At A, tubocurarine	0.10 mg. per kg.
	At B, edrophonium	0.50 mg. per kg.
(c)	At A, dihexaazonium	0.40 mg. per kg.
	At B, edrophonium	0.40 mg. per kg.

effect is also observed when tubocurarine is used to cause neuromuscular block. A typical experiment is shown in part 'a' of Figure 52 (page 196).

The effect of edrophonium on the neuromuscular block caused by dihexone and dioctasulphonium was not so striking as that seen when the other compounds were used. There was antagonism but bigger doses (1.0 to 2.0 mg. per kg.) were needed, and the recovery was gradual and incomplete. The antagonistic effect of edrophonium on the neuromuscular block caused by dioctasulphonium and dihexone has been shown in Figure 53 (page 197). Didecasulphonium, didecaazonium and trisdecatetrazonium on the other hand had entirely different effects. Neuromuscular block caused by didecasulphonium was either unaffected or slightly reduced by edrophonium in the dose range of 0.5 to 2.0 mg. per kg. The effect of didecaazonium and trisdecatetrazonium was actually potentiated when 0.5 to 2.0 mg. per kg. of edrophonium was injected at the point of maximal depression. These effects are illustrated in Figure 54 (page 198).

(ii) Neostigmine:

The antagonistic effect shown by neostigmine to the neuromuscular /

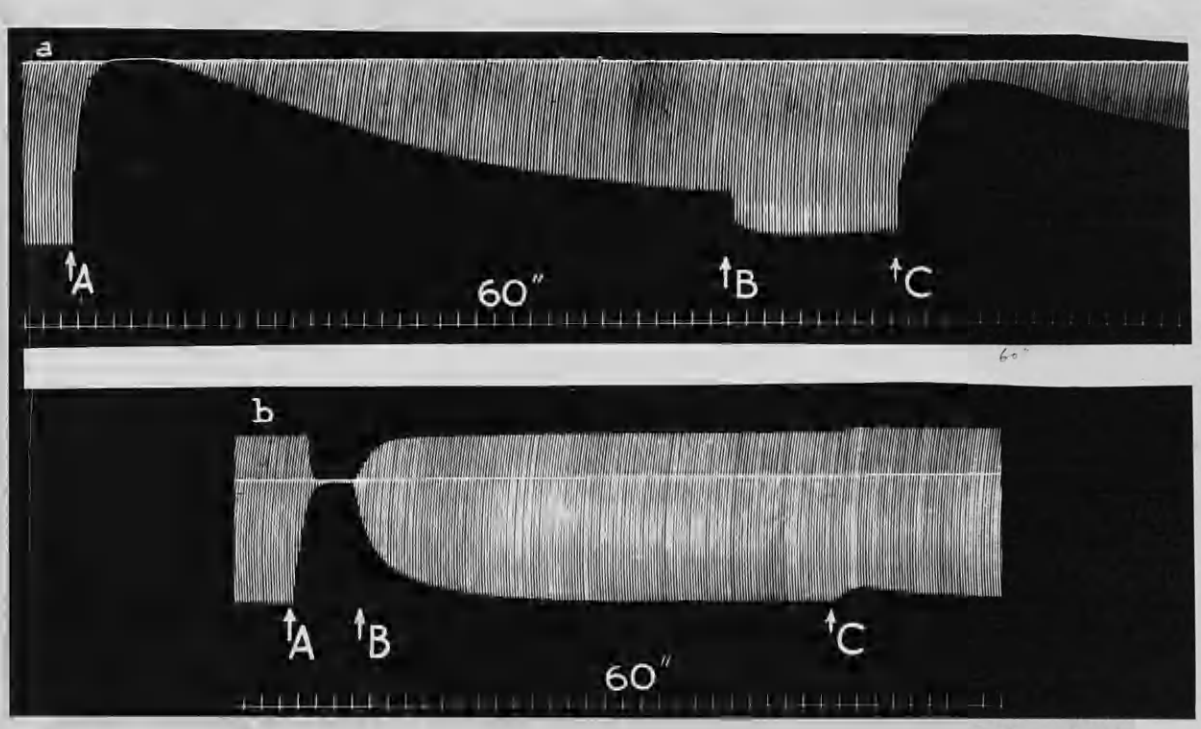

Figure 52. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A and at C, trishexatetrazonium 0.10 mg. per kg.
At B, edrophonium 0.5 mg. per kg.

(b) At A and at C, dihexasulphonium 0.50 mg. per kg.
At B, neostigmine .06 mg. per kg.

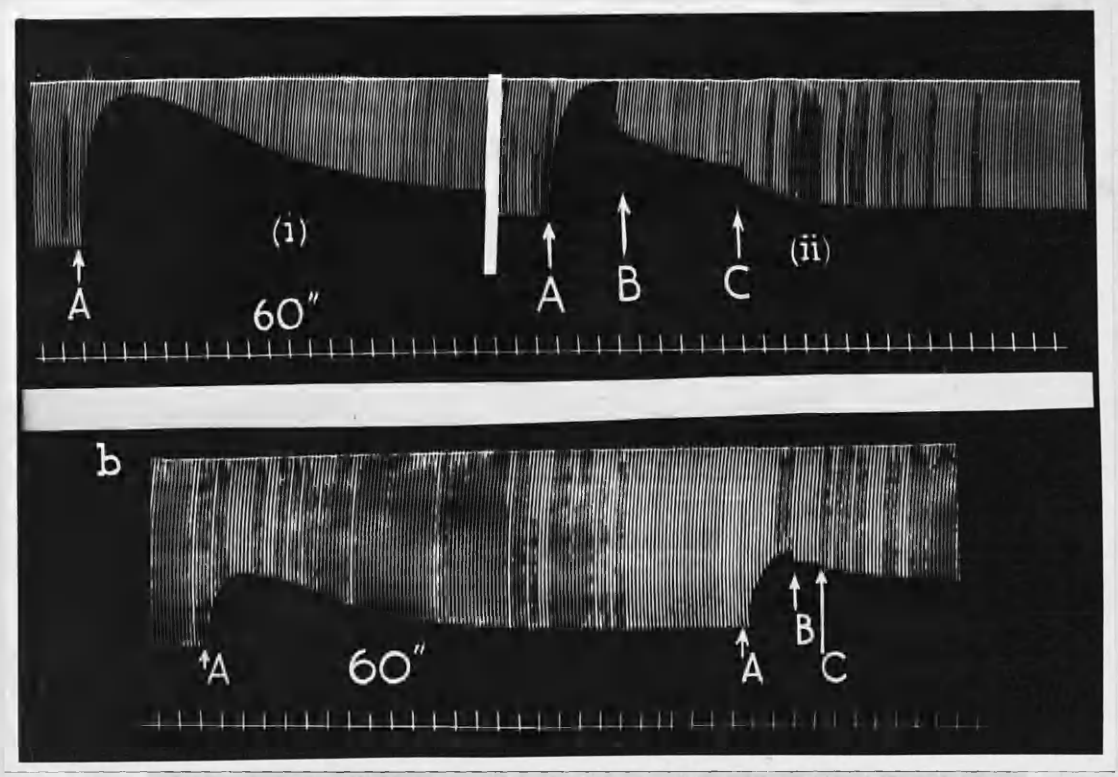

Figure 53. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	dioctasulphonium	0.30 mg. per kg.
	At B,	edrophonium	1.0 mg. per kg.
	At C,	edrophonium	0.75 mg. per kg.
(b)	At A,	dihexone	1.50 mg. per kg.
	At B,	edrophonium	0.50 mg. per kg.
	At C,	edrophonium	0.75 mg. per kg.

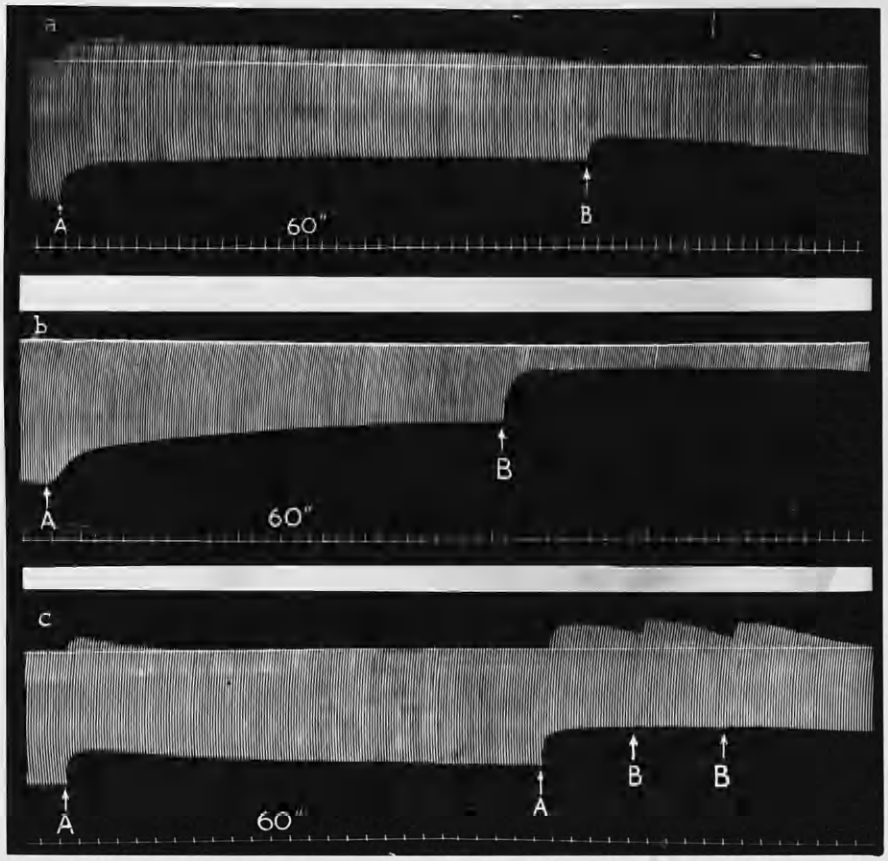

Figure 54. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	didecaazonium	0.20 mg. per kg.
	At B,	edrophonium	0.08 mg. per kg.
(b)	At A,	trisdecatetrazonium	0.20 mg. per kg.
	At B,	edrophonium	1.0 mg. per kg.
(c)	At A,	didecasulphonium	0.30 mg. per kg.
	At B,	edrophonium	0.50 mg. per kg.

neuromuscular block caused by different types of muscle relaxants is one of the major differentiating factors in the determination of their mechanism of action. Neostigmine antagonises the block caused by the competitive type of neuromuscular blocking agent[11,19,21], while it has a potentiating effect upon the effects of the depolarizing drugs[20]. The effect of this drug on the neuromuscular block caused by the compounds was studied by injecting neostigmine at the point of maximal neuromuscular depression and the recovery of the twitch height was compared with that of the control.

Partial or complete neuromuscular block caused by dihexasulphonium trimethiodide, dipentasulphonium, dihexasulphonium, dihexaazonium and trishexatetrazonium was completely antagonised by neostigmine although the process of antagonism was not so rapid as that seen following edrophonium. Neostigmine was injected in a dose range of 0.05 to 0.10 mg. per kg. and an effective antagonism to the characteristic effects of these four compounds was observed. The muscle twitch began to increase in amplitude within about 10 to 15 seconds after intravenous administration of neostigmine at the point of maximum depression of the twitch height. The twitch regained /

regained its original height in about 2 to 5 minutes depending upon the magnitude of the dose of neostigmine employed. The antagonistic effects of neostigmine to the neuromuscular block caused by trishexatetrazonium, dihexaazonium and dihexasulphonium are shown in parts b, c and d of Figure 55, page 201, in which the effect of neostigmine upon the block caused by tubocurarine is also shown (Fig. 55,a).

Didecasulphonium, didecaazonium and trisdecatetrazonium had different properties in this respect. 0.05 to 0.50 mg. per kg. neostigmine did not show any antagonism to the neuromuscular blocking effects of didecaazonium and trisdecatetrazonium whether the block was partial or complete. These two compounds share this property with decamethonium. Neostigmine at dose levels of 0.05 to 0.50 mg. per kg. showed either no effect or slight antagonism to didecasulphonium (Fig. 56,a, p.202).

Neostigmine in similar doses reversed the neuromuscular block caused by dihexone, dioctasulphonium and dioctazonium, but the reversal was not so complete as that observed in the cases of dipentasulphonium, dihexasulphonium, dihexaazonium and trishexatetrazonium. This is shown in Figure 56, /

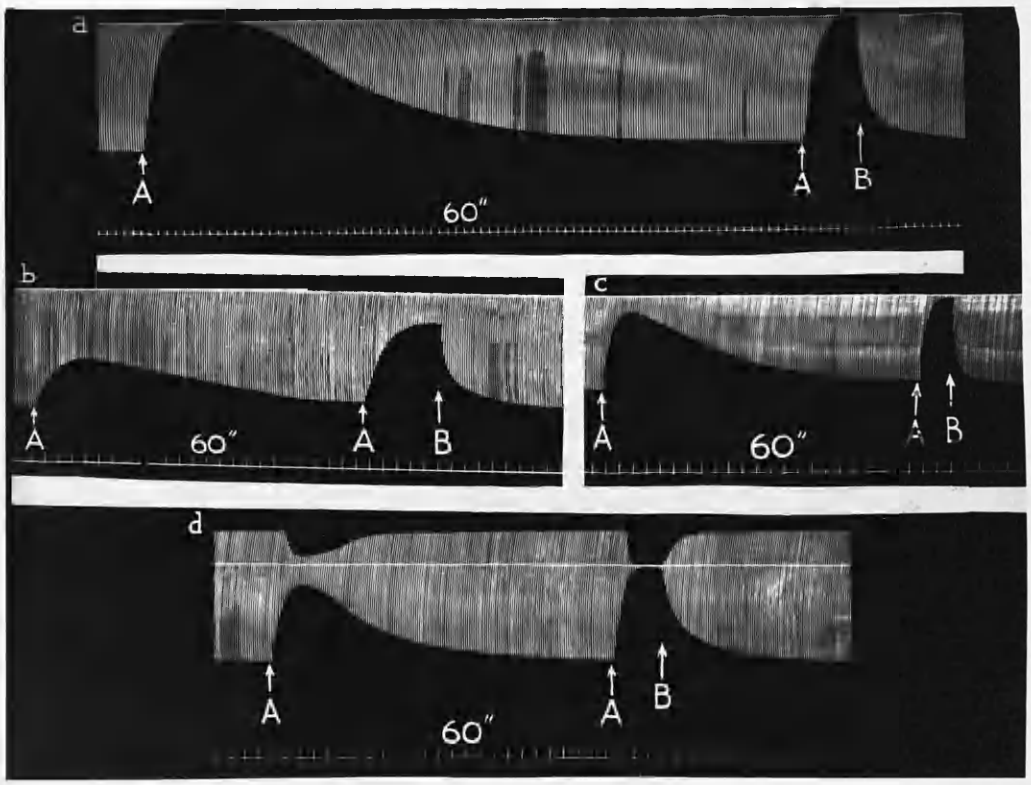

Figure 55. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	tubocurarine	0.20 mg. per kg.
	At B,	neostigmine	0.10 mg. per kg.
(b)	At A,	trishexatetrazonium	0.05 mg. per kg.
	At B,	neostigmine	0.10 mg. per kg.
(c)	At A,	dihexaazonium	0.20 mg. per kg.
	At B,	neostigmine	0.05 mg. per kg.
(d)	At A,	dihexasulphonium	0.50 mg. per kg.
	At B,	neostigmine	0.06 mg. per kg.

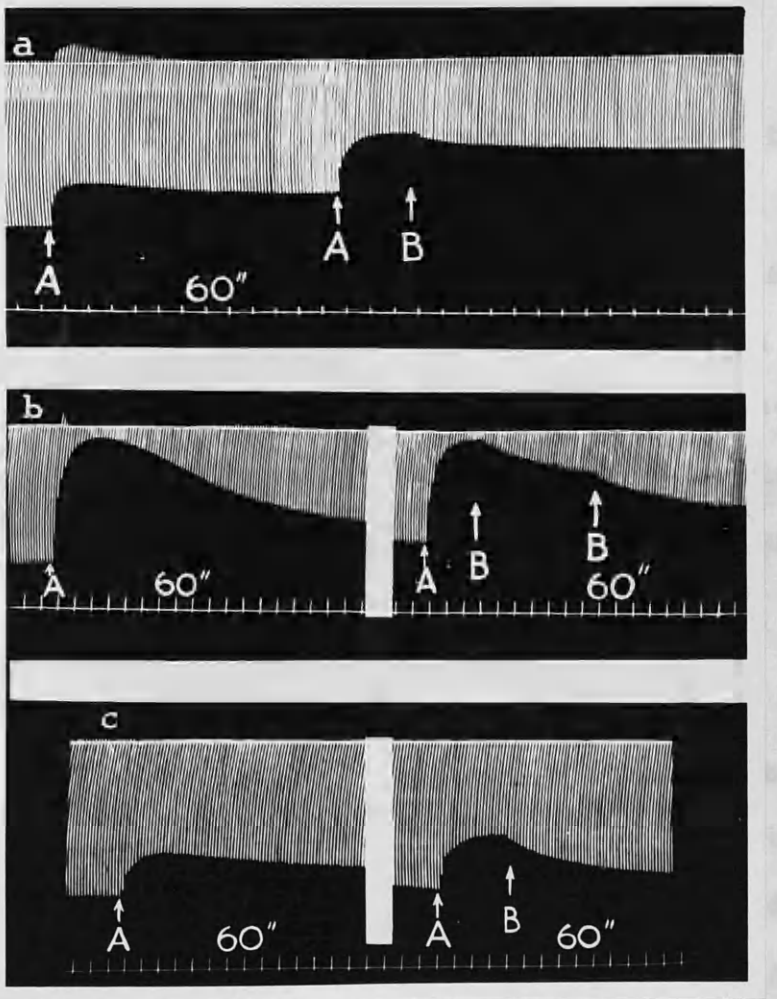

Figure 56. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	didecasulphonium	0.30 mg. per kg.
	At B,	neostigmine	0.075 mg. per kg.
(b)	At A,	dioctasulphonium	0.30 mg. per kg.
	At B,	neostigmine	0.10 mg. per kg.
(c)	At A,	dioctaazonium	0.15 mg. per kg.
	At B,	neostigmine	0.10 mg. per kg.

Figure 56, b and c. Even with a dose of neostigmine as large as 0.50 mg. per kg., the recovery of the muscle twitch amplitude depressed by dihexone, dioctasulphonium or dioctaazonium was only slight compared with 100 per cent recovery when 0.05 to 0.10 mg. per kg. neostigmine was injected at the point of maximal block caused by dihexasulphonium, dihexaazonium, trishexatetrazonium or tubocurarine (Fig. 55, p.201).

The antagonism shown by neostigmine is a fairly prolonged one and its effects persisted for about 30 minutes or more, as shown by the observation that a second dose of dipentasulphonium, dihexasulphonium, dihexaazonium or trishexatetrazonium given within a thirty minute interval produced very little effect (Fig. 52, part b, page 196).

(iii) Eserine:

The effect of eserine on the neuromuscular block caused by the compounds investigated was essentially similar to that observed with neostigmine but was less marked. Effective and prolonged antagonism to the neuromuscular block caused by dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexaazonium and /

and trishexatetrazonium was shown by eserine in the dose range of 0.50 to 1.0 mg. per kg. Figure 57 (p.205) shows a typical example of eserine antagonism to the effect of these drugs.

Eserine caused only a slight reversal of the neuromuscular block produced by dihexone, dioctasulphonium and dioctaazonium, even when the dose of eserine given was as great as 2.0 mg. per kg.

Neuromuscular block caused by didecasulphonium, didecaazonium and trisdecatetrazonium was not affected by eserine in the dose range of 1.0 to 2.0 mg. per kg.

(iv) Decamethonium:

Decamethonium is a typical depolarizing neuromuscular blocking agent. The effect of decamethonium is additive with those agents having a similar mechanism of action[6], while it antagonises the effects of tubocurarine or other competitive neuromuscular blocking agents[7].

Paton and Zaimis[22] observed that the injection of a small dose of tubocurarine rendered decamethonium less effective as a neuromuscular blocking agent in the cat. This observation has been confirmed by Macfarlane et alia[23] in /

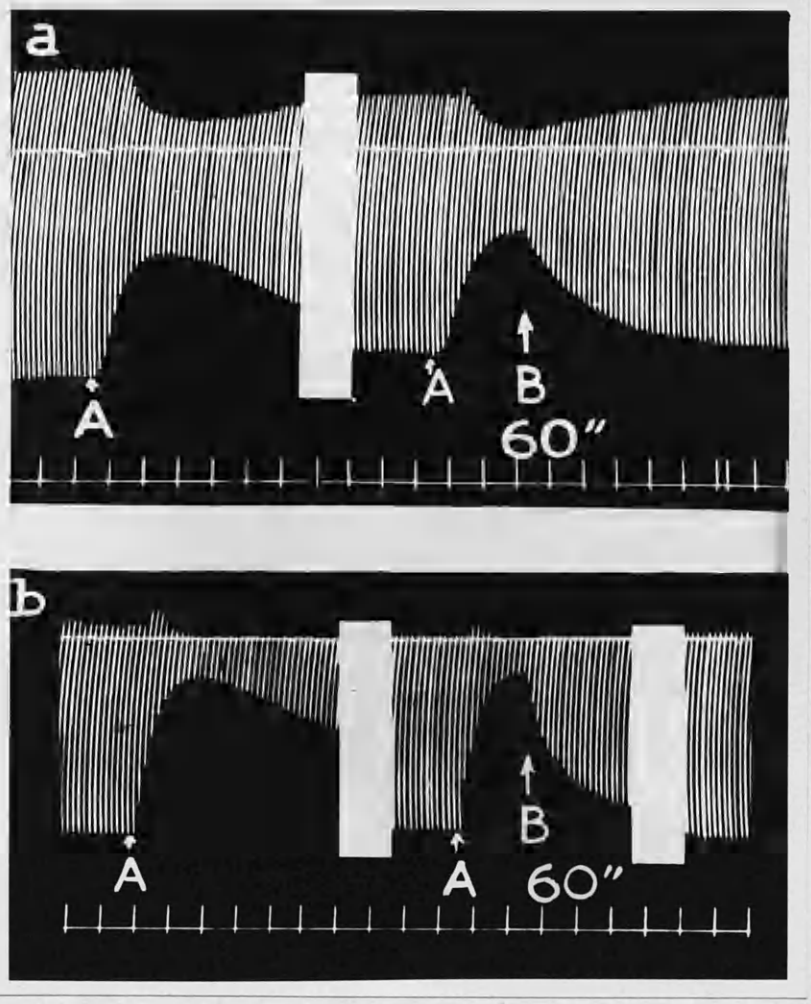

Figure 57. Cat gastrocnemius-sciatic preparation.

Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards.
Drugs administered intravenously.

(a)	At A,	dipentasulphonium	0.20 mg. per kg.
	At B,	eserine	0.50 mg. per kg.
(b)	At A,	dihexasulphonium	0.25 mg. per kg.
	At B,	eserine	0.75 mg. per kg.

in man. Paton and Zaimis[8] also found that when decamethonium was given before tubocurarine it did not greatly modify the neuromuscular blocking activity of the alkaloid. Depierre[24], however, observed a reciprocal antagonism between gallamine, a competitive neuromuscular blocking agent, and decamethonium. Hutter and Pascoe[6] showed that suitable doses of decamethonium were able to restore neuromuscular transmission after it had been blocked by tubocurarine. The reverse phenomenon, namely the antagonistic effect of tubocurarine to the neuromuscular block produced by decamethonium, was observed by Dallemagne and Philippot[25]. In all cases the observations were made using cats as the experimental animals.

Decamethonium antagonism to the effects of the new compounds which have been investigated was studied on the cat's nerve-muscle preparation, in an attempt to gain more information about their mechanism of action. The nature of the investigations and the results are described under the following different heads:

(a) Effects of decamethonium administered following a dose of one of the new compounds.

(b) Effects of the new compound administered following a dose of decamethonium.

(c) /

(c) Effects of decamethonium given at the point of maximal neuromuscular depression caused by the new compound.

(d) Effects of the new compound given at the point of maximal neuromuscular depression caused by decamethonium.

(a) The previous injection of a small dose of dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexaazonium, dihexone, dioctasulphonium, dioctsazonium or trishexatetrazonium modified the effects of later administration of decamethonium. A dose of 0.02 to 0.03 mg. per kg. decamethonium, when given alone, produced approximately 70 to 90 per cent neuromuscular block. A second and similar dose of decamethonium, given after complete recovery from the neuromuscular block caused by a small dose of any one of the new compounds, produced either no depression of the twitch height or only a slight depression. A similar effect was observed by Paton and Zaimis[22] and Hoppe[20] who showed that a dose of decamethonium, which when given alone produced neuromuscular block, was without effect in cat and dog nerve-muscle preparations which had just recovered from the effects of a small dose of tubocurarine. A typical experiment is shown in Figure 58, part 'a' (page 208). At A, the first dose of decamethonium /

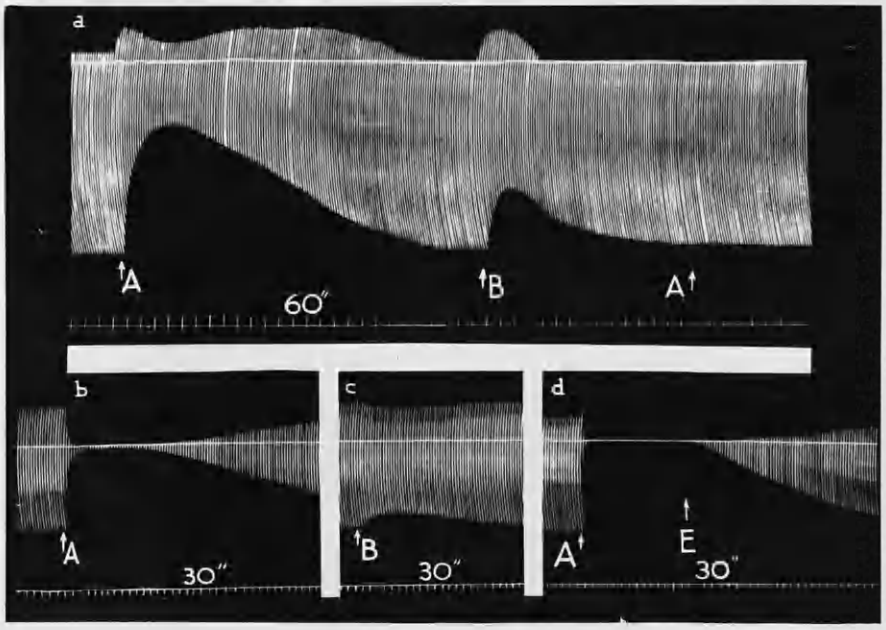

Figure 58. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via the sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	decamethonium	0.02 mg. per kg.
	At B,	trishexatetrazonium	0.04 mg. per kg.
(b)	At A,	dihexasulphonium	0.50 mg. per kg.
	At B,	decamethonium	0.06 mg. per kg.
	At E,	eserine	0.50 mg. per kg.

decamethonium, 0.02 mg. per kg., reduces the twitch height by about 70 per cent whereas the second and same dose of decamethonium at A, given after a dose of trishexatetrazonium (0.04 mg. per kg.), does not reduce the twitch height.

The effect of decamethonium given after a dose of didecasulphonium, didecaazonium or trisdecatetrazonium was different. The effect of a second and similar dose of decamethonium, given after a small paralysing dose of one of the compounds, was about the same as that caused by the first dose.

(b) The previous administration of a paralysing dose of decamethonium did not greatly modify the neuromuscular blocking properties of any of the new compounds. This effect was expected when didecasulphonium, didecaazonium or trisdecatetrazonium was given after decamethonium, as the effect of decamethonium itself was not modified by the prior administration of any one of these three compounds. The observation that the effect of the later administration of any of the other compounds following a paralysing dose of decamethonium was practically unaltered, was rather unexpected but was similar to the effect observed by Paton and Zaimis[22] when they administered a dose of tubocurarine /

tubocurarine following a previous injection of decamethonium in the cat nerve-muscle preparation. A typical experiment is shown in Figure 58, part b, c and d, page 208.

(c) Neuromuscular block, partial or complete, caused by dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexaazonium, dihexone, dioctasulphonium, dioctaazonium or trishexatetrazonium was rapidly and completely reversed by decamethonium injected during the block. The dose of decamethonium required was roughly about one-fourth of the dose of the compound used to cause paralysis. Immediately after injection of decamethonium at the point of maximal neuromuscular depression, the muscle twitch began to increase in amplitude and if the dose was correctly given, complete reversal was effected within 2 to 5 minutes and the twitch amplitude remained steady at the control level. If the dose of decamethonium given was bigger, there was a sharp increase of the amplitude of the muscle twitch which immediately afterwards began to decline in amplitude, rapidly causing a neuromuscular block which was more intense than that caused by the compound itself. This secondary block was perhaps due to the effect of decamethonium itself which was present in quantities more than were needed to counteract the effect of the other drug causing the initial neuromuscular depression.

If /

If, on the other hand, the dose of decamethonium chosen is less than optimal there is an increase of twitch height, but complete recovery is not observed. Hutter and Pascoe[4] found that decamethonium, in suitable doses, would restore neuromuscular transmission after neuromuscular block caused by tubocurarine. In Figure 59 (p.212) an example of decamethonium antagonism is shown.

The effect of decamethonium given during maximal depression of neuromuscular transmission caused by didecasulphonium, didecaazonium or trisdecatetrazonium was completely different. Decamethonium, instead of decreasing the block, actually increased its intensity as if another dose of the same drug had been given. This effect is shown in Figure 60, page 213.

(d) It has been shown by Dallemagne and Philippot[25] that when given in suitable doses tubocurarine can rapidly and completely reverse a neuromuscular block caused by decamethonium. The magnitude of the dose of tubocurarine needed has been shown to be critical, and must be very accurately gauged.

To investigate this particular point, partial or complete neuromuscular block was produced by giving a suitable /

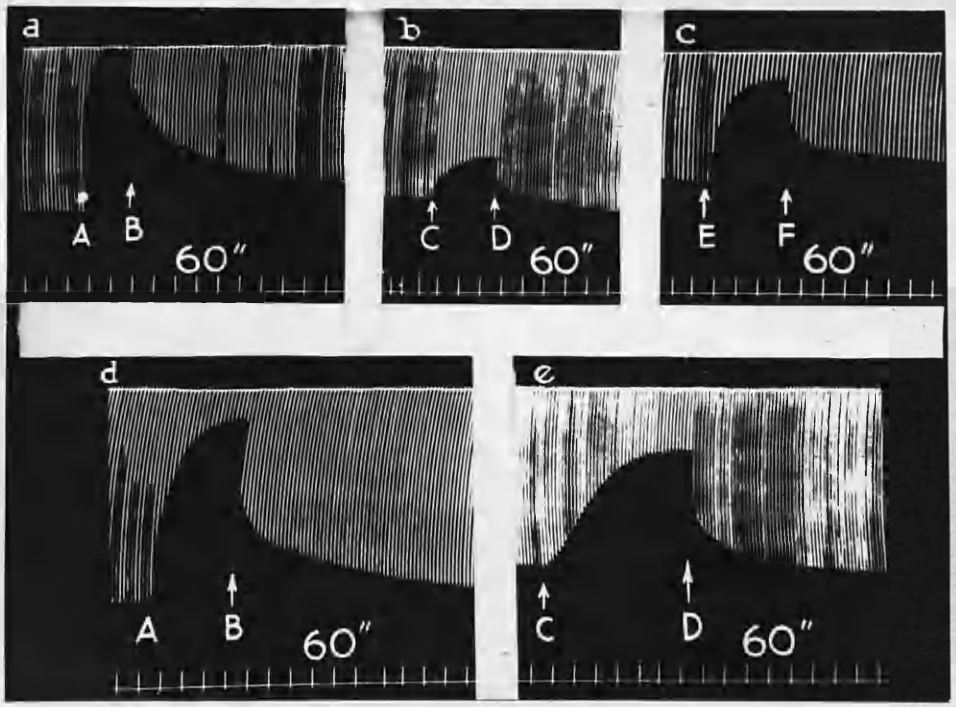

Figure 59. Cat gastrocnemius-sciatic preparation.

Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards.

Drugs administered intravenously.

(a)	At A,	dipentasulphonium	0.50 mg. per kg.
	At B,	decamethonium	0.15 mg. per kg.
(b)	At C,	dioctasulphonium	0.15 mg. per kg.
	At D,	decamethonium	0.07 mg. per kg.
(c)	At E,	dihexone	0.40 mg. per kg.
	At F,	decamethonium	0.06 mg. per kg.
(d)	At A,	tubocurarine	0.08 mg. per kg.
	At B,	decamethonium	0.07 mg. per kg.
(e)	At C,	trishexatetrazonium	0.06 mg. per kg.
	At D,	decamethonium	0.06 mg. per kg.

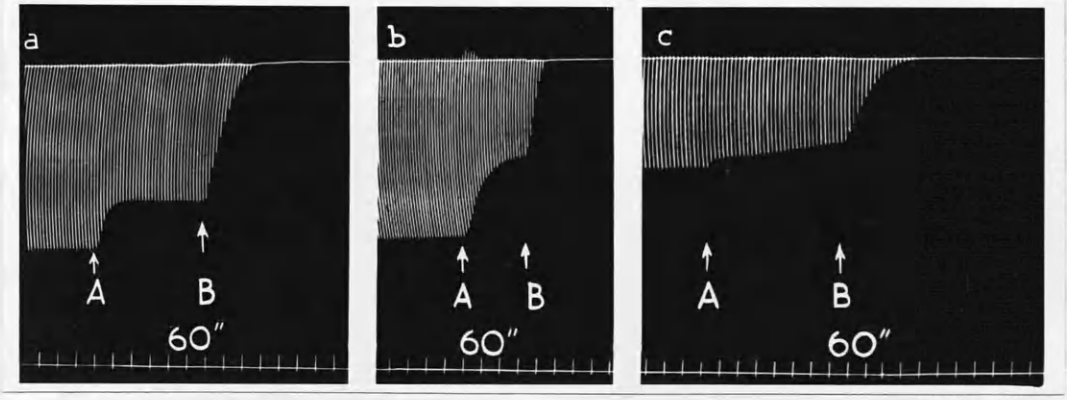

Figure 60. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	trisdecatetrazonium	0.10 mg. per kg.
	At B,	decamethonium	0.04 mg. per kg.
(b)	At A,	didecasulphonium	0.10 mg. per kg.
	At B,	decamethonium	0.05 mg. per kg.
(c)	At A,	didecaazonium	0.05 mg. per kg.
	At B,	decamethonium	0.02 mg. per kg.

suitable dose of decamethonium. Tubocurarine or one of the new compounds was injected at the region of maximal neuromuscular depression caused by decamethonium, and the recovery of the muscle twitch height was observed. All of the compounds tested, excepting didecasulphonium, didecaazonium and trisdecatetrazonium, reversed the block caused by decamethonium. The reversal was rapid and complete following the administration of dipentasulphonium, dihexasulphonium, dihexasulphonium trimethiodide, dihexaazonium, dioctaazonium and trishexatetrazonium but the antagonism shown by dihexone and dioctasulphonium was not very well marked, and complete reversal of the decamethonium block was not obtained. A typical experiment is shown in Figure 61, page 215.

If the dose of any one of the compounds employed to diminish the neuromuscular block caused by decamethonium was slightly larger than that actually needed, then there was an immediate increase in the intensity of the block already caused by decamethonium. If the dose was insufficient, the decamethonium block either remained unaffected or there was an incomplete reversal.

The effect of didecasulphonium, didecaazonium or trisdecatetrazonium on the neuromuscular block caused by decamethonium /

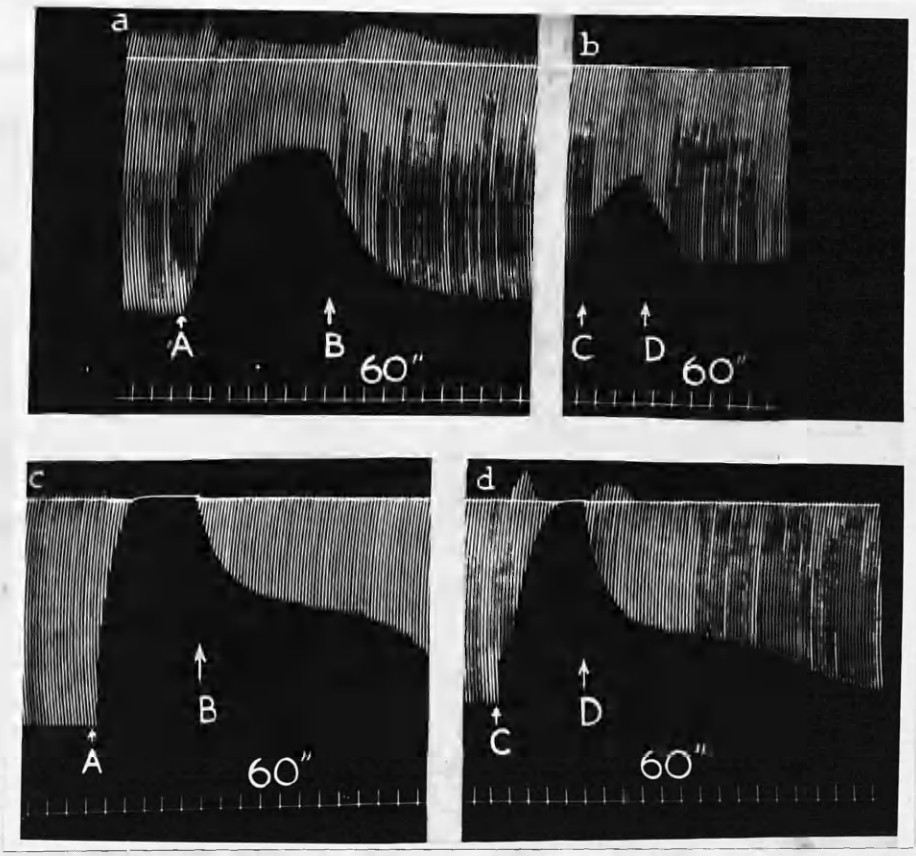

Figure 61. Cat Gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	decamethonium	0.025 mg. per kg.
	At B,	tubocurarine	0.125 mg. per kg.
(b)	At C,	decamethonium	0.04 mg. per kg.
	At D,	dihexaazonium	0.20 mg. per kg.
(c)	At A,	decamethonium	0.025 mg. per kg.
	At B,	trishexatetrazonium	0.10 mg. per kg.
(d)	At C,	decamethonium	0.05 mg. per kg.
	At D,	dihexasulphonium	0.30 mg. per kg.

decamethonium was an additive one. When any one of these three compounds were given (0.5 mg. to 1.0 mg. per kg.) at the point of maximal depression caused by decamethonium, the muscle twitch either remained unaffected or was reduced in magnitude depending upon the magnitude of the dose of didecasulphonium, didecaazonium or trisdecatetrazonium. Decamethonium block was never antagonised by these three compounds. A typical experiment is shown in Figure 62, page 217.

(v) Potassium chloride:

The potassium ion has been shown to antagonise neuromuscular block caused by tubocurarine[15] but decamethonium block is very little affected[4,7]. It was of interest, therefore, to investigate the effect of potassium chloride on the neuromuscular block caused by these new synthetic compounds. It was observed that the block caused by all the compounds (excepting didecasulphonium, didecaazonium and trisdecatetrazonium) was reversed by intravenous injection of 15 to 20 mg. per kg. of potassium chloride. The reversal was usually incomplete and did not persist (Fig. 63, p.218). This effect was similar to that observed with tubocurarine. Didecasulphonium, didecaazonium and trisdecatetrazonium, on the other hand, had different /

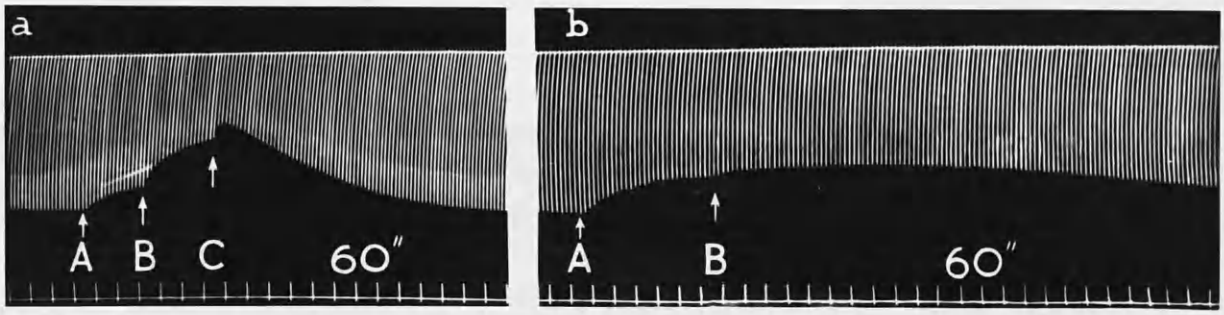

Figure 62. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	decamethonium	10.0 µg. per kg.
	At B,	didecasulphonium	0.10 mg. per kg.
	At C,	tubocurarine	0.10 mg. per kg.
(b)	At A,	decamethonium	10.0 µg. per kg.
	At B,	didecaazonium	0.10 mg. per kg.

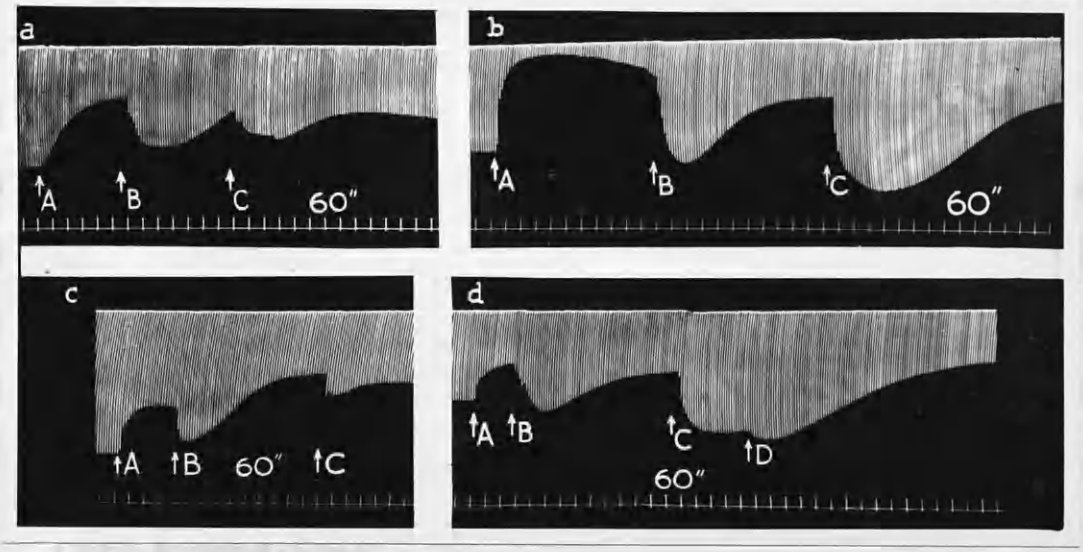

Figure 63. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A, trishexatetrazonium	0.10 mg. per kg.
	At B, adrenaline	0.05 mg. per kg.
	At C, potassium chloride	20.0 mg. per kg.
(b)	At A, dihexone	0.40 mg. per kg.
	At B, potassium chloride	20.0 mg. per kg.
	At C, adrenaline	0.08 mg. per kg.
(c)	At A, dioctaazonium	0.07 mg. per kg.
	At B, adrenaline	0.05 mg. per kg.
	At C, potassium chloride	15.0 mg. per kg.
(d)	At A, dihexaazonium	0.40 mg. per kg.
	At B, potassium chloride	20.0 mg. per kg.
	At C, adrenaline	0.10 mg. per kg.
	At D, adrenaline	0.05 mg. per kg.

different effects. Potassium chloride when injected in doses of from 15 to 20 mg. per kg. had either no effect on, or caused slight reversal of the neuromuscular block produced by these compounds (Fig. 64, a and c, p.220). A similar effect was observed when decamethonium was used (Fig. 64,b).

(vi) Adrenaline:

The decurarizing effects of adrenaline were first observed by Panella[14] and were later investigated by Camus and Porak[26]. Rosenblueth et alia[27] also showed the antagonistic effects of adrenaline to the neuromuscular block caused by tubocurarine in the cat nerve-muscle preparation. On the other hand, Paton and Zaimis[4] observed that adrenaline had no effect upon the block produced by decamethonium in the same preparation. As the effects of adrenaline on the neuromuscular block caused by tubocurarine and decamethonium were reported to be different, it was decided to investigate the effect of adrenaline on the block caused by the new synthetic compounds (see pages 189 and 192.

Adrenaline in doses ranging from 0.05 to 0.10 mg. per kg. caused a rapid but short lasting reversal of the block caused /

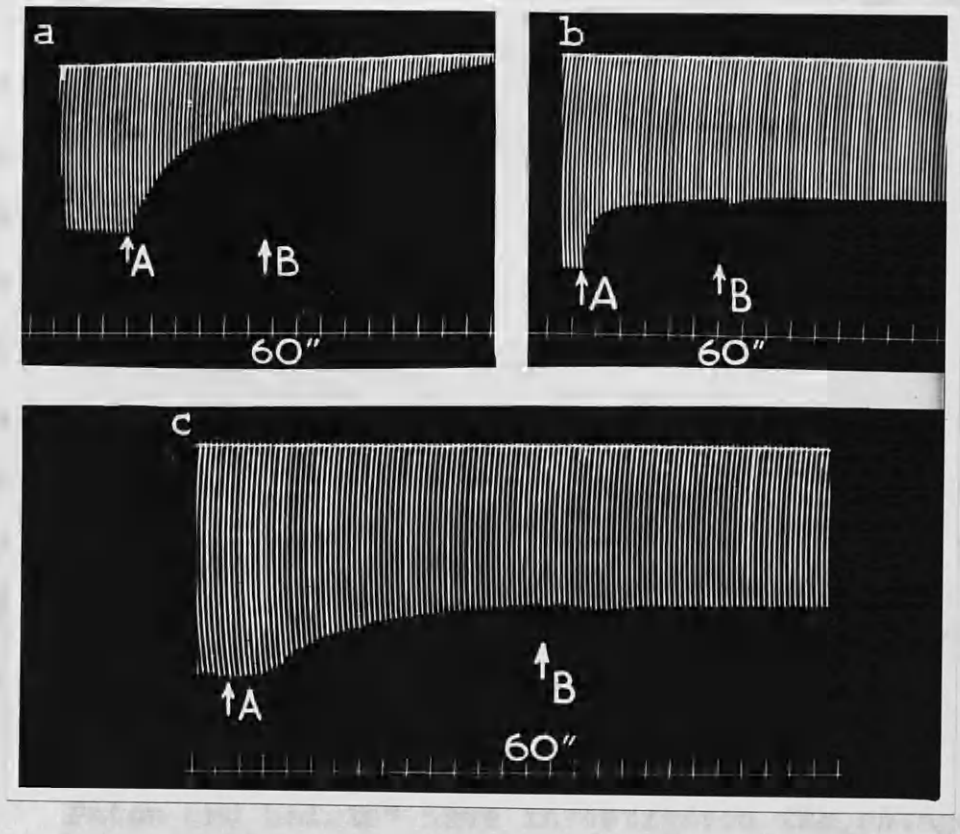

Figure 64. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	didecasulphonium	0.30 mg. per kg.
	At B,	potassium chloride	20.0 mg. per kg.
(b)	At A,	decamethonium	0.03 mg. per kg.
	At B,	potassium chloride	20.0 mg. per kg.
(c)	At A,	didecanazonium	0.20 mg. per kg.
	At B,	potassium chloride	15.0 mg. per kg.

caused by all compounds. In some cases the twitch amplitude became almost normal within a few minutes, but soon declined again to the level it had attained when the adrenaline was injected. Adrenaline had a similar effect when injected into the cat during neuromuscular blockade caused by tubocurarine and decamethonium, although Paton and Zaimis[4] observed that adrenaline was ineffective in reversing the neuromuscular block caused by decamethonium. A typical experiment is shown in Figures 63 and 65, pages 218 and 222.

(vii) Hexamethonium:

Paton and Zaimis[4] have investigated the antagonism between hexamethonium and decamethonium in the cat and rabbit. They observed that hexamethonium not only showed no antagonism to tubocurarine but actually potentiated its action to a small extent. When the effects of hexamethonium upon the neuromuscular blocking activity of the new series of compounds were investigated, it was found that dipentasulphonium, dihexasulphonium trimethiodide, dihexasulphonium, dihexaazonium, dihexone, dioctasulphonium, dioctaazonium and trishexatetrazonium had properties similar to those of tubocurarine. while didecasulphonium, didecaazonium and trisdecatetrazonium had /

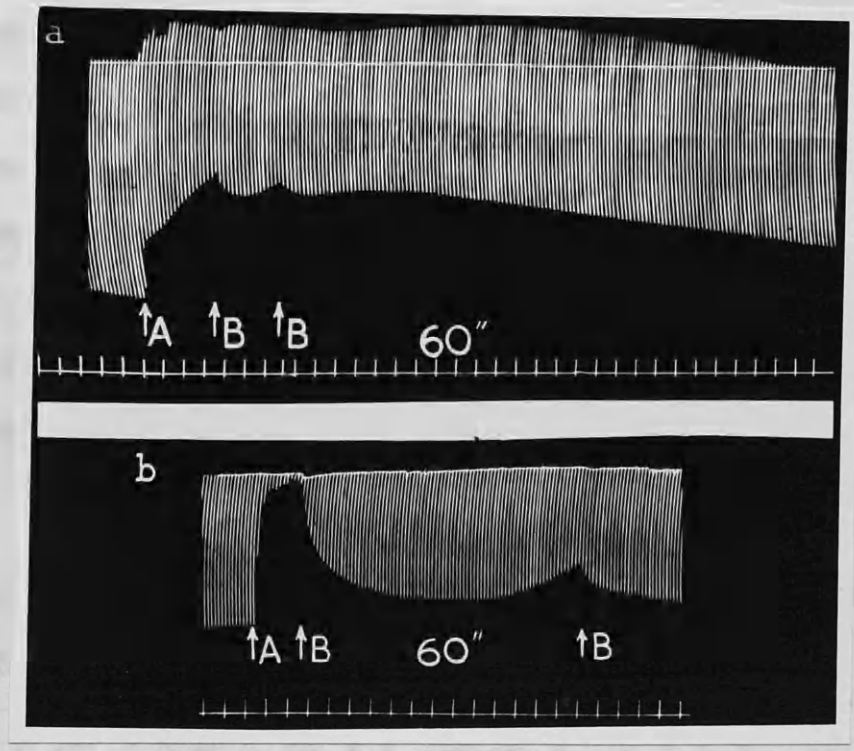

Figure 65. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At A,	decamethonium	0.04 mg. per kg.
	At B,	adrenaline	0.02 mg. per kg.
(b)	At A,	didecasulphonium	0.50 mg. per kg.
	At B,	adrenaline	0.07 mg. per kg.

had decamethonium-like properties. The neuromuscular block produced by the compounds of the former groups was either unaffected or potentiated by an intravenous injection of 2 to 5 mg. per kg. of hexamethonium. The compounds in the latter group had properties different from those of the first group in this respect. Hexamethonium either slightly increased the twitch height of the partially blocked muscle or had no effect upon it.

The properties of these compounds on the cat gastrocnemius muscle-sciatic nerve preparation are summarized in Table 2, pages 224 and 225.

2. (A) Rabbit Head Drop Test.

The mean head drop doses etc. of the new compounds as well as of tubocurarine and decamethonium are shown in Tables 3 and 4, pages 226, 227 and 228. Groups of 9 rabbits were injected in each test. The duration of head drop caused by dipentasulphonium, dihexasulphonium, dihexaazonium, dihexone and trishexatetrazonium varied from 10 to 15 minutes, and complete recovery from paralysis usually occurred within 30 minutes. 60 to 90 per cent of the rabbits used survived the administration of head drop doses of these 5 compounds, whereas when tubocurarine

was /

T A B L E 2.

A Tabular Comparison of the Properties of Dipentasulphonium, Dihexasulphonium, Dihexasulphonium trimethiodide, Dihexaazonium, Dioctasulphonium, Dioctaazonium, Dideca-sulphonium, Didecaazonium, Dihexone, Trishexatetrazonium and Trisdecatetrazonium with Tubocurarine and Decamethonium on the Cat Gastrocnemius-sciatic preparation.

	Tubocurarine	Dipentasulphonium	Dihexasulphonium	Dihexasulphonium trimethiodide	Dihexaazonium	Dioctasulphonium	Dioctaazonium	Dihexone	Trishexatetrazonium	Decamethonium	Dideca-sulphonium	Didecaazonium	Trisdecatetrazonium
Neuromuscular blocking activity (a) Time of onset of maximum paralysis (minutes)	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	1 - 3	5 -10	5 -10	5 - 10
(b) Duration of paralysis (minutes)	20 -30	20-30	20-30	15-25	20 -30	20 -30	20 -30	30-40	20 -30	15- 25	60 -120	90 -120	90 -120
(c) Potency (tubocurarine =100)	100	95	95	20	100	33	50	12	300	1000	100	100	50
Preliminary excitation of skeletal muscle	none	none	none	none	none	none	none	none	none	twitching and fasciculation	fasciculation	twitching and fasciculation	fasciculation
Effect of tubocurarine on block	additive	additive	additive	additive	additive	additive	additive	additive	additive	antagonistic	additive	additive	additive
Effect on tubocurarine block	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	antagonistic	antagonistic	antagonistic

/continued.

T A B L E 2 (continued).

	Tubocurarine	Dipentasulphonium	Dihexasulphonium	Dibexasulphonium trimethiodide	Dihexaazonium	Dioctasulphonium	Dioctaazonium	Dihexone	Trishexatetrazonium	Decamethonium	Dideca-sulphonium	Dideca-azonium	Trisdecatetrazonium
Effect of decamethonium on block	antagonistic	antagonistic	antagonistic	antagonistic	antagonistic	antagonistic	antagonistic	antagonistic	antagonistic	additive	additive	additive	additive
Effect on decamethonium block	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.
Effect on the block of													
(a) Neostigmine	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	no antagonism	no antagonism	no antagonism	no antagonism
(b) Eserine	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.	do.
(c) Edrophonium	do.	do.	do.	do.	do.	do.	do.	do.	do.	Potentiation	no antagonism	Potentiation	Potentiation
(d) Adrenaline	do.	do.	do.	do.	do.	do.	do.	do.	do.	antagonism	antagonism	antagonism	antagonism
(e) Potassium chloride	do.	do.	do.	do.	do.	do.	do.	do.	do.	no antagonism	no antagonism	no antagonism	no antagonism
(f) Ether anaesthesia	additive & prolonged	additive & prolonged	additive & prolonged	additive & prolonged	additive & prolonged	additive & prolonged	additive & prolonged	additive & prolonged	additive & prolonged	No effect or antagonism	no effect	*	*
(g) Indirect tetanization	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	antagonism	no effect	no effect	no effect	no effect
Response of partly blocked muscle to indirect tetanus	poorly sustained contraction	poorly sustained contraction	poorly sustained contraction	poorly sustained contraction	poorly sustained contraction	well sustained contraction	poorly sustained contraction	poorly sustained contraction	poorly sustained contraction	well sustained contraction	poorly sustained contraction	well sustained contraction	well sustained contraction

* Could not be investigated due to very prolonged effect.

TABLE 3.

Individual and Mean Head Drop Doses of Dipentasulphonium, Dihexasulphonium, Dihexaazonium, Dioctasulphonium, Dioctaazonium, Didecasulphonium, Didecaazonium, Dihexone, Trishexatetrazonium, Trisdecatetrazonium, Decamethonium and Tubocurarine in the rabbit.

Compound	HEAD DROP DOSES (HDD)		No.Died / No.injected.	Potency (tubocurarine = 100)
	Individual (mg.per kg.)	Mean ± s.e. (mg.per kg.)		
Dipentasulphonium	0.43, 0.42, 0.30, 0.37, 0.38, 0.40, 0.35, 0.40, 0.37.	0.38 ± 0.014	2/9	29
Dihexasulphonium	0.42, 0.27, 0.38, 0.40, 0.35, 0.40, 0.35, 0.35, 0.30.	0.36 ± 0.071	3/9	30
Dihexaazonium	0.57, 0.47, 0.77, 0.60, 0.58, 0.40, 0.38, 0.40, 0.45.	0.51 ± 0.057	2/9	21
Dioctasulphonium	0.26, 0.25, 0.22, 0.30, 0.25, 0.30, 0.20, 0.20, 0.23	0.24 ± 0.013	9/9	46
Dioctaazonium	0.25, 0.25, 0.28, 0.30, 0.25, 0.25, 0.26, 0.25, 0.28.	0.26 ± 0.014	9/9	42

T A B L E 3 (continued).

Compound	HEAD DROP DOSES (HDD)		No.died / No.injected.	Potency (tubocurarine = 100)
	Individual (mg.per kg.)	Mean ± s.e. (mg.per kg.)		
Didecasulphonium	0.13, 0.29, 0.25, 0.18, 0.16, 0.20, 0.20, 0.23, 0.25	0.21 ± 0.023	9/9	52
Didecaazonium	0.19, 0.26, 0.30, 0.30, 0.30, 0.30, 0.25, 0.34, 0.33.	0.28 ± 0.017	9/9	39
Dihexone	0.30, 0.40, 0.38, 0.43, 0.43, 0.40, 0.40, 0.36, 0.40.	0.39 ± 0.017	1/9	28
Trishexatetrazonium	0.21, 0.21, 0.21, 0.23, 0.20, 0.18, 0.16, 0.12, 0.20.	0.19 ± 0.014	3/9	58
Trisdecatetrazonium	0.25, 0.50, 0.35, 0.50, 0.32, 0.45 0.38, 0.40, 0.45.	0.40 ± 0.024	9/9	28
Decamethonium	0.20, 0.18, 0.17, 0.14, 0.13, 0.15, 0.15, 0.18, 0.18.	0.16 ± 0.008	3/9	69
Tubocurarine	0.10, 0.09, 0.10, 0.15, 0.11, 0.12, 0.10, 0.12, 0.12.	0.11 ± 0.01	6/9	100

TABLE 4.

Head Drop Doses of Dipentasulphonium, Dihexasulphonium, Dihexaazonium, Dioctasulphonium, Dioctaazonium, Didecasulphonium, Didecaazonium, Dihexone, Trishexatetrazonium, Trisdecatetrazonium, Decamethonium and Tubocurarine in the Rabbit Before and After Treatment with Neostigmine and the ratio: HDD (Neostigmine Treated)/ HDD (Control).

Compound	Mean Head Drop Dose (HDD) ± s.e. (mg. per kg.)		Ratio: $\frac{\text{Neostigmine treated}}{\text{control}}$
	Control	Neostigmine (0.10 mg. per kg. subcut.) treated.	
Dipentasulphonium	0.38 ± 0.014	0.57 ± 0.014	1.50 ($P = < 0.01$)
Dihexasulphonium	0.36 ± 0.071	0.59 ± 0.071	1.64 ($P = < 0.01$)
Dihexaazonium	0.51 ± 0.057	0.81 ± 0.057	1.56 ($P = < 0.01$)
Dioctasulphonium	0.24 ± 0.013	0.25 ± 0.013	1.04 ($P = > 0.90$)
Dioctaazonium	0.26 ± 0.014	0.33 ± 0.014	1.27 ($P = < 0.01$)
Didecasulphonium	0.21 ± 0.023	0.28 ± 0.023	1.30 ($P = > 0.05$)
Didecaazonium	0.28 ± 0.017	0.28 ± 0.017	1.00
Dihexone	0.39 ± 0.017	0.44 ± 0.017	1.12 ($P = > 0.40$)
Trishexatetrazonium	0.19 ± 0.014	0.31 ± 0.014	1.63 ($P = < 0.01$)
Trisdecatetrazonium	0.40 ± 0.024	0.33 ± 0.024	0.82
Decamethonium	0.16 ± 0.008	0.15 ± 0.008	0.93
Tubocurarine	0.11 ± 0.01	0.30 ± 0.01	2.72 ($P = < 0.01$)

was used 66 per cent died following a head drop dose (Table 3 ,pages 226 and 227). Death in each case was invariably due to respiratory paralysis.

The duration of paralysis caused by didecasulphonium, didecaazonium, dioctasulphonium, dioctaazonium and trisdecatetrazonium was more prolonged than that caused by the compounds of the former group. All of the rabbits which had received head drop doses of the compounds of the latter group died within 10 to 30 minutes (Table 3).

The potency of the new compounds together with that of tubocurarine and decamethonium as estimated by this test is shown in Table 3. All of the compounds were found to be less potent than tubocurarine. Pictures of the rabbit head drop are shown in Figure 34, page 148.

2. (B) The Effect of Neostigmine upon the Magnitude of the Head Drop Dose.

If 0.10 mg. per kg. of neostigmine was injected subcutaneously 15 minutes before the drug infusion was started then there was in some cases a change in the magnitude of the head drop dose. The doses of dipentasulphonium, dihexasulphonium, dihexaazonium and trishexatetrazonium were found to be appreciably raised,

whereas /

whereas those of didecaazonium, dioctasulphonium and trisdecatetrazonium remained either unaltered or reduced (Table 4 ,p.228). This property of didecaazonium, dioctasulphonium and trisdecatetrazonium was shared with decamethonium and that of the former group was shared with tubocurarine. The head drop doses after prior treatment with neostigmine, together with the ratio, head drop dose for the neostigmine pretreated rabbits/head drop dose for the untreated rabbits, are shown in Table 4.

3. Mouse Test.

Median Paralysing Dose (PD_{50}).

Five to ten minutes after intraperitoneal injection into mice, all of the new compounds caused increased movement and restlessness. These symptoms appeared at the same time as symptoms of respiratory embarrassment became apparent. The mice then became quiet and began suddenly to lose their ability to retain their position on the inclined screen and slid abruptly backwards off it. Recovery from paralysis was usually complete in 15 to 20 minutes and the mice had completely recovered in 30 to 45 minutes. The time of onset of action and the period required for recovery from paralysis were greater for didecasulphonium, didecaazonium and trisdecatetrazonium.

The /

the median paralysing doses (PD_{50}) of the compounds studied are shown in Table 5, page 232. The relative potency has been determined in terms of tubocurarine = 100 and is shown in the same table. It was observed that the compounds dioctasulphonium and trisdecatetrazonium were the most potent (2.5 times more potent than tubocurarine) and dihexazonium was the least potent.

4. Chick Paralysis Test.

It is well known that competitive neuromuscular blocking agents cause a flaccid paralysis in birds, whereas depolarizing agents cause a typical spastic paralysis[7,28]. It was of interest, therefore, to see the type of paralysis produced in chicks by the new compounds.

Following an intraperitoneal injection of from 10 to 40 mg. per kg. of dipentasulphonium, dihexasulphonium, dihexazonium, dioctasulphonium, dioctazonium, dihexone or trishexatetrazonium, a typical tubocurarine-like flaccid paralysis was seen in chicks. Didecasulphonium, didecazonium, dihexasulphonium trimethiodide and trisdecatetrazonium, caused different effects in chicks. Didecasulphonium, didecazonium and dihexasulphonium trimethiodide in the dose range of from 10 to 40 mg. per kg. /

TABLE 5

A Comparison of the Potency and Toxicity of Dipentasulphonium, Dihexasulphonium, Dihexaazonium, Dioctasulphonium, Dioctaazonium, Didecasulphonium, Didecaazonium, Dihexone, Trishexatetrazonium and Trisdecatetrazonium with Tubocurarine and Decamethonium in mice.

Compound	Approximate Median Paralysing dose (mg.per kg.)	Approximate Median Lethal Dose (mg.per kg.)	Paralysing Potency (tubocurarine = 100)	Median Lethal Dose / Median Paralysing Dose.
Dipentasulphonium	0.70	1.33	28	1.9
Dihexasulphonium	0.80	1.20	25	1.5
Dihexaazonium	1.20	2.25	17	1.9
Dioctasulphonium	0.08	0.40	250	5.0
Dioctaazonium	0.10	0.40	200	4.0
Didecasulphonium	0.10	0.20	200	2.0
Didecaazonium	0.16	0.18	125	1.15
Dihexone	0.60	2.30	33	3.8
Trishexatetrazonium	0.40	0.75	50	1.9
Trisdecatetrazonium	0.08	0.10	250	1.25
Decamethonium	3.65	4.10	5.4	1.12
Tubocurarine	0.20	0.28	100	1.4

kg. produced an initial decamethonium-like spastic paralysis with extension of the legs and retraction of the head, but within a few minutes the paralysis became flaccid in nature. This type of paralysis was observed in chicks following an injection of the bisquaternary methonium compound, tridecamethonium[7].

Trisdecatetrazonium at dose levels of from 10 to 20 mg. per kg. caused a flaccid paralysis, but when the dose was increased to 30 to 40 mg. per kg. an initial spastic paralysis was seen which rapidly became flaccid in nature.

Results of these experiments are summarized in Table 6, page 234.

5. Activity on the Rat Phrenic Nerve-Diaphragm Preparation.

All of the compounds tested in the dose range of 0.05 to 0.2 mg. per ml. reduced the twitch height of the diaphragm caused by indirect stimulation via the phrenic nerve. The effect was always reversible on washing. Tubocurarine was many times more potent on this preparation than the most potent of the new compounds. A typical experiment is shown in Figure 66, page 235. Tubocurarine was about 50 to 100 times more potent than dipentasulphonium /

T A B L E 6

Type of Paralysis in Chicks

Compound	Type of paralysis observed.
Dipentasulphonium	Flaccid
Dihexasulphonium	Flaccid
Dihexasulphonium trimethiodide	Spastic → flaccid
Dihexaazonium	Flaccid
Dioctasulphonium	Flaccid
Dioctaazonium	Flaccid
Didecasulphonium	Spastic → flaccid
Didecaazonium	Spastic → flaccid
Dihexone	Flaccid
Trishexatetrazonium	Flaccid
Trisdecatetrazonium	(Flaccid in smaller dose (Spastic in larger dose
Tubocurarine	Flaccid
Decamethonium	Spastic

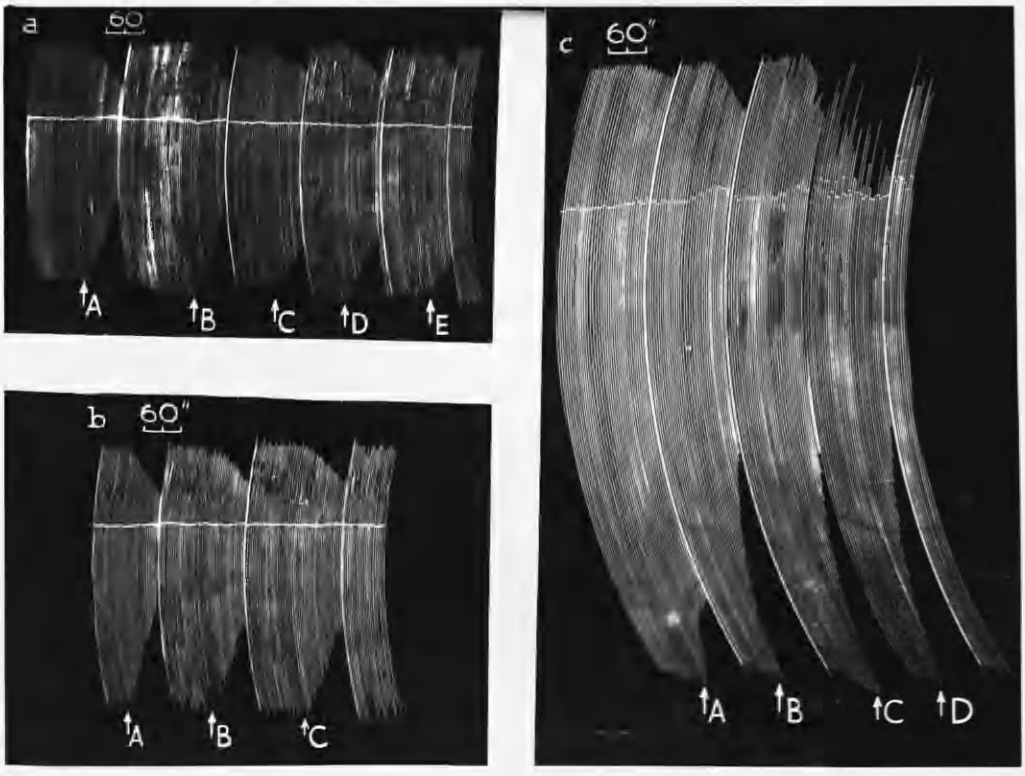

Figure 66. Isolated rat phrenic nerve-diaphragm preparation. Indirect stimulation via phrenic nerve at 10 volts, pulse width 1.5 msecs., 8 impulses per minute. W = wash out.

(a)	At A, tubocurarine	3.0 µg. per ml.
	At B, dioctasulphonium	100.0 µg. per ml.
	At C, dioctaazonium	100.0 µg. per ml.
	At D, trisdecatetrazonium	100.0 µg. per ml.
	At E, tubocurarine	2.0 µg. per ml.
(b)	At A, tubocurarine	3.0 µg. per ml.
	At B, trishexatetrazonium	100.0 µg. per ml.
	At C, dihexasulphonium	200.0 µg. per ml.
(c)	At A, dihexaazonium	30.0 µg. per ml.
	At B, dihexaazonium	100.0 µg. per ml.
	At C, tubocurarine	2.0 µg. per ml.
	At D, didecasulphonium	100.0 µg. per ml.

dipentasulphonium, dihexazonium, dihexasulphonium, didecasulphonium or didecazonium, while trishexatetrazonium, trisdecatetrazonium, dioctasulphonium and dioctazonium were about 30 to 50 times and dihexone about 150 times less potent than tubocurarine.

6. Activity on the Kitten Phrenic Nerve-Diaphragm Preparation.

This preparation was many times more sensitive to the neuromuscular blocking activity of the compounds under investigation than the rat diaphragm preparation. All the compounds tested reduced the height of the indirectly induced twitch, and the effect was always reversible on washing. There was no initial potentiation of twitch height. Although dipentasulphonium and dihexazonium were equipotent and dioctasulphonium and dioctazonium were less potent than tubocurarine on the cat gastrocnemius muscle-sciatic nerve preparation, these compounds were more potent on this preparation. Doses required to reduce the twitch height of the diaphragm by 50 per cent in three minutes varied very much from preparation to preparation and were usually between 0.01 to 0.1 mg. per ml. The results of some typical experiments are shown in Figure 67, page 237 . In some experiments complete block /

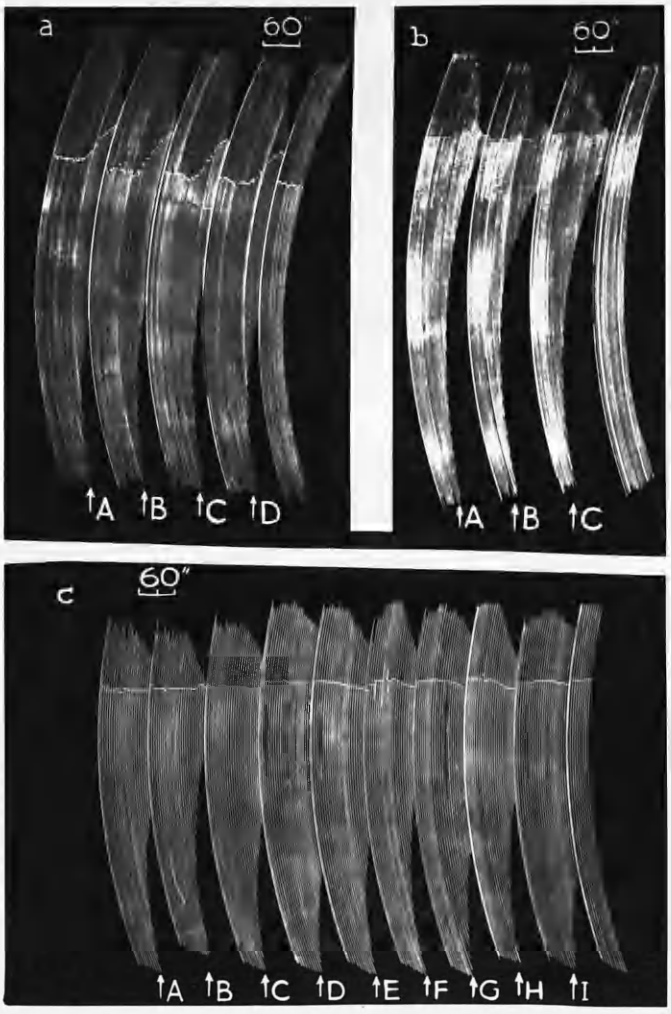

Figure 67. Isolated kitten phrenic nerve-diaphragm preparation. Indirect stimulation via phrenic nerve at 12 volts, pulse width 2.0 msecs., 8 impulses per minute. W = Wash out.

(a)	At A, dihexasulphonium	30 µg. per ml.
	At B, tubocurarine	30 µg. per ml.
	At C, trishexatetrazonium	10 µg. per ml.
	At D, dioctasulphonium	15 µg. per ml.
(b)	At A, tubocurarine	4 µg. per ml.
	At B, didecaazonium	60 µg. per ml.
	At C, didecasulphonium	65 µg.,per ml.
(c)	At A, tubocurarine	20 µg. per ml.
	At G, tubocurarine	10 µg. per ml.
	At B, dihexaazonium	10 µg. per ml.
	At C, dihexaazonium	5 µg. per ml.
	At D, dihexasulphonium	5 µg. per ml.
	At E, dihexasulphonium	10 µg. per ml.
	At F, dihexasulphonium	20 µg. per ml.
	At H, dipentasulphonium	10 µg. per ml.
	At I, dipentasulphonium	5 µg. per ml.

block was produced at dose levels of 0.015 to 0.1 mg. per ml. in 5 to 7 minutes. There was complete recovery of the twitch height on washing. This type of experiment is shown in Figure 68, page 239. The approximate potency of the compounds expressed in terms of tubocurarine is shown in Table 7, page 240.

7. The Frog Rectus Abdominis Muscle.

None of the new compounds had any direct stimulant effect on this preparation at doses of up to 25 µg. per ml. All of them in the dose range of 0.5 to 2.5 µg. per ml. caused graded inhibition of contractions induced by 0.10 to 0.25 µg. per ml. of acetylcholine or 1.5 to 2.5 µg. per ml. of decamethonium (Figs. 69 and 70, pages 241 and 242). In this respect all of the compounds showed properties similar to those of tubocurarine. The potency of these compounds as measured by the degree of inhibition of acetylcholine induced contractions is shown in Table 8, page 243.

Antagonism by some of these compounds to acetylcholine and decamethonium induced contractions of the frog rectus abdominis muscle is shown in Figures 71 and 72, pages 244 and 245.

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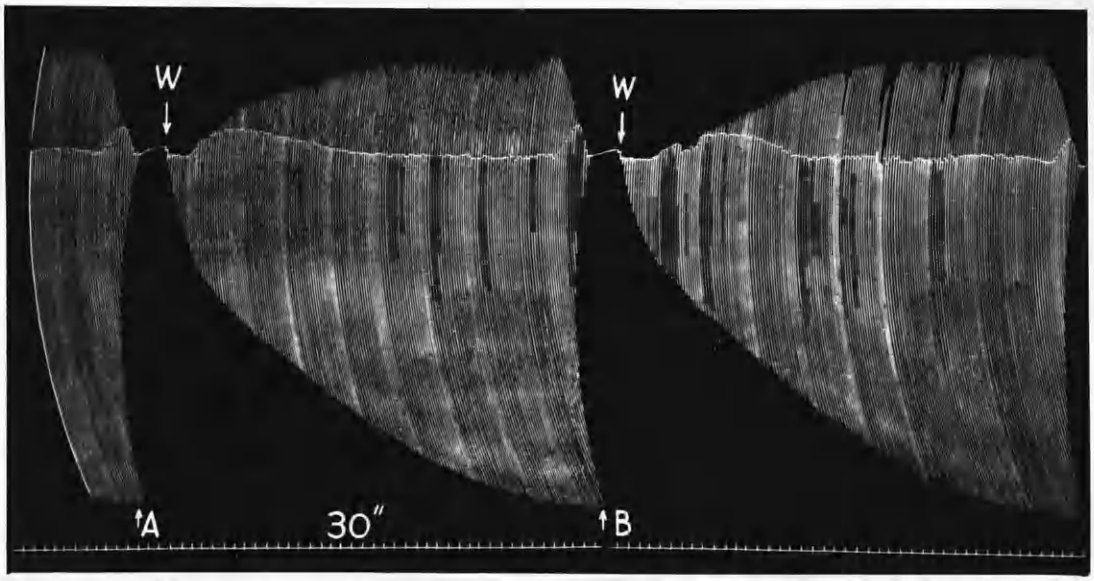

Figure 68. Isolated kitten phrenic nerve-diaphragm preparation. Indirect stimulation via phrenic nerve at 10 volts, pulse width 1.5 msecs., 8 impulses per minute.

At A, dihexaazonium 15 µg. per ml.
At B, dipentasulphonium 30 µg. per ml.
At W, wash out.

TABLE 7

The relative potencies of dipentasulphonium, dihexasulphonium, dihexaazonium, dioctasulphonium, dioctaazonium, didecasulphonium, didecaazonium, dihexone, trishexatetrazonium, trisdecatetrazonium and tubocurarine on the kitten phrenic nerve-diaphragm preparation.

Compound	Potency in terms of tubocurarine = 100
Dipentasulphonium	200
Dihexasulphonium	100
Dihexaazonium	300
Dioctasulphonium	200
Dioctaazonium	150
Didecasulphonium	15
Didecaazonium	15
Dihexone	7
Trishexatetrazonium	300
Trisdecatetrazonium	*
Tubocurarine	100
Decamethonium	75

* could not be compared.

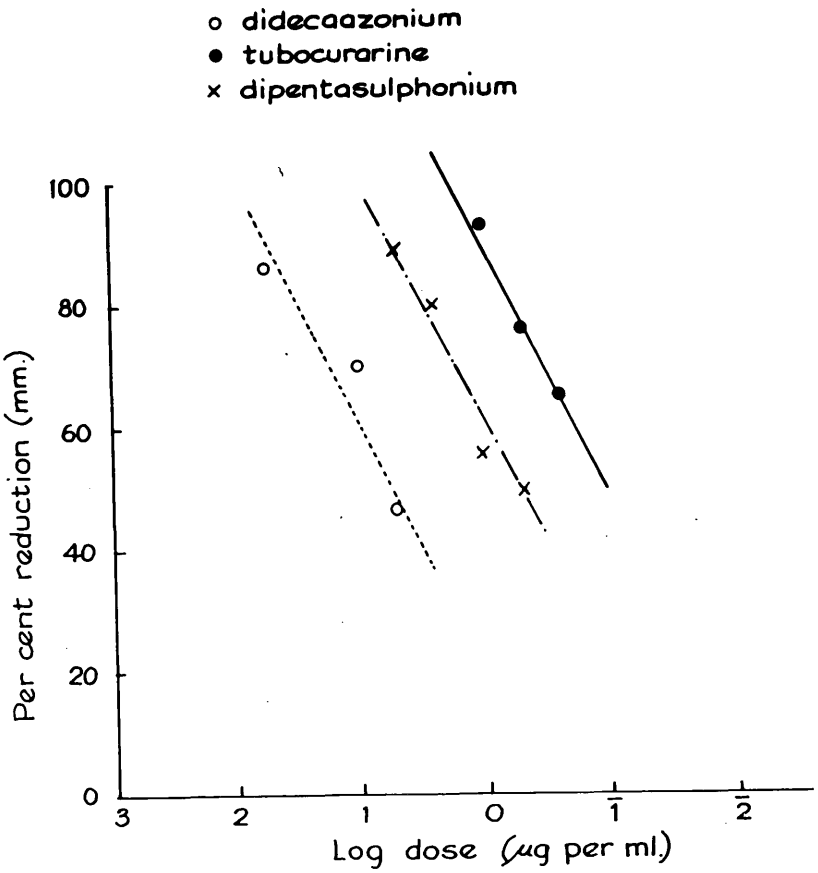

Figure 69. Isolated frog rectus abdominis muscle. Relation of log dose of didecaazonium, tubocurarine and dipentasulphonium (abscissa) to the percentage reduction (ordinate) of the height of contraction produced by the same dose of acetylcholine.

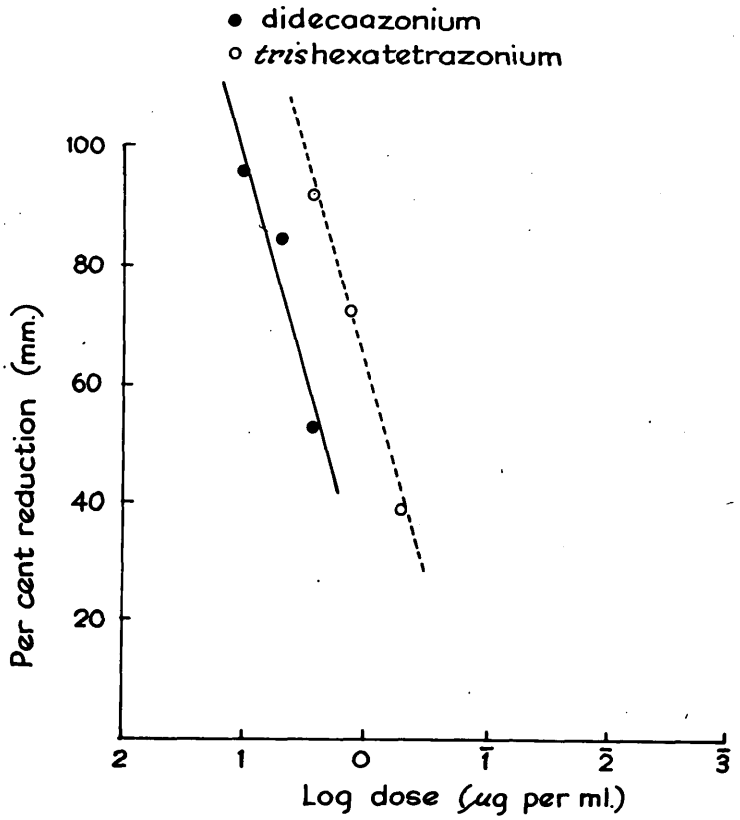

Figure 70. Isolated frog rectus abdominis muscle. Relation of log dose of didecaazonium and trishexatetrazonium (abscissa) to the percentage reduction (ordinate) of the height of contraction produced by the same dose of decamethonium.

TABLE 8.

Comparison of the potency of the compounds, dipentasulphonium, dihexasulphonium, dihexaazonium, dioctasulphonium, dioctaazonium, didecasulphonium, didecaazonium, dihexone, trishexatetrazonium, trisdecatetrazonium, with tubocurarine on the frog rectus abdominis muscle.

Compound	Potency in terms of tubocurarine = 100
Dipentasulphonium	25
Dihexasulphonium	25
Dihexaazonium	50
Dioctasulphonium	10
Dioctaazonium	20
Didecasulphonium	10
Didecaazonium	10
Dihexone	2
Trishexatetrazonium	50
Trisdecatetrazonium	20

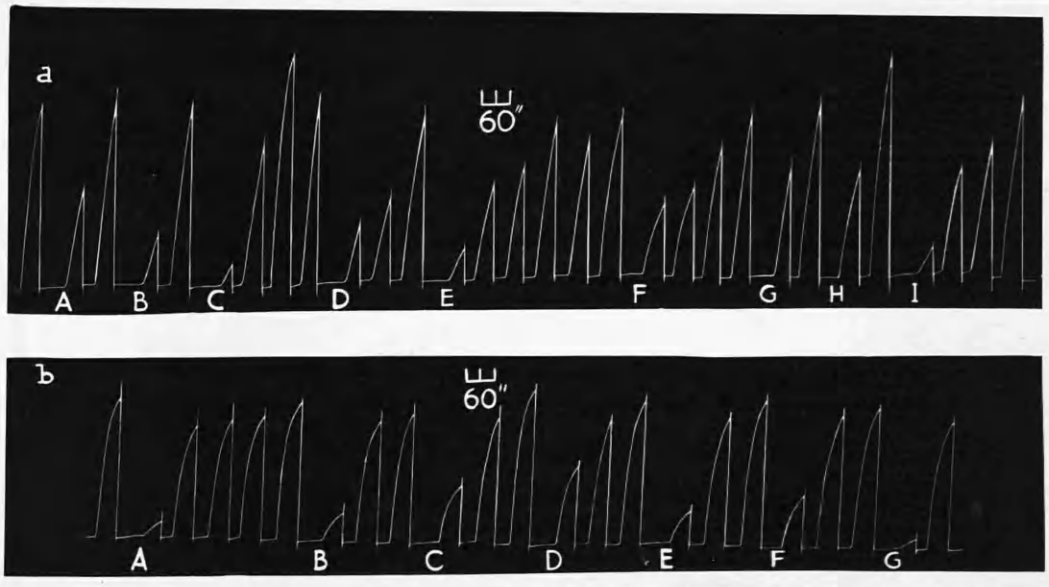

Figure 71. Isolated frog rectus abdominis muscle.

(a) All contractions were due to 0.20 µg. per ml. acetylcholine acting for 1.5 minutes. Labelled contractions were preceded one minute earlier by

At A,B and C, didecasazonium 5,10 and 15 µg. per ml. respectively.

At D,E and F, tubocurarine 1, 1.5 and 0.75 µg.per ml. respectively.

At G,H and I, didecasulphonium 5, 5 and 20 µg. per ml. respectively.

(b) All contractions were due to 0.10 µg. per ml. acetylcholine acting for 1.5 minutes. Labelled contractions were preceded one minute earlier by

At A,B,C and D,dipentasulphonium 5, 2.5, 1 and 0.5 µg. per ml. respectively.

At E,F and G, tubocurarine 0.5, 0.25 and 1 µg. per ml. respectively.

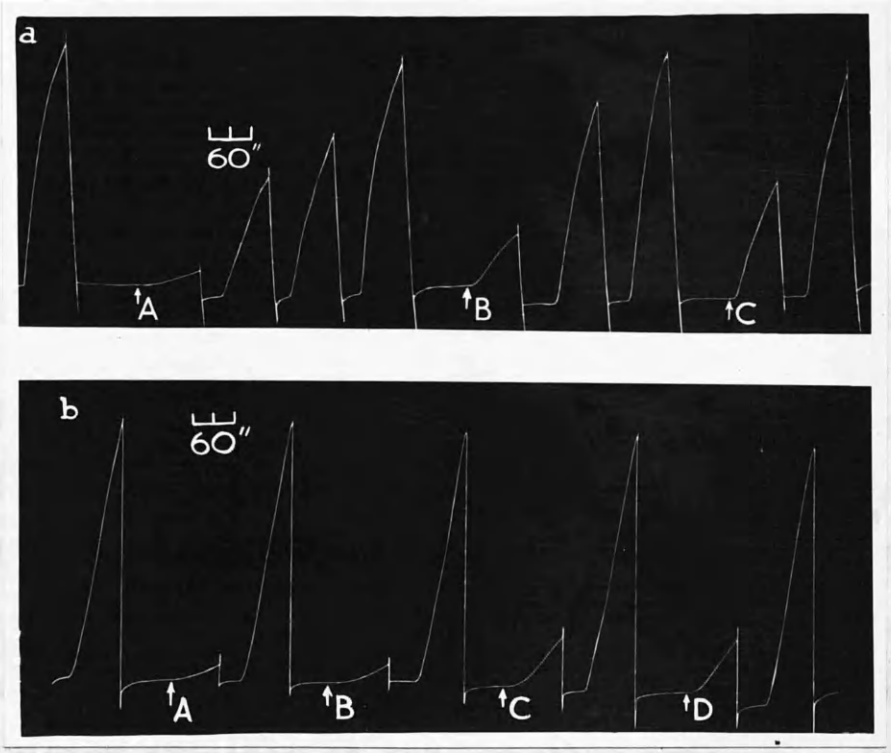

Figure 72. Isolated frog rectus abdominis muscle.

(a) All contractions produced by decamethonium 2.5 µg. per ml. acting for 2 minutes. The addition of decamethonium was preceded one minute earlier by

At A, B and C, didecaazonium 10, 5 and 2.5 µg. per ml. respectively.

(b) All contractions produced by decamethonium 2.0 µg. per ml. acting for 2 minutes. The addition of decamethonium was preceded one minute earlier by

At A and D, trishexatetrazonium 2.5 and 1.25 µg. per ml. respectively.

At B and C, dioctaazonium 2.5 and 1.25 µg. per ml. respectively.

The neuromuscular blocking potency of these compounds in different species is compared with that of tubocurarine and is shown in Table .

8. Effects upon the Blood Pressure of the Anaesthetised Cat.

Tubocurarine in doses of 0.1 to 1.0 mg. per kg. caused a fall of blood pressure of varying intensity in anaesthetised cats and dogs[29]. The fall in blood pressure which follows administration of tubocurarine is probably due to blockade of transmission of sympathetic vaso-constrictor impulses at the ganglion synapse. It may also be partly due to liberation of histamine[30]. In order to investigate whether the new compounds resembled tubocurarine in this respect, their effects upon the blood pressure of anaesthetised cats were studied. None of the compounds caused a significant rise or fall in the blood pressure when administered in doses ranging from 0.5 to 2.0 mg. per kg. excepting trisdecatetrazonium which always caused the blood pressure to fall when given at doses of from 0.5 to 1.0 mg. per kg. (Figs. 73,c and 74,a, pages 248 and 249). A moderate fall of blood pressure was observed in one cat following 0.4 mg. per kg. of didecaazonium (Fig.74,b). Tubocurarine (0.5 to 1.0 mg. /

TABLE 9

Comparison of Potency of Dipentasulphonium, Dihexasulphonium, Dihexasulphonium trimethiodide, Dihexaazonium, Dioctasulphonium, Dioctaazonium, Didecasulphonium, Didecaazonium, Dihexone, Trishexatetrazonium and Trisdecatetrazonium with Tubocurarine taken as 100 in different species.

Compound	CAT		RAT	RABBIT	MOUSE	FROG
	Gastrocnemius-sciatic	Phrenic diaphragm	Phrenic Diaphragm	Head Drop Dose	Approximate median paralysing dose	Acetylcholine antagonism.
Dipentasulphonium	95	200	1-2	29	28	25
Dihexasulphonium	95	100	1-2	30	25	25
Dihexaazonium	100	300	1-2	21	17	50
Dioctasulphonium	33	200	2	46	250	10
Dioctaazonium	50	150	2	42	200	20
Didecasulphonium	100	15	1-2	52	200	10
Didecaazonium	100	15	1-2	39	125	10
Dihexone	12	7	0.6	28	33	2
Trishexatetrazonium	300	300	2	58	50	50
Trisdecatetrazonium	50	-	2	28	250	20
Tubocurarine	100	100	100	100	100	100
Decamethonium	1000	75	-	69	5.4	contracture

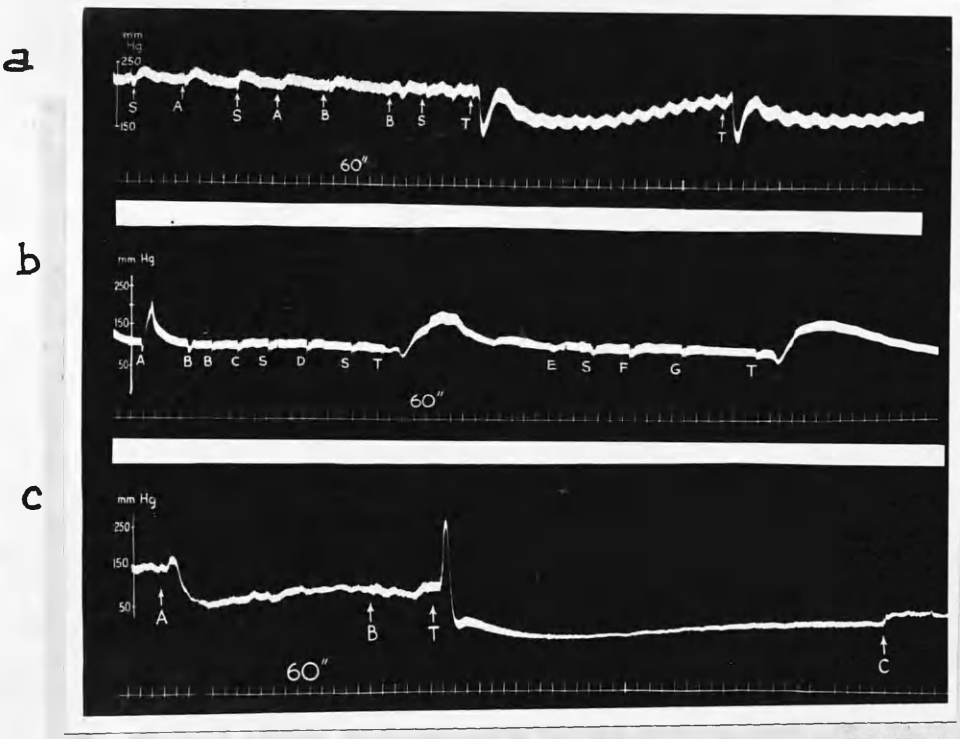

Figure 73. Cat. Pentobarbitone anaesthesia. Blood pressure record from common carotid artery. Drugs administered intravenously and followed by 4 ml. saline in each case.

(a)	At A,	dipentasulphonium	0.50 mg. per kg.
	At B,	dihexasulphonium	0.50 mg. per kg.
	At S,	saline 4 ml.	
	At T,	tubocurarine	0.50 mg. per kg.
(b)	At A,	adrenaline	1.0 µg. per kg.
	At B,	dihexaazonium	1.0 mg. per kg.
	At C,	dihexaazonium	2.0 mg. per kg.
	At S,	saline 4 ml.	
	At D,	dioctasulphonium	2.0 mg. per kg.
	At T,	tubocurarine	1.0 mg. per kg.
	At E,	trishexatetrazonium	2.0 mg. per kg.
	At F,	didecasulphonium	2.0 mg. per kg.
	At G,	dihexone	2.0 mg. per kg.
(c)	At A,	trisdecatetrazonium	1.0 mg. per kg.
	At B,	trishexatetrazonium	1.0 mg. per kg.
	At C,	didecaazonium	1.0 mg. per kg.

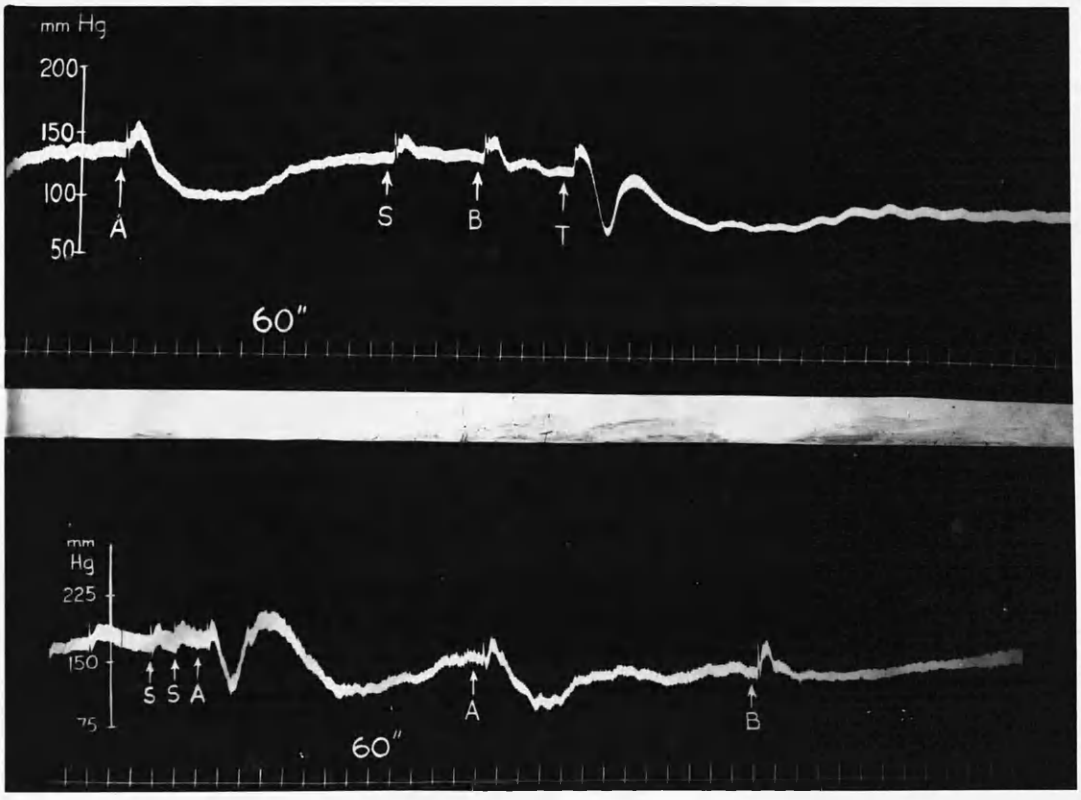

Figure 74. Cat. Pentobarbitone anaesthesia. Blood pressure record taken from the common carotid artery. Drugs administered intravenously and followed by 4 ml. saline in each case.

(a) At A, trisdecatetrazonium 1.0 mg. per kg.
At S, saline 4 ml.
At B, trishexatetrazonium 1.0 mg. per kg.
At T, tubocurarine 1.0 mg. per kg.

(b) At S, saline 4 ml.
At A, didecaazonium 0.40 mg. per kg.
At B, didecasulphonium 0.40 mg. per kg.

kg.) usually caused a well-marked fall of blood pressure in the anaesthetised cat. In some cats there was slight initial fall followed by a well-marked rise, and in some cats a well-marked rise and then a fall in the blood pressure level were seen following the injection of tubocurarine (Fig. 73,b and c). These effects of tubocurarine and the effects of some of the other compounds on the blood pressure of the anaesthetised cat are shown in Figures 73 and 74.

9. Ganglion Blocking Activity.

In order to study the ganglion blocking activity of the compounds under investigation, their effects were studied on the response of the nictitating membrane to stimulation of the preganglionic fibres of the cervical sympathetic. There was a slight reduction of the height of contraction of the nictitating membrane following the injection of 0.5 to 1.0 mg. per kg. doses of compounds, dipentasulphonium and dihexaazonium (Fig. 75, page 251).

Whereas dihexasulphonium trimethiodide, dihexaazonium, dioctasulphonium, dioctaazonium, didecasulphonium, dihexone, trishexatetrazonium and trisdecatetrazonium at doses of up to 3.0 mg. per kg. showed no activity /

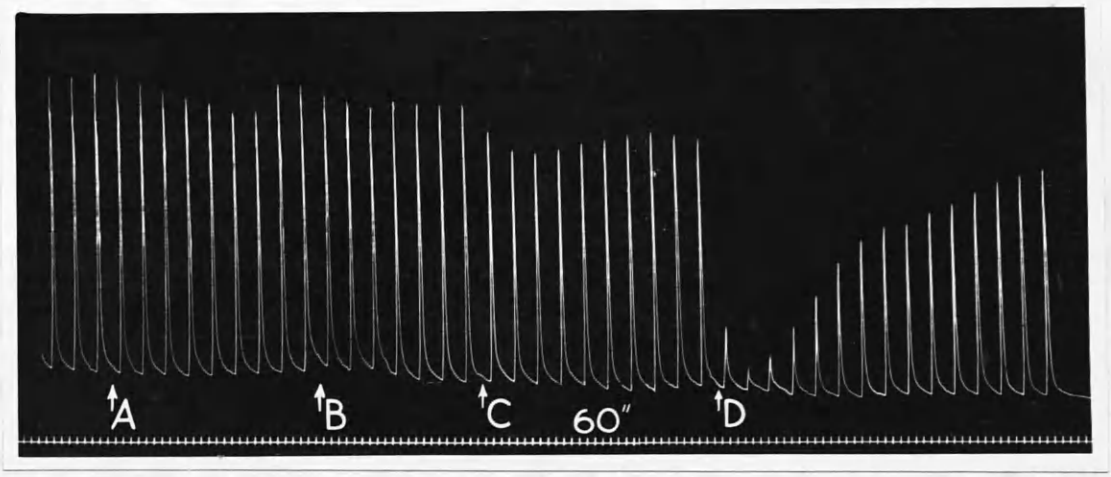

Figure 75. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Contractions of nictitating membrane elicited at 3 minute intervals by preganglionic stimulation at a frequency of 1,000 impulses per minute, 10 volts, 0.5 msec. for 15 seconds. Drugs administered intravenously one minute before the stimulation.

At A,	dihexasulphonium	1.0 mg. per kg.
At B,	"	0.50 mg. per kg.
At C,	dipentasulphonium	0.50 mg. per kg.
At D,	tubocurarine	0.50 mg. per kg.

activity, didecaazonium exhibited a stimulant effect. Following the administration of didecaazonium in the dose range of 0.25 to 1.0 mg. per kg., there was a significant contraction of the nictitating membrane and the effect was a long lasting one (Fig. 76, p.253). In contrast to these compounds, tubocurarine in doses as low as 0.2 mg. per kg. always caused a marked reduction of the amplitude of the contraction of the nictitating membrane. Some typical effects are shown in Figures 75, 76 and 77, pages 251, 253 and 254.

10. The Effect on the Respiration of the Anaesthetised Cat.

The continuous intravenous infusion of the new compound investigated, as well as of tubocurarine and decamethonium, always resulted in respiratory paralysis in the pentobarbitone-anaesthetised cat. If administration of the drug was stopped immediately upon cessation of the respiration, spontaneous respiration began again in a certain proportion of animals. As a rule, artificial respiration had to be given until spontaneous respiration began again. This always produced complete recovery. The dose required to cause respiratory paralysis was always higher than the dose required to cause /

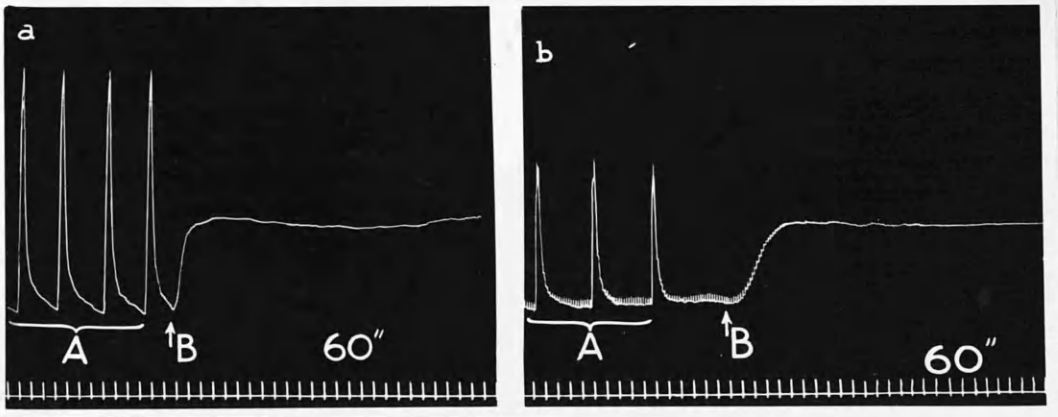

Figure 76. Cat. Pentobarbitone anaesthesia.

(a) At A, contractions of nictitating membrane elicited by preganglionic stimulation at 12 volts, 1,000 impulses per minute, pulse width 1.0 msec. for 15 seconds.
At B, didecaazonium 0.50 mg. per kg. intravenously.

(b) At A, contractions of nictitating membrane elicited by preganglionic stimulation at 15 volts, 800 impulses per minute, pulse width 1.5 msec. for 15 seconds.
At B, infusion of didecaazonium (0.20 mg. per ml.) at the rate of 0.80 ml. per minute for 10 minutes.

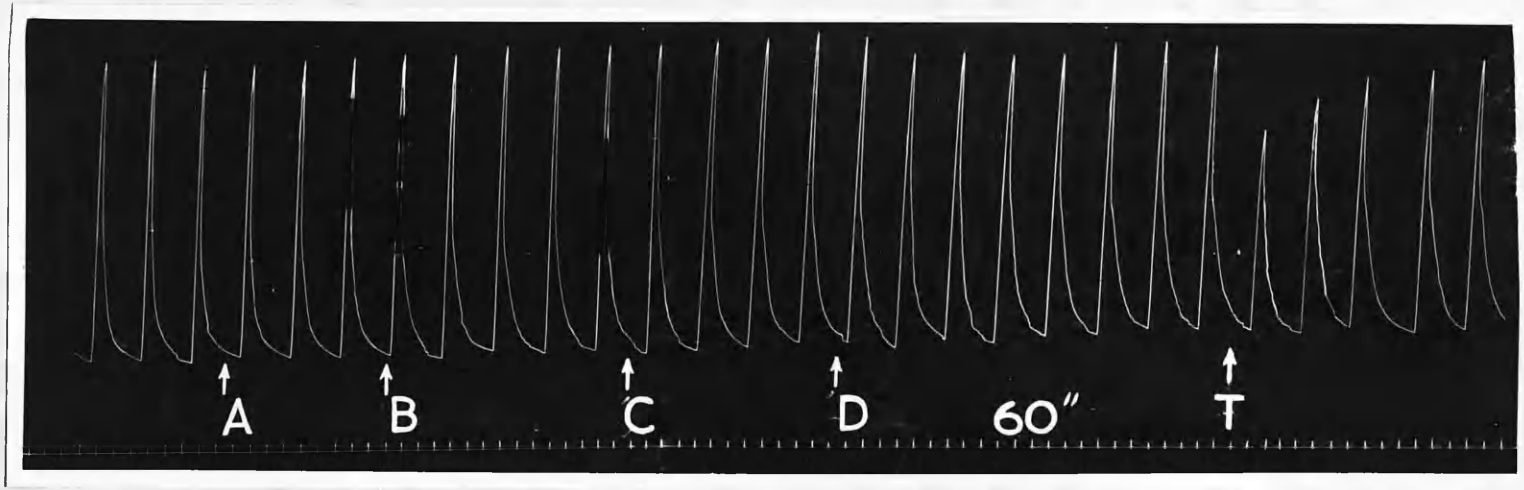

Figure 77. Cat. Pentobarbitone anaesthesia. Contractions of nictitating membrane elicited at three minute intervals by preganglionic stimulation at a frequency of 1,000 impulses per minute, 10 volts, pulse width 0.50 msec. for 15 seconds. Drugs administered intravenously.

At A,	trishexatetrazonium	2.0 mg. per kg.
At B,	dihexasulphonium trimethiodide	2.0 mg. per kg.
At C,	dihexaazonium	2.0 mg. per kg.
At D,	didecaazonium	2.0 mg. per kg.
At T,	tubocurarine	0.20 mg. per kg.

cause complete neuromuscular paralysis in the cat nerve-muscle preparation. The respiratory paralysing dose as well as the potency in terms of tubocurarine taken as 100 are shown in Table 10, page 256.

Tubocurarine was more potent than any of the compounds tested, excepting trishexatetrazonium. This compound was slightly more potent in paralysing respiration but was 3 times as potent as tubocurarine in causing neuromuscular block in the cat. Figure 78, page 257 shows respiratory paralysis due to trishexatetrazonium, dioctasulphonium and didecaazonium.

11. Experiments on isolated Perfused Rabbit and Kitten Hearts.

None of the new compounds in doses of up to 1.0 mg. had any depressant effect upon this preparation. A slight increase in the rate and amplitude of the heart beat was observed, and the outflow of the heart was slightly increased.

12. Experiments Carried Out Using the Isolated Rabbit Duodenum.

All the compounds showed stimulant properties on this preparation in dose range of 10 to 20 µg. per ml. This effect /

TABLE 10

Comparison of the Respiratory Paralysing Potency of Dipentasulphonium, Dihexasulphonium, Dihexaazonium, Dioctasulphonium, Dioctaazonium, Lidecasulphonium, Didecaazonium, Dihexone, Trishexatetrazonium, Trisdecatetrazonium and Decamethonium with Tubocurarine indicating the Individual and Average Respiratory Paralysing Doses in the Anaesthetised Cat.

Compound	Respiratory Paralysing Dose.		Potency (tubocurarine = 100)
	Individual (mg. per kg.)	Average (mg. per kg.)	
Dipentasulphonium	0.72, 0.80, 0.76	0.76	63
Dihexasulphonium	1.20, 1.15, 0.86	1.07	45
Dihexaazonium	1.0, 1.60, 0.96	0.85	56
Dioctasulphonium	1.10, 1.0, 0.68	0.93	51
Dioctaazonium	1.40, 1.08, 1.25	1.24	38
Didecasulphonium	1.28, 0.90, 1.40	1.19	40
Didecaazonium	0.64, 0.60, 0.48	0.57	84
Dihexone	1.12, 2.0, 2.0	1.70	28
Trishexatetrazonium	0.30, 0.28, 0.48	0.35	137
Trisdecatetrazonium	*	*	*
Decamethonium	0.15, 0.12, 0.17	0.15	320
Tubocurarine	0.40, 0.56, 0.50	0.48	100

* No estimate could be made due to violent muscular movements.

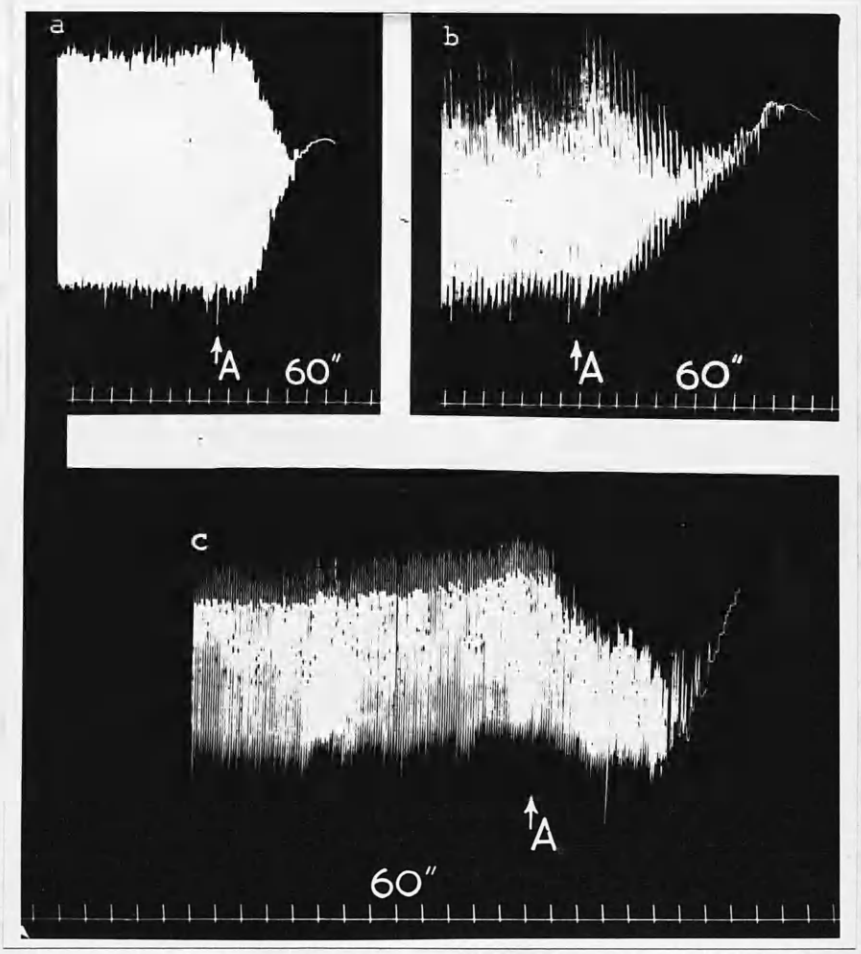

Figure 78. Cat. Pentobarbitone anaesthesia.

Record of respiratory movements of the epigastrium. Intravenous infusion of drug at the rate of 0.80 ml. per minute. Concentration of the drug, 0.20 mg. per ml.

(a) At A, infusion of trishexatetrazonium

(b) At A, " " dioctasulphonium

(c) At A, " " didecaazonium.

effect was effectively abolished by atropine (0.002 to 0.004 µg. per ml.). In some preparations dihexaazonium, dihexasulphonium, dioctasulphonium and dioctaazonium showed no stimulant effect at these dose levels. Tubocurarine (10 to 20 µg. per ml.) had no stimulant properties in any of the preparations. None of the compounds (10 to 20 µg. per ml.) had any observable effect on the peristaltic movements of the duodenum. Some of these results are shown in Figure 79 (page 259).

13. Experiments using Preparations of Isolated Guinea Pig Ileum.

Dipentasulphonium, dihexasulphonium, dioctasulphonium and dioctaazonium in doses up to 0.2 mg. per ml. had little or no stimulant effects upon this preparation. These compounds in doses of 0.10 to 0.20 mg. per ml. antagonised the contractions induced by 0.10 to 0.5 µg.per ml. of acetylcholine. Tubocurarine at similar dose levels had similar effects.

Dihexaazonium, dihexone, trishexatetrazonium, didecasulphonium, didecaazonium and trisdecatetrazonium showed direct stimulant effects at doses of from 0.10 to 0.20 mg. per ml. The stimulant properties of these compounds were /

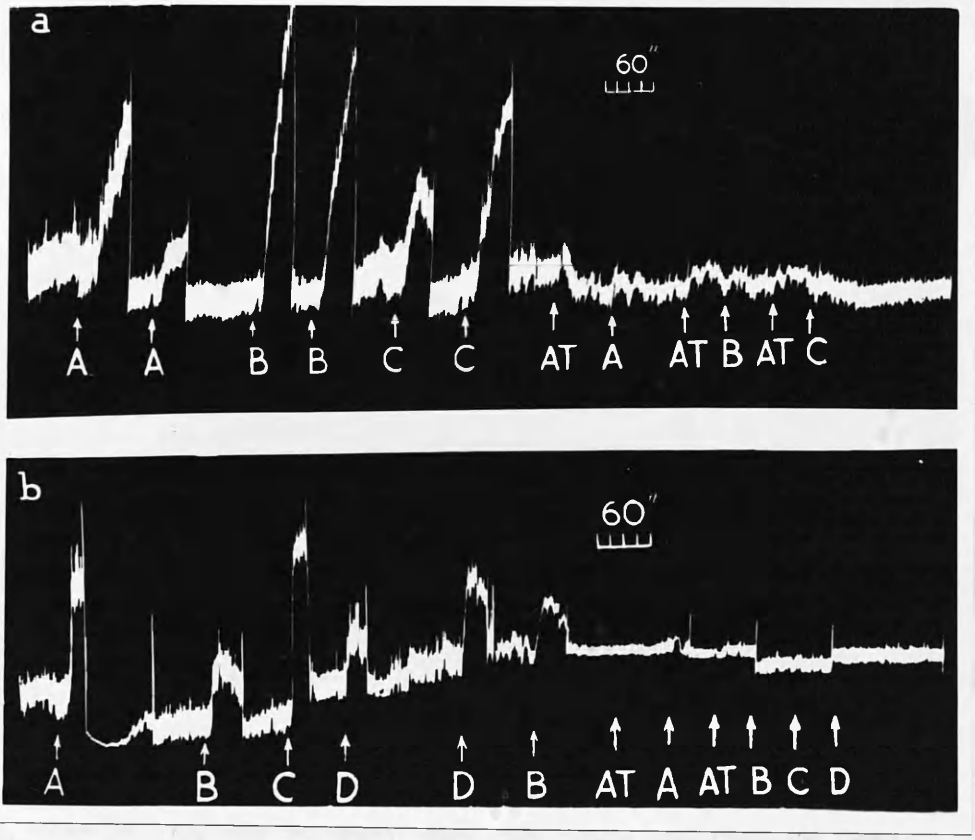

Figure 79. Isolated rabbit duodenum.

(a)	At A, trishexatetrazonium	10.0 µg. per ml.
	At B, dihexaazonium	10.0 µg. per ml.
	At C, dihexasulphonium	10.0 µg. per ml.
	At AT, atropine	0.002 µg. per ml.
(b)	At A, didecasulphonium	10.0 µg. per ml.
	At B, trisdecatetrazonium	20.0 µg. per ml.
	At C, acetylcholine	0.02 µg. per ml.
	At D, didecaazonium	20.0 µg. per ml.
	At AT, atropine	0.004 µg. per ml.

were blocked by atropine (0.002 µg. per ml.). Although they all showed stimulant properties, all of them antagonised acetylcholine-induced contractions of the guinea pig ileum when used in doses of 0.10 to 0.20 µg. per ml. Some typical results are shown in Figure 80 (page 261).

14. Isolated Perfused Rat Hindquarters.

Trisdecatetrazonium (0.20 to 1.0 mg.) caused constriction of the isolated perfused blood vessels of the hindquarters of the rat. Other compounds in the dose range of 0.25 to 1.0 mg. did not cause any significant alteration in the outflow of the perfusion fluid. Tubocurarine usually causes constriction of the blood vessels in this dose range. A typical experiment is shown in Figure 81 (page 262).

15. Mouse Test.

Median Lethal Dose (LD_{50})

The median lethal doses (LD_{50}) in mice following intraperitoneal injection of the compounds investigated, as well as of tubocurarine and decamethonium, are shown in Table 5, p.232. Following an intraperitoneal injection of /

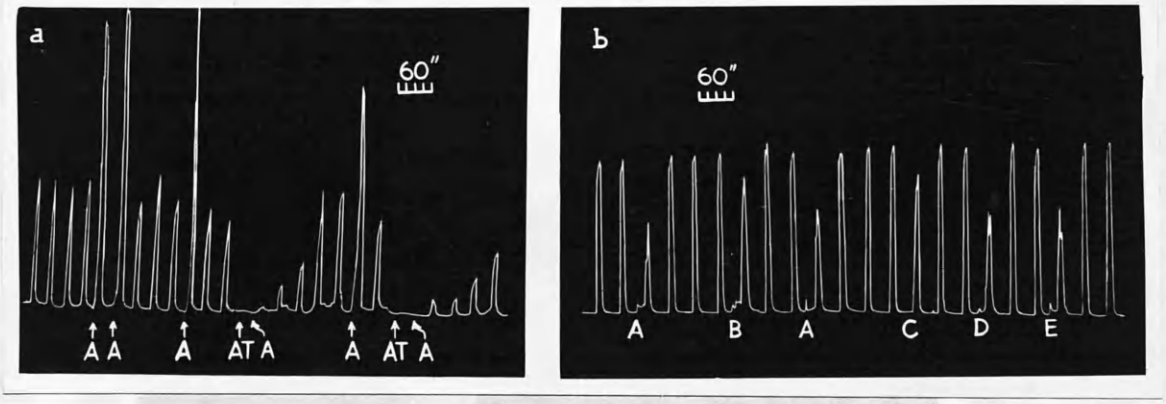

Figure 80. Isolated guinea pig ileum.

(a) Unlabelled contractions caused by acetylcholine 0.02 µg. per ml.

At A, trisdecatetrazonium 10 µg. per ml.

At AT, atropine 0.03 µg.per ml.

(b) All contractions caused by acetylcholine 0.20 µg. per ml. Addition of acetylcholine was preceded one minute earlier by:

At A, dihexasulphonium	0.20 mg. per ml.
At B, dihexasulphonium	0.10 mg. per ml.
At C, dipentasulphonium	0.10 mg. per ml.
At D, dipentasulphonium	0.15 mg. per ml.
At E, dipentasulphonium	0.20 mg. per ml.

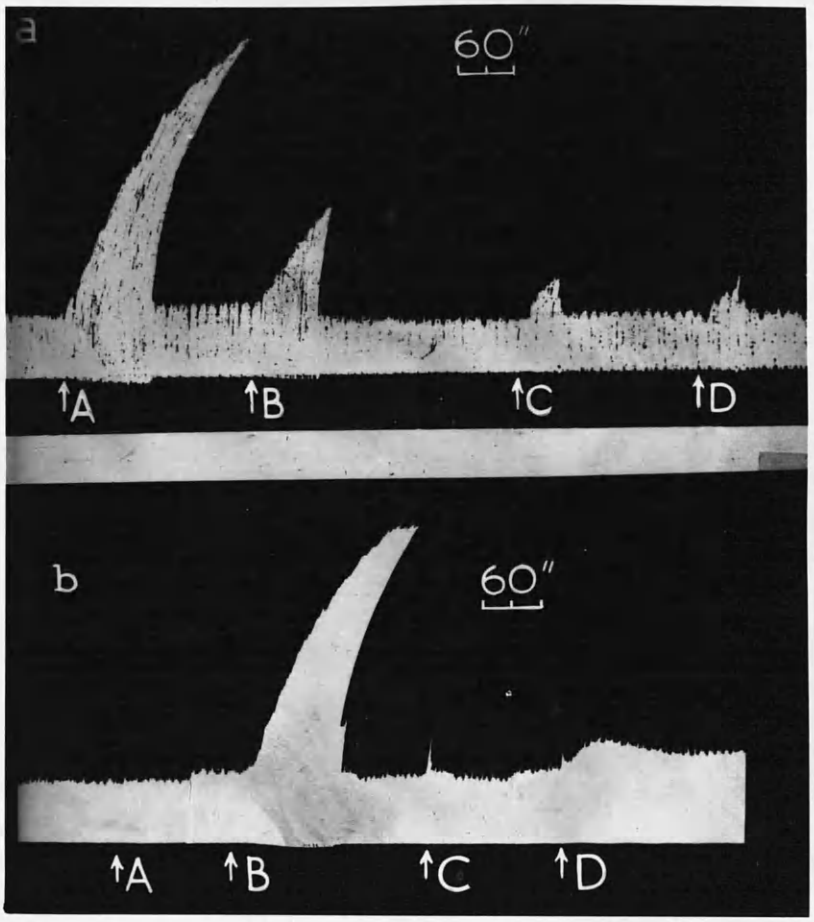

Figure 81. Perfusion of isolated rat hindquarters. Upstroke indicates vasoconstriction.

(a)	At A, trisdecatetrazonium	1.0 mg.
	At B, tubocurarine	1.0 mg.
	At C, trisdecatetrazonium	0.20 mg.
	At D, tubocurarine	0.50 mg.
(b)	At A, dipentasulphonium	1.0 mg.
	At B, tubocurarine	1.0 mg.
	At C, dihexasulphonium	1.0 mg.
	At D, tubocurarine	0.20 mg.

of a minimal lethal dose of all the compounds (excepting didecasulphonium, didecaazonium and trisdecatetrazonium), there was a rapid development of a typical flaccid paralysis. This was followed by failure of respiration which was the apparent cause of death because the heart continued to beat for some time after the respiration had ceased.

With didecasulphonium, didecaazonium and trisdecatetrazonium some stimulant properties were noticed. These accompanied the onset of paralysis. The mice had clonic convulsions and there was extensor spasm of the hindlegs before death.

The ratio of LD_{50} to PD_{50} as shown in Table 5, p.232 was highest for dioctasulphonium, whereas that of didecaazonium was the lowest.

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CHAPTER IV

DISCUSSION

The compounds investigated have been shown to possess neuromuscular blocking activity when tested upon different experimental preparations and intact animals. In the cat gastrocnemius muscle-sciatic nerve preparation, dipentasulphonium, dihexasulphonium, dihexaazonium, didecaazonium and didecasulphonium are about equipotent with tubocurarine. Trishexatetrazonium is three times more potent than tubocurarine, whereas dioctaazonium, dioctasulphonium, dihexone and tris-decatetrazonium are about one half, one third, one eighth and one half as potent as tubocurarine respectively. Dihexasulphonium trimethiodide, the methyl analogue of dihexasulphonium, is about one fifth as potent as the parent compound. In general, the potency of the trissulphonium compounds appears to be a little less than that of the corresponding trisazonium compounds when compared upon cat nerve-muscle preparations, although it is of interest that in the isolated kitten phrenic nerve-diaphragm preparation, dihexaazonium is about three times as potent as dihexasulphonium. The duration of the neuromuscular block in the cat gastrocnemius muscle-sciatic /

sciatic nerve preparation, caused by didecasulphonium, didecaazonium and trisdecatetrazonium - the compound in which the polymethylene chains separating the quaternary centres each consist of ten units - is from two to four times as long as the effect of tubocurarine given in equipotent doses. The other compounds appear to have approximately the same duration of effect as that seen with equipotent doses of tubocurarine. The reversibility of the neuromuscular block caused by the three compounds in which a decamethylene chain has been interposed between the quaternary centres was very often observed to be incomplete, but that obtained following the use of the other compounds was, like that following tubocurarine, completely reversible - even after the dose had been repeated several times.

The character of the neuromuscular block, produced by the individual members of this series of compounds in the cat, was not uniform. That the block caused by dipentasulphonium, dihexaazonium, dihexasulphonium and trisdecatetrazonium is entirely tubocurarine-like is shown by the following observations:

(1) Prior to depression of twitch height, there is no /

no evidence of muscular fasciculation or of potentiation of the muscle twitch amplitude.

(2) The block is effectively antagonised by edrophonium, neostigmine, eserine and decamethonium.

(3) Temporary but well-marked antagonism to the blockade is shown by adrenaline, potassium chloride and indirect tetanization of the muscle.

(4) The block is intensified by hexamethonium, ether and tubocurarine.

(5) The tension of a tetanus in the partly blocked muscle is poorly sustained.

(6) There is no evidence of a direct stimulant effect on the isolated rectus abdominis muscle of the frog, and antagonism is shown to acetylcholine or decamethonium-induced contractions of the rectus.

(7) A typical flaccid paralysis is seen when these compounds are injected into chicks.

The characteristics of the neuromuscular block produced in the cat by didecasulphonium, didecasazonium and trisdecatetrazonium are different from those described

above /

above.

(1) Neuromuscular block is always preceded by twitching and muscular fasciculation, and in the case of didecaazonium there is an increase in the twitch height.

(2) Edrophonium, neostigmine and eserine do not antagonise block due to these three compounds; edrophonium actually intensifies the block caused by didecaazonium and trisdecatetrazonium.

(3) Potassium chloride and indirect tetanization have no effect upon the intensity of the block.

(4) The block is reversed by hexamethonium and intensified by decamethonium.

(5) The tension of a tetanus is well maintained (except in the case of didecasulphonium where the tension is poorly maintained).

(6) Tubocurarine block is partially reversed by all of these compounds.

(7) An initial decamethonium-like contractural or spastic paralysis is seen in the chick.

In /

In the characteristics outlined above didecasulphonium, didecazonium and trisdecatetrazonium show typical decamethonium-like depolarizing properties. But in the isolated frog rectus abdominis muscle, these three compounds do not show any direct stimulant effects and all of them antagonise acetylcholine or decamethonium-induced contractions. When it can be demonstrated that these compounds possess all of the other typical depolarizing properties, lack of stimulant activity on the isolated frog rectus abdominis muscle is rather difficult to explain. Decamethonium which causes a marked nicotine-like contracture of the frog rectus has a much weaker stimulant effect than these compounds on the isolated rabbit duodenum and guinea pig ileum.

Dioctasulphonium, dioctazonium and dihexone have what appear to be predominantly tubocurarine-like properties and are similar to dipentasulphonium, dihexasulphonium, dihexazonium and trishexatetrazonium, whose quaternary centres are separated by polymethylene chains each consisting of five or six units. On the other hand, neuromuscular block caused by these compounds is not well antagonised by neostigmine and the tension of an indirect tetanus in the muscle, partly blocked

by /

by dioctasulphonium, is well sustained - a decamethonium-like effect.

Decamethonium and suxamethonium, and tubocurarine and gallamine all cause neuromuscular block, yet they bring about their effects by mechanisms which appear to be of two quite distinct types. Three of these compounds possess two quaternary centres, while gallamine has three. Decamethonium, suxamethonium and tubocurarine are methonium derivatives; gallamine is an ethonium compound. It has been suggested that the distance between the quaternary centres is critical and should be within the range of 12 to 14 Angström units. The function of the groups on the quaternary nitrogen atoms is less clear. It is tempting to suggest that methonium compounds are predominantly depolarizing and ethonium compounds have competitive (i.e. tubocurarine-like) properties, but tubocurarine, a bismethonium compound, causes neuromuscular block by competition, and some of the ethonium compounds described in this thesis cause block by depolarization in the cat. Several investigators have attempted to analyse what appears to be an important relationship between the ability to cause excitation /

excitation and the presence of methyl groupings. The early observations of Burn and Dale[1] revealed that a loss of excitatory properties, as well as the appearance of ganglion blocking activity, were associated with the replacement by ethyl groups of the methyl groups of the tetramethylammonium ion. The effect upon the mechanism of action of several series of neuromuscular blocking agents of ethyl substitution on the quaternary centres, as compared with that of methyl substitution, has been investigated by a number of workers[2] to 10. Riker and Wescoe[8] observed that replacement by methyl groups of the ethyl groups in the quaternary nitrogen centres of gallamine resulted in a loss of potency, but that this loss did not appear to be associated with a change in the basic mechanism of action. A significant loss of neuromuscular blocking potency did not take place until at least two of the ethyl groups upon each of the quaternary nitrogen atoms of gallamine had been replaced by methyl groups. Accordingly, the tris (diethylmethylaminoethoxy-) derivative, when tested upon the nerve muscle preparation of the cat, closely resembled gallamine in its neuromuscular blocking activity, whereas the tris (trimethylaminoethoxy-) derivative /

derivative had approximately one-fourth of the neuromuscular blocking potency of gallamine. Despite the alterations in potency produced by these changes, all of the compounds tested continued to exhibit (when given in appropriate doses) neuromuscular blocking activity indistinguishable in type from that shown by gallamine.

In another series of trisquaternary ammonium compounds, Kensler et alia[9] reported that the methonium derivatives were less potent than the ethonium derivatives, but did not observe any change from a uniform tubocurarine-like mechanism of neuromuscular blocking action to an effect resembling decamethonium. The ethonium derivative of benzoquinonium has been reported by Hoppe et alia[11] to be tubocurarine-like in its mechanism of action, whereas in the cat the methonium derivative was found to possess striking decamethonium-like properties.

In the polymethylenebisquaternary ammonium series, the effects of replacement of the methyl groups on the quaternary nitrogen atoms by ethyl groups were studied on the chicken nerve muscle preparation by Thesleff and Unna[7] /

Unna[7], and on the rat diaphragm and rabbit nerve-muscle preparation by Barlow, Roberts and Reid[5]. These investigators observed a reduction of potency as well as a change in mechanism of action when the methyl groups on the quaternary nitrogen atoms of decamethonium were replaced by ethyl groups.

In the series of polymethylene trisquaternary derivatives which the author of this thesis has investigated, the methyl analogue of dihexasulphonium was tested for neuromuscular blocking activity. While dihexasulphonium was found to be equipotent with tubocurarine on the cat, dihexasulphonium trimethiodide, the methonium analogue, was only one-fifth as potent as the parent compound or tubocurarine but the mechanism of action remained unaltered. This observation, which also shows that a striking change can take place in the properties of a neuromuscular blocking agent following an alteration in the nature of the substituents on the quaternary nitrogen atoms, is similar to that seen with gallamine and its methyl analogue but is contrary to that observed with the decamethylene bistrimethyl and triethyl compounds (decamethonium and decaethonium) of Barlow /

Barlow and his colleague.

In the polymethylene bisquaternary series studied by Theseleff and Unna[7] and Wien et alia[12], hexaethonium has been shown to have weak neuromuscular blocking activity when tested on the chicken gastrocnemius muscle-sciatic nerve preparation, and on the isolated rabbit phrenic nerve-diaphragm preparation. This compound has only weak ganglion blocking activity[12]. When the ethyl groups on the nitrogen atoms are replaced by methyl groups, the neuromuscular blocking activity is further reduced although the compound now becomes a potent ganglion blocking agent[12]. The molecules of dihexasulphonium and dihexasulphonium trimethiodide may be considered to be made up of two hexaethonium and two hexamethonium residues respectively, which are linked together through a sulphur atom. The reduction in the neuromuscular blocking potency of dihexasulphonium trimethiodide, the methyl analogue of dihexasulphonium, appears to be related to that observed when hexamethonium and hexaethonium are compared, although the absolute lack of ganglion blocking activity shown by dihexasulphonium trimethiodide (see page 250) is in sharp contrast to the potent ganglion blocking activity /

activity of hexamethonium.

In the polymethylene bisquaternary ammonium series the influence on the neuromuscular blocking potency of the length of the polymethylene chain separating the nitrogen atoms was studied by Barlow and Ing[13], Paton and Zaimis[14,15], and Thesleff and Unna[7] using the rabbit head drop method and the chicken and cat gastrocnemius muscle-sciatic nerve preparation. These workers observed that in the bisquaternary methonium series maximum potency was obtained with decamethonium. In the bistriethylammonium series Barlow and Ing[13] also observed that the activity gradually rose and was maximum in the compound having thirteen carbon atoms in the polymethylene chain. That the type of activity in the neuromuscular blocking agents is also influenced by the length of the polymethylene chains separating the quaternary centres was shown by Paton and Zaimis[15] in the cat, and by Thesleff and Unna[7] in the chicken gastrocnemius muscle-sciatic nerve preparation. They observed that pentamethonium and hexamethonium had tubocurarine-like activity, while decamethonium caused neuromuscular block by producing depolarization. A large series of bisquinoline and bis isoquinoline derivatives in which the nitrogen atoms were linked by a decamethylene chain /

chain has been studied by Collier and Taylor[16] and Taylor and Collier[17]. A study of the effects of altering the length of the polymethylene chain between the nitrogen atoms in the 6, 7-dimethoxy and 6,7,8-trimethoxy derivatives revealed that in rabbits and mice maximum neuromuscular blocking activity was obtained with the deca and undecamethylene members[18]. These compounds had a tubocurarine-like mechanism of action[19]. In the tris-sulphonium compounds, dipentasulphonium and dihexasulphonium, in which the quaternary centres are separated by polymethylene chains of five and six units respectively, both the mechanism of action and the potency are the same as those of tubocurarine in the cat. When each of the polymethylene chains is increased so as to contain eight units (as in dioctasulphonium) the compound still has predominantly tubocurarine-like properties, but a few transitional properties are seen. On the other hand the potency is diminished threefold when this compound is compared with compounds having five and six methylene units in between the two quaternary centres. When the polymethylene chains are further increased so that each contains ten units (as in didecasulphonium), the potency remains unaltered and is comparable with that of compounds having five and six membered /

membered polymethylene chains in between the quaternary centres, but the mechanism of action becomes entirely different. Neuromuscular block produced by these compounds in the cat is very similar to that caused by decamethonium except that the tension of an indirect tetanus in the partly blocked muscle is poorly maintained - a property shared with tubocurarine. In the trisazonium compounds a similar sequence of events takes place when the polymethylene chains separating the quaternary nitrogen atoms are increased in length. In dihexaazonium where the polymethylene chains each consist of six units, both the potency and mode of action are similar to those of tubocurarine. In dioctaazonium where the polymethylene chains each consist of eight units, the properties are similar to those of tubocurarine except that the block is not so well antagonised by neostigmine. On the other hand potency is reduced to about half of that of dihexaazonium. In didecaazonium, where the polymethylene chains are increased to contain ten units in each, the neuromuscular blocking activity is decamethonium-like. The potency, however, rises again and this compound is equipotent with the compounds which have five and six polymethylene units separating the quaternary centres.

In /

In the case of the compounds containing four nitrogen atoms similar phenomena can be observed when the polymethylene chain length is increased. Trishexatetrazonium has properties in the cat which are similar to those of tubocurarine, but is about three times more potent. This compound has polymethylene chains which consist of six units in each. Trisdecatetrazonium - another tetrazonium compound in which the quaternary nitrogen atoms are separated by polymethylene chains each consisting of ten units - has properties which are entirely different from those of tubocurarine and in the cat closely resembles decamethonium. The potency of this compound is about one half of that of tubocurarine. This difference in mechanism of action and potency in the tris and tetra-quaternary compounds appears to be due to the alteration in the length of the polymethylene chains between the nitrogen atoms. Whereas in the bistriethyl and bistrimethyl ammonium series, the potency increases from the compounds which have five or six membered polymethylene chains to reach a maximum in the compounds having ten (in the methonium series) and thirteen (in the ethonium series) carbon atoms separating the quaternary nitrogens[13,15], in the tris and tetraquaternary compounds which have been investigated, /

investigated, it has been observed that there is a decrease in potency with increase in chain length. This is apparently not in agreement with observations made in either the methonium or ethonium compounds of the bisquaternary series[13,14]. The change in the mechanism of action which is seen in the transition from the hexamethylene compound to the decamethylene compound in the trisquaternary series is similar to that observed in the transition from hexamethonium to decamethonium in the polymethylene bisquaternary trimethyl ammonium series[7]. The appearance of some transitional properties in dioctasulphonium and dioctaazonium - which have polymethylene chains of eight units - is a phenomenon similar to that observed in hepta- and octamethonium. In the bistriethyl ammonium series, however, the mechanism of action remains unaltered. Both hexamethonium and decamethonium possess tubocurarine-like properties. Since the compounds studied in this investigation were all ethonium derivatives, the type of activity was expected to remain unaltered in much the same way as in the bistriethyl series, and not to alter in the way observed in the bistrimethyl series of Barlow and Ing[13] and Paton and Zaimis[15].

These properties can be explained if it is assumed that the type of activity - decamethonium-like, transitional or tubocurarine-like - depends not so much on chain length or on the nature of the substituents on the quaternary nitrogen atoms as on the firmness with which the molecules are adsorbed on to the receptor surface. If depolarizing activity is associated with very firm adsorption, then compounds with several adsorbing groups might be expected to show decamethonium-like activity, while compounds with fewer strongly adsorbing groups should have tubocurarine-like activity. Even this theory does not explain everything but if a further assumption is made that the receptors on the post synaptic membrane are of two types, (a) of a strongly adsorbing type (S) and (b) of a less strongly adsorbing type (W), and that these follow a regular pattern then it is possible to speculate a little further. Supposing that the strongly adsorbing receptors are about 14 Å apart and that the less strongly adsorbing receptors be about midway between a group of four of the former arranged in a pattern as follows:-

S S

W

S S

and /

and that this pattern is reproducible indefinitely at the end-plate region. A compound like hexamethonium or hexaethonium might combine with one (S) and one (W), ((S) - (W)), - not a very strong adsorption and likely to be easily reversible. Decamethonium on the other hand should combine with two (S), ((S) - (S)), and so be firmly held to the receptor surface. The decamethylene compound with four quaternary nitrogen atoms would combine with four (S) receptors, ((S) - (S) - (S) - (S)). The hexamethylene compound with four quaternary nitrogen atoms should combine with two (S) receptors and two (W) receptors, ((S) - (W) - (S) - (W)) and would be more weakly held.

The functional relationship of tubocurarine to acetylcholine has focused attention upon the role played by the quaternary centres in the neuromuscular blocking agents. As has been mentioned in the introductory section many drugs such as atropine, quinine, strychnine etc. show a marked increase in neuromuscular blocking activity when their nitrogen atoms are quaternized. On the other hand, many nonquaternary (tertiary) ammonium compounds such as quinine, nicotine, erythroidine derivatives /

derivatives etc. have neuromuscular blocking activity. The occurrence of potent neuromuscular blocking activity in tertiary ammonium bases such as β-erythroidine, dihydro-β-erythroidine and the disappearance of this activity in β-erythroidine methiodide - the quaternary methonium salt of β-erythroidine - show that although the presence of quaternary centres in the molecule is often necessary for the neuromuscular blocking activity, their presence is not essential in every class of compound. While certain monoquaternary compounds (such as the C-toxiferines) can be shown to possess neuromuscular blocking activity, compounds having two quaternary centres have aroused the most interest in the study of the neuromuscular blocking agents. Some of the important compounds in this group (such as the straight chain compounds, quinoline and isoquinoline derivatives and others) have already been described in the introductory chapter of this section of the thesis.

A number of compounds with three and four quaternary centres and having neuromuscular blocking activity have been investigated, but all of the clinically useful neuromuscular blocking agents possess two quaternary centres (excepting /

(excepting gallamine which has three). Decamethonium, suxamethonium, benzoquinonium - all of which contain two quaternary nitrogens - are much more potent than gallamine which has three quaternary nitrogen atoms. Other compounds having three and four quaternary centres (see pages 116 and 117) have been examined only in experimental animals and found to be much less potent than tubocurarine. From these observations it is rather difficult to understand the role of the actual number of quaternary centres in neuromuscular blocking potency. On the other hand, when the number of quaternary centres in a particular series of compounds is increased there appears to be a definite relationship between the potency and the number of quaternary atoms in the molecule. Thus a series of ethers of β-hydroxyethyltriethyl ammonium, F.2512, F.2557 and F.2559 (gallamine) (Fig.82, page 287) having one, two and three quaternary centres respectively were shown by Bülbring and Depierre[20] to possess neuromuscular blocking activity in the rat phrenic nerve-diaphragm preparation in the ratio of 2: 20: 100, i.e. in this series there is an increase in potency with increase in the number of quaternary centres. In the series of compounds which have been investigated in this section /

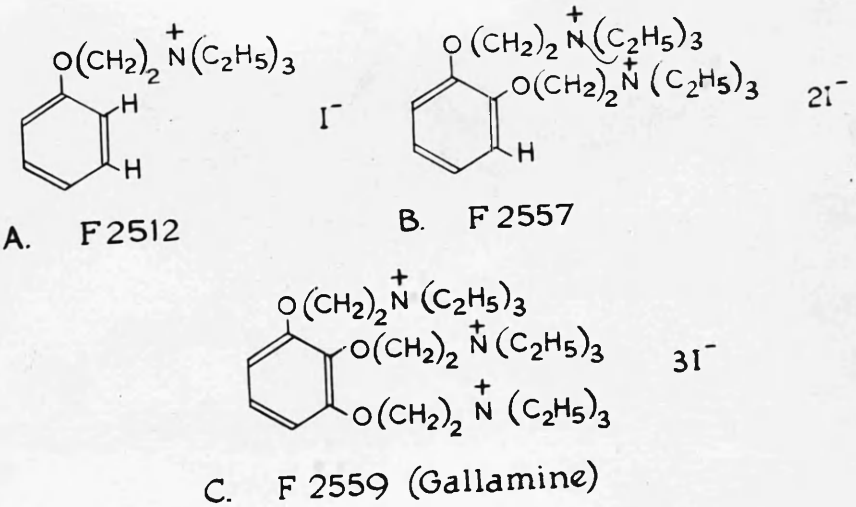

Figure 82. The structural formulae of F.2512, F.2557 and F.2559.

section of the thesis, the relationship between the number of quaternary centres and the neuromuscular blocking potency is not clear. In the hexamethylene members of the series, dihexone (a bisquaternary derivative with a sulphone ($-SO_2$) group in the centre) is about one-eighth as potent as tubocurarine in the cat. When the ($-SO_2$) group is replaced by $-\overset{+}{S}-$ or $-\overset{+}{N}-$, dihexasulphonium or dihexaazonium are produced respectively, both of which contain three quaternary centres and have a potency equal to that of tubocurarine in the cat. Further, when the overall chain length is increased so as to interpose another quaternary nitrogen atom, trishexatetrazonium is produced. This compound is three times as potent as tubocurarine in the cat. It has been shown that in the compounds dihexone, dihexaazonium and trishexatetrazonium (having two, three and four quaternary centres respectively), the ratio of neuromuscular blocking potency is of the order of 1: 8: 24, which is similar to that observed in the compounds F.2512, F.2557 and F.2559 (gallamine). On the other hand, in the decamethylene members of the present series trisdecatetrazonium - which has four quaternary nitrogen atoms - is only one half as potent in the cat as didecaazonium, a compound with three quaternary nitrogen centres./

centres. When the potency does appear to be related to the number of quaternary atoms as in the hexamethylene members of the series, it is rather difficult to explain why it should not also hold true for the decamethylene members.

The replacement of the central quaternary nitrogen atom in dihexaazonium, dioctaazonium and didecaazonium by a quaternary sulphur atom appears to have some slight influence on the neuromuscular blocking potency of these compounds. In the cat dihexasulphonium appears to be slightly less potent than dihexaazonium; it is more potent in the rabbit and mouse (Table 9, page 247). In the octamethylene compounds, the trissulphonium derivative, dioctasulphonium, is also slightly less potent in the cat than the trisnitrogen derivative, dioctaazonium, but is more potent in the rabbit and mouse (see Table 9). In addition, the well-maintained tension of a tetanus in the partly blocked muscle, and the less well-marked antagonism by neostigmine seen in dioctasulphonium may be due to the replacement of the quaternary nitrogen atoms by quaternary sulphur atoms. In the case of the decamethylene compounds, the trissulphonium compound, didecasulphonium, has some properties which enable it /

it to be distinguished from the trisazonium compound, didecaazonium. In the first place, following administration of didecasulphonium, there was no initial potentiation of the twitch height of the gastrocnemius muscle of the cat in response to indirect stimulation via the sciatic nerve, which was usually observed in the case of didecaazonium. Secondly, the tension of a tetanus in the muscle partly blocked by didecasulphonium was poorly sustained. This was in contrast to the well-maintained tension of a tetanus of the partly blocked muscle following administration of didecaazonium. Thirdly, the neuromuscular block caused by didecasulphonium was either slightly reversed or not influenced by the administration of neostigmine or edrophonium, whereas neostigmine had no effect upon, and edrophonium intensified the neuromuscular block caused by didecaazonium. Further, didecasulphonium was equipotent with didecaazonium in the cat, but slightly more potent in the rabbit and mouse (Table 9, page 247). These differences in the properties of didecasulphonium and didecaazonium appear to be due to the replacement of the central nitrogen atom by a sulphur atom, because the two compounds are otherwise structurally identical. The observed change in potency of the trissulphonium /

trissulphonium and trisazonium compounds, although only slight in different species, is not in entire agreement with the observation of Walker[21] who, using the rabbit head drop test, found a reduction in neuromuscular blocking potency by replacing the quaternary nitrogen atom in decamethonium by a quaternary sulphonium atom.

Other factors may come into play and it is possible that the degree of solvation of the quaternary centres is important in determining both potency and type of effect. Unfortunately, no data is yet available but it seems possible that a cationic group which carries a large number of molecules of water is likely to be held less firmly to the receptor surface than one which has a small "solvation shell".

From a pharmacological standpoint an ideal drug is one which exerts a specific effect upon a given organ or system without producing other effects in other parts of the body. Many compounds have been described which possess considerable neuromuscular blocking potency but, in most instances, they lack specificity of action. They may, for example, block nerve impulse transmission in the autonomic nervous system either at the ganglia or at the periphery, /

periphery, and they may cause liberation of histamine or have a marked depressant effect upon respiration. In some instances these secondary effects are of sufficient intensity to cause inconvenience in the routine clinical application of the drugs. Lack of complete specificity of neuromuscular blocking activity among the competitive type of neuromuscular blocking agents is illustrated by the fact that tubocurarine possesses not inconsiderable ganglion blocking activity[22] together with the capacity to liberate histamine[23,24,25]. These properties are quite marked in dimethyl tubocurarine, although they are less noticeable then in tubocurarine[26,27]. Although laudexium shows little clinical evidence of any histamine release and ganglion blockade[28], its neuromuscular blocking effect is more prolonged than that caused by tubocurarine[19,29], and neostigmine even in excessive doses is a less effective antagonist[30]. Gallamine, although lacking the properties which cause release of histamine[31] and ganglion blockade[20], has the disadvantage of causing sinus tachycardia due to its selective atropine-like parasympatholytic action on the cardiac vagus[32,33,34]. Decamethonium possesses no significant ganglion blocking[35] or histamine liberating activity[35] /

activity[35] at therapeutic dose levels, but it cannot be considered to be an ideal neuromuscular blocking agent due to its depolarizing action[15] and the lack of suitable antagonists or antidotes. Of the compounds investigated in this section none shows ganglion blocking activity as measured by the response of the nictitating membrane of the cat, even when given in doses more than five times those needed to cause complete neuromuscular block. Didecaazonium, however, causes a contraction of the nictitating membrane. Didecaazonium and trisdecatetrazonium cause a fall in the blood pressure level of the pentobarbitone-anaesthetised cat in a similar way to that observed when tubocurarine is used. Other compounds have no significant effects upon the blood pressure, even when doses many times more than those needed to produce complete paralysis in the cat's nerve-muscle preparation are used. A fall of blood pressure following tubocurarine is believed to be due to its histamine liberating property and also to its inhibitory effect on sympathetic ganglionic transmission[24,25,36].

Compounds having the following properties appear to be suitable for further investigation and clinical trial:

(1) Those /

(1) Those with potent tubocurarine-like neuromuscular blocking activity.

(2) Those which do not appreciably raise or lower the blood pressure level.

(3) Those which do not alter the heart rate.

(4) Those which do not cause histamine release.

(5) Those with less respiratory paralysing effects than tubocurarine.

Some of the compounds investigated, namely, dipentasulphonium, dihexasulphonium, dihexaazonium and trishexatetrazonium, the properties of which have been reported in this thesis appear to possess, in the cat, certain desirable features. On the other hand, the species variations in potency seen in the neuromuscular blocking agents[15] is a complicating factor, and their potency in man cannot be assumed from that obtained by experiments on animal. Species variations among the competitive neuromuscular blocking agents is relatively smaller than that found with depolarizing agents. There is, in addition, a considerable variation in the sensitivity of various muscles of the same species to the action of both competitive and depolarizing /

depolarizing neuromuscular blocking agents[15].

The considerable species variations in potency among the new compounds investigated and described in this thesis is shared with the other neuromuscular blocking agents (see Table 9 , p. 247). Of these compounds, only dihexasulphonium has been studied on dog nerve-muscle preparation by Forrester and his colleagues[37] who reported it to be equipotent with tubocurarine. Although this observation is similar to that found in the cat, recent clinical trials and trials upon human volunteers have shown dihexasulphonium to be much less potent than tubocurarine[38]. It, therefore, appears that the final answer with respect to potency of these compounds in man can only be obtained by careful evaluation in man himself.

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CHAPTER V

SUMMARY

In the introductory chapter of this section of the thesis, the history, sources, pharmacological properties and clinical uses of the alkaloids of curare have been described.

The mechanisms of neuromuscular block and the classification of the naturally occurring and synthetic neuromuscular blocking agents have been briefly reviewed.

A short account of the properties of the clinically useful neuromuscular blocking agents is also given.

The chemical structures of a new series of tris- quaternary and tetraquaternary ethonium compounds, the properties of which have been investigated in this thesis, are also described.

In Chapter II of this section, the various experimental procedures used in the investigation of the properties of these new compounds are described. These involve the use of anaesthetised and conscious animals, as well as isolated tissues.

In Chapter III of this section, the results of an

investigation/

investigation of the properties of the new trisquaternary and tetraquaternary compounds are described.

It has been shown that all of the compounds possess neuromuscular blocking activity on the cat, as well as on other preparations. Some of them possess a potency equal to, or greater than, that of tubocurarine itself.

While some of the compounds investigated shared the properties of tubocurarine, others showed typical decamethonium-like activity.

Like other neuromuscular blocking agents, the compounds investigated showed wide species variations in their activity.

In Chapter IV of this section, the influence of the length of the polymethylene chain separating the quaternary atoms, the effect of multiplication of the number of quaternary centres, and the effect of substitution of ethyl groups for methyl groups on these, is discussed. The effects of these changes and mechanism of action of these new compounds is also discussed and comparisons made with other series of compounds.

It has been shown that an increase in chain length in /

in the trisquaternary and tetraquaternary compounds investigated decreases the potency and alters their type of activity from being tubocurarine-like to being decamethonium-like.

The only methonium compound investigated, the methyl analogue of dihexasulphonium, shows less activity than the ethonium analogue, although the type of activity remains unaltered.

PART II

B. THE PHARMACOLOGY OF SOME HYDROXYBENZYL-ISOQUINOLINE DERIVATIVES.

CHAPTER I

INTRODUCTION

The compounds I and II (Fig.83, I and II, p.305) were synthesised by Jeffreys[1] during studies on the constitution of the ditertiary alkaloid isochondrodendrine (Fig.83,III). These compounds are related chemically to armepavine[2] (Fig.83, IV), coclaurine[3] (Fig.83,V), isococlaurine[3] (Fig.83, VI), and magnocurarine[4] (Fig.83,VII). Isococlaurine and magnocurarine have been reported to possess some curare-like neuromuscular blocking activity. Coclaurine is inactive. Compounds I and II are possible degradation products of isochondrodendrine which is chemically somewhat similar to tubocurarine (a bis-quaternary base). It was thought worthwhile to test these compounds for curare-like activity, and also to test the corresponding quaternary salts, especially since magnocurarine, which is a quaternary base, has been reported to possess some neuromuscular blocking activity[4]. Some comparisons have been made with tubocurarine and with a berberine-like compound (Fig.83, VIII) which, like tubocurarine, contains an isoquinoline ring system[5].

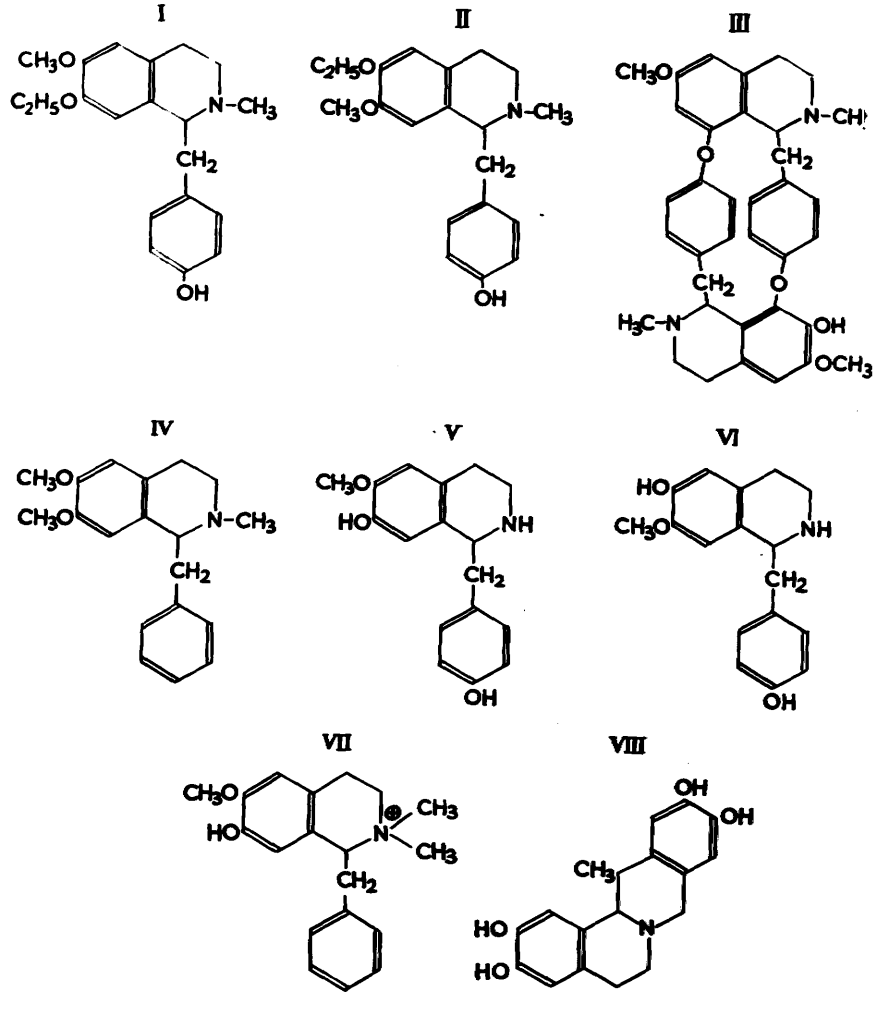

Figure 83. The structural formulae of I, compound I; II, compound II; III, isochondrodendrine; IV, armepavine; V, coclaurine; VI, isococlaurine; VII, magnocurarine and VIII, compound VIII.

The systematic chemical names of Compounds I, II and VIII are given on pages 307 and 308.

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CHAPTER II

A. MATERIALS.

Drugs used in the investigation described in this section of the thesis, together with their shortened names, were as follows:-

(1)	Acetylcholine chloride	is described as	acetylcholine
(2)	(-)-Adrenaline hydrochloride	" " "	adrenaline
(3)	(-)-Noradrenaline hydrochloride	" " "	noradrenaline
(4)	(+)-tubocurarine chloride	" " "	tubocurarine
(5)	Decamethonium iodide	" " "	decamethonium
(6)	Histamine acid phosphate	" " "	histamine
(7)	5-hydroxytryptamine creatinine sulphate	" " "	5-hydroxytryptamine
(8)	Phentolamine	" " "	phentolamine
(9)	Dibenamine	" " "	dibenamine
(10)	Hydergine	" " "	hydergine.

The isoquinoline compounds investigated were:-

(a) (i) (±)-1-(4^1-hydroxybenzyl)-2-methyl-6-methoxy-7-

ethoxy /

ethoxy-1 : 2 : 3 : 4 - tetrahydro-isoquinoline (Fig.83,I, p.305). This is described as Compound I.

(ii) The dextrorotatory isomer of Compound I is described as Compound ID.

(iii) The laevorotatory isomer of Compound I is described as Compound IL.

(iv) The quaternary derivative (methochloride) of Compound I is described as Compound IQ.

(b) (i) (±)-1-(4^1-hydroxybenzyl) - 2 - methyl - 7 - methoxy - 6 - ethoxy - 1 : 2 : 3 : 4 - tetrahydro-isoquinoline (Fig. 83,II, p.305) is described as Compound II.

(ii) The laevorotatory isomer of Compound II is described as Compound IIL.

(iii) The quaternary derivative (methochloride) of Compound II is described as IIQ.

(c) A compound related to tetrahydroworenine , 5 : 6 : 13 : 13a - tetrahydro - 2 : 3 : 10 : 11 - tetrahydroxy-8H - dibenzo - (a, g) - pyridocholine hydrochloride (Fig.83,VIII, p.305) /

is described as Compound VIII.

The tertiary compounds are insoluble in water but can be dissolved in dilute hydrochloric acid at about pH 5. The corresponding quaternary derivatives are freely soluble in water.

The composition of the different physiological saline solutions used is shown in Table 12, page 336.

B. EXPERIMENTAL

1. Frog Rectus Abdominis Muscle.

The experimental procedure was similar to that described on page 33 of this thesis. Reproducible submaximal contractions were obtained to acetylcholine (0.10 to 0.20 µg. per ml.), and to decamethonium (2.0 to 2.5 µg. per ml.) added at five minute intervals and left in contact with the tissue for 1.5 minutes. Drugs under investigation in doses of 12.5 to 250 µg. per ml. were added a half-minute before the next addition of acetylcholine or decamethonium, and left in contact with the tissue for two minutes. The next addition of the drug was not made until the response of the muscle to a similar /

similar dose of acetylcholine or decamethonium had regained its original magnitude.

2. Frog Sartorius Muscle-Ischiad Nerve Preparation.

METHOD.

The sartorius muscle of the frog lies immediately under the skin covering the ventral aspect of the thigh.

Frogs of either sex were decapitated, pithed and pinned in the supine position upon a dissecting board. The skin over both thighs was removed and the rectus abdominis muscles carefully freed at their points of insertion into the pelvis. Having thus exposed the upper tendinous attachments of the sartorius muscles, a blunt seeker was used to free both the proximal and distal attachments of the muscle from the underlying tissues. Cotton threads were tied around both ends of the muscle which was then cut free from its attachments to the femur and the pelvic girdle. A slight tension was applied to the muscle by means of the threads tied at each end. At this stage of the dissection, the muscle remained attached to the underlying tissues by virtue of the soft connective tissues running along its longitudinal axis. Using the blunt dissection technique, the main body of the /

the muscle was freed from the underlying muscles in the leg, except for the bridge of connective tissue in which runs the thread-like ischiad nerve.

The ischiad nerve enters the sartorius muscle roughly midway between the two tendinous attachments of the latter with the femur and pelvic girdle. Having freed and then tied the ends of the muscle, the path of the nerve from the muscle to its junction with the sciatic nerve was traced. At this point, a fine cotton thread was tied around the nerve which was then very gently freed from the connective tissue surrounding it. When the nerve had been freed, the preparation was removed from the leg. By means of a fine needle attached to the thread at its proximal end, about one cm. of the nerve was drawn through a rubber membrane into a J-shaped fluid electrode (Fig. 84, p. 312) filled with double glucose frog Ringer's solution (page 336). One end of the muscle was attached to an oxygen delivery tube, the other to a Starling-type heart lever fitted so as to record the contraction of the muscle upon a smoked surface. The completed preparation was then lowered into a bath (Fig. 84, p. 312) containing 30 ml. of double glucose frog Ringer's solution at room temperature. The muscle /

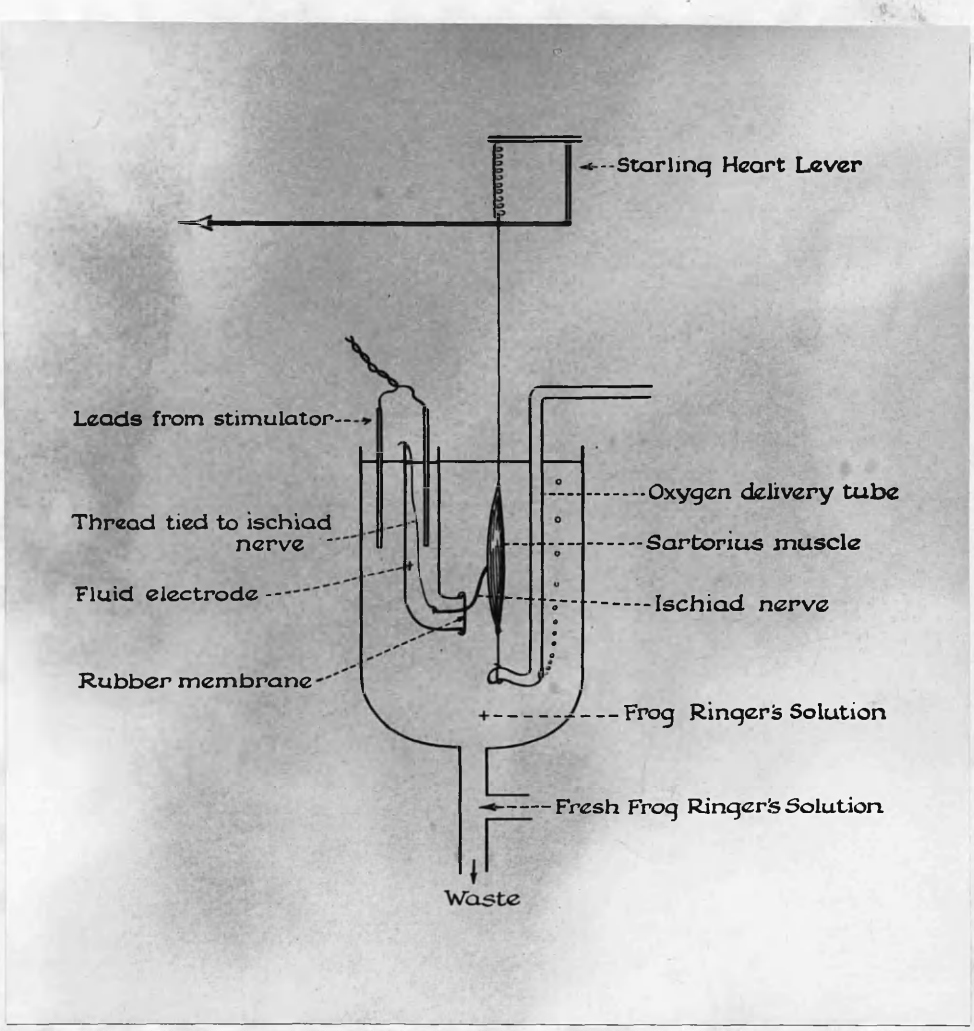

Figure 84. Diagram of the apparatus used for recording contractions of the frog sartorius muscle produced by electrical stimulation of the ischiad nerve.

muscle was stimulated indirectly through its nerve, and also directly by means of a Dobbie McInnes square wave stimulator delivering 12 impulses per minute. The duration and voltage of the impulses were 1.0 msec. and 10 to 15 volts respectively. One terminal of the stimulator was connected to an electrode immersed in the bath fluid, the other to the fluid electrode. It was found that this preparation could be stimulated continuously for up to 6 hours without any signs of fatigue becoming evident.

When the twitch height of the muscle in response to indirect stimulation was uniform, the drug under investigation or tubocurarine (in the appropriate dilution with the frog Ringer's solution) was added to the bath and the effects observed for a period of three minutes. The fluid in the bath was then replaced several times by fresh frog Ringer's solution from the reservoir. Sufficient time was allowed for the twitch height of the muscle to regain its original magnitude before the next addition of the drug. In any one experiment the voltage, pulse width and frequency of stimulation were constant.

3. Rat Phrenic Nerve-Diaphragm Preparation.

The /

The experimental procedure was similar to that described on page 152 of this thesis. The bath solution was gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The muscle was stimulated indirectly using square pulses at a frequency of 6 per minute at 12 volts; pulse width 1.0 msec. Drugs were added to the bath and kept in contact with the muscle for three minutes.

4. Isolated Perfused Kitten Heart.

The preparation was set up in a manner similar to that described on page 2[illegible] of this thesis. Drugs dissolved in Locke's solution were injected into the rubber tubing attached to the aortic cannula. The heart rate was counted by direct visual observation, and the outflow was measured by collecting the perfusate for a period of one minute at five minute intervals after addition of the drug.

5. Guinea Pig Ileum.

The preparation was set up in a manner similar to that described on page 29 of this thesis. About four cm. of the terminal ileum was suspended in a four ml. bath containing oxygenated Tyrode's solution at 31°C.

Reproducible /

Reproducible submaximal contractions were obtained by adding acetylcholine (0.05 to 0.20 µg. per ml.) or histamine (0.05 to 0.20 µg. per ml.) at three minute intervals and leaving them in contact with the tissue for ninety seconds. Drugs were added either thirty or sixty seconds before the next addition of acetylcholine or histamine. The total time of contact of drug with tissue was sixty or ninety seconds.

6. Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

The preparation was set up in a manner similar to that described on page 144 of this thesis. Anaesthesia was induced by ether and maintained by intravenous chloralose (80 mg. per kg.). Stimulation of the sciatic nerve was by means of square pulses at a frequency of 6 per minute at 12 volts; pulse width 2.5 msecs.

7. Cat Blood Pressure.

In cats weighing 2.0 to 3.5 kg. anaesthesia was induced by ether and maintained by intravenous chloralose (80 mg. per kg.). The preparation was set up in a menner similar to that described on page 11 of this thesis. The blood pressure was recorded from the common carotid artery, and drugs were administered *via* the cannulated external /

external jugular vein. In some experiments, spinal cats were used (see page 17).

8. Mouse Test.

Drugs were given by intraperitoneal injection into groups of mice weighing from 40 to 50 g. (see also page 164 of this thesis).

CHAPTER III

RESULTS

1. Frog Rectus Abdominis Muscle.

All the compounds tested in doses of from 12.5 to 250 µg. per ml. showed antagonism to acetylcholine-induced contractions of the rectus, and a graded effect was seen (see Figs. 85 and 86, pages 318 and 319). The potency of the compounds, as compared with that of tubocurarine, is shown in Table 11, page 320.

None of the compounds tested had any direct stimulant effects upon the rectus muscle. All of them antagonised contractions induced by decamethonium, but high doses of the order of 0.05 to 0.1 mg. per ml. were needed. The effects were additive with those of tubocurarine.

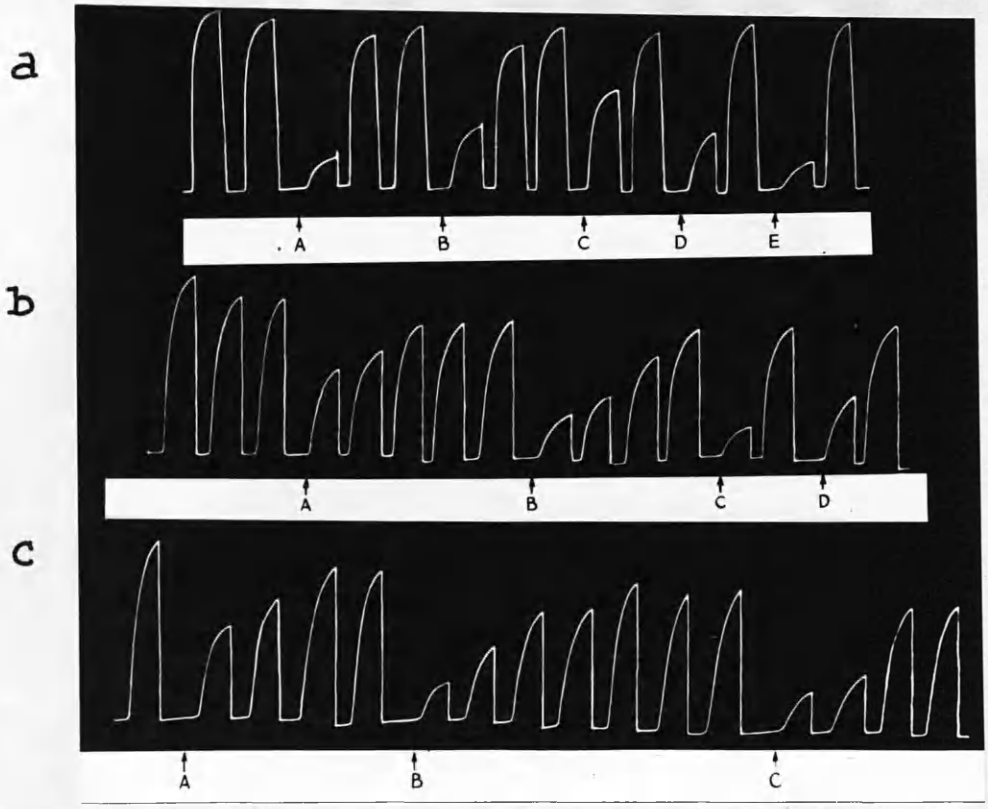

Figure 85. Isolated frog rectus abdominis muscle.

(a) At A, B and C Compound VIII 100, 50 and 25 µg. per ml. respectively.

At D and E tubocurarine 0.6 and 1.0 µg. per ml. respectively.

(b) At A and B Compound II 100 and 200 µg. per ml. respectively.

At C and D tubocurarine 0.4 and 0.25 µg. per ml. respectively.

(c) At A, B and C Compound I 25, 50 and 37.5 µg. per ml. respectively.

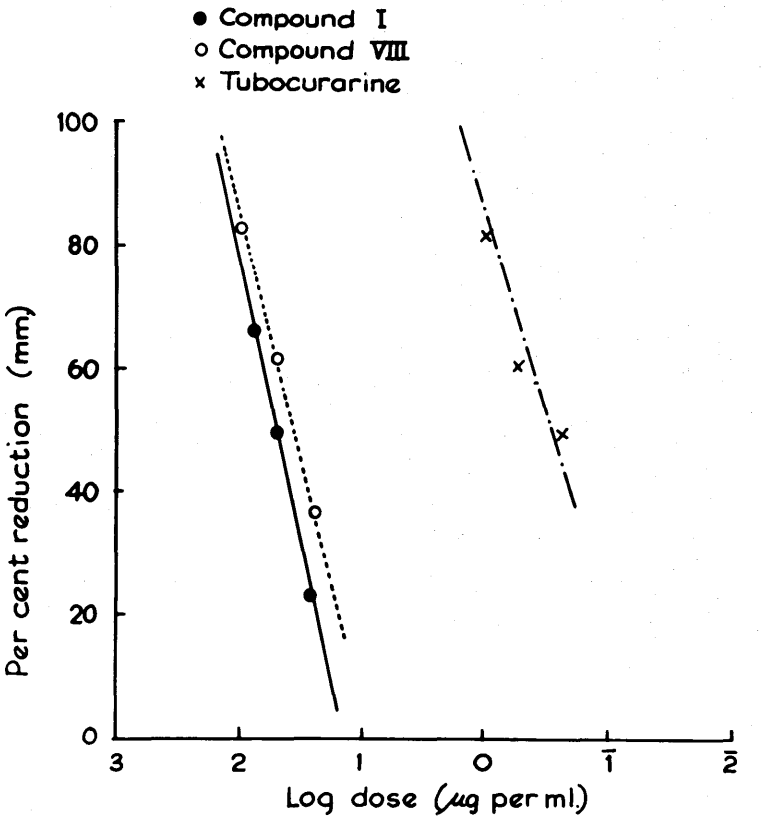

Figure 86. Isolated frog rectus abdominis muscle. Relation of log dose of compounds I, VII and tubocurarine (abscissa) to the percentage reduction (ordinate) of the height of contraction produced by the same dose of acetylcholine.

The Relative Potencies of Compounds I, IL, ID, IQ, II, IIL, IIQ and VIII and Tubocurarine on the Frog Rectus Abdominis Muscle

Compound	Potency in terms of Tubocurarine = 100
I	2.0
IL	0.25
ID	0.25
IQ	3.30
II	0.25
IIL	0.25
IIQ	2.0
VIII	1.0

2. Frog Sartorius Muscle-Ischiad Nerve Preparation.

Only Compounds I, II and VIII were tested on this preparation. These three compounds inhibited the response of the muscle to indirect stimulation. They were much less potent than tubocurarine. After washing, there was complete recovery of the twitch height (Fig.87, p.322).

3. Rat Phrenic Nerve-Diaphragm Preparation.

On this preparation, the compounds tested had only very weak curare-like neuromuscular blocking activity. Compounds IQ and IIQ, the quaternary salts of Compounds I and II, were about 5 times more potent than the tertiary bases from which they were prepared. Complete neuromuscular block could be produced, but high doses of about 0.25 mg. per ml. had to be used. After the response to indirect stimulation had failed, the muscle still responded to direct stimulation. The tertiary bases were about 250 times less potent (Fig. 88, p.323) and Compound VII had about one-fortieth of the potency of tubocurarine (Fig. 89, p.324).

4. /

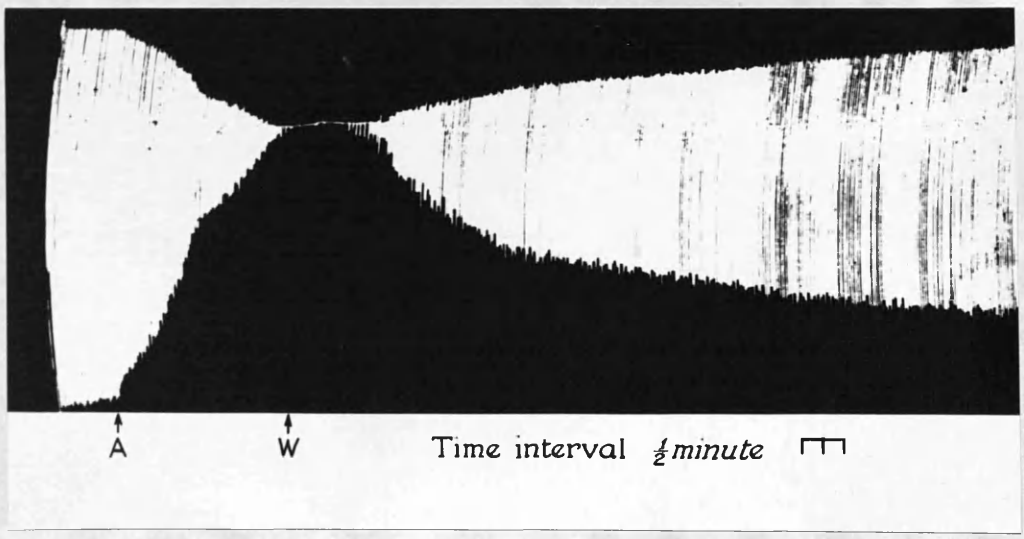

Figure 87. Isolated frog sartorius muscle-ischiad nerve preparation. Contractions due to indirect stimulation via the ischiad nerve at 12 volts, pulse width 0.5 msec., 12 impulses per minute.

At A, Compound II, 62.5 µg. per ml.

At W, Wash out.

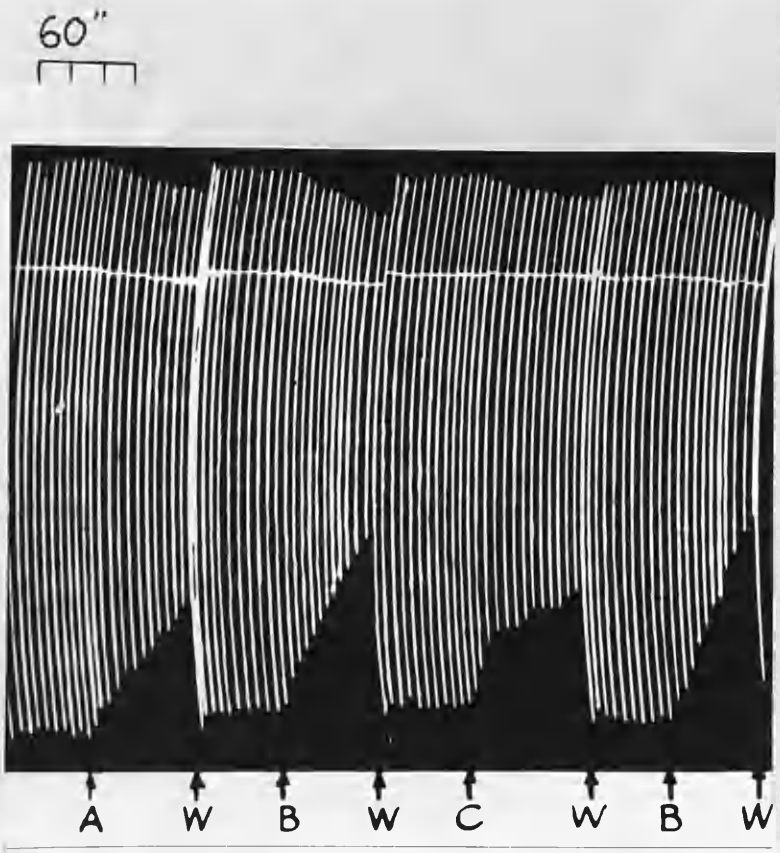

Figure 88. Isolated rat phrenic nerve-diaphragm preparation. Contractions due to indirect stimulation via the phrenic nerve at 12 volts, pulse width 1 msec., 6 impulses per minute.

At A, tubocurarine 2.5 µg. per ml.

At B, tubocurarine 3.0 µg. per ml.

At C, Compound I 0.60 mg. per ml.

At W, Wash out.

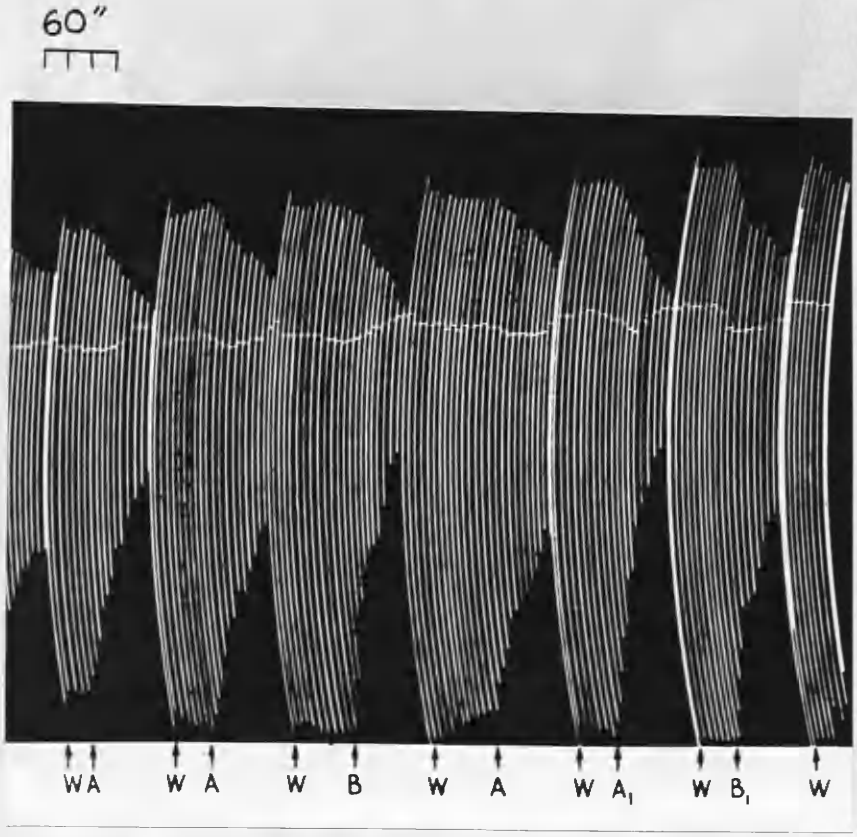

Figure 89. Isolated rat phrenic nerve-diaphragm preparation. Contractions due to indirect stimulation via the phrenic nerve at 12 volts, pulse width 1 msec., 6 impulses per minute.

At A, tubocurarine 5 µg. per ml.

At A_1, tubocurarine 10 µg. per ml.

At B, Compound II 0.30 mg. per ml.

At B_1, Compound II 0.25 mg. per ml.

At W, Wash out.

4. Isolated Perfused Kitten Heart.

10 to 20 µg. of Compound IIL increased the rate and amplitude of the heart; 20 µg. of Compound IL had a similar effect. These effects were reversible. Compounds II and VIII had no effects in doses of up to 0.5 mg.

5. Guinea Pig Ileum.

All of the compounds tested inhibited contractions of the ileum induced by acetylcholine or histamine, but doses of the order of 0.15 to 0.3 mg. per ml. were needed and a graded antagonism was not seen. No direct stimulant or depressant effects upon the ileum were seen.

6. Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

The quaternary bases (IQ and IIQ) at doses of 1 to 2 mg. per kg. did not depress the response of the muscle to indirect stimulation. Similarly, the tertiary bases (I, II and VIII) at 1 to 4 mg. per kg. had no neuromuscular blocking activity. In every case there was, however, some spontaneous muscular twitching following drug administration.

7. /

7. Cat Blood Pressure.

Compound I in doses of from 1.0 to 2.0 mg. per kg. always caused a biphasic depressor-pressor response. The fall in blood pressure was marked but the ensuing rise was small. (Fig.90, p.327). Compound ID and IL showed qualitatively similar effects, but both depressor and pressor components were less. When Compound I was given after bilateral mid-cervical vagotomy, the depressor component was lost and only a rise in blood pressure was seen (Fig.90, p.327). In a few experiments Compound IL caused only a rise in the blood pressure. Compound IQ (0.5 to 1.0 mg. per kg.) caused a rise in blood pressure (Fig.91, p.328). Compounds II and IIL, at dose levels of 1.0 to 2.0 mg. per kg., caused respectively a slight rise in blood pressure, and a biphasic depressor-pressor effect. Compound IIQ (0.5 to 1.0 mg. per kg.) also caused a rise in blood pressure (Fig.91, p.328). Compound VIII (1.0 to 2.0 mg. per kg.) had no effects on the blood pressure level. The pressor responses were not blocked by phentolamine (up to 5 mg. per kg.), dibenamine (up to 25 mg. per kg.) or hydergine (up to 0.4 mg. per kg.). These compounds did not antagonise the characteristic effects on the blood pressure of acetyl-choline /

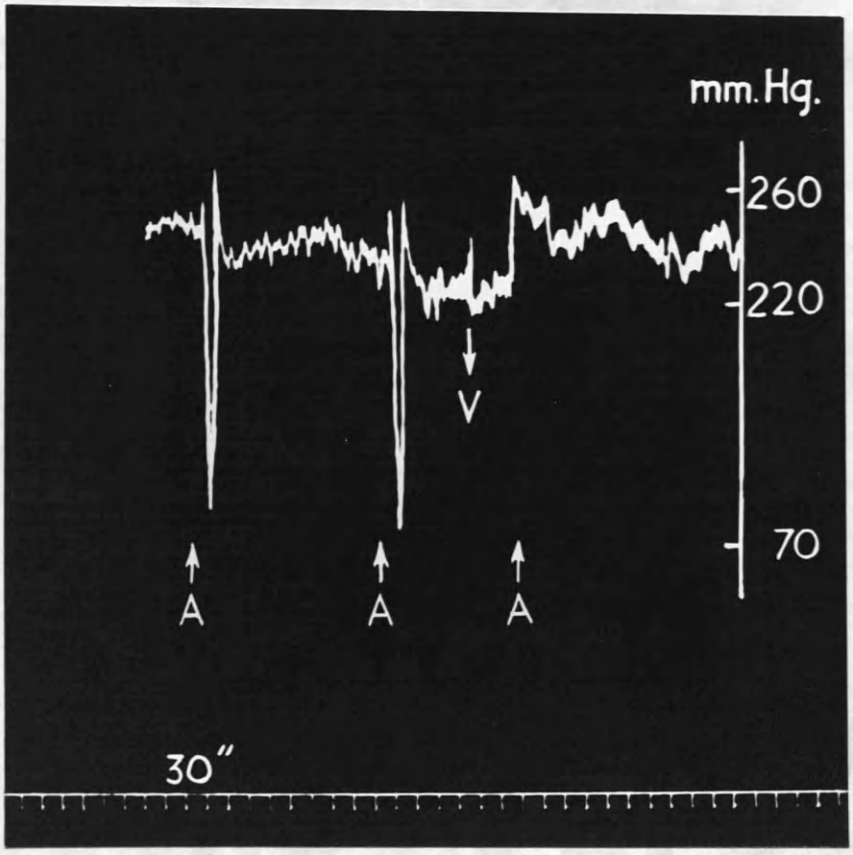

Figure 90. Cat. Chloralose anaesthesia. Blood pressure recorded from common carotid artery. Drugs administered intravenously followed by 4 ml. saline in each case.

At A, Compound I 0.80 mg. per kg.

At V, bilateral midcervical vagotomy.

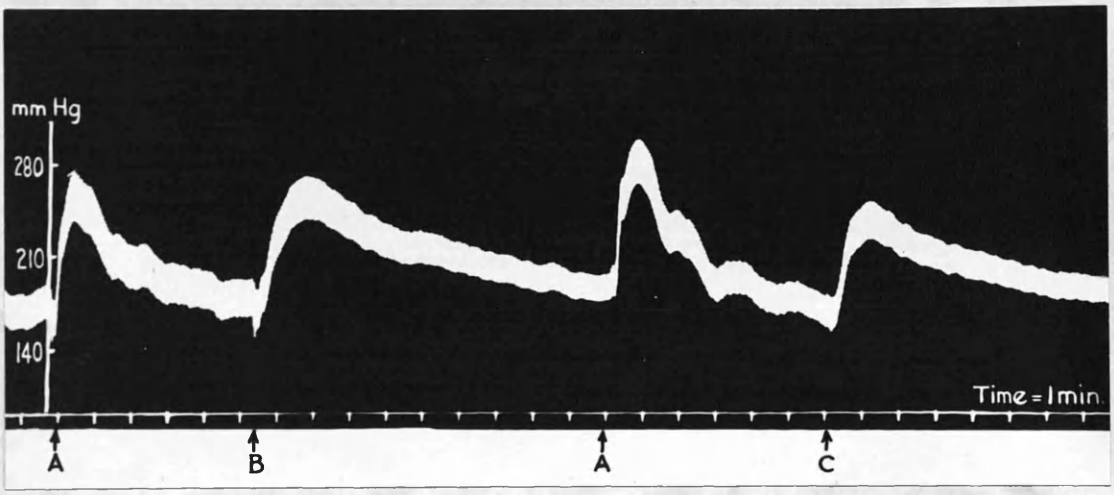

Figure 91. Cat. Chloralose anaesthesia. Blood pressure record from common carotid artery. Drugs administered intravenously followed by 4 ml. saline in each case.

At A, noradrenaline 0.20 µg. per kg.

At B, Compound IQ, 0.20 mg. per kg.

At C, Compound IIQ, 0.50 mg. per kg.

acetylcholine (0.5 to 1.0 µg. per kg.), histamine (0.5 to 1.0 µg. per kg.), adrenaline (1.0 to 2.0 µg. per kg.) or 5-hydroxytryptamine (1.0 to 2.0 µg. per kg.).

Higher doses when given repeatedly to anaesthetised cats caused respiration to stop, and artificial respiration had to be given.

When doses of 1.0 mg. per kg. or more were given to anaesthetised or spinal cats at intervals of one quarter to one half of an hour, convulsions were caused. Convulsant activity was not shown following the first dose, but on repeated administration of each drug convulsant activity became apparent. This appeared to be a cumulative effect. Convulsions were preceded by twitching movements which later became clonic convulsions. There were periods of quiet between the convulsions.

8. Mouse Test.

In mice Compounds I, II and VIII showed potent convulsant activity. These compounds were roughly equipotent with leptazol. The mice showed tonic-clonic convulsions with maximal hindleg extensor spasm.

CHAPTER IV

DISCUSSION

The compounds investigated in this section show little or no neuromuscular blocking activity in the cat gastrocnemius muscle-sciatic nerve preparation, but they block neuromuscular transmission in the isolated rat diaphragm and frog sartorius preparations, although the potency is very low. The lack of neuromuscular blocking activity in the cat may be a manifestation of the species variation in sensitivity which is so common in the neuromuscular blocking agents[1,2]. (This is discussed in Chapter IV, page 294 of this thesis.) The preparation of the quaternary salts of Compounds I and II increased their potency on the rat diaphragm and frog rectus muscle, and showed that quaternization of the tertiary bases had increased the neuromuscular blocking potency. Compound II is less potent than Compound I which would indicate that transposition of the ethoxy and methoxy groups had altered activity.

All the tertiary bases were convulsants and had convulsant activity in mice, as well as in anaesthetised and spinal cats. The quaternary derivatives also showed /

showed convulsant activity in anaesthetised and spinal cats (the convulsant activity of the quaternary salts could not be tested in mice due to insufficient supplies of the compounds). From the observation that the spinal cat had convulsions following the administration of these drugs, the site of convulsant action seems likely to be the spinal cord.

The chemical relationship between these compounds and tubocurarine and isochondrodendrine made it worth while investigating them for neuromuscular blocking activity. Some of them can be looked upon as being halved molecules of tubocurarine. In addition, they are monotertiary or monoquaternary bases, and it was of interest to see if these had any tubocurarine-like activity. On investigation no neuromuscular blocking activity was found in the cat nerve-muscle preparation. It seems that in these compounds one tertiary nitrogen or one quaternary nitrogen is not sufficient to confer neuromuscular blocking activity, but these compounds are convulsants and show some nicotine-like activity.

REFERENCES

1. Paton, W. D. M., and Zaimis, E. J., (1949),
Brit. J. Pharmacol., 4, 381.

2. Hoppe, J. O., (1955),
Anaesthesiology, 16, 91.

CHAPTER V

SUMMARY

(±)-1-(4^1-Hydroxybenzyl(-2-methyl-6-methoxy-7-ethoxy-1: 2: 3: 4-tetrahydro-isoquinoline and its dextrorotatory and laevorotatory isomers and methochloride, (±)-1-(4^1-hydroxybenzyl)-2-methyl-7-methoxy-6-ethoxy-1: 2: 3: 4-tetrahydro-isoquinoline, its laevorotatory isomer and methochloride and a berberine-like compound related to tetrahydroworenine have been tested for curare-like activity. Little activity was noted, but on the rat diaphragm and frog rectus muscle quaternisation of the racemic compounds increased this. The tertiary bases possessed potent convulsant properties.

APPENDIX I

APPENDIX I

In Table 12 (p.336) are given the formulae of the physiological saline solutions used in the experimental work described in this thesis. All the chemicals used were of "Analar" quality and only glass distilled water was used. In some cases aqueous stock solutions of certain salts were prepared to facilitate the rapid preparation of a saline solution. With the exception of sodium bicarbonate solution, these stock solutions could be used for at least two weeks after their preparation. Sodium bicarbonate stock solution was freshly prepared every three or four days. Glucose was added in the solid form to each batch of saline.

Preparation of Solutions of Rescinnamine from the Solid.

The rescinnamine was dissolved in a few drops of glacial acetic acid and sufficient distilled water added to give a final concentration of 2 mg. per ml. The pH of a solution of rescinnamine prepared in this way was about 3.0. Further dilution was made by the addition of the appropriate volume of physiological saline immediately before use.

The control solution was prepared by adding the same

volume /

volume of saline to the same volume of glacial acetic acid. The pH of the control solution was always checked and if necessary adjusted so as to correspond to that of the rescinnamine solution.

All other drugs and fine chemicals used were of the purest standard available from commercial sources.

TABLE 12.

Formulae of Physiological Saline Solutions

Salts (g. for 1 litre)	Frog Ringer's Solution	Tyrode's Solution	Locke's Solution
Sodium chloride	6.50	8.00	9.00
Potassium chloride	0.14	0.20	0.42
Calcium chloride (Anhydrous)	0.12	0.20	0.24
Sodium dihydrogen Phosphate (dihydrate)	-	0.05	-
Sodium bicarbonate	0.20	1.00	0.50
Magnesium chloride	-	0.10	-
Glucose	1.00	1.00	1.00

APPENDIX III

APPENDIX II

The Electrical Controlling Mechanism of the Semi-automatic Isolated Organ Bath.

The apparatus used was a modification of that suggested by Gaddum and Lembeck[1].

The flow of solutions into the isolated organ bath was regulated by two modified electro-magnetic relay switches (Fig.92, p.338). Solutions flowed only when these were activated. The remainder of the equipment which is illustrated diagrammatically in Figure 92 served to control the activation of the relay switches in a predetermined time cycle.

The time cycle was controlled by a Palmer time clock, supplied with a 60 volt D.C. input. The clock activated a switch relay, and thus the driving magnets of a uniselector, at 5 seconds intervals. The sweeper arms of the uniselector which were in two halves, thus giving a continuous activation over the semicircular bank of contacts, advanced one contact every 5 seconds. Two banks of contacts with 25 terminals in each, were connected and the opposite halves of the upper and lower sweeper arms were removed, so that the upper bank was activated /

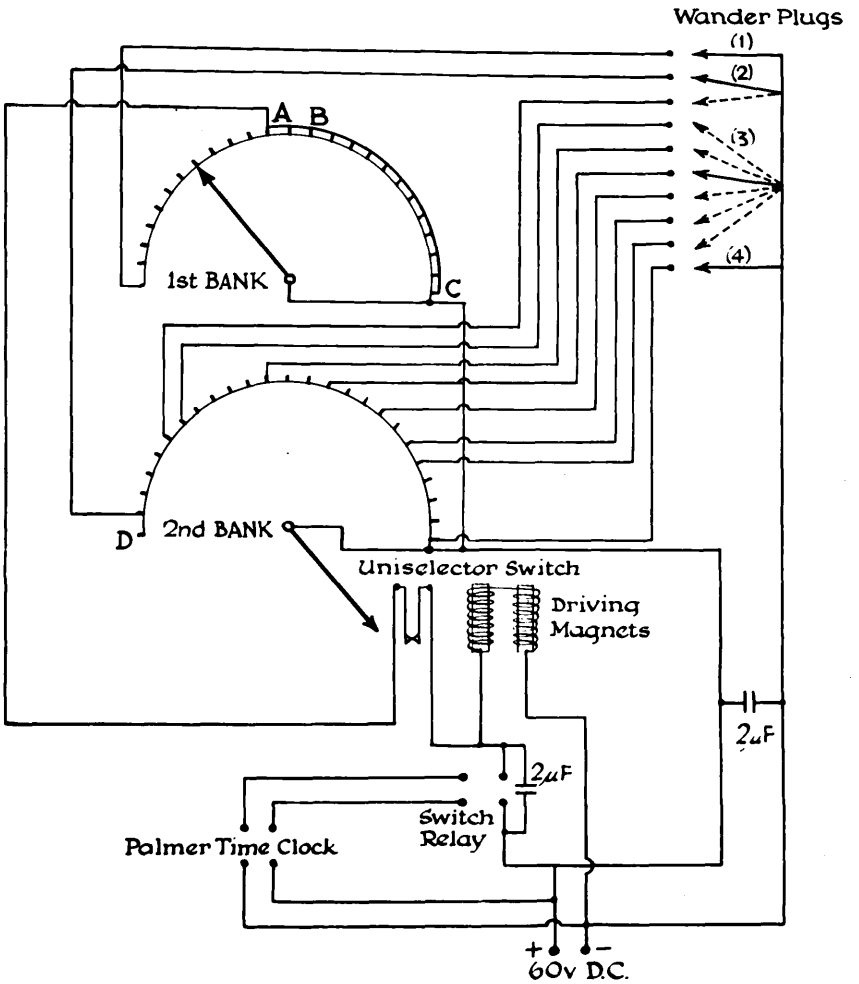

Figure 92. Circuit diagram of the electrical equipment used to control the semi-automatic isolated organ bath (see Fig. 3, page 30).

activated for half a revolution of the sweeper arm, while the second bank was activated for the next half of a revolution. The two banks thus served effectively as one complete circle of 50 contacts. To obtain a complete revolution of the sweeper arms in 3 minutes, 14 contacts in one bank were short circuited as illustrated in Figure 92. When the first bank sweeper arm reached contact A, positive current was fed through the uniselector switch (which was closed) to the driving magnets of the uniselector and moved the wiper arm, at the same time opening the uniselector switch. The first bank sweeper arm was moved to contact B and the process was repeated. This sequence of events was repeated very rapidly until the sweeper arm reached contact C (covering 14 terminals). At this stage, the sweeper arm of the second bank had reached point D. Thus 14 contacts were effectively removed from the circuit since, in practice, the time taken for the sweeper arm to pass from point A to C on the first bank was only a fraction of a second.

Certain contacts on both banks were connected to wander plugs. Negative current was led to the two modified relay switches and two indicator lights. Each was fitted with a connection to the wander plug board. By placing /

placing these plugs into the appropriate sockets (which were fed with negative current via the contacts of the uniselector), the events in the three minute cycle could be pre-set. The plugs were connected as follows:

Wander plug number 1 was connected to an indicator light which lit 5 seconds before the inflow of a stimulant drug solution.

Wander plug number 2 was connected to a light indicating the time for addition of an antagonist.

Wander plug number 3 was connected to the relay switch controlling the inflow of fresh physiological saline. This could be pre-set to take place 15, 20, 30, 45, 60 or 90 seconds after the inflow of a stimulant drug.

Wander plug number 4 was connected to the relay switch controlling the inflow of a stimulant drug.

REFERENCES.

1. Gaddum, J. H., and Lembeck, F., (1949).

Brit. J. Pharmacol., 4, 401.

These are described in Appendix III
and Appendix IV.

ADDITIONAL STUDIES

These are described in Appendix III
and Appendix IV.

APPENDIX III

SOME ASPECTS OF THE INFLUENCE OF pH ON DRUG ACTION.

APPENDIX III

I N T R O D U C T I O N

The influence of changes in the hydrogen ion concentration of the bath fluid upon the reactions to drugs of preparations of smooth muscle is of much interest and has been studied in some detail. In 1923 Evans and Underhill[1], using preparations taken from the intestine and uterus of the guinea pig, rabbit and cat, showed that the addition of dilute acid to the bath fluid always caused relaxation and the addition of alkali contraction. They also showed that the response of intestinal and uterine smooth muscle to stimulant drugs was reduced in a bath fluid of acid reaction and was abolished at pH 6. Gaskell[2] and Bayliss[3] investigated the reaction of vascular smooth muscle to small changes of pH and observed vasodilatation in the frog following the use of a dilute solution of lactic acid, and constriction with alkalis[3]. Dixon[4] investigated the reaction of intestinal smooth muscle over a greater range of pH values and stated that dilute lactic acid solutions produced relaxation of frog stomach. Fordon[5] observed that in preparations of the mammalian uterus the presence of alkali augmented tonus and caused

a diminution of the magnitude and frequency of the spontaneous contractions. Wild and Platt[6] found that acidity caused vasoconstriction in the frog but that very dilute acid sometimes caused a preliminary dilatation. Fleisch[7] perfusing the hind limb of the frog confirmed that treatment with very dilute acids caused the arterial smooth muscle to relax and that the use of a stronger solution caused it to contract. Hooker[8] observed 'rhythmic' intestinal muscle and 'non-rhythmic' vascular muscle to relax when treated with acid, while 'non-rhythmic' intestinal muscle was found to contract when treated with alkali. That acids as well as alkalis can cause vasoconstriction was shown by Ishikawa[9] who used the perfused hindquarters of the frog, and Frankel and Morita[10] showed that smooth muscle always contracts when treated with alkali, while there was usually a relaxation when acid was used. McSwiney and Newton[11] using preparations of the stomachs of rats, rabbits and cats showed that (a) moderate changes of the pH of the physiological saline solution towards the acid side (from pH 7.5 to pH 5.9) caused relaxation, and that with a further decrease in the pH to 2.4 there was contraction and finally relaxation, (b) moderate and

larger /

larger changes towards the alkaline side from pH 7.5 to pH 11.5 caused contraction and a further increase in pH was followed by relaxation.

These studies clearly indicate that the reaction of intestinal and vascular smooth muscle is very much influenced by changes in the hydrogen ion concentration of the bath fluid or perfusion fluid. While it is clear that the response of preparations of smooth muscle varies with the pH of the physiological saline solutions used, it is also to be expected that the response of these preparations to different drugs will be modified by changes in the alkalinity or the acidity of the perfusion fluid. Although it has been shown that the response of intestinal uterine and smooth muscle to pilocarpine and to histamine was reduced or abolished[1] according to the degree of acidity of the bath fluid yet there appears to be little or no published work upon this particular aspect of the mode of action of drugs. Recently Gillis[12], during studies on the mode of action of reserpine on the guinea pig ileum, noticed that the response of the tissue to various stimulant drugs was modified by the addition of the drug in an acid solution (pH 4). The reaction of the solution of reserpine which he used was also on the

acid /

acid side of neutrality. It seemed necessary, therefore, to investigate the response of smooth muscle preparations, especially those of the guinea pig ileum, to stimulant drugs such as acetylcholine, histamine etc. at different pH values. As a preliminary study it was decided to investigate the effect of acetylcholine and histamine on the guinea pig ileum in bath fluids having both acid and alkaline reactions.

This investigation was carried out as a preliminary to studies on the properties of rescinnamine and to aid in the interpretation of the results obtained by Gillis and Lewis[13] using reserpine. It seemed necessary to carry out this work since there was an obvious and significant variation in the response of the guinea pig ileum seen when reserpine and reserpine antagonists were used in more acid solution. It was noted for example that certain reserpine antagonists were rendered more potent when applied in a more acid solution. This effect might be due to potentiation of the response of the preparation to the stimulant drug or it might be due to an effect upon ionization, since it is well known that the non-ionized form of drugs are able to penetrate tissue better than the ionized ones. If increased

acidity /

acidity suppressed ionization, then the effect of the antagonist might be explained in this way.

Methods and Materials.

A piece of terminal guinea pig ileum was set up in a 2 or 5 ml. bath in a manner similar to that described on page 29 of this thesis. The perfusion fluid used was Tyrode's solution (see Appendix I, page 336), the pH of which was from 7.7 to 8.0, and this solution is referred to, for convenience, as "normal" Tyrode's solution. In order to alter the pH of the "normal" Tyrode's solution, hydrochloric acid (N/10, N or 5N solution) or sodium hydroxide (N solution or fused pellets) were used. The pH of the solution was adjusted to the required value by adding the minimum amount of acid or alkali and checked against a glass electrode using a Muirhead pH meter.

The stimulant drugs used in these experiments were acetylcholine chloride (acetylcholine) and histamine acid phosphate (histamine) and were employed in the dose range of from 0.01 to 0.20 µg. per ml. and the antagonists were atropine sulphate (atropine) and mepyramine maleate (mepyramine), used in the dose range of from 0.0001 to 0.005 µg. per ml. Acetylcholine and histamine were added /

added to the bath at three minute intervals from a semi-automatic apparatus (see Appendix II, page 337) and the bath fluid containing the drug was replaced and the tissue washed by means of "normal" Tyrode's solution thirty seconds after the drug solution was added to the bath. Solutions of atropine and mepyramine were added one minute before the inflow of the solutions of acetylcholine or histamine.

Standard reproducible contractions of the ileum were obtained to acetylcholine or histamine in "normal" Tyrode's solution. Tyrode's solution containing the same concentration of the drug but adjusted to different pH levels was then allowed to run into the bath and the experiment repeated at the new pH. The pH of the Tyrode's solution used to wash out the bath was within the "normal" range (7.7 to 8.0), but in some cases the pH was adjusted to that of the acid or alkaline Tyrode's solution containing the drug.

Demonstration of Drug Antagonisms and the Influence of pH Changes upon These.

Standard reproducible submaximal contractions of the ileum were obtained to acetylcholine or histamine (0.01 to 0.10 µg. per ml.) using "normal" Tyrode's solution and atropine /

atropine antagonism or mepyramine antagonism was demonstrated in the conventional way. Antagonists were added to the bath one minute before the inflow of Tyrode's solution containing acetylcholine or histamine. The experiments were then repeated in an exactly similar manner, but using Tyrode's solution at different pH values.

In some experiments dose-response curves were obtained. Drugs at different dose levels (acetylcholine or histamine) were added at three minute intervals to the bath containing the modified Tyrode's solution by means of a tuberculin syringe. Responses were observed for thirty seconds after which the tissue was washed with Tyrode's solution having the same pH as that used to dissolve the drug.

The effect of alterations of bath fluid pH without addition of drug was also studied. The tissue was allowed to remain in contact with the Tyrode's solution at different pH values for varying periods of time (two to ten minutes) and the response was observed.

RESULTS. /

RESULTS.

A. Acetylcholine.

(1) Effects of Altering the pH from Normal (7.7 to 8.0) to a Range of Values Between pH 2.1 and pH 7.5 or From pH 8.2 to pH 9.6.

(a) Raising the pH (Increased Alkalinity). As a rule the height of the contractions was increased (Fig. 93, p.350) but once or twice no modification from the normal was observed. This increase in magnitude was seen at all levels of pH from 7.8 to 9.6. The effects were reversible.

(b) Lowering the pH (Increased Acidity). When the pH was lowered gradually from normal (pH 7.7 to 8.0) to pH 5.8, the height of the contraction was usually increased and it was observed to remain steady at the new level (Figs. 94 and 95, pages 351 and 352). When the pH was further reduced to values of from pH 5.8 to pH 5.1, the first few contractions were greater than the control contractions but gradually the height was decreased and ultimately fell below the control level (Fig. 95). The effect was reversible on changing the bath fluid pH to normal (i.e. washing and replacement with "normal" Tyrode's solution. /

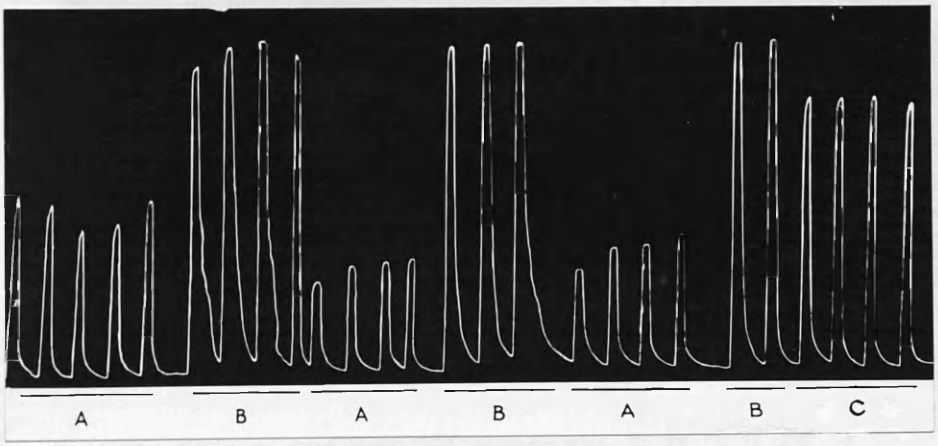

Figure 93. Isolated guinea pig ileum.

All contractions produced by acetylcholine, 0.20 µg. per ml.

At A, the pH of the bath fluid was 8.0

At B, " " " " " " " 9.4

At C, " " " " " " " 9.0

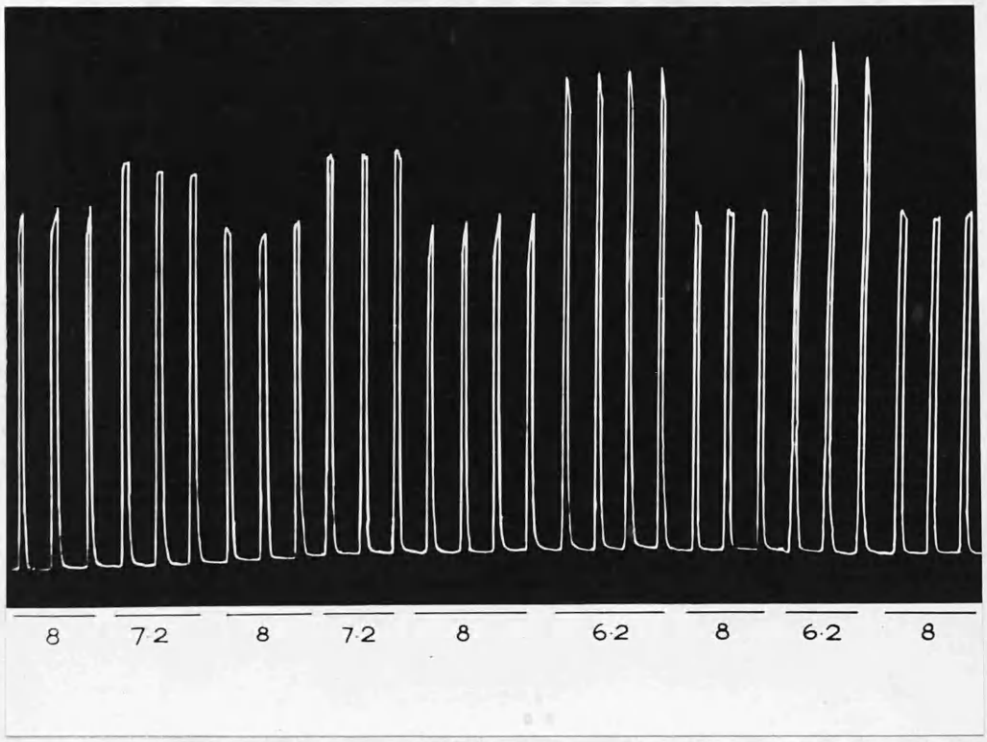

Figure 94. Isolated guinea pig ileum.

All contractions produced by acetylcholine, 0.15 µg. per ml. The figures at the bottom of the recording indicate the pH of the bath fluid.

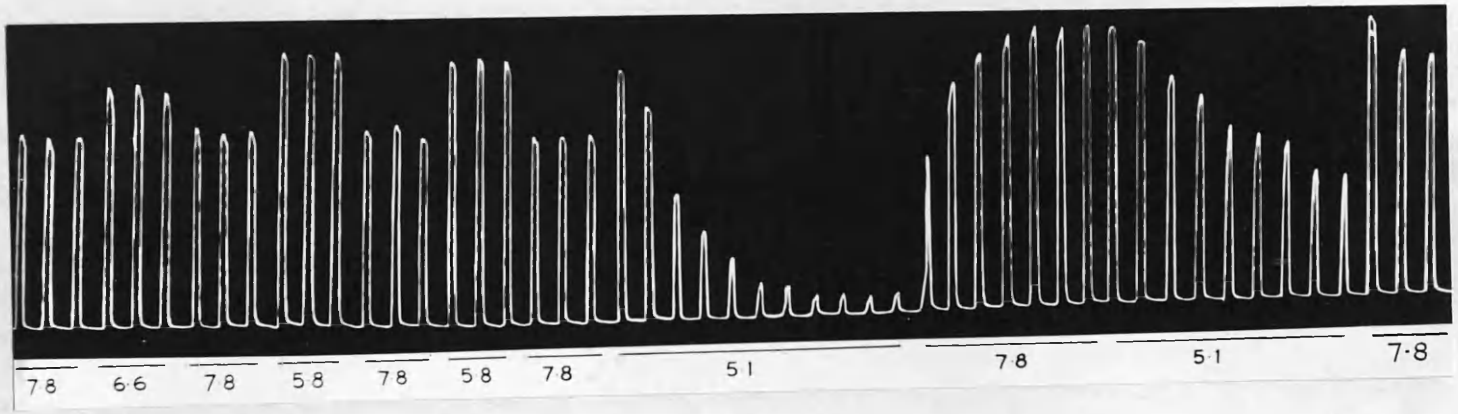

Figure 95. Isolated guinea pig ileum. All contractions produced by acetylcholine, 0.20 µg. per ml. The figures at the bottom of the recording indicate the pH of the bath fluid.

solution). No spontaneous activity or reduction of tone was noticed at any pH level between pH 7.7 and pH 5.1. Lowering the pH from pH 5.1 to pH 3.0 caused a gradual reduction in the height of the contraction and there was little or no spontaneous activity. The effect was still reversible on washing with "normal" Tyrode's solution. A reduction of pH to a level below pH 3.0 appeared to cause some irreversible change in the tissue and spontaneous activity became well marked. Ultimately spontaneous activity ceased and the tissue remained in a state of increased tone.

The response of the tissue to different doses of acetylcholine was studied at different pH levels between pH 9.2 and pH 6.1. At any particular pH level within this range the response to acetylcholine appeared to be related to the dose used (see Fig. 96, p.354).

(ii) Atropine-Acetylcholine Antagonism.

Atropine-acetylcholine antagonism was studied using bath fluids with reactions between pH 6.2 and pH 9.2. Atropine antagonism to acetylcholine was shown at all pH levels. At both the acid and alkaline sides of neutrality, the antagonism was seen to be proportional to the dose of atropine used (Fig. 97, p. 355) despite the fact that both the /

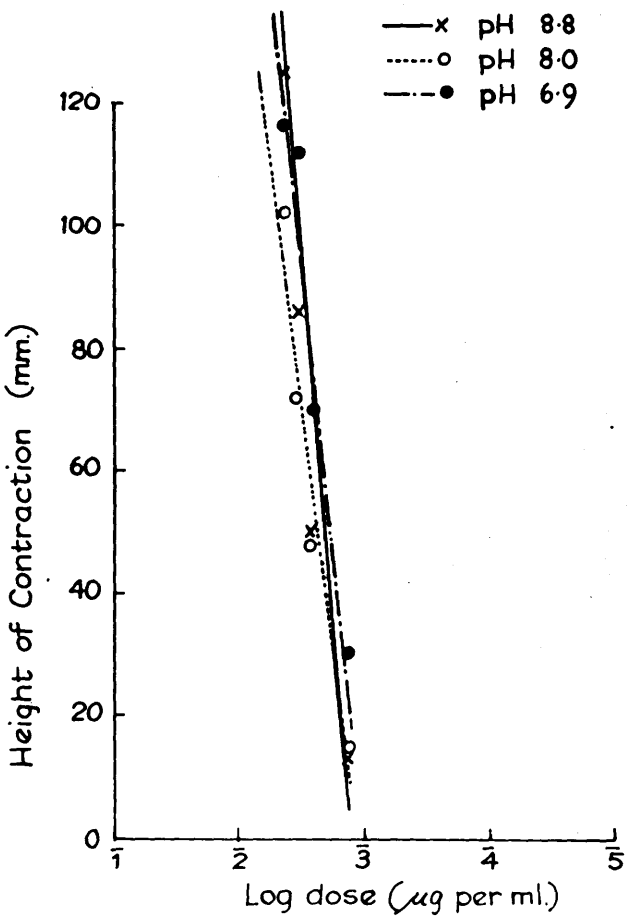

Figure 96. **Isolated guinea pig ileum.**

Relation of log dose of acetylcholine (abscissa) to the height of contraction (ordinate) at pH 8.8, 8.0 and 6.9.

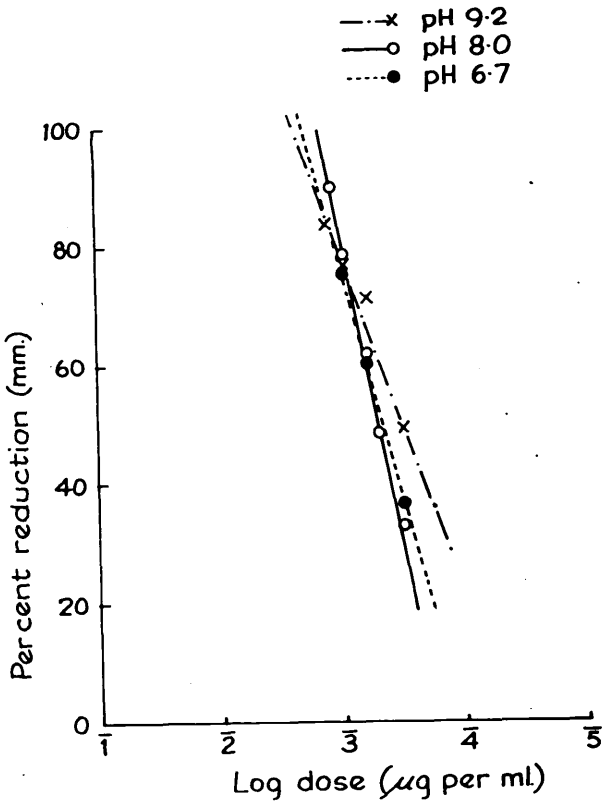

Figure 97. Isolated guinea pig ileum.

Log dose of atropine (abscissa) at pH 9.2, 8.0 and 6.7 plotted against percentage reduction (ordinate) of the height of contraction produced by the same dose of acetylcholine.

the lower and higher levels of pH increased the height of the contractions.

B. Histamine.

(i) Effects of Altering the pH From the Normal (7.7 to 8.0) to pH 5.3 on the Acid Side and to pH 9.2 on the Alkaline side of Neutrality.

(a) Raising the pH. This always increased the height of the contractions (Fig. 98, p. 357). No spontaneous activity was seen. Increase in the height of the contractions occurred at all pH levels on the alkaline side of neutrality within the range of pH 8.0 to pH 9.6. The effect was reversible after washing with "normal" Tyrode's solution.

(b) Lowering the pH. This always reduced the height of contractions, an effect seen at all pH levels from pH 7.4 to pH 5.3 (Fig. 99, p.358). No spontaneous activity was seen but at pH 6.8 or lower the tonus of the muscle appeared to be reduced and histamine-induced contractions could not be maintained (Fig. 99). When the pH of the bath fluid was lowered from normal pH to the acid side, the reduction in the height of contraction produced by /

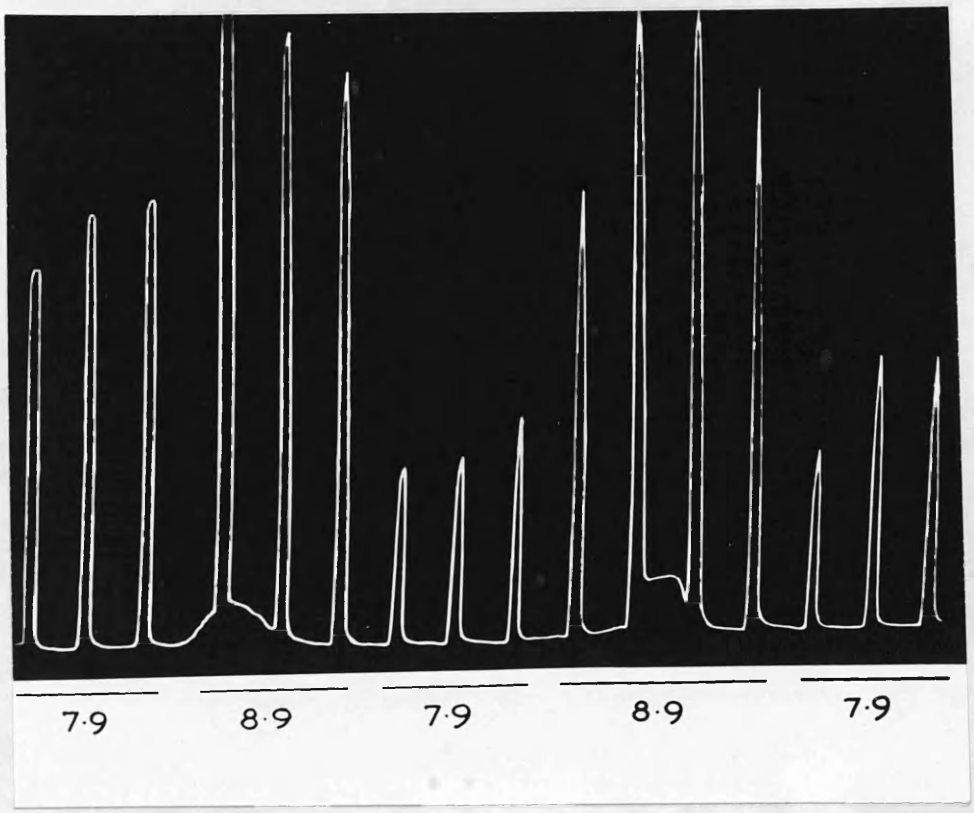

Figure 98. Isolated guinea pig ileum.

All contractions produced by histamine 0.10 µg. per ml. Figures at the bottom of the recording indicate the pH of the bath fluid.

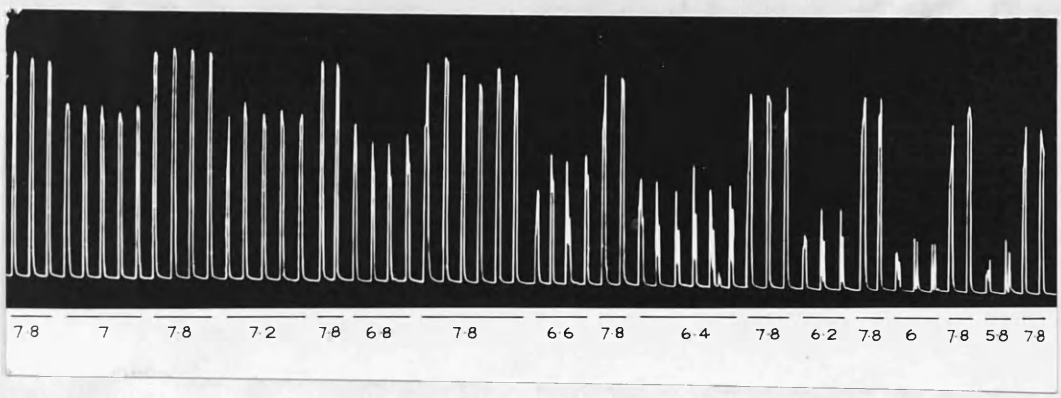

Figure 99. Isolated guinea pig ileum.

All contractions produced by histamine 0.50 µg. per ml. Figures at the bottom of the recording indicate the pH of the bath fluid.

by the same dose of histamine appeared to be related to the pH levels and this relationship was linear up to pH 6.0 (Fig. 100, p.360). No such linearity was observed to the increase in the height of contraction when the pH was raised.

A graded dose-response effect was observed at different pH levels ranging from pH 6.5 on the acid side and to pH 9.0 on the alkaline side of neutrality (Fig. 101, p. 361).

(ii) Histamine-Mepyramine Antagonism.

Antagonism by mepyramine to the response to histamine was obtained at pH levels from pH 6.5 to pH 9.0. At higher pH levels the contractions were increased in magnitude, and at lower pH levels there was a decreased response. In all cases there was inhibition of histamine induced contractions by mepyramine, although the inhibition appeared not to be proportional to the dose used.

C. The Effect of Altering Bath Fluid pH without Addition of Drugs.

No direct effect was observed when the tissue was suspended in Tyrode's solutions with reactions of from pH 9.6 to pH 5.0. No change in the activity was caused by sudden /

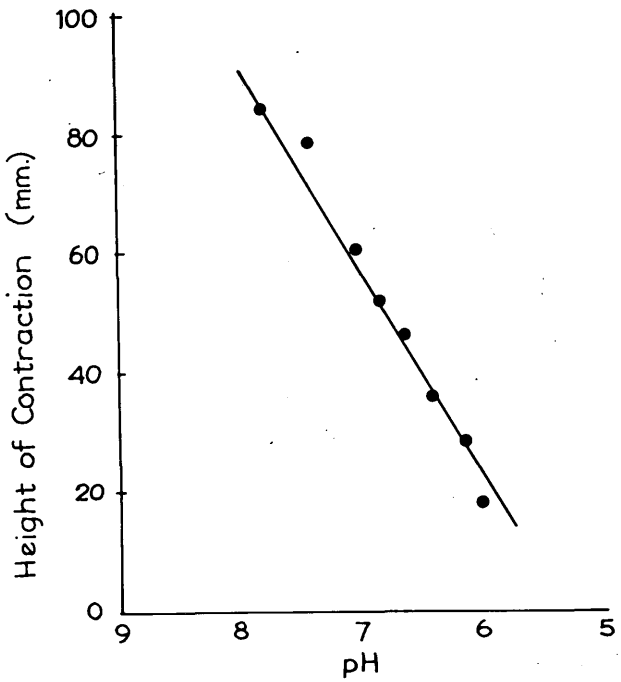

Figure 100. Isolated guinea pig ileum.
Relation of pH of the bath fluid (abscissa) to the height of contraction (ordinate) produced by a given dose of histamine.

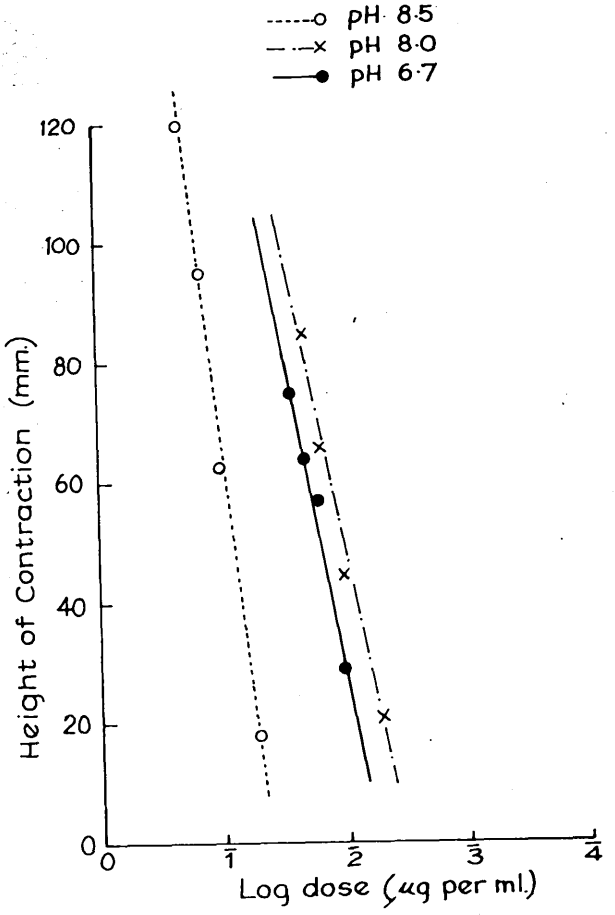

Figure 101. Isolated guinea pig ileum.

Relation of log dose of histamine (abscissa) to the height of contraction (ordinate) at pH 8.5, 8.0 and 6.7.

sudden changes of bath fluid pH from pH 9.6 or pH 5.0 back to a normal value (pH 7.7 to 8.0) or from normal to pH 9.6 or pH 5.0. When the tissue was kept in contact with Tyrode's solution with a reaction of less than pH 5.0 for one or two minutes and then the pH suddenly changed to normal pH (7.7 to 8.0), there was a spontaneous contraction which disappeared on washing with "normal" Tyrode's solution. If the tissue was treated with Tyrode's solution with a pH between 9.8 and 11.0 there was a large spontaneous contraction which was always reversible on washing with "normal" Tyrode's solution (Fig.102,p.363). These contractions due to a high pH of the bath fluid were reproducible and were not antagonised by atropine, mepyramine, lysergic acid diethylamide, papaverine hydrochloride, hexamethonium bromide or cinchocaine hydrochloride (Fig.103, p.364). At pH 12.2, a spontaneous contraction was obtained but this was reduced in magnitude.

DISCUSSION

The effects of changes in the reaction of the bath fluid upon the activity of preparations of smooth muscle has been investigated by many workers. It is known that moderate changes of pH towards the acid and alkaline sides cause /

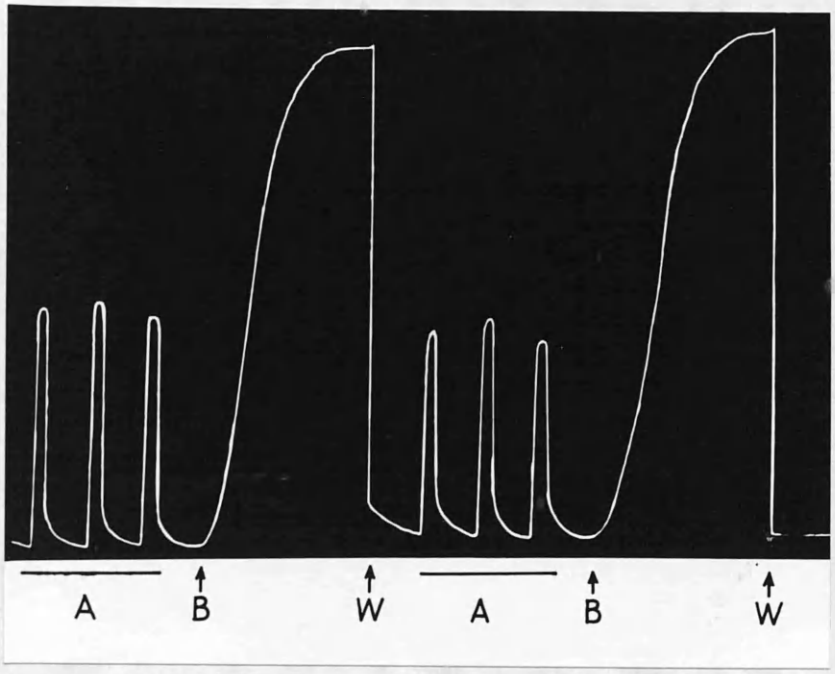

Figure 102. Isolated guinea pig ileum.

At A, contractions produced by acetylcholine, 0.20 µg. per ml. at pH 8.0.

At B, spontaneous contraction of the ileum at pH 9.9.

At W, wash out with normal Tyrode's solution.

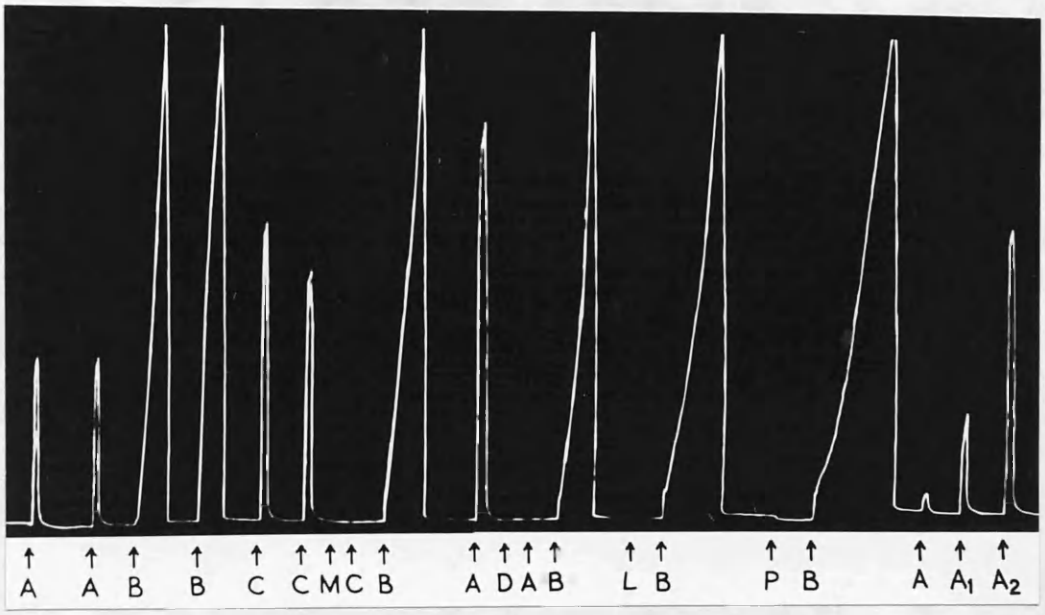

Figure 103. Isolated guinea pig ileum.

At A, acetylcholine 0.01 µg. per ml. at ph 8.0

At B, spontaneous contractions at pH 10.5

At C, histamine 0.05 µg. per ml. at pH 8.0

At M, mepyramine 0.10 µg. per ml. at pH 8.0

At D, atropine 0.10 µg. per ml. at pH 8.0

At L, lysergic acid diethylamide 0.10 µg. per ml. at pH 8.0

At P, papaverine 2.0 µg. per ml. at pH 8.0

At A_1 and A_2 acetylcholine 0.10 and 0.50 µg. per ml. respectively at pH 8.0.

cause relaxation and contraction[1,11,14] of the smooth muscle respectively, whereas large changes of pH towards the acid and alkaline sides always cause contraction[11]. Evans and Underhill[1] and McSwiney and Newton[13] have shown that rhythmic contraction of the smooth muscles of the intestine are slowed and depressed by acid and are quickened in rate and depressed in amplitude when the reaction becomes alkaline.

In an attempt to investigate the response of intestinal smooth muscle to drugs at different pH levels, moderate and large alterations have been made towards both the acid and alkaline sides of the normal pH of the physiological saline solution. At higher pH levels the responses of the guinea pig ileum to both acetylcholine and histamine are increased, i.e. they are greater than those produced with the same dose of acetylcholine or histamine at the "normal" pH value. At lower pH levels the magnitude of the acetylcholine contraction is increased, while that induced by histamine is decreased. Change in the pH of the bath fluid to a level as high as pH 9.6 or as low as pH 3.5 appears to have no direct effect on the smooth muscle of the guinea pig ileum, but the change in pH definitely alters the sensitivity to acetylcholine and histamine /

histamine and perhaps also to other drugs. Increased sensitivity of the tissue to acetylcholine and decreased sensitivity to histamine at lower pH levels may be due to the different mode of action of these two drugs upon the muscle cells. It has been pointed out by McSwiney and Newton[11], and Evans and Underhill[1] that alterations of pH have no effect on the local nervous mechanism but it is difficult to establish the validity of such a conclusion. Atzler and Lehman[16] suggested that hydrogen ions acted directly on the muscle protein and McSwiney and Newton[11] suggested that the reaction of the smooth muscle to changes of pH was of a physico-chemical nature. None of these assumptions appears to explain the difference in response to the ileum - suspended in bath fluids of lower pH to histamine and acetylcholine. Whatever may be the cause of this difference in response of the tissue to histamine and acetylcholine, a change in pH of the fluid bathing the tissue is undoubtedly an important factor in determining the response of the tissue to these drugs and should be taken into consideration in studying the action of drugs upon isolated tissues.

Although the sensitivity of the tissue to drugs is changed when pH is altered, the physiological functions of /

of the tissue do not appear to be irreversibly changed within the limit of the pH levels used. This is shown by the fact that in bath fluids of higher and lower pH the response of the tissue remains proportional to the dose of the drug used, and atropine and mepyramine show a graded antagonism to the responses to acetylcholine and histamine.

The spontaneous contraction of the ileum when suspended in bath fluids of higher pH levels is an interesting phenomenon because it is not inhibited by the usual antagonists which suggests that the mechanism of action may be of physico-chemical in nature involving a direct action on the muscle protein as suggested by Atzler and Lehman[16].

S U M M A R Y

Alteration of the reaction of the bath fluid from normal (pH 7.7 to 8.0) to pH 4.0 on the acid side or to pH 9.6 on the alkaline side has little or no direct effect upon the smooth muscle of the isolated guinea pig ileum.

No direct effect is observed when the pH of the bath fluid is lowered to pH 3.0, although there is a spontaneous /

spontaneous contraction on washing with "normal" Tyrode's solution. A further increase in alkalinity to pH 11.0 induces a large contraction, the magnitude of which becomes less if the pH is subsequently increased to 12.2. These changes in the activity of the tissue are reversible on washing with "normal" Tyrode's solution.

At higher levels of pH (increased alkalinity) the sensitivity of the ileum to acetylcholine and histamine is increased.

At lower levels of pH (increased acidity) the sensitivity of the tissue to acetylcholine is increased but the sensitivity to histamine is reduced.

Within the limit of a change from pH 9.6 to pH 6.1 there appears to be no irreversible change in the tissue.

Changes in pH of the fluid bathing the tissue is an important factor in the response of the tissue to drugs and should always be taken into account.

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A P P E N D I X IV

THE PHARMACOLOGY OF THE TOTAL ALKALOIDS OF GREENHEART (NECTANDRA RODIOEI).

APPENDIX IV

In 1843, Maclagan[1] investigated the constituents of greenheart bark (Nectandra rodioei), and isolated from it two amorphous alkaloid fractions which he named sepeerine and bebeerine. From the total alkaloids of greenheart Grundon[2] obtained two crystalline alkaloids for which the names sepeerine and ocotine were given.

The pharmacological properties of the alkaloids of greenheart have been reported by Pindel et alia[3], who have shown that although the crystalline dimethiodide obtained from the mixed amorphous alkaloids of greenheart bark possessed curare-like activity as measured by the rabbit head drop test, the mixed unquaternized alkaloids showed no such properties in the rabbit. Somewhat similar results were also reported by McKennis et alia[4] who showed that the crystalline methiodide of greenheart alkaloids had little or no effect upon the blood pressure of pentobarbitone-anaesthetised dogs. On the other hand the mixed amorphous alkaloids caused a marked hypotension.

The mixed amorphous alkaloids of greenheart were made available to the author for further pharmacological studies. Although the mixed unquaternized greenheart alkaloid /

alkaloid showed no neuromuscular blocking activity in the rabbit, it was felt that they might have some curare-like activity in preparations from other species. It was, therefore, decided to carry out a more extensive pharmacological investigation with the total alkaloids of greenheart on different isolated tissues and on the intact cat.

The main objective was to determine whether the greenheart alkaloids, which have been reported to have structure related to tubocurarine, were potent neuromuscular blocking agents and, if this was the case, to study their properties very fully.

Materials and Methods.

The composition of the perfusion fluids used in this investigation is given in Appendix I (Table 12, p.336).

Drugs used were as follows: acetylcholine chloride (acetylcholine), adrenaline hydrochloride (adrenaline), noradrenaline bitartrate (noradrenaline), histamine acid phosphate (histamine), atropine sulphate (atropine), mepyramine maleate (mepyramine), hexamethonium bromide (hexamethonium), and tubocurarine chloride (tubocurarine).

The substance investigated was the total alkaloid fraction /

fraction isolated from greenheart bark. The alkaloids were insoluble in water but soluble in dilute hydrochloric acid to give a dark brown solution with a pH of about 3.5.

The experimental methods used in this investigation were similar to those described in chapter II of part I (page 11) and chapter II of part IIA (page 144) of this thesis.

Results.

(1) Frog Rectus Abdominis Muscle.

The greenheart total alkaloids within the dose range of from 5 to 50 µg. per ml. had no direct stimulant effect upon the rectus, but antagonised contractions induced by 0.10 to 0.20 µg. per ml. of acetylcholine (Fig. 104, p.374) or 2 µg. per ml. of decamethonium. Tubocurarine was about 30 times more potent than the greenheart alkaloids on this preparation.

(2) Rat Phrenic Nerve-Diaphragm Preparation.

There was no depression of the twitch amplitude of the diaphragm when greenheart total alkaloids in the dose range of 5 to 10 µg. per ml. were added to the bath.

(3) Cat Gastrocnemius Muscle-Sciatic Nerve Preparation.

There /

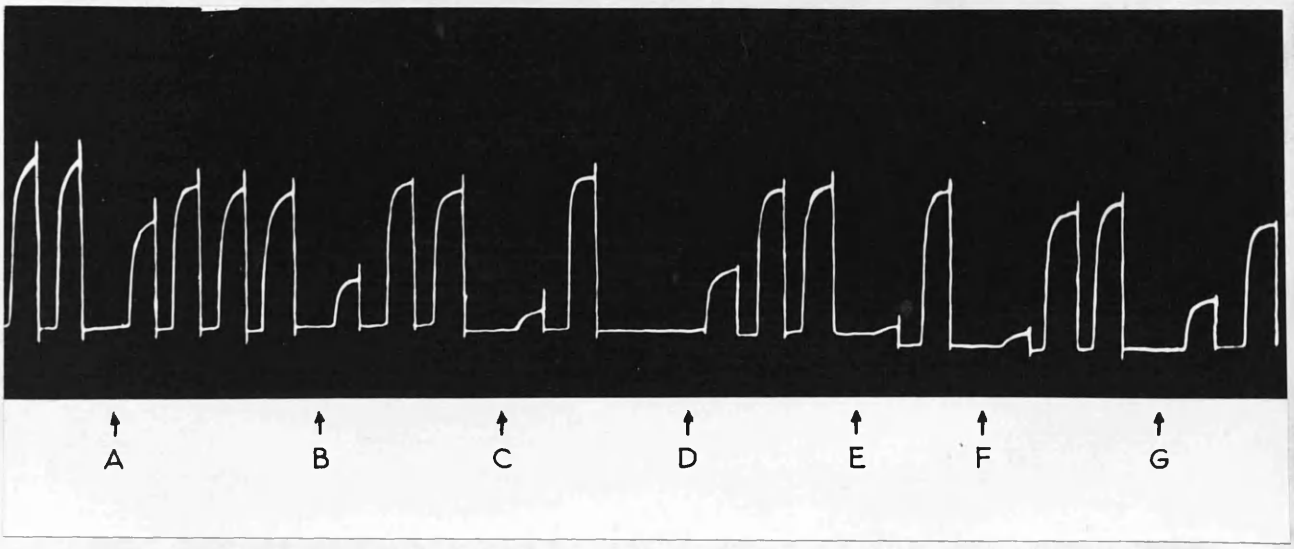

Figure 104. Frog rectus abdominis muscle.

All contractions produced by 0.20 µg. per ml. acetylcholine acting for ninety seconds.

Labelled contractions were preceded one minute earlier by

At A,	greenheart alkaloids	10 µg. per ml.
At B,	" "	20 µg. per ml.
At C,	" "	40 µg. per ml.
At D,	tubocurarine	0.50 µg. per ml.
At E,	"	1.5 µg. per ml.
At F,	"	1.0 µg. per ml.

There was no reduction in the twitch height following intravenous injection of 1 to 2 mg. per kg. of greenheart total alkaloids, but very large doses of 4 to 8 mg. per kg. of the alkaloids however caused about a 20 per cent reduction of the twitch height. The maximal depression was reached after about half an hour and recovery was not complete even after 2 hours elapsed.

(4) Rabbit Head Drop Test.

The intravenous injection of 4 to 10 mg. per kg. of the greenheart total alkaloids did not produce head drop, but a dose of 10 mg. per kg. caused death within 15 to 20 minutes. Death was associated with the sudden onset of convulsions. When a dose of 14 to 18 mg. per kg. was injected intravenously head drop was seen, but the animal convulsed and died after about 15 minutes.

(5) Guinea Pig Ileum.

The total alkaloids of greenheart in the dose range of from 50 to 100 µg. per ml. caused a direct contraction of the ileum. The stimulant action was blocked by atropine (0.50 µg. per ml.), mepyramine (0.50 µg. per ml.), and hexamethonium (20 to 50 µg. per ml.). Contractions of the ileum induced by 0.50 to 1.0 µg. per ml. of acetylcholine /

acetylcholine or 0.10 to 0.50 µg. per ml. of histamine were inhibited by 10 to 30 µg. per ml. of the total alkaloid (Fig. 105,p. 377).

(6) Rabbit Duodenum.

The total alkaloids of greenheart in doses of from 10 to 50 µg. per ml. reduced the tone and inhibited spontaneous activity of the duodenum (Fig.106a, p.378). This effect was preceded by a short period of stimulation. They also antagonised the stimulant effects of acetylcholine (0.01 to 0.05 µg. per ml.) when a dose of 20 µg. per ml. was used (Fig.106,b). The relaxant effect of adrenaline (0.04 µg. per ml.) remained unaffected when the greenheart alkaloids were added at the same dose levels (Fig. 106,c).

(7) Isolated Rat Hindquarters.

The total alkaloids of greenheart in the dose range of 20 to 200 µg. caused vasoconstriction in the isolated perfused rat hindquarters (Fig.107, p. 379).

(8) Isolated Rabbit Heart.

20 to 100 µg. of the greenheart total alkaloids reduced both the rate and amplitude of the heart (Fig.108, p.380). Atropine 100 µg. did not modify this effect and /

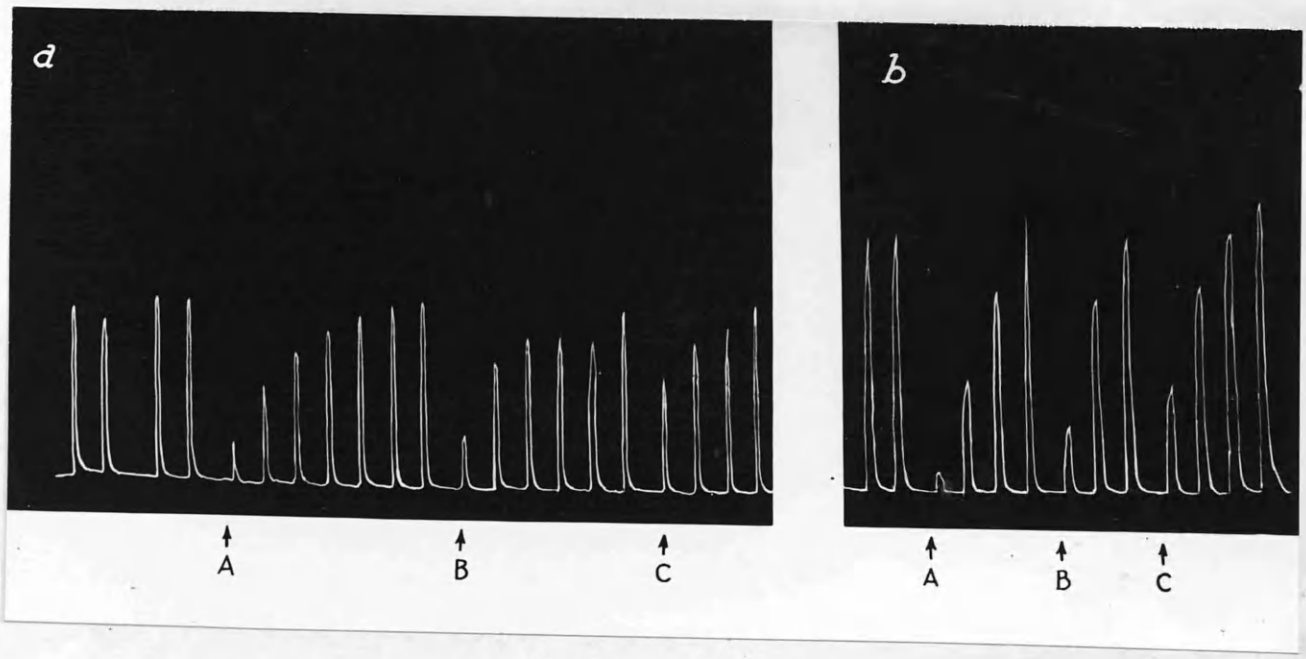

Figure 105. Isolated guinea pig ileum.

(a) All contractions produced by 0.50 µg. per ml. acetylcholine acting for 3 seconds. Addition of acetylcholine was preceded one minute earlier by

At A, greenheart alkaloids 50 µg. per ml.
At B, " " 25 µg. per ml.
At C, " " 12.5 µg. per ml.

(b) All contractions produced by 0.10 µg. per ml. histamine acting for 30 seconds. Addition of histamine was preceded one minute earlier by

At A, greenheart alkaloids 50 µg. per ml.
At B, " " 25 µg. per ml.
At C, " " 12.5 µg. per ml.

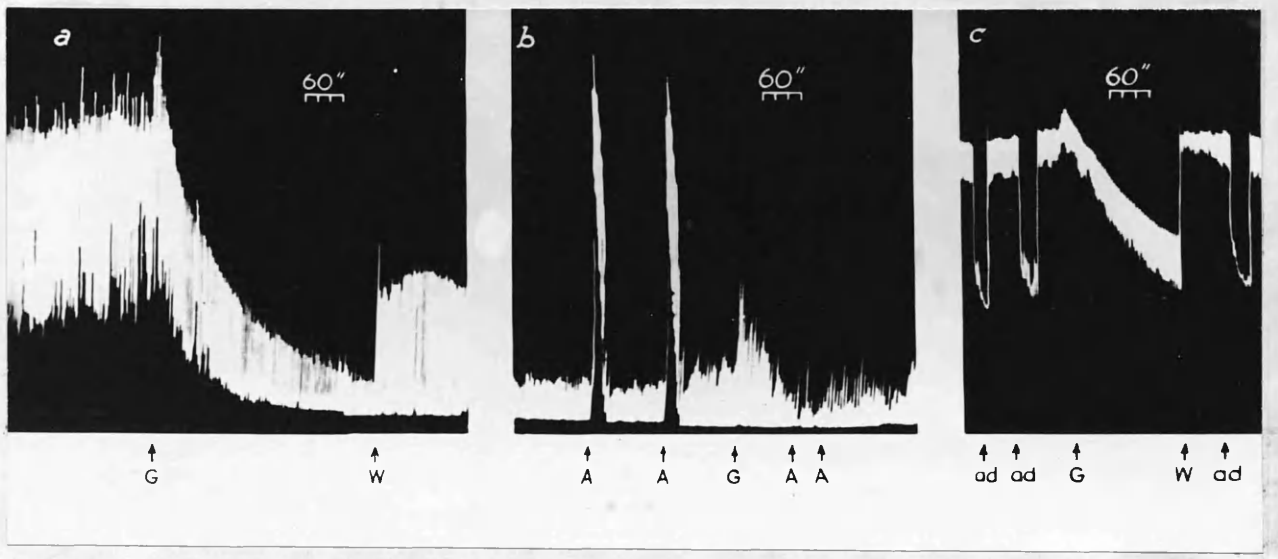

Figure 106. Isolated rabbit duodenum.

(a) At G, greenheart alkaloids 50 µg. per ml.
At W, wash out.

(b) At A, acetylcholine 0.02 µg. per ml.
At G, greenheart alkaloids 20 µg. per ml.

(c) At ad, adrenaline 0.04 µg. per ml.
At G, greenheart alkaloids 20 µg. per ml.
At W, wash out.

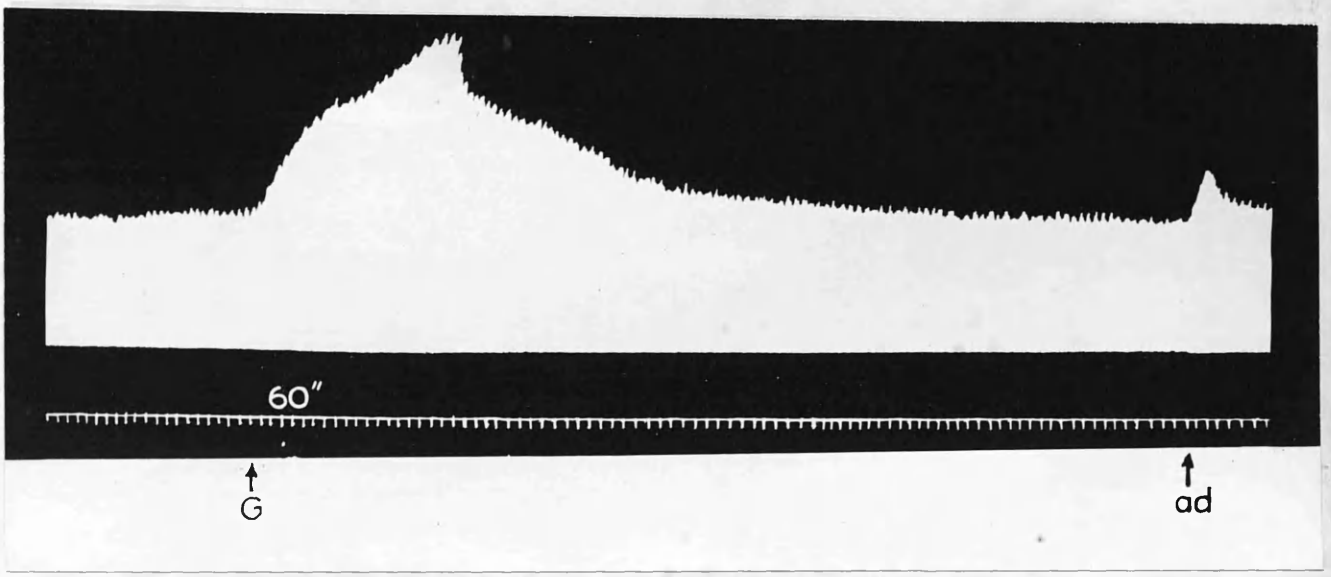

Figure 107. Isolated rabbit hindquarters.

Upstroke indicates constriction of the blood vessels.

At G, greenheart alkaloids 20 µg.

At ad, adrenaline 0.05 µg.

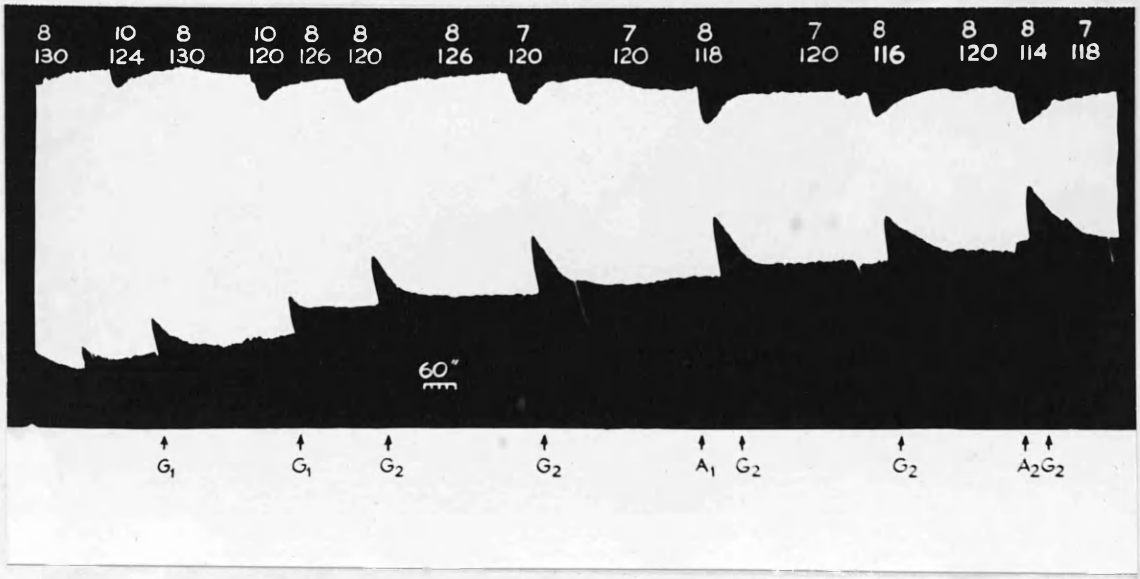

Figure 108. Isolated rabbit heart.

Figures above the recording refer to:

(a) Upper row, outflow in ml. per minute

(b) Lower row, number of beats per minute.

At G_1, greenheart alkaloids 50 µg.

At G_2, greenheart alkaloids 100 µg.

At A_1, atropine 10 µg.

At A_2, atropine 100 µg.

and the amplitude did not recover to its original level. There was no appreciable change in the output of the heart following administration of the alkaloids.

(9) Blood Pressure of the Anaesthetised Cat.

When given in the dose range of 0.20 to 1.0 mg. per kg. greenheart total alkaloids usually caused a marked fall in the blood pressure level of the anaesthetised cat (Fig. 109,a, p.382). In a few cats a rise in the blood pressure was seen (Fig.109,b). There was neither antagonism nor potentiation of the characteristic effects on the cat's blood pressure of acetylcholine (0.50 to 1.0 µg. per kg.), histamine (0.50 to 1.0 µg. per kg.), adrenaline (0.50 to 2.0 µg. per kg.), or noradrenaline (0.50 to 2.0 µg. per kg.).

The effect on the blood pressure of the anaesthetised cat was not blocked by atropine (1.0 to 5.0 mg. per kg.) or mepyramine (1.0 to 5.0 mg. per kg.).

In the spinal cats greenheart total alkaloids (0.50 to 2.0 mg. per kg.) had little or no effect upon the blood pressure level.

(10) Toxicity (in mice).

Very little effect was observed when mice were injected /

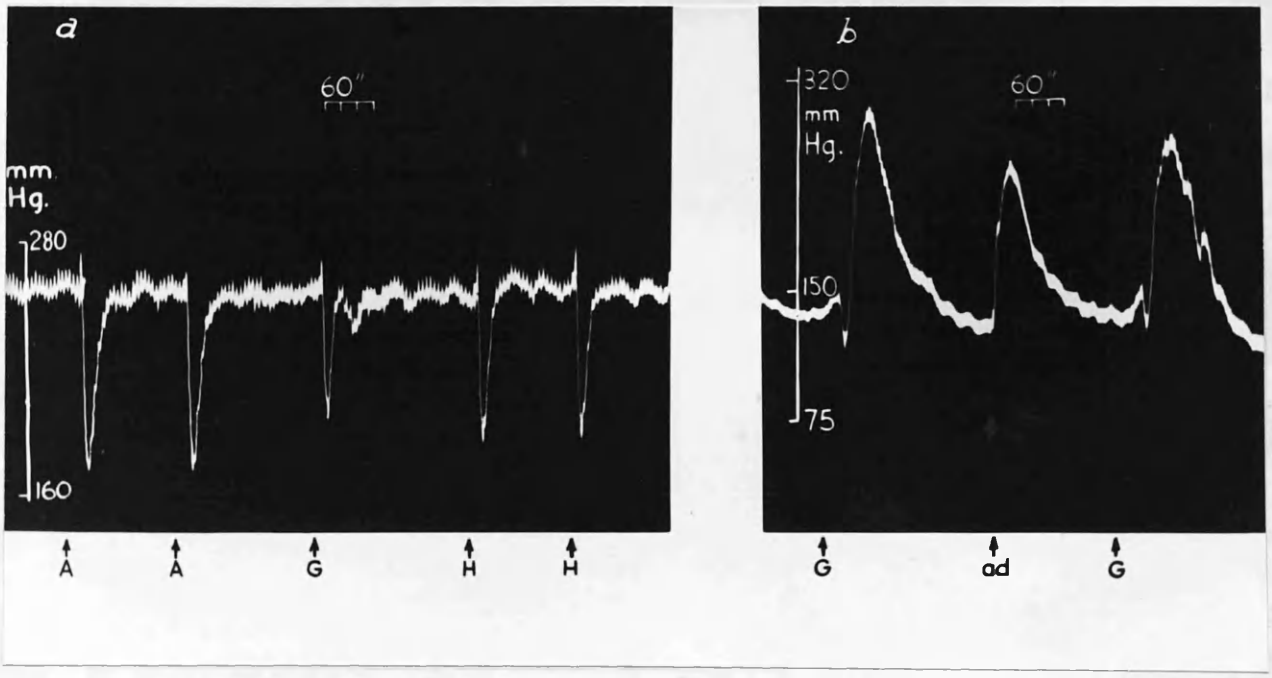

Figure 109. Cat. Chloralose anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously.

(a) At A, acetylcholine 0.50 µg. per kg.
At G, greenheart alkaloids 0.40 mg. per kg.
At H, histamine 0.50 µg. per kg.

(b) At G, greenheart alkaloids 0.80 mg. per kg.
At ad, adrenaline 0.80 µg. per kg.

injected intraperitoneally with 1.0g. per kg. of greenheart total alkaloids. 2.0g. per kg., however, killed the mice within 15 minutes. Before death there was excitement and convulsions which were perhaps due to respiratory failure.

Summary and Conclusions.

The pharmacological properties of the total alkaloid fraction of greenheart bark have been investigated on isolated tissues and in intact animals. Although they inhibit acetylcholine-induced contractions of the frog rectus, they otherwise show very little evidence of neuromuscular blocking activity on other isolated tissue preparations. In bigger doses (4 to 8 mg. per kg.) the greenheart alkaloids appear to have some depressant effects on the neuromuscular transmission in the cat gastrocnemius sciatic preparation. The alkaloids caused head drop in the rabbit only when given in lethal doses.

The total alkaloids of greenheart have some depressant activity on isolated cardiac smooth muscle and antagonise drug induced stimulation of intestinal smooth muscle. On the other hand the alkaloids themselves have some /

some stimulant activity when tested on an isolated vascular bed or on the isolated intestine.

Following the intravenous administration of the greenheart total alkaloids there was usually a fall in the blood pressure level of the anaesthetised cat.

Unfortunately the quaternized total alkaloids of greenheart were not available for testing at the time of writing this thesis nor were any of the purified single alkaloids, but it is hoped that it will be possible to complete this investigation in the future.

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SUMMARY

Studies on the Mode of Action of Pharmacologically Active Substances from Natural Sources with Additional Studies on Some Synthetic Neuromuscular Blocking Agents.

The thesis is divided into parts as follows:

Part I. Studies on the Mode of Action of Rescinnamine.

Part IIA. Studies on Some Synthetic Tris- and Tetraquaternary Neuromuscular Blocking Agents.

IIB. The Pharmacology of Some Hydroxybenzylisoquinoline Derivatives.

ADDITIONAL STUDIES.

Appendix III. Some Aspects of the Influence of pH on Drug Action.

Appendix IV. The Pharmacology of the Total Alkaloids of Greenheart (Nectandra Rodiaei).

Part I. Studies on the Mode of Action of Rescinnamine. In the normotensive anaesthetised cat, rescinnamine caused little or no fall in the level of the blood pressure. It depressed or abolished pressor responses elicited by various laboratory techniques. Hypertension in anaesthetised or spinal cats due /

due to the continuous infusion of adrenaline or noradrenaline was reduced by an injection of rescinnamine, but the pressor effects of single injections of adrenaline or noradrenaline were either unaltered or only slightly depressed.

Although its action is delayed in onset, rescinnamine has been shown to possess some ganglion blocking activity.

Rescinnamine depressed the activity of preparations of isolated cardiac muscle. In isolated vascular and intestinal smooth muscle rescinnamine reduced the magnitude of drug-induced contraction and had a direct relaxant effect on the former. In the isolated frog rectus muscle, although rescinnamine had some direct stimulant effect, acetylcholine induced contractions were inhibited. It is concluded that while the drug has a definite action upon the central nervous system, which probably contributes largely to its anti-hypertensive effect in man, peripherally induced relaxation of the vascular smooth muscle may also play a part.

Part IIA. Studies on Some Synthetic Tris- and Tetraquaternary Neuromuscular Blocking Agents.

The pharmacological properties of a new series of tris- and tetraquaternary ethonium compounds have been investigated and found to possess neuromuscular blocking activity. Some of them have a potency equal to, or greater than that of tubocurarine /

tubocurarine in the cat. Compounds in which the quaternary centres are separated by five, six or eight membered polymethylene chains had tubocurarine-like properties while others having ten membered polymethylene chains in between the quaternary centres showed typical decamethonium-like activity. Wide species variations in the potency of these compounds were noted.

It has been shown that an increase in chain length in the tris- and tetraquaternary compounds decreases the potency and alters the type of activity from being tubocurarine-like to being decamethonium-like.

The methyl analogue of dihexasulphonium, shows less activity than the ethonium analogue, although the type of activity (tubocurarine-like) remains unaltered.

Part IIB. The Pharmacology of Some Hydroxybenzylisoquinoline Derivatives.

The pharmacological properties of some hydroxybenzylisoquinoline derivatives were investigated and found to possess little or no neuromuscular blocking activity. Quaternization of the racemic compounds increased the neuromuscular blocking activity on preparations of the rat diaphragm and frog rectus muscle. The tertiary bases were found to possess potent convulsant properties.

Appendix III. /

Appendix III. Some Aspects of the Influence of pH on Drug Action.

Alteration of the reaction of the bath fluid from normal (pH 7.7 to 8.0) to pH 4.0, or to pH 9.6, has little or no direct effect upon the smooth muscle of the isolated guinea pig ileum. A further increase in alkalinity to pH 11.0 induces a large contraction, the magnitude of which becomes less if the pH is subsequently increased to 12.2. These changes in the activity of the tissue are reversible on washing with normal Tyrode's solution.

At higher levels of pH (increased alkalinity) the sensitivity of the ileum to acetylcholine and histamine is increased, whereas at lower levels of pH (increased acidity) the sensitivity of the tissue to acetylcholine is increased but the sensitivity to histamine is reduced.

Appendix IV. The Pharmacology of the Total Alkaloids of Greenheart (Nectandra Rodiaei).

The greenheart alkaloids inhibit acetylcholine-induced contractions of the frog rectus, but they show very little evidence of neuromuscular blocking activity on other isolated tissue preparations. In bigger doses (4 to 8 mg. per kg.) the greenheart alkaloids appear to have some depressant effects upon neuromuscular transmission in the cat gastrocnemius muscle-sciatic nerve preparation. The alkaloids caused head drop /

drop in the rabbit only when given in lethal doses.

The total alkaloids of greenheart have some depressant activity on isolated cardiac muscle and antagonise drug-induced stimulation of intestinal smooth muscle. They usually caused a fall in blood pressure level of the anaesthetised cat.